IFCC-WORLDLAB BERLIN 2011

21st International Congress of Clinical Chemistry and Laboratory Medicine
19th IFCC-EFCC European Congress of Clinical Chemistry and Laboratory Medicine
8th Annual Meeting of the German Society of Clinical Chemistry and Laboratory Medicine

Berlin, Germany, 15-19 May 2011

ICC Berlin, International Congress Center

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
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CLINICAL CHEMISTRY AND LABORATORY MEDICINE

DE GRUYTER

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PLENARY LECTURE ABSTRACTS

Molecular Tumor Diagnostic

Monday, 16 May 2011   11:30–12:45

THE POTENTIAL IMPACT OF INDIVIDUALIZED THERAPY APPROACHES ON PEDIATRIC CANCER CLINICAL RESEARCH: TECHNOLOGY VALIDATION AND GLOBAL ACCESS

G. H. Reaman

Abstract not received

CANCER GENE EXPRESSION: A SYSTEMS BIOLOGY APPROACH TO BIOMARKER AND DRUG DEVELOPMENT

W. Nicol Keith
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One of the fundamental changes required for tumourigenesis is escape from cellular senescence. Strategies to induce senescence in cancer cells might provide future therapies complementary to existing interventions aimed at apoptosis. Progress toward senescence targeted drug discovery could be accelerated by applying novel screening technologies and in particular, cell-based screening approaches to identify and validate small molecule agonists/effectors or stabilisers of senescence.

Whilst there is value in examining senescence in isolation, there may be benefits in considering it as part of an interconnected matrix of signalling pathways which together are capable of driving or restraining tumour development. Thus senescence can be viewed in the context of biological processes including apoptosis and autophagy. In tumour development there may be a dynamic balance of signalling pathways modulating cell death and senescence. During cancer progression, senescence bypass signalling may dominate. Through intervention, a shift to triggering or upregulating senescence signalling may dominate.

Our approach is based on integrating within a bioinformatic framework a wide variety of external and in-house data sources with high content screening technologies including cell-based assays, to deconvolute senescence signalling pathways and build a network of genes for target and biomarker discovery. At present, telomerase represents the best defined and validated target for clinical development within the biology of cellular immortality.

To demonstrate the tractability of this approach we carried out an unbiased small molecule screen using a cell-based assay to discover compounds that would regulate telomerase gene expression. In addition an siRNA library screen was applied to expand and validate the findings of the compound screen. This approach identified a role for GSK3B signalling in hTERT gene regulation. Cells treated with GSK3B inhibitors showed repression of endogenous hTERT expression, telomerase activity and decreased telomere lengths.

In summary, given the role played by GSK3B in a variety of disease states such as cancer, type 2 diabetes and of the nervous system as well as its role in stem cell pluripotency, these data link telomerase into the emerging picture of pathways regulated by GSK3B with implications for regenerative medicine and cancer therapeutics.

POSTTRANSLATIONAL MODIFICATIONS IN TUMOR DIAGNOSIS

C. Wagener and P. Nollau
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Posttranslational modifications of proteins are important effectors of growth control and cellular communication. The malignant transformation of cells and tissues is accompanied by dysfunction of posttranslational modifications such as tyrosine phosphorylation and glycosylation. Posttranslational modifications are recognized by special protein domains. SH2 domains bind phosphotyrosine residues, and carbohydrate recognition domains (CRDs) interact with defined glycostructures. The protein domains, which recognize posttranslational modifications, can be applied as analytical tools. We use recombinant SH2 domains as ligands of tyrosine phosphorylated proteins, and we characterize glycostructures by the CRDs of recombinant glycoreceptors in tumor cells and tissues. The human genome encodes about 120 SH2 domains. Of these, 70 domains could be expressed as functional recombinant proteins. Using the recombinant SH2 domains, patterns of tyrosine phosphorylated proteins as revealed by Far Western Blots were evaluated in human malignancies. A panel of domains was defined, which reliably grouped pediatric lymphoblastic leukemias. Importantly, leukemias with unfavourable prognoses could be differentiated. In a comparable approach, human recombinant glycoreceptors were used to characterize glycan structures of glycoproteins in human carcinomas. Some of the receptors bound glycoproteins, which were expressed preferentially in tumors. With a panel of glycoreceptors, node positive mammary carcinomas could be differentiated from node negative tumors. Though most tumor markers detected in body fluids are glycoproteins, it is rather an exception that the glycan structures are targets of monoclonal antibodies applied in the respective assays (for example the sialyl Lewis a epitope of the CA 19-9 marker). In the future, human glycoreceptors may be used as analytical tools to bind tumor associated glycostructures. Since glycoreceptors are expressed in the cells of the tumor microenvironment, the glycan structures of circulating cancer biomarkers recognized by the respective recombinant glycoreceptors may carry biological information and may increase the specificity of tumor diagnosis.

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Advances in Clinical and Diagnostic Immunology

Tuesday, 17 May 2011   11:30–12:45

CHRONIC INFLAMMATORY BOWEL DISEASE – FROM BENCH TO BEDSIDE

M. F. Neurath
Department of Medicine 1, University Hospital, Ulmenweg 18, 91054 Erlangen, Germany

Recent data have important implications for bench to bedsite research in IBD patients. For instance, various studies underline the pathophysiological relevance of barrier dysfunction for the development of chronic intestinal inflammation. Studies in mutant mice such as the NEMO-villin Cre line have unequivocally shown that alterations of the intestinal barrier and intestinal epithelial cells are sufficient to cause infiltration and expansion of antigen presenting cells and T cells in chronic intestinal inflammation. Furthermore, studies in patients with IBD reveal that changes in barrier function, antimicrobial defensins and tight junction proteins occur in inflammatory bowel diseases in man. Moreover, SNPs in certain barrier related genes appear to predispose for the development of human IBD. Collectively, these findings underline the role of the barrier as key pathophysiologic principle in IBD. Strategies to restore barrier function in IBD patients may be used to develop new therapeutic strategies for IBD patients. Similar principles apply to studies studying macrophage and T cell activation in mouse models of IBD, where several cytokines have been identified as crucial regulators of intestinal inflammation that hold promise for targeted therapies in IBD patients.

INTEGRATIVE GENOMIC ANALYSIS OF T CELL IMMUNITY IN HUMANS

Haining, WN.
Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston; Division of Pediatric Hematology/Oncology, Children's Hospital, Boston; Harvard Medical School.

We have used integrative genomic approaches to identify genes and pathways that impair T cell function in chronic viral infection in humans. Traditionally, immunologists have used flow cytometry and functional assays to define combinations of markers that are representative of specific lineages and functional states within the immune system. More recently, genome-scale technologies have become widely available and provide the ability to define with increasing resolution complex sets of markers – expression signatures – that represent discrete biological properties of cell populations. Moreover, because genomic signatures can serve as surrogates of a phenotype, function or cell state, they can integrate phenotypic information between experiments, cell-types and species. We have analyzed gene-expression profiles from HIV-specific CD8+ T cells in patients with HIV and integrated these data with transcriptional profiles from mouse models of chronic viral infection as well as in vitro experiments. We have used these orthogonal datasets to identify novel regulators of T cell function that would not have been apparent through the analysis of any single dataset in isolation. Integration of expression signatures across experimental conditions together with functional analysis of their component genes can therefore provide new opportunities to dissect the complexity of the adaptive immune response to chronic viral infection in humans.

GENE-ENVIRONMENT INTERACTION IN CHRONIC INFLAMMATORY DISEASE

Prof. Dr. Harald Renz

Chronic inflammatory diseases are on the rise worldwide: Particularly in westernised and industrialised regions a dramatic increase in prevalence and incidence is observed since the end of WW II. Although chronic inflammatory conditions including autoimmunity and allergies have a genetic composition, it is now well-established that environmental conditions contribute markedly to disease development. One of the best-studied diseases in this context is allergic asthma. Epidemiological and experimental studies indicate that the pre- and postnatal period represents an important "window of opportunity" for disease priming. A number of environmental factors including nutritional compounds and microbial exposures play an important role in this regard. A model situation represents traditional farming environment which provides protection for respiratory allergies to a large extent. Maternal exposure to a variety of gram-positive and gram-negative bacteria has been identified as an important mechanism which primes the foetal neonatal immune system for the development of clinical and immunological tolerance. Development of tolerance is an important prerequisite for disease prevention. These findings are now being translated into clinical studies in order to develop novel therapeutic approaches for disease protection. Genetic risk assessment as well as individualised immune monitoring will represent future fields for laboratory medicine.

The Brain: Biological and Cerebral Diseases

Wednesday, 18 May 2011   11:30–12:45

ALZHEIMER'S DISEASE: FROM MOLECULAR PATHOLOGY TO DIAGNOSIS AND TREATMENT APPROACHES

R. N Martins

Abstract not received

BIOMARKERS IN MULTIPLE SCLEROSIS

M. Comabella

Abstract not received

MECHANISMS OF NEUROTOXICITY AND NEURODEGENERATION IN PRION DISEASES

Adriano Aguzzi

Protein misfolding and aggregation (PMA) disorders, which include many neurodegenerative disorders, systemic amyloidoses, and possibly Type II diabetes, comprise a class of pervasive and devastating diseases with limited treatment options. One of the major roadblocks to the development of therapies for these disorders is a fundamental lack of understanding about the underlying mechanisms of cellular dysfunction and toxicity. Each of these disorders is characterized by the abnormal accumulation of a different endogenous protein and deposition into insoluble aggregates, sometimes in the form of amyloid. In most cases, it is not known what triggers the aggregation of these proteins. Furthermore, the mechanisms of cellular toxicity are similarly enigmatic. Prion diseases or transmissible spongiform encephalopathies (TSEs) comprise a class of infectious neurological PMA disorders which include Creutzfeldt-Jakob disease (CJD), kuru, fatal familial insomnia (FFI), and Gerstmann-Sträussler syndrome (GSS) in humans, scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids. Clinically, prion diseases are marked by rapid neurodegeneration and spongiform changes in the brain which are concurrent with the accumulation and deposition of an abnormal, β-sheet-rich, partially protease-resistant conformer (PrP^{Sc}) of the endogenous prion protein (PrP^{C}). PrP^{Sc} exerts neurotoxicity only onto cells expressing its normally folded endogenous conformer, PrP^{C}, and the physiological function of PrP^{C} is unclear. Demonstrated cases of iatrogenic transmission of CJD among humans, BSE transmission from bovines to humans, rising CWD incidence in cervids, and evidence that infectious PrP^{Sc} accumulates in extraneural tissues all combine to make prion diseases a serious medical concern. Several lines of evidence from our and other laboratories indicate that the physiological function of PrP^{C} is related to the mechanisms of prion-induced neurodegeneration. Normal PrP^{C} expression is required for prion replication and PrP^{Sc}-mediated neurotoxicity. This does not appear to be simply due to the requirement of PrP^{C} for the propagation of PrP^{Sc} infectivity, since *Prnp*$^{o/o}$ mice transplanted with wild-type CNS tissue grafts are resistant to neurodegeneration despite significant accumulation of PrP^{Sc} outside of the graft. The deletion of the C-terminal domain of endogenous PrP (PrP^{ΔF}) causes spontaneous cerebellar degeneration in the absence of PrP^{Sc} infection reminiscent of prion disease. These data raise the intriguing possibility that a specific PrP conformation or complex transmits a cellular cell death signaling cascade. Conversely, normally folded PrP^{C} may have neuro- and/or glia-protective functions.

New Avenues in Laboratory Diagnostics

Thursday, 19 May 2011   11:30–12:45

DEVELOPMENT AND CLINICAL IMPLEMENTATION OF NEXT GENERATION SEQUENCING FOR MULTI-GENE DIAGNOSTIC PANELS

K.V Voelkerding

Abstract not received

MASS SPECTROMETRY: ITS FUTURE IN SEVERAL DISCIPLINES OF LABORATORY MEDICINE

V. Viette, A. Cherkaoui, M. Fathi, S. Emonet, D.F. Hochstrasser and J. Schrenzel.
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Background. The aetiology of diseases is either genes or the environment, the later being divided into microbes and "toxics" in a large sense. Therefore, in laboratory medicine, the future relies into rapid gene sequencing and microbes and "toxics" identification. The recently improved mass spectrometry precision, accuracy and ease to use offer new avenue in many fields of clinical practice.
Methods. The performance of various LC-ESI-MS-MS instruments in clinical toxicology and MALDI-TOF MS in microbiology has been compared.
Results. LC-ESI-MS-MS offers excellent alternative to the old REMEDI system around the clock for ER or ICU patients for toxicology screening and TDM (1). MALDI-TOF-MS, MALDI-RE & PCR-ESI-MS dramatically speed up the identification & genotyping of microbes in every day clinical practice (2). Most methods lower the laboratory expenses while improving the diagnostic accuracy.
Perspective. Mass spectrometers are ideal detection tools. Their uses become easier and easier with ergonomic software such as SmileMS (3). The need of costly reagents is abolished. The impact of such technology will certainly rise rapidly in many fields including pathology.

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PROTEOMICS STRATEGIES LOOKING INTO CARDIOVASCULAR DISEASE; DISRUPTED SIGNALING PATHWAYS AS BIOMARKER

Arjen Scholten[1,2] and Albert J. R. Heck[1,2]
[1]Biomolecular Mass Spectrometry & Proteomics Group, Utrecht University, The Netherlands
[2]Netherlands Proteomics Centre
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Background. Proteins, the most functional and dynamic entities of cells and tissues, are the most useful indicators for studying the onset, progression, as well as the therapeutic intervention of the various forms of cardiovascular diseases (CVDs). This contribution will focus on the use of so-called shot-gun as well as targeted proteomics technologies to study cardiac related diseases at a global level, but also at the more targeted level of CVD related signal transduction.

Methods. Proteomics profiling in combination with mass spectrometry is used to evaluate qualitatively and quantitatively protein expression, protein association and the disruption of signaling pathways related to heart failure.

Results. The complexity of the human proteome is quite overwhelming. Not only the amount of proteins present in cells or tissue, but also the huge variability in protein expression over space and time create serious bottle-necks hampering comprehensive proteome analysis. To illustrate this complexity, we will present a semi-quantiative protein atlas of the human heart, providing the protein abundance of more than 3000 different proteins [1]. This data reveal that used CVD protein biomarkers belong nearly exclusively to the most abundant proteins present in heart tissue. A major bottleneck in shot-gun proteomics is the limited dynamic range in analyzing simultaneously high and low abundant proteins. Therefore, in standard proteomics analyses low abundant proteins, which may act as biomarkers with known functional roles in CVDs, such as certain kinases and their signaling scaffold are often missed.

To overcome these limitations sub-proteomic approaches have been introduced, whereby small messenger molecules are used as bait to selectively enrich for proteins that do interact with them, a technique termed chemical proteomics. Here, we explored immobilized cAMP-based chemical proteomics strategies to enrich for specific low abundant cyclic nucleotide signaling protein complexes from human left ventricular free wall tissue of healthy hearts and explanted hearts compromised through dilated cardiomyopathy. Our data reveal that significant redistribution occurs in cAMP related signaling scaffolds in the failing human heart, which undoubtedly results in altered cardiac molecular function, providing potentially a promising novel treatment repertoire.

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SYMPOSIUM ABSTRACTS

SYM 1 Biomarkers for the Diagnosis of Cardiovascular Diseases

Monday, 16 May 2011   09:00–11:00

CLINICAL IMPLICATION OF HIGH SENSITIVE TROPONIN TESTING

S. Blankenberg

Abstract not received

QUALITY SPECIFICATIONS FOR TROPONIN ASSAYS

R. Christenson

Abstract not received

CELLULAR AND MOLECULAR IMAGING

R. Choudhury
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Appreciation of the molecular and cellular processes of tumours, neurological disease, atherosclerosis, thrombosis, and vascular inflammation has identified new targets for imaging. The common goals of molecular imaging approaches are to accelerate and refine diagnosis, provide insights that reveal disease diversity, guide specific therapies, and monitor the effects of those therapies. Here we will undertake a comparative analysis of imaging modalities that have been used in a variety of disease areas. We will consider the elements of contrast agents, emphasizing how an understanding of the biology of a given disease and its complications can inform optimal design. We will address the potential and limitations of current contrast approaches in respect of translation to clinically usable agents and speculate on future applications.

CLINICAL USE OF BNP IN HEART FAILURE

Christian Mueller, Beatrice Drexler, Cathrin Balmelli

B-type natriuretic peptide (BNP) is a cardiac hormone which is predominately secreted from the left and right ventricle in response to cardiac hemodynamic stress mediated by volume and pressure overload. The predominant stimulus for the release of BNP is end diastolic wall stress. Multiple studies have confirmed that assays measuring either BNP, the active hormone, or NT-proBNP, the inactive peptide, have comparable performance in most indications. Therefore, we use the term "BNP" throughout to represent "BNP and NT-proBNP". In fact, recent evidence suggests that another natriuretic peptide, mid-regional proANP, also has similar diagnostic and prognostic performance as compared to BNP and NT-proBNP.

BNP can be seen as a quantitative marker of cardiac stress and heart failure summarizing the extent of systolic and diastolic left ventricular dysfunction, valvular dysfunction and right ventricular dysfunction. ia the severity of heart failure. As a sensitive and specific marker of cardiac stress and heart failure, BNP has gained widespread clinical use in the diagnosis, risk-stratification, and treatment monitoring of patients with heart failure. In addition, BNP is a powerful predictor of death in multiple other conditions, highlighting the close association between the extent of cardiac stress and mortality in multiple medical conditions including coronary artery disease, valvular heart disease, pulmonary embolism, pneumonia, and vascular surgery.

The therapeutic goals in the treatment of heart failure include a marked reduction of cardiac BNP secretion. The concept of BNP-guided HF management appears to improve clinical outcome by reducing rehospitalisation rate and possibly also by improving mortality.

Like any other clinical test, BNP as a cardiac biomarker cannot be used in isolation. BNP should be used as a complementary tool that augments physician skill and judgment.

Likely the most important indication for BNP is to help in the evaluation and management of patients with acute dyspnea. The differentiation of heart failure from other causes of acute dyspnea remains a clinical challenge, especially in the emergency department. Numerous studies in patients presenting with acute dyspnea to the emergency department have validated the use of BNP and showed that:

1) BNP has high diagnostic accuracy for the early diagnosis of acute heart failure

2) The additional use of BNP improves patient management

Interesting but still controversial indications include the use of BNP in the screening for patients with asymptomatic left ventricular systolic dysfunction. Regarding the use of BNP in the treatment monitoring of patients with chronic heart failure, technical innovation has allowed to develop simple patient-based devices ("fingerstick BNP") that ultimately might allow the patient to take over a much more prominent and powerful role in the management of his/her disease.

SYM 2 Diagnostic Challenges in Liver Disease

Monday, 16 May 2011   09:00–11:00

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

V. Ratziu

Abstract not received

BIOMARKERS OF FIBROGENESIS FOR THE DIAGNOSIS AND THE ACTIVITY OF THE FIBROSIS PROCESS IN LIVER DISEASES

M. Vaubourdolle[1]
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The extension of liver fibrosis represents the major complication observed in many liver diseases. The detection of liver fibrosis and the assessment of its severity are very useful for diagnosis and prognostics purposes, particularly concerning therapeutic decision and estimation of cirrhosis occurrence.

The assessment of the disease is carried out through liver biopsy, an invasive procedure used in assessing fibrosis stage so as to determine the need for therapy. However, regarding to the number of patients, the risk related to biopsy procedure and the cost, some non-invasive methods for fibrosis stage assessment have been reported, using serum markers.

Some usual parameters such as transaminases, platelets, prothrombin time, have been described as indirect fibrosis markers. More recently, improvement of knowledge about fibrogenesis mechanisms allowed identifying circulating substances able to become new direct liver fibrosis markers. Components of extra cellular matrix, degradation products or metabolism enzymes have been proposed. Among them, hyaluronic acid seems to be of great interest. Moreover, some scores have been calculated from these parameters and validated in clinical situations such as C hepatitis. Fibrotest®, Fibrometre® and Hepascore® are among the more often used. Finally, Fibroscan®, which is a medical device for diagnosis and assessment of liver fibrosis based on elastography, has been proposed. The relative interest of these markers has to be assessed.

HEPATOCELLULAR CARCINOMA: MOLECULAR PATHOGENESIS AND CLINICAL ASPECTS

H. Blum

Abstract not received

OCCULT HEPATITIS B VIRUS INFECTION: DIAGNOSIS AND SIGNIFICANCE

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Background. Hepatitis B virus (HBV) infection is detected by the assay of its surface antigen (HBsAg) in the serum. However, many persons harbour HBV DNA in the liver without detectable HBsAg in the serum. These persons have occult HBV infection (OBI) (1).

Occurrence. OBI may develop after resolution of HBsAg positive acute infection with or without recognised clinical symptoms. Persistent OBI may fluctuate and leads more often to detectable low-level viremia (<1000 IU/ml) in patients with HIV- or HCV-co-infection or mild immunodeficiency. The infection may be OBI-like from the beginning as detected by screening of blood donors for HBV DNA (2). Partial immunity after immunisation against HBV with low anti-HBs titers (<100 IU/L) favours the development of OBI, while non-immune persons usually develop a course with transient HBsAg (3).

Diagnosis. OBI is often defined as HBV DNA positive, HBsAg negative in the serum. This definition is problematic, because the levels of HBV DNA in the serum are in most cases too low to be detectable. Thus, a negative HBV DNA test can not exclude OBI. A surrogate marker for OBI is the antibody against HBV core antigen, anti-HBc. However, ca. 15 % of immunocompetent OBI carriers with detectable HBV DNA in liver are anti-HBc negative (1). Furthermore, patients with hematological disorders and OBI may be anti-HBs positive and anti-HBc negative (2). In conclusion, it is impossible to reliably diagnose OBI.

Significance. OBI has been connected with chronic liver disease, enhanced risk of hepatocellular carcinoma, and a worse prognosis of HCV and HIV coinfection, but all this is not well established (1). OBI is definitely a problem in liver transplantation. and transfusion medicine. Livers of anti-HBc positive donors show severe HBV reactivation in the immunosuppressed recipients unless pre-emptive antiviral therapy is employed. Low-viraemic blood donors with anti-HBc but without anti-HBs transmit frequently HBV. In most cases (22/25) the infection remains asymptomatic but in immunocompromised recipients it may lead to liver failure (2). Thus, blood and liver donors should be screened for HBV DNA with very sensitive tests and for anti-HBc. The biggest problem of OBI is the reactivation of HBV under severe immunosuppression. This is relatively frequent in haematological disorders but rare in cancer therapy or solid organ transplantation. Reactivation remains asymptomatic under immunosuppression but may cause liver failure under immune reconstitution due to re-appearing immunopathogenesis. Thus, all patients should be tested for anti-HBc and anti-HBs before immunosuppression and positive patients should be careful monitored for appearance of HBV DNA (2). A special feature of many OBI is the selection of HBV mutants leading to HBeAg-negative or core promoter variants. The HBs antigen loop is also a target of selection leading up to 16 in 65 aminoacid changes (2). These mutants may be undetectable by HBsAg tests even in high concentrations after reactivation, and they may escape the HB vaccine induced immunity.

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YR 1 Clinical Chemistry 2010 and Beyond: Opportunities and Challenges for the Next Decade

Monday, 16 May 2011   09:00–11:00

NEXT-GENERATION SEQUENCING OF PLASMA DNA FOR MOLECULAR DIAGNOSIS

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Background. Cell-free nucleic acids have been found in the plasma of human subjects. Two applications of this phenomenon have particularly caught the attention of workers in the field, namely the detection of tumor DNA in the plasma of cancer patients and fetal DNA in the plasma of pregnant women.
Methods. The development of next-generation DNA sequencing has permitted millions of plasma DNA molecules to be investigated per analysis. This has allowed plasma DNA to be analysed with unprecedented sensitivity, specificity and precision.
Results. The analysis of serum DNA from cancer patients has identified characteristic alterations that might be used for cancer screening or monitoring. The use of next-generation DNA sequencing for the plasma of pregnant women has allowed fetal chromosomal aneuploidies to be detected with high sensitivity and specificity. Plasma DNA sequencing has also revealed that circulating DNA molecules possess nucleosomal features which might shed light on their mechanism of production.
Conclusions. Next-generation sequencing of plasma DNA might become an increasingly important tool in the future developments of molecular diagnostics.

DRUGS OF ABUSE TESTING, FROM URINE TO ORAL FLUID: SCIENTIFIC AND LEGAL RAMIFICATIONS

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Monitoring an individual's drug use is an important aspect of clinical medicine, and driving under the influence of drugs (DUID), drug treatment, pain management, workplace and forensic drug testing programs. Urine, the most common and well-established biological matrix for testing, offers standardized techniques, ample specimen volume, high drug concentrations and numerous controlled drug administration studies, providing a scientific database for result interpretation. However, specimen collection is problematic due to privacy issues, ease of specimen adulteration, and need for specialized collection facilities and same-sex collectors. In addition, urine drug concentrations do not reflect concurrent blood concentrations or drug impairment. A new biological matrix, oral fluid (saliva), was investigated to provide unique data on an individual's drug usage, windows of drug detection, and collection procedures. Recently, oral fluid testing increased tremendously, primarily due to ease of observed specimen collection, reduced adulteration potential, need for a rapid, roadside drug test for DUID, and potentially, a better correlation with blood concentrations and performance impairment. Challenges of this new testing matrix in comparison to urine are reduced specimen volume, especially after ingestion of stimulant drugs, lower drug concentrations, potential contamination of oral fluid by smoked, inhaled, or oral drugs, and lack of controlled drug administration studies to guide result interpretation. The increasing prominence of oral fluid drug testing is changing and challenging drug monitoring in the clinical laboratory. First, there are many issues related to oral fluid collection; expectoration or spitting is disliked by donors and collectors, and new evidence indicates that drug concentrations may be lower in these specimens than in those obtained with oral fluid collection devices. Currently, there is little standardization on the amount and precision of oral fluid collected, and drug recovery from commercial collection devices. An elution/stabilization buffer improves recovery from the device, but also dilutes drug concentrations, and frequently contains chemicals that interfere with confirmation testing. Due to the spectrum of oral fluid analytes requested and low available specimen volume, liquid chromatography tandem mass spectrometry (LCMSMS) is becoming the instrument of choice for oral fluid testing. Whether or not LCMSMS can handle the volume of testing required in US workplace drug testing programs or the required turnaround time is still an open question, while the ability to analyze up to 29 different target analytes in a single assay is clearly documented. Additional challenges to this new technique include automating specimen analysis, developing collection devices with required specimen volume and drug recovery specifications, and importantly, conducting the required controlled drug administration research to define analytes of interest, windows of drug detection, appropriate cutoff concentrations, and perhaps correlations with performance impairment. Oral fluid testing offers clinical laboratories a new testing paradigm requiring experienced analysts, LCMSMS technology, a new revenue stream and interpretation challenges. Multiple European Union states, Australia and the US have approved oral fluid as an appropriate matrix for monitoring drug use; clinical laboratories have experienced a decrease in urine drug testing and need to meet the opportunities and challenges of oral fluid testing in the next decade.

LABORATORY AND CLINICAL IMPLICATIONS OF HIGH-SENSITIVITY TROPONIN ASSAYS

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Cardiac troponin (cTn) I and T play pivotal roles in clinical decisions regarding diagnostic accuracy for acute myocardial infarction (AMI) and risk outcomes assessment for the triage and management of patients presenting to acute care centers to rule in and rule out AMI. International guidelines (Global Task Force, NACB, WHO, ESC Acute Cardiac Care) have emphasized the importance of a number of assay-related analytical issues that may markedly influence cTn test performance and clinical care. The goal of this presentation will be to provide up to date evidence-based information on how specifications for both high-sensitivity (hs) and contemporary sensitive cTn assays will impact clinical practice. Specifically the following areas will be addressed: a) how a normal reference population should be defined to determine the 99th percentile cutoff value; b) the influence of biological variation and imprecision characteristics on the 99th percentile; c) information on assay specific diagnostic accuracy for clinical sensitivity and specificity; d) the role of cTn delta changes and serial orders for improving clinical specificity; e) the role of understanding how different cTn assays may affect risk stratification based on appropriate cutoff concentrations in both ACS and non-ACS patients. Both laboratorians and clinicians need to better understand how to use recommended cutoffs (99th percentile vs. ROC curve derived) as well as the high sensitivity assays that are being implemented into clinical practice. The clinical laboratory needs to become a strong partner with all aspects in healthcare in educating all medical disciplines on how to use appropriate cTn assays in clinical practice. Every effort needs to be made to make sure the right patients get the right treatment.

THE DIAGNOSTIC PROTEOME: PROSPECTS FOR BIOMARKER DISCOVERY AND VALIDATION IN PLASMA

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Background. The current clinical plasma proteome consists of approximately 200 different proteins or about 1% of the baseline human proteome: 109 proteins measured by FDA-cleared or approved assays and 96 proteins measured using widely-available laboratory developed tests. However the rate at which new protein analytes achieve FDA approval has remained essentially constant over the past 15 years at 1.5 proteins per year, insufficient to meet important diagnostic and prognostic needs and suggesting a failure of the clinical validation process for novel candidate biomarkers.

Methods. New approaches to protein measurement based on mass spectrometric detection have been developed to allow rapid creation of panels of biomarker assays with very high structural specificity and true internal standardization. Specific enrichment (e.g, of tryptic peptides with anti-peptide antibodies in the SISCAPA method) can provide sensitivity equivalent to sandwich immunoassays.

Results. SISCAPA-MS assays have been implemented for more than 200 candidate biomarker proteins, and a multiplex panel using monoclonal anti-peptide antibodies to capture 11 biomarker peptides characterized in detail.

Conclusions. In the clinical laboratory, affinity enrichment with MS quantitation can provide absolute specificity, true internal standardization, and facile multiplexing – thus transcending the major limitations of conventional immunoassays. The generality, strengths and limitations of immuno-MS point to a growing role in replacing interference-prone immunoassays, in implementing assay panels, and in validating novel candidate biomarkers prior to clinical use. An extension of this approach, the hPDQ project, could provide the larger biomedical research community with direct quantitative access to all 21,300 human proteins.

SYM 3 Tumor Markers in Cancer

Monday, 16 May 2011   09:00–11:00

CIRCULATING TUMOR CELLS AS DIAGNOSTIC AND THERAPEUTIC TARGETS

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Early spread of tumor cells is usually undetected by current imaging technologies. Therefore, in patients with cancer and no signs of overt metastases, sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood and disseminated tumor cells (DTC) in the bone marrow. These technologies can be classified into cytometric and/or immunological and molecular approaches. Interestingly, the bone marrow seems to be a common homing organ for cells derived from various epithelial tumors, and level 1a data from European and US groups have sustained the prognostic impact of DTC in the BM of breast cancer patients. However, a significant fraction of DTC remain over years in a "dormant" stage, and little is known about the conditions required for the persistence of dormancy or the escape from the dormant phase into the active phase of metastasis formation. Sequential peripheral blood analyses, however, are more convenient for patients than BM analyses in patients with solid tumors and many research groups are currently assessing the clinical utility of CTC for assessment of prognosis and monitoring of systemic therapy. In view of the plethora of prognostic indicators—especially in breast cancer—monitoring of CTC during and after systemic adjuvant therapy might provide unique information for the clinical management of the individual cancer patient and allow an early change in therapy years before the appearance of overt metastases signals incurability. There is an urgent need for biomarkers for real-time monitoring of the efficacy of systemic adjuvant therapy in individual patients. At present, the success or failure of anti-cancer therapies is only assessed retrospectively by the absence or presence of overt metastases during the postoperative follow-up period. However, overt metastases are, in general, incurable by most current therapies. The monitoring of CTC as "liquid biopsy" will provide new insights into the selection of tumor cells under biological therapies. Molecular characterization of DTC and CTC opens a new avenue for understanding metastatic spread of tumor cells with important implications for future therapies. The use of CTC analyses in clinical trials testing new anti-cancer agents as companion diagnostics has the potential to speed up the cumbersome and expensive drug validation process in oncology.

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MEASUREMENT AND DIAGNOSTIC USE OF PROSTATE SPECIFIC ANTIGEN

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Prostate specific antigen (PSA) is produced by the prostatic epithelium and excreted at high concentrations into the prostatic ducts. Only a minor part of PSA leaks out into circulation from the normal prostate but, when tissue architecture is deranged in cancer and the tumor loses contact with the prostatic duct, PSA is secreted into circulation. Therefore, PSA is a very sensitive marker for prostate cancer and a tumor of 0.5 - 1 g size usually causes elevated serum concentrations. Elevated PSA values may also be caused by benign diseases like benign prostatic hyperplasia (PBH) and prostatitis. Thus the cancer specificity of PSA is low at cutoff values giving high sensitivity. PSA is a serine protease and, when reaching circulation, enzymatically active PSA forms complexes with protease inhibitors. Alpha-2-macroglobulin (A2M) is the major inhibitor but PSA in complex with A2M is not detected by conventional immunoassays. PSA also forms complexes with alpha-1-antichymotrypsin (ACT) and alpha-1-protease inhibitor (API). Most of PSA in plasma occurs in complex with ACT (PSA-ACT), less than 5 % consists of PSA-API while a 5 – 40 % is free (fPSA). Patients with prostate cancer have a higher proportion of PSA-ACT and a lower proportion of fPSA (%fPSA) than men with BPH (1). Immunoassay of fPSA and total PSA (tPSA) is technically favorable and determination of %fPSA is widely used to improve diagnostic accuracy. Further improvement can be achieved by calculating various indexes based on prostate volume, results of digital rectal examination, age and rate of PSA increase. Algorithms incorporating these parameters have been developed using logistic regression and neural networks. The impact of %fPSA on cancer probability is considerable. When tPSA is in the range 4 – 10 μg/l an fPSA value of 7% is associated with a 10-fold higher risk than a value of 35%. fPSA is also important when PSA is in the range 2 – 3 μg/l, fPSA values below 15 % are associated with a 30% risk of prostate cancer within the next 5 years (3). This is important because early detection of prostate cancer is likely to reduce mortality especially in younger men, who have relatively low reference values for PSA. A low %fPSA is also associated with aggressive disease and predicts that tumor grade determined in a biopsy will be upgraded by examination of the surgically removed prostate. This information can be utilized to select patients for active surveillance or curative therapy. A further improvement in diagnostic accuracy can be achieved by determination of some variants of fPSA, but assays for these are not yet commercially available.

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BIOMARKERS AND PERSONALIZED MODELS IN ONCOLOGY DRUG DEVELOPMENT

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Biomarker discovery in oncology has been robust but development has been plagued by the need to validate these markers at various key phases. Target discovery in tumors and or in cell lines with differential sensitivity usually starts the process. Then, simple cutoffs must first be identified and established in samples of convenience. Robust technology assessment and implementation must take place to ensure reliable and accurate results. Retrospective clinical analysis must be done, testing the biomarker in key studies where clinical drug sensitivity is established. Eventually, a prospective clinical analysis must be performed to validate use of the marker, though this can be done in prospectively collected samples. Finally, either a laboratory or a commercial entity must offer the predictive biomarker to ensure its integration in the clinic. We will discuss various biomarkers including key genetic and epigenetic markers in development and those already in the clinic. We will also discuss the development of new predictive personalized models which are at the nexus of integrating biomarkers and drug testing.
Preclinical oncology drug development typically originates from high passage number immortalized cell lines. While information from these models is useful in discovery and initial proof-of-concept studies, their clinical relevance is often limited due to alterations and adaptations from successive passages in tissue culture and animals. Preclinical personalized models established from donor patient tumor fragments passaged only a few times in vivo may better represent clinical disease. Following establishment, models can be characterized at the molecular level and then correlated with in vivo sensitivities of various agents and clinical information from patient donors as well as current standards of care. Molecular characterization studies identified known mutations in several signaling molecules important in cancer progression as well as novel markers of sensitivity and resistance to standard agents. These low passage models offer an alternative to standard xenografts and may be more representative of clinical disease. Data collected from molecular characterization and in vivo evaluation of these models will aid greatly in development of novel agents and predictive biomarkers.

PROTEOMIC APPROACHES FOR NOVEL BIOMARKER DISCOVERY

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Background. Current cancer biomarkers suffer from low diagnostic sensitivity and specificity and have not yet made a major impact on reducing cancer burden. Proteomic methods based on mass spectrometry have matured significantly over the past few years and hold promise to deliver candidate markers for diagnosis, prognosis or monitoring therapeutic response.

Methods. A number of mass spectrometry-based cancer biomarker discovery strategies have been employed. These strategies could be broadly grouped into two categories: an unbiased approach or a targeted discovery approach. The former strategy includes: two-dimensional gel electrophoresis, MS-based profiling techniques and MS-imaging of frozen tissues. Furthermore, the analysis of peptides or fragments originating from proteins which could serve as novel cancer biomarkers have also been explored. Other strategies include a more global quantitative analysis involving isotopic labels such as SILAC, iTRAQ or iCAT. Finally, tandem mass spectrometry (LC-MS/MS) analysis of serum, cell lines, relevant biological fluids and tissues from normal and cancer patients have revealed a number of potential cancer biomarkers. On the other hand, a targeted discovery approach could consist of post-translational modification analysis with the hypothesis being that cancer cells may produce proteins with a different modification such as phosphorylation or glycosylation that differs from the form found in normal individuals. Other approaches under this category include the utilization of different MS instruments and their capabilities to perform multiple reaction monitoring (MRM) to measure specific analytes in complex mixtures or employing a hybrid technology such as immuno-MS. Each approach to cancer biomarker discovery has its own advantages and disadvantages and it seems likely that a global biomarker discovery platform that mines all possible sources (such as proximal fluids, tissues, human cancer cell lines, plasma) for biomarkers might be more useful. Such data could be combined with information from relevant microarray data, bioinformatic analyses and literature searches to yield highly promising candidate biomarkers.

Results. Using pancreatic cancer as the example, over 3,400 proteins were confidently identified by tandem mass spectrometry in human pancreatic cancer-related cell line conditioned media and pancreatic juice of cancer patients. Preliminary verification of 5 proteins by immunoassay in serum of pancreatic cancer patients and healthy individuals demonstrated a higher area under the curve compared to CA 19-9 alone.

Conclusions. This proposed integrated systems biology approach has the potential to yield promising cancer markers for diagnosis, prognosis and monitoring of patients during therapy. Further validation studies of these proteins are warranted.

IFCC 1 IFCC EMD/CPD Joint Symposium: ELearning and Distance Education in Clinical Chemistry and Laboratory Medicine

Monday, 16 May 2011   09:00–11:00

WEBINAIRS, STEAMING MEDIA, BLOGS AND OTHER E-COMMUNICATIONS

A. Lyon

Background. A wide variety of e-communication tools have emerged with potential of improving information exchange related to the practice of Laboratory Medicine. There are opportunities to apply these tools in your practice: to teach, to learn, to conduct business and to build relationships with others. Like all tools, these new methods do require some skill to be used most effectively.
Materials and Methods. This session will depict software, hardware and network issues related to the use of webinars, podcasts, blogs, electronic black boards, list-servs and social media websites that relate to the practice of Laboratory Medicine.
Results. Trends in the use of various electronic media will be discussed. Barriers and concerns related to privacy, confidentiality, information integrity, "electronic" medical practice will be reviewed. The interface between electronic patient records, lab reports and various internet tools will be considered. The use of electronic information exchange often removes barriers of time and location for educational events, providing new opportunities. Examples depicting use of social media to foster affiliation in Lab Medicine organizations will be shown.
Conclusions. Use of electronic communication tools will continue to develop and become integrated into clinical practice. Laboratory Medicine professionals have opportunities to adopt these technologies as aides to education and laboratory function.

LABTESTSONLINE: TRANSMITTING LABORATORY MEDICINE IN THE CONTEXT OF NATIONAL AND INTERNATIONAL GUIDELINES

M. Klouche

Abstract not received

IFCC EXPERIENCES WITH DISTANCE LEARNING

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Distance learning has existed, in one form or another, for over a century when reliable postal services became available around 1840. Over the years it has expanded with the introduction of audio, visual, motion, and more recently, hypermedia, interactive multimedia and the internet. The internet, the largest, most powerful computer network in the world, encompasses more than1.3 million computers with internet addresses used by up to 30 million people in over fifty countries. As more and more colleges, universities, schools, companies, and private citizens connect to the Internet, either through affiliations with regional not-for-profit networks or by subscribing to information services provided by for-profit companies, more possibilities open for distance educators to overcome time and distance and reach students. At the same time travel costs have increased and budgets have decreased. Additionally, a shrinking labor force and associated staffing issues has reduced the ability of individuals to take time off and attend continuing education programs.   Internet distance learning is categorized as either synchronous, where all participants are "present" at the same time, or asynchronous, where participants access course materials on their own schedule. Whereas synchronous programming is dynamic in nature, asynchronous programming is either dynamic or static. An organization's website can be an educational portal, a consolidator of distance learning resources, by providing either direct or indirect access to distance learning material. It is more than maintaining a digital library. There are dynamic elements; blogs, listservs (moderated or unmoderated), webinars, databases, and there are static elements; documents, eJournals, eNewsletters, podcasts, and presentations/ lecture. Different types of presentations can be made available ranging in sophistication from simple PowerPoint presentations to audio or audio/video presentations. Technology (software) is available that has simplified videoconferencing and the ability to create distance learning programming in various formats incorporating multimedia resources such as *graphics, video or audio (e.g., MP3 files), PowerPoint, or Flash-based* applications. Regardless of the type of difference learning, effective distance teaching, for the most part, requires the enhancement of existing skills, rather than the development of new abilities. The IFCC, through its website, is providing access to a variety of distance learning opportunities.

E-LEARNING EXPERIENCES OF NATIONAL SOCIETIES OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

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Background. eLearning and distant education in clinical chemistry by member national societies of the IFCC was first reviewed in 2005 by the Working Group on Distance Education (WG-DE) chaired by D. Juretic, under the auspices of the Committee on Education and Curriculum Development (C-ECD) chaired by L.C.Allen. The second survey of national societies was carried out in 2010 by the Committee on Education and Curriculum Development (C-ECD) chaired by P.Kocna and the Working Group on Internet-Distance Learning (WG-IDL) chaired by V.T.Thanh, covered by Communication & Publications Division (CPD) chaired by E.Jacobs. This presentation summarises changes and trends in the use of eLearning and distant education of clinical chemistry in this 5-year period.

Results. The survey of national societies was performed in 2005 using paper-printed questionnaires, in 2010 by on-line internet questionnaire forms. Analysed responses were 34 and 42 in 2005 and 2010 respectively. National society websites increased from 70.6% (24 of 34) in 2005 to 90.5% (38 of 42) in 2010. The educational sections of websites increased from 41.2% (14 of 34) in 2005 to 57.1% (24 of 42) in 2010. Lectures and presentations published on websites are still the most widely used form of educational resource, 79.2% (19 of 24) in 2010 and 71.4% (10 of 14) in 2005. IFCC C-ECD published in 2008 an on-line internet available Educational Resource Database recommending 254 resources for education in clinical chemistry - URL *http://eduweb.virt.cz* and was visited more than 1790 times by visitors of 86 countries. This database is now used by 12 national societies and 40% (10 of 25) of societies recommended distant education on their websites. The best internet educational resource recommended by national websites was NLM database (mean of marks 2.3) followed by Google (mean of marks 2.38). Most national societies, 76.2% (32 of 42), preferred a unified IFCC educational strategy and mentioned in responses the concept of IFCC credits, 59.5% (15 of 42) of responding national societies.

SYM 4 Kidney Diseasy – Acute and Chronic

Monday, 16 May 2011   09:00–11:00

UNMET CLINICAL NEEDS IN ASSESSING CKD

G. Jones

Abstract not received

CYSTATIN C FOR ESTIMATION OF RENAL FUNCTION IN CKD

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Information on GFR is required, not only to detect and follow impaired renal function, but also to allow correct dosage of drugs and contrast media cleared by the kidneys. Correct determination of GFR requires slow and expensive invasive techniques. Therefore, GFR-prediction equations based upon cystatin C alone or creatinine and anthropometric data or upon cystatin C, creatinine and anthropometric data have been developed. The combined prediction equations display the best diagnostic performance, but in several easily identifiable clinical situations prediction equations based upon either cystatin C or creatinine are better than the combined equations. Cystatin C has been used as a GFR-marker in Lund since 1994, and a strategy based upon accumulated clinical experience has been developed (www.egfr.se, http://www.egfr.se/eGFRStrategy.pdf) It comprises simultaneous use of a cystatin C- and a creatinine-based GFR-prediction equation. If the GFRs predicted agree, the mean value is used as a reliable GFR-estimate. If the GFRs predicted do not agree, clinical data are evaluated to identify reasons for not using one of the two prediction equations and the GFR predicted by the other one is used. If no reasons for the difference in predicted GFRs are found, an invasive gold standard determination of GFR is performed. Recent introduction of an international calibrator for cystatin C and results demonstrating no relation between cystatin C and inflammation (http://informahealthcare.com/eprint/NBnrzEpRUrXj5CyB-nfcb/full?tokenKey=) will facilitate the general introduction of cystatin C as a marker of GFR.

BIOMARKERS OF ACUTE KIDNEY INJURY

C. Ronco

Abstract not received

DYSLIPIDEMIA OF CHRONIC RENAL DISEASE

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Background. Chronic kidney disease (CKD) is associated with typical metabolic alterations. Specific alterations of the lipoprotein metabolism are inherent features of the two major syndromes that develop in kidney disease, i.e. nephrotic syndrome and chronic renal insufficiency. The lipid abnormalities of these two syndromes differ in causes and lipoprotein pattern. This presentation will only discuss the profile and pathophysiology of renal dyslipidemia of chronic renal insufficiency.

Renal dyslipidemia. The dyslipidemia is characterised by an accumulation of intact or partially metabolised triglyceride-rich apoB-containing and apoC-III-containing lipoproteins. These lipid abnormalities become more pronounced with lower renal function. Thus, with lower GFR the lipid profile changes with increasing levels of TG and non-HDL-cholesterol, decreasing levels of HDL-cholesterol, whereas LDL-cholesterol usually remains in the normal range. However, before marked alterations in the plasma lipid levels can be detected characteristic changes develop in the plasma apolipoprotein pattern. The typical alterations in the spectrum and composition of individual lipoprotein particles occur already at GFR levels at or just below 50 ml/min also in normo-lipidemic renal patients. The hallmark of renal dyslipidemia is the early and marked increased concentration of apolipoprotein CIII. This is explained by the accumulation of lipoprotein particles in VLDL and IDL that contain apoB and apoC-III, so called LP-B:C particles, and particles that contain apoAII, apoB, apoC-III, apoD and apoE, so called LP:AII:B:C:D:E particles. At the same time the concentrations of apoA-containing particles, particularly LP-AI particles, are decreased.

Causes of renal dyslipidemia. Kinetic studies have shown that the main underlying mechanism is a decreased catabolism and accumulation of the apoB-containing lipoproteins, which also have apoC-III and apoE on their surface, whereas the hepatic synthesis of lipoproteins is not increased. ApoC-III is a powerful inhibitor of lipoprotein lipase and the presence of apoC-III on the surface of the lipoprotein particles also render them less attractive as substrate for lipolysis. The alterations of the lipoprotein particles also result in a prolonged residence time in the circulation. This makes them accessible for modifications by glycation, oxidation, carbamylation or other alterations. The underlying cause of the increase of apoC-III in CKD is presently unknown. However, there is a link between apoC-III and insulin resistance, and patients with impaired renal function have reduced insulin sensitivity. Both synthesis and release of apoC-III and apoC-III containing lipoproteins are upregulated in insulin-deficient states and suppressed by the administration of insulin. One may speculate whether this is due to a decreased PPAR-α activity since it has been shown that PPAR-α agonism stimulates lipoprotein lipase and inhibits apoC-III expression.

Consequences of renal dyslipidemia. The lipoprotein profile of CKD patients is of atherogenic nature and may contribute to the accelerated atherosclerosis that frequently develops in this patient category. Furthermore, it has been associated with progression of renal damage. Neither statin nor fibrate therapy can normalise renal dyslipidemia. Dual PPAR-agonism has the potential to beneficially affect the catabolism of apoB- and apoC-III containing triglyceride-rich lipoproteins, but presently there is no such drug under clinical development for this indication.

DGKL 1 Role of Non-Medical Scientists in the Medical Laboratory (DGKL)

Monday, 16 May 2011   09:00–11:00

SCIENTIFIC METHODS IN MEDICINE

W. Bauersfeld

Abstract not received

THE ADVANCE OF SCIENCE IN MEDICINE

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Natural sciences including physics, chemistry and biology, form the basis of modern medicine. The importance of natural science in medicine is emphasised by the number of Nobel laureates in Medicine over the past decade, who have a background in a natural science, for example genetics.

Great achievements in the field of modern medicine have been made due to advances in technical and natural science and their practical application. In Germany this success is associated with clinical chemists, human geneticists and medical physicists, and must continue to be a part of future strategies in which both medical practitioners and natural scientists play an active role.

Optimal patient care requires a team of medical practitioners and natural scientists who each has his own responsibilities. For example, the final decision in the course of medical treatment forms part of the doctor-patient relationship and must be taken by a physician, whereas the responsibility in the diagnostic laboratory may be taken by a qualified natural scientist. The current practice in Germany, that a medically qualified person must take the final responsibility for the laboratory results hinders an optimal cooperation between the two disciplines and should be discontinued in order to optimise patient care and treatment as well as to ensure future development and implementation of relevant scientific discoveries.

MEDICAL LABORATORY MANAGER – NON-ANALYTICAL WORK

R. Lichtinghagen

Abstract not received

REQUIREMENTS FOR EXCELLENCE IN LABORATORY MEDICINE – A MELTING POT OF BIOSCIENCES AND MEDICINE

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Laboratory medicine is common divided in some sections, like Clinical Chemistry or Clinical Biochemistry, Haematology, Clinical Microbiology and Transfusion Medicine and Hemotherapy. The laboratories are almost hospital laboratories often separated into each section or centralized and private laboratories mostly with a wide offer of special tests.

Inside these laboratories the sample proceeding usually starts with a request form and some different samples from a patient. Then the samples, resp. specimens were analyzed in different automated analyzers or by staff, for example to differentiate the cells in a blood count with a microscope.

All results must be verified by a competent professional according to ISO 15189. The medical technologists give answers to the majority of questions like a result given by phone or technical problems. The most work with abnormal results referred to the relevant pathological pathway is done by clinical scientists or in some laboratories only by medical staff. Both, non-medical and medical specialists, have the right to interpret und discuss pathology results. To do this work at all levels in various fields of biology and medicine, esp. for application to human systems, the methods and tools in Biosciences and Medicine must be known. Some important key features are:

• Methodology details
• Alternative methodology approaches
• Critical test evaluation
• Interference factors
• Result validation
• Explaining pathologic results.

For the academic staff in a medical laboratory it is very important for all this aspects to have knowledge in Biosciences and Medicine.

WS 1 Reporting HBA1C for Monitoring and Diagnosis: The Devils's in the Detail

Monday, 16 May 2011   09:00–11:00

ESTIMATED AVERAGE GLUCOSE 2 YEARS ON

E. S. Kilpatrick

Haemoglobin A1c gives an indication of average glycaemia over at least the last 1-2 months in patients with diabetes. It has been suggested that this means there may be merit in expressing HbA1c as an average plasma glucose equivalent, or 'estimated average glucose' (eAG) rather than in the traditional way of being reported as a percentage or in mmol/mol.

There has been an ensuing debate as to whether such a move will be a help or hinderance in the management of diabetes patients. This workshop will describe the evidence for implementing eAG as well as some of the problems that can arise in its use. This will hopefully allow delegates to reach an informed decision about whether, on balance, eAG will be more of a benefit or detriment to healthcare staff and patients.

STATUS OF HBA1C MEASUREMENT AND GOALS FOR IMPROVEMENT

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Background. The Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) established hemoglobin A_{1c} (HbA_{1c}) as an important predictor of risks for complications in patients with diabetes mellitus. Based on the results of these studies, many clinical diabetes organizations have recommended specific HbA_{1c} targets or 6.5 or 7% HbA1c for most people with diabetes. However, lack of comparability among assays limited the ability of clinicians to use these targets. The National Glycohemoglobin Standardization Program (NGSP) was implemented in 1996 to standardize HbA_{1c} results to those of the DCCT/UKPDS. There has been considerable improvement in HbA_{1c} measurement as a result of NGSP activities. This is especially important in light of recent recommendations to use HbA1c for screening and diagnosis of diabetes.

Methods. The NGSP certifies manufacturers of HbA_{1c} methods as traceable to the DCCT. The certification criteria have been tightened over time and the NGSP has worked with the College of American Pathologists (CAP) in tightening proficiency testing requirements. The International Federation of Clinical Chemistry (IFCC) has developed a reference system for HbA_{1c} that facilitates metrological traceability of HbA1c results to a higher order reference system. The NGSP maintains traceability to the IFCC network via ongoing sample comparisons. The controversy over whether to report HbA_{1c} results in IFCC or NGSP units or as estimated average glucose (eAG) is being decided by individual countries.

Results. Most countries are choosing to dual report HbA1c in % (NGSP) and in mmol/mol (IFCC) before switching to IFCC results only. The US will continue to report %HbA1c and has also recommended reporting of eAG. The NGSP and IFCC programs are complimentary in their roles of maintaining and improving the quality of HbA1c testing. As a result of NGSP efforts in tightening NGSP certification criteria and CAP criteria for proficiency testing, variability of HbA_{1c} results among clinical laboratories has been considerably reduced. Based on CAP data, all-method CVs have decreased from approximately 7% to 4.0% between 2000 and 2010 in the low (non-diabetic) sample range.

Conclusions. The quality of HbA1c measurements continue to improve. Goals of within and between-laboratory CVs of $\leq 2\%$ and $\leq 3.5\%$, respectively, have been proposed. Not all countries will report HbA_{1c} in the same units, but there are established equations that enable conversion between different units. The NGSP will continue efforts to improve HbA_{1c} testing to ensure that clinical needs are met.

EQA: HbA1c FIT FOR THE DIAGNOSIS OF DIABETES?

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Background. Throughout the years the concept and golden standard for the diagnosis of diabetes have changed. In 1979 the National Diabetes Data Group in the US and the WHO proposed glucose concentrations as leading concept with the oral glucose tolerance test as the gold standard. In 1997 the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus advocated long term complications as leading concept with fasting plasma glucose as gold standard. In 2009, guided by the successful standardisation of HbA_{1c}, an international expert committee (installed by ADA, EASD and IDF), investigated HbA_{1c} for diagnosis and concluded "HbA_{1c} is the gold standard for diagnosis and screening". Meanwhile HbA_{1c} has been included in the guideline of the ADA; decision limits are a) diabetes (>46 mmol/mol;>6.4%), b) increasing risk of diabetes (40-46 mmol/mol; 5.8 – 6.4%) and c) low risk of diabetes (<40 mmol/mol;<5.8%). The difference in HbA_{1c} between the categories are small (6 mmol/mol; 0.6%) and it is questionable whether HbA_{1c} can be measured accurately enough to warrant reliable diagnosis of diabetes.

Methods. The federal EQA programme of national organisers in Belgium, Greece, Finland and the Netherlands provides real life, state of the art performance of routine methods for HbA_{1c} in terms of trueness (bias from target established with the IFCC Reference Measurement Procedure) and reproducibility (intralab CV and interlab CV).

Results. In 2010, at the 42 mmol/mol (6.0%) HbA_{1c} level, the mean bias of 475 laboratories was 0.0 mmol/mol (0.0%) with a mean intralab CV of 1.8% and an interlab CV of 2.8%. 95% of the participating labs correctly classified this sample as "increasing risk"; 4% concluded "low risk" and 1% came to "diabetes". These results are quite reassuring and seem to validate the use of HbA_{1c} to diagnose diabetes. However it should be considered that there are differences in performance in the various methods, ranging from excellent for last-generation HPLCs to poor for a number of POCT instruments. In the example above, a bias of +3 mmol/mol (0.3%) along with an intralab CV of 6% will lead to 45% misclassification (5% erroneously "low risk" and 40% erroneously "diabetes"). In addition, not measured in EQA programmes, the biological (inter-individual) variation, related to erythrocyte lifespan might affect the validity of HbA_{1c} for diagnosis.

Conclusion. HbA_{1c} can be used for diagnosis of diabetes, provided a well standardised, highly reproducible analytical method is used and erythrocyte lifespan is normal.

SYM 5 From Bench to Bedside in Autoimmunity

Monday, 16 May 2011   14:30–16:30

ANTIPHOSPHOLIPID SYNDROME: PATHOGENESIS

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In 1990, three groups, including our group, independently reported the necessity of a cofactor for the binding of autoimmune anticardiolipin antibodies (aCL) to the solid phase phospholipids. β2-glycoprotein I (b2GPI) was identified as this cofactor. Subsequently, we showed that the epitope for aCL develops when b2GPI is adsorbed on polyoxygenated polystyrene plates. We also described antiprothrombin antibodies bind to prothrombin exposed to immobilized phosphatidylserine and established a phosphatidylserine dependent monoclonal antiprothrombin antibody.

We found that this nicked b2GPI is a marker of cerebral infarct and has a novel role in the negative feedback pathway of extrinsic fibrinolysis. Very recently, we showed that plasmin-nicked b2GPI promotes angiogenesis by interacting with plasmin-generated angiostatin4.5.

We demonstrated that monocytes stimulated by monoclonal anti- b2GPI antibodies from APS patients induce phosphorylation of p38 MAPK, a locational shift of NFkB into the nucleus and up-regulation of TF expression. We clarified the profile of complement activation in patients with APS. The serum complement levels were clearly lower in patients with primary APS than in healthy persons or in controls having non-SLE rheumatic diseases. In addition, hypocomplementemia was correlated with anticoagulant activity and TNFa levels, suggesting that antiphospholipid antibodies are potent to activate monocytes/macrophages via complement activation. Monokines are activators of procoagulant cells. Therefore, we may conclude both the complement system and the direct effect of antiphospholipid antibodies on monocytes or endothelial cells are most important for the pathophysiology of APS. Very recently, we observed that STAT4 single nucleotide polymorphism, rs7574865 G/T, is a risk for antiphospholipid syndrome. The positive correlation between STAT4 SNP and APS are evident. Moreover, the correlation was even enhanced when focusing at primary APS, indicating that this SNP is also associated with APS itself. BANK1, BLK and SNP in 1q25.1 region were associated with not only SLE but also APS in Japanese population. TNFSF4 association was found only in APS population whereas TNFAIP3 association was found only in SLE. These results suggest that APS and SLE share a common genetic background.

NOVEL STRATEGIES FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

S. Gay

The successful use of the novel "Biologicals" has revolutionized the treatment of rheumatoid arthritis (RA) over the last two decades. Nevertheless, despite the tremendous progress made in drug development, we cannot cure the disease yet and not more than 60% of the treated patients achieve an ACR 70. This fact has prompted the pharmaceutical industry to enter a quite competitive race illustrated by the presently ongoing clinical trials exceeding over 400 entries world wide to improve this still limited outcome. By looking at the cells targeted with the current therapeutic strategies, it needs to be acknowledged that the achievements were obtained by reducing and/or eliminating certain cell types intimately involved into the pathogenesis of the disease, such as macrophages, as well as certain subsets of B and T cells. However, since it is known that synovial fibroblasts in RA are not only effector cells, but also represent an endogenously activated phenotype involved at least in part by spreading the disease (1), novel strategies have to be developed to target specifically the activity of this type of cells (2).
Our laboratory has been searching for the factors leading to the activation and persistence of activities by studying the epigenetic modifications responsible (2). We could show that the cells reside in a hyperacetylated synovial environment and are characterized by to be hypomethylated. Moreover, the synovial fibroblasts from patients with RA display a specific set of modulated miRNAs (3) responsible for the overproduction of inflammatory cytokines, like TNFa and IL-6 as well as chemokines like CXCL12. Based on these findings novel targets are currently evaluated.

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THE MANY CHALLENGES OF AUTOANTIBODY TESTING

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Detection and quantification of IgG autoantibodies against normal components of tissue are important tests in the diagnosis and management of a variety of autoimmune diseases. Historically, manual, qualitiative or semi-quantitative indirect immunofluorescence assays have been used for detection or "screening" of autoantibodies. ELISA based assays have been used as follow-up investigations to confirm and quantify the concentration of the autoantibody. These investigations were typically requested in small numbers and were suited to specialised or "manual" areas of pathology. Consolidation of services and the continual increase in demand has pushed these tests towards automated platforms and a perceived requirement for quantitation. These ELISA based tests are now widely available on automated immunoassay analysers and these assays are now being moved from dedicated specialised labs into high throughput routine service laboratories. The next large marked for autoimmune serology testing is the general public with some home or pharmacy testing kits already available. The detection and quantification of auto antibodies however, presents a selection of analytical problems. These are related to (1) the variability in the antigens used as reagents to bind to the auto antibodies in the patients samples (2) the lack of reference material and standardisation (3) the variability in the affinity and avidity of the autoantibodies in the patient sample and (4) the variability in the methods used. The implications of these variations are clearly seen when a patient for example with SLE is monitored in more than one lab; in one situation the tests may show that the disease is under control while the same sample analysed in a different lab may show that the disease is active. There is an important clinical risk so we must understand the underlying science in order to generate, evaluate and introduce some harmonisation in autoimmune serology testing.

NEW CLINICAL ASPECTS IN RHEUMATOID ARTHRITIS - GUIDELINE BASED MANAGEMENT

J.S. Smolen

Abstract not received

SYM 6 Diabetes

Monday, 16 May 2011   14:30–16:30

IFCC STANDARDISED HBA1C: SHOULD THE WORLD BE AS ONE?

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Following the introduction of HbA_{1c} methods into routine use during the early 1970's, it quickly became apparent that there was a significant difference in the results produced by different laboratories due to the lack of a standardisation. The lack of international standardisation resulted in several countries developing National Standardisation Programs. A common feature of these National programs is the absence of internationally recognised and accepted reference materials and measurement procedures to assure the accuracy and comparability of HbA_{1c} measurements at a global level.

To address these shortcomings, and to achieve a uniform international standardisation of HbA_{1c} measurements, the IFCC established a Working Group (WG) on HbA_{1c} Standardisation with the aim to develop a complete reference measurement system based on the concepts of metrological traceability. In addition to reference methods and materials, essential elements of a comprehensive reference measurement system include the definition of the measurand (including the unit) and the establishment of a network of reference laboratories. For this project, the decision was made to define HbA_{1c} as haemoglobin molecules having a stable adduct of glucose to the N-terminal valine of the haemoglobin b chain (bN-1-deoxyfructosyl-haemoglobin). Two equivalent reference methods specifically measuring this hexapeptide were then developed, with a combination of high-pressure liquid chromatography (HPLC) and electron-spray mass spectrometry (MS) or, alternatively, a two dimensional approach using HPLC and capillary electrophoresis (CE) with UV detection. In 2001, the IFCC reference methods were unanimously accepted by the National Societies of Clinical Chemistry, a network of laboratories was then established, using either the HPLC-MS or the HPLC-CE option.

During the past years the use of IFCC traceable calibrators has become universal. Several countries have moved to implement the 2007 consensus statement[1] but the detail of reporting units and timescale remains variable. Unlike many countries, the American Diabetes Association, later backed by the American Association for Clinical Chemistry, advocate reporting of estimated average glucose (eAG) in addition to HbA_{1c} [2].

In an attempt to facilitate greater harmonisation in the reporting of HbA_{1c} a further consensus statement on the worldwide standardization of haemoglobin A_{1c} measurement was published in July 2010[3]. The new statement reinforces the proposals in the earlier statement except reporting of eAG is no longer recommended. It remains to be seen whether this second consensus statement will lead to more consistent practice in the reporting of HbA_{1c}.

In the interests of patient safety there is an urgent need for diabetic physicians and laboratory medicine professionals to agree and implement a harmonised procedure for reporting HbA_{1c} measurements. IFCC recommends the 2010 consensus statement for this purpose[4].

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GENETICS OF TYPE 2 DIABETES

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Lecture Speaker: A. Rosengren

During the last 30 years the world has faced a formidable epidemic of obesity and type 2 diabetes. It is clear that the genes have not changed during this short period of time. The logical conclusion would then be that it is all due to the change in the environment. But genes determine how we respond to the environment and genes change in response to the environment as seen for the lactase gene. This gene started to mutate with the domestication of cattle to allow adults to be able to utilize energy from cow milk. The picture of the genetics of type 2 diabetes (T2D) has dramatically changed with the introduction of whole genome wide association studies (GWAS). Today we know about 40 gene variants which are consistently associated with T2D. This list includes among others TCF7L2 (the No 1 T2D gene),PPARG, KCJN11, CDKAL1, IGFBP2, CDKN2A/CDKN2B,HHEX, SCL30A8, FTO, WSF1, MTNR1B etc.. Also studies on glucose-related traits have shown that variants in G6PC, GCK, GCKR and GIPR are associated with glucose and insulin levels. Common to most of these variants is that they seem to result in impaired beta-cell function. Individuals who carry these variants seem to be unable to increase their insulin secretion in response to an increase in BMI and insulin resistance to maintain glucose tolerance normal. Only variants in two genes, IRS1 and PPARG seems to influence insulin sensitivity and only one increases risk of T2D by increasing obesity, FTO. It is premature to start to use these genetic variants for prediction of T2D as we have only been able to explain a small proportion of the familial risk of T2D. We shall though not underestimate the importance of dissecting novel biological pathways for the pathogenesis of T2D, some of which might become potential new drug targets. An important step will also be to use the genetic information for prediction of disease progression, development of complications and response to treatment, i.e. a step towards individualized medicine.

HBA1C IN THE DIAGNOSIS OF DIABETES: OPPORTUNITIES AND PROBLEMS

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The laboratory performs a fundamental role in the diagnosis and management of patients with diabetes mellitus. Measurement of haemoglobin A1c (HbA1c) is used extensively in individuals with diabetes mellitus to monitor long-term glycaemic control and adjust therapy. Large prospective randomised clinical trials, most notably the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), documented that HbA1c is a measure of the risk of developing microvascular complications in type 1 and type 2 diabetes, respectively. Based on these data, many diabetes organisations around the world recommend a HbA1c value of 6.5 to 7% (48 to 53 mmol/mol) as the target for glycaemic control. The HbA1c assay is thus an integral component of diabetes management.

Glycated haemoglobin (GHb) consists of HbA1a, HbA1b and HbA1c. Almost 100 methods are commercially available to measure GHb. These factors have led to considerable variation among results reported by different laboratories. Efforts by the NGSP, which uses a standardisation process based on the DCCT reference method, and the IFCC, which developed a reference method for HbA1c, have significantly reduced interlaboratory variation over the last few years.

Detection of increased glucose concentrations has been used to identify individuals with diabetes mellitus for over 3,500 years. Measurement of glucose in the blood – either after a glucose challenge or in the fasting state - has for many years been the sole criterion to diagnose diabetes. However, this approach is limited by several factors, including the need for the subject to be fasting, large biological variability and a lack of reproducibility of the oral glucose tolerance test. HbA1c is both an indicator of long-term glycemic control and predicts risk for the development of microvascular complications. These attributes, combined with low intra-individual variability and the lack of influence of food ingestion (a fasting sample is not necessary), make HbA1c a conceptually appealing marker for the diagnosis of diabetes. Importantly, improvements in harmonization of HbA1c assays has eliminated one of the major impediments to its use as a diagnostic criterion. Based on these characteristics, HbA1c has been recommended by the American Diabetes Association - and is expected to be recommended by the World Health Organisation - for screening and diagnosis of diabetes. These new developments are likely to have considerable impact on clinical laboratories.

MOLECULAR AND BIOCHEMICAL MECHANISMS OF DIABETIC COMPLICATIONS

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The evolution of diabetes mellitus is characterized by severe degenerative long-term complications due to hyperglycemia and related metabolic disorders, which involve enzymatically controlled pathways (polyol, hexosamine, specific protein kinase C isoforms). However, the most prominent feature is the increased rate of protein nonenzymatic glycation, which refers to the spontaneous binding of reducing sugars (mainly glucose) to free amino groups. This cumulative and irreversible reaction is amplified by oxidative processes (the whole reaction is often referred to as "glycoxidation"), generates a variety of intermediate reactive by-products such as aldehydes, and leads to the formation of stable, generally fluorescent, compounds named "advanced glycation end products" (AGEs) together with products of other oxidative pathways, *i.e.* "advanced oxidation protein products" and "advanced lipoxidation end products" (AOPPs and ALEs). AGEs (*e.g.* : pentosidine) accumulate in tissues and generate abnormal protein crosslinks that alter tissue organization and interactions with cells, mainly in long-lived proteins of extracellular matrix. Their amount is related to degenerative complications of diabetes mellitus. Various enzymatic mechanisms that mediate protein repair (*e.g.* : amadoriases such as fructosamine-3-kinase) or degradation of reactive intermediates (*e.g.* : glyoxalases) are altered during the course of diabetes mellitus.

The deleterious effects of glycoxidation products involve direct actions on cells (toxic effects, oxidative damages) or on proteins (structural and functional alterations), and receptor-mediated actions. Many cell types express membrane receptors that bind AGEs, such as RAGE, receptor of AGEs, which triggers intracellular signalling pathways stimulating specific cell functions (*e.g.,* synthesis of inflammatory cytokines, oxidative stress, migration).

Besides glycation, diabetes mellitus is characterized by other metabolic disorders involving protein modifications, especially when patients develop glomerulopathy and chronic renal failure. Firstly, AGEs are retained in blood and tissues because of renal impairement, and generate a major oxidative stress. Secondly, another nonenzymatic modification of proteins, carbamoylation, is increased. Carbamoylation is due to the protein binding of isocyanic acid, formed *in vivo* either by spontaneous dissociation of urea or by the enzymatic action of myeloperoxidase on thiocyanate in presence of H_2O_2. It may occur at the α-NH_2 extremity of proteins or aminoacids, or on ε-NH_2 lysine groups, generating homocitrulline. Carbamoylation of proteins leads to alterations of their structural characteristics, biological properties or bioavailability. For example, carbamoylation inhibits the capacity of collagen to stimulate oxidative functions of polymorphonuclear neutrophils. Besides, carbamoylated collagen exhibits an altered resistance to enzymatic digestion and stimulates matrix metalloproteinase-9 production by monocytes, thus increasing their ability to remodel arterial walls. Carbamoylation occurs concurrently with glycoxidation and competes for the same protein sites.

Thus, the metabolism of these numerous nonenzymatic post-translational modification derived products (PTMDPs) constitute a promising field of investigation in basic research and laboratory medicine, especially for preventing diabetic long-term complications which constitute an increasing problem of public health worldwide.

WS 2 Emerging Infections at AFCB Countries

Monday, 16 May 2011   14:30–16:30

ANTIMICROBIAL RESISTANCE: AN EMERGING PUBLIC HEALTH PROBLEM

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Antimicrobial Resistance (AMR) which is a natural process has been greatly augmented by human actions.

AMR is becoming a global public health problem which is undermining efforts to control infectious diseases. Unregulated use of antimicrobial, both in humans and in animals is a major cause behind the increasing magnitude of the AMR. Use of antimicrobials in humans suffer from overuse, misuse and underuse. It is estimated that in developed countries, human use of antimicrobials constitute 50% of all production while the other 50% is used in animals mostly as a gross promoting factors in food producing ones. In addition to its deleterious effect on health, the economic impact of AMR has recently become a real concern. The monetary cost of treating AMR infections worldwide is estimated to be many billions of US dollars per year. It's known that treatment of drug resistant infections cost as much as 7-90 times more than non resistant ones. This increase in cost might even reach thousands of times more in the case of multi drug resistance as noticed in MDR- TB.

World Health organization (WHO) in 2001 issued the first global strategy for combating the serious problems caused by the emergence and spread of AMR. The strategy recognized AMR as a global problem. No country on its own can successfully protect itself from resistant organisms. Unfortunately, this strategy was not translated into concrete action, particularly in developing countries. Many public health experts refer to the past twenty years as the years of neglect of drug resistance.

Establishing lab-based networks for surveillance of AMR is a WHO priority. WHO theme for the World Health Day in 2011 is containing AMR through development of a comprehensive policy package for health ministries. This will be an opportunity to launch sustainable efforts to contain resistance. If prompt action is not taken in the near future particularly in the developing world, the problem will go out of hand, especially when the big pharmaceutical companies are showing no interest in developing new antimicrobials.

VISCERAL LEISHMANIASIS HIV, HEPATITIS B AND HEPATITIS CO-INFECTIONS

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Background. Visceral leishmaniasis (VL) is an opportunistic febrile parasitic disease that is inevitably fatal if not treated. In Sudan the disease mainly affects children. Since its first description in 1904, Sudanese VL wiped large groups of the population in endemic areas. Currently, VL is endemic over large areas of Sudan, with new foci continuously emerging with a prevalence of 60-80 patients/1000 individuals/year. In 1988, 1998 major epidemics claimed hundreds of thousands of individuals in Kordofan, Gedarif and Western Upper Nile States. Successful treatment is followed by a dermatosis called post kala-azar dermal leishmaniasis (PKDL) in more than 50% of cases. PKDL is believed to be an important disease reservoir. Case control and drug treatment is the most effective method of disease control. The main treatment is sodium stibogluconate (SSG) and AmBisome, these drugs are either toxic or expensive. Protective VL vaccines studies have been going on for the last few years, first generation vaccines proved inefficacious in their present form. Modulation of the whole parasite vaccines by addition of Alum as adjuvant showed promising results in Phase I/II studies. Emerging VL HIV, HBV and HCV co infection markedly changed the epidemiology and clinical presentations of VL and dictated modifications to treatment protocols to reduce accompanying drug/disease morbidity and mortality. *L.donovani, HBV and HCV* and SSG produce hepatocellular damage evidenced by increased serum bilirubin, transaminases and alkaline phosphatase in patients with VL/HBV/HCV co-infection. The prevalence of HBV/HCV in Sudan ranges from 5% to 10%, while HIV sero-positivity is ~0.01% among healthy blood donors.

Methods. In 2010, nine hundred and fifty patients with febrile illness (fever> 3 weeks duration) were were parasitologically positive in lymph node/bone marrow aspirates. The parasite isolates were characterized as *L.donovani* using species specific kDNA primers. VL patients were screened for HBsAg, HCV and HIVI/II. Transaminases (ALT & AST) levels were determined in the sera of all patients. HIV I/II seroreactivity was seen in one patient, while HBsAg and HCV was detected in 5% of patients. Transaminases levels were > 3 folds of the upper range of normal in all VL /HBV/HCV co-infected patients. Three VL /HBV/HCV co-infected patients who were on SSG treatment failed to show improvement in temperature and general condition and they continued to feel ill with considerable increases in the transaminases levels, and finally died despite stopping SSG treatment. For the rest of the patients, ambisome was substituted for SSG and patients were cured without further complications. Counselling was provided for all patients and were advised to report for follow up.

Conclusions. VL HIV, HBV and HCV co-infections are emerging as important causes of morbidity and mortality in the Sudan. The treatment protocols were changed to cater for HBsAg/HCV reactive VL patients and alternative regimens of ambisome were given.

TUBERCULOSIS IN THE AFCB COUNTRIES; RE-EMERGING DISEASE

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Background. Each year over nine million people become ill with TB and nearly two million people die. In a single minute, three people die from TB. In a single day, TB causes the equivalent in lives lost of 15 jetliner crashes.

People with TB often suffer from discrimination and stigma, rejection and social isolation. The disease is a major cause of poverty because people with TB are often too sick to work, and they and their families may have to pay for treatment.

TB is the number four cause of death among women worldwide.

People living with HIV are up to 50 times more likely to develop TB than people free of HIV infection; TB is the leading cause of death among people living with HIV in Africa.

In this paper we are trying to shed some light about the current situation of tuberculosis in AFCB and EMRO region.

In 2008, the estimated number of prevalent tuberculosis cases in the Eastern Mediterranean Region was 929.166. The estimated number of incident tuberculosis cases in 2008 was 674.585. Of these, 294.866 were sputum smear positive pulmonary tuberculosis. The below figure shows that 7 countries contribute to 92% of the tuberculosis burden in the Region. These are Afghanistan, Pakistan, Iraq, Morocco, Somalia, Sudan, and Yemen. Pakistan alone shoulders 61%the burden of the Region. Another figure shows the progress in reducing tuberculosis burden in 2008 compared to 1990. It shows that 11 countries reduced the tuberculosis burden to rates below 25 per 100.000 population compared to one country in 1990. The estimated number of TB deaths in 2008 was 115.137 deaths.
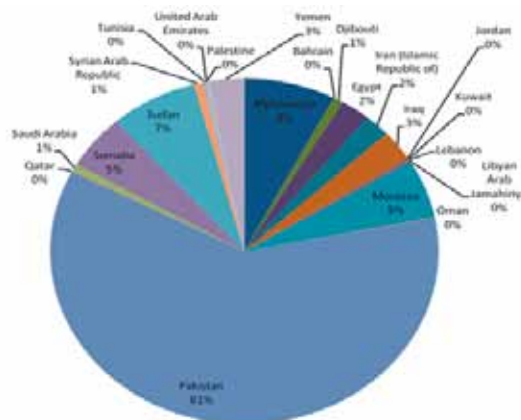
The figure below shows the development of treatment success rate and case detection rate from 1995 till 2007.
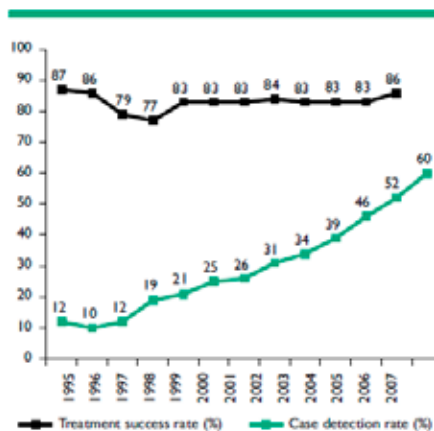
Figure 6. Case detection and treatment success rates for smear-positive tuberculosis in countries in the Region, 2007

EFCC 1 How to Assess the Clinical Effectiveness of Laboratory

Monday, 16 May 2011   14:30–16:30

INDUSTRY AND REGULATORY PERSPECTIVE: CLINICAL EVIDENCE FOR IVDS

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Background: Defining and using clinical evidence for in vitro diagnostic assays, has always been complex not least because often a marker is correlated to a condition before the actual mechanisms which explain that correlation have been fully understood. Three related developments have brought to the forefront of the regulatory discussions the development of clinical evidence for IVDs, namely the increasing role of genetic assays in the clinical laboratory, the development of new generations of cancer markers and the increase in the use of companion diagnostics.

Regulators and industry have been discussing this issue within the framework of the Global Harmonization Task Force (GHTF), however it is apparent that the answer to the complex question of how clinical evidence for IVDs is developed, understood and used is not something that can be fully understood without the input from clinical laboratory professionals themselves.

The state of the discussions at this point will be covered by the this presentation as well as trying to identify the concerns of industry and the way in which the process of development and use of clinical evidence for IVDs can be better defined.

TESTING A TEST – PHASES OF BIOMARKER EVALUATION

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In the era of "omics" and drug discovery we are witnessing the emergence of new potential biomarkers and companion diagnostics. Translational research is the process between biomarker discovery and clinical use. Currently the analytical validity of new tests is more thoroughly assessed during this process than their clinical validity or impact on patient-relevant or organizational or economic outcomes. The European Commission, government and IVD regulatory bodies have recently proposed that the clinical effectiveness of biomarkers (i.e. the extent to which the test improves health outcomes) also needs to be appropriately addressed before the test is released to the market. These initiatives call for standards and harmonization of biomarker evaluation studies. To meet these challenges, EFCC has involved laboratory professionals, researchers, the IVD industry and epidemiologists in its Test Evaluation working group in order to provide an evidence-based framework and practical tools for the assessment of new biomarkers.

The journey of a biomarker in the research translation continuum starts with the identification of a link between the test and certain diseased states. These early research questions are best addressed by case-control studies, which, however, are known to produce a biased estimate of diagnostic accuracy in real life settings. Once the link is proved to be strong, the next step is to investigate how the new test fits into existing clinical pathways (i.e. an add-on, triage or replacement) and what the purpose of testing will be. Only those tests that have a potential to improve existing practices should enter the next phase of analytical validity assessment according to well-defined IVD regulatory requirements. The purpose of testing will also define the most appropriate study designs for the assessment of clinical validity. Whilst the STARD initiative provides standards for diagnostic accuracy studies, these criteria will not be appropriate when the biomarker is intended to be used for screening for a disease, monitoring therapy, or assessing risk or prognosis.

The assessment of the clinical effectiveness of a diagnostic test is more complex than that of drugs and is determined by the diagnostic or prognostic accuracy of testing, its impact on management decisions, and the effectiveness of treatment. Ideally, randomised controlled trials provide direct evidence of clinical effectiveness. 'Direct evidence' comes from trials that compare groups of people receiving either the existing or the proposed diagnostic test/test strategy and measure the differential impact of the new method on outcomes. These studies are rarely available and in some cases may not even be feasible or justified in practice. When direct evidence on outcomes is unavailable an alternative approach is 'linked evidence' of test effectiveness which connects the evidence of comparative test accuracy to separately sourced evidence of treatment effectiveness in order to approximate the likely clinical impact of the new test/testing strategy.

The framework presented highlights the need for clear definition and demarcation of the steps to be taken pre- and post-market during the development of biomarkers. The criteria described intend to increase the quality and clinical applicability of translational research findings on the utilization of biomarkers.

EVALUATING THE EFFECTIVENESS AND COSTS OF MONITORING

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In an era of evidence-based medicine and increasing scrutiny of the use of health care resources, decisions about monitoring should also be based on solid evidence of its effectiveness and costs. A specific monitoring strategy should only be used in practice if there is evidence that it improves or maintains patients' health, and that this gain in health outcome is obtained at reasonable balance with the additional resources needed.

Whenever we evaluate the effectiveness of monitoring, we assess more than the monitoring test itself. What we will evaluate is not the monitoring test, but a specific monitoring strategy. Such a strategy has a protocol for repeated testing, defines action thresholds, and specifies the actions to be taken if any of the thresholds is reached.

RCT's have become the cornerstone of evidence of medical effectiveness. Monitoring RCT's will always be trials of monitoring strategies. Because of the interactions between tests, repeated tests, test results, and the decisions based on these results, monitoring RCT's will usually require large sample sizes. There are a number of other challenges in and disadvantages to RCT's of monitoring strategies. One of them is that RCT's are usually limited to comparisons of two or three monitoring strategies, whereas in many circumstances an infinite multitude of monitoring strategies can be developed, differing in the type of assessment, the persons who are doing the assessments, the timing, the decision limits, and the changes in interventions. Because of these limitations, modeling may be a useful alternative for evaluating monitoring strategies. Modelers look at a hypothetical cohort of patients and try to estimate the changes in health outcome from implementing one or more monitoring approaches. In the model several pieces of evidence, coming from a variety of study designs, are incorporated to estimate the final health outcomes: the natural course of the condition that is being monitored, the accuracy of the monitoring tests, and the effectiveness and risks of treatment. One should not immediately jump to RCTs or models. There are several reasons to make a plea for a staged approach, similar to those that have been proposed for the evaluation of pharmaceuticals and diagnostic tests. In a first stage, researchers will try to collect evidence about the variability and predictability of the condition that is to be monitored, and the performance of the monitoring test. This will be followed by and identification of the most promising monitoring strategy and competitors, usually based on modeling. Evaluation of monitoring is not easy, but in an era where chronicity is growing in health care, monitoring should be carefully evaluated, as all other interventions in health care.

CLINICAL PRESPECTIVES: CLINICAL EFFECTIVENESS OF SELF-MONITORING ORAL ANTICOAGULANT TREATMENT

C. Heneghan

Abstract not received

DGKL 2 Laboratory Medicine – Quo vadis?
Conditions, Structure and Developmental Trends in Europe

Monday, 16 May 2011   14:30–16:30

CONCENTRATION IN GERMAN LABORATORY MEDICINE – WHERE ARE WE HEADED?

M. Müller

Abstract not received

LABORATORY MEDICINE AS A MEDICAL DISCIPLINE – IS THE PHYSICIAN IN THE LABORATORY (IN)DISPENSABLE?

R. Klakow-Franck

Abstract not received

LABORATORY MEDICINE IN COMPETITION – THE SWISS EXPERIENCE

A. R. Huber

Abstract not received

FRANCE ON ITS WAY TO CENTRALISED LABORATORY DIAGNOSTICS

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The lecture, entitled "France on its way to Centralised laboratory diagnostics" will look in comparison with the Federal Republic of Germany especially on the changes and trends in our neighboring country of France. It will indicate the resulting developments and differences with a main focus on quality in laboratory medicine and patients welfare or risk.

INT 2 Pitfalls in the Hemostasiologic Diagnostics: Clinical Cases

Monday, 16 May 2011   14:30–16:30

CURRENT ASPECTS AND FUTURE TRENDS IN THE ANALYSIS OF BLOOD COAGULATION

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Background. Several established laboratory tests for coagulation factors, platelet and vascular components as well as for specified pathological conditions exist that are frequently used in the routine laboratory. Moreover, analytical parameter settings have been defined that are characteristic for certain hereditary or acquired disorders of the haemostatic/coagulation system or are associated with severe pathological situations such as sepsis. In this presentation, mechanistic aspects in the onset and regulation of the haemostasis/coagulation system are covered that will introduce novel factors and molecular connections, eventually leading to new diagnostic tools.

Results. The following molecular and cellular systems will be discussed: Protein disulfide isomerase and tissue factor activation; intravascular tissue factor pathway and innate immunity; the extracellular RNA/RNase system in thrombosis; platelets and angiogenesis; microparticles as biomarkers.

Conclusions. Further understanding of new molecular pathways in haemostasis/coagulation and their respective links to vessel formation, inflammation or vessel degeneration will allow to define new diagnostic parameters that may help to specifically analyse patients with thromboembolic complications or vascular diseases.

NEW ANTICOAGULANTS AND ROUTINE COAGULATION TESTS

A.C. Haushofer[1], V. Aliskanovic[1], T. Mayerhofer[1], D. Trubert-Exinger[1], G. Weigel[2], L.J. Loacker[2], M. Schnapka-Köpf[2], A. Griesmacher[2], J. Tomasits[3], G. Aspöck[4], P. Quehenberger[5], W.M. Halbmayer[6]
[1]Institut für Laboratoriumsmedizin, Landesklinikum St. Pölten, St. Pölten, Austria, [2]Zentralinstitut Med. Chem. Labordiagnostik, Medizinische Universität Innsbruck, Innsbruck, Austria, [3] Zentrallabor, Allgemeines Krankenhaus Linz, Linz, Austria, [4]Institut für Laboratoriumsmedizin, Klinikum Wels-Grieskirchen, Wels, Austria, [5]Klinisches Institut für Med. und Chem. Labordiagnostik, Medizinische Universität Wien, Vienna, Austria, [6]Institut für Laboratoriumsmedizin, Krankenhaus Hietzing und NRZ, Vienna, Austria

Background. New oral anticoagulants such as Dabigatran (F IIa inhibitor, prodrug Dabigatran etexilate) and Rivaroxaban (F Xa inhibitor) are approved for prophylaxis in thromboembolism in patients undergoing total knee or hip replacement and has also shown promising results for treatment of VTE and the prevention of stroke in arterial fibrillation. The extent of interaction with routine coagulation tests is not extensively evaluated. Interaction with this tests can perhaps lead to misinterpretation of coagulation tests, in the worst case even to wrong medical decision. To investigate the extent of interaction of Dabigatran with coagulation tests the task-force-group on new-oral-anticoagulants of the Austrian Society of Lab Med & Clin Chem started a multicenter pilot-trial with the first CE-labelled dabigatran–spiked plasma samples (Hyphen BioMed, kindly gifted by CoaChrom Diagnostica, Austria); intended use of this spiked samples is the possibility for Dabigatran activity measurement in plasma samples.
Methods. For Dabigatran the first CE-labelled spiked plasma samples (0.04 – 0.5 µg/ml) and for Rivaroxaban Rivaroxaban-spiked normal plasma samples (Coagulation Control N, Technoclone, Austria; plasma samples were "home brewed" spiked in our laboratory from 0.125 – 1 µg Rivaroxaban/ml plasma) were tested with routine coagulation tests.
Results. Dabigatran and Rivaroxaban showed dose-dependent influences reflected as prolongation of the clotting times of the majority of the measured coagulometric assays. Dabigatran showed the strongest influence on aPTT, thrombin time, Protein S activity, APC-Resistance followed by interferences (reduced results) with prothrombin time (PT, %) and fibrinogen (Clauss). Thrombin-dependent amidolytic (colorimetric) activity assays for F XIII and AT III showed interferences, for F XIII in decreased activity, for AT III increased activity. Not influenced were immunologic and non-thrombin-dependent assays (F XIII latex particle based, Plasminogen, Protein C, AT III F Xa based). Rivaroxaban showed the strongest dose-dependent influence on PT and Thrombotest (Axis-Shield, Technoclone, Austria; much higher extent compared to PT) and in a lower extent on aPTT. Factor Xa-dependent amidolytic (colorimetric) activity assays for AT III showed increased activities. Uninfluenced were the assays for fibrinogen (Clauss and PT-derived), thrombin time, reptilase time, D-Dimer, AT III (F IIa based).
Conclusions. New anticoagulants show a broad range of influence on routine coagulation tests and laboratories have be aware of this. Information on the drug used is essential for the coagulation laboratory. It is recommended that the best time for blood collection for coagulation tests under new anticoagulants is before the next dosage will be given, but in elderly or in liver or renal impaired patients a prolonged activity must be concerned. Much more evaluation is therefore needed and also activity tests for the new anticoagulants - for discrimination between coagulation disorder or drug interaction - have to be introduced.

TWO PUZZLING CASES

B. Lämmle

Abstract not received

GENERAL CASE REPORT

G. Weigel

Abstract not received

SYM 7 Pharmacogenetics and Pharmagocenomics

Tuesday, 17 May 2011   09:00–11:00

PHARMACOGENETICS IN THE TREATMENT OF PSYCHIATRIC DISEASES

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For the treatment of psychiatric diseases pharmacogenetic tests have promising clinical relevance. Drug-metabolising enzymes, especially cytochromeP450 (CYP) isoenzymes exhibit genetic variability, and recent investigations indicate that the drug efflux transporter P-glycoprotein (P-gp) in the intestinal mucosa and blood-brain-barrier is also relevant for the pharmacokinetic variability of psychotropic drugs. Moreover, multiple target structures involved in the mechanism of action of psychotropic drugs, such as dopamine receptors blocked by antipsychotic drugs or serotonin transporters (SERT) blocked by antidepressant drugs, genetic variants have been identified. Best evidence is given so far for a genetic association of a functional SERT polymorphism and the efficacy of selective serotonin reuptake inhibitors (SSRIs). We have recently observed a significant association of SSRI serum concentration and treatment outcome in long but not in short allele carriers of the SERT. This gave evidence that dose increase of SSRIs should be recommended only for patients with the long allele variant.

In conclusion, genotyping is not yet an element of every day psychopharmacotherapy. However, there are a number of special indications where genotyping is most helpful. For CYP enzymes, genotyping should be applied when the drug is characterized by a small therapeutic index with a risk of toxicity in case of a genetically impaired metabolism, or on the other hand, risk of treatment failure due to an ultra rapid metabolism and the inability to reach therapeutic drug levels. Another indication for genotyping of metabolic enzymes is given when the patient presents unusual plasma concentrations of the drug or its metabolite(s) and genetic factors are suspected to be responsible. With regard to the genetic variability of targets for psychotropic drugs, the short/long polymorphism of the serotonin transporter is so far the only candidate for which genotyping may be useful to optimize the psychopharmacotherapy.

PHARMACOGENETIC PREDICTORS OF DRUG INDUCED LIVER INJURY

M. Pirmohamed

Abstract not received

THE RAPIDLY EVOLVING STATE-OF-THE-ART IN CLINICAL PHARMACOGENTIC APPLICATIONS

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There are currently a limited number of clinical pharmacogenetic diagnostic services which have made substantial impact on global healthcare. However, several recent developments in the areas of programmatic integration of pharmacogenetic diagnostics into healthcare benefit plans as well as standard of care, development of clinical application information technology support systems, and, novel scientific discoveries are now poised to accelerate demand of existing services as well as to facilitate more rapid growth of newly established genetic biomarkers. The author will discuss the rationale and benefits with respect to the integration of CYP2C19 and HLA genotyping by health care benefit management organizations and academic institutions to escalate the standard of care in anti-platelet and anti-retroviral therapies. Progress towards the integration of CYP2C9:VKORC1 and other pharmacogenetic biomarkers into information technology systems will be discussed to demonstrate the potential for these clinical decision support systems to facilitate actionable alternative therapeutic strategies in the areas of oncology and anticoagulation. The session will conclude with a discussion of recent advances in the understanding of existing markers, such as Kras codon 12 vs codon 13 mutations as well as newly discovered pharmacogenetic markers which promise to make significant contributions to the evolution of healthcare from intuitive to precision medicine.

PHARMACOGENETICS OF ANTI-CANCER THERAPY

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One of the most challenging fields for optimizing drug therapy, is oncology. Not only because the therapeutic window of most drugs in this field is narrow, but also because of the mostly lethal consequences of both over- and undertreatment. One of the most prominent pharmacogenetic markers at the moment is the CYP2D6 genotype to predict outcome on tamoxifen therapy. Currently, results seem to indicate that this marker could be used fro thsi purpose, but controversial data have been published, placing regulatory bodies like FDA and EMEA, oncologisst and patients for difficult choices. In addition, data are emerging taht also genetic polymorphisms in CYP2C19 and UGT2B15 play a role in predicting the response to tamoxifen.
Other areas in which pharmacogenetics was thought to be a tool for optimizing therapy, was UGT1A1 genotyping for irinotecan, DPD testing for 5-FU treatment, CYP3A5 analysis for vincristine therapy and CYP2B6/CYP2C19 testing for cyclophsophamide treatment. What has happened to these potential markers in the last years?
A new area in which pharmacogenetics could possibly contribute, is in the treatment with the taxanes paclitaxel and docetaxel. Genetic polymorphisms in CYP2C8 and CYP3A4, but also in drug transporters may affect the level of side effects on these drugs. In our current study, the use of the Affymetrix DMET Chip, containing 1,936 SNPs is 225 drug metabolizing enzyme and drug transporter genes, may reveal new markers for optimizing taxane therapy.

YR 2 Infectious Diseases

Tuesday, 17 May 2011   09:00–11:00

NEW MARKERS FOR ACUTE KIDNEY INJURY

C. Ronco

Abstract not received

BEYOND CRATININE STANDARDISATION

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Background. The recent global campaign for standardisation of creatinine measurements has been promoted to allow the widespread use of formulas for estimating the glomerular filtration rate (GFR). However, studies on trueness verification and measuement interferences still show a disappointing interassay variation of serum creatinine results.
Methods. The clinical consequences of creatinine standardisation (SRM 967) are evaluated.
Conclusions. The creatinine recalibration has major clinical consequences. In particular in pediatrics, where reference ranges for serum and plasma creatinine are low, calculation of GFR is problematic when based on alkaline picrate methods because of method unspecificity and the lack of appropriate GFR estimating formulas. Therefore, enzymatic creatinine assays are to be preferred. In the near future, cystatin C determination might offer an interesting alternative for GFR estimation. As the lion share of literature dealing with drug dosage in renal insufficiency is based upon older (often poorly characterized) methods, the effects of creatinine standardisation on the calculation of drug doses are an important topic. The Modification of Diet in Renal Disease (MDRD) study formula generally offers reliable data. However, attention has to be paid to the aged. Also the calculation of the Model for End-Stage Liver Disease (MELD) score, which is used to prioritise patients for liver transplantation, may significantly be influenced by the recent recalibration of creatinine assays. Creatinine restandardisation may also affect the current guidelines for referral of CKD patients to nephrologists and the reimbursement criteria for a number of drugs (e.g. erythropoietin).

POCT GLUCOSE MEASUREMENT AND ITS LIMITATIONS

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Background. Uses of POCT glucose measurements range from home testing to guiding intensive insulin therapy (IIT). Early studies of IIT found reductions in mortality with use of an accurate glucose assay. Most subsequent studies used POCT glucose, and subsequent meta-analyses found no benefit of IIT. Recently, a very large study showed increased mortality with IIT that used primarily POCT glucose measurements. The effect of measurement error on glycemic control is unknown.
Methods. We and others performed computer simulations of patients whose treatment depends on glucose measurement. In our most recent studies of IIT we estimated hourly glucose concentrations based on the effects of (1) IV glucose administration, (2) gluconeogenesis, (3) insulin infusion rates as determined by each of 2 published algorithms, and (4) errors in glucose measurements. For each of 45 total-error conditions, we simulated 100 patients, and each patient was followed for 100 h (450,000 glucose results).
Results. We and others have shown that errors in measurement of glucose degrade the ability to select the appropriate insulin dose (in home use) or rate of insulin infusion (in IIT). Our most recent studies showed that (1) mean glucose was inversely related to assay bias, (2) glucose variability increased with negative assay bias and assay imprecision, (3) frequency of severe hypoglycemia increased with negative assay bias and assay imprecision, and (4) frequency of hypoglycemia (plasma glucose <3.3 mmol/L) increased with positive assay bias and assay imprecision.
Conclusions. Errors in glucose measurement compromise glycemic control and may compromise patient safety.

THE D-LIGHTFUL VITAMIN D FOR HEALTH

Michael F. Holick

Vitamin D is recognized as the sunshine vitamin because most of our requirement for vitamin D comes from exposure to sunlight. Vitamin D deficiency is associated with rickets in children and osteomalacia and osteoporosis in adults. Vitamin D deficiency and insufficiency has also been linked to a wide variety of chronic illnesses including the autoimmune diseases type I diabetes, multiple sclerosis and rheumatoid arthritis, cardiovascular disease, type II diabetes, cancer, neurocognitive dysfunction and infectious diseases. The recent Institute of Medicine report noted that children 13 years and older and all adults up to the age of 70 should increase their vitamin D intake threefold from 200 IU to 600 IU per day. To obtain all of the health benefits of vitamin D there is no downside to increasing vitamin D intake for children to 1000 IU and adults to 2000 IU a day not only to maximize bone health but also to reduce risk of many chronic illnesses that have been associated with vitamin D insufficiency. Although patients with chronic kidney disease require an active vitamin D analogue to reduce risk of secondary hyperparathyroidism it is also recommended that all CKD patients maintain a blood level of 25-hydroxyvitamin D> 30 ng/ml. Although there has been skepticism about the non-skeletal benefits of vitamin D recent RCTs have supported many of the association studies especially as they relate to vitamin D's benefit for reducing risk of infectious diseases and cardiovascular disease.

WS 3 Oxidative Stress in Diabetes Mellitus

Tuesday, 17 May 2011   09:00–11:00

ROLE OF NRF2 AND THE ANTISTRESS GENE RESPONSE IN COUNTERING OXIDATIVE STRESS IN DIABETES MELLITUS

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Transcription factor nuclear factor E2-related factor-2 (nrf2) regulates gene expression through the antioxidant response element (ARE) promoter signaling system. Nrf2 regulates the transcription of a battery of protective and metabolic enzymes. Activation of the nrf2 system enhances the protection against damage to proteins by oxidation and glycation, increases proteasomal removal of damaged proteins, down regulates lipogenic enzyme expression to correct dyslipidaemia, conflicts protectively with inflammatory cell signalling and counters metabolic dysfunction by increasing pentosephosphate pathway activity. This may potentially reverse activation of multiple pathways of biochemical dysfunction during development of diabetes and those induced by hyperglycaemia in vascular and other cells. Protective effects to be potentially realised, therefore, are slowing the decline of glucose tolerance in development of diabetes and reversal and prevention of vascular complications associated with diabetes. In experimental models, nrf2 is important in resisting loss of glycaemic control and chemical induction of diabetes, countering diabetic dyslipidaemia, preventing development of diabetic nephropathy and preventing dysfunction in hyperglycaemia of cardiac myocytes, mesangial cells, vascular endothelial cells and other cells. In initial investigations, we assessed if activation of nrf2 by the dietary bioactive compound, sulforaphane (SFN), prevented metabolic dysfunction induced by hyperglycaemia in human microvascular endothelial cells *in vitro*. Activation of nrf2 by SFN induced nuclear translocation of nrf2 and increased ARE-linked gene expression. For example, 3 - 5 fold increased expression of transketolase and glutathione reductase. Hyperglycaemia increased the formation of reactive oxygen species (ROS) – an effect linked to mitochondrial dysfunction and prevented by SFN. ROS formation was increased further by knockdown of nrf2 and transketolase expression. This also abolished the counteracting effect of SFN, suggesting mediation by nrf2 and related increase of transketolase expression. SFN also prevented hyperglycemia-induced activation of the hexosamine and protein kinase C pathways, and prevented increased cellular accumulation and excretion of the glycating agent, methylglyoxal. Treatment with nrf2 activators, such as bardaloxone methyl, SFN and others, is under experimental and clinical investigation for treatment of diabetic nephropathy.

OXIDATIVE STRESS MARKERS IN DIABETIC COMPLICATIONS

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Recent studies have indicated that a hyperglycemia-induced overproduction of superoxide seems to be the first and main event in the activation of all pathways involved in the pathogenesis of complications of diabetes. Superoxide overproduction is accompanied by increased generation of nitric oxide and, consequently, formation of the strong oxidant peroxynitrite and by poly(adenosine diphosphate-ribose) polymerase activation, which in turn further activates the pathways involved in the pathogenesis of diabetes-related complications. This process results in acute endothelial dysfunction and activation of inflammation in blood vessels of patients with diabetes, and these factors contribute to the development of complications of diabetes. Furthermore, studies also suggest that fluctuating glucose produces an increase of free radicals as well an endothelial dysfunction which are worse that those produced by stable high glucose.
Antioxidant therapy, therefore, may be of great interest in diabetic patients. However, the classical antioxidants, like vitamin E and C, do not seem to be helpful. New insights on the mechanisms leading to the generation of oxidative stress in diabetes are now available. As reported above, clinical trials with antioxidant vitamins has been unsuccessful in preventing cardiovascular disease also in diabetes, while it is well recognized that the consumption of fresh fruit and vegetables is particularly helpful. Foods of plant origin, despite plenty of nutrients contain many non-nutrition compounds, which may prevent oxidative stress-induced damage. There are at least two hypothetical new beneficial mechanisms which can be related to vegetables consumption: one is the possibility that compounds such as alpha-lipoic acid may regulate free radical over-generation at the mitochondrial level, a second is the possibility of increasing the own intracellular defences with isothiocyanates present in some vegetables.This new approach seems very promising and it seems reasonable that it could be also helpful in diabetes.

OXIDATIVE STRESS AND CALORIC RESTRICTION

Škrha, J.

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Background. Advanced oxidative stress characterized by increased reactive oxygen species (ROS) production and their impaired removal may be changed by different processes including overfeeding or caloric restriction. The antioxidative scavenger enzyme activities may be modulated by the level of oxidative stress. Possible effects of low calory diet on the protein expression and thus on scavenger enzymes have been suggested.

Methods. In a pilot study plasma malondialdehyde (MDA), ascorbic acid (AA), alpha-tocopherol (AT) concentrations and superoxide dismutase (SOD) activity in erythrocytes were evaluated before and after 8 days of very low calory diet (600 kcal) in obese diabetic (BMI 35.9 ± 1.9 kg/m^2, n=9) and non-diabetic (BMI 37.3 ± 2.1 kg/m^2, n=9) persons.

Results. A decrease of body mass index (BMI), plasma total and LDL-cholesterol were observed in both groups following very low calory diet. Significant decrease of plasma MDA ($p<0.01$) and serum AT ($p<0.01$) together with an increase of AA ($p<0.01$) and SOD activity ($p<0.01$) was found in control persons whereas only increase of SOD ($p<0.01$) was observed in diabetic patients. Positive correlation was found between plasma non-esterified fatty acids or beta-hydroxybutyrate and SOD activities ($p<0.01$) as well as between BMI and MDA changes.

Conclusions. Very low calory diet decreases oxidative stress in obese non-diabetic but only partly in obese diabetic persons. Diabetes causes therefore greater resistance to the effect of caloric restriction on oxidative stress. The analysis of other factors contributing to insulin resistance may further elucidate how to enlarge beneficial effect of caloric restriction on oxidative stress more significantly in different populations.

SYM 8 Infectious Diseases

Tuesday, 17 May 2011   09:00–11:00

IMPACT OF GENOMICS ON MOLECULAR EPIDEMIOLOGY OF MRSA

W. Witte

Abstract not received

HELICOBACTER PYLORI – A GLOBAL CARCINOGENIC PATHOGEN

S. Suerbaum

Abstract not received

INFLUENZA PANDEMIC, VACCINATION AND PITFALLS OF COMMUNICATION

Prof. Dr. Stephan Becker, Philipps-Universität-Marburg, Institut für Virologie

In April 2009, a new influenza A virus, H1N1, was isolated from patients with severe pneumonia in Mexico that during the next weeks rapidly spread worldwide and caused the first influenza pandemic of this century. Although in most cases, the new influenza virus caused mild diseases, severe courses were observed especially among children and young adults. Vaccine production started immediately after the pandemic potential of the new influenza virus became obvious and seed vaccines were available few weeks after the virus had been isolated from the first German patients.
The public perception of the influenza pandemic in Germany was coined by ambivalent emotions. During the first weeks of the outbreak, fears were prevalent. Later, when it turned out that the course of the pandemic was rather mild, the public opinion switched and media became more critical towards the preventive measures of the government and also towards vaccine manufacturers. The tenor of the headlines was set by the opinion that the pandemic was a good opportunity for vaccine manufactures to make money. When the first fatalities occurred, the public opinion switched again, and media asked why the pandemic vaccine was ordered too late. Finally, the pandemic vaccine became available and the first clinical studies were performed. Simultaneously a discussion started around the safety of the pandemic vaccine; especially the employed adjuvants were highly debated. As a result of the controversies, large parts of the German society developed a negative attitude towards vaccination against pandemic influenza. This was aggravated by the fact that general practitioners often were indecisive towards vaccination and many opinion leaders did not support vaccination against pandemic influenza at all. Interestingly, vaccination against seasonal flu was still accepted. The lessons learnt from the pandemic for future outbreaks of emerging pathogens will be discussed.

THE FOURTH DIMENSION: COMBINING EPIDEMIOLOGIC AND TYPING DATA FOR THE TRANSITION FROM DATA TO KNOWLEDGE FOR INFECTIOUS DISEASES

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Background. Epidemiologic data (time, place, and person) in combination with informatics and molecular laboratory techniques (type, i.e. the fourth dimension) makes fast and accurate early warning outbreak detections for infectious diseases on different geographic levels possible.

Results. We have recently shown that rapid MRSA outbreak detection, based on epidemiological and spa typing data, can be applied on hospital and pan-European level for the identification of potential sources of infection in a hospital (Mellmann A et al. PLoS Med 2006 & Grundmann H et al PLoS Med 2010). We have also shown in an international multicenter study that high inter-laboratory reproducibility of DNA sequence-based typing of bacteria can be achieved due to the unambiguous nature of sequence data. By using dedicated client/server software, Ridom StaphType (Harmsen D et al. J Clin Microbiol 2003), a worldwide uniform terminology ("molecular Esperanto") can be ensured. Only high-quality sequence data are automatically accepted by the server (www.SpaServer.ridom.de) and, therefore, no curator is needed for administration of the database. Finally, we are currently busy in a concerted action of European laboratories to build capacities and to harmonize technology for sequence-based typing of microorganisms (www.SeqNet.org). -The German National Reference Centre for Meningococci (NRZM, Würzburg) stores information on analyzed meningococcal samples in a central database. The recorded information includes high-resolution typing data, obtained by serogrouping and epitope sequence typing of porA and fetA. We have assembled a server that receives an anonymized subset of the NRZM data. A custom developed software combines and controls the database and additional open source software components (UNM MapServer and OpenLaszlo) to build an epidemiological geographical information system (GIS). The user accesses the automatically generated maps via the Internet, using a Flash-based application (www.EpiScanGis.org; Reinhardt M et al. Int J Health Geogr 2008). The server utilizes the open source software SaTScan to detect significant spatio-temporal clusters, taking the typing-, epidemiologic-data and population-at-risk into account. The SaTScan output is finally visualized within the GIS by depicting significant cluster of cases within the maps.

Conclusions. Thus, the application of interactive, Internet-based tools can help achieving better quality control and faster cluster detection and allows for turning surveillance data into knowledge. Community building in an environment of mutual trust and sustainability of such services is crucial for long-term success.

IFCC 2 Biobanking and Laboratory Medicine: Two Resources for Modern Health Care

Tuesday, 17 May 2011   09:00–11:00

BIOBANKING AND LABORATORY MEDICINE: TWO RESOURCES FOR MODERN HEALTHCARE

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Biobanking is increasingly becoming recognized as a corner stone in life sciences and future medical care. It is foreseeable that it will connect the scientific progress with personalized medicine of modern health care systems, and thus will have important impact on modern societies. Most classical biobanking designs represent large resources of archived biomaterials aiming at epidemiological studies. Disappointingly, these have so far been of little value for anything than the analysis of the (near indestructible) DNA. While large-scale studies - e.g. GWAS - performed with them have provided us with important statistic information on genetic risk factors in disease predisposition, the diagnostic powers of genetic variants calculated from them are not sufficient to guide individual medical decisions. For this goal, a much closer link to the biochemical and metabolic phenotpye must be achieved for biobanks requiring us to turn to more delicate bioanalytes like RNA species, proteins and post-translational protein modifications, metabolites or cells. It is increasingly appreciated that appropriately biobanking such specimens will set enormous challenges on different levels ranging from preanalytical factors that influence the biomaterial quality to particular methods of monitoring biospecimen integrity. Only this will avoid the garbage biobanks often encountered in the past and lead to a superior biobanking design. Additionally and quite importantly, current lack of standardisation in biobank design and biobanking maintenance seriously limits the interconnectivity of these resources that is required for complex multicentric projects.

For the objectives mentioned, Clinical Chemistry represents an ideal scientific and professional platform to support clinical biobankers. Clinical laboratories are vital in the analysis of all bodily fluids for the characterization of the genotypes and (biochemical) disease phenotypes, because they are closely imbedded as integral part of most clinical procedures into hospital structures and their operational procedures. Furthermore, modern clinical laboratories use highly standardised procedures and an substantial and increasing number of them operate as accredited medical laboratory units. Also, today´s clinical laboratory "assay landscape" is – through IVD directives, guideline activities and rigorous internal and external quality management - highly homogeneous with only a very limited number of vendors of reagents and hardware systems. Together with highly trained staff, they are provide best preconditions for high-quality clinical biobanking.

Clinical Chemistry/Laboratory Medicine - as a technical and a professional medical discipline - should therefore make an programmatic effort to help solving several of the current serious shortcomings in clinical biobanking: Firstly, Order Entry and LIS systems allow time stamps to record the sampling time at the sampling point. Time stamps within the laboratory allow the documentation of a) the transport time to the lab, b) the time needed for processing and clinical lab analysis and c) the time to storage. IT-based processing of lab orders prior to sampling allows the assessment of volumes required for routine analyses. Measurement of specimen volumes in the lab´s specimen reception area would then allow a preemptive sample splitting, thereby reducing preanalytical time to storage for research aliquots. d) Systematically connecting LIS and HIS system would complement the high-quality archived biomaterial and its comprehensive routine laboratory context data with medical context data in an interdisciplinary fashion.

ETHICAL AND BIO-LEGAL ASPECTS: CROSSBORDER EXPERIENCES IN EUROPE

M. Kiehntopf

Abstract not received

PUBLIC HEALTH AND BIOBANKING – THE ROLE OF PUBLIC HEALTH GENOMICS

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The current shift in healthcare towards a systemic and holistic understanding of the aetiology of diseases ("systems thinking") is a scientific revolution. Systems biomedicine triggered by next-generation sequencing technologies is likely to become the leading healthcare paradigm in the next decades. It will help to reshape research, policy-making and practice in a fundamental way.

The rapidly evolving field of epigenomics is contributing to the understanding of genome-environmental interactions and the biological phenotype. It suggests measurable mechanisms whereby environmental factors such as stress, nutrients, toxic agents or a virus can influence gene expression. These potential epigenetic modifications can occur throughout the lifetime of the organism, beginning as early as the intrauterine environment, and can accumulate in tissues and cells over time. They may also help to explain the differences in health or disease risk patterns between individuals.

In addition, recent advances in systems biology indicate that specific cellular functions are infrequently carried out by single genes, but rather by groups of cellular components. This network-based research is already starting to change nosology. Seemingly dissimilar diseases and health outcomes are being lumped together. What were thought to be single diseases are being split into separate ailments ("diseasomes"). The approach offers a novel method for human disease classification. It defines disease expression on the basis of its molecular and environmental elements in a holistic way. This knowledge will not only enable clinical interventions but also disease prevention programmes to be targeted at susceptible individuals as well as subgroups of the population (personalized healthcare).

Thus, a comprehensive model of future healthcare taking into account integrative genomics alongside with environmental, social and life style factors will become essential to realize the P4 Medicine as the future paradigm of healthcare systems being predictive, personalized, preemptive and participatory. At this moment, the biggest challenge is the interoperability and interpretation of a huge amount of data gathered around an individual: What information is at what time relevant for the individual? Computational bioinformatics and mathematics (e.g. fuzzy logic or chaos theory) may provide us with solutions in the near future. Hence, biobanks and surveillance systems have a key role in providing the research infrastructure needed to be in place. Until now these systems have been looked at independently from each other. The success of surveillance systems including genome-based biobanks will heavily depend on the quality of the information into the system and on the value of the information to its intended users. So far, all stakeholders including policy-makers and the private sector are struggling to translate the emerging knowledge into clinical, public health and technological applications. Public Health Genomics (PHG) is the new area of public health, vital if it has to be ensured that scientific advances in genomics ("from cell...") triggered by innovative technologies are timely, effectively and responsibly translated into health policies and practice ("...to society"). The upcoming era of knowledge implementation requires increased concerted activities in this field such as the Public Health Genomics European Network (PHGEN) in Europe (www. phgen.eu) or GRaPHInt on the global level.

THE KEY ROLE OB BIOBANKING FOR THE STUDY OF COMPLEX DISEASES

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The practice of medicine continues to evolve in the modern era at an exponentially faster rate through advances in technological science underpinned by greater computing speed, and progress in molecular scientific disciplines such as genomics and proteomics.

This can only be realized through greater understanding of many and adaptive complex patho-physiological molecular pathways, and hence the importance of the "bench work" of molecular science.

The rapid improvement of genomics research has unveiled the secrets of the human genome, which enabled us to get access the cause of disease more efficiently. The progress along with the development of high-throughput analytic techniques forced many researchers to perform more large-scale genomic association studies. Consequently, there has been an increasing need of human genome data, especially from the individuals with diseases of multifactorial origin.

Because more genomic data sample usually means more probability of obtaining meaningful results, researchers have made an effort to insure large genomic dataset with greater clinical information. Moreover in the post genomic era, the screening of many different genetic polymorphisms and copy number variations in large populations represents a major goal that will facilitate the understanding of individual genetic variability in the development of multifactorial diseases and drug response and toxicities.

The last few years have witnessed an important expansion of human DNA sampling and data collecting in order to exploit and study the genetic information collected. The strategic importance of this activity for genetic research and its applications is obvious. Human DNA, tissue or cell collections, as well as databases which are attached to such biological resources are necessary for a wide range of purposes and these collections have been extensively exchanged for scientific purposes.

Biobanks should not be considered a static activity. On the contrary, biobanking is a young discipline which need continuously evolve according to the permanent development of new techniques and new scientific goals. To accomplish current requirements of the scientific community, biobanks need to face some essential challenges including an appropriate design, harmonized and more suitable procedures, and sustainability, all of them in the framework of their ethic, legal and social dimensions.

Systematic clinical biobanking could become a major asset for clinical research and public health if biobanking is considered as a routine part of everyday clinical practice, and the science of biobanking is considered an essential part of the science of laboratory medicine.

SYM 9 Haematology and Anaemia

Tuesday, 17 May 2011   09:00–11:00

DIAGNOSTIC OF THALASSAEMIA

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Thalassaemias are among the most common genetic disorders worldwide. The classic long known tools have been replaced by modern technologies step by step. Among them we find standardized HPLC, IEF and different molecular biology approaches. New technologies have made significant progress in this field. Most recently, the use of next generation sequencing, CGH-Chip-Technology and novel real time PCR approaches have slowly but surely moved from bench to bedside. The newest technologies have the advantage of being highly specific and sensitive yet the are demanding for sophisticated skills, validation, reporting and of course – at least at this time – significant higher financial resources. This lecture will present the pros and cons of different diagnostic approaches and will propose a prevalence and economic adapted approach. It can be anticipated that laboratory testing for Thalassaemia will become more economical. However, standardization, center of excellence formation, tele-hematology and most important general counseling will be important.

HOW CAN HEPCIDIN HELP US IN THE DIAGNOSIS OF IRON DISORDERS?

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The discovery of the peptide hormone hepcidin is among the most important breakthroughs in understanding iron homeostasis. This master iron regulator is produced by the hepatocytes and the primary determinant of the level of dietary iron absorption and of body iron distribution.
 Hepcidin inhibits the export of iron from certain cell populations, particularly reticulo-endothelial macrophages (the primary site of body iron turnover) and duodenal enterocytes (the primary site of body iron absorption). It does so by causing the internalization and degradation of the cellular iron exporter ferroportin, which is highly expressed in these cells. The net effect of hepcidin is to increase intracellular iron stores, decrease dietary iron absorption, and decrease circulating iron concentrations.
Certain physiologic and pathologic processes regulate the synthesis of hepcidin. Situations in which demand for circulating iron is increased (particularly erythropoietic activity) bring about a decrease in hepcidin synthesis. These include iron deficiency, hypoxia, anemia, conditions characterized by ineffective erythropoiesis (e.g. hemoglobinopathies), or the use of erythropoiesis stimulating agents (ESA, such as recombinant human erythropoietin, EPO). The decrease in hepcidin results in the release of stored iron and an increase in dietary iron absorption. On the other hand, infection or inflammation cause an increase in hepcidin synthesis. This causes a decrease in circulating iron, thought to protect the body from extracellular proliferating pathogens.
Since the discovery of hepcidin a decade ago, multiple *in vitro* and animal studies have contributed insights into the regulation of hepcidin and its functional properties. The first reliable assays to quantify hepcidin in human body fluids have recently been developed and have been followed by first steps towards its world -wide harmonization. These achievements led to several clinical studies that added to our understanding of iron metabolism and allowed us to speculate on its future application for several diseases: 1. *Hereditary hemochromatosis* (innate low hepcidin levels which may be compensated by iron overload over time): i) screening test in the diagnosis of various forms, ii) prediction of biochemical penetrance of C282Y- homozygosity and iii) targeting phlebotomy protocols. 2.*Iron loading anemia's* (low hepcidin levels which may be compensated by iron overload over time): predicting and monitoring iron overload. 3. *Iron refractory iron deficiency anemia* (inappropriately high hepcidin levels): screening for an inherited defect in hepcidin regulation . 4. *Anemia of chronic disease* (elevated hepcidin levels): differential diagnosis of anemia of chronic disease and iron deficiency anemia, i.e. guide in iron treatment. 5. *Anemia of chronic kidney disease* (elevated hepcidin levels): i) marker of iron deficient erythropoiesis and ii) prediction of long-term EPO response.
Further studies are now awaited to firmly establish hepcidin as a novel tool in diagnostic medicine.

HEPCIDIN FUNCTION AND REGULATION

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Imbalances of iron homeostasis account for some of the most common human diseases. Pathologies can result from both iron deficiency or overload and frequently affect the hepcidin/ferroportin regulatory system that balances systemic iron metabolism. The small hepatic peptide hormone hepcidin orchestrates systemic iron fluxes and controls plasma iron levels by binding to the iron exporter ferroportin on the surface of iron releasing cells, triggering its degradation and hence reducing iron transfer to transferrin. Hepcidin thus maintains transferrin saturation at physiological levels assuring adequate iron supplies to all cell types. Research into the molecular mechanisms that underlie hereditary hemochromatosis (HH) in patients and mice identified HFE, transferrin receptor 2 (TfR2) and hemojuvelin (HJV) as important activators of hepcidin transcription. Low hepcidin expression and iron overload further hallmark mouse models with deficiencies in Bmp6 or hepatic Smad4, suggesting that the Bmp/Smad signaling pathway is indispensable to maintain physiological hepcidin mRNA levels. The BMP/Smad signalling pathway is impaired in several iron-related disorders such as HH or the iron refractory iron deficiency anemia (IRIDA) and is subject to regulation by erythropoietic activity. IRIDA is characterized by mutations in the serin protease TMPRSS6 which suppresses hepcidin levels by targeting hemojuvelin for degradation. By contrast, inflammatory cytokines induce hepcidin transcription via the IL6/STAT signalling pathway. Some of our recent work shows that in addition to proteins tissue iron levels and hepcidin mRNA expression is maintained by the liver-specific microRNA 122. Efficient and specific depletion of the liver-specific miR-122 in mice increases mRNA expression of genes that control systemic iron levels (*Hfe*, *hemojuvelin*, *Bmpr1a* and *hepcidin*) by directly targeting the 3' untranslated regions of the Hfe and hemojuvelin mRNAs. These data establish a direct mechanistic link between miR-122 and the regulation of systemic iron metabolism. In my presentation I will present an overview of regulatory mechanisms involved in the control of hepcidin levels and their link to frequent iron related disorders.

LABORATORY DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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Background. Hereditary spherocytosis (HS) is an inherited hemolytic anemia with a frequency of 1 in 2000 to 1 in 5000 births in Northern Europe (higher than other ethnic groups). Diagnosis of HS is straightforward when a patient with family history presents spherocytes on blood smear, low hemoglobin, hyperbilirubinemia, reticulocytosis, and ± splenomegaly [1]. However, some HS patients can have asymptomatic parents. The shortened life span of HS red cells is due to fragile cytoskeleton, which maintains the integrity and deformability of red blood cell (RBC). The cause is a quantitative or a quality defect(s) in the membrane proteins involved in the vertical interaction of the cytoskeletn, namely, spectrin, ankyrin, protein 4.2, and band 3 [2]. HS is not a single gene disorder [3]. The patient management is not influenced by the knowledge of protein and molecular defect(s).

Methods. The laboratory screening tests for diagnosis of HS are the osmotic fragility (OF) test [4], the acid glycerol lysis time (AGLT) test [5], and flow cytometric analysis of eosin-5-maleimide (EMA) labeled RBCs [6] . Further investigation includes SDS-polyacrylamide gel electrophoresis (PAGE) of membrane proteins and molecular analysis of the affected protein genes.

Results. The OF and AGLT tests measure the rate of RBC lysis in different incubation media as an indicator for the degree of red cell fragility. In the EMA binding test, the mean channel fluorescence (MCF) readings for HS are 20% - 30% lower than those obtained for normal adults. However, Southeast Asian Ovalocytosis, Cryohydrocytosis, and some cases of Congenital Dyserythropoietic Anemia type II also give MCF results in the same range as HS [6]. Hereditary pyropoikilocytosis (HPP, severe hereditary elliptocytosis) gives MCF readings about 25% lower than those of HS [7]. SDS-PAGE of erythrocyte membranes determines specific protein deficiency. DNA or cDNA sequencing is performed when the different clinical phenotypes among family members can not be explained by SDS-PAGE result.

Conclusions. A screening test with 100% sensitivity and specificity for HS has yet to be found. The EMA binding test has a high predictive value for this red cell disorder. SDS-PAGE can not detect about 10% of HS patients. Molecular diagnosis of HS is not a common practice because the affected protein genes are large, between 19 and 52 exons. Over 95% of the protein gene mutations are private, i.e., associated with a specific kindred. In some cases, the primary protein gene defect is unravelled only after sequencing more than one protein gene.

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WS 4 Update on Cardiac Troponin – Clinical and Laboratory Issues

Tuesday, 17 May 2011   09:00–11:00

A ROADMAP FOR CARDIAC TROPONIN I STANDARDIZATION AND TRACEABILITY

D. Bunk

Abstract not received

THE ROUND ROBIN STUDY FOR CARDIAC TROPONIN – PROGRESS REPORT

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Background. Currently, the various cardiac troponin I (cTnI) assays produce results that are non comparable and, consequently,
need different clinical cut-off values for biomarker interpretation. To improve this situation, suitable commutable reference materials that are assigned cTnI concentrations by a higher-order measurement procedure are required. They can be used by industry to
assign values to working and product calibrators through a value-transfer process. Using routine procedures with these validated
calibrators to measure patient samples, clinical laboratories will obtain standardised and traceable values. The IFCC Working
Group on Standardization of cTnI (WG-TNI) has recently undertaken a project to address the establishment of a secondary
reference immunoassay measurement procedure for cTnI of a higher metrological order than current commercial immunoassay
methods, and the development of a serum-based commutable reference material for cTnI to which companies can reference their
calibration process. A pilot study has been planned to compare the candidate immunoassay reference measurement procedure
for cTnI (cRMP) with commercial assays and to investigate the feasibility of preparing a commutable and stable secondary reference material for cTnI by use of serum pools.
Methods. The cTnI pools are prepared in three different ways, namely: 1) by the addition of individual cTnI-positive native patient
samples; 2) by dilution of a high cTnI concentration pool with low and medium concentration pools; and 3) by dilution of a high
and medium pool with a normal pool. The commutability of these pools is tested between routine assays and cRMP utilising sets
of individual patient samples at different cTnI concentrations. At the same time, the short term stability of cTnI in the pool materials will be evaluated by assay before and after the addition of a cocktail of protease and phosphatase inhibitors. A preliminary
phase preceding the study is planned to enable participant companies to actively familiarise themselves with the protocol. Sample
handling and preparation of frozen materials prior to the duplicate assay of patient specimens, pools and quality control materials
during the study are designed to ensure standardised procedures.
Results. The pilot study has started early in 2011 and involves the National Institute of Standards and Technology (US), the
National Physical Laboratory (UK), the Institute for Reference Materials and Measurements (EU), the Department of Pathology,
University of Maryland School of Medicine (US), and nine diagnostic companies. Method comparability, commutability and stability
results from the evaluation of individual patient samples and pools by 23 commercial assays and by cRMP are planned.
Conclusions. The IFCC WG-TNI is presently investigating the feasibility of preparing a commutable reference material and its
value assignment using a higher-order cRMP (1). Although this is a research project and there is no guarantee of success, we
think that such experimental work is needed if there is to be progress in the standardisation of cTnI.
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CLINICAL USE OF HIGH-SENSITIVITY TROPONIN ASSAYS

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For years cardiac troponins (cTn) have been regarded as the preferred biomarkers for the diagnosis of myocardial infarction and for the risk stratification of patients with acute coronary syndromes (ACS), as well as for the selection of patients who need an early invasive strategy, and for the guidance of adjunctive pharmacological therapy. Now, novel more sensitive and appropriately precise troponin assays have been introduced that comply with the Universal MI definition. This Universal MI definition recommends the use of the 99th percentile, and troponin assays should allow measurement at that cutoff with an imprecision (CV) of 10% or less. In clinical practice, these new assays allow detection of MI earlier and more frequently. However, due to lowered diagnostic threshold the prevalence of troponin-positive patients not due to ACS has increased dramatically. Strategies to increase clinical specificity include strict guideline adherence, particularly awareness that MI diagnosis requires a clinical context of myocardial ischemia and a typical dynamic change (rise and/or fall). For the latter the exact magnitude has not been established yet.

As even low-level elevations of troponin are almost always associated with adverse outcomes, regardless the presence of ACS, the differential diagnosis of a troponin elevation should be sought actively to ensure timely and specific treatment. Possible diagnoses include conditions with acute and chronic myocardial damage such as stable CAD, chronic heart failure, pulmonary embolism, and chronic pulmonary arterial hypertension. More sensitive troponin assays may help to diagnose subclinical stages earlier. In addition, for most of these diagnoses an elevated troponin has been linked to adverse prognosis.

Conclusion: High-sensitivity troponins do not only allow an earlier and more accurate diagnosis of MI but could be useful for earlier identification and risk stratification of acute or chronic cardiovascular disease not due to ACS.

SYM 10 Diagnostic Use and Value of Function Test in Endocrinology

Tuesday, 17 May 2011   14:30–16:30

MOLECULAR GENETIC WORK UP FOR THYROID AND PARATHYROID DISEASES

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Background. Molecular genetic analysis of RET-gene in hereditary medullary thyroid carcinoma (MTC) as part of the multiple endocrine neoplasia syndrome (MEN2) helps to further classify patients in different risk groups. Hereditary primary hyperparathyroidism (HPT) as part of the MEN 1, MEN 2, hyperparathyroidism -jaw tumor syndrome (HPT-JT), familial hypocalciuric hypercalcemia (FHH) could be classified by mutation analysis of their respective genes.
Methods. Molecular genetic analysis for RET-gene was done in 190 patients with MEN 2, and in 80 patients with hereditary HPT analyzing MEN1-, RET-, CaSR-, and CDC73-genes in the corresponding diseases.
Results. In MEN2 148 missense mutations in the RET gene were found, the most common codons were 634(n=52), 804(n=28), 790(n=13), 791(n=12), 618(n=11), 918(n=6) and others (n=26). Classification of *RET* mutations into 4 risk levels according to genotype-phenotype correlations was done allowing adequate treatment in the patients and affected family members. On the one side of the spectrum there is the most aggressive and early onset MTC in patients with MEN2B having a mutation in RET codon 918 and on the other side are patients with codon 791 mutation some of them never developing MTC during their live time. Of 80 patients with hereditary HPT 52 had MEN1 with 29 different MEN1 mutations, 15 had MEN2 with 2 different RET mutations, 7 had JT-HPT with 5 different CDC73 mutations and 4 had FHH with 4 different mutations in the CaSR, in one family with 2 members no mutation was found (isolated familial hyperparathyroidism). The youngest patient with HPT was found in the MEN2 / HPT-JT group with 7/16 years, the highest serum-calcium (3,6mM) in the HPT JT group, the highest recurrence rate of HPT in the MEN1 group. Parathyroid cancer was found only in the HPT-JT group. In 3 of 4 patients with FHH and HPT, serum calcium could be normalized.
Conclusions. The specific RET mutation suggests a predilection towards a particular phenotype and clinical course, with strong genotype–phenotype correlations. The MEN 2 syndrome gives a unique model for early prevention and cure of cancer and for stratified roles of mutation-based diagnosis of carriers. Earlier identification of patients with hereditary MTC by DNA screening results in a high cure rate of affected patients by presymptomatic treatment with improved life expectancy and quality of life. In hereditary HPT the availability of DNA testing for the 4 syndromes has improved diagnostic accuracy and simplified family monitoring in many cases.

DIAGNOSTIC WORKUP OF PRIMARY ALDOSTERONISM

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Primary aldosteronism (PA) is much more common than previously thought, accounting for up to 5-10% of hypertensives with most normokalemic. Aldosterone excess has adverse cardiovascular consequences independent of hypertension development. Because specific surgical (unilateral adrenalectomy) and medical (aldosterone antagonist) treatment effectively abrogates the morbidity associated with PA, this condition should be systematically sought and specifically treated.

The aldosterone/renin ratio (ARR) is the most reliable method of screening for PA but is not without false positives and negatives. Dietary salt restriction, concomitant malignant or renovascular hypertension, pregnancy and treatment with diuretics (including spironolactone), dihydropyridine calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists can all lead to false negative ratios by stimulation of renin secretion. We recently found treatment with selective serotonin reuptake inhibitor antidepressants to lower the ARR. Because K^+ is a chronic regulator of aldosterone secretion, false negatives may also occur in the setting of uncorrected hypokalemia.

Beta-adrenoceptor blockers, alpha-methyldopa, clonidine and NSAIDs suppress renin and have the potential to cause false positive ratios. False positives may also be seen in patients with impaired renal function or advancing age. We have recently shown that (1) females have higher ratios than males, (2) false positives can occur during the luteal phase of the menstrual cycle or while taking an oral ethinylestradiol/drospirenone contraceptive preparation but only if renin is measured as direct renin concentration and not plasma renin activity, while (3) subdermal insertion of an implantable form of etonogestrel did not affect the ARR.

Where feasible, diuretics should be ceased for at least six weeks and other interfering medications for at least two (preferably four) before measuring the ratio, substituting other medications which have a lesser effect on results, such as verapamil slow-release, hydralazine and prazosin. Hypokalemia should be corrected and the patient encouraged to follow a liberal salt diet before ratio measurement. Sensitivity is maximized by collecting blood midmorning from seated patients who have been upright (sitting, standing or walking) for 2-4 hr. The ratio should be regarded as a screening test only, and should repeated at least once (serially if conditions of sampling, including medications, are being altered) before deciding whether or not to go on to a reliable suppression test (e.g. fludrocortisones suppression testing) in order to definitively confirm or exclude PA. Because computed tomography frequently misses aldosterone-producing adenomas and yet detects non-functioning nodules, the only reliable means of differentiating unilateral (surgically correctable) from bilateral (usually treated medically) forms of PA is by adrenal venous sampling. For the glucocorticoid-remediable familial form of PAL (familial hyperaldosteronism type I, FH-I), genetic testing for the causative "hybrid" 11beta-hydroxylase/aldosterone synthase gene has greatly facilitated detection. It is important to recognize the potential inaccuracies of many currently available methods for aldosterone and renin (except in very well established and experienced laboratories). New, high-throughput mass spectrometric methods of measuring aldosterone have proven highly reliable and reproducible and represent a major step forward. Validation of new assays of plasma renin activity using similar technology is awaited with interest.

VITAMIN D: CLINICAL RELEVANCE, ANALYTICAL ISSUES

Cavalier E.

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"Vitamin D" (VTD) is a lipophilic pro-hormone that can be found in two forms. Indeed, vitamin D3 (cholecalciferol), the "natural" form, can be synthesized after sun exposure of the skin or found in some (rare) foods whereas vitamin D2 (ergocalciferol) is the form found in different plants. In some countries the two forms can be found in pharmacological supplements whereas vitamin D3 is preponderant in some others. The role of VTD in maintaining bone health has been known for decades. Recently, however, observational studies, and more and more interventional studies, have raised the importance of a significant VTD supplementation for not-only skeletal benefits. For example, VTD has been found to play an important role in preventing cancers, risk of falls in the elderly, blood pressure, cardiovascular diseases, diabetes, infections and even total mortality.

Everybody agrees now to use serum 25(OH)-vitamin D (25(OH)D) as the correct functional indicator of VTD status and to use a cut-off target instead of heath-based reference range. However, the opinion of the experts is not unanimous on the value of this cut-off (20 ng/mL or for the majority of them, 30 ng/mL). According to these cut-offs values, VTD deficiency is very common in our populations. The recently published studies are clearly now in favour of a systematic vitamin D supplementation in some categories of patients at high risk to be deficient. Thus, renal insufficient patients, dark-skinned or veiled subjects, individuals ≥ 65 years old and institutionalized subjects should be systematically treated. Some other categories of patients would benefit from a 25(OH)D determination in order to adapt their dose, or monitor their 25(OH)D level. This mainly concerns patients at risk of osteoporosis, pregnant or lactating women, transplanted patients or patients suffering from chronic kidney disease, obese individuals, patients suffering from diabetes, primary hyperparathyroidism, hypertension or auto-immune diseases, patients with bone/muscle pain or under corticosteroid treatment. From our point of view, newborns, children and teenagers should also benefit from a systematic supplementation. Generally, a daily dose of 800 IU of vitamin D3 is recommended. However, this dosage can be largely increased (up to 5000 IU/day) in some particular situations. However, one should not forget that all these cut-off values have been established with the DiaSorin RIA kit. Their extrapolation to other techniques remains questionable.

From an analytical point of view, 25(OH)D determination is a difficult task. Amongst others, we have recently highlighted different problems, like a lack of 25(OH)D2 recognition for some kits or a clear overestimation in the highest range of the values with others. Unfortunately, these analytical problems have led to different hazardous or irrelevant clinical decision. Nevertheless, according to the UK-DEQAS proficiency testing, the largest trial to date, the discrepancies observed between the different techniques are not (globally) so bad. This should, of course, be interpreted in the light of other situations where cut-offs values have been established, whatever the assay used (growth hormone or parathormone, for example).

HORMONAL CHANGES AFTER GASTRIC SURGERY AND OBESITY INTERVENTIONS

C. Le Roux

Abstract not received

SYM 11 Point of Care Testing – Practical Aspects

Tuesday, 17 May 2011   14:30–16:30

MANAGEMENT OF POCT PROGRAM IN A TEACHING HOSPITAL

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Point of care testing (POCT) requires reliable methods and technology, as well as trained operators to ensure a good quality service. It also represents a nowadays rare opportunity for laboratory staff to interact with clinical collegues personaly instead of virtualy. However, the benefit of POCT implementation is demonstrated only if the obtained result triggers some kind of action along the clincal pathway. In a hospital setting, that usualy means that central laboratory TAT has to be a proven rate limiting step in the patient care process, which might conceivably be ameliorated by the introduction of POCT. Hospital staff convenience, although not completely negligible, should normaly not be high on a list of considerations when thinking about the introduction of near patient testing. Calculations should be made regarding the cost benefit of POCT in terms of any kind of desired outcome, since a near patient testing possibility and its reliable maintenance, costs considerably more than the same test result obtained from the central laboratory. Thus, we should be aware of the fact that the introduction of POCT does not bring the same kind of benefit in each hospital or healthcare environment. Sometimes (the probability being approximately inversely proportional to the country GDP) hiring more auxilliary staff for quick sample transport might be a viable financial alternative to obtaining a near patient testing analyzer with expensive reagents whose shelf life expires after predetermined amount of time. In case of blood glucose meters, utilising the interference sensitive test strips in certain particular wards might prove not only to be counterproductive, but downright dangereus. On the other hand, if introducing the POCT device redirects patients from inpatient to outpatient status, reduces hospital stay, improves medical decision making and compliance or prevents complications, any of those scenarios clearly represent a case in favor of implementation. However, the implementation is only the first step – maintenance of the high quality service is a *conditio sine qua non*, thus requiring motivated, expert and gentle laboratory staff with excelent interpersonal skills and preferably a sense of humour. The main issue is keeping the clinical personnel educated and re-educated about the preanalytical procedures as well as constantly aware of possible causes of erroneus results. The real life examples from our university hospital will be presented.

UPDATE ON CURRENT AND DEVELOPING POC APPLICATIONS WITH A GLOBAL AND EMERGING COUNTRY FOCUS

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Background. Rapid diagnosis and effective treatment of life-threatening and chronic diseases in all economic, geographic, and technological environments is a global healthcare goal. Considerable advancements have been made in diagnostic point-of-care (POC) technologies. POC devices that provide reliable and consistent test results, can be performed in a wide range of environmental conditions by individuals that lack technical training, and that are at low cost continues to be a challenge for resource poor countries.
Discussion. The largest segment of POC testing is blood glucose monitoring for both in home self-testing and professional (e.g. healthcare) settings. POC testing for acute coronary syndrome (e.g. troponin), coagulation (e.g. PT INR), hemostasis (e.g. platelet function) and acquired immunodeficiency syndrome (HIV) continues to grow. Examples of other POC tests with potentially high growth rates for use outside of the hospital setting include influenza virus types A and B, ketones and B-hydroxybutyrate, mycobacterium tuberculosis, and biodefense for biological threat agents. The menu of POC tests, technological advancements, and information management continues to grow. Access to these tests is constrained by cost, availability, and geography. This talk will focus on the technologies available, their clinical applications, challenges, and examples of how some of the challenges have been overcome. The need for consistency in testing and tools to assist with implementing quality driven POC testing in these settings will be discussed.

THE OPERATOR IS KEY IN DELIVERING QUALITY IN POINT OF CARE

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Point of Care (PoC) has been with us for many years but the issue of how well it is used, while the subject of many anecdotes, is often not operationally addressed to ensure that the quality of the system around the use of the analytical device matches, or seeks to achieve, that which is expected of laboratories. With the increasing reliability and sophistication of PoC devices and the desire to bring care closer to the patient, the operation of quality PoC is now focused on the operator. There are three elements in a quality PoC system: regulation, accreditation and training.

Regulation. Countries have different requirements on regulation of PoC, however the ISO 22870 applies to PoC systems and is the bench-mark for any PoC quality system. Additionally national agencies may issue guidance or regulate.

Accreditation. Different countries approach PoC accreditation in different ways but where there are standards these may utilise ISO 22870 to write standards to accredit a service as a measure of the quality of the service. However, as PoC is typically performed by non-analytically trained staff, there may be local acceptance of relatively low skill sets in the delivery of the PoC analytical service; this may in some cases extend to the clinical interpretation.

e-Learning. Training the operator to understand the analytical principles that are required for satisfactory performance is key in any analytical system; this is just as true in PoC as it is in a central laboratory. The knowledge level required will consist of a basic set of knowledge around core topics such as: quality control, safety, documentation etc., there is then a need for analyte specific information e.g. PoC glucose analysis for monitoring diabetes, blood gases in Critical Care. Use of e-learning packages is clearly the way forward to enable local determination of knowledge and skill levels attainment and will help inform the skill set mix required for delivery of PoC compatible with local expectations of a satisfactory output. The attainments may be used to define local accreditation standards; these may be an integral part of a wider accreditation system relating to medical laboratories i.e. ISO 15189. Such approaches ensure appropriate quality is delivered wherever and whenever any analysis is done.

DESIGNING A QUALITY POCT PROGRAM USING CLSI DOCUMENTS

L.A. Wyer

Abstract not received

WS 5 Lessons from the Asian-Pacific Multicenter Reference Interval Study

Tuesday, 17 May 2011   14:30–16:30

STUDY DESIGN: STRATEGY FOR COLLABORATION IN THE DEFINITION OF REFERENCE INTERVALS

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Background. Multicenter studies for derivation of common reference interval (RI) rely on two main assumptions: comparability of assay results across different laboratories and lack of regionality in test results. To test the validity of the second assumption, we conducted a multicenter feasibility study involving 550 healthy individuals from six laboratories in East and South-East Asia in 2006, targeting 40 commonly measured standardized analytes. Using assays in a central laboratory to assure result comparability, the study revealed existence of regionality in test results for some analytes. In addition, comparison of the central laboratory results with those of local assays demonstrated the difficulty of attaining comparability without proper efforts for standardization. Based on these experiences, a third multicenter study was conducted in 2009 to derive common RIs in an efficient and reliable manner, targeting 72 commonly measured analytes including non-standardized analytes measured by immunoassays.

Methods. 69 laboratories in East and South-East Asia (55 from 7 areas in Japan, 14 from 7 cities from outside) took part in the study and 3541 well-defined healthy volunteers, mostly hospital workers, were recruited. Although all the specimens were sent to Tokyo at –80°C for the centralized assays, as a new strategy to accommodate non-standardized analytes, we asked each laboratory to retain and make local measurements on specimen aliquots to allow conversion of centrally derived RIs to laboratory-specific RIs based on cross-check comparison of the test results. Other important strategies were (1) to use a multivariate iterative method, called latent abnormal value exclusion (LAVE), to minimize the influence on RIs of frequent abnormal results representing common disorders like metabolic syndrome, (2) to seek traceability of values to reference measurement systems (RMS), and (3) to explore sources of variation, including regionality, in reference values by means of the nested ANOVA and multiple regression analysis using information obtained from a detailed questionnaire.

Results. The ANOVA revealed between-gender differences in 36 analytes and age-dependent differences in 32 analytes. Between-region differences, requiring specific RIs, were observed for 14 analytes, including HDL-cholesterol, PTH, folate, and inflammatory markers. However, when the data were limited to those from Japan, regionality became practically negligible across all the analytes. The RIs of 30 analytes were made "universal" by ensuring traceability of values to RMS. The cross-check testing results of each laboratory with the central laboratories demonstrated close linear relationships in test results for most of the non-standardized analytes, except some of tumor markers and free thyroid hormones. Thus, RIs specific to reagents used by each collaborating laboratory were successfully derived.

Conclusions. The strategies employed in the study allowed us (1) to efficiently derive RIs in a reliable manner even for non-standardized analytes, and (2) to present a broad range of findings regarding sources of variations in test results, including region, gender, and age related changes, all relevant for clinical laboratory diagnosis.

STATISTICAL CONSIDERATIONS IN THE GENERATION OF REFERENCE INTERVALS

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Background. Reference intervals are the most commonly used tool for the clinical interpretation of laboratory results. Development of reference intervals has relied on classical methodology described decades ago for use with small reference data sets. Newer multicenter reference interval studies, however, have resulted in larger reference data sets and allowed utilization of more sophisticated statistical methods for data analysis that can take into account the effects of data covariates, both categorical (such as gender, pregnancy status, smoking status) and continuous (such as age and body mass index).

Content. Whereas classical reference interval studies have largely been confined to analysis of data from approximately 120 individuals, newer multiregional studies gather reference data from thousands of persons. The detection of outlying observations in these new large data sets presents both an important and a challenging problem. Because most current statistical methodologies for the detection of outliers rest on the tenuous assumption that the observed reference values are Gaussian distributed, elimination of outlying values for purely statistical reasons (e.g., >4 SDs away from the mean, outside the Tukey fence, exceeding the Dixon criterion) can be risky without corroborative supporting information that could explain why such values might be considered outliers. Better methods for detecting outliers are needed. Under the assumption that there are no analytically- or biologically-based between-region differences in mean laboratory values, new multi-regional reference data sets offer the possibility of developing common reference intervals that can be adjusted for the effects of various covariates. Methods to determine the need for such reference interval adjustment or partitioning are relatively straightforward for categorical covariates like gender with two possible subcategories (male and female), but become more difficult when more subcategories are involved (e.g., blood group, ethnic group, underlying diet, geographic location). Nested analysis of variance methodology has been proposed recently as a promising approach to determine the need for partitioning reference intervals by multi-category covariates, but this partitioning method will require corroboration and additional evaluation in further studies. Various statistical methods also are available to adjust reference intervals for the effects of continuous covariates such as age. The ease of use and general applicability fractional polynomial curve fitting make this technique a particularly attractive method to use in adjusting reference intervals for continuous covariate effects.

Summary. As larger multi-regional reference interval studies are conducted, the need to apply more sophisticated statistical methods will continue to grow. Additionally, the implementation of multi-regional reference intervals will increase the need for better assay standardization and between-assay result comparability. Clinical laboratorians will play a central role in both of these activities.

OBTAINING REFERENCE INTERVALS TRACEABLE TO REFERENCE MEASUREMENT SYSTEMS

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An issue associated with the standardization efforts is the need to develop scientifically sound and globally useful reference intervals. Lack of proper reference intervals may indeed hamper the implementation of standardization in Laboratory Medicine as: a) the implementation of standardization can modify analyte results, b) without adequate reference intervals, this situation can impair the interpretation of the results and, paradoxically, worsen the patient's outcome, c) the absence of reliable reference intervals for newly standardized commercial methods may hamper their adoption, and d) usually, a single clinical laboratory or manufacturer may not have the means to adequately produce reference intervals.

The reference measurement system represents a trueness-based approach. With this approach, different commercial methods that provide results traceable to the system are able to produce comparable results in clinical laboratories using these assays. Thus, reference intervals obtained with analytical procedures that produce results traceable to the corresponding reference system can be transferred among laboratories (becoming "common" or "universal"), providing that they use commercial assays that produce results traceable to the same reference system and populations have the same characteristics or, alternatively whether it is known that the specific analyte is not influenced by ethnicity or environmental factors. The definition of common reference intervals should hopefully cause the disappearance of different intervals employed for the same analyte, providing more effective information to clinicians.

Using the approach described above, some examples of common reference intervals can be found in literature (1, 2). Large multicenter studies are needed for a robust definition of common reference intervals, using a protocol for collaborative experiments that include well defined prerequisites. The difficulties are related to the need for verifying traceability of participating laboratories by the distribution of commutable frozen sera, with values assigned by the reference measurement procedure. Other difficulties include the co-ordination among participating centers for the performance of thousands of tests and enrolment of hundreds of individuals, which entails considerable cost. Particularly, in the development of reference intervals, the methods that are employed must produce results that are traceable to the reference measurement system for that specific measurand. For this reason, the trueness of participating laboratories should be verified and, if necessary, experimental results corrected in accordance with correlation results with the reference procedure. Alternatively, the samples from reference individuals can be collected at the different centers, frozen and then shipped to a central laboratory where all the analyses are performed. The latter approach is simpler and allows better control of the analytical phase. However, this approach uses frozen samples and thus introduces a variable not typical for the clinical laboratories.

In summary, the production of common reference intervals may pose numerous practical problems to solve. However, the possibility of providing reference intervals that are applicable to any laboratory, able to produce results traceable to the reference measurement system, seems to be quite realistic.

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EFCC 2 Accreditation Should Be Focused on More Than Well Organized Examinations With the Laboratory

Tuesday, 17 May 2011   14:30–16:30

IVD DIRECTIVE 98/79 CE IN RELATION TO ISO 15189

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Despite the Vitro Diagnostic Medical Devices directive is essentially intended to create a free European market, also consumer (in this case: patient) protection was intended.

Therefore the directive includes a) quality requirements and b) mandatory information to be provided by manufacturers to the users of the devices.

These requirements are listed in annex I: essential requirements of the directive, especially under item B8.

Since 2003, medical laboratories can be accredited according to the universally accepted standard for such activities: ISO 15189 (2). Validation, verification and documentation of these are cornerstone issues in the accreditation requirements.

Clause 4.6.2 of ISO 15189 requires that purchased equipment and consumables that affect the quality of the service shall not be used until they have been verified as complying with standard specifications. Consequently this means that the user must be able to trust the validation done by the manufacturer and that he has al needed information to verify this.

Clause 5.5.2 states that only validate procedures shall be used. Here again, in the case of IVD's, the primary validation must be done by the manufacturer and the user must only prove implementation verification.

The clear interaction in the requirements laid done in both documents, resulted in requests for assistance and documentation from laboratories to distributors and manufacturers of in vitro medical devices.

Some laboratories mistakenly demanded that their suppliers would be responsible for preparing their whole quality documentation but other based questions were not always understood by manufacturers.

We could define following problems:
• some IVD's are put into the market without all needed documentation related to the requirements from the directive;
• some calibration procedures from manufacturers were not correct or were not acceptable for accreditation;
• there is a confusion between IVD's and RUO (research use only) device;
• there is a need for more transparency in field service and installation reports of equipment;
• traceability and evidence of traceability of measurement equipment used for the calibration of instruments is often lacking

The lecture give concrete examples of these problems.

Many problems and misunderstanding could be solved in a dialogue between users, providers and accreditation bodies.

The EFCC WG on IVD's tries to set up a dialogue between users and manufacturers by submitting concrete examples of problems to EDMA, the European Diagnostic Manufacturer Association. This will result in some guidance documents.

Conclusions. A continuous dialogue between users, accreditation bodies and manufacturers is still needed. The EFCC WG on IVD's tries play a role in this process of understanding between laboratories and manufacturers.

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INFLUENCE OF PRE-EXAMINATION ASPECTS ON RESULT´S VALIDITY – ARE ISO 15189 REQUIREMENTS SUFFICIENT AND CLEAR?

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The standard EN ISO 15189-2007 is a basic standard of clinical laboratories competence evaluation. In brief the laboratory's ability of issuing valid results is also to be considered within the process of accreditation. Question is whether the above mentioned standard is sufficient to consider all the processes involved in results' creation. And also whether the fulfilment of all its requirements can be influenced by laboratory. In this point of view I will follow the preanalytical phase out of laboratory – collection of sample in GP office or policlinic - in my message. What does the standard declare? First of all, it defines the preanalytical phase (pre-examination procedures) in article 3.11.: steps starting, in chronological order, from the clinician´s request and including the examination requisition, preparation of the patient, collection of the primary sample, and transportation to and within laboratory, and ending when the analytical examination procedure begins. Requirements for this part of the process are to be found in article 5.4.3. – requirements for content of Primary Sample Collection Guide. Next articles attend to proper identification and transport of the sample. It is also connected to articles 5.4.5. – identification of sample and 5.4.6. – transportation of sample.

From the above mentioned it is obvious, that almost all aspects influencing validity and sequence of results are included in the standard, but often just as requirements for documentation. Important agent – preparing a patient and method of collection – is just an obligatory part of Primary Sample Collection Guide. Its fulfilment depends only on doctor's responsibilty in terrain. A laboratory is able to verify a proper utilization of sampling tubes, transportation (temperature, time) and consesnus of data on list of requirements and on sampling tube. Preparing a patient (diet, urine taking entirety...) and method of sample collection (lying or sitting position; with tensed of released arm) cannot be verified and controlled.

That means that the standard is clear and its requirements are understandable and sufficient. Nevertheless a fulfilment of some of them out of laboratory is difficult to be controlled – controllable is only a proper documentation (guides) and its comprehensibility for doctors and patients. And how does the doctor in terrain (or phlebotomist) act? Does he instruct a patient properly? Does he verify the preparing of patient? And how? This part of pre-analytical phase cannot be controlled, not even during an audit.

It results in a fact, that the standard defines the requirements for pre-examination processes sufficiently but some of the parts of non-analytical phase cannot be controlled. Therefore following the standard EN ISO 15189-2007by the laboratory does not necessary warrant the validity of results in cases of tests with more complicated preparation for collection of more complicated sample collection, as well as in cases of test of collected urine.

COVERAGE OF VALIDATION OF RESULTS AND POST-EXAMINATION ASPECTS: IS IT SUFFICIENT?

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Medical laboratories play an essential role in health care (diagnosis and therapy). More than 60% of all medical decisions are based on medical laboratory results. The full medical laboratory process covers everything from the pre-analytical phase to the post-analytical phase, and the importance of the post-analytical phase is detailed in the standards for accreditation document ISO/EN/15189[1].

In particular Chapter 5 of the Standards - Biological validation of results and post-examination aspects – will be discussed. This covers competencies for validation, likelihood and compatibility with the patient history, reporting of results within agreed time intervals, release of results, « alert » or « critical » intervals, recording of all verbal results provided, emergency cases, responsibility for authorisation, complete interpretation of the results, requirement for the clinical context, and respect for confidentiality. It also covers transmission of results, detailing the requirement for clearly documented procedures for release of results, including details of who may release results and to whom. Record-keeping and sample storage are also part of the post-examination aspects.

In addition:

· a medical laboratory should be accredited according to ISO/EN15189 for the whole process
· it must be clear that a recognised specialist (EC4 European Register of Specialists in Clinical Chemistry and Laboratory Medicine)[2] has overall responsibility and offers clinical advice and consultation.

The current French experience will be discussed. According to the French Law[3], all laboratories shall prove that they are in the process of accreditation according to ISO/EN/15189 by 2013, directed by highly qualified professionals, and shall be accredited for their whole repertoire and processes by 2016.

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THE SPECIFIC ROLES OF ASSESSORS DURING ACCREDITATION AND BEYOND

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Accreditation has as purpose that a competent third party registers that the laboratory fulfils all the requirements stated in a standard. For medical laboratories this is the ISO 15189:2007.In Europe the only institutes which are allowed to perform accreditations are the National accreditation Bodies who are members of the European cooperation for Accreditation. Within the Health Care committee of EA we try to harmonise the way assessment is done to warrantee that the mutual recognition also in practice is accomplished.

The lead assessor has as primary function to judge the quality system. The different items are presented in chapter 3 of ISO15189. Points of attention are: continuous quality improvement, the PDCA cycle, really working internal audit, documentation, complaints system, management review etc. The results of the questionnaire relating to assessment of medical laboratories, showed that the lead assessor does not have to be a medical laboratorcavay professional. Knowledge about quality systems is essential, although familiarity with medical laboratories is needed as well.

The professional assessor needs not only to be a medical laboratory specialist, but competent in the scope which is assessed as well. The number of professional assessors needed is strongly depended upon their training and the extensiveness of the laboratory. This was shown quite clearly in the questionnaire. Their role is primarily to judge the competence of the laboratory as given in chapter 4 of the ISO15189. Not only related to the examination aspects as validation of methods, traceability, internal and external quality control, up to date methods and adequate standard operating procedures, turn-around time, but as well to the pre-examination aspects, even if the phlebotomy is not done by the laboratory, and post-examination aspects especially the consultancy function of the laboratory specialists. Part of the assessment is the competence of laboratory technicians and the laboratory specialist. This in relation to their number, specialities in extensive laboratories, their continuing education and renewal of registration. Also the contacts with the physicians who order the tests should be evaluated.

A combined responsibility if the assessment team is to establish confidence in the reliability of the service offered by the laboratory. This means not just the presence of competent people, but real cooperation between them. Many non-consistencies are not just mistakes, but are related to deficiencies in the system. For solving them a root analysis important. It relates to Root cause of the non-consistency, Extensiveness of it, Solution of the problem and Operationallity of the solution proved by internal audit. It is helpful if the assessment team already tries to combine non-consistencies.

Re-registration of laboratory professionals is not the competence of accreditation bodies. Registration of attendance in congresses, post doctoral education, publications, is helpful, but assessments can be used for this as well. The route for the decision and the responsibility is different. However, there is an overlap between both aspects, and the information from one is helpful for the other. Structured training of laboratory professionals to become assessors is needed for both aspects.

DGKL 4 Quality Management according to the German Rilibaek: An International Perspective

Tuesday, 17 May 2011   14:30–16:30

GERMAN MEDICAL ASSOCIATIONS DIRECTIVE FOR QUALITY ASSURANCE FOR MEDICAL LABORATORY TESTS, HISTORY AND LAST ISSUES

W. Vogt

Abstract not received

QUALITY MANAGEMENT AND QUALITY ASSURANCE IN ITALIAN MEDICAL LABORATORIES

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Quality management systems are obviously applied in many public and private clinical laboratories in Italy; but the general situation is very heterogeneous in different regions of the country. This is due to the application of federal laws issued already in the nineties, which established the general principles for accreditation of health institutions (and among them, also of medical laboratories) all over the national territory. However, the setting of the rules and the choice of the required quality level was assigned to local authorities. The consequence was that all regional authorities issued different rules and standards, and the degree of application of these rules to existing institutions was and is very uneven. There is no authority comparable to the *German Bundesärztekammer*, who issues the *RiliBÄK*-rules, which are valid in the whole national territory. This leads to the fact that in the North of the country, where the public medicine is promoted much more than the private one, the quality levels in laboratories tend to be higher than in the South of the country, where there are many more private institutions. There is actually no association between quality performance tested in audits or by participation to external quality assessments and the reimbursement of laboratory testing.

In the past, QM-systems in Italian medical laboratories were mainly realised in accordance to the ISO 9001-guidelines; certifications were issued by local, national and international bodies. Since the ISO 15189 was published in 2007 in his last version, more and more laboratories get accredited according to this standard. The only national accreditation authority, called ACCREDIA, which was established in 2009 from the fusion of SINCERT, active in the North, and SINAL, active in the South, should get in the future the role of a supervisor for all these quality activities. ACCREDIA has as full members 9 ministries, as sponsor members 8 national public entities,13 Business and Labor Associations, 2 National Standardisation Bodies (CEI and UNI) and 2 Major Client Organisations, and 26 other organisations as ordinary members. In 2009 nearly 7.000 health and other social services were accredited, and more than 800 testing laboratories (mainly chimical or microbiological) operated under ACCREDIA acrreditation. However, for the time being the accreditation/certification of an existing medical laboratory is mostly voluntary, whereas new opening laboratories, who look for reimbursement from the National Health Service, normally must get authorisation and accreditation after a quality assessment performed by a body which is recognized either by ACCREDIA or by the competent regional accreditation authority.

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QUALITY MANAGEMENT AND QUALITY ASSURANCE IN AUSTRIAN MEDICAL LABORATORIES

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Quality Management. Whearas quality management (QM) is defined as "coordinated activities to direct and control an organization", quality assurance (QA) can be considered as a "part of quality management focused on providing confidance that quality requiremenets will be fulfilled" (1). Based on this differentiation, QM in Austrian medical laboratories is mainly realised by the implementation of quality management systems (QMS) in accordance to ISO 9001 and certification by one of the national or international certification bodies (2). Certification of laboratories started in 1995 and in contrast to other European countries kept also on when ISO 15189 had been published in 2003 (3). This opposing trend can be explained by peticular accreditation regulations, no national impact of other previous standards (e.g. EN 45001 or ISO 17025) and the focused activities on ISO 9001 of the main laboratory societies under the guidance of the Austrian Society for Good Analytical Practise (GALP), founded in 1994. Publishing an interpretation guideline of ISO 9002:1994 for medical laboratories had an additional major impact in terms of usabilty and understanding of the very general requirements of this standard and its acceptance in the special field of laboratory medicine (4, 5). On the other hand, the Excellence Model of the Euopean Foundation for Quality Management (EFQM) is not applied in Austrian laboratories.

Since 2005, accreditation according to ISO 15189 gained more importance and is exclusively administered by the accreditation authorities of the Federal Ministry of Economy, Family and Youth. Accreditation is actually confined to only a few laboratories. It is currently and in the near future not mandatory.

Quality Assurance. QA is required by various laws and in particular for medical devices, as well as pharmaceutical and blood products. Therefore, specific requirements exist for laboratories designing inhouse in vitro diagnostic devices, participating in clinical trials or performing test for bood banks. Periodical inspections and audits in this field are done by the Austrian Agency for Health and Food Safety (AGES).

Further requirements are stipulated from national and provincial social insurances as well as medical associations. In general, they are basic, mainly focused on the periodic participation of external quality assessment schemes and associated with the reimbursement of laborartory testings.

Finally, the Austrian Standards Institute has implemented a specific task force for standardization in the national health care system (ON committee 250) and publised two relevant standards representing the state of the scientific and technical knowledge. The ON K1950 standard (6) is very similar to part A of the German RiliBÄK (8) and also based on the content of ISO 15189. The ON K 1361 deals with validation and documenation of inhouse tests (7).

Conclusion and future aspects. QM became an integrated part of daily laboratory business in Austria. ISO 9001 and 15189 are the most relevant standards and they will be continuously supplemented by other topics (e.g. safety or risk management). Procedures of certification and accreditation authorities as well as the auditors of QMS must be adapted in time to follow this development.

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ROOT MEAN SQUARE OF MEASUREMENT DEVIATION: A NEW APPROACH IN GERMAN MEDICAL ASSOCIATIONS DIRECTIVE FOR QUALITY ASSURANCE FOR MEDICAL LABORATORY TESTS

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Background. Quality assurance for quantitative investigations in laboratory medicine is an important aspect of general quality management in medical laboratories. Important components of internal and external quality assurance in this context are frequent metrological controls in combination with comparison measurements with reference laboratories or interlaboratory surveys. Since more than 30 years, minimum requirements for internal and external quality assessment of quantitative measurements in medical laboratories in Germany are committed by the *Guidelines for Quality Assurance of Medical Laboratory Examinations of the German Medical Association.*

Methods. A novel approach has been introduced in the recent version [1] of the *Guidelines for Quality Assurance of Medical Laboratory Examinations of the German Medical Association* to check for the quality of quantitative results of investigations in laboratory medicine by assessment of the root mean square of measurement deviation [2].

Summary. The concept required in the new *Guidelines for Quality Assurance of Medical Laboratory Examinations of the German Medical Association* 2008 for internal quality control is discussed. It is shown how the accuracy of quantitative analytical results is validated against a (single) maximum permissible (rms-) value for the deviation of measurement instead of having two control rules for random and systematic errors separately. The new approach allows, in particular, an improved real-time assessment of the accuracy of quantitative results in laboratory medicine.

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WS 6 The 2009 Influenza Pandemic – Lessons Learned

Tuesday, 17 May 2011   14:30–16:30

THE 2009 INFLUENZA PANDEMIC - THE EXPERIENCE IN THE SOUTHERN HEMISPHERE

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After its first detection in Mexico and the USA in April 2009, the A(H1N1) 2009 pandemic virus took only a short time to reach the southern hemisphere. Many countries of the northern hemisphere experienced summer and autumn outbreaks, in some cases in two waves. By contrast, in the southern hemisphere, the new virus generally caused a single wave of infections which peaked in the winter months of June – August and had subsided by October 2009. Only sporadic cases were then detected until the next winter season in mid-2010. Despite these differences in the shape of the epidemic curve, the circulating pandemic viruses and the epidemiological and clinical features of disease were similar in the two hemispheres. Young people were preferentially infected and the disease was mild in most people but severe in some – particularly those with certain other conditions, in pregnant women and in indigenous populations. In Australia this presented the paradox of a largely unconcerned community at the same time that intensive care units were under intense pressure managing unprecedented numbers of patients requiring extended ventilation or extracorporeal membrane oxygenation for severe lung disease. As foreseen in pandemic plans, the many months required to develop, register, produce and distribute a specific pandemic vaccine presented a major issue for governments, public health authorities and the community. Australia, with an on-shore vaccine manufacturer, was in the fortunate position of having sufficient vaccine available for its population of 21 million from late September but, with the first pandemic wave largely ended by that time, uptake was initially modest. Serological surveys measuring serum antibodies to the pandemic virus in various populations in Australia and New Zealand have since shown that 10 – 20% of people had been infected with the pandemic virus by late 2009 while many older people had cross-reactive antibodies apparently induced by exposure to previous seasonal influenza viruses. A concerted immunisation campaign in early 2010 brought the proportion of Australian adults with specific antibodies up to about 40%. Perhaps reflecting this degree of population immunity, the Australian influenza season in 2010 was relatively mild and characterised by co-circulation of type B and A(H3N2) viruses with the former pandemic strain. Overall the behaviour of the A(H1N1) 2009 virus in the southern hemisphere in 2010 was consistent with the decision of the World Health Organisation to announce the post-pandemic phase in August 2010. By late 2010, A(H1N1) viruses were showing increasing genetic diversity but little evidence of antigenic drift. A major focus now is on surveillance to detect the emergence of variants which can escape antibody neutralisation, requiring a change in vaccine composition.

IMPACT OF PANDEMIC A/H1N1 VIRUS MUTATIONS ON DRUG RESISTANCE AND VIRULENCE

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Background. The 2009 pandemic influenza A/H1N1 (pH1N1) virus was associated with significant morbidity in young individuals and mortality in patients with underlying diseases or pregnancy. We assessed the role of specific neuraminidase (NA) and hemagglutinin (HA) mutations in conferring antiviral drug resistance and altered pathogenicity, respectively.

Methods. We generated recombinant mutants and used several in vitro methods (plaque and enzymatic assays) as well as two animal models (BALB/C mice and ferrets) to characterize the pH1N1 H275Y NA mutation conferring resistance to oseltamivir (OS) in previous seasonal A/H1N1 strains and the D222G HA mutation associated with increased mortality in humans.

Results. The H275Y NA mutant conferred high levels of resistance to OS with cross-resistance to peramivir but susceptibility to zanamivir. Compared to the wild-type (WT) virus, the H275Y mutant had slightly delayed replication in vitro but similar pathogenicity in mice and ferrets when evaluating weight loss, pyrexic response and lung or nasal wash viral titers. Both the WT and H275Y mutant were efficiently transmitted by direct contact but only the former was consistently transmitted by the airbone route in the ferret model. The D222G HA mutant had increased tropism for alpha 2,3 sialic acid receptors compared to the WT virus in plaque assays and HA tests. This switch in receptor specificity correlated with higher lung viral titers and alveolar inflammation in mice but not in ferrets.

Conclusions. The H275Y NA mutant associated with OS resistance is as virulent although somewhat less transmissible than the WT pH1N1 virus whereas the D222G HA mutant is associated with a greater risk of pneumonia. Monitoring for both mutations should be performed in clinical samples of pH1N1-positive patients with less favourable outcomes.

THE ROLE OF PATHOGENIC IMMUNE COMPLEXES IN SEVERE DISEASE CAUSED BY PANDEMIC INFLUENZA

F.P. Polack (Argentina)

Abstract not received

SYM 12 Biomarkers in Acute and Chronic Brain Diseases

Wednesday, 18 May 2011   09:00–11:00

ALZHEIMER DISEASE

G. Casadeus

Abstract not received

MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic central nervous system disorder that affects 2.5 million people worldwide. Several striking new findings are notable. Although traditionally considered to be primarily a disease affecting those of northern European ancestry, the disease is increasingly being diagnosed in other populations, such as those of Middle-eastern, African and Asian heritage. The female to male ratio appears to be increasing worldwide. Low serum vitamin D levels have been associated with increased risk of developing MS. World-wide efforts to determine the genetics of susceptibility to MS using genome-wide association studies have revealed associations with several genes, most of which are related to the immune system. In the past, the pathology of MS was thought to primarily affect CNS white matter. However, recent studies have demonstrated the considerable damage to the CNS grey matter that may occur in this disease. Diagnosis of MS still rests on the demonstration of CNS lesion dissemination in space and time, for which no alternative diagnosis is likely. The diagnosis is typically made by magnetic resonance imaging of the brain and spinal cord. Laboratory testing may include cerebrospinal fluid (CSF) analysis for cell count and differential, immunoglobulin levels and the presence or absence of oligoclonal (IgG, IgM) bands specific to the CSF and not found in the blood. Evoked potential studies are occasionally used as well. MS must often be differentiated from other diseases that can appear like MS, such as neuromyelitis optica, central nervous system vasculitis, lupus cerebritis, neuroborreliosis, etc. MS has been divided into clinical subtypes, which are primarily relapsing-remitting, secondary progressive and primary progressive subtypes. The first two subtypes display relapses and remissions (partial or full), whereas in primary progressive MS, dissemination in time is increasing symptoms or signs over a one-year period. Within the past two decades, treatment has changed dramatically for those with relapsing subtypes MS subtypes. Treatments that alter the course of the disease, reducing relapse rate and long-term disability for relapsing clinical subtypes of MS are now available (beta-interferons and glatiramer acetate). Accumulating data indicate that the treatment of patients with beta-interferons leads to less disability and reduced numbers of patients transitioning to the more severe secondary progressive subtype. Several newer medications, including natalizumab, fingolimod, oral cladribine, dimethyl fumarate, laquinimod, teriflunomide, alemtuzumab, and several others are either approved or under study.

IDENTIFYING BLOOD BIOMARKERS TO IMPROVE THE MANAGEMENT OF STROKE

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A blood test to confirm a clinical or imaging diagnosis of stroke (or to aid risk-stratification in confirmed cases), based on simple and low-cost near-patient technology, could be extremely useful.

Identification of potential biomarkers. There are 21 studies of biomarkers for the diagnosis of stroke testing 58 single markers and 7 panels constructed with a number of markers. Several markers had the potential to be useful for the diagnosis of stroke, though many studies had deficiencies in their design, which may have explained the apparently – and perhaps spuriously - impressive diagnostic performance.

The assessment of promising diagnostic biomarkers. 19 markers of several processes disturbed after stroke showed promise: inflammatory: thrombosis, cardiac strain: and cerebral damage. We prospectively recruited patients where an emergency department clinician suspected stroke or TIA as a cause of ongoing symptoms. Each patient was assessed with the clinical 'face, arm, speech' test (FAST). We measured the improvement in diagnostic performance by adding blood markers to the FAST in logistic regression models to predict a diagnosis of stroke or TIA. 405 patients had suspected stroke: 285 with TIA or stroke (230 ischaemic, 40 TIA, 15 haemorrhagic) and 120 with other diagnoses. Only the markers tPA and NT pro-BNP were positively and significantly associated with a diagnosis of TIA or stroke, though neither improved the sensitivity or specificity of the FAST to a clinically and statistically significant degree.

Blood markers for the prediction of recurrent stroke and MI after stroke. I used data from the Edinburgh Stroke Study which prospectively recruited stroke patients, drew markers of inflammation soon after stroke onset and followed them for up to 4 years for: fatal or non-fatal recurrent stroke, myocardial infarction or fatal vascular events, and death from any cause. The adjusted incidence of the outcome cluster 'recurrent stroke, myocardial infarction or vascular death' after stroke was significantly higher with higher levels of IL-6 (75th to 25th centile: hazard ratio [HR] 1.56, 95 % CI 1.37–1.77), CRP (75th to 25th centile HR 1·08, 95% CI 1·04–1·11) and fibrinogen (75th to 25th centile HR 1.45, 95% CI 1.24–1.72). However, no inflammatory marker improved the prediction of recurrent vascular events over the clinical variables: age; prior TIA, myocardial infarction or stroke; and atrial fibrillation.

Conclusions. Although several markers of different physiological processes were associated with a diagnosis of TIA or stroke, and with recurrent vascular events after stroke, no marker improved the diagnosis of stroke or prediction of recurrent events over established clinical variables. Major challenges in the diagnosis of stroke are both the variety of conditions that mimic stroke, and the heterogeneity of stroke itself. It is very difficult to imagine a pathophysiological process that is unique to stroke or one of its subtypes and not found in a stroke mimic. This is quite unlike the situation for myocardial infarction where very few conditions, other than cardiac ischaemia, cause severe acute chest pain and lead to a rise in markers of myocardial necrosis.

S100B PROTEIN : A SCREENING TOOL FOR THE DIAGNOSIS OF MINOR HEAD INJURY

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Background. Incidence of traumatic brain injury (TBI) ranges between 200 and 400 for 100,000 persons, and more than 80 percent are minor (Glasgow Coma Scale - GCS - score: 13 to 15) or moderate (GCS score: 9 to 12) head injuries. Computed tomography currently is the "gold standard" for the diagnosis of minor/moderate TBI, but is not always available, costly and exposes subjects to X-ray. S100B protein is a calcium binding-glial protein whose blood measurement is proposed as a screening tool of brain damage with a high negative predictive value. We aimed to verify usefulness of early and/or 3h-delayed blood measurement of S100B protein in a large cohort of French patients suspected of minor/moderate TBI.

Methods. Five hundred patients suspected of minor/moderate TBI were consecutively recruited in 7 French hospitals: Cochin (Paris), Lariboisière (Paris), Pontoise, Orléans, Reims, Créteil, Poitiers. Patients (>18 year-old) presented to the Emergency Department within 3h of head trauma, with a GCS of 9 to 15. Neurological examination, head computed tomography scan, and blood S100B level determination (at admission and 3h later) were systematically performed. S100B concentration was measured using two commercially available automated immunoassays: Diasorin (Liaison® analyzer) and Roche Diagnostics (Modular E® or Elecsys® analyzer) according to manufacturers' recommendations.

Results. Both sensitivity, specificity, negative and predictive values for S100B measurement are being determined and are discussed at the congress. Expected results are as follows: (1) approx. 8-10% of positive cases of TBI as revealed by head computed tomography scanner; (2) significantly higher blood S100B levels in subjects with TBI when compared to subjects without TBI; (3) a high (>99%) negative predictive value that will confirm the interest of S100B blood level determination in subjects suspected of moderate/minor injury, that allows to exclude the diagnosis of traumatic brain damage when S100B blood concentrations remain below the cut-off value specifically defined according to the analytical assay.

YR 3 Developments in Clinical Laboratory Practice

Wednesday, 18 May 2011   09:00–11:00

TRANSLATIONAL MEDICINE: THE CASE OF LABORATORY MEDICINE

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The implementation of basic and clinical research findings into health care is a challenging and slow task that is often left incomplete, to the extent that these findings are often "lost in translation". In particular, the chasm is wide between new discoveries in "omics" and their clinical validity and utility in health care (1). Current expansion of direct-to-consumer marketing of personal genome profiles for risk assessment and disease prevention is symptomatic of a larger problem that is the relatively low importance given by most stakeholders to evaluate diagnostic tests. The journey from the discovery of a biomarker to its implementation in clinical practice is too long and complex. Failure to provide convincing evidence of the clinical and financial benefits of introducing a new laboratory test and the weakness of many published studies were identified as major factors relevant to the slow uptake of new laboratory tests, namely new biomarkers (2) . Serious limitations include deficiencies in study designs leading to different types of bias (e.g. inappropriate selection of cohorts of patients and controls and/or specimens (3), lack of rigorous investigation of pre-analytical factors (i.e. biomarker stability and specimen requirements, diurnal and intra-individual variation, etc), absence of independent verification of results, and failure of investigators to evaluate whether the application of the biomarker would change and improve clinical practice to the benefit of patients and/or the health service as a whole. Recently some recommendations and some proposals of key requirements for the successful introduction of new biomarkers into routine practice have been released, and the role of laboratory professionals highlighted thus paving the way to a more efficient translational process (4-7).

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RISK MANAGEMENT IN THE PRE-ANALYTICAL PHASE

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Medical errors typically fall into four categories, i.e., errors of diagnosis, treatment, prevention, and an other "diverse" category. Due to the increasing number and complexity of laboratory testing, mistakes in the total testing process might occur with a relatively high frequency, fall within all four types of medical errors and might seriously jeopardize patient health. Several lines of evidence now attest that although great progresses have been made in the analytical phase of testing, the preanalytical phase is the most vulnerable throughout the total testing process, preanalytical errors representing up to 70% of all mistakes in laboratory diagnostics. As such, the positive trends towards reduction of laboratory errors over the past decade has only little involved the preanalytical phase, which actually represents the most critical area to pursue. Most preanalytical errors result from system flaws and insufficient audit with operators involved in specimen collection/handling responsibilities. In particular, the high frequency of errors still attributable to processes external to the laboratory (i.e., collection, handling and management of the specimens) requires additional efforts for the governance of this mistreated phase of the total testing process. As for any other type of human errors, the most effective approach for improvement is the implementation of a total quality management system, encompassing a multifaceted strategy encompassing reassessment and rearrangement of quality requirements, process analysis, implementation of error-tracking systems, reduction of complexity and error-prone activities, education and training of healthcare professionals, dissemination of operative guidelines and best-practice recommendations, continuous monitoring of performances. Finally, the introduction of event reporting policies meeting reliable criteria for reviewable sentinel events should be firmly encouraged by the IFCC as well as by other national and international scientific societies, to facilitate learning, develop solutions and establish a more positive safety culture.

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RISK ANALYSIS OF THE ANALYTICAL TESTING PROCESS

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Background. The comparability of laboratory test results is a serious issue for the safety of patients who are diagnosed, monitored, and treated on the basis of test results obtained from different laboratories within a healthcare system or different laboratories throughout a geographical region. HbA1c and glucose testing illustrate the difficulties of achieving comparable test results and the need for a well developed analytic quality system.
Methods. The review of recent publications and guidelines provides examples of problems and improvements in analytical quality management, as well as the introduction of risk analysis for the development of Analytic QC Plans and Quality Systems.
Results. New guidelines for the use and interpretation of HbA1c results provide a good example of the clinical definition of the quality required for intended use (1). Guidelines for the development of error grids (CLSI EP 27) are useful for comparing diagnostic and treatment criteria, as well as criteria for glucose testing and the reporting of estimated glucose from HbA1c measurements. The establishment of outcome-related analytic performance goals has been reviewed by (2) and specific recommendations for HbA1c and glucose testing have been discussed (3). Application of such requirements has been demonstrated in a detailed method validation study for point-of-care HbA1c methods (4). The quality of point-of-care glucose testing has also been studied (5) and the application of glucose meters for tight glycemic control has been critically evaluated (6). Improvements in analytic performance are still needed, as well as continuing efforts to monitor performance via internal quality control. The development of Analytic QC Plans on the basis of risk analysis is described in a new guideline (CLSI EP23). The issue of commutability of control materials has also received attention (7) and it was recommended that changeovers between reagent lots should be monitored by measuring fresh patient specimens. The critical importance of commutability in EQA programs has also been emphasized (8), particularly for establishing traceability and estimating measurement uncertainty. A renewed interest in traceability was evidenced by a special conference on harmonization that was organized by AACC in late 2010. HbA1c again provides examples of ongoing work on reference methods (9) and materials (10).
Conclusions. Many improvements are still needed to assure comparability of test results. HbA1c provides a model for understanding both the problems and the solutions (11).

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REFERENCE VALUES AND BEYOND

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A PubMed search on the last three years (2008 to 2010) of papers on humans, English language, having in the title the terms "reference values" or "reference intervals" or "reference limits" or "normal values" yielded 344 titles. Of them, 178 dealt with clinical laboratory tests, almost evenly distributed in the 3 years (66, 54 and 58 respectively). Why such a relatively large number of papers specifically dedicated to a quite topic? The reply to this question can be found analyzing the typology of the papers. Twenty eight % (50) dealt with pediatric or neonatal values and 16 are related to pregnancy, but only 2 to elderly people. All these fields are lacking reliable reference values (RV). Thirty studies are on a single analyte or on panels of common analytes in specific populations (mainly from Asia and Africa); 22 are related to novel biomarkers and 10 to the reevaluation with new analytical methods of existing RV. Thyroid hormones are the preferred subject (20 papers), creatinine, iron metabolism and micronutrients are other RV studied by several papers. At least 10 papers use data mining statistical methods to derive RV from the data present in the large laboratories' databases and two of them are proposing newly developed methods; strength and pitfalls of this approach will be discussed. From the analysis of the literature two aspects appear clearly: analytical and pre-analytical standardization are often insufficient and this makes impossible the application of the result of a study to different contexts, moreover is impossible to demonstrate if different populations have different RV or it is just the effect of the study design. For many "less common" analytes RV are lacking and for sure genetic-environment interaction can influence RV, but only two papers were on this argument.

RV have several limits both intrinsic (represent the central 95% of the population, analytes with low individuality index), and extrinsic due to the way they are calculated (wrong reference population, wrong statistical method, bias of the analytical method, etc.) but their relevance in transforming "numbers" into "information" can be improved through the use of better standardized analytical methods and well defined and correctly partitioned RV. In this last 3 years the concept expressed by the IFCC of making RV the fourth pillar of the Reference System (together with reference methods, materials and laboratories) has been presented by the IFCC Reference Intervals Committee (C-RIDL) with the practical examples of creatinine, AST and ALT (1,2). Moreover the need and the importance of large multicenter studies has been demonstrated by several papers and a large Asian study in press. Decision limits are a further interpretative tool, but an analogous PubMed search returned only 5 papers, none of which really presenting original data on the topic. Likelihood ratio and reference change values are other ways to go beyond RV, but significant experimental data are lacking.

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WS 7 Clinical Pharmacogenetics

Wednesday, 18 May 2011   09:00–11:00

CYP2D6 GENOTYPING TO GUIDE USE OF TAMOXIFEN IN BREAST CANCER

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Background. CYP2D6 is important for metabolism of tamoxifen to its active metabolite, endoxifen. Women with breast cancer who are CYP2D6 poor metabolizers (PM) may have suboptimal response to tamoxifen. Intermediate metabolizers (IM) may also have reduced clinical response. We examined the feasibility of using CYP2D6 genotyping to determine optimal tamoxifen dose and investigated whether the key active tamoxifen metabolite, endoxifen, could be increased by genotype-guided tamoxifen dosing in patients with reduced CYP2D6 metabolism.

Methods. Breast cancer patients on tamoxifen and not on any strong CYP2D6 inhibitors were assayed for CYP2D6 genotype and plasma concentration of tamoxifen metabolites. Patients found to be CYP2D6 extensive metabolizers (EM) remained on 20mg of tamoxifen daily and those found to be IM or PM were increased to 40mg daily. Four months after tamoxifen dose adjustment, measurement of plasma concentration of tamoxifen metabolites was repeated.

Results. As expected, the median baseline endoxifen concentration was higher in EM (34.3 ng/mL) compared with either IM (18.5 ng/mL, p=0.0045) or PM (4.2 ng/mL, p<0.0001). When tamoxifen dose was increased from 20mg to 40mg in IM and PM patients, the endoxifen concentration rose significantly: in IM there was a median intrapatient change from baseline of +7.6 ng/mL (-0.6 to 23.9, p=0.00040), and in PM there was a median change of +6.1 ng/mL (2.6 to 12.5, p=0.020). After the dose increase, there was no longer a significant difference in endoxifen concentration between IM and EM patients (p=0.84), demonstrating that a doubling of tamoxifen dose normalized endoxifen concentration in IM; however, the PM endoxifen concentration was still significantly lower.

Conclusions. This study demonstrates the feasibility of genotype-driven tamoxifen dosing and demonstrates that doubling the tamoxifen dose can increase endoxifen concentrations in CYP2D6 IM and PM. The clinical utility of pharmacogenetic CYP2D6 genotyping to guide use of tamoxifen in breast cancer will be discussed.

PHARMACOGENETICS OF COUMARINS

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Coumarins like warfarin or phenprocoumon are the most commonly used oral anticoagulants for treatment of arterial or venous thromboembolism. Adjustment of the dose is challenging due to large inter-individual and interethnic variation in dose requirement. The target of coumarin type anticoagulants is vitamin K epoxide reductase complex subunit 1 (VKORC1). VKORC1 represents a small ER membrane bound enzyme and recycles vitamin K 2,3 epoxide to vitamin K hydroquinone which functions as essential cofactor for γ-carboxylation of Gla-domains of coagulation factors II, VII, IX, X, protein C, and protein S. Inhibition of VKORC1 activity by coumarin based drugs reduces vitamin K recycling efficacy which limits carboxylation of vitamin K dependent proteins. In VKORC1, frequent single nucleotide polymorphisms form four haplotypes which have been identified as main pharmacogenetic determinants for coumarin dose. Haplotype VKORC1*2, comprising a functional promoter polymorphism was shown to be associated with a 50% reduced mRNA transcription rate and enzyme activity. This is reflected by cut to half dose warfarin requirement in patients being homozygous for the VKORC1*2/*2 genotype compared to patients with homozygous for VKORC1 wild type alleles. Thus, VKORC1*2 is responsible for a significant part of normal interindividual dose variation. With a prevalence of roughly 40% in European populations, but approximately 90-95% in cohorts with Asian ancestries and roughly 10-15% in Africa, this haplotype is also the main reason of interethnic coumarin dose differences.

Another well studied hereditary pharmacokinetic factor influencing coumarin metabolism is cytochrome p450 2C9 (CYP2C9) genotype. Several CYP2C9 variants with reduced metabolizing ability for warfarin (but not phenprocoumon) so far have been identified. The CYP2C9*2, CYP2C9*3, and CYP2C9*11 variants exhibit residual enzyme activities between 12% and 5% and show allele frequencies relevant for warfarin dosing in the general population. The Cyp2C9 haplotype composition accounts for about 10-15% of the variability of coumarin dose.

Mutations in VKORC1 are quite rare, but determine two different phenotypes: a homozygous missense mutation in the VKORC1 gene was shown to cause combined deficiency of vitamin K dependent coagulation factors type 2 (VKCFD2) whereas heterozygous missense mutations are responsible for hereditary warfarin resistance (1,2). In most cases of partial coumarin resistance a therapeutic INR can be achieved by administration of high coumarin doses. Therefore, the knowlegde of the underlying mutation may contribute to successful coumarin treatment of patients with mutations in VKORC1.

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CAN DRUG INDUCED LIVER INJURY BE PREDICTED BY PHARMACOGENETICS?

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Pharmacogenetic tests help to predict drug actions in individuals prior to therapy by testing genetic variants which are associated with impaired transport and metabolism of different drug. Not only drug metabolism, but also detoxification processes influence the susceptibility of liver tissue towards drug induced adverse effects. Drug induced liver toxicity can be caused by different reactions such as impaired pharamcokinetics or immunoallergic reactions. Genomwide studies identified a number of genetic variants, which are associated with hepatotoxicity. We pinpoint relevant target genes in order to identify patients receiving different drugs which are at risk for the development of liver injury. Risk stratification should help to personalize drug treatment by identifying low, intermediate and high risk patients. Prior to treatment the selection of drugs and drug doses could minimize risks or side effects and optimize positive drug effects. Target genes include genes encoding for a number of functional proteins, such as metabolizing, detoxificating or conjugating enzymes, cytokines, transporters or human leukocyte antigens. However, not all variants have clinical significance and might therefore be tested. Some targets might only be of scientific importance at the moment. Nevertheless, with improved analytical possibilities and the knowledge of relevant genetic variants, pharmacogenetic testing might optimize drug treatment and help to prevent patients from drug induced liver injury.

SYM 13 Prenatal and Neonatal Screening

Wednesday, 18 May 2011   09:00–11:00

PRE-ECLAMPSIA: GENETIC AND EPIGENETIC ASPECTS

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Pre-eclampsia is a maternal disease with placental origin. Deregulation of placentation in early pregnancy causes changes in circulating placental antiangiogenic factors, i.e. soluble FMS-like tyrosine kinase 1 (sFlt1), soluble endoglin (sEng) and placental growth factor (Plgf), that occur months before the clinical appearance of maternal symptoms (hypertension, proteinuria) in late pregacy induced by TGFβ-mediated NOS-dependent vasodilation.

The maternal phenotype in pre-eclampsia is controlled by the fetal (epi)genotype. The consistent observation in monozygotic parous twins that lack of concordancy for proteinuric hypertension is the rule rather than the exception implicates that placental contributions are essential for any genetic as well as epigenetic basis of pre-eclampsia and related syndromes (HELLP).

Pre-eclampsia is not a single entity. Early-onset (<34 weeks) pre-eclampsia has a high risk for adverse outcome, is associated with intrauterine growth retardation, a clear familial component and abnormal placental morphology. Late-onset (>34 weeks) has a negligible risk of adverse otucome, is not associated with intrauterine growth retardation, no strong familial component and normal placental morphology.

The genetics of the different pre-eclampsia forms are different. Late-onset pre-clampsia is associated with a predisposed maternal constitution reflecting microvascular disease or predisposed genetic constitutions with cis- or transacting genomic variations subject to interaction. Their identification is ongoing using genome wide case-control association studies (GenPe Study) (Wellcome Trust Case Control Consortium). Early-onset pre-eclampsia with grandmaternal origin is -in Dutch families- associated with a gain-of-function varation in the DNA binding domain of the STOX1 transcription factor. The mutant STOX1 allele -by increased CTNNA3 expression- leads to reduced trophoblast invasion. The involvement of the parologous STOX2 gene is suspected to be involved in other populations (Norway).

Given the epigenetic features (hypomethylation, repressive histone modifications, imprinting, chromosome-specific microRNA clusters, etc), that control placental development, and given the fact that 90-99% of the mammalian genome is transcribed into RNA, including non-coding RNA, at some time in life, wheras less than 2% of the genome encodes proteins, pre-symptomatic, patient-specific diagnosis of pre-eclampsia should be pursued by molecular approaches such as genome-wide RNA-seq of exosomal RNA in first trimester maternal plasma.

AN UPDATE ON ANTENATAL SCREENING FOR DOWN'S SYNDROME

N.J.Wald

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Background. Efficacy and safety in screening programmes arise from maximising the detection rate (DR) for a given false positive rate (FPR) or minimising the FPR for a given DR.

Methods. Using published data different methods of screening were compared to determine the method of screening with the greatest efficacy and safety.

Results.

Currently available test:	Screening performance
	DR for a 1% FPR or less
Screening in 1st and 2nd trimester	
1. Integrated test	88%
2. Integrated test with ductus venosus pulsaticity index (DVPI)	91%
1st Trimester	
1. Combined test	76%
2. Combined test with DVPI	82%
2nd Trimester	
1. Triple test	57%
2. Quad test	67%
Possible Future test:	
Maternal plasma DNA sequencing	≥99%

Conclusions. Of the currently available tests considered, the Integrated test with DVPI is the most effective. Maternal plasma DNA sequencing has a greater efficacy but the test is not ready for routine use.

CIRCULATING CELL-FREE FETAL DNA IN MATERNAL PLASMA: PROSPECTS FOR NON-INVASIVE PRENATAL ASSESSMENT OF FETAL ANEUPLOIDIES

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Screening for, and prenatal diagnosis of, fetal aneuploidy have been an evolving and integral component of obstetric practice for 5 decades, beginning in the 1960's and 70's with invasive detection of fetal Down syndrome and neural tube defects, respectively. Continuous process improvement has been an integral part of the evolution of both diagnostic and screening approaches. For example automated cytogenetic analysis is commonplace, and multi-analyte risk assessment used in screening for fetal Down syndrome has evolved from the initial approach advocated by Merkatz and coworkers, who first demonstrated an inverse correlation between low maternal serum alpha-fetoprotein (MSAFP) and risk. Recently, there has been yet another significant research development in identifying pregnancies at increased risk for fetal aneuploidy (and, perhaps more importantly, the ability to better identify among high-risk pregnancies, those *not* at increased risk). This advance is based upon the relatively recent discovery by Lo and coworkers, that fetal nucleic acids are circulating in the maternal blood stream.

Since this discovery multiple researchers have evaluated the prospect to identify fetuses with trisomy 21 utilizing circulating cell-free fetal (ccff) nucleic acids isolated from the plasma of pregnant women using a variety of methods and assay formats involving RNA and DNA. For example, investigators employed a single nucleotide polymorphism (SNP)-based approach using ccff RNA. This approach proved effective in identifying fetuses affected with trisomy 21 in parents demonstrating SNP heterozygosity in the PLAC4 gene on chromosome 21. This process, however, was limited clinically to those fetuses who were heterozygous for the target SNPs. More recently, researchers have shifted their attention to assay formats where fetal heterozygosity is not essential. The currently favored target is DNA, and the process by which the status of chromosome 21 and other aneuploidies is elucidated uses next-generation, massively parallel "shotgun" sequencing (MPSS) of fetal DNA extracted from maternal plasma.

DNA of fetal origin is typically 10% of the total circulating cell-free DNA found in maternal plasma at varying gestational ages. Unlike intact fetal cells which may persist in maternal blood beyond term, the circulating cell-free fetal DNA is cleared from the maternal bloodstream within hours after birth, thus, carry-over from a previous pregnancy is unlikely. One major advantage of DNA as an analyte is that it is much less readily degradable under physiological or laboratory conditions than RNA. Further, MPSS allows for broad ethnic coverage of the global population as it is not dependent on intra individual variable genetic markers such as SNPs. In MPSS, the fraction of the sequence reads that map to unique sites on the human genome are used for data analyses. Detection of a relative overabundance of sequence reads from the target chromosome (e.g., 21, 18 or 13) is the basis for detection of the relevant trisomy using a maternal blood sample.

We will review the history and current status of the utility of circulating cell-free fetal DNA based methods for non-invasive prenatal assessment of fetal aneuploidies.

RECOMMENDATIONS IN PRENATAL SCREENING IN THE WORLD AND CONNECTIONS TO OTHER DISEASES LIKE THYROPATHY

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Background. The basic postulate of high quality maternal-fetal care is the uncomplicated birth of a healthy baby to a healthy mother at term. There is a large range of other screening tests in pregnancy: gestational risk in diabetes, infection (HIV, hepatitis and syphilis), rhesus incompatibility and various other tests. Thyroid dysfunction in pregnancy is known to cause problems not only in the outcome of pregnancy, also in the development of the fetus.

Screening pregnant women for the presence of genetic aberration (Down's or Edwarsd's syndrome) is possible in the first or second trimester or in both of them. New screening tests benefits from presence of fetal DNA in the mother's blood.

The management of screening is very similar all over the world, in USA, Europe, Australia and Asia. Systems differ by economy and availability of health care, but everywhere the best strategy of screening is attempted. Screening results are also influenced by other factors, e.g.ethnic origin.

Methods. In the beginning the risk of Down's syndrome was based on maternal age. The eighties brought in second trimester screening AFP (alpha fetoprotein) and total hCG, later also uE3 (unconjugated oestriol) and inhibin. In the nineties usage of new markers PAPP-A (pregnancy-associated plasma protein-A) and free b-hCG in the first trimester was started. Scan in 11-14 weeks of pregnancy confirmation of viability, accurate date of pregnancy and mainly is used for measuring nuchal translucency (NT).

The thyroid markers TSH, TPOAb and FT4 are used to investigate, as each test yields important information, independently.

Results. The first-trimester combined screening is better than second trimester screening, both stepwise sequential screening and fully integrated screening have higher rates of detection of Down's syndrome, with low false positive rates.

In our group of 7,530, 9-11 week pregnant women, were determined TSH, anti TPOAb and FT4. In a region with sufficient iodine supplementation, a raised concentration of TSH was found in 5.14% of pregnant women; a suppression of TSH in 2.90%. Further there were 11.5% TPOAb positivity. Family or personal history of thyroid or autoimmune diseases were present in 58.3% women with any thyroid test positivity, in case of subclinical hypothyroidism it was only in 21.9% of them. Minimally 40% of pregnant women with positive results only in TPOAb, have some of thyroid disorders after delivery.

Conclusions. The full integrated test or the sequential integrated test is the safest, most cost-effective test currently available. If a NT measurement is not available, it is the serum integrated test. First trimester combined screening is an effective test for women who seek earlier diagnosis. For women who do not attend antenatal care until 15 weeks gestation the second trimester test is recommended.

World guidelines for management of thyroid dysfunction during pregnancy and postpartum recommend not universal but only case finding screening. Our results show a relative high risk of thyreopathy in pregnancy and we strongly support screening programs for pregnant women early in gestation. The screening of thyroid dysfunction should be joined with sampling for screening in the first trimester.

IFCC 3 Standardization in Molecular Diagnostics

Wednesday, 18 May 2011   09:00–11:00

THE NEED FOR STANDARDIZATION IN MOLECULAR DIAGNOSTICS

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Recent advances in molecular science and technology have been translated into diagnostic, prognostic, and therapeutic tools, and medical tests using these developments are being applied to a wide variety of clinical conditions. These include detection of viruses, bacteria, and other pathogens in patient samples, as well as tests included in important clinical settings in genetics, oncology, pharmacogenetics and haematology. Molecular diagnostic methods can be performed by using protocols developed and validated within the medical laboratory or by implementing and verifying test kits developed by manufacturers for commercial distribution. Commercially distributed test kits usually include all reagents and instructions needed to complete the test procedure and interpret the results, and are currently regulated by the IVD directive or FDA as in-vitro diagnostic devices. Laboratory developed molecular tests (LDMTs – or LDTs- Laboratory Developed Tests) on the other hand, are developed within laboratories performing the tests and intended for use solely in the test developer's laboratory. Regulation of medical laboratories and the tests they provide differ by country and region. The majority of molecular tests that are considered clinically relevant, are available as LDMTs and some are offered without a clearly defined evaluation of the analytic validity, clinical validity, and clinical utility. Analytic validity encompasses the evaluation of performance characteristics such as accuracy, precision, analytic sensitivity, analytic specificity and linear range. Clinical validity includes determination of reference ranges or cut-off points. Though the definitions may be debated, these are the minimum and foundation evaluations of medical laboratory testing required by globally-accepted and regional standards and regulations. Due to the rapid application of highly complex and continuously developing technologies used in molecular testing, laboratories struggle to find guidance and material resources to establish these parameters. There are endeavours to address these obstacles. Among them, the Clinical and Laboratory Standards Institute (CLSI), has published a series of molecular-methods guidelines in the areas of genetics, hematopathology, and infectious diseases covering relevant technologies such as microarrays, nucleic acid amplification, fluorescence in-situ hybridization, sample preparation and multiplex nucleic acid assays. They also provide best practice strategies for validation, implementation and quality assurance (www.clsi.org). Those attempting to tackle the challenges regarding the limited availability of reference materials, reference methods and PT programmes, are evaluating approaches such as synthetic materials, laboratory exchange programmes, and technique-based proficiency testing. Whether they are as effective, or can fill the gaps left by traditional programmes, is uncertain. These aspects and the evaluation of possible solutions will be presented as well as the role of scientific, governmental and industrial organizations in contributing to the standardization of the molecular diagnostics.

STANDARDIZATION IN MOLECULAR DIAGNOSTICS: DEFINITIONS AND USES OF NUCLEIC ACID REFERENCE MATERIALS

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Background. Molecular diagnostics is one of the most rapidly growing areas of laboratory medicine. This rapid growth of clinical molecular tests has outpaced the availability and development of reference methods and reference materials. Such methods and materials are important for the development, validation, and interpretation of diagnostic methods and tests.
Methods. Definitions of reference materials were reviewed by the Committee-Molecular Diagnostics, IFCC.
Conclusions. A lack of harmonization between the numerous international organizations currently either certifying or defining reference materials was identified. The following presentation (supported by the Committee-Molecular Diagnostics, IFCC) will review and clarify the definition, attributes and applications for the use of reference materials in the context of molecular diagnostics.

THE IFCC NETWORK OF MOLECULAR DIAGNOSTIC CENTRES

F. Rousseau[1], on behalf of the IFCC Committee on Molecular Diagnostics (C-MD).
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Background. In the rapidly evolving field of molecular diagnostics, with the building up of the evidence-base showing clinical utility of specific molecular diagnostic tests and methods, there is an increased need for introducing molecular diagnostic tests into the clinical laboratory diagnostic test offer.
However, despite their apparent simplicity, molecular diagnostic tests have been shown to be challenging for many clinical laboratories, for various reasons. Establishment of a molecular diagnostics quality system is also an important component of the test offering, as for any clinical laboratory test.
There is thus a need for sharing of information and expertise between more experienced laboratories and laboratories interested in implementing new molecular tests, or facing troubleshooting problems with existing assays.
Intervention. To provide a solution to some of these multiple challenges, the IFCC Committee on Molecular Diagnostics has established a Network of Molecular Diagnostic Centres (MDCs). This Network is comprised of both Expert Laboratories in specific fields of application, and of Member Laboratories.
Expert Laboratories have been selected by a peer-review committee after an initial call for applications, using a specific set of objective criteria. They accepted to fulfill a mandate for helping other laboratories in the Network to solve technical problems, or to implement new molecular diagnostic tests in their respective area of expertise.
This is performed through different types of activities such as 1) offering expert advice on the conduct and interpretation of molecular diagnostic tests; 2) acting as a point of reference for analysis of difficult samples; 3) offering expertise on genotype/phenotype correlations. In doing this, these laboratories can share protocols, share internal control specimens, analyze samples as a referral laboratory, and providing expertise for implementing or troubleshooting molecular diagnostic assays. IFCC MDC Expert Laboratories can also be called-upon by the Committee on Molecular Diagnostics to perform other tasks that will be described. Examples of the activities of this Network of MDCs up to now will be provided.
Conclusions. It is expected that the expansion and operation of this Network of Molecular Diagnostic Centres will contribute to increasing both the accessibility to molecular testing worldwide as well as to increase the quality of molecular diagnostic results.

THE PHARMACOGENETICS REFERENCE LABORATORY: AN IFCC MOLECULAR DIAGNOSTIC CENTER IN ACTION

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The clinical chemistry laboratory plays an important role in introducing new markers and laboratory tests for supporting diagnosis and optimizing patient care. Important questions for the introduction of new tests are issues like when a test should be ordered, how the test should be performed (which components to test, which platforms to use, which assay conditions), which quality issues should be met and how test results should be interpreted and reported. The strenght of the IFCC is the network of laboratories that can address these questions. The Committee Molecular Diagnostics has inited Laboratories to be candidates for Reference Centers for different fields. The Pharmacogenetics Core Laboratory of the Dept. Clinical Chemistry of the Erasmus MC Rotterdam appleid, and got recognized by the IFCC/C-MD as an IFCC Molecular Diagnostic Center for Pharmacogenetics.

Since this valuable recognition, the Pharmacogenetics Core Laboratory has developed several activities. We now act as the reference center for proficiency testing for pharmacogenetics (SKML) and have an important role in validating new platforms and tests for pharmacogenetic analyses (a.o. Roche AmpliChip, Affymetrix DMET, Infinity-system, XMAP/Luminex, LightCycler and Applied Biosystems DME-assays). For patient diagnostics, we are involved in writing guidelines and act as a referral site for difficult samples. On the educational level, students from several European countries have been visiting for periods of 2-12 months to be trained in pharmacogenetics. We have been active in organizing lectures and symposia on pharmacogenetics for clinical specialists, for pharmacists, for general practisioners and for clinical chemists. In the Netherlands, we participate in an national working group that translates scientific literature in an evidence based manner into specific drug dose recommendations, base don genotype. A start has been made to create a European Network for Pharmacogenetics, in order to share information and promote exchange of experiences. Important interactions have been established with the European Network for Pharmacogenetic Research (steering committee), the European Society for Personalized Medicine (board), the European Society for Clinical Diagnostics (board), the European Medicines Agency (EMEA) (member Pharmacogenetics Working Party), the FDA (advisor) and EuroGenTest. Also with other profesional organiszation like the ASCPT and the IATDMCT, a role in the pharmcogenetic working groups was obtained.

The aim of the Pharmacogenetic Core Laboratory for the coming years is to further expand its role in promoting the proper integration of pharmacogenetics to improve drug therapy.

SYM 14 Allergy Diagnostics

Wednesday, 18 May 2011   09:00–11:00

EMERGING CONCEPTS IN INDIVIDUALIZED THERAPY OF ATOPC DERMATITIS

T. Bieber

Atopic dermatitis (AD) is a chronic inflammatory skin disease which can be considered as a paradigmatic genetic complex disease. AD condition emerges on the background of complex gene-gene and gene-environmental interactions which explain its large clinical spectrum. Thus due to its great clinical variability, physician taking care of AD patients have in a sense, always practiced personalized medicine. Individual allergy profiling based on specific IgE or severity adapted therapeutic regimens are in some extent considered as "pre-genomic" personalized medicine. On the hand, it is now assumed that at least 2 sets of genes should be considered: one related to structural proteins involved in the epidermal barrier function and one related to immunological mechanisms involved in increased IgE synthesis. Much progress has been made in understanding the genetic background of the disturbed epidermal barrier function highlighting mutations and variants of the gene encoding for e.g. Filaggrin. Similarly, the role of the adaptive immune system and the putative genetically determined variations have been highlighted. However, the cross-talk between both innate and adaptive immune system is still poorly understood as well as the putative impact of chronic cutaneous inflammation for the mechanisms driving of IgE sensitization. While recent progress in genetics and immunology have tremendously contributed to a better understanding of the pathophysiology and natural history of atopic dermatitis, there is some room left for other yet-to-be-explored mechanisms such as epigenetics events which may help to complete the molecular puzzle underlying atopic dermatitis. With regard to the high clinical variability of AD, recent progress in biomedical sciences could provide a solid background for a more tailored approach in the management of AD patients. Thus the genomic-based identification of putative subgroups of patients at high to develop a severe and chronic form could be detected at an earlier time and would allow the initiation of adequate preventive and therapeutic activities. Similarly, responders and non-responders to established therapy based on pharmacogenomic, transcriptomic and metabonomic approaches as well as informations about the epigenetic reprogramming of pathophysiologic relevant genes could lead to a significant improvement of our management strategies in this condition.

COMPONENT-BASED DIAGNOSTICS-RECOMBINANT ALLERGENS AS DIAGNOSTIC TOOLS

R. Valenta

Abstract not received

CARBOHYDRATE ALLERGENS

T.A.E. Platts-Mills

Abstract not received

CARBOHYDRATE ALLERGENS

T. Werfel

Abstract not received

DGKL 5 Development of a Transcripting, Proteomic and Metabolomic Database for Human Blood Cells

Wednesday, 18 May 2011   09:00–11:00

PROBLEMS AND SOLUTIONS FOR CONSOLIDATED BLOOD CELL PROTEOME ANALYSIS

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A principal goal of blood cell proteome analyses is to identify new disease specific protein biomarker. Since the dynamic range of protein concentrations in serum or plasma samples is very high exceeding the covered range of the applied proteomic technologies one step to overcome this bottleneck is to do the proteome analyses with isolated blood cell isolates.

However, the right strategy is extremely important to prevent an unwanted activation of the cells of interest like in the case of platelets. Another hurdle is the high bio-variability from individual to individual. Since pooling of such samples can't be applied as a solution for this problem, a minimum number of individual samples have to be analyzed individually to get statistically valid data, demonstrating which of the proteins of interest might be differentially expressed in disease states.

How to choose the right strategy will be presented in this lecture.

A DATA STORAGE AND RETRIEVAL SYSTEM FOR ACROSS-OMICS DATA INTEGRATION IN EUKARYOTIC SYSTEMS

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Background. Omics experiments such as proteomics and transcriptomics observe either the proteome or the transcriptome respectively. Although their experimental methodologies to generate transcript and proteome maps are completely different, their underlying biology is heavily interrelated. It is therefore important to integrate data from across-omics experiments via bioinformatics methods, and thus study the synergistic information derived from integrated experiments as a whole.

Methods. Both proteomics and transcriptomics data were collected from public databases and literature, and these were integrated onto a shared reference system. The resulting integrated dataset was then used for various data mining analyses ranging from set operations to multidimensional scaling.

Results. The results start by clarifying our bioinformatics strategy to integrate across-omics data into a shared reference system. On the one hand, integrated across-omics data enables straightforward studies, for instance to investigate the correlation between protein and transcript abundance measurements. On the other hand, integrated across-omics data enables the investigation of more complicated research questions. We will therefore present our results from integrating platelet protein sets with transcriptome analyses on megakaryocytes. Following the integration of these data, we made use of transcriptome information to divide the platelet proteins into internal and external classes, respectively differentiating between proteins that origin from the parental megakaryocytes and proteins associatied with external processes.

Conclusions. This study illustrates how to integrate across-omics exeriments onto a shared reference system, and shows the resulting synergy of performing across-omics experiments.

THE PROTEOME OF RESTING AND ACTIVATED PLATELETS

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Background. Quantitative and time-resolved phosphoproteomics is conducted to study the phosphorylation dependent activation and inhibition of human platelets in detail.
Methods. Human platelets from healthy donors are isolated using an established protocol yielding highly pure samples without pre-activation. After proteolytic digestion, changes in phosphorylation patterns are quantified by mass spectrometry either (a) sub-sequent to enrichment of phosphopeptides or (b) using established targeted single reaction monitoring (SRM) methods applied to complete digests.
Results. A reproducible enrichment of phosphopeptides from minute sample amounts has been established. So far, ~1,000 phosphopeptides have been quantified in a time-resolved manner after activation as well as inhibition. Among them, ~15% show significant up-/down regulation under the applied conditions, many of those involved in cytoskeletal (re-)organization. SRM transitions for >300 peptides have been established using synthetic analogs to allow for more accurate quantification from lyates as well as enriched samples. For proteins such as VASP and LASP, quantitative data obtained from mass spectrometry corresponds well to data obtained from alternative assays. Currently, numerous biological replicates are conducted to enable better statistics (error <15%) for sound systems biology modeling. Additionally, selected candidate proteins are subject to further biochemical characterization.
Conclusions. Quantitative phosphoproteomics can be a powerful approach to identify novel candidates for treatment as well as diagnosis of cardiovascular diseases.

TRANSCRIPTOMICS, PROTEOMICS AND LIPIDOMICS FROM MEGAKARYOPOESIS TO PLATELET SENESCENCE

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Blood platelets are anucleate cells released from bone marrow platelet podia of megakaryocytes into primary blood sinus. We showed that the ATP-binding cassette transporter ABCA1 as key regulator of reverse cholesterol transport linked to genetic HDL deficiency syndromes, promotes cargo packaging of platelet granules and platelet shedding from megakaryocyte platelet podia, without being itself transferred to shedded platelets. After maturation platelet membrane homeostasis critically depends on plasma lipoproteins, which exchange lipids with the platelet cell membrane. Compared to other circulating blood cells, platelets are char-acterized by a unique lipid class and species pattern, which reflects that of plasma. Together with the fact that transcriptomic data of megakaryocytes and the proteome of platelets and plasma show that ~ 90 % of platelet proteins are also found in plasma, indicate that platelets trap proteins and lipids in the open canalicular system, constituting up to 30% of the platelet volume, which makes it difficult to comprehensively understand the proteome and lipidome of platelets. During platelet senescence protein and lipid species composition significantly changes, with alterations of the "ceramide (Cer)-sphingosine-1-phosphate rheostat" towards Cer, and loss of proteins and lipids into the surrounding plasma through shedding of heterogenous MPs differing from platelets in their protein and lipid species pattern. In summary, only combined transcriptomic, proteomic, and lipidomic analysis of the megakaryocyte lineage and platelet senescence and plasma may help to enhance our understanding of thrombopoiesis and the platelet storage lesion of platelets in transfusion and to open new opportunities for anti-atherosclerotic drug and biomarker development.

WS 8 Molecular Diagnosis of Infection

Wednesday, 18 May 2011   09:00–11:00

MOLECULAR DIAGNOSIS OF TUBERCULOSIS

E. Domann

Abstract not received

CHALLENGES OF MOLECULAR DIAGNOSIS AND EPIDEMIOLOGY: THE LEPROSY MODEL

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Background. Leprosy is still detected even after years of global multidrug therapy programs. Lack of *in vitro* cultivation methods for the pathogen *Mycobacterium leprae*, the long incubation period and the wide range of clinical presentations [paucibacillary, PB to multibacillary, MB] in skin and peripheral nerve together with diminishing control programs pose challenges in achieving elimination. Therefore molecular methods are being investigated for early case detection, drug resistance surveillance (DRS) and tracing transmission.

Methods. Skin biopsies and slit skin smears (SSS) from newly diagnosed patients were preserved in 70% ethanol and processed for total DNA and RNA. Multiplex PCR was used to amplify panels of *M. leprae* specific DNA and RNA targets. Conventional and real-time PCR (RT-PCR) assays were tested: 23 variable number tandem repeat (VNTR) loci followed by fragment length analysis (FLA) and 4 single nucleotide polymorphisms (SNP) for strain typing; 16S rRNA for detection and quantitation of *M. leprae*; *rpoB* and *folP1* for rifampicin and dapsone DRS. Sensitivity, specificity and utility of these methods were evaluated in relation to the SSS bacteriological index [BI, scale of 0-6] determined microscopically.

Results. SSS and biopsy DNA are suitable and stable for amplification of multiple *M. leprae* targets following extraction using Qiagen DNeasy Kit. Multiplex PCR kits (Qiagen) provide robust, facile, and reproducible PCR tests. After testing >300 clinical specimens with >20 *M. leprae* primer pairs, PCR positivity was found to be correlated with BI of SSS. Specimens with BI>1 are PCR positive, while those with <1 BI are PCR negative or weakly positive in a subset of samples. Short amplicons (<200 bp) allow detection in few but not all low BI cases. With VNTR-FLA, the population structure of *M. leprae* has been discovered for Cebu, Philippines. Multicase families and community linked residents carry closely matching strains. Primary drug resistance to rifampicin was not observed in 112 *rpo*B PCR and sequence positive tests; only two patients (out of 115) carried *folP*1 mutations related to dapsone resistance. Two novel RT-PCR assays have been validated for DRS based on high resolution melt (HRM) analysis and altered Ct values for *rpo*B and *folP*1 in reference isolates. Multiplex RT-PCRs have been standardized to quantitate expression of metabolic genes and 16S rRNA.

Conclusions. A suite of molecular assays for leprosy have been produced. Multiplex RT-PCR for DNA and RNA targets are available. RT-PCR HRM is high throughput one step assay for DRS and SNP typing, FLA-VNTR assays are discriminating, universal and portable;. However, for routine new case identification, particularly PB forms and pure neuritis, these techniques are not 100% sensitive. PCR for host genes implicated in leprosy, immune assays such as serology and biomarker screens of tissue and blood metabolites are in development to complement the molecular diagnosis for early case detection and studying disease progression.

MOLECULAR DIAGNOSIS OF VIRAL INFECTIONS: ARBOVIRUSES AND RODENT-BORNE VIRUSES

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Molecular techniques appeared in the last 30 years as a great advancement in diagnostic methods in virology. Polymerase chain reaction, nucleotide sequencing and cloning/expression of viral proteins are nowadays a fundamental part on every day life in virology laboratories. These methods have enabled great progress on the knowledge about arboviruses (arthropod-borne) and rodent-borne viruses, including details of their maintenance cycles in nature and the diseases they cause. It is important to emphasize that most arboviruses and rodent-borne viruses of relevance in human public health are zoonotic RNA viruses.
Four types of dengue virus (*Flaviviridae*) cause the most important arboviral disease worldwide. Dengue virus infection can cause acute febrile illness and occasionally a severe, sometimes fatal, disease characterized by hemorrhage and plasma leakage that can lead to shock, known as dengue hemorrhagic fever/dengue shock syndrome. Other important arboviruses in South America are yellow fever virus, also a Flavivirus, that causes outbreaks of severe hepatitis and can also lead to hemmorhagic fever; and Mayaro (*Togaviridae*) and Oropouche (*Bunyaviridae*), both causing outbreaks of acute febrile illness with sporadic cases of meningitis. In this Lecture, I focus on genera-specific RT-PCR assays that we have developed for flavivirus, alphavirus and orthobunyavirus. These assays are coupled with nested-PCRs to allow for virus species identification. I will also show phylogenetic analysis done with nucleotide sequences of flavivirus that we obtained, and how we sequenced the complete genome of Oropouche virus. Recently, we have developed a simple and fast one step real-time RT-PCR for dengue and I will show results obtained with this technique in samples from different regions in Brazil as well as some findings about molecular epidemiology of dengue types 3 and 4 in Brazil.
Hantaviruses (*Bunyaviridae*) are rodent-borne agents that produce human infection usually by inhalation of aerosolized excreta of infected sylvan rodents. In the Americas, since 1993, Hantavirus Pulmonary Syndrome (HPS) has been recognized as an important public health problem. In Brazil, more than 1300 HPS cases have been reported with a 39% case fatality rate. In this Lecture, I will talk about the development of an RT-PCR method for the diagnosis of human and rodent hantavirus infections and the recent isolation of hantavirus Araraquara. Finally, I will show data on Brazilian hantaviruses and how we have produced, in eucariotic and in insect cells, a recombinant nucleocapsid protein of hantavirus Arararaquara and its use as antigen in serologic diagnostic tests. Probably, in the near future, the progress of molecular techniques will facilitate the use of microarrays for multiple arbovirus and rodent-borne simultaneous diagnosis and the development of DNA vaccines for protection against these pathogens.

SYM 15 New Developments in Haemostasiology

Wednesday, 18 May 2011   14:30–16:30

COAGULATION FACTOR XIII DEFICIENCY

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Factor XIII (FXIII) circulates in the plasma as a tetrameric zymogen (pFXIII; FXIII-A_2B_2). Its potentially active A subunit (FXIII-A) is synthesized in cells of bone marrow origin; FXIII-A is also present in platelets and monocytes/macrophages in dimeric form (FXIII-A_2). The non-catalytic B subunit (FXIII-B) is synthesized in the liver and in the plasma it is in excess of FXIII-A. pFXIII is converted into an active transglutaminase (FXIIIa) by thrombin and Ca^{2+} in the terminal phase of clotting cascade. FXIIIa catalyzes an acyl transfer reaction. In the first step a peptide-bound glutamine residue forms a thioester with the active-site cysteine and ammonia is released. Then, in the second step the glutamine residue becomes covalently linked to a primary amine through isopeptide bond. If the primary amine was provided by a peptide-bound lysine residue, the end-result is the cross-linking of peptide chains. FXIIIa stabilizes fibrin and protects it from fibrinolysis by cross-linking fibrin chains and α_2-plasmin inhibitor to fibrin.

Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiencies. Deficiency of the potentially active FXIII-A is a rare, but severe hemorrhagic diathesis. Delayed umbilical stump bleeding is characteristic and in non-supplemented patients subcutaneous, intramuscular and intracranial bleeding occurs with relatively high frequency. Impaired wound healing and spontaneous abortion in women are also features of FXIII-A deficiency. Type 1 deficiency (low activity and antigen) is more frequent than type 2 (low activity with normal or moderately decreased antigen concentration). B subunit (FXIII-B) deficiency results in milder bleeding symptoms. Various forms of severe acquired deficiencies due to auto-antibody have been described. Neutralizing antibodies usually inhibit FXIIIa, but interference with FXIII activation has also been reported. In a few cases non-neutralizing anti-FXIII-A or anti-FXIII-B antibodies that bind to pFXIII and increase its rate of elimination from the circulation were also detected. A significant portion of patients with an autoantibody against FXIII is suffering from autoimmune disease. Virus inactivated pFXIII concentrate is now available for treatment and prophylaxis.

A quantitative FXIII activity assay is to be used as first line (screening) test for the diagnosis of FXIII deficiency. The traditional qualitative clot solubility assay is now obsolete and should not be used as screening test. Quantitative FXIII assays are based on two principles: 1/ measurement of ammonia released during the transglutaminase reaction, 2/ the incorporation of labeled substrate amine into a glutamine donor substrate protein. The former methods are easy to perform, quick kinetic assays, while the latter ones are more sensitive, but time-consuming laborious methods. For classification FXIII-A_2B_2 antigen in the plasma is to be determined, and if it is decreased, measurement of individual subunits in the plasma and FXIII-A in platelet lysate is recommended. Other techniques like the examination of fibrin cross-linking by SDS PAGE, mixing studies and binding assays to detect neutralizing and non-neutralizing auto-antibodies against FXIII subunits are useful additions to establish the correct classification of the deficiencies. An algorithm and a scheme for the diagnosis and classification of FXIII deficiencies are presented in the lecture.

GENETICS AND EPIDEMIOLOGY OF THROMBOPHILIA

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Venous thrombosis is a common multicausal disease occurring due to interacting genetic, environmental and behavioral risk factors, at a rate of 1-3 per 1000 per year. Around two-thirds manifests as deep vein thrombosis of the leg, and one third as pulmonary embolism. Around five percent of venous thromboses prove fatal, with deaths predominantly among the elderly and patients with severe underlying disease, notably cancer. The recurrence rate of thrombosis is high at several percent per year. Individuals from families with inherited thrombophilia tend to develop thrombosis at a young age with frequent recurrences.

Venous thrombosis occurs when several risk factors are present simultaneously in a particular combination . This often concerns the simultaneous presence of long-term risk factors, e.g. genetic defects, and short term acquired factors. Some of the acquired risk factors are very strong, causing thrombosis in several percent of those afflicted, which implies a relative risk of 50 or higher. These are orthopaedic, neurosurgical and major abdominal interventions, major trauma with multiple fractures, central venous catheters and metastasised cancer, particularly adenocarcinomas. Moderate risk factors are antiphospholipid antibody syndrome, puerperium, prolonged bedrest and non-metastasised cancers, while pregnancy, oral contraceptive use, hormone replacement therapy, obesity and long-haul travel constitute mild risk factors, with a 2- to 5-fold increased risk..

Some factors have been known since medieval times, such as the increased risk due to immobilisation in pregnancy and after childbirth. The immobilisation in puerperium in the past has in modern times be replaced by immobilisation after surgery or with plaster casts, or by long-haul travel in airplanes, or even by traffic jams and excessive electronic gaming. Pregnancy and puerperium still cause thrombosis, as do exogenous hormones in oral contraceptives and hormonal replacement therapy.

Over the last decade many new risk factors for venous thrombosis have been identified. This has advanced our knowledge of its aetiology, because more determinants have been described, and because the underlying concepts have received a new and broader understanding.

While pedigrees with abundant thrombosis were observed in the early 1900s, the first cause of heritable thrombophilia was discovered in 1965, with the subsequent identification of deficiencies of protein C and protein S in the early 1980s. Heterozygous antithrombin deficiency and homozygous factor V Leiden are the strongest genetic risk factors, increasing the risk of thrombosis 20- to 50-fold. Heterozygous protein C and protein S deficiencies are moderate contributors to risk, with a relative risk of 10. Other genetic factors that are associated with venous thrombosis are mild and increase the risk at most 2- to 5-fold, as is the case for factor V Leiden, prothrombin 20210A and non-0 blood groups. Mildly increased risks are also present for abnormalities in the coagulation system of which the origin is unclear, such as elevated levels of procoagulant factors (fibrinogen, II, vWF, VIII, IX, X, XI) and anti-fibrinolytic factors (TAFI), and low levels of anticoagulant factors (TFPI). Finally, there are many very common and weak risk factors. These genetic factors act epistatically and jointly with environmental factors in causing thrombosis.

PLATLETS

U. Walter

Abstract not received

MONITORING OF ANTICOAGULANT THERAPY

B. Lämmle

Abstract not received

SYM 16 Contribution of Laboratory Medicine in Leukaemia

Wednesday, 18 May 2011   14:30–16:30

ACUTE MYELOID LEUKEMIA: MOLECULAR DIAGNOSTICS AND MRD

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Acute myeloid leukemia (AML) can be considered a model disease for cancer. Several molecular aberrations lead to malignant transformation of hematopoietic stem cells or progenitor cells. It is commonly believed that at least two different signaling pathways need to be mutated for a full malignant phenotype: A so called class I mutation activating the RAS-RAF-MEK-ERK-pathway, and a class II genetic lesion involving a nuclear protein, for instance a transcriptions factor / repressor (i.e. translocation t(8;21) resulting in the fusion of AML1 and ETO, or translocation t(15;17) yielding the PML-RARa leukemia fusion protein). Numerous data show that these different aberrations can result in different responses to chemotherapy, and patients are treated differently when a given molecular lesion is detected. For instance, AML patients whose leukemic blast harbour AML1-ETO and CBF-MYH11 or AML with mutations involving the NPM1-gene are considered favourable, and thus these patients are treated with conventional chemotherapy and not subjected to allogeneic stem cell transplantation in first remission. Also, there are some data that AML with oncogenic RAS mutations benefit most from high-dose cytarabine as post-induction therapy. In contrast, patients with high-risk cytogenetic lesions such as complex aberrations or monosomy 7 are considered high-risk AML and therefore, allogenetic stem cell transplantation in first remission is recommended. In between are AML with normal cytogenetics. Here, different subgroups can be found, such as the favourable AML with mutations in NPM1. In contrast, AML with an internal tandem duplication in the FLT3 gene (FLT3-ITD) is considered high-risk. Fusions genes or specific mutations (i.e. NPM - or FLT3-ITD mutations) can be detected by PCR, and in many studies detection of minimal disease have identified these patients at higher risk for relapse.

Taken together, in AML, many different molecular lesions are known, most of which can be used to detect minimal disease within complete remission. AML patients are routinely diagnosed and monitored with modern molecular means, which may be important for individual treatment planning.

IDENTIFICATION OF PROGNOSTIC SUBGROUPS IN ACUTE LEUKEMIA BY NOVEL PHENOTYPIC PATTERNS AND MDR ASSAY

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Several decades ago acute leukemias were considered a devastating disease in practically all patients. In the past years a significant improvement was observed in overall and event-free survival rates, particularly in pediatric ALL cases. This was achieved both by therapy improvement and more advanced laboratory methods for diagnosis and monitoring. An important aspect of a laboratory assay in this disorder is its usefulness as a prognostic indicator. In AML, cytogenetic anomalies are considered a major prognostic factor and their importance is also mirrored in the WHO classification of the disease, while in ALL cytogenetics is of less importance in prognostication.

We have investigated two prognostic factors measured by flow cytometry in acute leukemias, the multidrug resistance (MDR) activity and a newly described leukemia associated immunophentotype the cellular expression of coagulation factor XIII.

The MDR proteins Pgp and MRP1 were investigated by a flow cytometric activity assay in 93 untreated AML patients in 2 hematological centers. The assay is based on the measurement of calcein fluorescence in the presence and absence of verapamil an inhibitor of MDR proteins. For quantitave analysis a multidrug resistance activity (MAF) was calculated and defined in all patients upon diagnosis as well as in Pgp positive and negative cell lines. It was found that the non-responder rate in the MDR positive group was 69%, while only 28% % in the MDR negative group. On Kaplan-Meier plots the 50% survival of the MDR negative patients were 3-times higher than observed in MDR positive cases. Upon relapse the MDR activity either remained elevated in blasts or the MDR activity was induced in previously low MDR activity cases[1].

In acute leukemias the most reliable markers are often intracellular and their detection may not be straightforward in all cases[2]. Coagulation factor XIII subunit A (FXIII-A) is an intracytoplasmic marker of the platelet/megakaryocyte and monocyte lineages that was shown to be also a sensitive marker of AML[3]. More recently we identified FXIII-A as novel leukemia-associated immunophenotype in acute promyelocytic leukemia and in common ALL[4,5]. We explored the prognostic impact of this novel leukemia-associated phenotype in small cohorts of patients with ALL (n=42) and with promyelocytic leukemia all bearing the 15:17 translocation (n=13). We found that FXIII-A+ pediatric ALL cases (n=23) had a 96% overall survival (only 1 patient died), while in FXIII- cases (n=19) this value was only 58% (8 patients died). In AML M3 we had a more impressive difference between FXIII+ and FXIII- cases. The FXIII+ group (n=9) showed 100% overall survival while all the patients corresponding the FXIII- group (n=4) died within a relative short treatment period (no survivors). In summary the FXIII expression proved to be more sensitive for favourable disease outcome prediction than cytogenetic, FISH, PCR and DNA index parameters in our cALL patients. Our preliminary results suggest the possible role of FXIII expression in prognostic grouping of cALL however in vitro experiments must be performed aiming the susceptibility testing of FXIII positive and negative blasts to chemoterapeutic drugs.

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DIAGNOSIS OF AML BETWEEN MORPHOLOGY AND NEXT-GENERATION SEQUENCING

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Background. The diagnosis of acute myeloid leukemia (AML) has been changing over the last decades tremendously. Based on cytomorphology and cytochemistry and supplemented by immunophenotyping, the standard techniques since the early 70's. Cytogenetics based on chromosomal-banding analysis led to much important further information not only for diagnosis but for classification and prognostication. This was complemented by many molecular techniques in the last 15 years.

Methods. The diagnosis of AML today and for the next future is based on cytomorphology, cytochemistry, immunophenotyping, standard chromosomal-banding analysis supplemented by fluorescence in-situ hybridization (FISH) and several molecular techniques. The latter one use standard Sanger sequencing but in the near future also next-generation sequencing to approach the several genes of interest to be investigated in parallel.

Results. The WHO classification from 2008[1] is classifying AML based on its cytogenetic background and also includes several molecular markers as measured by standard PCR or by respective gene-expression of genes of interest. In addition, immunophenotyping and also several morphological aspects such as dysplasia are needed to classify patients with AML according to these newest standards. The now increasing needs for molecular markers and, in parallel, more and more sophisticated techniques including gene-expression profiling and next-generation sequencing, can be demonstrated best in the diagnostic scenario of AML today and in the future: For the diagnosis, and direct clinical decision-making, still morphology and immunophenotyping are necessary. In addition, chromosomal-banding analysis is mandatory. However, more and more molecular markers are of importance not only for classification but especially for prognostication and guidance of treatment. This can be demonstrated by genes such as *NPM1*[2] or *CEBPA*[3] or *DNMT3A*[4]. To follow-up with these breathtaking inventions, the method of next-generation sequencing will more and more replace todays molecular techniques in the investigation of AML and other hematological diseases.

Conclusions. The AML classification and prognostication clearly demonstrates that any laboratory approach ranges between standard cytomorphology using Pappenheim staining and next-generation sequencing. Only by including all these different aspects, the diagnosis, the classification and the prognostication of patients with AML is state of the art today.

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MYELOPROLIFERATIVE NEOPLASIA: DIAGNOSTIC TOOLS FOR CLASSIFICATION AND THERAPY GUIDANCE

J. Westermann

Abstract not received

WS 9 What Papers Will Be Preferred in the Future Related to Laboratory Medicine? Editors Point of View

Wednesday, 18 May 2011   14:30–16:30

ETHICAL ISSUES INVOLVING PUBLICATIONS

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There are many ethical issues that authors must consider when conducting and disseminating scientific studies. Improperly conducted or reported studies can have serious consequences to the investigator, such as loss of scientific integrity and professional reputation, discontinuation of funding for studies, inhibition of academic promotion, fines, and even criminal charges of fraud. In recent years, there have been reports where scientific data was shown to be fabricated. This may have been motivated by studies that have failed to meet goals. When discovered, it will jeopardize future funding and career promotion. Plagiarism is another unethical practice that has occurred since the very beginning of investigational science. The use of another author's written text or even an investigator's own words when uncited, is easier now because of the existence of electronic media. The motivation for cheating here is to increase the number of publications credited to an investigator, an important criteria for professional advancement.

In the medical field, there are additional ethical issues that must be considered. Prior to initiation, all studies involving patient materials or clinical information must be reviewed and approved by local ethics committee or institutional review boards for human subjects research. Manuscripts cannot be reviewed unless they acknowledge approval from these bodies. For interventional studies, the health and welfare of participants must be ensured with regular review by an independent Data Safety and Monitoring Board. This committee has the authority to inspect, audit and terminate clinical trials if patient safety is determined to be at risk. Clinical trials of diagnostic tests usually pose no health risk to participants. However, there is an increasing emphasis of the in vitro diagnostics industry to develop novel laboratory tests that can direct therapy (e.g., "companion diagnostic tests"), making trials involving safety boards relevant to clinical chemistry.

The privacy of patient health information must be maintained in research studies and in the corresponding published reports. In the US, there are new tougher laws that govern accidental or purposeful loss of patient confidentiality. Violators have been imprisoned for unauthorized acquisition or disseminating of health information. Authors and editors must be particularly careful in case report publications. Displaying radiographic or cardiographic tracings may have names or medical record numbers electronically encoded onto the image and must be de-identified. There may also be inadvertent loss of confidentiality with the reporting of an uncommon generic description of a particular subject (e.g., "the patient was a 105-year old transgender female") especially when this is also linked to the admission date and medical facility location.

The important role that the in vitro diagnostics industry plays in clinical chemistry studies present additional ethical issues regarding publications. Recently a Consortium of editors of laboratory medicine journals have simultaneously published an editorial regarding studies that involve commercial assays. For scientific integrity, authors must disclose industry study sponsors and potential conflicts of interests between sponsors and investigators. The identity of any commercial assays should also be disclosed. Complicance to these and other ethical issues will accelerate publication of scientific investigations.

POSITION IN YOUR JOURNAL ABOUT THE PEER REVIEW? IS THERE ANY ALTERNATIVE?

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Editorial peer review is an extension of basic principles of science and scholarship. It has existed for more than 200 years and has achieved near universal application for assessing research reports before publication. Despite its wide acceptance, peer review has been subjected to a variety of criticisms, and, in particular little is known about its effects on the quality and utility of published information, much less about its beneficial or adverse social, psychological, or financial effects. However, according to an Editorial published in Nature Neuroscience "so far, new approaches do not seem to be achieving better-or even much different results than does traditional peer review" (1). In fact, it was demonstrated that the term "peer review" is used to describe a number of processes, most commonly gathering opinions from external experts, but also review by in-house editors, and that it may not always be possible to make a clear distinction between peer review and technical editing. However, the main problem seems to be to properly define the aims of the peer review process and a proposal suggested to categorize its aims in two groups that are a) selecting submission for publication and rejecting those with irrelevant, trivial, weak, misleading or potentially harmful content, and b)improving the clarity, transparency, accuracy, and utility of the selected submissions (2). It should be underlined that reviewers are authors wearing a different hat and that possible conflicts cannot be solved simply changing to open peer-reviewing, or hybrid systems of peer review. The growth of electronic publishing has increased the urgency of establishing an effective and efficient system for evaluating scientific information, but also offer opportunities to explore alternatives to the current peer-review system. Authors, reviewers and editors must act to protect the quality of research and avoid the increasing risk of fraud, plagiarism, redundant publication, authorship problems and unethical research. No cookbook solution still exists but collaborative and co-operative efforts should be effective in improving the quality of scientific publications.

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EDITORS ROLE IN IMPROVING LABORATORY SCIENCE

N. Rifai

There is an increased pressure on researchers to publish their work to inform their peers of their findings, support their grant applications, and enhance their chances for promotion. As a result, the number of scientific publications is ever increasing and editors have the challenging task of identifying and publishing the most relevant reports to their readers with the highest scientific impact while staying within their page allotment. Furthermore, editors of societal journals have to reach a broad segment of the membership to assure the viability of their journals. This latter issue can possibly be accomplished by a greater emphasis on publishing educational materials and improving the readability of the journal by increasing the variety in which scientific information is delivered. Despite these challenges, some journal editors strive not only to reflect the current scientific interest in their areas but also to define and drive their fields. The latter task may be accomplished by defining the areas that the editor believes hold most promise and recruit the leading expert editors and researchers to serve on the editorial board and submit to the journal. Editors should also entice leading researchers to submit to their journals by providing a quick review of their work, a fast-track possibility, and other means to promote their findings. In this presentation, the experience with the journal Clinical Chemistry will be used to illustrate some of these points

EFCC 3 A Scientific Approach to the Post-Analytical Phase

Wednesday, 18 May 2011   14:30–16:30

BIOLOGICAL VARIATION DATA: THE NEED FOR APPRAISAL OF THE EVIDENCE BASE

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Results of laboratory investigations are utilised in diagnosis of disease, determination of prognosis, monitoring the clinical course of disease, screening, and assessment of risk. The clinical utility of the result of a measurement depends upon metrology, an understanding of its relativity of the result to a defined point of reference, and an understanding of the significance of change between serial results. Knowledge of biological variation enables laboratory specialist to apply objective criteria in these areas. Biological variation data have many established applications ranging from the setting of analytical goals for imprecision and definition quality specifications of total error, to the assessment of significance of change in serial results (reference change values (RCV)). Given the importance of these applications, and many others, there is an imperative that these fundamental data are fit for purpose. If the data are flawed in any way, or inapplicable to the population to which a measurement is being applied, then the application must be considered to be erroneous. These data, like all data, will be subject to uncertainty that will impact on their usefulness. Uncertainty arises from design of the experiments from which the data are derived, the assay characteristics and integrity of the data analysis. Furthermore, extrapolation of published data to local populations of interest requires an understanding of the factors affecting commutability of those data and a clear understanding of the characteristics of the population originally studied. Review of the literature on biological variation identifies a significant volume of work stretching back some 40 years. Much of these data have been incorporated into published reviews and web based databases that make them accessible to laboratory specialists. Indiscriminate use of biological variation data from poorly characterised, or executed studies, may be problematic. Application of these data must follow an objective and critical assessment of the data on a case by case basis. Ideally the data sets need to be accompanied by adequate descriptions of the populations studied, derived using an appropriate experimental design, accompanied by details of analytical methods used in terms of analytical performance and specificity, along side evidence of application appropriate statistical methods to the component data sets. There are parallels here to the requirements for the production and reporting of reference values as identified by the IFCC. The complexity of biological variation data should not be underestimated by users. To enable an appropriate evaluation of biological variation data a critical appraisal checklist is required to enable veracity of existing and newly published biological variation data in the growing evidence base.

REFLECTIVE TESTING – IS THERE EVIDENCE THAT IT IS WORTHWHILE AND FOR WHICH CLINICAL PROBLEMS?

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Background. "Reflective testing" is a procedure, where the laboratory specialist after inspection (reflection) of the report, adds additional tests and comments. It can be considered as an extension of the authorization process where laboratory tests are inspected before reporting. The authorizing laboratory specialist will inevitably find inconclusive results, where additional testing can be a solution. Reflective testing differs from "reflex testing", where a prefixed protocol is followed. Both reflective testing and reflex testing could be effective means to implement evidence based recommendations. In some studies the effect of reflective testing was evaluated in specific groups of patients (1,2). There is, however to date scarce scientific evidence of the effectiveness of this procedure.

Methods. In the first study, general practitioners (GPs) received 200 historical laboratory reports from their own patients to which additional tests and comments had been added. They were asked to indicate whether reflective testing had had a positive, negative or neutral influence on patient management. In a second study, a randomized trial has been started to evaluate the effects of reflective testing on patient treatment and outcome. Reports of 600 consecutive patients were subjected to reflective testing, randomized and additional tests and comments reported to half of the GPs (intervention group). In the other half, the original results were sent to the GPs without any additional information (control group). After a follow-up period of six months, differences in diagnostic and therapeutic actions of the GPs were investigated. Information was collected about following laboratory reports, following treatments, referrals or other diagnostic procedures. The patient data were evaluated to answer the following questions: 1) how useful were the additional tests and/or comments for the patient? 2) Was there an improvement in the care process of the patient related to reflective testing? 3) Was the treatment adequate in the control group, without additional information reported to the GP?

Results. Our investigation using questionnaires showed that GPs highly appreciated this service: 53% indicated a positive influence of reflective testing on treatment (e.g. earlier diagnosis, treatment or referral; adjustment of medication). Only 1% reported a negative influence. Overall, 99% of the GPs indicated that the additional information had been useful.

Preliminary results of the trial indicate that less than 4% of the reflective testing was considered not useful; 48% of the GPs (intervention group) and 15% (control group) had stated the intention of an adequate treatment action; 42% (intervention group) and 27% (control group) actually documented a adequate action.

Conclusion. Reflective testing can be seen as a new dimension in the service of the clinical chemistry laboratory to primary health care. GPs highly appreciated this service. Preliminary results show a positive effect on patient treatment.

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POST-ANALYTICAL EXTERNAL QUALITY ASSESSMENT – A WAY TO ASSESS TEST REQUESTING AND INTERPRETATION

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Background. Much attention has been paid to secure the quality of the analytical phase whereas less attention has been paid to the pre- and post-analytical phase where most errors occur. It is therefore a need to try to implement methods to improve these phases.

Methods. Post-analytical external quality assessment surveys have been performed on a European basis both on the post-analytical phase (how results are handled in the laboratory) and the post-post-analytical phase (how the results are handled by the clinicians). For the post-analytical survey a printout from a 5-part cell counter, a short case history and a questionnaire were circulated in 9 European countries. The participants (medical laboratory technologists responsible for the instrument) were asked to interprete the counts and the plot corresponding to their main haematology instrument and they answered some questions in relation to this. For the post-post survey, case histories on glucose, HbA1c, u-albumin and PT-INR have been circulated to from 4 – 10 000 general practitioners in Europe and some overseas countries. The participants dealt with questions related to the clinical interpretation of the results of the constituent in questions.

Results. The surveys showed a wide dispersion of actions among the participants with regard to how they would handle a "pathological" plot from an automated haematology analyzer, e.g. concerning follow up routines, what – and if- additional parameters were requested and what persons that handled the results. The interpretation of the analytical results by general practitioners also varied showing that that they adhered to some guidelines whereas others seemed unknown.

Conclusions. It is necessary to establish structures that can assess the post and post-post analytical phase. Quality specifications for performance should be set for these phases. EFCC and EQALM have in cooperation established a working group to address these topics.

POSTANALYTICAL ERRORS IN LIPID & LIPOPROTEIN TESTING

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In Europe, the risk for developing cardiovascular disease (CVD) is traditionally assessed using the SCORE charts estimating 10-year risk of fatal CVD based on age, gender, systolic blood pressure, smoking status and total cholesterol (TC) concentration. In high-risk patients, prevention strategies with lipid-lowering agents (mainly statins) are primarily targeted to LDL-cholesterol (LDL-C). Thus, beyond TC and LDL-C, other lipid tests do not have a major impact in medical decision making. However, although increased LDL-C is a major risk factor for CVD, many individuals with CVD have normal or low LDL-C. Treatment goals are not defined for HDL-cholesterol (HDL-C) and triglycerides (TG) in the European guidelines. Furthermore, the existence of heterogeneous LDL subclasses may contribute to inaccurate prevention strategies based on "LDL-C" alone. The occurrence of small dense LDL particles, together with low HDL-C and high TG concentrations (lipid triad), is a common feature of the metabolic syndrome (MetS), a prediabetic condition with central obesity and dyslipidemia frequently existing without elevated TC or LDL-C. Risk of many individuals including MetS patients (20-30% of Europeans) can be underestimated using European SCORE charts.

To improve the (post-)post-analytical phase of lipid testing in CVD prevention (accurate risk estimation, appropriate treatment options), current research is now focusing on novel biomarker discovery and validation for:
• Improving risk assessment in subjects with apparently low or intermediate risk SCORE;
• Estimating "residual risk" in statin-treated patients who have achieved their LDL goal.

Non-HDL-C and apolipoprotein (apo)B/A-I are useful as alternative targets to LDL-C. The calculated parameter "non-HDL-C" represents the cholesterol content in LDL, IDL, VLDL and Lp(a), and it is superior to calculated LDL-C particularly for patients with hypertriglyceridemia wherein the Friedewald equation is unreliable. However, like LDL-C, non-HDL-C might underestimate CVD risk in patients with small cholesterol-depleted LDL particles. Furthermore, increasing HDL-C values as observed with the introduction of direct homogeneous assays (positive bias) might also result in CVD risk underestimation based on calculated LDL-C and non-HDL-C.

ApoB concentration is a representative risk indicator of the total number of atherogenic lipoproteins, regardless of their size. ApoB (and apoA-I) measurements are more accurate than traditional LDL and HDL assessments, and are internationally (IFCC) standardized. On-treatment apoB adds prognostic information to LDL-C by indicating LDL particle number in patients with "normal" LDL-C.

High-sensitive C-reactive protein (hs-CRP) measurement is a candidate risk marker of subclinical inflammation in atherosclerosis. The results of the JUPITER trial with statins in subjects with normal LDL-C and elevated hs-CRP open new perspectives for CVD risk stratification using inflammation markers to identify additional patients who are at high risk, particularly those with MetS characterized by inflammatory ectopic fat.

In conclusion, apoB and hs-CRP add significant diagnostic and prognostic information to traditional lipid testing, which might reduce (post-)post-analytical errors by integrating these markers in CVD prevention strategies.

DGKL 6 Research Funding by the Foundation of Pathobiochemistry and Molecular Diagnostics of the DGKL – 10 Years on

Wednesday, 18 May 2011   14:30–16:30

CHARACTERIZATION OF THE NEUTROPHIL-TCR IMMUNE RECEPTOR IN PATIENTS WITH SEPSIS OR SIRS

Tina Fuchs, Kerstin Püllmann[1], Olivia Scharfenstein, Elfi Stobe, Romy Eichner, Alexander Beham[2], Michael Neumaier, Wolfgang E. Kaminski
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Sepsis or SIRS (Systemic Inflammatory Response Syndrome) is an acute bacterial systemic inflammation that evokes a rapid neutrophil-dominated host response. As we recently identified a variable immune receptor in neutrophils that is based on the T cell receptor (TCRαβ), we sought to determine whether the novel myeloid host defense system is implicated in these important inflammatory diseases. To address this question, a cohort of patients with sepsis or SIRS (n = 30) and an age- and gender-matched control cohort of patients without overt signs and symptoms of inflammatory disease (n = 30) were recruited at the Universitätsmedizin Mannheim.

Here, we demonstrate that neutrophils from peripheral blood of all 30 patients with sepsis or SIRS consistently express the TCRαβ. These findings rely on combined evidence from immunocytochemistry, immunoblot and TCR repertoire analyses by CDR3 spectratyping. In the age- and gender-matched control cohort, we observed an increasing loss of TCRVβ diversity with advancing age. This finding is in accordance with the reported decrease of TCR repertoires in T cells. Importantly, the patterns of the expressed TCRVβ repertoires differed significantly between the patients with sepsis/SIRS and the control group. Detailed analysis of TCRVβ gene usage identified several specific TCRVβ chains that display significant positive or negative correlation with the prevalence of sepsis/SIRS. Our results suggest that systemic bacterial inflammation is characterized by the preferential expression of defined chains of the variable neutrophil-TCR immune receptor.

ANTIPHOSPHOLIPID-ANTIBODIES INDUCE TRANSLOCATION OF TLR7 AND TLR8 TO THE ENDOSOME IN HUMAN MONOCYTES AND PLASMACYTOID DENDRITIC CELLS.

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Background. The antiphospholipid syndrome (APS) is an autoimmune disease characterized by thromboembolic events and/or abortions in the presence of antiphospholipid antibodies (aPL). The mechanisms underlying the pathogenicity of aPL are still poorly understood but recent data support the idea that the thrombotic activity in APS patients is attributed to enhanced TNFa release. To investigate this mechanism more precisely, we employed three human monoclonal aPL and APS patients' IgG fractions for stimulation of human plasmacytoid dendritic cells (pDC) and monomac1 cells

Methods. Subsequent to stimulation, expression of toll-like receptor (TLR) 7 in pDCs or TLR8 in monomac1 cells was quantified using real-time RT-PCR, westernblot technique and flow cytometry. To evaluate activation, flow cytometry assays were carried out measuring cytokine and superoxide production. Translocation of TLR7/8 was visualized by confocal microscopy.

Results. We could show that three human monoclonal aPL as well as IgG fractions from patients with the APS increase mRNA expression of the intracellular TLR7 in pDC and TLR8 in monocytes. Simultaneously they induce the translocation of TLR7 or TLR8 from the endoplasmic reticulum to the endosome. These effects depend on the uptake of aPL into the endosome, subsequent activation   of endosomal NADPH oxidase, and generation of superoxide. The exact mechanism, how aPL activate endosomal NADPH oxidase is not known yet, but is likely to be related to interaction of the antibodies with endosomal LBPA. Activation of NADPH oxidase leads to activation of NFkB and a subsequent increase in the expression of the genes for TLR7 and TLR8. While the activation of NFkB by superoxide is not surprising, the involvement of ROS in the translocation of TLRs from the ER to the endosome was unexpected. The latter process is obviously independent of NFkB activation. Translocation of TLR7/8 to the endosome dramatically sensitizes cells to natural ligands of these TLRs, i.e. single stranded RNA.

Conclusion. This observation delineates a novel signal transduction pathway in innate immunity originating from the endosome. As the overexpression of TLR7 can also be detected in pDC from patients with the APS ex vivo our results provide an explanation for proinflammatory and procoagulant effects of aPL. Since inappropriate expression of TLR7 has been implicated in the development of systemic autoimmunity, these findings may also be relevant for the understanding of autoimmunity.

ROLE OF PROTEASE SIGNALLING IN VASCULAR DISEASE

B. Isermann

Abstract not received

SPHINGOID BASES - A NEW CLASS OF BIOMARKERS?

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Sphingoid bases are the building blocks for sphingolipids and commonly formed from the precursors L-serine and palmitoyl-CoA - a reaction which is catalyzed by the serine-palmitoyltransferase (SPT). Several SPT missense mutations are associated with the inherited sensory neuropathy HSAN1 (OMIM162400). It was reported recently that SPT is not strictly depending on serine but also metabolizes alanine and glycine to a certain extent. This promiscuous activity is greatly increased in HSAN1. The conjugation of palmitoyl-CoA with alanine or glycine results in the two atypical deoxysphingoid bases (DSB) deoxy-sphinganine and deoxymethyl-sphinganine. Both metabolites lack the C1 hydroxyl group and are therefore not converted into complex sphingolipids nor degraded by the normal catabolic pathway. Consequently, these lipids are elevated in cells which express the mutant forms of SPT. Highly elevated DSB levels were found in plasma and from HSAN1 patients and in plasma and nerves of transgenic HSAN1 mice. The neurotoxic properties of DSBs were confirmed *in-vitro* and *in-vivo*. HSAN1 is clinically very similar to the diabetic sensory neuropathy (DSN) - the most common chronic complication of Diabetes mellitus (DM). Alanine and serine metabolism is functionally linked to the carbohydrate metabolism. We therefore analyzed normal and deoxy-sphingolipid levels in two independent studies to see whether sphingoid base levels are altered in DM. In both studies we found significantly elevated DSB levels in DM but no difference in normal sphingoid bases. This suggests that DSBs are relevant biomarkers in DM and DSN but might also contribute to the course and progression of DSN.

INT 1 Challenges at the Clinical Interfaces

Wednesday, 18 May 2011   14:30–16:30

INTERPRETATIVE PARADIGM 1: ENDOCRINOLOGY CASE

Freedman, D.[1]; Horvath, A.[2]; Hallworth, M.[3]; Kilpatrick E.[4]
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Interactive clinical cases. A laboratory result is only as good as the understanding of its meaning and the action that is taken upon its receipt. As more and more diagnostic data becomes available, the role of the laboratory is becoming one of a provider of KNOWLEDGE (including interpretation), rather than simply a results factory. Specialists in laboratory medicine must be able to assess the clinical importance of data produced in their laboratories, and there are always new and challenging developments and research findings that need to be incorporated into good practice. Developing and maintaining clinical interpretative skills is an essential element of laboratory work which assists clinicians and health care institutions to deliver quality care and is paramount for patient safety.

 As an educational tool, these genuine interactive clinical case presentations with anonymous electronic voting will enable participants to develop a systematic approach to patient history and data interpretation and to extend their understanding of the laboratory's role in diagnosis, treatment and management of patients. The cases presented will be on electrolytes, acid-base metabolism, endocrinology and the effects of drugs. They have been selected from routine practice and therefore contain the clinical nuances that reflect the reality of service delivery. Where applicable, references will be given to key recommendations, actions and learning points. Sessions such as these have been found to be of great value at national meetings of societies such as the ACB(UK) and AACC (USA).

References
1. Freedman DB, Hooper J, Wood PJ, Worthington DJ, Price CP. Challenges at the Clinical Interface. Washington DC: AACC Press. 2001.

INTERPRETATIVE PARADIGM 2: WATER AND ELECTROLYTES CASE

A.R. Horvath

Abstract not received

INTERPRETATIVE PARADIGM 3: TOXICOLOGY CASE

M. Hallworth

Abstract not received

INTERPRETATIVE PARADIGM 4: DIABETES CASE

E. Kilpatrick

Abstract not received

SYM 17 New Strategies in Molecular Diagnostic

Thursday, 19 May 2011   09:00–11:00

DNA MELTING ANALYSIS: FROM FUNDAMENTALS TO PRACTICAL APPLICATIONS

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Background. Separation of the two strands of DNA with heat (melting) is a fundamental property of DNA that is conveniently monitored with fluorescence after PCR amplification. Recent advances include high-resolution instruments and saturating DNA dyes that distinguish many different species without expensive fluorescent probes.
Methods. Heteroduplex scanning is easily performed by high resolution melting. A public web application (uMelt, http://www.dna. utah.edu) was developed to predict amplicon melting curves with multiple domains. Comparison of predicted vs experimental melting curves helps to develop and optimize scanning assays. Targeted towards practical PCR applications, melting curve calculations incorporate the free {Mg++], after chelation of dNTPs. In addition to scanning, genotyping can be performed on small amplicons, with unlabeled probes, or with snapback primers.
Results. Consensus is building that high resolution melting is a better mutation scanning technique than methods that require physical separation, such as dHPLC. uMelt accurately predicts the shape and position of melting curves. Most, but not all, single base variants and small insertions or deletions can also be genotyped by amplicon melting. More complex regions can be typed with unlabeled probes or snapback primers. Mutation scanning and genotyping can be performed in the same tube, eliminating most re-sequencing needs.
Conclusions. High-resolution DNA melting is homogeneous, closed-tube, rapid (1-10 min), non-destructive and does not require fluorescent probes or real-time PCR. Its simplicity suggests that high-resolution melting will become the method of choice for heteroduplex scanning and genotyping.

QUALITY ASSURANCE AND QUALITY CONTROL IN THE MOLECULAR DIAGNOSTIC LABORATORY

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Molecular diagnostics through nucleic acid amplification techniques has become one of the dominant platforms in clinical laboratory medicine. Because suitability of a laboratory technique does not necessarily prove that it will be performed correctly and provide valid results, validation and verification procedures must be introduced.
Validation focuses on the competence of the laboratory providing reliable test results and their correct interpretation. For validation work, harmonization among the framework of common standards (ISO 9000 and ISO 15189) exists. In the laboratory, a distinguished quality control system must be established. Components of validation include: (1) internal and external quality controls, (2) use of international standards and reference materials if available, (3) validation of the employee expertise, (4) calibration procedures of the instruments, (5) correlation of test results with clinical findings regarding the diagnostic sensitivity and the diagnostic specificity of the molecular test or test system.
In contrast, no harmonization exists for verification work currently; however, verification is mandatory if any new molecular test or test system for routine clinical testing is introduced. Verification helps to ensure reliable test results and contributes to a better comparability of molecular tests and test systems. Components of verification of IVD/CE-labeled and/or FDA-approved molecular tests and test systems include testing of accuracy, imprecision (between day and within-run), and analytic measuring range (linearity). If a sample matrix is intended to be introduced for which the manufacturer has not verified the assay, recovery testing should be performed additionally. Laboratory-developed and research-use-only tests or test systems and the combination of different IVD/CE-labeled tests without recommendation of the manufacturer are subject to extended verification including testing of reproducibility and specificity and determination of the limit of detection.

NEXT-GENERATION SEQUENCING IN LEUKEMIA DIAGNOSTICS

A. Kohlmann
MLL Munich Leukemia Laboratory

Next-generation sequencing (NGS) platforms have evolved to provide an accurate and comprehensive means for the detection of molecular mutations in heterogeneous tumor specimens. Here, potential applications of this novel laboratory technology will be discussed. In particular, an update will be provided on the utility of amplicon deep-sequencing assays in characterizing myeloid neoplasms where the number of molecular markers applied for disease classification, patient stratification and individualized monitoring of minimal residual disease is constantly increasing. The potential of this technology will be demonstrated by discussing data from a recent study on chronic myelomonocytic leukemia (CMML). Although many facets of this assay need to be taken into account, e.g. the preparation of sequencing libraries with molecular barcodes, specific experimental design options when considering sequencing coverage to calculate diagnostic sensitivity, or the use of suitable software and data processing solutions to obtain accurate results, amplicon deep-sequencing has already demonstrated a promising technical performance that has initiated development efforts towards a routine application of this technology in diagnostic laboratories so that an impact on clinical praxis can be achieved. Moreover, data will be presented from a collaborative study termed IRON (Interlaboratory RObustness of Next-generation sequencing), a ring trial that will help to establish consensus processes and acceptance criteria in terms of primer design, diagnostic sequencing coverage and bioinformatic data processing.

HIGH THROUGHPUT QPCR EXPRESSION PROFILING, FROM TISSUE SAMPLES TO SINGLE CELLS

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Background. Real-time quantitative PCR (qPCR) has rapidly become the preferred technique for quantitative analysis of nucleic acids. It has sensitivity to detect a single target molecule with high specificity, good accuracy and essentially unlimited dynamic range. This opens for detailed characterization of expression in biological samples and can also be used for analysis of individual cells.

Method. We have developed platform for high throughput single cell expression profiling based on streamlined quantitative real-time PCR. Dissociated cells from tissue, culture or blood are collected by fluorescence assorted cell sorting (FACS), lyzed in CelluLyser (TATAA) that releases the mRNA, while being compatible with downstream reactions, the mRNA is reverse transcribed, the cDNA is pre-amplified with PCR based technology, quantified in a BIOMARK (Fluidigm) or OpenArray (Life Technologies) high performance, high throughput platforms, and the data are analyzed using GenEx (MultiD).

Result. We show gene expression can be measured in high throughput and high multiplicity with excellent precision in single cells. Large variations in mRNA levels, consistent with log normal distribution, are observed among seemingly like cells. This seems to be a general phenomenon observed for all cells with active transcription and for all the genes studied. The variations are most likely temporal caused by transcriptional bursting. In fact, a different behavior suggests different cell types are present. Expression levels of functionally related genes often correlate on single cell level, suggesting that the genes' have common regulatory mechanism. This reflects genes' roles in expression pathways and can be used for powerful finger printing of cell subtypes. The approach is used to identify two previously unknown subtypes of astrocytes.

Conclusions. High throughput single cell expression profiling is a forthcoming technique that will lay the ground for single cell systems biology. Hitherto unprecedented information about genes interactions within cells can be extracted, which reveals intricate correlations and relations. The platform can also be usedHitherto to determine the presence and abundance of different phenotypic cells and cell subtypes in clinical samples, which can lead to greatly improved therapies, and opens for powerful individualized drug treatments in oncology that targets only the particular cancer cells present.

SYM 18 Standardization and Quality Control Issues in Emerging and Developing Countries

Thursday, 19 May 2011   09:00–11:00

ANALYTICAL QUALITY IN LATIN AMERICA AREA

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Introduction. At this conference we will address different aspects related to analytic quality in Latin America. When we talk about analytic quality we refer to different links which are related to each other and seek the same goal, to generate clinically useful results in patient health care.

Development. The links that make up the analytical quality chain are: Instrument qualification, quality requirements, methods assessment, internal quality control planning and external quality control. In the different countries that are part of the region we can find laboratories of different dimensions which handle different sample volumes. In recent years, analytical quality has improved a great deal, although we should not forget there is still a lot to do in that area. In several countries in the region, there are regulations, even though they are not too strong, and analytical quality guidelines are very elemental. Instrument qualification is carried out during the installation, although it is not usual to generate the corresponding records. Not many laboratories have quality requirements for their essays, and in other cases those are wrongly selected. Without quality requirements we can hardly talk about quality control planning. As we all know, in order to plan quality control we must consider on one hand, how much margin of error we can allow the essay to have without letting that error nullify the clinical usefulness of the generated results, and on the other hand, we must know the method performance under stable conditions. Not too long ago, methods assessment was very rare in the different countries in the region, therefore methods were controlled without actually knowing them. If we go through several laboratories we will observe that most of them will be able to identify the coefficient of variation of their tests. If at each one of these laboratories, we try to have access to systematic error estimation for each of these tests, the matter gets quite complicated. There are different models available for estimating the bias which yield different results. Luckily, the peer group comparison schemes are more and more frequent in the region. These schemes allow for an easy estimation of the bias and coefficient of variation; therefore, by selecting only the quality requirements we could have everything necessary to plan our quality control and to perform follow-up in terms of our essays analytical performance time.

Conclusions. To say that the analytical quality of our essays is ensured in the region is a mere perception. We need to prove this with objective evidence. There is still a long way to go but the situation is improving. The financial situation in Latin America is sometimes against us. Sometimes it is difficult to negotiate with management the necessary resources to do what we need. At other times, it is very difficult for management to administer resources they do not have.

QUALITY ASSURANCE PROGRAMS IN ARAB COUNTRIES

Dr. Fouad Harb
Damascus – Syria

The concept of Quality Assurance, Quality Assessment, Quality Management, and Accreditation in clinical laboratory is relatively new.
Since 1988 WHO – Regional office try to implement Quality Assessment program in public sector, cooperation with ministries of health. Many regional meeting 1988- 2000, and many recommendations, many plan pf action, training courses organized by WHO in Arab countries.
In 1994 only Syria and Tunisia implement its national program in private & public sectors, with government legislation, national committees, external quality scheme, and official audit.
Since 1998 many Arab countries implement quality assurance program at public health laboratories.
Know there is many efforts in many Arab countries to start its quality assurance program: Sudan , Jordan , Palestine , Algeria , and Morocco
There is an urgent need for government legislation for many Arab countries .
60-70 % of laboratory services in Arab countries implement by private labs, which most of them are small (50-60 %) and it's very important to protect the patient by quality assurance program at private sector.
Many private labs (Big labs) join quality program scheme outside Arab countries (BioRad- CAP- NEQAS- Randox …..)
Arab Federation of Clinical Bioloy try to raise the awareness about the importance of quality assurance program, and tried to help the Arab societies to organize its national program for their members, and to advice the government bodies about legislation and licensing.

STANDARDIZATION AND QUALITY CONTROL ISSUE IN EMERGING AND DEVELOPING COUNTRIES

E. Frank

Abstract not received

CURRENT STATUS OF QUALITY MANAGEMENT IN LABORATORY MEDICINE IN CHINA

S. Hong

Abstract not received

WS 10 Standardisation of Complex Analyte Measurements: The Clinbiotrace Project

Thursday, 19 May 2011   09:00–11:00

THE CASE OF HUMAN GROWTH HORMONE: MEASUREMENT LIMITATIONS AND DESIRABLE PERFORMANCE FOR CLINICAL USE

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Measurement of human growth hormone (GH) concentrations is essential in diagnosis of both, growth hormone deficiency and growth hormone excess (acromegaly). In clinical practice, immunoassays are routinely used to this purpose. Many different GH assays are available on the market today, but agreement between assay results is poor. Over many years variability between assay results exceeding 200% has been documented in external quality assessment schemes (EQAS) worldwide. Therefore, the actual value reported for the GH concentration in a specific patient's sample mainly depends on the assay used by the respective laboratory. The discrepancies in assay results prevent harmonization of clinical decisions in the field because implementation of guidelines on diagnosis and treatment of GH related disorders is impossible: As log as assay results are not comparable, the universal applications of cut-off levels mentioned in any guidelines are useless or even dangerous.

Among the reasons for the heterogeneity in GH assay results is the heterogeneity of the analyte itself - GH is not a single, defined chemical entity but consists of several molecular isoforms. This inherently leads to discrepancies in immunoassay results as antibodies – depending on their respective epitopes - recognize different subsets of molecular isoforms. Furthermore, the interference from matrix components (mainly GH binding protein) has an influence on assay results. Another important issue is that different reference preparations for calibration are available (a pituitary derived extract 80/505 and the recombinant preparations 88/624 and 98/574). Differences in reporting units (mass units or international units) together with the application of variable conversion factors between both units also contributed to the problem.

Several groups proposed measures to improve the comparability of assay results. Some progress has been made, as the use of international units meanwhile has been largely abandoned. However, there is still a problem with use of different calibrators, with a problematic situation for assays from specific manufacturers which are distributed with different calibrators in Europe and the US. The Growth Hormone Research Society (GRS) in collaboration with the Working Group on GH of the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) as well as the International Society for IGF Research and the Pituitary Society organized an expert workshop to define criteria, strategies and ways to implement harmonization of GH and IGF-I assays. The combination of forces between clinical chemists and endocrinologists is mandatory as quality requirements for laboratory methods only can be defined from a clinical context. As first steps for improvement the use of a single, recombinant calibrator for all assays and reporting in mass units only has been agreed. Obviously, the commutability of such a standard preparation should be demonstrated, but the available data in this respect are promising. In terms of clinical decision limits, there is agreement that assays should ideally be specific for the 22 kD GH isoform only, as no clinical condition is known where measurement of other isoforms would add clinical information. Continuous efforts from the laboratory and the clinical side are required to improve GH assay comparability.

MAKING LABORATORY MEDICINE RESULTS COMPARABLE: UNDERSTANDING THE MEASUREMENTS

H. Schimmel

Abstract not received

NOVEL MASS SPECTROMETRY APPROACHES TO DETECT STRUCTURAL VARIABILITY IN CLINICALLY USEFUL PROTEINS: IMPACT ON THE IMPLEMENTATION OF REFERENCE SYSTEMS

G. O'Connor

Abstract not received

SYM 19 Current Challenges in the Pediatric Laboratory

Thursday, 19 May 2011   09:00–11:00

THE IMMATURE PLATELET FRACTION AS A NOVEL DYNAMIC CELLULAR PARAMETER FOR PREDICTING AND MONITORING THE COURSE OF NEONATAL THROMBOCYTOPENIA

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Background. Newly released platelets, referred to as immature platelets, can be reliably quantified based on their RNA-content after addition of a fluorescent dye by fluorescence flow cytometry in an automated blood analyzer. The so-called "immature platelet fraction" (IPF) is suitable to distinguish between insufficient production in the bone marrow or an increased destruction of platelets. About 30% of the patients, admitted to neonatal intensive care, suffer from thrombocytopenia.

Methods. We determined IPF in three different clinical situations: (1) IPF for evaluating the course of thrombocytopenia in unselected neonates admitted to intensive care (n=612). (2) IPF for differential diagnosis in thrombocytopenic neonates with intrauterine growth restriction or bacterial infection (n=857). (3) IPF in neonates with culture-proven sepsis (n=28) or surgical necrotizing enterocolitis (NEC)(n=10). Full blood count and IPF were quantified with the fully automated blood analyzer XE-2100 (Sysmex, Japan).

Results. An increase of IPF anticipated the increase of the platelet count. Neonates with IPF <8% had a 4.7 fold (95% CI = 1.9-11.8) increased risk for a severe drop of the platelet count on the following day. Independently of the cause of thrombocytopenia (growth restriction or infection) IPF was lower (8.5 ± 2.7/nl) in thrombocytopenic infants compared to non-thrombocytopenic infants (9.5 ± 3.6/nl; p<0.05). In infants with sepsis or NEC, IPF decreased in parallel with decreasing platelet counts during the disease.

Conclusions. We conclude that IPF is suitable to predict the course of thrombocytopenia and to quantify platelet production rate.

1. Cremer M, Paetzold J, Schmalisch G, Hammer H, Loui A, Dame C, Weimann A. Br J Haematol. 2009;144:619-21.
2. Cremer M, Weimann A, Schmalisch G, Hammer H, Bührer C, Dame C. Thromb Haemost. 2010;103:1016-21.

LABORATORY MARKERS FOR DIFFERENTIAL DIAGNOSIS OF BACTERIAL AND VIRAL INFECTIONS IN CHILDREN WITH FEVER

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Discrimination between viral and bacterial infections in pediatric febrile patients is often a puzzle, and correct solution is a prerequisite to adequate patient care. Verification of diagnosis and substantiation of treatment rely on a clinical evaluation in conjunction with laboratory tests. Available panel of the laboratory markers that are used for screening in febrile diseases does not always give sufficiently clear results. This warrants a search for additional markers and their effective combinations. Febrile infections are accompanied by alteration in cytokine production. By means of a Bio-plex microbead suspension array system we measured serum concentrations of a wide cytokine panel and found that the patients with bacterial and viral infections, as well as healthy children, all have distinct cytokine profiles. Triggering acute inflammation in febrile infections, cytokines mediate changes of homeostasis that are reflected by modifications of the laboratory variables such as white blood count with differential, C-reactive protein, procalcitonin, etc. One of the inflammatory cytokines, IL-6, has been validated as a diagnostic marker for neonatal sepsis, and IL-6 immunoassay is now available in an automated analyzer format. The cytokine production alteration during acute inflammatory response, particularly elevation of IL-6 concentration, modifies iron metabolism stimulating synthesis of hepcidin. Being a key regulator of the iron metabolism, hepcidin diminishes the availability of iron for erythropoiesis. This functional lack of iron affects hemoglobin synthesis that is promptly reflected by reduction of the hemoglobin content in reticulocytes. A test for reticulocyte hemoglobin equivalent that is available on several hematological analyzers could be an early and easy marker of infection. Inflammation and cytokines influence also neutrophil granulocytes, inducing elevation of the neutrophil counts as well as modifying their function. The latter is mirrored by change of granularity and cellular biosynthesis/nucleic acid content in the neutrophils that could be measured by an automated hematological analyzer. It is worthwhile to speculate that a combination of several laboratory parameters will possess higher discriminative ability for bacterial and viral infection than a single marker alone. Elaboration of the infection "laboratory fingerprint" will promote development of more accurate diagnostic tools that will help physicians to make important clinical decisions.

CHILDHOOD METABOLIC SYNDROME: PATHOPHYSIOLOGY AND LABORATORY ASSESSMENT

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Over the past few decades, there has been an alarming rise in incidence of insulin resistant states such as obesity and type 2 diabetes worldwide in both adult and pediatric populations. Although not formerly considered a disease of childhood, type 2 diabetes has begun to present with increasing frequency in the pediatric population. It is feared that the disease progression begins early in life, and persistence from childhood to adulthood produces type 2 diabetes and cardiovascular disease in early adulthood. Insulin resistance (or "Prediabetes) is a central pathophysiological feature of type 2 diabetes and abdominal obesity, and is commonly associated with metabolic dyslipidemia. The current consensus is that obesity and insulin resistance may be part of a common pathologic state termed the "metabolic syndrome". The metabolic syndrome, formerly referred to as insulin resistance syndrome or syndrome X, is characterized by a constellation of pathologies that include glucose intolerance, insulin resistance, obesity, dyslipidemia, and hypertension. Insulin resistance generally develops as the first indicator of type 2 diabetes and manifests as a decreased biological response to normal levels of circulating plasma insulin. Indicators of insulin resistance include impaired glucose tolerance, hyperglycaemia, and elevated plasma insulin levels. As long as the pancreas can compensate for the decreased insulin response by increasing insulin secretion, the individual is able to control blood glucose level. Allowed to continue untreated, however, the pancreas eventually fails to produce sufficient insulin, and type 2 diabetes occurs.

Mechanisms leading to development and progression of metabolic syndrome are not fully known but are under intense investigation. Childhood exposure to high calorie foods and high fat/ high fructose diets and a sedentary lifestyle are key contributors to the surge in obesity and insulin resistance. At the cellular/molecular level, a central initiating factor appears to be defects in free fatty acid (FFA) storage and oxidation, leading to enhanced FA flux in key insulin sensitive tissues such as the liver and muscle. It is also known that part of the pathogenesis of type 2 diabetes includes a manifestation of subclinical inflammation with physiologic alterations including increased lipid peroxidation, increased TNF-alpha and reduced mitochondrial rates of beta-oxidation. Patients who are overweight possess increased adipose tissue, which is involved in the modulation and regulation of fatty acids and inflammatory markers – contributing to the augmented secretion of proinflammatory adipokines, particularly TNF-alpha and reduced secretion of anti-inflammatory cytokines such as adiponectin.

The main objectives of the present workshop are to review the pathophysiology of the metabolic syndrome, and the role of the clinical laboratory in the diagnosis and monitoring of patients with insulin resistant states. A number of new and emerging laboratory biomarkers of insulin resistance and prediabetes, and their diagnostic value will be reviewed.

DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC RELEVANCE OF ASSAY OF B-TYPE NATRIURETIC HORMONE AND RELATED PEPTIDES IN CHILDREN WITH CONGENITAL HEART DISEASES

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Background. The aim of this article is to review the diagnostic and prognostic relevance of measurement of
Brain Natriuretic Peptide (BNP) and N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) in pediatric patients with congenital cardiac diseases (CHD).
Methods. A computerized critical literature search in the National Library of Medicine using the keywords "BNP assay" and "NT-proBNP assay" + neonate/s and newborn/s was performed. We then refined the analysis to include only the studies specifically designed to evaluate the clinical usefulness of BNP and NT-proBNP assays in children with CHD.
Results. Several Authors suggested that BNP/NT-proBNP assay is clinically helpful as a diagnostic and prognostic marker for children with suspected CHD. BNP values are closely age-dependent, even in paediatric age. Unfortunately, accurate reference values of BNP and NT-proBNP assays for neonatal age only recently become available. As a result, the lack of homogenous and accurate decisional levels in the neonatal period greatly limited the clinical impact of BNP assay and also contributed to the production of conflicting results. Regardless of age, there is a great variability in BNP/NT-proBNP values among CHD characterized by different haemodynamic and clinical conditions. In particular, cardiac defects characterized by left ventricular volume and pressure overload usually show a higher
BNP response than CHD characterized by right ventricular volume or pressure overload.
Conclusions. BNP and NT-proBNP may be considered helpful markers in the integrated clinical approach for patients with CHD, especially in the neonatal age. BNP assay cannot replace cardiac imaging (including echocardiography, angiography and magnetic resonance), but provide independent, low cost and complementary information for the evaluation of cardiac function and clinical patient status.

EFCC 4 Standardization of the Pre-Analytical Phase for Markers

Thursday, 19 May 2011   09:00–11:00

EU PROJECT SPIDIA – STANDARDISATION AND IMPROVEMENT OF GENERIC PREANALYTICAL TOOLS AND PROCEDURES FOR IN-VITRO DIAGNOSTICS

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Background. Molecular *in vitro* diagnostics have allowed great progress in medicine. Further progress is expected by new technologies analyzing cellular biomolecule profiles such as nucleic acids, proteins, and metabolites. Studies have demonstrated that profiles of these molecules can change drastically during sample transport and storage thus making a reliable diagnostic or research unreliable or even impossible(1). The lack of international guidelines in sample collection, stabilisation, transport and storage as well as missing technologies enabling the improvement of pre-analytical workflows are meanwhile recognized as a significant limitation to further progress in molecular diagnostics. The European Commission has therefore launched the four-year large-scale integrating research project SPIDIA within the European Union FP7 programme in October 2008 (grant agreement no. 222916).

SPIDIA Programme. The SPIDIA research consortium is build by seven public research organizations, eight companies and an official European standards organization. The project is organised into three activities. The first activity will lead to pan-European guidelines for pre-analytical workflows of *in vitro* diagnostics. Such documents will be based on evidence gathered during ring trials in order to elucidate problematic steps in pre-analytical procedures. These procedures will have a specific focus on DNA, RNA, proteins, and metabolites isolated from tissue, tumour, whole blood, serum and plasma samples. In addition, quality assurance biomarkers will be discovered to serve as indicators for artificial, post collection changes of clinical and biological samples. The second activity is dedicated to the discovery and integration of breakthrough technologies that strengthen weak steps in pre-analytical workflows. This work includes the discovery of novel stabilisation technologies for tissues, blood, and non-invasive samples. The third activity focuses on the spreading of excellence to the clinical, research and biobanking communities.
A project introduction and an overview on its progress will be presented.

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PRE-ANALYTICAL PARAMETERS IMPACTING ON TISSUE-BASED BIOMARKERS

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Background. Molecular characterization of human cancer requires analysis of multiple parameters ranging from classical histo-pathological features to a broad spectrum of molecular biomarkers. The morphological characterization of tumors is based on the analysis of formaldehyde-fixed and paraffin-embedded (FFPE) tissues, whereas analysis of molecular biomarkers typically requires frozen tissue samples, the quality of which is affected by a variety of pre-analytical parameters. Furthermore, in the context of personalized medicine there is increasing need for combined morphological and molecular diagnostics from the same tissue sample, especially when collection of freshly frozen material is impossible for medical, ethical or logistic reasons.

Methods. To assess pre-analytical variables related to tissue quality such as ischemia, sample processing, fixation method, fixation time, embedding and storage, tissue specimens were divided in several aliquots and processed in parallel according to different protocols. FFPE samples were compared with PAXgene fixed and paraffin-embedded (PFPE) samples. The PAXgene Tissue System is a novel fixative that should simultaneously preserve morphology, antigenicity and biomolecules. Snap-frozen tissue served as a reference. Comparative studies of morphology, antigenicity and nucleic acids preservation were performed with a focus on RNA quality using electropherograms, spectroscopy, a qRT-PCR assay based on different amplicon lengths, and gene signature arrays for 92 cancer-related genes.

Results. Results demonstrated that established methods for quality control of RNA, such as the ratio of the ribosomal 28s:18s RNA or the RIN value are good parameters for frozen tissue but do not readily correlate with PCR amplification efficacy of RNA isolated from paraffin-embedded tissues. Quantitative assessment of RT-PCR efficacy for different amplicon lengths was better suited to the assessment of RNA quality isolated from paraffin-embedded tissues. FFPE tissues showed ct values varying between 4 to up to 15 cycles (depending on the amplicon length) from the corresponding ct value for snap-frozen samples, reflecting RNA fragmentation and modification. Furthermore, the 92 cancer-pathway associated gene signature arrays (TaqMan) revealed major gene-to-gene variations (up to 7 cycles) between FFPE and freshly frozen tissues that could be attributed to RNA modification during formaldehyde fixation whereas the downstream pre-analytical processes had only minor effects. In contrast to FFPE, PFPE tissues constantly demonstrated excellent RNA quality, similar to RNA from freshly frozen tissues, and at the same time resulted in good preservation of morphology and antigenicity.

Conclusion. Pre-analytical parameters have a major impact on molecular tissue diagnostics and assay results. Consequently, the quality of a tissue-based biomarker can only be defined in the context of the pre-analytical procedures. The excellent preservation of biomolecules in PFPE samples besides well-preserved morphology and antigenicity provides the opportunity to analyze a broad spectrum of biomarkers and perform classical histopathological diagnosis from the same tissue sample. This markedly facilitates biomarker research and the future application of molecular biomarkers in a routine clinical context.

PRE-ANALYTICAL ASPECTS OF THE CARMAGUE STUDY. ADHERENCE TO THE AMI AND CHF GUIDELINES

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Background. Uniform guidelines for diagnostic criteria of acute myocardial infarction (AMI) and investigation of patients with suspected acute coronary syndromes (ACS) have been established in collaboration with clinical cardiology (ACC/ESC) and laboratory organizations (IFCC/NACB) in 2007. Guidelines for the diagnosis of acute chronic heart failure have been updated by ECS in 2008. The aim of this survey was to document the present situation of the use and implementation of cardiac markers of ACS and CHF in European countries.

Methods. All member societies of European Federation of Clinical Chemistry and Laboratory Medicine were invited to participate. A link to the audit questionnaire was sent either directly to the laboratories in their countries By the National Society or via a national quality assurance provider. The questionnaire requested information on hospital type, type of clinical units, type of tests performed, clinical protocols, reference limits, decision limits for diagnosis and management of ACS and heart failure.

Results. The questionnaire was sent to 39 countries and responses were received from 303 laboratories in 28 countries. 34% (101) of the responders were from university hospitals, 25% (75) from central and 36% (107) from district hospitals. In 2006 26.7% of laboratories were in university hospitals and 69% were either central of district hospitals. In 2010 94.7% of laboratories offer troponins as preferred marker for routine diagnosis of ACS, which has not changed since 2006. 282 of 309 laboratories using troponins report the origin of their decision limits, thus 27 laboratories do not know the origin of their decision limits. Forty-one percent (116/282) of laboratories use assay imprecision (concentration at 10% CV) and 38% (n=107) reference interval (99th percentile limit of reference population) as their decision limits, similar to the results obtained in 2006. Ninety-one percent (n=145) of laboratories reporting troponins use either data supplied by manufacturer or published literature for the upper limit of reference population. Twenty-four percent (67/283) of laboratories report that they have implemented AMI guidelines and recommended decision limits for troponin. The guideline implementation has significantly improved since 2006 (9%). Seventy-six percent (n=210) of laboratories measure natriuretic peptides as markers of heart failure. In 90 laboratories the decision limits were derived from upper reference limit and in 74 (35%) laboratories they were determined by reference interval (99th percentile limit of reference population). The source of the decision limits was in most cases (119/214) the assay package information sheet, which situation has not changed since 2006.

Conclusions. In this recent study troponin was the preferred biomarker for diagnostics of AMI but was not the only marker used by all respondents. A significant proportion of laboratories continue to use other cytoplasmic biomarkers in addition to troponin. There was little independent verification of manufacturers claims for the 99th percentile and analytical imprecision. There is still a gap in incorporating guidelines and the most recent evidence into routine laboratory practice.

EVIDENCE-BASED QUALITY GUIDELINES FOR THE PRE-ANALYTICAL PHASE OF BLOOD SAMPLES

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Background. Molecular Diagnostics have allowed a great progress in medicine but their use can be limited by the lack of guidelines for collection, handling, stabilisation and storage of biosamples. The development of evidence-based quality guidelines for blood samples requires the identification of critical steps in the pre-analytical procedure that need further development.

Methods. To reach this goal, within the EU granted project SPIDIA (www.spidia.eu) it was planned the implementation of a panel of Pan- European external quality assurance schemes (EQAs) specifically designated for blood DNA, cell-free DNA and blood RNA. With the EFCC support, 322 applications have been collected from 219 laboratories of 30 different European countries. The participants to the SPIDIA EQAs received the same sample/s (whole blood, plasma) and performed the extraction procedure using their own protocol and reagents. Participants then sent back the extracted DNA/RNA to SPIDIA for further analysis, plus details about reagents and protocols used for the extraction phase.

Results. At SPIDIA facilities, the extracted samples have been investigated for quality/quantity/integrity and stability and then the participants have received a report and a qualitative "score" which includes the comparison of the performance of the single laboratory with that of the other participants. A more detailed description of the project and an overview on the SPIDIA-EQAs results will be presented. From the analysis of the three proposed SPIDIA-EQAs, it is assumed that the critical steps of the pre-analytical procedure can be identified. This evaluation will be the basis for the preparation of a draft for the guidelines for the pre-analytical phase. Based on this work, improved protocols will be sent to the participating laboratories to be used for the second run of the SPIDIA-EQAs programmes.

Conclusions. This project will be the basis for the preparation of evidence-based Guidelines for the pre-analytical phase and for the preparation of documents scientifically mature enough to serve as a basis for assessment of a CEN/TC official document and potentially serve as a basis for standardisation activities.

SYM 20 Vitamin D: Myth or Magic

Thursday, 19 May 2011   09:00–11:00

VITAMIN D REQUIREMENTS FOR HEALTH

H. Bischoff-Ferrari

Abstract not received

A NEW PARADIGM FOR VITAMIN D ACTIVITY IN BONE: AUTOCRINE: PARACRINE ACTIONS

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Vitamin D contributes to the maintenance of calcium, and phosphate homeostasis as well as exerting a wider range of biological activities including regulation of cellular differentiation and proliferation. The endocrine action of circulating 1,25 dihydroxyvitamin D (1,25D) at the intestine and kidney are the major organs controlling calcium and phosphate homeostasis. Serum 25-hydroxyvitamin D (25D) levels below 20 nmol/L decrease this endocrine activity resulting in hypocalcaemia and hypophosphataemia. 1,25D is also synthesised in a wide range of tissues including bone cells where it is under investigation as an autocrine or paracrine agent. Significant clinical data indicate that serum 25D levels higher than those required to maintain calcium homeostasis provide benefits for the skeleton. For example the elderly with mean serum 25D levels of 40 nmol/L have increased risk of hip fracture due to osteoporosis and vitamin D and calcium supplementation can reduce this risk of hip fracture.[1]. Mean serum 1,25D levels are not statistically significantly lower in these patients.[2] A major question is the cellular and molecular mechanisms by which depleted levels of vitamin D produce osteoporosis. Preclinical studies with low vitamin D diets demonstrate that serum 25D levels between 20 and 80 nmol/L result in trabecular and cortical bone loss without any evidence of osteomalacia.[3] This bone loss is largely due to increased bone resorption. No relationship is evident between bone volume and either serum 1,25D or parathyroid hormone in these animals. Another approach is the use of transgenic mouse models to increase vitamin D activity solely in mature osteoblasts and osteocytes whether through over expression of the gene for the vitamin D receptor[4] or the 25D-1 hydroxylase enzyme which is responsible for synthesis of 1,25D.[5] Both interventions significantly increase bone volumes. 25D is metabolised to 1,25D by each of the major bone cell types which is essential for its anabolic bone cell activities including promotion of bone cell maturation, mineralisation by osteoblasts and modulating bone resorption by osteoclasts. These preclinical data provide evidence for an autocrine / paracrine action of vitamin D within bone to maintain optimal bone structure at serum 25D levels above 80 nmol/L.

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BIOCHEMISTRY AND METABOLISM OF VITAMIN D

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For almost four decades, we have known that vitamin D needs to be activated by two successive hydroxylations in liver and kidney, catalyzed by the 25-hydroxylase (CYP2R1 or CYP27A1) and 1α-hydroxylase (CYP27B1) respectively, to become 1α,25-dihydroxyvitamin D, the circulating hormonal form responsible for the biological actions in calcium/phosphate homeostasis & cell differentiation/cell proliferation. Most, if not all, of these biological actions of 1α,25-dihydroxyvitamin D are achieved through a transcriptional mechanism involving a nuclear DNA-binding protein, known as the vitamin D receptor (VDR), which regulates gene expression all around the body, controlling genes from those coding for calcium transporting proteins to those involved in cell division. The recent renaissance in vitamin D metabolism has been mainly through our new understanding that the CYP27B1 enzyme, responsible for 1α-hydroxylation, is not confined to the kidney, as first thought, but is found extra-renally, in many of vitamin D- target tissues making it likely that 1α,25-dihydroxyvitamin D can be synthesized at its site of action and thus be considered an autocrine/paracrine factor. Furthermore, new knowledge around the degradatory enzyme, 24-hydroxylase (CYP24A1) also found in extra-renal tissues, argues for its important role in controlling the levels of the hormone inside target cells. The application of gene array technology has revealed that 1α,25-dihydroxyvitamin D regulates hundreds of genes in a variety of cell types making its spectrum of biological roles far wider than first thought and associated with roles in cancer prevention, in the immune system and in the health of the vascular tree in addition to its classical roles in Ca and PO_4 homeostasis. These new developments have also raised the profile of the main circulating metabolite, 25-OH-D and the genome-wide factors that regulate serum 25-OH-D which include 7-dehydrocholesterol reductase, DBP (Gc), CYP2R1 and CYP24A1. Serum 25-OH-D has become a surrogate for locally-synthesized 1α,25-dihydroxyvitamin D as a predictor of health outcomes or alternatively the diseases thought to result from vitamin D deficiency. This lecture will review our current knowledge of the structure, properties and regulation of the three major vitamin D hydroxylases, the current evidence for the existence of extra-renal 1α-hydroxylase and the involvement of 1α,25-dihydroxyvitamin D in a variety of non-classical VDR-mediated actions around the body.

25-HYDROXYVITAMIN D ASSAYS; ARE THEY FIT FOR PURPOSE?

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Background. The concentration of serum 25-hydroxyvitamin D (25-OHD) is universally used as an index of vitamin D nutrition. Measurement of serum 25-OHD presents unique analytical challenges, and the only objective means of assessing the reliability of results and the methods used is for laboratories to participate in an external quality assessment (proficiency testing) scheme. The DEQAS international quality assessment scheme for 25-OHD assays was started in 1989 after regional and international surveys revealed serious shortcomings in the reliability of 25-OHD results.

Methods. Five samples of liquid human serum of varying concentrations are distributed quarterly to participants, who are given approximately 5 weeks to submit their results. The distribution of 5 samples to a large number of participants (over 1000 in January 2011) allows an indepth investigation of methods that would be difficult or impossible in smaller schemes. The data are analysed by the method of Healey to produce an All-Laboratory Trimmed Mean (ALTM) and a robust estimator of Standard Deviation (SD). The accuracy of an individual result is defined by its % bias from the ALTM. The data are also grouped by method to produce a trimmed Method Mean (MM) and SD, and the relative accuracy of each method is estimated by its bias from the ALTM. Changes in method performance over time have been assessed by plotting % bias of the MM over consecutive distributions, and by sending out the same pool on more than one occasion (e.g., July 2008/October 2009). In July 2009, the influence of blood collection tubes on 25-OHD results was assessed by distributing serum previously stored for 24 hours in tubes containing a serum separating gel and EDTA. The extent to which differences in assay standardization contribute to inter-laboratory variability among LC-MS/MS users was investigated in January 2008. Participants were asked to calculate their results conventionally, and again with a commercial calibrator sent with the DEQAS samples.

Results. Inter-laboratory precision (CV) has improved gradually over the last 15 years, from approximately 32% (1995) to 16% (2010). In a recent distribution cycle (2009/10), most method results were within 10% of the ALTM and 15% of each other, but the long-term bias of individual methods is inconsistent. Results from two immunoassays, the IDS iYS® and the Roche 25-OHD3 were lower in serum containing EDTA (28.4 and 25.7% respectively), and a serum-separating gel caused interference in some HPLC and LC-MS/MS assays. Use of a common calibration material improved agreement among users of LC-MS/MS assays, with the inter-laboratory CV decreasing from 16.4% (in-house calibrators) to 10.4% (common calibration material).

Conclusions. The performance of 25-OHD assays has improved and, when used by experienced analysts, most analytical methods are probably suitable for routine clinical purposes. However, attempts to compare results from different data sets in epidemiological and national diet and nutrition surveys are handicapped by the lack of a common method, means of calibration, or an accepted reference method, a problem currently being addressed by the US Office of Dietary Supplements.

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IFCC-WASPaLM WS Chronic Kidney Disease – Developing a National Testing Program

Thursday, 19 May 2011   09:00–11:00

A GLOBAL CLINICAL PERSPECTIVE

F.F. Alcantara

Central Laboratory Division- Department of Clinical Pathology Hospital das Clinicas University of Sao Paulo – School of Medicine Brazil has close to 20.000 laboratories (public and private) performing assays for several analites, including common kidney function assays (creatinin, urea, albumin, both on urinary and serum/plasma samples). Two non governmental institutions, connected to scientific societies provide and evaluate most of the locally available quality controls for the private laboratories, and also for some governmental laboratories. The evolution of quality control use and practices for such assays has evolved gradually over years, leading to a steady fall in the Coefficient of Variation in the last decade, coming from creatinin %CVs above 20% to levels bellow 5%. A survey was conducted during 2010 for analysis of practices related to such assays (methods and controls used for creatinin, values reported, reporting of estimated Glomerular Filtration Rate, etc.). This survey generated data from the major laboratories in the country, reaching close to 4 millions creatinin asays per year.

WHY A NATIONAL PROGRAM FOR CKD TESTING?

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The problem of non-standardized testing is well recognized among laboratory professionals. Unfortunately this is not the case with those who fund healthcare and the professionals that work within it. There is an underlying assumption within healthcare that lab tests are standardized and that lab to lab variability in test results is minimal and may be ignored. With the expanded use of evidence based treatment guidelines that rely on laboratory test results for triage and clinical decision making, there is an increasing awareness of the clinical and economic impact that non-standardized testing can have when these guidelines are implemented at the national level. It is now recommended for adults that clinical laboratories routinely report an estimation of glomerular filtration rate (eGFR) with creatinine test results. The serum creatinine test results that are used in the calculation of eGFR should be standardized and traceable to the ID/MS reference method for this analyte. There are many problems in addition to the standardization of creatinine that may be encountered with implementation of the routine reporting of eGFR. This presentation will draw upon experiences obtained with implementation of the routine reporting of eGFR by 123 independent clinical laboratories serving a population of 4.5 million people. The benefits of a National Program for CKD Testing will be discussed within the context of the pre-analytical, analytical and post-analytical problems that were encountered.

KEY FACTORS IN SUCCESSFUL NATIONAL CKD PROGRAMS

G. Jones

Abstract not received

WS 11 Biomarkers of Acute and Neurodegenerative Brain Damages

Thursday, 19 May 2011   09:00–11:00

DIAGNOSIS VALUES OF BIOMARKERS FOR ALZHEIMER DISEASE

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Accurate diagnosis in dementia is becoming a requirement in order to optimize patient healthcare, caregivers' burden and clinical trials, especially in Alzheimer disease (AD) and other dementia such as Fronto-Temporal Dementia (FTD). Recently, the scientific community revisited AD diagnosis criteria by including neuroimaging and cerebrospinal fluid (CSF) biomarkers (total and phosphorylated Tau (p-Tau), Aβ42). Other CSF biomarkers like the soluble amyloid precursor protein (sAPP)α and β isoforms, and additional Aβ peptides (Aβ38, Aβ40) have been also identified as potentially interesting in dementia diagnosis.

We use in our routine practice with patients with cognitive impairment the three "classical" CSF biomarkers: Tau, p-Tau and Aβ42. We analyzed retrospectively the sensitivity and specificity of these biomarkers on the various subgroups of dementia. They globally were coherent with the clinical diagnosis in 80% of the cases, p-Tau being the most interesting maker that showed a high specificity for AD.

We then analysed in different clinical context, and using a multiplex approach, the values of Aβ peptides (Aβ38, Aβ40, Aβ42) and the sAPPα and β isoforms. We first confirmed a strong correlation between sAPPα and β values and we observed a correlation between sAPPs and Aβ peptides, as well as between Aβ peptides themselves. We then investigated the diagnosis interest of these markers especially for the diagnosis of FTD which often present misleading clinical presentations.

We observed that Tau concentrations were increased in FTD, but less than in AD. We also observed in FTD a significant decrease of sAPPβ, Aβ38 and Aβ40. Aβ38 and the Aβ38/Aβ42 ratio were in particular interesting for FTD diagnosis. We then combined p-Tau and Aβ38 values in a simple decision tree model which led for FTD detection to a high sensitivity of 91% and a specificity at 78%.

All these results enlighten the complex relationships between these molecular markers in both physiological and pathological situations. The data thus generated are important for the further use of these analytes for AD diagnosis, as well as, to validate cell biological hypotheses of APP processing and Aβ fragment production.

CSF biomarkers are now currently used as an aid in diagnosis of Alzheimer Disease (AD) in atypical clinical phenotype, for stratification of patients in clinical trials and for predicting prodromal AD in MCI subjects using Tau, pTau181 proteins and Aβ1-42 peptide. Due to important between-center variability, there are no consensus medical cutoff values for a CSF AD signature. Between-centre variability can be explained by analytical and pre analytical factors.

Based on literature analysis, most of laboratories are measuring these biomarkers using assays provided by a main manufacturer. Even if we cannot exclude heterogeneity introduced by variability in the application of the commercial instructions (where for example, the user is free to use a washer …), impact of using a multiplex approach against using three conventional ELISA could be a source of heterogeneity of cut-offs since lower values were measured in the multiplex approach.

NO FUTURE FOR CSF ALZHEIMER DISEASE BIOMARKERS WITHOUT STANDARDISATION OF PREANALYTICAL STEPS

A. Perret-Liaudet

Before the assay, guidelines covering all the different steps since lumbar puncture modalities to the analytical ELISA, are strictly needed to manage the major critical points impacting on the clinical cut-off.

CSF sampling is a major critical step impacting the biomarker titration. Due to the hydrophobic character of amyloid peptides, choosing a sample tube avoiding non specific adsorption is clearly needed. Traditionally, polypropylene (PP) tubes are systematically recommended instead of polystyrene or glass, well know able to adsorb these molecules.

But, as generic PP denomination hides a high heterogeneity of plastic polymer composition, we analyzed the influence of 25 PP tubes on measured concentrations of Aβ1-42, Tau and pTau181 concentrations, using 6 newly collected CSF samples and 3 supernatants of centrifuged CSF stored frozen at -80°C. Our experiments showed a highly significant effect of tube type on CSF measured concentrations, differences among tube types ranged up to 25 % for Tau, pTau181 and up to 65 % for Aβ1-42. Furthermore, the precision of measurements differed greatly among tubes. We statistically found that loss of amyloid peptides was increased when CSF presented a low total amount of proteins (< 0.30 g/L). The effect of tube type can be attributed to non-specific adsorption of biomarkers onto the tube surface occurring within 15 minutes of contact.

Finally, these 3 biomarkers showed different adsorption ability and may cause difficulty to choose a unique tube with low adsorption for all biomarkers. Composition analysis of PP tubes revealed that only 3 tubes were pure PP, all others presenting copolymers with at least polyethylene. Surprisingly, pure PP tubes did not give the best results. This first comparative analysis of «PP» tubes strongly suggests the importance of tube types used in the observed between-center effects. We can assume that the" tube effect" is a major critical point to resolve urgently. Optimization of tube quality by surface pre-treatment is a hypothesis able to lead standardization eventually leading to consensus medical decision points used in clinical routine or drug trials. Without this standardization, clinicians could fall in the suspicion of the real value oft these biomarkers.

CEREBROSPINAL FLUID BIOMARKERS FOR THE DIAGNOSIS / PROGNOSIS OF NEUROLOGICAL DISEASE

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In the field of neurological diseases, the analysis of cerebrospinal fluid (CSF) appears as a central tool very helpful for etiological diagnosis.

The main progress in the recent years has consisted by the inclusion of neuroimaging and three CSF biomarkers in the new criteria of Alzheimer disease (AD): the total Tau protein (tTau), its phosphorylated form (pTau) and the amyloid peptide Ab 1-42 (Ab1-42). This panel of CSF biomarkers is now recognised as a major component of AD diagnostic due to its contribution in the etiological diagnosis: an increase of tTau and pTau associated with a decrease of Ab1-42 are related to the elementary lesions of AD, respectively neuronal damage, neurofibrillary tangles and amyloid plaques. CSF tTau, pTau and Ab1-42 determination permit also the diagnosis of atypical focal presentations of AD not involving at the beginning prominent amnesia and so misleading clinicians. This corresponds to atypical AD cases with frontal presentation, progressive aphasia or visual disturbance. We recently reported that using CSF AD biomarkers, posterior cortical atrophy (Benson syndrome) can be related in a high percentage to AD typical lesions. Moreover, the physiopathological modifications highlighted by the alteration of the CSF biomarkers are detectable very early in the time course of clinical symptoms, allowing the diagnosis of AD since its prodromal stage.

Unfortunately, it does not exist yet equivalent etiological biomarkers useable for routine diagnosis for the others neurodegenerative disease clinically overlapping with AD, like Lewy Body Dementia (LBD), Fronto-Temporal Dementia (FTD) or prion diseases (PD). For the latter, lots of tentative to detect the pathological form of the prion protein, the brain hallmarks of the disease, in CSF have failed. The protein 14.3.3, witness of neuronal damage, remains the best supportive diagnostic tool in the appropriate clinical context. For LBD and dementia linked to Parkinson Disease, CSF determination of alpha synuclein seems to be a good candidate but lots of preanalytical difficulties avoid at this time its common use. For FTD, no satisfactory CSF specific marker emerges yet. In amyotrophic lateral sclerosis (ALS), linked with FTD in a significant percentage of cases, quantification of TDP-43 protein in CSF recently appears to be an early stage biomarker candidate.

Finally, for acute neurological diseases including stroke and subarachnoid hemorrhage (SAH), if promising CSF tools are still in validation for diagnosis of cerebral vasospasm, only pigments detection in CSF is currently in use for diagnostic of cerebral hemorrhage. Determining biomarkers of glucose metabolism (lactate/pyruvate ratio, glucose) and excitatory neurotransmitter (Glutamate) in cerebral extra cellular fluid is useful at bedside in the multimodal monitoring of SAH patients and Traumatic Brain Injury to prevent ischemic complications.

SYM 21 New Technologies in Clinical Application

Thursday, 19 May 2011   14:30–16:30

NMR-SPECTROSCOPY IN THE DIAGNOSIS OF METABOLIC DISEASES

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Background. Metabolic diseases are due to specific perturbations of metabolism and often lead to abnormally high and/or abnormally low concentration of metabolites in body fluids. With conventional laboratory screening techniques for such inborn errors of metabolism groups of metabolites are measured (amino acids, sugars, organic acids etc). Proton NMR spectroscopy can detect protons and thus will be able to provide a holistic view on metabolism.
Methods. Proton NMR spectroscopy using 500 and 600 MHZ spectrometers was used to analyze body fluids of patients suspected to suffer from inborn errors of metabolism.
Results. Quantitative analysis of a broad range of metabolites is possible in complex matrices like urine, serum or cerebrospinal fluid. Sample pretreatment is minimal and derivatisation or extraction procedures are not required. The sensitivity of the technique is in the low micromolar range. A standard 1-dimensional proton spectrum takes 30 minutes measurement time. Relevant databases are available for metabolite identification. Endogenous metabolites but also metabolites deriving from food or medication will show up in the spectra. NMR spectra also contain structural information on each molecule in the sample. This allows identification of metabolites that normally do not occur in body fluids which often is the cases in inborn errors of metabolism. The NMR technique has enabled us to define 8 novel inborn errors of metabolism. The "Handbook of ^{1}H NMR spectroscopy in inborn errors of metabolism" from our group gives the typical NMR characteristics for 82 different inborn errors of metabolism. The book is freely available on request via the email address in this abstract. Also the technique is suited for metabolomics studies.
Conclusions. NMR spectroscopy provides an overall view on metabolism and may be used to simultaneously assess a broad array of metabolites in complex matrices like body fluids.

STRATEGIES FOR PROTEIN QUANTIFICATION BY INTEGRATED ELEMENTAL AND MOLECULAR MASS SPECTROMETRY

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Background. Not only the kinds but also the amounts of proteins expressed under certain physiological conditions in a cell or a tissue are necessary to elucidate and understand life process and mechanism, especially for a biomarker, the information of its quantity is crucial for early diagnosis of a disease.

Methods. Element-labeling strategies together with stable isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS) and ESI/MALDI-MS were developed for protein quantification. Several examples demonstrated the feasibility and effectiveness of the developed methods.

Results. 1) Selenoproteins such as GPX and SelP in human plasma have been determined using HPLC/ID-ICPMS through the determination of selenium (a natural element-tag). Detection limits (DLs, 3s) for GPX and SelP reached to 0.59 and 1.7 pmol/mL.[1] 2) A dynamic labeling strategy of CH_3Hg^+ releasing from a synthesized methylmercurithiosalicylic acid to some model peptides and proteins was developed, demonstrating that the strong and specific interaction between CH_3Hg^+ and the sulfhydryls in the protein benefit the quantitative labeling of the protein and thus a subsequent quantification of the protein using ID-ICPMS. DLs (3s) for GSH, BLG and OVA reached 45.4, 45.4 and 15.8 pmol/L, respectively.[2-4] 3) A bifunctional molecule 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-acetic acid-10-maleimidoethylacetamide (MMA-DOTA) were used to label a protein via the conjugation of MMA to the sulfhydryls in the protein and loading lanthanide (Ln) into DOTA, achieving sensitive protein quantification. DLs (3s) for lysozyme, insulin and ribonuclease A reached down to 0.819, 1.638 and 0.819 fmol.[5] 4) Multiplex protease assay was performed by novel synthesized Ln-coded peptide NPs probes using ICPMS.

Conclusions. Element-labeling strategies demonstrated the ability of ICPMS for high sensitive protein quantification, and will find its applications to clinical practice, especially for early diagnosis of a disease in the near future.

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SCREENING, IDENTIFYING AND QUANTIFYING SMALL MOLECULES BY HYPHENATED MASS SPECTROMETRY IN TOXICOLOGY & DRUG MONITORING – AN UPDATE

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Background. Reliable analytical data are important prerequisites for competent assessments in clinical and forensic toxicology as well as clinical pharmacology. The analytical strategy may include screening, confirmation and identification (in toxicology) followed by quantification of relevant compounds and pharmacokinetics-based interpretation of the results.
Methods. Mass spectrometry coupled to gas chromatography (GC-MS) or liquid chromatography (LC-MS) is the gold standard in clinical and forensic toxicology because of its universality, reliability, high sensitivity and specificity. For the same reasons, LC-MS has also become the gold standard in drug monitoring.
Results. GC-MS and more and more LC-MS are used for target and comprehensive screening, library-assisted identification, and validated quantification of drugs, poisons and their metabolites in blood, urine or alternative matrices. Concepts and procedures using GC-MS or LC-MS techniques in the areas of toxicology and drug monitoring with special focus on multi-analyte procedures will be presented and discussed (1-5). The presentation will close with a short discussion of the future position of GC-MS and LC-MS in these fields.
Conclusions. The reliability of GC-MS and LC-MS helps to ensure the quality of analytical data needed for correct interpretation of analytical findings thus helping to avoid wrong treatment of the patient or analytical data being contested in court.

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RECENT DEVELOPMENTS IN THE BIOLOGY AND APPLICATIONS OF PLASMA NUCLEIC ACIDS

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Background. Cell-free nucleic acids have been found in the plasma of human subjects. This talk illustrates the diagnostic applications of this phenomenon using fetal DNA that is present in the plasma of pregnant women as an example. The discovery of circulating cell-free fetal DNA has opened up new possibilities for noninvasive prenatal diagnosis.
Methods. The development of methods that would allow the analysis of single plasma DNA molecules, especially massively parallel sequencing, has opened up exciting diagnostic possibilities and has allowed us to study these molecules with unprecedented detail.
Results. The use of massively parallel sequencing of maternal plasma DNA has allowed us to detect fetal trisomy 21 with high sensitivity and specificity. This technology has also allowed us to construct a genome-wide genetic map of an unborn fetus from maternal plasma. This latter development has enabled us to perform the noninvasive prenatal diagnosis of monogenic disorders, such as beta-thalassemia. The use of paired-end sequencing of maternal plasma DNA has also allowed us to study the size profile of circulating DNA molecules with very high resolution.
Conclusions. Analysis of maternal plasma nucleic acids will likely play an increasingly important role in prenatal diagnosis and monitoring.

SYM 22 Intertwining Emergency Medicine and Laboratory Medicine

Thursday, 19 May 2011   14:30–16:30

MEDICAL EMERGENCIES: WHAT IS THE LABORATORY'S ROLE?

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Medical emergencies arise in many different situations – in a hospital when a patient's status deteriorates, in Emergency Departments when patients present themselves with a medical problem or when people injured in accidents or poisoned in some manner are delivered to an Emergency Department. Unique situations occur as a result of war or natural disasters. This talk will focus on emergency issues arising in patient-care units and in the Emergency Room, although drug-related medical emergencies will not be discussed. The most common emergency situations to be discussed are common to hospitals throughout the world and require the same tests to be available. The most common causes of visits to the Emergency Room are primarily medical issues and include chest pain and related symptoms, abdominal, back or other pains, headaches, and respiratory problems. Dehydration is also a common problem. The most common causes of surgical visits to the Emergency Room include falls, motor vehicle accidents and unintentional injuries.

Laboratory support for management of emergencies needs to be developed to ensure that appropriate tests are available to support clinical needs for the most common cause of emergencies. Necessary requirements for tests are rapid reporting of results and precise analyses since test results performed in emergency situations frequently are used for clinical decision-making and errors are particularly dangerous. In the Emergency Room laboratory test results are commonly used by physicians to determine whether a patient is admitted to hospital, held for observation, or discharged home. The most commonly ordered laboratory tests include a complete blood count, electrolytes and measures of renal function, blood glucose, measures of cardiac damage, liver function tests, blood cultures, prothrombin times and INR and arterial blood gases. Urinalyses and pregnancy tests are frequently performed in Emergency Rooms. The types of tests needed are independent of the age of the typical patient and must be available 24 hours per day every day of the week. Within hospital emergencies require the same tests as those arising in the Emergency Department, but are most often performed in a central clinical laboratory or one dedicated to the performance of Emergency or Stat tests.

Special precautions must be taken in the hospital Emergency Room to guard against patient misidentifications and hemolysis of blood specimens since Emergency Departments are usually associated with a high level of staff activity with multitasking and distractions common, and the absence of skilled phlebotomists. Since patients must be triaged rapidly the reporting of results to the most appropriate caregivers complicates laboratory reporting procedures. The merits of point-of-care testing versus clinical laboratory testing will be discussed. Even though a laboratory may not perform tests at the point-of-care it must assume responsibility for the quality of testing performed in the Emergency Room.

WHAT SHOULD THE CLINICAL LABORATORY AND THE TOXICOLOGIST-PHARMACOLOGIST OFFER THE POISONED PATIENT?

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In the past clinical toxicology has primarily been related to suicidal attempts. Nowadays, drugs and toxic substances are less available and people are inclined more and more to attempt mechanically suicide instead of chemically. On the other hand unknown recreational drugs or unknown drugs from Internet are taken for excitement and self-care, or given to victims for drug facilitated crimes. Some patients are using poisons for attracting attention, like syndrome of Munchausen or Munchausen by proxy. An increasing number of severe overdose are caused by iatrogenic poisonings. Patients are becoming more vulnerable by age (neonates, seniors), transplantation, severe illness (HIV, cancer, diabetes), a combination of diseases and pharmacotherapies, congenital or acquired organ failure, or fast or slow liver enzymes (CYP 450 status). The number patients administered to the emergency clinic with a possible iatrogenic poisoning is dramatically increased. However, the cause of these severe clinical situations is often not recognised as such.

It is obvious that the toxicologist-pharmacologist has to recognise the different possible causes of the clinical picture of the patient. Knowing only the conventional suicidally used substances and the classic drugs of abuse is not sufficient any more. It is clear that therapeutic drug monitoring and clinical toxicology are merging. The clinical toxicologist-pharmacologist have to take this in mind by choosing the right qualitative and quantitative assay. Convulsion can be caused by many drugs, by under or overdose of an antiepileptic, by drug interaction, wrong route of administration or patient noncompliance.

The modern liquid chromatography with triple quad mass selective detection (LC-MS/MS) is offering the clinical laboratory, and thus indirectly the patient, a fantastic tool to exclude or include a drug and to clarify the poisoning within a short time. However, analytical tools are not always sufficient, then a creative and experienced clinical toxicologist- pharmacologist is required to solve the sometimes remarkable case.

In this lecture some interesting and instructive cases will be presented and discussed.

THE CLINICAL LABORATORY AND ACTS OF CHEMICAL OR BIOLOGICAL TERRORISM…CAN WE BE PREPARED?"

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Background. The complexity of preparing for and dealing with weapon of mass destruction events puts most clinical chemists and health care providers into a state of denial. The range of possible weapons is practically infinite and the capability to predict the magnitude of the event or have foreknowledge to prepare is very limited at the level of the individual hospital.   For most biological weapons, and many chemical weapons, the local hospital may be the first location that the detection of an event will occur. It is critical that the clinical laboratory be prepared and ready to assist in dealing with such catastrophic events. While training for first-responders, hospital emergency rooms and infectious disease specialists has created a substantial knowledge base within most communities, the clinical laboratory has largely ignored the issues of preparing for weapons of mass destruction.   If samples contaminated with chemical agent make their way into the fluidics of laboratory equipment, what is the risk to testing personnel? If biological agent is aerosolized in a centrifuge during sample prep, how does the laboratory protect personnel and decontaminate equipment? The questions a clinical laboratory must ask are numerous, but manageable.
Discussion. Comprehensive planning based on risk assessment and management practices are crucial to addressing the threats associated with weapons of mass destruction. The individual hospital must be integrated into a network of resources that scale from the local to regional to national governmental levels. These networks must work together to assess the risks that would be present at each level and provide education, training and resources to those individuals potentially affected by those risks. Clinical Chemists are critical players in the planning process and must be proactive, insisting on clinical laboratory involvement in preparation planning. Clinical chemists can provide expertise to properly assess methods used to detect chemical and biological weapons. Knowledge of the proper chemical handling provides wisdom to an often exaggerated risk and subsequent response plan(s). The laboratory must also be prepared to provide information for appropriate testing as an event progresses. The large number of patients and samples may quickly overwhelm a facility, but once an agent has been identified the need for testing needs to be addressed as symptomology may dictate clinical intervention and reserving laboratory resources for the truly critical testing needs will be important to proper utilization of resources. Exercises are an important tool in the process to evaluate facilities capabilities to respond and recover from a WMD event. Well planned exercises provide personnel hands on experience that improve ability to respond in real events and helps identify potential weaknesses that can be addressed before actual needs arise.
Conclusion. The entire clinical laboratory, including clinical chemists must be involved and prepared for the events associated with a weapon of mass destruction attack. Planning, practice and review at the local, regional and national level is necessary to assure the ability to respond to and recover from a terrorist attack that utilizes chemical, biological or nuclear agents.

LABORATORY RESOURCES NEEDED TO ADDRESS UNANTICIPATED DISASTERS

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For Médecins Sans Frontières [MSF], an organization that has specialized in responding to emergencies as well as working in fragile and unpredictable contexts, no two years are the same. MSF is active in >65 countries: examples of different emergencies faced in the last 2 years are:

- Palestinian Territories: the military offensives in the Palestinian Territories left 1,300 people dead, 5,300 injured and an entire population dependent on medical aid and relief.
- Democratic Republic of the Congo: ongoing conflict continues to cause huge critical needs, prompting one of MSF's largest interventions year after year. Again, we try to carry out a full range of medical activities there in the midst of what has become a chronic health emergency.
- Pakistan: In Mardan, Pakistan, where around one million displaced people settled, having fled fighting in the Swat district, our teams supported referral hospitals, health centers and mobile clinics.
- Sri Lanka: The conflict came to a climax, leaving many civilians in a vulnerable state as they tried to reach safe zones, but they all too often found themselves trapped by the violence. Access to medical facilities and aid was extremely difficult. More often than not, by the time they finally reached those who could help them, their health needs were critical.
- Haiti: largest emergency operation in MSF history following the devastating earthquake that ruined large parts of Haiti and claimed many thousands of lives. Within the first four months MSF teams assisted 173,000 patients and performed more than 11,000 surgeries.
- Burkina Faso: 150,000 people were made homeless by floods in the Burkina Faso capital of Ouagadougou, in September 2009, when the amount of rain that usually falls in a year fell in one single day.
- Bangladesh: assistance to 75,000 people hit by Cyclone Aila.
- HIV/AIDS globally: In 2009 over 190,000 people were being treated by MSF for HIV, and some 160,000 were on antiretroviral treatment.
- TB: In 2009, MSF treated over 21,000 TB patients.
- Neglected diseases: sleeping sickness, Kala Azar and Chagas leave more than 500 million people at risk from infection.

As the nature of disasters with its emerging medical needs differ drastically, the laboratory response has to as well. MSF opted for pre-packed laboratory modules which can be flown out within hours to unanticipated disaster zone.

Pre-packed packages are available for meningitis, malaria, microscopy, blood transfusion, cholera etc. Depending on the nature of the disaster a module or combination of modules can be chosen.

WS 12 Joint Session with IATDMCT Trends in Therapeutic Drug Monitoring: Personalized Immunosuppression

Thursday, 19 May 2011   14:30–16:30

TDM OF IMMUNOSUPPRESSANTS: CURRENT PRACTICE AND GUIDELINES

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Before 1995 the number of immunosuppressive drugs available for maintenance treatment of solid organ transplant recipients was limited to cyclosporine, prednisone and azathioprine. Since then several other immunosuppressive drugs have entered the field, and there is now something to choose. In 2009 the Clinical Practice Guidelines for the care of kidney transplant recipients were published [1]. Although these guidelines do not take into account the needs for individual patients, they do give guidance to clinicians in deciding what combinations of immunosuppressive drugs work best in the majority of patients. An important recommendation in these guidelines is to use a combination of a calcineurin inhibitor and an antiproliferative agent, with or without corticosteroids as maintenance therapy. Induction therapy is recommended before, or at the time of transplantation.

In the quest of the best immunosuppressive regime many randomized trials with a follow-up time of one to three years have been performed. However, the long term effects of these regimens have to be studied more extensively to be able to decide on the optimal maintenance treatment. Although it is understandable that within one transplant center a uniform immunosuppressive regimen is used for most of the patients, there should always be room for adjustments in individual patients depending on drug toxicity and side effects, and considering co-morbidity: a patient (and kidney) tailored approach. Adjusting the immunosuppressive regimen according to risk stratification is often limited to changing induction treatment from anti-interleukin 2 monoclonals to antithymocyte polyclonals in high risk patients, and to aiming for higher target concentrations for the CNIs. There clearly is a need for randomized trials that show the benefits of certain regimens in low, medium or high risk patient populations.

Therapeutic drug monitoring offers an easy way of achieving personalized immunosuppression, the topic of this workshop. Biomarkers and pharmacogenetics (discussed by the two other speakers) offer other potential routes to tailor the amount of immunosuppression to the individual patient. In my presentation will focus on the current clinical practice regarding immunosuppressive therapy, the recommendations given in the Clinical Practice Guidelines (mentioned above), and to methods to further achieve personalized immunosuppression.

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USE OF ENDOGENOUS BIOMARKERS TO ACHIEVE PERSONALISED IMMUNOSUPPRESSION IN TRANSPLANT RECIPIENTS

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Background. Pharmacokinetic monitoring of immunosuppressive drugs does not entirely predict pharmacological effects on immune cells and, ultimately, clinical outcome. Appropriate peripheral blood biomarkers could be useful to individually tailor immunosuppression. The development of "tolerance permissive" immunosuppressive regimens would be desirable.

Methods. Calcineurin activity has been proposed to assess the efficacy of different calcineurin inhibitor-based regimens. The assay is, however, technically demanding and its usefulness has been questioned. IMPDH activity in peripheral blood mononuclear cells or $CD4^+$ cells has been proposed to assess mycophenolic acid (MPA) pharmacodynamics. Patients receiving a dose reduction while having a high IMPDH activity showed the highest incidence of acute rejection. Another approach to assess the pharmacodynamic effects of calcineurin inhibitors has been to study cytokine production by T cells (IL-2, IFN-g, TNF-α). An optimal pharmacodynamic target range, however, is not known. It could be demonstrated that the proportion of IL-2-producing $CD8^+$ T cells after ex-vivo stimulation in whole blood over 4h was pre-operatively elevated in liver recipients with acute rejection. This biomarker shows potential for identifying patients, who would require more potent immunosuppression. In a further approach mitogen-stimulated lymphocyte proliferation was measured through flow cytometric determination of the expression of proliferating cell nuclear antigen in the S/G2-M phase and T-lymphocyte activation by determination of the expression of CD25, CD71 and CD134. In these assays blood cultures have to be incubated for three days rendering such tests less useful for routine monitoring. A more practical immune cell function assay (Cylex® ImmuKnow®) is commercially available in which the increase in intracellular adenosine-triphosphate (ATP) after T-lymphocyte activation by mitogenic stimulation is measured, requiring only an overnight incubation of the patient's blood sample.

Results. From a meta-analysis a target immunological response zone was derived for assessing relative risks of infection and rejection. Recent investigations have shown that ATP production of $CD4^+$ T cells was significantly decreased in liver or lung transplant recipients with infection. A strong immune response was observed in the majority of patients with allograft rejection following heart, liver, or renal transplantation. In one study, however, acute rejections in liver transplant recipients were not associated with an increased immune response. Individual baselines, however, were not established in this investigation. The usefulness of sequential determinations with the Cylex® ImmuKnow® test was demonstrated in a retrospective study for immunosuppression monitoring during immunosuppressant weaning in patients after small bowel transplantation. According to our experience the Cylex® assay may also be useful to detect non-compliance and irregular drug intake. Biomarkers such as $CD4^+CD25^{high}FOXP3^+$ regulatory T cells could be helpful to identify tolerant patients, particularly after liver transplantation. For monitoring Tregs in human peripheral blood recently a novel method can be used, which is based on Treg-specific DNA demethylation within the *FOXP3* locus. A tolerance-specific signature of 49 genes has recently been described in kidney transplant recipients.

Conclusions. Such approaches may be helpful to identify stable patients under conventional immunosuppression, who could qualify for controlled reduction in immunosuppression. Future prospective outcome studies are necessary to assess the potential of the discussed biomarkers to complement immunosuppressive drug level monitoring.

IMPACT OF PHARMACOGENETICS TO OPTIMIZE IMMUNOSUPPRESSIVE THERAPY

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Background. Most of the immunosuppressive drugs in current use have a narrow therapeutic index and wide variation between individuals in the blood concentration achieved by a given dose. Therapeutic drug monitoring is used routinely for a number of drugs but has limitations where either early achievement of therapeutic blood concentrations is important or where the association between pharmacokinetics and pharmacodynamics is imperfect. Genetic predictors of pharmacokinetics or pharmacodynamics have the potential to allow the development of pharmacogenetic dosing strategies to individualise treatment.

Azathioprine. The purine antagonist azathioprine is used as an antiproliferative agent in solid organ transplantation and the treatment of a number of autoimmune diseases. Approximately 1:300 individuals is homozygous for mutant alleles in the gene encoding thiopurine-S-methyltransferase (TPMT) the enzyme that metabolises azathioprine prior to elimination. These individuals are susceptible to severe myelotoxicity. Determination of TPMT phenotype or genotype has become standard practice prior to treating with azathioprine in a number of disease areas but has not been widely adopted by the transplant community, possibly due to lack of necessity with frequent monitoring of the full blood count on starting treatment.

Calcineurin inhibitors. Tacrolimus and ciclosporin are the main-stay of most immunosuppressive regimens for solid organ transplantation. Failure to achieve minimum target blood concentrations within 2-3 days after transplantation results in an increased incidence of acute rejection and over-exposure results in susceptibility to infection and a number of drug-specific toxicities including nephrotoxicity and diabetes mellitus. Both drugs are metabolised by cytochrome P4503A4 and 3A5 (CYP3A4, CYP3A5) and are transported by P-glycoprotein, the product of the *ABCB1* gene. Individuals predicted genetically to express functional CYP3A5 by possession of at least one wild-type *CYP3A5*1* allele require approximately two-fold higher doses of tacrolimus to achieve target blood concentrations than individuals homozygous for the *CYP3A5*3* allele who are functional CYP3A5 non-expressers. Dosing tacrolimus based on the CYP3A5 genotype has been shown in one clinical trial to allow earlier attainment of target blood concentrations in patients where tacrolimus was commenced 7 days after transplantation but there was no difference in efficacy failure or toxicity. A further study in patients treated with tacrolimus from the time of transplantation is needed. This association does not apply for ciclosporin and the *CYP3A4* genotype does not reliably predict pharmacokinetics for either drug.

While the *ABCB1* genotype has minimal impact on pharmacokinetics it may influence intracellular drug concentrations to enable the identification of patients particularly at risk of rejection or nephrotoxicity.

Sirolimus. The *CYP3A5* genotype appears to have a similar influence on sirolimus pharmacokinetics to tacrolimus but pharmacogenetic strategies remain to be tested.

Mycophenolate. The purine antagonist mycophenolate is widely used in solid organ transplantation and in a number of autoimmune diseases. SNP in the target enzyme, inosine monophosphate dehydrogenase have been associated with increased risk of transplant rejection. The genotype for the drug metabolising enzyme UGT1A9 has been associated with both pharmacokinetics and incidence of rejection in renal transplant recipients. However, there is so much variability in mycophenolate exposure determined by non-genetic factors that a pharmacogenetic approach is unlikely to be useful clinically.

IFCC 4 Traceability, Standardisation, Harmonisation: Concepts and Implementation

Thursday, 19 May 2011   14:30–16:30

TRACEABILITY IN LABORATORY MEDICINE: A REVIEW OF THE INTERNATIONAL ACTIVITIES

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The implementation of the concept of traceability probably provides one of the most important strategies to achieve standardization in laboratory medicine aiming at reliable and comparable test results independent of the analytical principle of measurement, test procedure or commercial test kit and the laboratory where such clinical chemical testing is performed.

So, the In-vitro Diagnostica Directive of the European Union requires that *".. the traceability of values assigned to calibrators and control materials must be assured through available reference measurment procedures and/or reference materials of higher order..."*.

The establishment of a reference measurement system consisting of reference materials, reference measurement procedures and reference laboratories provides metrological traceability to routine clinical analysis, linking the patient's laboratory measurement results to an established higher-order standard (ideally, an SI-unit such as the mole or katal) through an unbroken chain of comparisons.

The Joint Committee on Traceability in Laboratory Medicine (JCTLM), a cooperation between the BIPM (International Bureau of Weights and Measures), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the International Laboratory Accreditation Cooperation (ILAC) publishes data bases of available higher-order reference materials and higher-order reference measurement procedures as well as reference laboratories on the website of the JCTLM (www.bipm.org/jctlm). These data bases can be used by the IVD industry and other users to meet requirements for traceability for in vitro diagnostic and laboratory medicine measurements.

The reference measurement laboratories listed have to establish an appropriate quality system via accreditation according to ISO 17025 and ISO 15195. Furthermore they have to demonstrate their competence trough their performance in international comparisons.

IFCC provides such surveys for reference measurement laboratories, called RELA.

Organised by the Reference Institute for Bioanalytics and advised by the IFCC Committee on Traceability in Laboratory Medicine these RELA ring trials are performed for measurands in the fields of metabolites, enzymes, electrolytes, hormones, drugs, and proteins annually.

For well characterized measurands, the global agreement on the reference measurement system will improve accuracy in laboratory medicine by providing a rational basis for standardization – which will ultimately be of benefit to patient care.

For many groups of substances in laboratory medicine, the measurands are not exactly known regarding their chemical or conformational structure. Before the concept of traceability to SI units can be established for these measurands, scientific work is necessary to define the measurands regarding their molecular structure and to develop reference measurement procedures.

STANDARDIZATION VERSUS HARMONIZATION

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The concepts of traceability, standardization and harmonization aim at comparable measurement results in laboratory medicine.
To date, standardization of measurement procedures using reference materials and reference methods is available for some 40 to 60 measurable quantities.
It can be demonstrated from External Quality Assessment that – after implementation of this concept of standardization –agreement of results from different laboratories using commercial test kits of different manufacturers has been significantly improved.
What about the large number of measurands in laboratory medicine where no standardization using the principles of traceability is available?
The main problem here is the fact that the "measurand" is not clearly defined. Although different suppliers of test procedures use the same name for a "measurand", different measurable quantities are actually determined by their test procedures.
In order to achieve comparable results for such "heterogeneous" measurands the concept of harmonization has been proposed, e.g. recalibration of individual test procedures to an overall average regression of all test procedures by using panels of native samples. However, it must be pointed out that this can be very dangerous if the different "measurable quantities" determined by the different manufacturers' test kits have a different clinical significance.
Whenever harmonization is used to improve comparability of test results it should be demonstrated that the different forms of heterogeneous measurands determined by the different procedures to be "harmonized" have the same clinical significance.

TRACEABILITY, STANDARDISATION, HARMONISATION: CONCEPTS AND IMPLEMENTATION FOR HETEROGENEOUS ANALYTES

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Background. Heterogeneous analytes, such as glycoproteins, cardiac and tumor markers, are an important part of the test menu of medical laboratories. However, today, the inter-assay comparability of measurement results is so poor that clinical interpretation against common reference intervals or decision limits is hampered. From the metrological point of view, this problem can be solved by supporting measurements by an SI-traceable reference measurement system (RMS) comprising the definition of the measurand, reference materials and reference measurement procedures (RMPs). The biggest hurdle to achieve this for heterogeneous analytes is the current confusion about the meaning of mixture analysis in biological fluids for in-vitro diagnostic use. In my opinion, this is due to a lack of understanding of basic metrological concepts and the willingness to accept pragmatic traceability approaches.

Methods. In my presentation I will describe the metrological concepts that apply to measurement of heterogeneous analytes by immunoassays. I will take the glycoprotein TSH as prototype. Currently, for TSH, the amount-of substance concentration in a given sample is expressed in arbitrary units with reference to the WHO International Reference Preparation 80/558. However, on longer term, it is the aim to make the transition to SI-units possible. Until this becomes reality, I propose to use a pragmatic approach to traceability of immunoassays that respects the continuum and, therefore, makes use of a dynamic RMS that can be updated according to scientific progress and technical possibilities.

Results. In the proposed RMS, the definition of the measurand should be commensurate with its realization in a measurement standard at any stage in the continuum. This may require that a quasi surrogate component-mixture is defined by recommendations for epitopes that immunoassays should recognize. Moreover, the RMS should apply a combination of commutable materials assigned with values by a RMP. Instead of utilizing an instrumental RMP as typically done for SI-analytes, a surrogate RMP should serve the purpose throughout the continuum, i.e., the all-procedure trimmed mean, derived from a method comparison of immunoassays with use of a panel of human samples. Further, the establishment and continuity of the traceability approach should follow the WHO process for establishment and transfer of the IU. Of course, the approach will be applicable only under certain medical, physiological and analytical circumstances. It will also require approval by regulatory authorities such as the Food and Drug Administration and the European Commission, because the current traceability statements of in-vitro diagnostic assays may have to be adapted. I will use investigations by the IFCC Working Group for Standardization of Thyroid Function Tests to demonstrate that the approach may be feasible for TSH.

Conclusions. The proposed pragmatic concept would be a major step towards traceability of measurements of heterogeneous analytes by immunoassays, without loosing the current traceability to measurement standards such as those from WHO. It would allow a staged introduction of standardization during the continuum from discovery of the analyte to full scientific understanding and transformation to the SI-unit. Once this switch is possible, also instrumental RMPs should be introduced for measurement of heterogeneous analytes.

IMPLEMENTATION OF STANDARDIZATION IN CLINICAL PRACTICE: NOT ALWAYS AN EASY TASK

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As soon as a new reference system (RS) is adopted and implemented, validation of the correctly calibrated commercial methods should take place. Tracing back the calibration of routine tests to a RS (i.e. implementing standardization) can actually modify the analyte results and this may invalidate some of the clinical decision-making criteria currently used. In order to maintain the clinical experience, the quantitative relationship to the previous calibration system should be established and, if necessary, the clinical decision-making criteria should be adjusted accordingly. Measurement of serum creatinine and its use for estimating glomerular filtration rate provide a good example. Following implementation of creatinine methods with calibration traceable to the isotope dilution-mass spectrometry, equations used to estimate kidney function will give values that are higher than the values obtained using traditionally calibrated creatinine assays. This calibration change may significantly affect interpretative criteria based on these estimates of kidney function and in order to avoid this risk a reexpression of equations with standardized creatinine results is required. A MDRD equation has indeed been reexpressed for standardized creatinine results with the best approximation. Conversely, this has never been done for Cockcroft-Gault equation resulting in a worsening of its clinical performance. The prostate-specific antigen (PSA) is another example. Two sources of calibration are in use, one based on the traditional calibration scheme that produces results consistent with the first PSA assay marketed by Hybritech, used to establish the clinically relevant PSA cutoff of 4.0 mg/L, and the second providing traceability to the WHO Reference Preparation 96/670. Recalibrating a PSA assay from an original 'Hybritech' calibration to the new WHO calibration results, however, in ~20% lower PSA values indicating that the 4.0 mg/L cutoff would not provide optimal clinical efficacy for the WHO standardized assays, but a newly recalculated cutoff of 3.1 mg/L must be employed. In the case of HbA1c, reliable linear relationships between results traceable to the IFCC RS and previous national recommended methods have been demonstrated allowing the conversion of analytical and clinical data from one system to another. In practice it is therefore possible to translate HbA1c target values generated in previous landmark clinical studies, using methods not traced to the RS.

All in all, the implementation of standardization should take place in a concerted action of laboratorians, clinicians, manufacturers, EQAS organizers, and other stakeholders. Dedicated meetings with manufacturers should be organized to discuss changes, e.g. process of assay recalibration, and studies should be performed to obtain convincing evidence that the standardization works improving comparability in clinical setting. Another important issue relates to the post-market surveillance of the performance of standardized assays through the organization of appropriate EQAS, requiring the availability of commutable control materials with target values assigned by accredited reference laboratories. Last but not least, uncertainty of measurement that fits for purpose must be defined across the entire traceability chain, starting with the provider of reference materials, extending through the manufacturers and their processes for assignment of calibrator values, and ultimately to the final result reported to clinicians by laboratories.

WS 13 Vitamin Deficiency in Elderly People: Diagnosis, Prevention and Effect on Age-Related Diseases

Thursday, 19 May 2011   14:30–16:30

B VITAMINS AND NEURODEGENERATION

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Background. Disorders in C1-metabolism have been related to brain disorders. This link might be mediated by deficiency of B-vitamins, or by disturbed methylation. Neurodegeneration is characterized by accumulation of modified functional proteins in the brain. Amyloid precursor protein (APP) is an intra-membrane protein that is processed via the amyloidogenic or the nonamyloidogenic pathway. Another brain protein, Tau, can accumulate when hyperphosphorylated. Methylation and dephosphorylation play an important role for removal of P-tau. We studied the link between neurodegenerative biomarkers and C1-metabolism in different models.

Methods. The clinical part of the study included 88 patients with different neurological disorders with exception of dementia and Parkinson disease (PD). Concentrations of amyloid beta 1-42, P-tau, and soluble APPs (sAPP a and b) were assayed in CSF samples in relation to blood and CSF concentrations of vitamins and related metabolites.

We also tested the effect of adding concentrations of Hcy, SAM, and SAH on protein level of APP and its degradation product, C99 in Down syndrome fibroblasts expressing 3 copies of APP gene (carried on Chromosome 21). We further tested the effect of hyperhomocysteinemia on the accumulation of tau protein in rat brain.

Results. Concentrations of sAPP a and b correlated to that of P-tau (correlation coefficient r=0.502 for sAPP a and r = 0.436 for sAPP b, p<0.001), amyloid beta 1-42 (r=0.571 for sAPP a and r = 0.527 for sAPP b, p<0.001), and CSF-SAH r=0.394 for sAPP a and r = 0.289 for sAPP b, p<0.01). sAPP a was negatively related to CSF folate (r=-0.300, p=0.004) with no significant relationship to CSF homocysteine (tHcy).

Compared to people in the lowest tertile of CSF-sAPP a, those in the highest tertile had significantly higher CSF-SAH (10.9 vs. 14.7 nM), CSF-tHcy (0.085 vs. 0.110 μM), P-tau (29.8 vs. 52.8 ng/L), amyloid beta (538 vs. 839 ng/L), CSF-folate (9.3 vs. 8.5 ng/ml). Predictors of sAPP b were CSF-SAM (regression coefficient R = -0.330), P-tau (R = 0.262), and amyloid beta (R = 0.685). Predictors of sAPPa were CSF-SAM (R = -0.415), CSF-SAH (R = 0.341), P-tau (R = 0.279), and amyloid beta (R = 0.647). Therefore, we proposed a role for C1-metabolism in APP processing.

In a medium that was free of the B-vitamins, SAM (100-300 μM) caused lowered protein expression of APP and SAH (50-150μM) caused increased APP protein expression in DS fibroblasts. SAH lowered C99 in cells incubated in a vitamin-free medium.

Our studies on hyperhomocysteinemic rats have shown that the expression of P-tau was increased in brain. This was probably related to the effect of insufficient folate on lowering PP2A activity.

Conclusion. Our results suggest that the amyloidogenic pathway is modified by C1-metabolism. The methylation-dependent regulation seems to be complex leading to enhanced amyloid production in non-demented people. Hypomethylation can enhance beta amyloid production that in turn accelerates Tau protein phosphorylation. Low folate can cause P-tau accumulation probably independent on tHcy elevation.

VITAMIN D AND CARDIOVASCULAR DISEASE: A REVIEW OF THE EPIDEMIOLOGICAL AND CLINICAL EVIDENCE

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Background. Until the 1970s, researchers and clinicians commonly believed that oral vitamin D was a cause of cardiovascular (CV) disease. However, the development in the 1970s of an assay for 25-hydroxyvitamin D [25(OH)D], the main marker of vitamin D status, led to studies showing that most vitamin D (>80%) came from sun exposure rather than diet, and small case control studies which found (unexpectedly) that cases of CV disease had similar or lower levels of 25(OH)D compared with matched controls. Since the 1980s, research by epidemiologists, laboratory scientists and clinicians has resulted in a 180^0 turn in opinion to where the predominant scientific viewpoint now holds that vitamin D may protect against CV disease. Key developments have been the publication of the hypothesis that ultra-violet radiation (through increasing body vitamin D levels) may reduce the risk of CV disease [1]; the identification of a vitamin D receptor in cardiac muscle [2]; a meta-analysis of randomised controlled trials showing that vitamin D supplementation reduces all-cause mortality [3]; and the publication of the first cohort study showing that low baseline blood levels of 25(OH)D are associated with an increased risk of CV disease during the follow-up period [4]. This presentation aims to provide an update on the epidemiological and clinical studies of vitamin D and CV disease, and to quantify the avoidable burden of disease from low vitamin D status.

Methods. Recent reviews and PubMed were searched up to November 2010 for community-based cohort studies of vitamin D and CV disease and all-cause mortality, and pooled regression coefficients (change in disease risk per nmol/L increase in 25(OH)D) were estimated from 11 studies of CV disease and 10 studies of all-cause mortality. Avoidable fractions of mortality were estimated for deaths from CV disease and all-causes in New Zealand using the WHO Global Burden of Disease method, which compares the current 25(OH)D distribution in New Zealand (mean 50 nmol/L) with higher counterfactual distributions.

Results. The pooled regression coefficients showed that 25(OH)D levels were associated inversely with both CV disease and all-cause mortality. A 25 nmol/L (10 ng/mL) increase in 25(OH)D was associated with a relative risk of 0.87 (95% CI: 0.83, 0.91) for CV disease and 0.89 (95% CI: 0.85, 0.92) for all-cause mortality. Avoidable mortality fractions in New Zealand for an increase in 25(OH)D by 10 nmol/L, and an increase to a mean of 100 nmol/L were, respectively, 5% and 25% for cardiovascular disease, and 4% and 21% for all-causes.

Conclusions. These calculations indicate that strategies to increase vitamin D levels, such as supplementation or increased safe sun exposure, could result in significant public health gains. However, clinical trials showing a benefit from vitamin D supplementation are required before such public health strategies can be supported by health agencies and clinicians.

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VITAMIN K AND BONE HEALTH

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Vitamin K occurs as the plant form phylloquinone (vitamin K1) and a series of mainly bacterial menaquinones (MKs) (vitamin K2). They act as an essential cofactor for a post-translational modification in which peptide-bound glutamate (Glu) residues are converted to γ-carboxyglutamate (Gla) residues in target proteins (Gla proteins). The rationale for a role of vitamin K in bone health is based primarily on the presence in bone matrix of at least five Gla-proteins of which osteocalcin (OC) is the most abundant and best studied in the context of health outcomes. In addition, vitamin K acts directly on bone by mechanisms not attributable to γ-carboxylation, with evidence of molecular specificity for MK-4 which can be synthesised *in vivo* from K1 via a newly identified enzyme UBIAD1.

The most practical way to assess vitamin K status is to measure serum K1 and undercarboxylated factor II (PIVKA-II), combining an indicator of tissue reserves with a sensitive functional marker of activity in coagulation factor synthesis. Although the vitamin K status of bone can be specifically assessed by measurements of undercarboxylated OC (GluOC) there are interpretive problems associated with different methodologies and the known association of OC with bone turnover. Expressing GluOC as a proportion of total OC gives a better measure of bone vitamin K status. The finding that even healthy people have detectable circulating GluOC has led to the concept that higher dietary amounts of vitamin K are required to maintain γ-carboxylation of bone than of haemostatic Gla-proteins. Several studies have shown that higher proportions of GluOC constitute an independent risk factor for bone fracture and low BMD and that GluOC can be readily lowered by vitamin K supplementation.

A recent systematic review and meta-analysis of several randomized controlled trials with K1 or MK-4 supplementation at a range of doses and time periods show mixed results. For K1 there was no effect on BMD and insufficient data for analysis of fracture incidence. Data for MK-4 seem more promising with overall significant associations with both increased BMD and reduced fracture risk. Caveats for the MK-4 data include potential bias with most studies in Japanese populations using very high (45mg) doses in excess of those needed for γ-carboxylation and problems with trial design and reporting. In their favour is evidence that MK-4 displays biological actions on cells not shown by K1, one example being that MK-4 specifically upregulates two extracellular matrix-related genes involved in bone signalling pathways.

Interpretation of any effects mediated via Gla-proteins such as osteocalcin is hampered by the lack of knowledge of their molecular function(s) and how their undercarboxylation can impact on BMD. Evidence that vitamins K (and osteocalcin) promote subtle changes in mineralization that do not show up in conventional BMD measurements is supported by reports that vitamin K improves hip bone geometry and bone strength indices.

In summary, except in certain clinical conditions (e.g. cystic fibrosis) in whom all indices of vitamin K status are commonly impaired, there is presently insufficient evidence to support routine vitamin K supplementation for bone health.

WS 14 Novel Insights in Iron Metabolism: Implications for Diagnostic Medicine

Thursday, 19 May 2011   14:30–16:30

NEWLY IDENTIFIED INHERITED DISEASES OF IRON METABOLISM

R. Fleming

Abstract not received

ANAEMIA OF CHRONIC DISEASE

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Anaemia of chronic disease (ACD), also termed as anaemia of inflammation, is the most frequent anaemia in hospitalised patients and is found with a high frequency in subjects suffering from auto-immune disorders, severe infections and cancer. ACD is an immune-driven disease which is induced by several cytokine-mediated pathways. First, cytokines as well as the acute phase protein hepcidin induce iron retention within cells of the reticuloendothelial system (RES), resulting in an iron-restricted erythropoiesis. This effect may be further aggravated by a inflammation mediated shortening of erythrocyte life span and stimulation of erythrophagocytosis. Second, mainly pro-inflammatory cytokines directly inhibit the proliferation and differentiation of erythroid progenitor cells while at the same time they inhibit the formation and biological activity of the erythropoiesis-stimulating hormone erythropoietin. In addition, several erythropoiesis driven hormones may contribute to ACD by modulating the expression of hepcidin.

While anaemia per se causes morbidity due to impaired cardio-vascular performance and tissue oxygenation, the development of ACD may harbour also some advantages, especially when infections or cancer underlie ACD. The retention of iron within monocytes and macrophages results in a reduced availability of the metal for invading pathogens which need iron for their growth and proliferation. Furthermore, due to the negative regulatory effects of iron on cell-mediated immune function, iron restriction leads to strengthening of innate immune effector pathways directed against invading pathogens. While this is an effective defence strategy in infection and cancer, ACD has to be considered as a side effect in association with auto-immune disorders. Accordingly, while the therapeutic application of iron for the treatment of ACD is risky and may cause exacerbation of the underlying disease in infection and cancer, treatment of ACD with iron in rheumatic disease may reduce disease activity due to its negative effects on pro-inflammatory immune pathways, a concept which has to be proven clinically in the future. In addition, due to our expanding knowledge on the regulation of iron homeostasis and specifically the elucidation of the complex network underlying the regulation of hepcidin expression, novel therapeutic concepts aim to modify hepcidin formation, thereby counter-acting iron restriction in the RES and mobilising the metal for erythropoiesis.

HEPCIDIN-25, A NEW BIOMARKER FOR DIFFERENTIATION OF IRON-RESTRICTED ERYTHROPOIESIS

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Hepcidin is a disulfide-rich peptide produced by hepatocytes as prohepcidin consisting of 84 amino acids. After posttranscriptional processing it is secreted into the blood in its biologically active form of 25 amino acids (hepcidin-25). Hepcidin is the central regulator of iron metabolism and iron is a main regulator for hepcidin expression. Hepcidin responds to stimulatory and inhibitory signals. The former include inflammatory stimuli and iron overload, the latter hypoxia, anemia and iron deficiency. Iron-sensing in blood occurs through a multiprotein complex at the hepatocyte plasma membrane.

Anemia of inflammation or anemia of chronic disease (ACD) is highly heterogeneous. Biochemical markers hardly allow to distinguish the combined state of iron-restricted erythropoiesis (IRE) and ACD/IRE from iron deficiency anemia (IDA), especially in patients with inflammation. The mediators of iron metabolism are genetically preserved and involve a number of critical proteins, including ferritin, transferrin, transferrin receptors, iron-regulatory proteins, divalent metal transporter-1, HFE, ferroportin and hepcidin. Common features in anemia of inflammation include iron sequestration, inadequate production of erythropoietin, inhibited proliferation and maturation and increased turnover of red cells. As a consequence inflammation causes decreased circulating iron and impaired iron distribution within the body for which the IL-6-hepcidin-axis appears to be responsible. Among the available hematological and biochemical markers of iron metabolism no single parameter exists that serves as a comprehensive indicator of iron status. Serum hepcidin-25 might be a marker resolving this problem.

The objective of studies was (i) to investigate the association of hepcidin-25 with biochemical markers and hematological indices of iron status and (ii) to evaluate the extent to which ACD can be differentiated from ACD/IRE and IDA based on hepcidin-25 alone or on its combination with other markers.

Hepcidin-25 significantly correlates with ferritin and transferrin saturation (TSAT) and concentrations are below the detection limit of 0.2 nmol/L at ferritin ≤9 μg/L and TSAT ≤14.3%. Correlations with soluble transferrin receptor, ferritin index, and CRP are weaker but still significant. Median hepcidin-25 concentrations are lowest in IDA patients, high in ACD and at a high or in-beteween level in ACD/IRE depending on the study. Studies of our working group indicated weak discriminatory power of hepcidin-25 to differentiate ACD from ACD/IRE, especially in patients with high-grade inflammation. For differentiation of ACD and ACD/IRE the relationship between hepcidin-25 and the CHr (hemoglobin content of reticulocytes) was used. In a diagnostic plot divided into quadrants that corresponded to the four states ACD, ACD/IRE, IDA (classic) and IDA (latent state). Data points in the hepcidin-25 range ≤4 nmol/L reflected IRE whereas data points in the range >4 nmol/L were consistent with ACD. Patients with data points in the CHr range ≥28 pg (normal CHr) did not have IRE, whereas those with a CHr <28 pg (reduced CHr) had IDA or ACD/IRE.

The ability to differentiate states of anemia using hepcidin shows great promise, however its implementation into the everyday clinical practice addresses limitations. Recently results of the first international round robin informed the medical community on the status of the current hepcidin methods.

INDUSTRY SPONSORED WORKSHOP ABSTRACTS

Clinical and Economic Advances in Prostate Cancer Diagnosis

Monday, 16 May 2011   13:00–14:00

PROSTATE CANCER DETECTION: THE PATIENT'S VIEW

Dr. Vincent Griesser
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In developed countries, prostate cancer (PCa) is the third most common cause of death from cancer in men. Therefore early detection of PCa is essential to offer more treatment options to patients to ensure the best possible outcomes. Unfortunately, the limitations of PSA testing can result in significant consequences on men's lives. The goal of this presentation is to share the patients' experience of PCa detection using PSA and its human consequences but also to call for an intelligent screening for PCa.

IMPROVING PSA-BASED PROSTATE CANCER DETECTION WITH THE PROSTATE HEALTH INDEX (*PHI*)

PD Dr. Carsten Stephan
Charité University Hospital, Berlin, Germany

Beckman Coulter recently developed the "Prostate Health Index" (*phi*)[*] which mathematically combines tPSA, fPSA and [-2]proP-SA values. The results of a multicenter study evaluating the clinical performance of *phi* for the detection of PCa on 905 patients confirmed the benefit of this test. The use of *phi* improves the clinical specificity for PCa detection over tPSA and %fPSA and could reduce significantly the number of unnecessary prostate biopsies. This index, which may identify preferentially aggressive PCa, could constitute a major progress for PCa detection.
[*]*Not available in the United States*

QUANTIFYING THE ECONOMIC VALUE OF *PHI*

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In clinical studies, the Prostate Health Index (*phi*)[*] has demonstrated improved specificity for prostate cancer detection when compared to PSA. A simulation model was conducted to evaluate the economic performance of *phi* when introduced to current European prostate cancer screening practice. Based on a 4-year screening interval, we found that the introduction of *phi* may reduce unnecessary biopsies by 29%. This suggests a potential 21% reduction in prostate cancer diagnosis cost, and a 12% gain in life-time cost-effectiveness.
[*]*Not available in the United States*

The Value of Improved Workflow Solutions

Monday, 16 May 2011   Hall 9, 15:00–16:00

THE VALUE OF IMPROVED WORKFLOW SOLUTIONS

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Background. This workshop will focus on meeting the challenges of supervising a clinical laboratory in a continuously transforming healthcare environment, which includes changes in demographics, reimbursement and increased focus on cost containment while continuously striving to maintain high standards of quality.
Methods. We will share the Siemens laboratory automation and IT solutions vision and explore how it is designed to help labs meet their challenges. We will review actual experiences from a clinical laboratory and reveal how automation made it possible for a laboratory to achieve its goals for productivity and workflow in spite of changing environmental pressures.
Results. Siemens is uniquely positioned to offer customized solutions through our comprehensive range of products and services to laboratories. By listening to the specific needs of laboratories all over the world, we are developing new and more innovative solutions to adapt to customers' needs. These innovative, performance-driven solutions help streamline workflow, enhance operational efficiency, and support improved patient outcomes. Direct customer experience will demonstrate that we are building on the strengths of our expertise in developing, installing, and positively impacting how laboratories work.
Conclusions. Adaptable automation and diagnostics IT solutions are critical to helping labs manage their workload and business requirements in both the short- and long-term. Siemens, in partnership with its customers, can meet the challenges that laboratories are facing.

Autonomous Biosensors – Technologies that Help to Bring Biomarkers to the Patient

Monday, 16 May 2011   13:00–14:00

AUTONOMOUS BIOSENSORS – TECHNOLOGIES THAT HELP TO BRING BIOMARKERS TO THE PATIENT

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Progress in molecular medicine will benefit patients with any kind of disease. The increase in knowledge of molecular constitution and regulation not only helps to find better suited therapies for many diseases but also gives access to more precise analysis and diagnostics. Therefore diagnosis based on molecular markers is starting to become available even in early stages of diseases as well as for the stratification of the individual patient for medication and for therapy control. Molecular diagnostics has already entered the clinical laboratory especially with regard to genomic analysis. But protein biomarkers and more complex signatures of metabolites in blood or other body fluids like saliva and urine have already been found and will be accessible for future diagnostics. However, to gain all the benefits that arose from the molecular knowledge diagnostics has to move as near as possible to the point of need, i.e. to the doctor´s office or even to the patient at home. To achieve this goal several technological hurdles have to be surmounted.
Lab-on-Chip technology has been well investigated during the recent years and many assays for various parameters have been demonstrated on various transducers and with various microfluidic systems. Only very few of them have been shown to proof routine diagnostic´s needs. Only lateral flow devices, that usually are very much limited with regard to the number of analytes and to the performed accuracy, have made their way to the market.
The main technological challenge is to guarantee the same accuracy for a single use lab-on-chip device compared to today´s routine clinical laboratory. Also mass production of such devices has to be performed with the same accuracy and reliability. Even more the device has to be handled by non trained users. Last but not least the readout of the data of any kind has to be connected to the patient´s record at his or her doctor´s office or the clinic. Therefore point-of-care devices have to be linked to telecommunication. All these challenges have been addressed in the work of the Centre for Molecular Diagnostics and Bioanalysis (ZMDB). A complete concept has been developed to provide a platform for future diagnostics that will be performed directly at the point of need to gain the relevant data within minutes.

A GLYCAN MICROARRAY PLATFORM FOR THE HIGH-THROUGHPUT ANALYSIS OF BLOOD SERA

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Carbohydrate arrays using defined, synthetic oligosacharides that are covalently linked to the surface of a chip by depositing with high-precision printing equipment are now standard tools for glycomics research. [1]
The utility of the microarrays now depends on the content that is placed on their surface and is a function of either glycan isolation or chemical synthesis of the carbohydrates. Our automated oligosaccharide synthesis platform [2] has fueled screening interactions of glycans with proteins, RNA and cells. Heparin arrays have revealed insights into the oligosaccharide sequences recognized by growth factors. [3]
A high throughput glycan microarray platform using synthetic GPI glycans served to determine the antibody titres against the GPI toxin in large numbers of people from endemic and non-endemic countries. This approach uses minimal amounts of valuable glycans and human sera while providing a detailed epitope analysis. [4]
In this lecture the screening platform will be introduced and applications to the discovery of anti-glycan antibodies associated with human disease will be described. The glycan array platform is now ready for development for clinical use.

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The Clinical Utility of Multiplex Biochips for the Detection of Multiple STI's and Respiratory Pathogens

THE CLINICAL UTILITY AND PRACTICAL IMPLICATIONS OF A 10-PLEX SEXUALLY TRANSMITTED INFECTION (STI) BIOCHIP ARRAY

Dr Peter V Coyle
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Background. Over the past decade there has been a considerable increase in the incidence of sexually transmitted infections (STIs) in the UK and worldwide. In order to address the increased demand for STI testing and to aid in the prevention of spread of these diseases,we describe a rapid, cost effective array that can detect all common STIs simultaneously.

Methods. Previously screened clinical samples were obtained from a large regional hospital and were tested for the presence of sexually transmitted infections (STIs) using a Multiplex STI biochip array (Randox Laboratories). The results obtained were compared to those derived from in-house developed and commercially available PCR.

Results. Data from representative samples revealed that the Biochip array produced results that concurred with routinely used PCR and culturing techniques, including the detection of Chlamydia trachomatis, Neisseria gonorrhoea, Herpes Simplex 1 and Herpes Simplex 2. Pathogens were successfully detected in a variety of sample matrices including mucosal swab and urine samples. The multiplex approach of the Biochip array also allowed detection of additional pathogens not routinely screened for by PCR; Ureaplasma urealyticum.

Conclusions. A multiplex biochip array with the capacity of testing 10 common STI pathogens may represent a more rapid, cost effective and comprehensive method of STI detection than routinely used PCR systems.

ABILITY OF A MULTIPLEX BIOCHIP ARRAY TO SIMULTANEOUSLY DETECT UP TO 8 BACTERIAL RESPIRATORY PATHOGENS IN A CLINICAL SETTING

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Background. In many laboratories, diagnosis of respiratory infection relies on direct fluorescent antigen (DFA) assays or culture procedures. Traditional culture methods are slow, taking 2-7days for a positive result and up to 14 days for negative. Multiplex amplification procedures, coupled with microarray detection of pathogens may provide the most sensitive, rapid diagnostic tool to identify respiratory pathogens as a cause for individual cases as well as for outbreak investigation. This will allow severely infected patients to be treated quickly with the correct treatment, thus reducing infection risk and minimising treatment with unnecessary antibiotics.

Methods. Previously screened clinical samples were obtained from the Scottish Meningococcus and Pneumococcus Reference laboratory and were tested for the presence of respiratory pathogens using a Multiplex RP Biochip array (Randox Laboratories). The results obtained were compared to those derived from real time PCR systems routinely used by the National Health Service.

Results. Data from 100 samples revealed that the Biochip array correctly identified all pathogens and displayed 100% agreement with PCR, with the same level of sensitivity and specificity. Eight different bacterial pathogens were successfully detected including Haemophilus influenzae, Moraxella catarrhalis, legionella pneumonia and Staphylococcus aureus from a variety of sample matrix such as sputum, nasal secretions and throat swabs.

 Conclusions. A multiplex biochip array with the capacity of testing for multiple common respiratory pathogens may represent a more rapid, cost effective and comprehensive method of STI detection than routinely used PCR systems.

Diagnostic Accuracy of the New Zenit RA System for Detection of Celiac Disease-Related Antibodies and Automated Application of a Reflex Algorithm in IgA-Deficient Celiac Patients

DIAGNOSTIC ACCURACY OF THE NEW ZENIT RA SYSTEM FOR DETECTION OF CELIAC DISEASE-RELATED ANTIBODIES AND AUTOMATED APPLICATION OF A REFLEX ALGORITHM IN IGA-DEFICIENT CELIAC PATIENTS

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International guidelines recommend quantitative testing for class IgA anti-tissue transglutaminase (anti-tTG) antibodies as the first step in the diagnosis of celiac disease (CD). The anti-tTG IgA assay must be associated with determination of total serum IgA, since patients with absolute IgA deficits are 10 to 20 times more at risk for CD than is the normal population. An alternative strategy to total IgA assay of all the samples is to use the anti-tTG IgA "signal" (which is very low in sera not containing IgA) to identify sera with probable deficits.

In subjects with absolute class IgA deficit (<5 mg/dl), the class IgG anti-tTG or antibodies against deamidated gliadin peptides (anti-DGP) must then be assayed. The anti-DGP antibodies are also of significant diagnostic utility in children under 4-5 years of age: not infrequently, children affected with CD will test positive for anti-DGP (prevalently class IgG) and negative for anti-tTG IgA. One drawback of the diagnostic procedure outlined above is that it is complex and not easily applicable in all laboratories, since it calls for sequential analyses that must be run on different instruments and employing different methods.

The Zenit RA Analyzer (A. Menarini Diagnostics, Florence, Italy) is a random access instrument capable of conducting fully-automated immunometric assays of the anti-tTG IgA and anti-tTG IgG autoantibodies and the anti-DGP IgA and anti-DGP IgG antibodies.

The assay methods are based on use of a solid phase consisting of microparticles sensitized with specific antigens (human transglutaminase and deamidated gliadin peptides) and of tracers, specific antibodies labeled with acridine esters. The signal is constituted by the light emitted by the tracer after appropriate sensitization. The quantity of light emitted is measured by a photo-multiplier and expressed in arbitrary units (RLU).

The high uniformity and stability of the reagents and use of methods featuring constant reaction and measurement conditions permit making use of stored calibration curves, on which any variables are normalized through use of 2 calibrators for each test. The core of the diagnostic system is the anti-tTG IgA assay kit.

This kit features a unique capability to provide an assessment of the quantity of total IgA antibodies present in the analysis sample at the same time that it reports the anti-tTG IgA concentration.

This result is obtained through use of a solid phase constituted by microparticles sensitized with the tissue transglutaminase antigen to which microparticles sensitized with monoclonal anti-human IgA antibodies are added.

Use of monoclonal anti-human IgA antibodies marked with acridine ester as the tracer in the second reaction phase makes it possible to also assay anti-tTG IgA negative samples by a "2-step sandwich" method; the response in this case varies only in relation to the total IgA concentration of the sample. In these conditions, the microparticles sensitized with tissue transglutaminase do not take part in the reaction.

In the presence of anti-tTG IgA antibodies, the assay method will provide a response that will vary only in relation to the anti-tTG IgA concentration and not to the total IgA concentration. The quantity of anti-IgA microparticles added to the reaction system is calculated so as to provide a calibration curve that is sensitive only in the presence of low concentrations of IgA and that "saturates" in the presence of normal or high total IgA concentrations.

A standard curve capable of linking the responses obtained with the two types of microparticles has been calculated for normalizing and interpreting the responses.

This makes it possible to identify samples containing a total IgA concentration of less than 25 mg/dl and to then proceed automatically (if desired) with assay of the anti-tTG IgG and/or anti-DGP IgG antibodies.

By using the diagnostic tests on the Zenit RA Analyzer it is therefore possible to set a customized profile for serological diagnosis of CD for each serum: from the stand-alone test for anti-tTG IgA to a profile also including the anti-DGP IgA and IgG antibodies, as suggested for example for pediatric patients. What is more, thanks to specific interpretation software, sera with a very low signal for the anti-tTG IgA test can be re-analyzed for anti-tTG IgG and anti-DGP IgG via a "reflex" test.

We conducted an assessment study to evaluate Zenit RA analysis system performance in determination of the anti-tTG IgA and IgG antibodies and the anti-DGP IgA and IgG antibodies, and another study to evaluate performance of the management software for verifying the applicability of the reflex test on sera from subjects with IgA deficiency. The study was conducted on serum samples from 40 subjects diagnosed with CD, 15 subjects with IgA deficits (of whom 6 were celiac subjects), 29 celiac subjects on dietary regimes (3-35 months), and 60 healthy non-celiac subjects.

All the sera were assayed with the Zenit RA tests for anti-tTG (IgA and IgG) and anti-DGP (IgA and IgG); the results were correlated with the results obtained via testing with the anti-tTG IgA and IgG kits manufactured by Orgentec (Mainz, Germany) and the anti-DGP IgA and IgG kits manufactured by INOVA (San Diego, CA).

During another analysis session, the reflex software was applied to testing anti-DGP IgG and anti-tTG IgG in sera with IgA values <25 mg/dl.

The correlations of the antibody concentrations obtained with the Zenit and Orgentec anti-tTG tests was $r^2 = 0.95$ for the class IgA antibodies and $r^2 = 0.72$ for the class IgG antibodies; the correlation between the Zenit and Orgentec anti-DGP tests was $r^2 = 0.88$ for class IgA and $r^2 = 0.73$ for class IgG. The sensitivity in newly-diagnosed CD patients was 100%, 57.3%, 77.5%, and 70%, respectively, for the Zenit anti-tTG IgA, anti-tTG IgG, anti-DGP IgA, and anti-DGP IgG kits, and 97.5%, 60%, 77.5%, and 72.5%, respectively, for the Orgentec anti-tTG IgA and anti-tTG IgG kits and the INOVA anti-DGP IgA and anti-DGP IgG kits. The sensitivity of all the different test methods was comparable in the gluten-free-diet patients. Performance in the case of the 15 IgA-deficient patients was excellent: all 7 of the sera from patients with CD and IgA deficit gave positive results with the Zenit anti-tTG IgG test and 6 also gave positive results with the Zenit anti-DGP IgG test. Specificity was 100% for the Zenit anti-tTG IgA, anti-tTG IgG, and anti-DGP IgA tests, and 96.5% for the Zenit anti-DGP IgG test.

The results of this study indicate that the diagnostic performance of the Zenit anti-tTG and anti-DGP IgA and IgG test kits is excellent. The Zenit RA instrument is capable of supplying rapid, fully-automated responses for the autoimmunology laboratory. The algorithm used by the Zenit RA instrument was shown to be capable of efficaciously handling all the steps involved in celiac disease diagnosis and thus of reducing costs and reporting times.

Molecular and Serological HBV Markers as Drivers of Individualized Patients Care

MOLECULAR AND SEROLOGICAL HBV MARKERS AS DRIVERS OF INIVIDUALIZED PATIENTS CARE

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Since the '60 serum HBsAg is the cornerstone of HBV infection diagnosis. After the characterization of HBsAg circulating forms where defective particles (HBV-DNA free spheres and filaments) exceed HBV-DNA competent virions by a factor of 10^2-10^5 it became evident that serum HBsAg does not merely reflect the production of virions but rather the overwhelming production of HBV envelope proteins. Thus, virologic information provided by quantifications of HBsAg and HBV DNA differs. HBsAg can be considered as a hallmark of "transcriptionally active cccDNA" that indirectly reflects the degree of control of HBV infection achieved by the host's immune-system, whereas HBV DNA levels beckons the level of viral replication. HBsAg and HBV DNA serum levels and their relationship vary during the different phases of HBV infection: HBsAg serum levels decline progressively from HBeAg positive to HBeAg negative phase, the lowest levels being found in inactive HBV infection.

The combined quantitative measure of HBsAg and HBV DNA provides complementary information and may contribute to a better management of asymptomatic HBV carriers by the identification of the candidates to antiviral therapy and of CHB patients providing a useful means for treatment tailoring. Accordingly in HBeAg negative CHB, it has been recently shown as during the 1 year of treatment serum HBsAg declines in pegylated interferon (Peg-IFN) treated patients but not in those receiving lamivudine, in spite of similar HBV DNA decline. Indeed in responders to Peg-IFN therapy HBsAg decline is associated with sustained immune control, defined as the maintenance of low viremia levels 6 or 12 months after the end of treatment and/or HBsAg loss during long term post-treatment follow-up. The evidence that on treatment kinetics of HBsAg, at variance with HBV-DNA, differ in patients who maintain viral response after the end of treatment from relapsers qualifies quantitative HBsAg as a new tool for the treatment optimization of HBeAg negative CHB. However, on treatment early HBsAg kinetics appears to be influenced by HBV genotypes: the slowest decline was shown in genotype D infected patients. Thus, to optimize treatment in the single patient level HBV genotype specific algorithms could be required together with HBV-DNA monitoring. Indeed, recent reports suggest that combining both HBsAg and HBV DNA could warrant an early and accurate identification of HBeAg negative CHB patients who are primary non responders to therapy. This would allow on the one hand early treatment discontinuation or optimization and on the other hand, in case of early on-treatment serum HBsAg decline, could motivate patients to complete therapy and achieve sustained immune control. Finally, a progressive HBsAg decline has been described also in a proportion of patients undergoing long term effective nucleos(t)ide analogues (NA) treatment; future studies should address the question whether HBsAg quantification could play a role in identifying patients who might stop NA treatment without major risk for hepatitis B relapse. In conclusion, the combined monitoring of HBsAg and HBV-DNA serum levels is going to play an important role in the clinical management of HBV infection and to provide a useful response-guided therapy approach for tailoring antiviral treatment.

REAL-TIME PCR BASED VIRAL LOAD ASSAY AND INCREASING COMPLEXITY OF HCV PATIENTS MANAGEMENT

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Background. Chronic hepatitis C virus (HCV) infection affects 0.5-1.5% of the population in the Western Countries. Progression to liver cirrhosis is observed in a significant proportion of patients. HCV induced liver cirrhosis and liver cancer is one of the most frequent indications for liver transplantation. Treatment consists of pegylated interferon-alfa in combination with ribavirin and in the near future also the application of direct antiviral agents blocking HCV replication (protease inhibitors telaprevir and boceprevir).

Methods. Analysis of importance of HCV RNA measurement for management of antiviral therapy.

Results. Commercially available, third generation HCV antibody assays display high sensitivity and specificity for screening of chronic hepatitis C. However, as characteristic symptoms are often absent, diagnosis of chronic hepatitis C is only established in a minority of patients. Determination of HCV genotype provides important information about the chance of viral eradication and the duration of antiviral therapy. With the current standard treatment approx. 50% of patients achieve a sustained virologic response and this will increase with the approval of direct antiviral agents in 2011/2012 to approx. 75%. The key parameter for assessment of virologic response during therapy is HCV RNA. Precise and reliable quantification and highly sensitive detection of HCV RNA before, during and after therapy is crucial for determination of virologic response, development of resistance and determination of optimal treatment duration.

Conclusion. Sensitive and precise HCV RNA measurement is crucial for management of treatment of chronic hepatitis C with direct antiviral agents.

HbA$_{1c}$: New Breakthrough Technology for Laboratories Facing a Change of Paradigm

Monday, 16 May 2011

HBA$_{1c}$: CHANGE OF PARADIGM

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HbA$_{1c}$ is considered the "gold standard" for monitoring metabolic control in diabetes. An International Expert Committee has recently recommended HbA$_{1c}$ as a better method respect to fasting glucose for the diagnosis of diabetes, based on its strong association with microvascular complications, a lower day-to-day variability and easiness to use, not necessarily in the fasting state. Measuring HbA$_{1c}$ has several advantages over glucose measurements, but its exclusive use should only be considered if the test is conducted under standardized conditions and its limitations are taken into due account. A Consensus Statement on the Worldwide Standardization of the Hemoglobin A$_{1c}$ Measurement was issued in 2007, and later on reinforced in 2010. The transition to the new IFCC reference system is a good opportunity to focus on some pre-analytical, analytical and post-analytical aspects, not particularly studied up to now. Particular attention will be dedicated to the issue of the biological variability of HbA$_{1c}$, since there is an urgent need to determine biological variability of HbA$_{1c}$ using a specific and traceable assay, appropriate protocol and appropriate statistical evaluation of data. Another point concerns the impact of the change of units on the definition of the analytical goals. Indeed, the use of the IFCC units (mmol/mol), instead of the NGSP units (%) has an impact both on the imprecision of the methods, as well as on the total error. In conclusion, the need for a more accurate and reproducible methods for HbA$_{1c}$ will be highlighted.

CURRENT TECHNIQUES: WHAT IS REALLY BEING MEASURED

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At present, more than 70 different assays are available to measure HbA$_{1c}$ in human blood. In Europe, at least from the data available from the main EQAS providers, approximately 30 of them are on the market, mostly being highly automated in response to the increased worldwide incidence of diabetes and the increased use of the HbA$_{1c}$ test for the routine monitoring and for the diagnosis of these patients. Point-of-care systems are a recent new entry in the field.
Almost all HbA$_{1c}$ assays are based on three main principles: *a)* separative techniques (such as HPLC and capillary electrophoresis), based on the difference in the isolelectric point between HbA$_{1c}$ and other red cell hemoglobins; *b)* affinity chromatograpy methods, based on the specific binding of cis 1,2 diol groups of glucose molecules bound to hemoglobin to m-amino-phenyl boronate resins; *c)* immunochemical methods, based on the binding of antibodies raised against various groups in the N-terminal domain of the beta globin chain. Different pre-analytical variables may interfere with the different methods, and this point has to be carefully evaluated by laboratory professionals. HbA$_{1c}$ standardization has been achieved according to the IFCC metrological strategy, and all the commercial methods have been calibrated against this new reference system. However, the analytical performance of the various methods and their alignment to the IFCC reference system is variable, and in many cases not in line with the actual criteria of acceptability, either derived from the clinical needs, or from preliminary data on biological variability.

NEXT GENERATION OF SEPARATION METHOD FOR HBA$_{1c}$: FIRST EVALUATION OF THE CAPILLARY ELECTROPHORESIS TECHNIQUE

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HbA$_{1c}$ is a key biomarker for the monitoring of glycemic balance in diabetic patients. It may be measured by various methods, including high-pressure liquid chromatography (HPLC) and immunoassays. We evaluated the Capillarys 2 Flex Piercing$^{\lozenge}$, a new analyzer using capillary electrophoresis for the separation and the quantification of HbA$_{1c}$ from whole blood in primary capped tubes. 8 capillary channels in parallel are used simultaneously for separation, allowing high throughput. The analytical performances of the assay have been tested and HbA$_{1c}$ values obtained were compared to those of a HPLC assay routinely used in the lab (Variant II$^{\lozenge}$, Bio-Rad). The influence of the most frequent analytical interferences on HbA$_{1c}$ assay (*e.g.* hemoglobin variants, labile HbA$_{1c}$ and carbamoylated hemoglobin) was also studied. The use of external quality control samples indicated a good accuracy of the method, since the results are in agreement with IFCC targets. This evaluation showed that the analytical performances of Capillarys 2 Flex Piercing$^{\lozenge}$ analyzer for HbA$_{1c}$ assay allow to recommend its implementation in clinical chemistry laboratories for a routine practice.

Using Active B12 (holotranscobalamin) to Diagnose Vitamin B12 Deficiency

Monday, 16 May 2011   Hall 10, 16:45–17:45

USING ACTIVE B12 (HOLOTRANSCOBALAMIN) TO DIAGNOSE VITAMIN B12 DEFICIENCY

Edward Valente
Axis-Shield Diagnostics
E-mail: Edward.valente@axis-shield.com

It is essential to have accurate diagnostic tests to identify vitamin B12 deficiency because of the often diffuse and non specific symptoms of this condition and because damage to the nervous system might be irreversible as a consequence of delayed diagnosis and treatment. Currently, no consensus exists concerning the efficient diagnosis of vitamin B12 deficiency, the current front-line test, total serum B12, is not reliable. This workshop will focus on the clinical use and value of Active B12 (holotranscobalamin).

New Breakthrough Technology for Laboratories Facing a Change of Paradigm

THE DIAGNOSTIC UTILITY OF HEART-TYPE FATTY ACID BINDING PROTEIN IN PATIENTS WITH ACUTE CORONARY SYNDROME

Dr Julian Barth MD FRCP FRCPath
Consultant Chemical Pathologist, Leeds General Infirmary, C-NET Group, Department of Clinical Biochemistry, West Yorkshire, United Kingdom

Background. The diagnosis of acute coronary syndrome (ACS) has been defined by the measurement of serum cardiac markers of which troponin has been the preferred agent. Despite this many other potential markers have been evaluated. This paper will present the data on heart-type fatty acid binding protein (FABP) that we have accrued in two large studies of ACS patients over the past years
Methods. Two phase 2 studies have been performed. In the first 2,499 subjects with proven ACS were studied and in the second, a cohort of 1080 consecutive patients presenting with chest pain considered to be cardiac in origin. In the first study FABP was measured with the Dianippon FABP assay and the second by the Randox assay.
Results. Both studies have demonstrated a clear relation between FABP and prognosis with up to 6 years follow-up. FABP identifies subsets of troponin negative patients with good and bad prognosis.
Conclusions. We conclude that the use of FABP in addition to troponin markers in patients with acute coronary syndrome improves the identification of subjects with poor prognosis and warrants its introduction in clinical practice.

HEART-FATTY ACID BINDING-PROTEIN MAY ENABLE IMMEDIATE EXCLUSION OF ACUTE MYOCARDIAL INFARCTION IN THE EMERGENCY DEPARTMENT

Dr Richard Body
NIHR Clinical Lecturer in Cardiovascular Medicine, Cardiovascular Sciences Research Group, 3rd Floor Core Technology Facility, University of Manchester, Manchester

Objective. Suspected cardiac chest pain accounts for over 25% of medical admissions but, as only a minority have acute coronary syndromes, there is tremendous potential to reduce unnecessary admissions. We evaluated six novel biomarkers for enabling immediate exclusion of acute myocardial infarction (AMI) using a single sample at the time of ED presentation.
Methods. We recruited patients who presented to the ED with suspected cardiac chest pain occurring within 24 hours. Blood was drawn at the time of presentation. Diagnostic value was assessed by calculating the area under the ROC curve (AUC). The primary outcome was a diagnosis of AMI, established by ≥12-hour troponin testing in all patients.
Results. 697 included patients underwent venepuncture a median of 3.5h after symptom onset. The AUCs for each biomarker were: heart fatty acid binding protein (H-FABP) 0.91, CK-MB 0.82, myoglobin 0.80, Randox troponin I, GPBB 0.65 and CA3 0.59. At a 2.5ng/ml cut-off, H-FABP had a sensitivity of 82.4%, specificity 83.6%, PPV 53.5% and NPV 95.4%. The combination of the ECG, Roche troponin T (which was used in practice during the study period) and H-FABP had a sensitivity of 98.5%, specificity 66.6%, PPV 39.7% and NPV 99.5%.
Conclusions. Using the Randox H-FABP assay at the time of presentation enables the positive identification of 82.4% of AMIs using a single sample. The combination of H-FABP, ECG and troponin T may enable immediate exclusion of AMI although the miss rate is not zero. Further research including combination with high sensitivity troponin assays is warranted.

From Clinical Chemistry to Molecular Diagnostics: Innovative Solutions for Laboratory Automation and Organisation

Tuesday, 17 May 2011   13:00–14:00

REACHING OPTIMAL EFFICIENCY IN A HIGH VOLUME LABORATORY

Dr. Dr. Dieter Münstermann
Labor Krone, Bad Salzuflen, Germany

The ongoing trend to consolidation in laboratory testing is creating the need to integrate and optimize the processes and information systems of different laboratory structures.
In our laboratory we needed to integrate seamlessly a pathology lab and a typical community laboratory structure, residing in different locations without going out of production. The lecture is going to present our approach this task with the concurrent need to increase sample throughput and volume and the streamlining of laboratory processes with the support of Beckman Coulter instrumentation and data management systems.

AU5800: THE NEW ULTRA HIGH THROUGHPUT CHEMISTRY ANALYSER - FIRST EXPERIENCE

Prof. Dr. Tammo von Schrenck
GLP laboratories, Hamburg, Germany

Beckman Coulter is introducing a new high speed analyzer for Clinical Chemistry, the AU5800. Consolidation of laboratories requests instruments with high analytical throughput.
We have been evaluating the new system, comparing an AU5822 with one of our routine systems, AU5422, focusing on the quality of the results as well as on turnaround-time. Using the same reagents, results are consistent, while throughput was significantly higher.
Our staff was trained on AU5800 without any problem; the convenience and ease of use are an important advantage.

UNICEL DXN, THE NEXT GENERATION IN CLINICAL MOLECULAR DIAGNOSTICS AUTOMATION

Michael Topham
Beckman Coulter Inc., Chaska, MN, USA

With increasing labor shortages in laboratory medicine, molecular diagnostics instrumentation will need to evolve toward central lab-like automation. In a recent report, Piper Jaffrey described a recipe for success in the molecular market as requiring a solid core technology, instrumentation, and menu. Few platforms today meet those requirements. The Beckman Coulter UniCel ® DxN will automate the entire real-time PCR procedure, providing real "sample to answer" capability, integrating the process of producing and reporting a result, with a diverse menu.

New Horizons in Cellular Analysis

Tuesday, 17 May 2011   Hall 14.2, 14:15–15:15

ANEMIA – NEW INSIGHTS

R. Simon
Scientific Director Global, Beckman Coulter, Nyon, Switzerland
E-mail: rlsimon@beckman.com

Anemia is one of the more common clinical problems with approximately 40% of all blood analyses indicating anemia.

Anemia of Chronic Disease (ACD) is the second most frequent cause of anemia in the general population and the first in the elderly, being many times under diagnosed. Some new parameters and insights about ACD have been developed recently, as Soluble Transferrin Receptor, IL-6 and parameters that permit to evaluate red cell hypochromia as a way to suspect iron deficient erythropoiesis.

We now have some evidence that patients with ACD, treated with recombinant Human Erythropoietin and intravenous iron, may have iron overload. The paradigm, assuming that in ACD Ferritin was an acute phase protein, not related to the amount of iron deposits, have been lately criticized, as well as the doses of intravenous iron in these patients.

Megaloblastic anemia and other causes of ineffective erythopoiesis are the third cause of anemia. B12 deficiency and folate deficiency represent a challenge, because many times come together with Iron deficiency and MCV is normal.

Some new tools have been developed based on the physical properties of the leukocytes, called: Cell Population Data. These data, when used as an in-laboratory flag, may help to identify samples with anemia, trigger a review in cases where abnormal cells are present and generate the determinations of B12, folate or other tests as required.

Algorithms or decision trees, combining hematological and immunochemistry parameters may increase the efficiency for the correct approach to anemia.

DOES THE MICROSCOPIC WBC DIFFERENTIAL COUNT HAVE A FUTURE IN A HEMATOLOGY LAB: THE BENEFITS OF HEMATOFLOW CONCEPT

Z. Kassengera, I. Beukinga and, O. Pradier
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Background. Automated differential white blood cell count (WBC diff) cannot count certain cell populations passing through the blood (immature granulocytes, blasts, atypical lymphocytes, lymphoma cells) which must be analyzed secondarily by optical or digitized microscopy. In hospitals, these reviews represent 10-25% of cases, according to established criteria. In this context, Beckman Coulter proposed the new HematoFlow™ concept using flow cytometry of 20 000 cells stained by the CytoDiff™ monoclonal antibodies combination prior to microscopy in a fully automatic process.

Method. Observational study of 10 days routine work in the academic hospital with 825 beds, including 22 beds of hemato-oncology, and 37 intensive care beds. Flagged WBC diff passed first through CytoDiff staining and flow cytometry analysis using the HematoFlow workflow, and then detected abnormal cell populations are controlled using microscopy according to a local algorithm and after microscopic confirmation the diff provided by the CytoDiff or microscopy is validated.

Results. HematoFlow includes two UniCel® DxH™ 800 Coulter Cellular Analysis Systems, FP1000 Cell Preparation System and FC 500 Flow Cytometer from Beckman Coulter controlled by the middleware REMISOL Advance (Normand Info SAS). The 12.56% review rate in stand-alone is reduced to 7.8% after application of the rules in REMISOL. In an average of 577 WBC diff per day, a median of ±40 samples are reviewed by CytoDiff (37 to 63) requiring 4 to 14 (average 9) controls by microscopy of which less than 2 require a count of 100 cells (atypical lymphocytes and CD16 low neutrophils with dysplasia essentially). Inter-day CV for blast count below 10% is about 3%.

VOLUME, CONDUCTIVITY AND SCATTER PROPERTIES OF LEUKOCYTES (VCS TECHNOLOGY) IS A HIGHLY SENSITIVE AND SPECIFIC PREDICTOR OF BLOOD CULTURE PROVEN NEONATAL SEPSIS

M. Bhargava[1], U. Sindhuri[1], S. Saluja [2], A. Saraf[1].
[1]Hematology Department, Sir Ganga Ram Hospital, New Delhi, India, [2]Neonatology Department, Sir Ganga Ram Hospital, New Delhi, India.
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Background. In spite of the availability of potent and specific antimicrobial therapy, neonatal septicemia remains as the major cause of mortality. We evaluate here the volume, conductivity and scatter properties of leukocytes (VCS technology) as predictor of neonatal sepsis.

Methods. 133 neonates with suspected sepsis and 36 gestation-matched controls were subjected to blood culture(gold standard), CBC, peripheral blood smear, I/T ratio and CRP. Blood samples were run on the Beckman Coulter® LH750 and LH755 hematology analyzers to provide individual cell volume, high frequency conductivity and laser light scatter for generating a research population data (RPD).

Results. ROC curves were made based on I/T ratio, immature neutrophils, band cells+immature neutrophils, CRP, mean neutrophil volume (@MNV), and @MNV+CRP. @MNV showed the best ROC curve results (sensitivity 95.5% ,specificity 82.1%, cut-off value >154.2 with AUC of 0.925). CRP showed sensitivity 78.9%, specificity 96.3%, cut-off value >7 with AUC of 0.891. @MNV and CRP combined showed sensitivity 100 %, specificity 85.7%, cut-off value of 154.8 with AUC of 0.968. These values were superior to Immature neutrophils alone (sensitivity 27.3%, specificity 89.3%, cut-off>2 with AUC 0.55) and immature neutrophils+band cells combined (sensitivity 22.73%, specificity of 96.4%, cut-off of >12 with AUC 0.597). I/T ratio showed (sensitivity 66.7%, specificity 67.9%, cut-off >0.09 with AUC 0.634). Compared to @MNV+CRP, the I/T ratio + CRP (Rochester criteria) showed lower performance (sensitivity 84.2%, specificity 92.9%, cut-off>7.04 with AUC 0.908).

Conclusion. The combination of @MNV+CRP may dramatically improve the early detection of neonatal sepsis.

@-For Research Use Only

ANEMIA – NEW INSIGHTS

R. Simon
Scientific Director Global, Beckman Coulter, Nyon, Switzerland
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Anemia of Chronic Disease (ACD) is the second most frequent cause of anemia in the general population and the first in the elderly, being many times under diagnosed. Some new parameters and insights about ACD have been developed recently, as Soluble Transferrin Receptor, IL-6 and parameters that permit to evaluate red cell hypochromia as a way to suspect iron deficient erythropoiesis.

We now have some evidence that patients with ACD, treated with recombinant Human Erythropoietin and intravenous iron, may have iron overload. The paradigm, assuming that in ACD Ferritin was an acute phase protein, not related to the amount of iron deposits, have been lately criticized, as well as the doses of intravenous iron in these patients.

Megaloblastic anemia and other causes of ineffective erythopoiesis are the third cause of anemia. B12 deficiency and folate deficiency represent a challenge, because many times they come together with Iron deficiency ,and MCV is normal.

Some new tools have been developed based on the physical properties of the leukocytes, called: Cell Population Data. These data, when used as an in-laboratory flag, may help to identify samples with anemia, trigger a review in cases where abnormal cells are present and generate the determinations of B12, folate or other tests as required.

Algorithms or decision trees, combining hematological and immunochemistry parameters, may increase the efficiency for the correct approach to anemia.

For Research Use Only. Not for use in diagnostic procedures.

Diagnostic and Clinical Importance of Measuring Vitamin D Total

Tuesday, 17 May 2011   Hall 4/5, 14:15–15:15

DIAGNOSTIC AND CLINICAL IMPORTANCE OF MEASURING VITAMIN D TOTAL

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Globally, more than a billion people are vitamin D deficient[1] and, in the United States, the NHANES III study from 2001 to 2004 indicated that 77% of U.S. adults are insufficient. Vitamin D deficiency has long been associated with bone diseases and more recently this marker has become known as a general health indicator. Associations with major conditions (eg autoimmune diseases, cancer and heart diseases etc) have been reported in epidemiologic, clinical and observational studies[2].

As a result, vitamin D testing has been increasing rapidly. Laboratories need to stay informed about how they can provide accurate and reliable vitamin D measurements to ensure their results properly distinguish patients across the spectrum from deficiency to toxicity.

During this workshop we will discuss why both vitamin D_2 and D_3 should be measured in an equimolar relationship to report a total vitamin D value and also how measurements can be obtained with different methodologies. Expected circulating levels and the consequences of deficiency and overdose will be discussed. Also, understanding how to improve the consistency of measurements will be addressed as well as reporting of studies linking vitamin D to multiple disease states and evaluating new assays.

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Troponin T High Sensitive: Applying the Guidelines to Hospital´s Daily Life

Tuesday, 17 May 2011   Hall 4/5, 14:15–15:15

TROPONIN T HIGH SENSITIVE: APPLYING THE GUIDELINES TO HOSPITAL´S DAILY LIFE

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For years cardiac troponins (cTn) have been regarded as the preferred biomarkers for the diagnosis of myocardial infarction and for the risk stratification of patients with acute coronary syndromes (ACS), as well as for the selection of patients who need an early invasive strategy, and for the guidance of adjunctive pharmacological therapy. In addition, measurement of cTn has been found useful for detection of myocardial necrosis in conditions unrelated to myocardial ischemia including acute pulmonary embolism, myocarditis, heart failure, sepsis, and endstage renal disease. In these conditions, an unfavourable prognosis is unequivocally associated with detectable concentrations of cTn.

A major limitation of most currently available cTn assays is the lack of adequate precision, i.e. to measure cTn concentrations at the 99thpercentile value with a coefficient of variation below 10% coefficient of variation (CV<10%). As a consequence, many manufacturers have developed more sensitive cTn assays that now comply with precision criteria required by the Joint European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation Task Force for the redefinition of acute myocardial infarction.

A major advantage of more sensitive assays is that a diagnosis of MI is made earlier and in a higher number of patients than with the use of standard assays. However, the number of cases with troponin elevation in the absence of ACS will also increase. As an elevation of troponin is related to an adverse prognosis, the causes of such a troponin elevation must be investigated. Possible reasons include acute, subacute and chronic cardiac disease such as heart failure, chronic pulmonary hypertension, pulmonary embolism, or cardiomyopathies.

FUTURE CALLS: CAN GDF-15 BE OF ADDED VALUE TO ACS MANAGEMENT?

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Background. Growth-differentiation factor (GDF)-15 is a distant member of the TGFß cytokine superfamily. GDF-15 is expressed in association with oxidative stress and inflammation and has been detected in human atherosclerotic plaque macrophages.

Methods. An immunoradiometric assay has been established to measure GDF-15 in patients. Recently, the assay has been adapted to the Roche Elecsys platform.

Results. The circulating levels of GDF-15 increase with age and are related with cardiovascular disease burden. The highest levels are found in patients with advanced heart failure, moderately elevated levels are detectable in non-ST-elevation acute coronary syndrome (NSTE-ACS). In contrast to cTnT and NT-proBNP, GDF-15 levels do not show the typical rise and fall pattern during an episode of NSTE-ACS but remain constant from admission up to several months after the event, indicating that GDF-15 reflects chronic (cardiovascular) disease rather than acute injury. GDF-15 levels on admission with NSTE-ACS are associated with the risk of death and recurrent MI. The prognostic information provided by GDF-15 is independent of clinical risk markers, the electrocardiogram, cTnT, and NT-proBNP. GDF-15 substantially enhances the predictive value of the GRACE (Global Registry of Acute Coronary Events) risk score. Data from the FRISC 2 trial indicate that NSTE-ACS patients with elevated GDF-15 levels on admission benefit from an invasive treatment strategy, whereas patients with normal GDF-15 levels do not. A recent analysis of more than 10,000 contemporary NSTE-ACS patients from the PLATO trial confirms this interaction of GDF-15 with treatment strategy.

Conclusions. GDF-15 enables enhanced risk assessment and may support treatment decisions in NSTE-ACS.

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NT-PROBNP GUIDED HEART FAILURE THERAPY

James L. Januzzi, Jr, MD, FACC
Associate Professor of Medicine, Harvard Medical School; Director, Cardiac Intensive Care Unit, Massachusetts General Hospital

The natriuretic peptides have been shown to be useful for diagnostic evaluation of the patient with suspected heart failure, and also afford strong prognostic value in affected patients. Regarding prognosis, while a single measurement of amino-terminal pro-B type natriuretic peptide (NT-proBNP) offers important prognostic value, serial measurements appear to provide incremental information, even in the absence of obvious clinical change. This observation is important, as a widely available, low cost, and reproducible objective tool for monitoring stability in heart failure is lacking; in addition, the observation that therapeutic interventions with favorable effects on heart failure risk may result in reductions in NT-proBNP values, in parallel with improvements in outcome suggests the marker may not only be used to "monitor" heart failure stability, but may actually be used to objectively "guide" therapy.

The intriguing concept of "guiding" heart failure therapy using NT-proBNP together with clinical judgment is a viable hypothesis that has been examined by recent studies. Through these trials (including notable prospective randomized studies from Vienna, as well as the TIME-CHF, BATTLESCARRED, and recent United States-based PROTECT study), a clearer understanding of the optimal patient for application of "guided" therapy, as well as the potential benefits of this approach have been derived.

This lecture will review the background information regarding NT-proBNP as a prognostic biomarker in heart failure, the design and results of "guided therapy" trials (including up-to-date results from PROTECT), and generate recommendations regarding the appropriate application of NT-proBNP for this monitoring and management of patients with heart failure.

Utility of Reflex Urine Culture Based on Results of Urinalysis and Automated Microscopy Using the Iris IQ200 Platform

UTILITY OF REFLEX URINE CULTURE BASED ON RESULTS OF URINALYSIS AND AUTOMATED MICROSCOPY USING THE IRIS IQ200 PLATFORM

Stefan Riedel, M.D., Ph.D., D(ABMM)
Johns Hopkins Bayview Medical Center, Department of Pathology, 4940 Eastern Avenue, Baltimore

Background. Specimens submitted for urine culture in hospital settings are frequently negative for bacteria. At a time of concern for cost containment, utilization of a reflex testing policy using specific screening criteria would be beneficial to eliminate unnecessary urine cultures.
Methods. We prospectively collected and reviewed 1248 clinical urine specimens submitted for urinalysis and/or urine culture. Specimens were collected from patients admitted to one general medical floor and the emergency department in our hospital. Urinalysis and automated microscopy was performed using the IQ200 (Iris Diagnostics, Chatsworth, CA). All specimens were cultured on sheep blood agar and MacConkey agar following standard laboratory urine culture guidelines. Cultures were considered positive if at least 10,000 colonies of a uropathogen were identified. Numbers of white blood cells (WBC) per high power field (hpf), results for nitrite, leukocyte esterase (LE), and bacteria were reported.
Results. 181 urine cultures were positive for a uropathogen. Organisms included *E. coli*, *E. cloacae*, *K. pneumoniae*, and *P. mirabilis*. 1067 cultures were considered negative. 966 specimens had a count of 0-5 WBC/hpf; 282 specimens had >5 WBC/hpf. For the WBC count sensitivity was 63%, specificity 84%, PPV 41%, and NPV 93%. The results for the other parameters were the following: sensitivity: LE 69%, nitrite 22%, bacteria 66%; specificity: LE 78%, nitrite 98%, bacteria 74%; PPV: LE 34%, nitrite 70%, bacteria 29%; NPV: LE 94%, nitrite 88%, bacteria 92%.
Conclusions. These test parameters separate or combined may be a useful screening method to determine the need for a reflex urine culture.

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TLA – The 'Lean' Alternative

TLA – THE 'LEAN' ALTERNATIVE

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Background. Over recent years Lean manufacturing principles have been applied to Healthcare. Focusing on the elimination of waste, a variety of tools and techniques are provided to achieve this aim. The workshop will briefly describe Lean principles and specifically focus on its practical application from the experiences of a UK hospital laboratory group.

Abstract. A fundamental goal of Lean is to achieve a continuous flow of work through a process. Batch processing is inherently inefficient causing delays and bottlenecks. Simply applying Lean to improve work flow, Path Links has achieved dramatic improvements to productivity and quality.

Path Links has implemented a Lean workcell concept as an alternative approach to deploying highly automated 'tracked' systems. Based on proven manufacturing production design, the Lean Workcell brings together all major laboratory analysers in a U-shaped configuration. Whilst providing most (if not all) of the benefits of a Total Laboratory Automation solution, the Lean Workcell has advantages in providing greater flexibility, significant reduction in space requirements and avoids the large investment and ongoing maintenance requirements of a tracked system.

The strength of a Lean approach is to fully understand and optimise work processes and use this knowledge to guide the correct choice of equipment. In other words, to make the equipment fit the process rather than fitting a process around the equipment. Lean processes aim for right-sized equipment which is usually smaller, less complex, more flexible and less expensive than highly automated systems.

Increasing Testing Efficieny in Laboratory Organizations from Pre to Post-Analytics

INCREASING TESTING EFFICIENCY IN LABORATORY ORGANIZATIONS FROM PRE TO POST-ANALYTICS

C. Brown
Head of Roche Professional Diagnostics, Roche Diagnostics Ltd., Rotkreuz, Switzerland.

Today, laboratories are challenged to deliver consistent high-quality analyses to support medical decision making while at the same time ensuring efficient analytical workflows by addressing costs, speed and quality of results. To meet all of these demands, Roche has developed the *cobas*® Modular Platform concept that delivers individualized solutions based on a common architecture for various workloads and testing requirements. We offer instruments to match different throughputs: low with the *cobas* 4000 analyzer series, medium with the *cobas* 6000 analyzer series and high with *cobas* 8000 modular analyzer series. We also offer a complete menu for these instruments to cover the entire Serum Work Area (SWA) spectrum.
With true SWA consolidation, 99% of samples can be handled without the need for manual intervention, additional aliquoting, or use of extra stand-alone analyzers.
From routine assays to innovative biomarkers, all *cobas* instruments use the same standardized reagents and the *cobas* c pack and *cobas* e pack cassette concept ensuring full parameter menu availability and improved consistency of results across the entire *cobas* (analyzer portfolio for increased confidence when supporting medical decision making.
Another critical issue we are addressing is helping laboratories improve all elements of workflow and data integration.
Connection to pre-and post-analytics greatly reduces hands-on time and opportunities for error allowing a laboratory to be significantly more efficient
Roche has a very strong offering in both Total Lab Automation (track) and Task Targeted Automation (stand-alone) pre- and post–analytics. Examples of Laboratory Organizations using *cobas* Modular Platform solutions together with pre and post analytics from Roche will be presented in this session.

OPTIMIZING A UNIVERSITY LAB

Dr. U. Steigerwald, MD
Director of Laboratory Medicine, University Würzburg, Institute of Clinical Biochemistry and Pathobiochemistry, Laboratory Medicine

Background. The new building of the Central Laboratory of University Hospital Würzburg was combined with the revision of all laboratory processes. Main objectives were the automation of routine processes with high quality and standardization, especially improved pre- and postanalytical quality and procedures, a significant reduction of allocated personnel, reduced TAT and covering future increases of performance.
Results. Different concepts were investigated, evaluated and decided through a tender process. The University Hospital has chosen a solution from Roche Diagnostics. The analysis of clinical chemistry, immunology and serology is processed on the consolidated systems cobas 6000 and cobas 8000. For these systems a high amount of parameters, in easy to handle reagent cartridges, is available. Thus, many tests can be performed on a system at the same time. The modular design offers a variety of expansion options. As a central sample entry an aliquoting system RSA Pro with integrated double centrifuges is used. RSA Pro checks the receipt of all samples, the required volumes and distributes the samples to the different working places. Afterwards, serum samples are automatically processed by the system MODULAR PRE-ANALYTICS with the connected systems cobas 6000 and cobas 8000. An integrated backup concept for all components ensures the 24 / 7 availability of the laboratory.
Conclusions. The automation of routine processes has led to higher quality and a reduction of allocated personnel. Rapid result availability at the wards and high-quality analysis show the efficiency of the laboratory. Flexible expansion options allow quick adjustments on site. The professional implementation of the project supported by Roche Diagnostics has led to a smooth transition to routine.

THE EVOLUTION OF A HANDS-ON TOWARDS A HANDS-FREE LABORATORY

F. Winnock
Laboratory association ASZ-AZO, Algemeen Stedelijk Ziekenhuis, Aalst, Belgium

The laboratory association ASZ-AZO is a hospital lab working with the ASZ group with hospitals in three Belgian cities (Aalst, Geraardsbergen, Wetteren) and the AZO hospital in Oudenaarde. Together these hospitals represent almost 850 beds. A special situation in our lab is the collaboration with a large number of general practitioners. This represents about a quarter of the workload. In contrast with the hospital work, general practitioners' samples are processed in the afternoon, which permits an optimal utilization of the laboratory equipment.

At the beginning of 2002, after the association of the hospitals of Aalst and Wetteren-Geraardsbergen, the workload in the core lab Aalst was exploded, which drove us towards automatisation of the pre-analytical phase. We implemented the RSD800 (PVT) in 2004. At that time we worked with two Integra800 analyzers and a Modular EE for chemistry and immunochemistry. The Integras were substituted by two Cobas6000 <501> analyzers in 2007 at the time of the association with the AZO hospital.

The introduction of the RSD800 in 2004 resulted in an acceptable turn-around time (one hour for most of the chemistry tests). However, due to a continuous growth of the number of requests in the last three years the turn-around time of the labtests deteriorated significantly. At peak moments more than 1500 chemistry tests per hour were demanded, which was more than the capacity of the chemistry analyzers. The implementation of a Cobas 8000 <701-701-502> and a Cobas 8000 <602-602-602> in December 2010 provided a solution for the capacity problem and resulted in a reduction of the turn-around time for chemistry parameters to about 15 minutes. No significant reduction in answering time for immunochemistry tests was noted (due to the intrinsic time necessary for immunochemistry tests). A diversification of the immunochemistry testpanel has no consequences for the turn-around time.

In order to meet the growing need for a shorter turn-around time we were forced to revise our work method for the pre-analytical phase. At peak moments only serum tubes are put on the RSD800, whereas at non-peak times all tubes are put onto the RSD (archiving included). This way of working gives us acceptable turn-around times for both chemistry and immunochemistry tests and gives us the opportunity to consolidate more immunochemistry tests.

Counting the Cost of the Preanalytical Phase

COUNTING THE COST OF THE PREANALYTICAL PHASE

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The Impact of Preanalytical Errors on Patients Treatment. Various studies that have investigated the extent of errors in laboratory diagnostics have concluded that those arising in the preanalytical phase are prevailing, up to 70%. Although most of these did not influence the managed care, in up to one-fifth of the cases they might be associated with inappropriate investigations, inappropriate care or unjustified therapeutically changes, thereby jeopardizing patients' health and increasing healthcare expenditure.
Understanding the Cost of the Preanalytical Phase in an Outpatient Setting. Normally in the process of reorganization of Laboratory Medicine, costs are compared before and after the reorganization. However, they almost never highlight the costs relating to the preanalytical processes of phlebotomy and the subsequent transportation of biological samples to the laboratory. Data will be presented from a collaborative survey of 20 laboratories on the cost of the preanalytical phase. Based on the survey, the average cost for the preanalytical phase represents 10% of the total cost for instrumentation, reagents and personnel of the entire analytical process.
Discovering the Opportunity Cost of Preanalytical Errors. We have used a cost model that allows investigation of the impact of repeated blood collections. The central measure of this model is the extended length of patient stay or the delay in hospital processes due to preanalytical errors. In an evaluation study of the model conducted in other hospitals, it was revealed that the opportunity cost associated with preanalytical errors can be as much as 372 € per hospital bed and year.

New High-Performance Assays for the Determination of Free Light Chains Kappa and Lambda – Adding Consistency to Monitoring and Screening of Multiple Myeloma

Tuesday, 17 May 2011   Hall 9, 16:45–17:45

NEW HIGH-PERFORMANCE ASSAYS FOR THE DETERMINATION OF FREE LIGHT CHAINS KAPPA AND LAMBDA – ADDING CONSISTENCY TO MONITORING AND SCREENING OF MULTIPLE MYELOMA

C. Pruemper[1], H. te Velthuis[2], R.M.J. Hoedemakers[3]
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Background. High serum levels of free light chain (FLC) kappa or lambda are a marker of plasma cell dyscrasia. Serum assays for automated measurements are commercially available; however the technical performance of these assays is under debate.
Methods. In cooperation with Siemens Healthcare Diagnostics we developed new, latex-enhanced, specific nephelometric assays based on monoclonal antibodies for the determination of FLC kappa and lambda in serum, EDTA plasma and Li-heparin plasma for use on the Siemens BNä systems.
Results. Reference ranges were determined from testing 369 serum and plasma samples from healthy donors: FLC kappa 6.7-22.4 mg/L, FLC lambda 8.3-27.0 mg/L and FLC kappa/lambda ratio 0.31-1.56. Antigen excess is secured with the built-in pre-reaction in the assay protocols. Lot to lot consistency between three different reagent lots, including reagent lot independent standards and controls, showed a normalized difference below 7.5%. The total reproducibility of serum samples varied between 4 to 7%. Method comparison with Freelite™ assays (The Binding Site, Birmingham, UK) included 647 samples of 541 patients. When classifying the results as abnormal low, normal, and abnormal high, concordance was 91.3% for the FLC kappa assays, 85.0% for FLC lambda and 94.8%, for the kappa/lambda ratio. Correlation coefficients (r_s) were 0.92 for FLC kappa, 0.91 for FLC lambda, and 0.77 for kappa/lambda ratio.
Conclusion. N Latex FLC demonstrates high precision and lot to lot consistency. Comparison with Freelite™ showed a high agreement rate for result interpretation despite a moderate numerical result correlation.

Clinical MSMS – New Developments in Today's Laboratory

CLINICAL MSMS – NEW DEVELOPMENTS IN TODAY'S LABORATORY

A. Cohen[1], H.J. Roth[2] and L. Thienpont[3].
[1]Hormone/Neonatal Mass Spectrometry, Statens Serum Institut, Copenhagen, Denmark. [2]Endocrinology and Oncology Department, Limbach Laboratory, Heidelberg, Germany. [3]Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium.

Background. There is growing interest in the potential of tandem mass spectrometry for routine analysis in clinical laboratories. Its benefits include improved specificity and the ability to multiplex. However, for the method to gain wide application, IVD medical device standardization and quality is essential.

Methods. In both steroid assay and vitamin D assay we describe experiences in implementing tandem mass spectrometry technology. Although the direct immunoassay techniques currently widely used in steroid assay have merit, they suffer from lack of specificity in the low concentration range. Similarly, in Vitamin D analyses, immunoassays, though still the most widely used technique, may not be ideal.

Results. In transitioning from immunoassays to LC-MSMS in steroid analyses, a number of challenges have been faced, but it has been possible to increase the steroid panel and implement on-line extraction of the samples.

The discovery that most cells in the body have a vitamin D receptor and are able to convert 25-hydroxyvitamin D [25(OH)D] to its active form has provided new insights in the non-musculoskeletal function of this vitamin. However, measurement of 25(OH)D is not an easy task. Due to continuing development of equipment and methods, tandem mass spectrometry is increasingly becoming accepted in clinical laboratories.

Conclusions. Mass spectrometric assays that compete with established in vitro diagnostic assays, typically based on immunochemistry offer confirmed benefits in both steroid profiling and in Vitamin D assay. However, with regard to CE-marking or FDA-approval, the new generation products need to be treated with the same scrutiny as the old.

The Use of Multiplex Assays for Determining Predisposition to Hypertension

THE USE OF MULTIPLEX ASSAYS FOR DETERMINING PREDISPOSITION TO HYPERTENSION

Dr Sophie Visvikis-Siest, BSc PhD
Université Henri Poincaré, Nancy, France.

Background. In spite of numerous candidate gene and genome-wide association studies (GWAS), only limited progress has been made in discovering the genetic background explaining the high heritability of blood pressure (BP) interindividual variability and of hypertension (HTN). A possible explanation is that studies until now have not incorporated ,in parallel, the various candidate genes already established to participate in the different biological pathways involved in BP regulation. Therefore, performing multiplex analyses taking into account these genes may be essential in identifying the genetic component of HTN.
Methods. Using a multiplex technology, twelve genetic variants in 11 candidate genes from various biological pathways (BP regulation, inflammation, lipid metabolism, cellular adhesion, and thrombosis) were genotyped in 3,433 French middle-aged individuals in order to study their effects on BP and HTN.
Results. Two among the 12 studied SNPs were significantly associated with BP traits (Systolic and Diastolic blood pressure) and HTN, whereas one was found specifically associated with BP.
Conclusions. The present findings provide insight into potential candidate pathways that may modify hypertensive risk.

Novel Markers in Reproductive Endocrinology

Wednesday, 18 May 2011   13:00–14:00

ANTI MULLERIAN HORMONE: A MARKER OF OVARIAN RESERVE

Professor Richard Anderson
Edinburgh, UK

Anti Müllerian Hormone (AMH) is produced by granulosa cells of preantral and antral ovarian follicles. AMH concentrations in females are maximal following puberty and decline until the menopause, when AMH is immeasurable. Profiles of the AMH* decline through reproductive life appear to mirror the decay in the number of non-growing primordial follicles – the ovarian reserve. Correspondingly, one of the main clinical utilities of the assay is in the assessment of ovarian reserve, which allows prediction of response to controlled ovarian stimulation and may have a role in predicting time to the menopause.

ANTI MULLERIAN HORMONE: DOES IT HAVE A ROLE IN POLYCYSTIC OVARIAN SYNDROME (PCOS)?

Dr Julian Barth
Leeds, UK.

PCOS is a relatively common syndrome of ovarian dysfunction affecting 5-10% of women. It has cardinal features of hyperandrogenism and polycystic ovary morphology. However there is no single diagnostic criterion which is sufficient for diagnosis. Serum AMH* is markedly elevated in women with PCOS and increased concentrations have also been reported in the healthy pre-pubertal daughters of PCOS mothers, suggesting that AMH may be a marker of developing ovarian follicles from an early age. There is a growing debate of the role and value of AMH in PCOS.

EXPERIENCE WITH NEW BIOMARKERS OF PREECLAMPSIA (PE)

Professor Vassilis Tsatsaris
Paris, France

PE is a major cause of maternal, fetal and neonatal morbidity and mortality. Clinical evidence is beginning to demonstrate that early detection may enable early intervention to prevent or reduce the severity of the condition. Data will be presented to show that, in a general population where the prevalence of PE is low, free PlGF**, Inhibin-A* and PAPP-A* (assessed between 14 and 18 weeks of gestation) appear to be useful markers in differentiating women who later develop PE from those with a normal pregnancy.
*Not intended as off-label promotion of any Beckman Coulter products.
**In development, pending achievement of CE compliance; not yet available for in vitro diagnostic use.

FreeliteTM and HevyliteTM in Monoclonal Gammopathies: Improving Diagnosis, Monitoring and Prognosis Assessment

FREE LIGHT CHAIN MEASUREMENT IN SERUM - CLINICAL USE AND LABORATORY HANDLING

Giampaolo Merlini M.D.
Director Amyloidosis Research and Treatment Center, University of Pavia

Providing an up to date background on *Freelite* serum free light chain measurement.

WHY SERUM FREE LIGHT CHAIN TESTING IS CRITICAL IN PATIENTS WITH RENAL IMPAIRMENT

Dr Colin Hutchison
Clinical Lecturer, Renal Institute of Birmingham, University of Birmingham and University Hospital Birmingham

Detailing how *Freelite* serum free light chain testing can identify patients at risk of kidney failure and be used in association with a ground-breaking new treatment combination which may enable some patients to regain dialysis independence.

HEAVY/LIGHT CHAIN ANTIBODIES FOR MONITORING AND PROGNOSTICATION IN PATIENTS WITH MYELOMA

Prof. Dr. Heinz Ludwig
1st Department of Medicine, Center for Oncology and Haematology

Showing the evidence for use of the Hevylite assay in myeloma patients for both prognostic assessment and monitoring of therapy.

Vitamin D Dietary Reference Intakes: Implications and Significance in Clinical Practice

Bruce Hollis[1], Stefan Pilz[2], Reinhold Vieth[3]
[1]Darby Children's Research Institute, Medical University of South Carolina, Charleston, SC, USA; [2]Medizinische Universität Graz, Klinische Abteilung für Endokrinologie & Nuklearmedizin, Graz, Austria; [3]Department of Nutritional Sciences, and Department of Laboratory Medicine and Pathobiology, University of Toronto Director, Bone and Mineral Laboratory, and Director, Point-of-Care Testing Pathology and Laboratory Medicine, Mount Sinai Hospital Toronto, Ontario.

The much anticipated United States IOM (Institute of Medicine) report on Calcium and Vitamin D has set forth new dietary reference intakes which take into account nearly 1,000 published studies and testimonials from scientists and stakeholders. The report confirms the role of Vitamin D and Calcium in promoting skeletal growth and the amount needed to maintain bone health. At present there still remains a lack of consensus about the amount of Vitamin D necessary to avoid deficiency and whether higher levels are required for sufficiency. The IOM report focused on dosing rather than the attainment of adequate serum levels of Vitamin D. Certainly, a "one size fits all" DRI cannot assure that targeted serum levels of Vitamin D are realized in individual subjects. Physicians need accurate serum Vitamin D measurements to implement and promote effective, personalized medical care.

Roche – Delivering on the Promise of Personalised Healthcare

ROCHE – DELIVERING ON THE PROMISE OF PERSONALISED HEALTHCARE

T. S. Gutjahr
Roche Diagnostics; F. Hoffmann-La Roche Ltd, Basel, Switzerland

The rapidly increasing ability to identify the root cause of diseases at the molecular levels has ushered in an era of targeted drug development. New compounds can be customized to match disease specifics, offering an alternative to the traditional broad-spectrum approach when creating treatment concepts. The interplay of latest technology and biology is at the core of Roche's strategy for Personalised Healthcare (PHC).

The 'Personalised Healthcare' approach to understanding disease nuances and creating accompanying therapeutic answers is the highest priority at Roche, now and into the future. *Fitting the treatments to the patients*, the leading principle Roche associates with PHC, adds medical value to patients and physicians as well as economical value to society and insurers in a dimension never before seen in the healthcare industry. With thriving Diagnostics and Pharma divisions, Roche is already delivering on its promise to make Personalised Healthcare a reality, e.g. in breast- and gastric cancer, hepatitis B and C infections or osteoporosis. The current pipeline is poised to continue delivering novel tests and solutions in 2011 and beyond: currently six 'New Medical Entities' for the treatment of breast-, lung, and skin cancer, hepatitis C infection and asthma are in Roche's late-stage pipeline, along with corresponding patient stratification tools.

BIOMARKERS FOR MANAGEMENT OF BREAST CANCER AND OTHER TUMOR ENTITIES

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Background. Nowadays, biomarkers are becoming of key players for management and drug development in cancer patients. In this respect breast cancer (BC) provides a paradigm where biomarker analyses contribute both to molecular sub-grouping and therapy decision making [1].

Methods. Current status of established biomarkers and test platforms will be demonstrated based on data from literature and own experience with Her2 testing in BC and gastric cancer (GC) [2,3]. The importance of standardization, quality control, training and improvement of test platforms will be discussed. Finally, future developments based on multigene analyses as well as *-omics* technologies will be addressed.

Results. Hormone receptor testing and determination of Her2 status have become mandatory and the standard of practice for invasive breast cancer [1]. However, application of the same marker in a different tumor such as GC needs adaptation of instructions [4]. Besides updated guidelines [5,6] quality measures such as QuiP in Germany and training efforts have to be taken. Recently, multigene analyses, such as Oncotype Dx® and Mammaprint®, have been used to tailor chemotherapy in ER+ Her2 negative patients. Prospective studies are underway to validate these first generation genetic signatures for clinical use. Finally, developments in optimization of platforms for single and multi-molecular markers and high-throughput approaches related to –omics technologies will be addressed.

Conclusions. Biomarkers play a key role for the concept of personalized medicine. However, prior to inclusion in daily patient´s management they need to be validated in clinical trials and should be affordable by health care systems. Whether in the near future biomarker testing will be performed in a more centralized (reference lab based) or decentralized fashion, e.g. in the context with routine diagnostic pathology, will largely depend on reliability of testing and cost effectiveness of available test platforms.

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Photometric Assays for Determination of Sodium, Potassium, Chloride and Lithium

PHOTOMETRIC ASSAYS FOR DETERMINATION OF SODIUM, POTASSIUM, CHLORIDE AND LITHIUM

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Determination of electrolytes in serum and urine is usually performed via ion selective electrode (ISE) measurement. This method pays off for laboratories with high test throughput but may be less cost-effective for laboratories with lower test quantities due to high maintenance costs. Hence photometric electrolyte assays are an alternative to ISE equipment, provided that test performances like precision and sensitivity are comparable. Additionally, the replacement of separate ISE units by photometric tests could improve work-flow and reduce data transfer error.
DiaSys Diagnostic Systems GmbH will introduce photometric assays for determination of sodium, potassium, chloride and, additionally, of lithium. The tests can be applied on automated and semi-automated systems, as well as on photometers. They provide high test precisions, comparability to ISE measurement and traceability to respective reference methods.

HANDS-ON EXPERIENCE WITH RESPONS®910 DURING EXTERNAL EVALUATION

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Background. Routine versatility of a new bench top clinical chemistry analyzer by cross checking several items.
Methods. Technical inspection, method comparison and analytical view within a clin lab workflow.
Results. By checking motors, drives, pipettes and pumps it is a solid engineered and clearly constructed system. In ari-conditioned facilities reagents can be hold on board during the stability time, 21 up to 56 days, without any cooling or bottle-recapping. Nevertheless the intra-assay precision is <3% CV (such as Crea-J 0,78%; ALT p5p 0,90%; Triglyceride 1,29%) and recovery in inter-assay precision measured very well (such as CRP 100,9%; GGT 96,9%; Gluc-HK 100,1%).
In method comparison we found out fine accordance in clinical important areas (such as Gluc-HK Passing Bablok; y=0.981, n=100; UREA Passing Bablok; y=1,059, n=100; CRP Passing Bablok; y=0.960, n=100).
Specimens could be drawn in standard serum tubes or several other cups, barcode can be read and asked to LIS (host-query).
The software allows starting STAT samples during a run gives overview about the running status completely and makes use of quality control management.
The photometer (12 wavelengths) is quickly ready to use after system start and reaction measured points can be displayed.
Conclusions. respons®910 shows a nearly equal behaviour in comparison to established clinical chemistry analyzers. This random access system is characterized by excellent performance data, minimal energy consumption, basic overview and good walk away capacity.
It is easily to handle and well-suited for small clin labs (bigger labs for flexible backup) or doctors' offices.Current and future perspectives in drugs of abuse screening; The multiplex approach

Current and Future Perspectives in Drugs of Abuse Screening; The Multiplex Approach

THE EVALUATION OF THE FIRST MEPROBAMATE, ZOPICLONE, ZOLPIDEM AND ZALEPLON ANTIBODIES USING BIOCHIP MULTIPLEXING TECHNOLOGY FROM RANDOX IN PLASMA, SERUM AND WHOLE BLOOD

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Objectives. Meprobamate is probably the molecule that causes most trouble today in French Hospital Toxicology. Indeed, this compound is often prescribed, and as a result often found in attempted suicides and suicides due to its high toxicity levels. However there is no immunochemical test that allows it to be quickly detected. In the same manner the "3 z's" zolpidem, zopiclone and zaleplon (still not sold in France), which are hypnotic molecules similar to benzodiazepines, don't give any positive results with any of the immunochemical tests sold today for the detection of benzodiazepines. The goal of the study is to test 4 kits developed at our request by Randox®, which are to be carried out against these molecules.

Methods. For meprobamate: 20 negative and 108 positive samples were analysed (38 samples of Post-Mortem whole blood coming from patients and 70 samples of blood plasma, blood serum and whole blood spiked with 5, 10, 25, 50, 100, 250 and 333 mg/L). The samples are all diluted by 1/200 before they are Biochip tested on the semi-automated Evidence Investigator analyser (Randox®). A 9-point calibration curve is made on the machine between 0 and 500 ng/mL. For zolpidem, 65 treated patient samples, 51 spiked samples and 20 negative samples were analysed. For zopiclone, 35 patient samples, 65 spiked samples and 10 negative samples. Finally for zaleplon, 51 spiked samples and 10 negative samples. The samples are diluted by 1/20 before Biochip analysis. A 9-point calibration curve is made between 0 and 100 ng/ml. All the samples are simultaneously confirmed either in GC/MS for meprobamate or in LC/MS/MS for the "3 z's".

Results. All samples without meprobamate are negative. The highest result obtained on a sample without meprobamate is 0,32 mg/L. A threshold of 0,5 mg/L for Positive results can therefore be used. With this threshold of 0,5 mg/l, the sensitivity of the test is 97,2% and the specificity is 100%. Only 3 samples of subtherapeutic concentration (3 to 5 mg/L) were not detected. A good correlation is obtained between the GC/MS and the Biochip Technique (r=0,84) allowing for a very interesting semi quantitative result for this molecule where the toxicity is in correlation with the blood concentration. For the "3 z's" with the low threshold still used of 0,5 ng/mL, the sensitivity of the test is 99,1% for zolpidem, 100% for zopiclone and 98% for zaleplon. The specificity is 85% for zolpidem, 60% for zopiclone and 91% for zaleplon. This relatively low specificity is explained by 3 "false positives" in zolpidem and 4 in zopiclone but only in the real samples of treated patients. Although, the antibody tested against these 2 molecules also intersects with their metabolites (71% with phenyl carboxylic zolpidem, 55% with N-oxide zopiclone and 112% with N-desmetyl zopiclone) allowing to explain the positive patient results while the parent compound is totally eliminated.

Conclusion. The 4 tests evaluated show excellent results with very low positivity thresholds allowing their use in Hospital Toxicology from today.

Traceability and Uncertainty of Catalytic Activity Measurements

Wednesday, 18 May 2011   15:30–16:30

UNCERTAINTY OF THE PRIMARY REFERENCE SYSTEMS

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Background. The ISO 18153 describes the way to achieve standardization of enzyme measurements via the development of a reference measurement system. Reference laboratories (accredited according to ISO 15195) provide results of measurement with an associated uncertainty using primary reference measurement procedures and certified reference materials, key elements of the reference measurement system.
Methods. Reference laboratories have to establish their uncertainty budget estimating the contribution of different components to the measurement uncertainty. The steps to follow are: identification of the possible sources; estimation of standard uncertainties and sensitivity coefficients; determination of combined standard uncertainty; and expression as expanded uncertainty. g-Glutamyl-transferase (GGT) has been used as example to illustrate the estimation of measurement uncertainty using a primary reference procedure and to describe the certification of catalytic concentration value in a reference material.
Results. The main uncertainty components were: inter-assay variation 0.41 %, lot of the reagents 0.29 %, wavelength 0.16 %, absorbance 0.15 %, pH 0.04 %, linearity 0.04 %, and volume fraction of sample 0.02 %. The estimated expanded uncertainty for GGT measurement was 1.91 % with a coverage factor corresponding to a level of confidence of 95 %. The certified value assigned to the ERM-AD452/IFCC reference material was (1.90 ± 0.04) mkat/L (n=12 laboratories). Components of uncertainty were inter-laboratories variation 0.37 %, homogeneity 0.08 % and long-term stability 0.61 %.
Conclusions. Reference laboratories are able to measure catalytic concentrations for certification of reference materials using primary reference measurement procedures and with a very small expanded uncertainty of measurement.

TRACEABILITY AND UNCERTAINTY OF THE VALUES ASSIGNED TO COMMERCIAL CALIBRATORS

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Background. The European Directive 98/79/EC requires that the metrological traceability of values assigned to commercial calibrators be assured through available reference systems (materials and procedures) of a higher order. ISO 18153 describes the traceability chains and the procedures to assure traceability of catalytic concentration measured values. The Committee on Reference Systems for Enzymes (IFCC) has described primary reference procedures and has certified reference materials for several enzymes. Valid traceable calibration also requires adequate calibrator commutability.
Methods. Human serum samples from local laboratories. Calibration materials: ERM-AD452 (GGT) and BioSystems Biochemistry Calibrators (bovine and human). Selected measurement procedure: adaptation of the IFCC primary reference procedure for GGT to an analyser (BioSystems A25).
Results. A value of GGT catalytic concentration was assigned to the commercial calibrators (bovine and human) by using a certified reference material (ERM-AD452) as calibrator and a selected measurement procedure. The corresponding expanded uncertainty (with a coverage factor corresponding to a level of confidence of 95 %) was 1,9 and 2.2 %. Both calibrators demonstrate to be commutable with human serum samples.
Conclusions. The use of calibrators that are commutable and traceable to reference systems allow to obtain measurement results of catalytic concentration using routine procedures, that are equivalent to those that would be obtained using the primary reference measurement procedure.

TRACEABILITY AND UNCERTAINTY OF THE PATIENT MEASURED VALUES

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There are mainly three types of properties examined in the clinical laboratory: nominal properties (e.g. the taxon of bacteria in urine), ordinal quantities (e.g. arbitrary concentration of *Brucella abortus* antibody in plasma), and scalar quantities (e.g. catalytic concentration of g-glutamyltranferase in plasma).

Measurements are made to know the values of scalar quantities. According to the last edition of the International Vocabulary of Metrology, it is important to distinguish between measured value and measurement result: a measured value is the numerical value (and a unit) obtained from the measurement, and it becomes a measurement result when the measurement uncertainty is included.

To estimate measurement uncertainty it is necessary to know the day-to-day imprecision of the measuring system and the uncertainty of the calibrator-assigned values.

Measurement uncertainty is important in clinical practice by two reasons: (i) when two results of the same samples are obtained by two different measuring systems, lower measurement uncertainty indicates higher metrological quality; and (ii) it facilitates the detection of significant changes between consecutive measurements of the same quantity in the same patient.

Two precautions should be taken into account for estimating the measurement uncertainty of catalytic concentration measured values: the commutability of the calibration and control materials and the heteroscedasticity related to the measuring system. Also, as measurement uncertainty is theoretically not related to systematic error, measurement results should be traceable to reference material to ensure the absence of bias. Fortunately, such a reference materials have been produced to standardize measuring systems for catalytic concentrations.

POSTER ABSTRACTS

Ageing

0001

COMPARISON OF OXIDATIVE DAMAGE PARAMETERS IN KIDNEY BETWEEN D-GALACTOSE INDUCED AND NATURALLY AGED RATS

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Background. The aging related renal insufficiency has important implications with regard to impaired redox homeostasis. Current study focused on protein, DNAand lipid oxidation products as well as protein bound sialic acid (SA) in kidney tissue of D-galactose induced agingrats, naturally aged rats and their respective young control group.
Methods. Intraperitoneal injection of d-galactose (60 mg/kg/day) for six weeks to young male Sprague-Dawley rats (20-week-old) used to establish mimetic-aging model. In the current study, we investigated the tissue levels of protein carbonyl groups (PCO), *various* thiol fractions like total(T-SH), protein(P-SH) and non-protein thiol groups(NP-SH),lipid oxidation parameters such as lipid hydroperoxides (LHP) and malondialdehydes (MDA). Our study also covered SA and 8-hydroxy 2'deoxiguanosine (8-OHdG) parameters in kidney tissue.
Results. In D-galactose induced aged ratsPCO, MDA, LHP, and 8-OHdG concentrations were significantly higher than young control group, whereas T-SH, P-SH levels were significantly lower than young rats.In addition,Np-SH and SA concentrations were not found to be different between mimetic-aging and young control groups. In naturally aging rats PCO and MDA levels were found to be significantly higher, T-SH, P-SH, NP-SH concentrations were lower than young controls. On the other hand, SA and 8-OHdG levels were not found to be different between naturally aging group and young control group.
Conclusions. Our results that those in the mimetic aging group share significant similarities in terms of impaired redox homeostasis with the naturally aged rats and can be used as a reliable animal model of kidney aging.

0002

CLUSTERIN/APOJ EXPRESSION IN PLASMA SAMPLES OF AN ELDERLY SARDINIAN POPULATION

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Background. Clusterin (CLU) is a glycoprotein with a nearly ubiquitous tissue distribution that has been reported to be implicated in several physiological processes as well as in many pathological conditions including ageing, diabetes, atherosclerosis, degenerative diseases and tumorigenesis. We studied how clusterin protein expression varies with age in a sardian population charachterized by a great number of centenarians.
Methods. A proteomic approach was used with 2D-PAGE-MS analyses, western immunoblotting and ELISA to perform comparisons in 68 individuals divided by chronological age into four groups.
Results. The typical train of spots of the clusterin protein in a 2D plasma map was examined and according to statistical analyses, 5 spots were detected as being differentially expressed within the four groups (ANOVA p-value<0.05) and their identity confirmed by MS analyses. Regarding total clusterin the results obtained with a commercially available ELISA kit agree with the data shown by literature with an increase of protein expression until 90 years. We extended the analyses to older people showing that after 90 years a decrease in plasma clusterin levels seems to occur expecially in centenarians.
Conclusions. The proteomic approach used allowed us to highlight differences in the clusterin isoforms between people of different ages. Sardinian centenarians showed a decrease in clusterin protein levels in plasma samples. Considering that clusterin is a sensitive biosensor of oxidative stress it could be in accordance with the fact that centenarians, "a model of successful ageing", seems to be characterized by low levels of ROS (radical oxygen species).

0003

PROTEIN AND DNA OXIDATION IN DIFFERENT ANATOMIC REGIONS OF RAT BRAIN IN BRAIN AGING MODEL INDUCED BY D-GALACTOSE

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Background. It has been reported that D-galactose administrationlead to increase in both oxidative and osmotic stress in various tissues of rodents.
Methods. In our study, we established brain aging model induced by D-galactose and investigated the concentrations of oxidative stress markers on hippocampus, parietal and frontal lobs of male Sprague-Dawley rats. Intraperitonal injection of D-galactose (60 mg/kg/day) to the experimental group for 42 daysuses to establish mimetic aging model. At the end of this period, we tested spatial memory with Morris Water Maze test. To investigate the magnitude of oxidative protein, lipid and DNA damage, we studied the concentrations of various oxidative stress parameters in tissues of hippocampus, parietal and frontal lobs. Glial and neuronal damage was histologically observed in each of the three anatomic regions of brain.
Results. Protein carbonyl groups and advanced oxidation products concentrations of D-galactose administrated group were found significantly higher than in the control group for each of the three brain lobs. Total thiol concentrations were found to be decreased in the parietal lobe. A concurrent increase in lipid hydroperoxides was also observed in this lobe of brain. On the other hand, 8-hydroxy2'-deoxyguanosine concentrations were significantly increased in hippocampal tissue of experimental group rats when compared to controls.
Conclusions. Our current results demonstrated greater susceptibility to oxidative protein, lipid, and DNA damage in different brain regions of D-galactose-induced aging rats. It is necessary to clarify optimal D-galactose dosage and duration of administration for *the establishment* of an *effective aging model in futurestudies.*

0004

SERUM RESISTIN IN ELDERLY PERSONS WITH OR WITHOUT METABOLIC SYNDROME

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Background. A population of elderly people is growing. Researches concerning metabolic syndrome elements are currently discussed. The analysis of adipose tissue hormones in elderly persons are proposed to mediate biochemical status between abdominal fat and other tissues. *The aim* was to investigate serum resistin concentration of elderly persons in with or without metabolic syndrome.
Methods. 234 Caucasians, healthy elderly persons, from Poznań metropolitan area, with no acute disease or severe chronic disorder were assessed. All persons were qualified for an oral glucose tolerance test (OGTT). During OGTT fasting (G 0') and 2h-glycemia (G 120') (bioMérieux, France) were determined, and type 2 diabetics excluded. After overnight fast plasma lipids: total cholesterol (T-C), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triacylogliceroles (TAG) (bioMérieux, France), fasting insuline (Ins0') and 2h-insulinemia (Ins 120') and resistin (Res) (Biocom, Belgium) were estimated. Finaly groups: metabolic syndrome, MetS (n=29; 70,5±4,8 years), and non-metabolic syndrome, non-MetS (n=25, 70,3±8,2 years).
Results. The studied groups did not differ in their age, Ins 0' and Ins 120' and lipid profile. Resistine was higher in MetS (11,9±3,8 ng/ml) vs. non-MetS (9,6±2,6 ng/ml) (p=0,01696) group. The MetS group had possitive corelation for Res&T-C (R=0,46; p=0,011), Res&LDL-C (R=0,40; p=0,03), Res&TAG (R=0,49; p=0,007) and negative for Res&age (R=-0,47; p=0,01). No correlation in non-MetS group were observed.
Conclusions. Different metabolic factors seem to contribute serum resistine concentration in elderly in the presence or absence of metabolic syndrome. Unraveling the pathophysiological roles of resistin in metabolic syndrome in elderly people may result in new pharmacotherapeutic approaches.

Ageing

0005

CANDIDATES FOR A TELOMERE POSITION EFFECT IN HUTCHINSON-GILFORD PROGERIA FIBROBLASTS

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Background. Most normal human cells lack the enzyme telomerase, which maintains telomere length. As a consequence, progressive telomere shortening occurs with each cells division, and eventually leads to replicative senescence. Much controversy exists if transcriptional silencing of genes adjacent to telomeres, called telomere position effect (TPE), may trigger replicative senescence in human cells.

Methods. Using Affymetrix gene expression chips, we compared the gene expression profiles of three different types of senescence: fibroblasts from patients with Hutchinson-Gilford progeria (HGP) as a model for replicative aging; fibroblasts, in which senescence was induced by UV radiation; and HGP-fibroblasts transfected with the catalytic subunit of human telomerase (TERT) to overcome and revert effects of replicative senescence.

Results. Gene expression of 10.047 genes was significantly altered in the HGP subgroup, compared to healthy control cells. Gene expression of 9813 genes was altered in the UV subgroup, with an overlap of 4049 genes. Within these subsets we found 11 potential candidates for a TPE, matching the following criteria: i) regulated in HGP cells only, ii) distance to chromosome end less than 100.000 bp, and iii) gene expression changes at least partially reversible in TERT immortalized HGP cells. To confirm these preliminary results we repeated gene expression analysis in senescent and TERT-immortalized HGP cells, using quantitative real time PCR.

Conclusions. The HGP model w/wo TERT is helpful to find TPE candidates. Current work includes expression analysis of candidate genes in normal cells at varying population doubling numbers and functional assays with genes involved in cell cycle regulation.

0006

DOES LINE-1 METHYLATION PLAY A ROLE IN HUMAN AGEING?

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Background. Advancing age is an important risk factor for many diseases such as cardiovascular disease and cancer. DNA damage accumulates during ageing and plays a role in the etiology of these diseases. Long interspersed nuclear element-1 (LINE-1) is a endogenous retrotransposon that comprises approximately 20% of the genome. Hypermethylation of CpG islands in the promoter region silences LINE-1 acitivity whereas hypomethylation leads to LINE-1 activation with genomic instability and mutations.

Methods. Blood samples from 82 subjects aged 18 to 99 years (63 women and 19 men) were collected. Subjects were divided into three groups according to age: younger, middle-aged and elderly (19 to 43, 65 to 75 and 85 to 99 years respectively; n= 26; 28; 28). LINE1 methylation of genomic DNA from peripheral blood was determined using a bisulfite pyrosequencing technique. In addition, plasma concentrations of homocysteine, B vitamins and lipids were measured.

Results. The mean LINE-1 methylation increased significantly with age in the three age-dependent groups (76.4%; 77.2% and 78.5% respectively; p=0.007). In addition, age was significantly correlated with LINE-1 methylation (r=0.390; p<0.001) and plasma homocysteine (r=0.565; p<0.001). A correlation between LINE-1 methylation and homocysteine (r=0.355; p=0.002) did not remain significant after adjusting for age (r= 0.067). LINE-1 methylation did not depend on sex and the biochemical parameters.

Conclusions. LINE-1 hypermethylation is probably associated with longevity because it protects DNA from genomic instability. However, it is not clear if LINE-1 methylation changes during lifetime. This and the effect on longevity have to be investigated in a prospective study.

Ageing

0007

THE ROLE OF TRANSTHYRETIN (TTR) IN THE LONGEVITY IN AN ELDERLY SARDINIAN POPULATION

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Background. Transthyretin (TTR) is a plasma transport protein of the thyroid hormone and forms a complex with retinol-binding protein; the presence of different molecular variants might be useful for scientific and diagnostic analysis. We investigated the genetic and proteomic profile of TTR in an elderly sardinian population.
Methods. The plasma proteins have been analyzed through 2D-PAGE and TTR spots identified by western blot and mass spectrometry. The DNA sequencing was performed on the four exons of the TTR gene.
Results. We have considered 40 DNA and plasma samples taken from individuals belonging to different ages, (80-89years, 90-99years, 100-104years) and to a control group (20-50years). 2D-PAGE/MS analysis of the plasma samples revealed 3 TTR spots. The results of qualitative and quantitative analysis carried out using the PD QUEST BASIC software showed a significative absence of one spot of TTR in about 60% of the groups 90-99y and 100-104y and a significative decrease in the intensity of the same spot only in the group 100-104y. The statistical analysis were performed using the Student's t-test. The four exonsof the TTR gene analysed by DNA sequencing didn't evidence any genetic mutations in the entire population including mutations usually involved in amyloidosis.
Conclusions. The Sardinian population investigated is charachterized by an intensity decrease only in the 100y-104y group ofone of the TTR spots identified suggesting a possible involvement in longevity. The absence of genetic mutations in the entire population studied could have a positive effect on successful ageing.

0008
THE EFFECTS OF AGE AND GENDER ON LABORATORY PARAMETERS

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Background. The population of individuals aged 65 years or older in most industral countries increased very rapidly in the 1980s. Aging is associated with a progressive reduction in functional reserve. Most laboratories have do not reference intervals for older men and women (aged from 60-65 years). The aim of the present study was to evaluate the reference ranges at various ages (aged from 60-98 years) in males and females.
Methods. Between 1994 and 2010, we tested 62,183 healthy subjects. Leukocyte count, platelet count and hemoglobin were measured by Coulter STKS (Beckman Coulter, USA) and plasma fibrinogen concentration was determined by the Clauss method (Stago Compact, France). The plasma total antioxidant (TAS) status was measured with the ABTS method (Randox, UK). Urinary N-terminal telopeptide with VITROS ECi ISM (Ortho-Clinical Diagnostics, USA). Plasma beta-CrossLaps, N-MID osteocalcin, calcium, parathormone, C-reactive pro- tein (CRP), total and free prostate-specific antigen (tPSA and fPSA), albumin, glucose, testosterone (T), gamma-glutamyltransferase (GGT), iron (Fe), ferritin and dehydroepiandrosterone-sulphate (DHEAS) test reagents were obtained from Roche (Roche Ltd, FRG).
Results. Age and gender have very different effects (significantly increased: CRP, uric acid, glucose, tPSA and fPSA, GGT, etc.; or decre-ased: albumin, TAS, T, DHEAS, Fe, etc.; or the elevations are not significant: hematological parameters, etc.) on the biochemical markers.
Conclusions. Laboratory physicians must help geriatric medicine with the new reference ranges of laboratory parameters for the older pop-ulation, but the levels vary considerably between and within the various countries.

Ageing

0009

INFLAMMATION AND FRAILTY INDEX IN OLDER ADULTS

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Background. Clinical diagnosis of frailty is often tedious and cumbersome, recent studies suggest a biochemical phenotype associated with this condition. The aim of this study was to identify an inflammation and frailty index (IFI) to be used as screening tool of frailty in older adults (OA).
Methods. We analyzed the results of inflammation biomarkers (IB) in a Spanish cohort of 443 subjects (age≥70) included in a population study of frailty and dependence. The IB studied were: erythrocyte sedimentation rate (ESR), C-Reactive protein (CRP) and neutrophil count. Frailty was defined according to Fried´s criteria. Clinical data regarding age, gender, comorbidity and BMI were collected. The association between IB and clinical variables was assessed by Pearson´s correlation and multivariate regression analysis. The diagnostic performance of IFI was studied by ROC curve analysis.
Results. 47.6% of the population were men (n=210) with a mean age of 76.9 (SD:4.8). IB was positively correlated with age, gender and comorbidity but not with BMI. Multivariate regression analysis adjusted by age, gender and comorbidity, showed that IB were associated with an increased risk of frailty (OR_{ESR}:1.03; OR_{CRP}:1.02; $OR_{neutrophils}$:1.32). The cut-off points from ROC curves were: CRP≥1mg/dl, ESR≥7mm/h and neutrophils≥2400cells/μl. The number of IB over the cut-off points were used to calculate the IFI (from 0 to 3). Pearson´s chi-square revealed a positive association between IFI and frailty (p<0.005). Sensitivity of IFI was 73% with a NPV of 94%.
Conclusions. IFI represents an easy and inexpensive biochemical tool to select OA at risk of frailty.

0010

DETERMINANTS OF OXIDATIVE STRESS RELATED TO GENDER: RELEVANCE OF AGING AND SMOKING HABIT

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Background. Magnitude and major causes of oxidative stress may be different between men and women. Gender effects on oxidative status have been addressed in clinical studies only to a limited extent with controversial Results. In the present study, we aimed to determine whether any gender-related difference exists concerning oxidative stress in a population of 332 subjects of both sexes, in a wide age-range, with and without cigarette smoking habit.
Methods. The Oxidative-INDEX was calculated after evaluation of serum hydroperoxides (ROMs) and total antioxidant capacity (OXY) by means of commercial kits (d-ROMs and Oxy-adsorbent Tests, Diacron, Italy) subtracting the OXY standardized variable from the ROMs standardized variable, and expressed as arbitrary units (AU).
Results. The Oxidative-INDEX resulted higher in women respect to men (0.37±0.1 *versus* -0.13±0.1 AU, p<0.001), in smokers than in non-smokers (-0.13±0.1 *versus* 0.5±0.1 AU, p<0.001), and correlated with number of cigarettes (r= 0.18, p<0.01) and age (r= 0.24, p<0.001). The multivariate analysis identified age (Odds Ratio, OR: 3.7, p<0.05), high blood pressure (OR: 7.2, p<0.05), and smoking habit (OR: 6, p<0.01), as factors independently associated with the Oxidative-INDEX in men, whereas cigarette smoking (OR: 2, p<0.05), and age (OR: 2, p<0.05), represented the independent risk factors for an elevated oxidative stress status in women.
Conclusions. Gender-based differences in oxidative stress levels may provide a biochemical basis for the epidemiologic differences in the disease susceptibility between sexes, and suggest different strategies for risk assessment, diagnosis and treatment specifically targeted to men and women.

Allergy

0011

SPECIFIC RESPONSE OF ANTIOXIDANTS AND CYTOKINES TO MITOGENS IN LYMPHOCYTES OF PATIENTS WITH BRONCHIAL ASTHMA

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Background. It is believed that oxidant stress, cytokines and their modulators play a key role in the initiation and the development of asthma. The aim of this study is to evaluate response of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity, as well as some Th1 (TNF-a, INF-g) and Th2 (IL-4, IL-13) cytokines to different mitogens, Concanavalin A (Con A) and Phormal myristate acetate (PMA) in lymphocytes of patients with newly diagnosed bronchial asthma. It is known that Con A is reported to stimulate the proliferation of cytotoxic T cells,while PMA stimulates some kind of oxidative stress.

Methods. The antioxidants were determinate in the patients' cultures of lymphocytes treated with Concanavaline A (Con A) and Phorbol 12-myristate 13-acetate (PMA) by colorimetric commercial tests (Ransod and Ransel) while cytokines were measured by ELISA tests.

Results. Unstimulated lymphocytes did not show any significant changes between lymphocytes of patients and healthy control. But after stimulation with PMA we noted the increase in SOD activity (p<0.01), together with increase in IL-13, TNF-a, INF-g concentrations (p<0.001) in lymphocytes of patients with newly diagnosed bronchial asthma compared with control group and these changes were associated with significant decrease in GPx activity (p<0.01). Also, the increase was noted in concentration of only IL-13 (p<0.01) after stimulation with Con A compared with lymphocytes of control.

Conclusions. The explanation of the reason why different mitogens stimulate the cells to produce different levels of cytokines is not clear, although thespectrum of target cells for each mitogen is known to be somewhatdifferent. The results from our study suggest that there is disturbance of specific cytokine and antioxidant pattern as well as on specific answer to different mitogen in patients with newly diagnosed bronchial asthma.

0012

RECOVERYELISA – A NEWLY DEVELOPED IMMUNOASSAY FOR MEASUREMENT OF THERAPEUTIC ANTIBODIES (MAB) AND THE TARGET ANTIGEN DURING ANTIBODY THERAPY

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Background. Certain therapeutic antibodies are already in clinical use. These antibodies are directed against specific proteins to inhibit their disease-causing effect. The common problem is the insufficiency for laboratory control because traditional assays are failed due to the occurrence of assay compartments in the sample already.

Methods. The intention of the study was to develop an in-vitro procedure for the estimation of both the therapeutic antibody and the antigen in serum samples by use of a sandwich immunoassay. An internal calibration procedure was developed to enable the estimation of both components in unknown samples, the therapeutic antibody and the target antigen. By measurement of the recovery of a standard addition the free antigen in the sample could be calculated with respect to the reaction velocity and binding characteristics.

Results. The application of the *recovery*ELISA is shown in patients treated with Omalizumab (Xolair)/IgE and with Adalimumab (HUMIRA)/TNF-a. It was possible to demonstrate therapeutic levels of antibodies, e.g. Xolair or Humira, and free antigen in serum.

n=7, bronchial asthma	IgE (IU/mL)	IgE Recovery (%)	Omalizumab (μg/mL)
before Treatment	783 $\pm$ 864	100 $\pm$ 0	0 $\pm$ 0
4 weeks treatment	14 $\pm$ 23	3 $\pm$ 2	32 $\pm$ 26

n=5, rheumatoid arthritis	Free TNF-α (ng/mL)	TNF-aμ- recovery (%)	Humira (μg/mL)
Before Treatment	4,3 + 6	100,3 + 0,7	0 + 0
4 weeks treatment	4,2 + 9	1,1 + 0,9	7.6 + 6,3

Conclusions. The *recovery*ELISA enables the control of therapeutic antibodies and of the target antigen.

Allergy

0013

THE ROLE OF TRYPTASE IN THE MANAGEMENT OF ALLERGIC OR HYPERSENSITIVE PATIENTS

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Background. During anaphylaxis the release of huge amounts of mediators like tryptase produced by basophil granulocytes and mast-cells can cause severe alterations in several organs. Tryptase measurements can be used for anaphylaxis diagnosis. Theaim ofthisstudy is to evaluate the role of the tryptase measurement in the emergency department and in the managementof allergic subjects with a history of anaphylactic reactions
Methods. The tryptase measurement was performed by ImmunoCap 250 (Phadia). During a two year period (2007-2009), we analyzed 95 serum samples from the Emergency Department, collected within120 minutes from an anaphylactic reaction and 1048 samples from patients with a clinical history of anaphylactic reactions. The cut off limitof tryptase (<11.4 ng/mL) was previously calculated in 40 healthy subjects (95 percentile).
Results. Serum tryptase >11.4 ng/mL was found in 28 patients from the Emergency Department (29.5%) and in 41 subjects with a clinical history of anaphylaxix (4%). In 30 of these subjects (73%), mastocytosis was diagnosed by bone marrow biopsy; the clinical sign of urticaria pigmentosa was evident only in 50% of patients. For both sets of patients, the most common precipitating event was a Hymenoptera sting (33%).
Conclusions. These results indicate that among the patient presenting at the Emergency Department with anaphylactic reactions, approximately one third need to be prescribed specific immunotherapy and/or adrenaline auto-injection. Furthermore, the majority of anaphylatic patients with high tryptase concentrations (>70%), have an underlying mastocytosis and need to be treated accordingly.

0014

A NEW EMERGING ROLE FOR THE CLINICAL CHEMIST: MANAGEMENT OF MULTIPLE FOOD ALLERGY

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Background. Allergy to Lipid-Transfer-Protein (LTP) is associated with severe clinical symptoms but it is relatively rare in Belgium. Indeed, Belgian people are more often sensitized to less dangerous proteins (PR-10 proteins) as they are mainly birch pollen allergic patients. It is also quite surprising to encounter a multiple sensitization profile to LTP in a young Belgian child. The aim of our study was to explore an 8-year-old-boy presenting multiple food allergies associated with severe reactions and to show the interest of the new in-vitro diagnostic tools for his management. We wanted to show that clinical chemists have a role to play in a multidisciplinary team.
Methods. We realized the measurement of specific IgE (sIgE) for recombinant allergen components thanks to the traditional ImmunoCAP_250 and thanks to the microarray ImmunoCAP_ISAC technology. Immunoblots and Immunoblot-inhibition were performed to estimate the involvement of LTP in food allergic reactions.
Results. The sIgE measurements were positive (>10kUA/L) with the ImmunoCAP_250 as well as with the ImmunoCAP_ISAC for 13 LTP from different kind of plants out of the 15 that we tested. The Immunoblot-inhibition allowed us to challenge the LTP presence in some food in order to reintroduce them in the diet.
Conclusions. Our experience shows that clinical chemists can give a new dimension to the management of multiple food allergy to LTP.

Allergy

0015

COMBINED ALLERGY TESTING FOR THE MANAGEMENT OF ASTHMA IN CHILDREN

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Background. Asthma is the most frequent cronic disease in children. Laboratory tests are used in order to demonstrate the atopic field(total serum Ig E), the organism reaction (,blood eosinophilia) and the etiologic agent of the allergy (allergen specific Ig E - a combination of 36 respiratory and food allergens)

Methods. We took into this study 56 patients, aged between 2-16 years, who were admitted in the National Programm for Asthma during 2 years period, 2009-2010... , Total serum IgE was measured with a Vidas system, Blood eosinophilia was measured with a Sysmex XS-1000i analyzer and IgE specific allergens were made on a MAST CLA system.

Results. Between the 56 patients in the study ,36 were males and 19 females.

44/56 patients(82%)had high levels of total serum IgE ,37/56 patients(70%), had high levels of blood eosinophilia and 31/56 patients(60%) had . high scores (3 and 4) for allergen specific IgE for more then 7 allergens.

Conclusions. We thing that the combination of these 3 lab tests, offers a clear picture of asthma in clindren.Using only blood samples is the easiest way to investigate a child(one visit to the doctor, easy sample collection, harmless than the " prick test"). The use of MAST CLA system with 36 allergens panel gave the possibility to investigate a large number of allergens without the need of a large sample(very convenient for pediatric studies) and within a reasonable price..

0016

COMPONENTS-ARRAY TECHNOLOGY DIAGNOSTIC, ASTEPFORWARDINTHE STUDY OF THESENSITIZATION PROFILE OF ALLERGIC PATIENTS

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Background. The separation of components diagnosis (Component Resolved CRD) is based on the use of purified recombinant allergen or native molecules for the diagnosis "in vitro" of allergy. InmunoCAP ISAC (Phadia) Array technology is the first multiplex "in vitro" diagnostic tool based on allergen components either specific markers or cross-reactivity markers. The CRD identifies individual allergens that cause the disease in each patient, revealing their sensitization profile. Many biological products contain cross-reactive allergens. Panallergens sensitization produce positive results when tested against many allergen extracts. The study of panallergens by array allows knowing the true cross-reactivity and identify co-sensitized patients.

Methods. ISAC InmunoCAP technology is based on the biotechnology of biochips that allows the study of 118 allergenic proteins simultaneously in one biochip.

Results. We studied 254 patients with complex clinical from the allergy department to assess the presence of panallergens in them. The results were as follows: 204 had panallergens (cross-reactiveallergensmarkersfrom plants:LTP81, PR1090, Polcalcina35, CCD/Bromelain 20 andProfilin88;cross-reactiveallergensmarkers notfrom plants:Cysteineprotease24, NPC226, Parvalbumin47, Tropomyosin 34 andSerum albumin55), 29 had only specific markers and 22 did not have any reactivity.

Conclusions. CRD attached to the array technology: facilitates the differentiation of sensitization to panallergens, identifies co-sensitized patients and improves the diagnosis by allowing to know the senstization profile.

Allergy

0017

RELATIONSHIP BETWIN SERUM FOLATE LEVEL LEVELS AND IGE IN PATIENTS WITH ALERGIC DISEASES

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Background. Folic acid (FA) is known to be associated with inflammatory diseases, but the relationship between folic acid and allergic diseases (AD) is still unclear. The aim of study was to assess FA in serum samples from patients with AD and correlation between FA and IgE levels.
Methods. 25 patients were included in our study, 8 of them suffered from asthma, 5 from allergic rhinitis (AR) and 12 from atopy (AT). Controls were 10 healthy individuals. Serum FA and IgE concentration were determined by electrochemeluminescent method. An IgE level ≥ 100 IU/mL was considered positive in adult and ≥90 IU/mL in 6-9 years old patients.
Results. FA serum level in asthma patients was from 0.8 ng/ml to 9.35 ng/ml (means = 3,1ng/ml), in patients with atopy or allergy rhinitis was from 5.94 ng/ml to 13.36 ng/ml (means = 9.6 ng/ml) and in healthy group was from 5.02 ng/ml to 18.36 ng/ml (means= 11.6 ng/ml). Serum IgE level in all asthma patients were >100 IU/mL (309.4-914.7 ng/ml) and in 9 patients with AT or AR (from 118.5 IU/mL to 508.1 IU/mL). All healthy individuals had total IgE level <100 IU/mL. Serum FA were inversely associated with total IgE levels (p=0034) only in asthma patients.
Conclusions. We found that FA deficiency was associated both with asthma and inversely with total IgE levels, but not with atopy. This consideration warrants caution in interpreting results and suggests further studies to determine the diagnostic accuracy of FA in allergic diseases.

0018

DETECTION OF AYTOANTIBODIES IN PATIENTS WITH ASTHMA

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Background. The object of study was to determine autoantibodies inasthmatics and their correlation with clinical manifestation and medication.
Methods. 3 adults and 3 children aged 28-58 vs 2-8 years old with asthma and 4control subjects were including in our study. In all patients were determined 24 autoantibodies (aAb) type G (dsDNA, rheumatoid factor, b2-glycoprotein I, LuM-membrane and LuS-cytoplasmic lungantigen, KiS-cytoplasmic and KiM-membrane kidneyantigen, GaM-stomachmembrane antigen, ItM-small intestinemembrane antigen, HeS-livercytoplasmic antigen, HMMP-antigen of liver mitochondria, b-adrenoreceptors, CoM-myocardial cellsantigen, TrM-platelets'antigen,ANCA,Thyroglobulin, TSH receptor, Insulin,Insulin receptors (IR), Adr-adrenal glandsantigen, Membranous antigen of prostate/spermatozoids, S100, GFAP,Myelin basic protein) and mean individual immune reactivity byE-LISA (ELI viscera 24 test, Immunculus, Russia).
Results. The mean individual immune reactivity in asthmatics was 42% (from 20% to 68%) and was significantly higher than in the controls subjects 9% (p<0,005) and was associated with serum level of total IgE. Serum level of aAbs to LuM, LuS, CoM and dsDNA in all asthmatics significantly increased, compared with the controls. Level of CoM and dsDNA depended on age and disease duration (in adults was higher than in children (32% vs 17%) p< 0,05. Anti-Adr aAbs was higher in patients than in controls, but only with steroidsmedication. Anti-HMMP, Insulin and IR aAbs were inversely associated with asthma (p<0,05).
Conclusions. Our results demonstrated that autoantibodies were found in asthmatics implies that the autoimmunity play an important role in asthma pathogenesis.

0019

DEVELOPMENT OF COMBINED MEASUREMENT OF SQUAMOUS CELL CARCINOMA ANTIGENS 1 AND 2 ASA POTENTIAL COMPANION DIAGNOSTIC FOR ANTI-IL-4/IL-13 THERAPIES IN ALLERGIC DISEASES

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Background. Several trials are now under way to develop antagonists against interleukin-4 (IL-4) and/or IL-13 for allergic diseases.However, no diagnostic to select patients in whom such drugs would be effective isavailable. We previously found that the squamous cell carcinoma antigen 1 (SCCA1) and SCCA2 are the most strongly induced products by IL-4 or IL-13 using DNA microarray. In this study, we investigated whether measurements of serum SCCAs were useful for diagnosing atopic dermatitis (AD) as potential companion diagnostics for anti-IL-4/IL-13 therapies.
Methods. We established novel ELISA systems to measure SCCA1 and SCCA2 specifically. We investigated the correlation between serum SCCA levels in AD patients and their clinical severity.
Results. Serum SCCA1 (2.9±2.4 ng/ml) and SCCA2 (3.2 ± 5.6 ng/ml) in AD patients (n = 188) were significantly increased compared to non-AD controls (n = 49; 0.8±0.2 ng/ml, SCCA1; 0.6±0.2 ng/ml, SCCA2). The serum SCCA levels were directly proportional to eczema severity. SCCA2/SCCA1 ratios in the AD patients (1.4 ± 0.4) were significantly higher than those of the non-AD controls (0.8 ± 0.1), due to the propensity of IL-13 to induce production of SCCA2 rather than SCCA1. Receiver operating characteristic analyses demonstrated high specificity (0.86, SCCA1; 0.88, SCCA2) and sensitivity (0.86, both) with the large areas under the curves (0.92, SCCA1; 0.94, SCCA2) in diagnosing AD patients.
Conclusions. Combined measurement of serum SCCA1 and SCCA2 levels is useful for diagnosing AD. It can be a companion diagnostic for anti-IL-4/IL-13 therapies against allergic diseases.

0020

AGE, SEX AND PLACE OF RESIDENCE AND POSSIBLE RELATIONSHIP WITH ALLERGIC STATES

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Background. The word allergy conveys all the ways of exaggerated congenital or ill-gotten hypersensitiveness to several substances in whose genesis there is a proved antigen – antibody immune reaction. We tried to conclude the relationship between the allergic state, diagnosed by quantification of total IgE by the sex, age and geographical region.
Methods. We collected serums in our service during six months, establishing three age groups (<10y.;
11-30 y.; >30 y.) and three geographical regions: industrial, agricultural and mountain area. Determinations of IgE were made through Abbot IMx Total IgE (based on MEIA technology). We did the study using a contrast of hypothesis in which we used Pearson's statistic Chi square for independence between two qualitative variables: sex-allergy, age – allergy, geographical area-allergy.
Results. There is no significant relationship regarding sex and possible allergopathy. We could state basically the same regarding geographic area, although there are relevant differences between those living in an industrial or mountain area, because the first ones have higher prevalence of allergies (p< 0.06). There is a meaningful relationship regarding age (p<0.01), appreciating that the group between 11 – 30 years old has a higher number of positive IgE quantifications than the rest of the groups.

Autoimmune disease

0021

ASSOCIATION OF ANTIBODIES TO CYCLIC CITRULLINATED PEPTIDES AND RHEUMATOID FACTOR WITH C-REACTIVE PROTEIN IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background. evaluate the frequency of autoantibodies against cyclic citrullinated peptides (anti-CCP) and rheumatoid factor (RF) and their correlation with C reactive protein (CRP) in a group of rheumatoid arthritis (RA) patients.
Methods. 70 patients with RA (50 female and 20 male) were included in the study. Serum samples were collected and studied at the time of appointment. RF and CRP concentrations were assessed by nephelometry (BNII nephelometer, Dade Bering, Germany), anti-CCP were measured by ELISA (Euroimmun, Germany). Cut-off values of positiveness were >5 RU/ml for anti-CCP, >5 mg/l for CRP and >20U/ml for RF, as declarated by the manufacturer.
Results. anti-CCP positivity was detected in 51% RA patients, positive RF – in 47% and CRP positivity was observed in 84% RA patients. Significant correlation was detected between anti-CCP and RF (r=0.58, p<0.05). There was no correlation between these autoantibodies and CRP.
Conclusions. significant association was estimated between antibodies against cyclic citrullinated peptides and rheumatoid factor in a group of rheumatoid arthritis patients. So these autoantibodies may be assessed together as markers of autoimmune disease progression. Data obtained suggest that were no association in the influence of CRP to the development and frequency of anti-CCP and RF.

0022

FLUOROIMMUNOASSAY VERSUS NEPHELOMETRY TO MEASURE RHEUMATOID FACTOR

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Background. About 80% of people with rheumatoid arthritis have detectable rheumatoid factor (RF). RF is an important predictor of more severe disease. However, it appears in other autoimmune diseases (Sjögren, Lupus). The aim of our study was to compare the new EliA™ RF IgM assay on Unicap250 (Phadia, fluoroimmunoassay) to Nephelometer Dade-Behring BN-II (Siemens).
Methods. One hundred and seventy five patients were enrolled. Tests were performed by Unicap250 and Nephelometer Dade-Behring BN-II. Results were considered as: Unicap250 Phadia (UI/mL) negative <3.5, grey zone 3.5–5, positive >5; Nephelometer Dade-Behring BN-II (UI/mL) negative <15, positive ≥15. Kappa index (agreement strength) was used as statistical method.
Results. Fourty results (22.86%) were discordant. Only 2 patients (7%) of the 26 with discordant results (BN-II positive, Phadia negative) had other various autoimmune diseases (age: 34-89 y; mean=64). Four patients (50%) of the 8 with discordant results (BN-II negative, Phadia positive) had other various autoimmune diseases (age: 57-83 y; mean=69). According to disease status, EliA™ RF IgM assay on Unicap250 and Nephelometer Dade-Behring BN-II yielded a specificity of 96.5% (CI 95%: 90.8–98.9) and 73.9% (CI 95%: 64.8–81.5) and a sensitivity of 96.7% (CI 95%: 87.5–99.4) and 93.3% (CI 95%: 83.0–97.8), respectively. Kappa index=0.541 (CI 95%: 0.416–0.666), which indicates a moderate agreement between the methods compared.
Conclusions. According to sensitivity, specificity and Kappa index, EliA™ RF IgM assay on Unicap250 showed good performance as method of screening of autoimmune disease and it has better sensibility-specificity than the method used previously.

Autoimmune disease

0023

VALUE OF ELECSYS ANTI-CCP ASSAY (COBAS E411, ROCHE) IN THE STUDY OF RHEUMATOID ARTHRITIS

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Background. The CCP test enables clinicians to distinguish Rheumatoid Arthritis(RA) patients from other RA-resembling diseases. Anti-CCP antibodies can be detected very early in the course of RA. The aim of our study was to compare the new Elecsys anti-CCP assay on the Cobas e411 analyzer (Roche) based in electrochemiluminescence, to EliA CCP on the Unicap250 (Phadia, fluoroimmunoassay).
Methods. Twenty RA patients (Phadia positive) who were diagnosed according to the American College of Rheumatology criteria and 102 healthy subjects (Phadia negative) were enrolled in this study. Tests were performed by Unicap250 (Phadia) and Cobas e411 analyzer (Roche). Results were considered as: Unicap250 Phadia (UI/mL) negative <7, positive ≥7; Cobas e411 Roche (UI/mL) negative <17, positive ≥17. Kappa index (agreement strength) was used as statistical method.
Results. Twenty RA patients (Phadia positive) were positive by Cobas e411 analyzer. One hundred and one out of the 102 (Phadia negative) yielded negative Cobas e411 Results. Kappa index=0.971 (CI 95%: 0.913–1.028), which indicates a very good agreement between the methods compared. Elecsys anti-CCP assay on the Cobas e411 analyzer yielded a specificity of 99.0% (CI 95%: 93.9–99.9) and a sensitivity of 100% (CI 95%: 79.9–99.5).
Conclusions. According to sensitivity, specificity and Kappa index, the new Elecsys anti-CCP assay on the Cobas e411 analyzer, based in electrochemiluminescence, showed a very good performance as method of Rheumatoid Arthritis assessment.

0024

SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR LEVELS IN ANKYLOSING SPONDYLITIS: THE IMPACT OF INFLIXIMAB THERAPY

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Background. Recent studies suggest that levels of soluble urokinase Plasminogen Activator Receptor (suPAR) may be useful for the characterization of inflammation in immune-mediated disorders. The aim of our preliminary work was to obtain data regarding suPAR levels in patients with ankylosing spondylitis (AS) and to test whether suPAR levels are affected by infliximab therapy used for AS treatment.
Methods. We enrolled 9 AS men (aged 41.2±10.4 years). Serum was obtained just before administration of infliximab (IFX); then 2 and 6 weeks after the initiation of IFX. suPAR levels were measured with commercially available ELISA kits and were to those of 8 healthy controls. CRP levels were also determined.
Results. suPAR levels were higher in AS compared to controls (2.09±0.42 vs. 3.07±0.80, p<0.01). Later, 2 and 6 weeks after initiation of IFX therapy, suPAR levels significantly decreased (2.61±0.68 and 2.68±0.25, p<0.05 and p<0.05, respectively), but remained still higher than normal (p<0.05). CRP levels were very high before IFX, then normalized with IFX [median (interquartile range)]: 15.0 (10.3 – 44.1), 1.5 (0.7 – 2.1) and 1.4 (0.7 – 2.4) mg/ml, p<0.01 and p<0.01, respectively. No difference between IFX treated and control subjects was present. No association between CRP and suPAR was observed.
Conclusions. These results raise the notion that it would be worth to test the role of suPAR as a possible biomarker for inflammation in AS and for the monitoring IFX therapy. suPAR may be sensitive for low-grade inflammation in treated AS patients with otherwise normal CRP values.

Autoimmune disease

0025

CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) IN RHEUMATOID DISEASES

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Background. The rheumatoid diseases may occur the changes in the glycosylation of plasma proteins. Therefore, the aim of this study was to evaluate and compare the concentrations of serum carbohydrate-deficienttransferrin (CDT) in non-drinking patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).
Methods. CDT concentration (absolute and relative units) in serum of 39 patients with RA, 11 with SLE, 32 with SSc and 52 healthy subjects was measured by immunononephelometry method using N Latex CDT test on the BN analyzer.
Results. The absolute values of CDT concentration in the sera of RA, SLE and SSc patients, and healthy subjects were (median and range): 40.7 mg/L (22.3-64.8), 41.2 mg/L (34.5-53.9), 42.0 mg/L (30.1-58.4) and 43.3 mg/L (23.0-76.2), respectively. There were no significant differences in CDT absolute values between RA, SLE, SSc patients and controls ($P=0.2022$; $P=0.9855$; $P=0.9339$, respectively) and between tested groups of patients ($P=0.6631$). The concentrations of %CDT were significantly higher in RA (1,96%; 1.10-3.55), and SLE (2.37%; 2.15-2.48) patients than those in controls (1.82%; 1.40-3.86)($P<0.01$ for both comparisons).The SLE patients had a significantly higher level of %CDT compared with patients with RA ($P<0.05$). Correlation analysis revealed that in the control group, levels of CDT and %CDT correlated positively with each other ($P<0.05$). But, there were no correlations between levels of these measures in RA and SLE patients ($P=0.9411$ and $P=0.2000$, respectively).
Conclusions. We conclude that in the patients with rheumatoid diseases the alterations in the glycosylationof plasma transferrin occurs.

0026

DIAGNOSTIC ACCURACY OF COELIAC SEROLOGICAL TESTS

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Background. The current guidelines, as a first step diagnostics of coeliac disease (CD), still recommend use of either IgA anti-transglutaminase (TTG) or IgA antiendomisial (EMA) antibodies as well as total serum IgA antibodies.
Methods. 50 patients of both sexes (21 male and 29 female), average age (39 years and 47.4 years) with clinical features of coeliac disease were tested for IgA anti-TTG (BioSystems, Barcelona, Spain on ChemWell Awareness Tech, analyzer.) and endoscopic biopsy was done. Basic biochemical and hematological parameters were done using standard Methods.
Results. CD was confirmed by endoscopic biopsy in 4 patients (2 male & 2 female) while IgA anti-TTG were positive in 3 patients (1 female was falsely negative due to hypoproteinemia and IgA deficiency). In our risk group sensitivity was 75%, specificity 100%, positive likelihood ratio was 75% while negative likelihood ratio was 25%. Prevalence of CD in our group was 8,2%. In male subgroup significant differences between patients with and without CD were present in mean values of erythrocyteparameters MCV (96.5±7.7 vs. 78.6 ±11.3; $p<0.05$), MCH (36.9±4.6 vs. 25.9±4.9; $p<0.01$) and MCHC (382.5 ±16.3 vs. 326.9±19.1; $p<0.005$) as well as in mean levels of total proteins (47.5 ±16.3 vs. 68.3 ± 7.6; $p<0.01$) and albumins (24.6±9.5 vs. 42.1 ± 6.9; $p<0.01$). Levels of HDL-cholesterol were also significantly lower in male patients with CD (0.42.±0.12 vs. 0.90±0.30; $p<0.05$). In female subgroup there were no significant differences between patients with and without CD.
Conclusions. Our results have shown high correlation IgA anti-TTG with gold standard (endoscopic biopsy).

Autoimmune disease

0027

COMPARATIVE STUDY OF THYROID PEROXIDASE ANTIBODIES BY IMMUNOCHEMOLUMINESCENCE AND ENZYME IMMUNOASSAY

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Background. The presence of thyroid peroxidase antibodies (TPOAb) suggests that the cause of thyroid disease is due to an autoimmune disorder such as Hashimoto's disease or Graves' disease. There is considerable intermethod variability of current TPOAb assays that precludes the numeric comparison of serum TPOAb values reported by different tests.
Methods. We analized TPOAb in100 seraof patients byan immunochemiluminometric automatedassay(ICMA)inan Immulite2000(Siemens)(negative:<35 IU/mL, positive<35 IU/mL)and by a manual enzymeimmunoassay (EIA) in an UniCAP®100 (Phadia)(negative:<60 IU/mL, positive>100 IU/mLand grey zone: 60-100 IU/mL).
Tocategorize the results, negative values areconsideredTPOAb<35 IU/mLin the ICMA andTPOAb<60UI/mL in the EIA. Other resultswere considered positive.
Results. Theobserved agreementwas 93%(expected bychance:56%),yieldinga kappa index of 0,848 (95% CI:0,731-0,966).The Spearman´s coefficient of correlation between the ratios (value/upper reference limit)of both methods was 0,850 (95% CI: 0,781 to 0,899), and the Passing-Bablok equation was:
*Immulite 2000 = 0.875*UniCAP100 – 6.78*, (slope 95% CI:0,7680 to 0,9228; intercept 95% CI: -7.6282 to -5,5378).
Conclusions. According to the results, the strengthof categorical agreement between bothmethods can beconsidered"very strong" (LadisandKoch criterion). That is, the results arequalitativelycomparable. However, the resultsare notquantitatively inter-changeable. In addition, ICMAtechnique providesresultswithin an hour,reducessample handlingand is not cumbersome.

0028

THE *PADI4* HAPLOTYPES ARE ASSOCIATED WITH ANTI-CCP LEVELS IN RHEUMATOID ARTHRITIS FROM THE WESTERN, MEXICO

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Background. Anti-cyclic citrullinated peptide (anti-CCP) antibodies are specific serologic markers in rheumatoid arthritis (RA). RA susceptibility has been associated with single-nucleotide polymorphism (SNP) in the peptidyl arginine deiminase 4 gene (*PADI4*) in Asianand Europeanpopulations. This study was undertaken to determine whether anti-CCP levels and RA are associated with *PADI4* haplotype in Mexican population.
Methods. We recruited 203 RA patients and 206 control individuals from Western Mexico. Three nonsynonymous SNPs in *PADI4* (padi4_89G/A, padi4_90T/C, and padi4_92G/C) were genotyped by PCR-RFLP and anti-CCP levels were measured by enzyme-linked immunosorbent assay (Diastat Anti-CCP kit, Axis-Shield, UK). The statistical analyzed was made using STATA v.9.2, and Arlequin v.3.1 software.
Results. We identified six different haplotypes, however the most frequent haplotypes were ACC and GTG. The comparison between RA and control individuals showed that the GTG (padi4_89*G, padi4_90*T, padi4_92*G) haplotype was associated with RA *vs* the ACC haplotype (patients 54.4%; controls 47.8%, OR=1.37 $_{95\%}$IC=1.02-1.83 p=0.025). In addition, when we compared the homozygous haplotypes (GTG/GTG *vs* ACC/ACC), we identified that the homozygous GTG haplotype is a risk factor to RA (OR= 1.84 (1.02-3.32) p=0.029). The anti-CCP levels were significantly higher in patients carrying the *PADI4* GTG *vs* ACC haplotype homozygous carriers (p=0.002).
Conclusions. The *PADI4* haplotype is a risk factor to RA associated with anti-CCP levels in Mexican population.

Autoimmune disease

0029

THE ANTI-CCP ANTIBODIES CORRELATED WITH RHEUMATOID FACTOR, ERYTHROCYTE SEDIMENTATION RATE AND DISEASE ACTIVITY INDEX IN RHEUMATOID ARTHRITIS

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Background. Antibodies to citrullinated proteins have been described in patients with rheumatoid arthritis (RA) and these appear to be the most specific markers of the disease. This study was undertaken to determine the association of anti-CCP antibodies with clinical markers and disease activity.
Methods. 239 patients with RA were includes in this study. Antibodies to cyclic citrullinated protein (Anti-CCP), was measured by enzyme-linked immunosorbent assay, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), were analyzed by baseline blood-samples. The disease activity score (DAS), visual analogue scale (VAS) and Health Assessment Questionnaire Disability Index (HAQ-DI) were too determined. The relationships between anti-CCP levels and clinical markers were analyzed statistically using STATA v.9.2.
Results. There was significant lineal correlation between anti-CCP antibody and RF (r^*=0.45, p <0.001) and ESR (r^*=0.15, p=0.042), but no significant correlation between anti-CCP antibody and CRP (r^*=0.12, p=0.10), DAS28 (r^*=0.08, p=0.30), VAS (r^*=0.07, p=0.34) and HAQ-DI (r^*=0.04, p=0.53) assessment, when we analyzed the anti-CCP levels by DAS 28 category, higher anti-CCP levels was found in patients with median activity (p=0.04) and high activity (p=0.04) *vs* patients in remission, but not correlation between anti-CCP and categories HAQ-DI.
Conclusions. The anti-CCP antibodies correlated with RF, ESR and DAS 28 as important markers clinical, which must be potential impact on the disease course and should have impact on considerations about treatment strategies in RA patients.

0030

FREQUENCY OF ANTINUCLEAR ANTIBODIES IN RHEUMATOID ARTHRITIS SEEMS TO BE RELATED TO PEPTIDYLARGININE DEIMINASE TYPE 4 GENOTYPE AND HLA-DRB1 SHARED EPITOPE

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Background. While HLA-DRB1 shared epitope (SE) represents the most prominent genetic risk factor for rheumatoid arthritis (RA), which clearly is involved in formation of anti-citrullinated peptide antibodies (ACPA), impact of peptidylarginine deiminase type 4 (PADI4) genotype on RA is still unclear. However, PADI4 is involved in generation of citrullinated proteins, which are essential targets of ACPA. Objective of this study was to analyse the influence of SE and PADI4 on autoantibody profile in RA.
Methods. SE, padi4_94C>T, rheumatoid factor (RF), ACPA, and antinuclear antibodies (ANA) were determined in 215 RA patients. The relation of SE and PADI4 on the autoantibody profile was statistically assessed.
Results. ACPA clearly was dependent on SE but independent on PADI4. A significant heterogeneity existed between SE+ and SE- patients with respect to association of PADI4 with ANA (P=0.006) with a positive association in the SE- stratum and a negative association in the SE+ stratum. In SE+ patients padi4_94T allele number was negatively associated with ANA (P<0.02), and in SE- patients there was a positive association (P<0.03). The heterogeneity of this allele-dose dependent effect between both SE-strata was highly significant (P=0.0002) (3).
Conclusions. PADI4 and SE seem to interactively modulate serological characteristics of RA.

Autoimmune disease

0031

PEPTIDYLARGININE DEIMINASE TYPE 4 GENOTYPE AS A POTENTIAL DISEASE MODIFIER IN RHEUMATOID ARTHRITIS

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Background. Recent evidence suggests that distinction of subsets of rheumatoid arthritis (RA) depending on anti-citrullinated peptide antibodies (ACPA) status may be helpful in distinguishing different aetiopathologies and in predicting course of disease. HLA-DRB1 shared epitope and peptidylarginine deiminase type 4 (PADI4) genotypehave been implicated in ACPA generation and protein citrullination. Both are assumed to be associated with susceptibility to RA. The aim of this study is to elucidate whether PADI4 affects clinical characteristics of RA, and whether it modulates the effect of ACPA on clinical course.
Methods. In 373 RA patients padi4_94C>T and ACPA were determined. Disease severity was characterised by cumulative therapy intensity (CTI) classified into 3 ordinal categories, disease activity score (DAS) 28, and by erosive joint disease (Steinbrocker score).
Results. CTI was significantly associated with disease duration, erosive disease, DAS28 and ACPA. Association of ACPA with CTI was decisively dependent on padi4_94C>T genotype (C/C: OR=0.93, p=0.92; C/T: OR=2.92, p=0.093; T/T: OR=15.3, p=0.002). Carriage of padi4_94T exhibited a significant trend towards higher Steinbrocker scores in univariate and multivariate analyses.
Conclusions. PADI4 in combination ACPA seems to modulate clinical characteristics of RA.

0032

AUTOANTIBODIES TO GP2 - A NEW MARKER FOR CROHN'S DISEASE

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Background. The etiopathogenesis of Crohn´s disease (CD) is not yet fully understood. Autoimmune mechanisms may play a pivotal role in triggering chronic inflammation in CD patients. Membrane glycoprotein 2 (GP2) localized in exocrine pancreas and intestinal Peyer's patches and overexpressed in inflamed CD intestinal biopsies has been identified as autoantigenic target for CD-specific antibodies to exocrine pancreas recently.
Methods. Novel enzyme-linked immunosorbent assays (ELISAs) for the detection of IgA and IgG autoantibodies to GP2 were evaluated using recombinant GP2 expressed in the Bacculovirus system. Autoantibody reactivity to GP2 and exocrine pancreas tissue (PAB) were assessed in CD patients (n=178), ulcerative colitis (CU) patients (n=100), and blood donors (n=162) by ELISA and indirectimmunofluorescence test (IFT), respectively.
Results. Anti-GP2 IgG and IgA were found in 48/72 (66.7%) and 23/72 (31.9%) PAB positive and 5/106 (4.7%) and 1/106 (0.9%) PAB negative CD patients (p < 0.0001), respectively. CD patients displayed significantly higher reactivity to GP2 than UC patients and BD (p < 0.0001), respectively. Occurrence of anti-GP2 antibodies correlated with PAB reactivity (Spearmen's rho = 0.493, p < 0.00001). There was a significant relationship between the occurrence of ASCA IgG and anti-GP2 IgG (p = 0.0307).
Conclusions. Anti-GP2 autoantibodies constitute novel CD-specific markers, the quantification of which could improve the serological diagnosis of inflammatory bowel diseases. Detection of anti-GP2 by ELISA is a readily available and robust method for the assessment of CD-specific autoantibodies. Further evaluation studies with disease controls are warranted.

Autoimmune disease

0033

THE DIAGNOSTIC SIGNIFICANCE OF DISEASE SPECIFIC ANTIBODIES TO NUCLEAR ANTIGENS IN PRIMARY BILIARY CIRRHOSIS

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Background. Primary biliary cirrhosis (PBC) is characterized by the presence of anti-mitochondrial antibodies (AMA) directed against M2 (AMA-M2) and also disease specific antibodies to nuclear antigens (ANA). The aims were to study the presence of AMA and multiple nuclear dots (MND) or rim like membranous (RLM) ANA pattern at indirect immunofluorescence, to detect the PBC-specific antibodies using immunoblot and to estimate their diagnostic value in PBC.
Methods. We used Liver Mosaic 8 for testing AMA and ANA patterns, Profile Autoimmune Liver Diseases EUROLINE for testing anti-native M2, anti-BPO, anti-Sp100, anti-PML and anti-gp210 (EUROIMMUN, Germany).
Results. In 12 (60%) of 20 PBC patients were ANA IF positive. MND pattern was determined in 6 (30%) and RLM in 5 (20%) PBC patients. Anti-Sp100 was positive in 4 (20%), anti-PML in 3 and anti-gp210 in 5 (25%) patients. All former antibodies were negative in 11 patients. MND pattern was associated with anti-Sp100 or anti-PML in 5 cases (83 %), in 2 cases were both antibodies positive. RLM pattern was present only in 2 anti-gp210 positive cases, 2 ANA-negative patients were also anti-gp210-positive.
AMA was found in 19 (95%), AMA-M2 was negative in 3 cases: first patient had MND pattern, anti-Sp100 and anti-PML positive, second RLM pattern but anti-gp210 negative, third all negative.
Conclusions. Testing for ANA is not alternative for detection of AMA and AMA-2. Although the diagnostic sensitivity of PBC-specific ANA is low, it is a useful additional test to AMA in PBC diagnostics, especially in AMA-M2 negative cases.

0034

SERUM LEVEL OF IL-18 IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background. Cytokines play an important role in pathogenesis of rheumatoid arthritis (RA). The objective of this study was to assess serum level of IL-18, a newly recognized proinflammatory marker in RA, in relation to other laboratory parameters.
Subjects and Methods. 75 patients aged 20-73 years that fulfilled ACR-criteria for RA, pharmacologically treated with DMARDs and NSAIDs, were divided into four groups according to Steinbrocker' scale: I°- 11 subjects, II°- 35, III°- 21 and IV°- 8 subjects. Twenty six age-matched healthy individuals were included as controls. Serum IL-18 (Bender MedSystems), RF IgM (Immundiagnostika GmbH), anti-CCP (Euroimmun) and CRP concentration by high-sensitivity Dade Behring test were determined.
Results. In seropositive RA patients (RF IgM >15 IU/mL or anti-CCP > 5 RU/mL) significantly higher concentrations of IL-18 were observed (368 pg/mL and 392 pg/mL) as well as hs CRP (5,46 mg/L and 4,96 mg/L) if compared to RF IgM (-) or anti-CCP (-) patients (132 pg/mL and 133 pg/mL; 1,59 mg/L and 2,36 mg/L, respectively) and to controls (39 pg/mL and 0,62 mg/L). Higher median values of IL-18 were found in patients with III° and IV° RA (329 and 284 pg/mL) than in patients with I° and II° (235 and 201 pg/mL). Positive correlation was found between IL-18 and RF IgM (r=0,54; p=0,0001) and anti-CCP (r=0,35;p=0,002). ROC analysis confirmed better diagnostic utility of IL-18 over hsCRP independently of degree of disease activity (AUC=0,810-0,892 vs 0,619-0,966).
Conclusions. Serum level of IL-18 could be regarded as a good indicator of disease activity in RA patients.

Autoimmune disease

0035

HARMONIZATION OF CUTOFF VALUE FOR RHEUMATOID FACTOR (RF) AS 15 IU/ML AT POSITIVE IN 5% OF HEALTHY SUBJECTS

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Background. Cutoff (CO) value of RF kits is not systematically defined. We have been promoting the standardization of RF in cooperation with JCCLS (Japanese Committee for Clinical Laboratory Standards). To harmonize the CO value of RF and to standardize values up to 3 times of CO value.
Methods. 892 healthysera selected using the latent abnormal value exclusion method proposed by K. Ichihara from 1,000 subjects who had a medical examination. 200 sera from RA patients and WHO standard sera were also measured for RF by 17 different RF kits. Eleven pooled sera were made by collecting RF positive sera in order of the titer of RF.
Results. 1) RF values was inconsistent among the 17 kits and could not be compensated by WHO standard sera. 2) The mean CO value for RF kits defined by ROC analysis was 16.0 IU/ml. 3) The mean value for each RF kits positive in 5 % of 892 healthy sera were 14.8, and the CO value could be set as 15 IU/ml at this point. 4) After harmonizing the CO value to 15 IU/ml, CV among the kits for pooled sera of 40.1 IU/ml were reduced from 44% to 17%.
Conclusions. Harmonization of CO value and standardization of low RF titers could be made by setting the CO value for any RF kit as 15 IU/ml where 5% of healthy subjects are positive. This idea will be soon approved as a guideline for RF kit by JCCLS.

0036

XANTHINE AND MALONDIALDEHYDE AS MARKERS IN EARLY DIAGNOSIS OF FETAL DISEASES

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Background. Amount of experimental data emphasizes the role of hypoxia and lipid peroxidation in the pathogenesis of fetal diseases. In connection with these facts, we watched a screening level of degradation products of purine nucleotides (xanthine, uric acid) and the end product of lipid peroxidation (malondialdehyde) in amniotic fluid in second trimester of pregnancy to assess the diagnostic value of biochemical markers for clinical practice.
Methods. We measured levels of malondialdehyde (MDA), xanthine (XAN) and uric acid (UA) by means of HPLC method in the amniotic fluids from subjects (n=241, 16 – 21 gestation week) screened for risk pregnancies.
Results. Significantly increased concentrations of malondialdehyde (MDA) were observed in amniotic fluids in risk pregnancies with high maternal serum alpha-fetoprotein (0.215 ± 0.17 (n=40) vs. 0.148 ± 0.1 μmol/L MDA (n= 48); $p<0.05$), high human chorionic gonadotropin (0.293 ± 0.25 μmol/L MDA (n=16); $p<0.05$), low serum estriol ($0.241 \pm 0.17\mu$mol/L MDA (n=14); $p<0.05$) and also in amniotic fluids with increased xanthine levels (0.369 ± 0.16 (n=14) vs. 0.132 ± 0.08 μmol/L MDA (n=35), $p<0.0001$). Patients with elevated levels of xanthine in the amniotic fluid had uric acid (xanthine degradation product) at physiological levels, which may be caused by free radical scavenger activity of uric acid.
Conclusions. We conclude that xanthine and malondialdehyde may have clinical usefulness as markers of increased fetal risk. Our data also support the presumption of increased oxidative stress and lipid peroxidation in the above mentioned pathologic situations.

Autoimmune disease

0037

THE RELATIONSHIP BETWEEN SERUM COENZYME Q10 AND CEREBROSPINAL FLUID DEGRADATION PRODUCTS OF PURINE NUCLEOTIDES IN PATIENTS WITH MULTIPLE SCLEROSIS

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Background. Coenzyme Q10 (CoQ10) is a vitamin-like nutrient that plays a vital role in cellular energy production. It is a critical component of mitochondria where biologically energy called ATP (adenosine triphosphate) is produced. CoQ10 is also a potent antioxidant and it helps protect the tissues and the cellular components in the body from free radical damage. In connection with these facts, we studied the relationship between serum CoQ10 and cerebrospinal fluid (CSF) levels of purine degradation products in patients with multiple sclerosis (MS) in order to evaluate suitability of parameters in other clinical studies.
Methods. Plasma concentrations CoQ, TBARS and CSF levels of degradation products of purine nucleotides (adenosine, inosine, hypoxanthine, xanthine, uric acid) were measured using high performance liquid chromatography (HPLC). Samples were obtained from 30 MS patients (average age 37.3 ± 11.6 years) in the early stage of the disease.
Results. Plasma CoQ10concentrations are significantly correlated withCSF levels of hypoxanthine ($r = 0.4017$; $p=0.028$), xanthine ($r = 0.369$; $p= 0.044$) and adenosine ($r = 0.358$; $p=0.0508$). Plasma CoQ10 levels were significant lower in 40% (12/30) MS patients. All MS patients had elevated plasma levels of TBARS, which correlated with *CSF* levels of *adenosine* ($r = - 0.5459$; $p= 0.0037$)**.**
Conclusions. We conclude that plasma CoQ10 deficiency has been at least partly implicated in the increased degradation of purine nucleotides in CNS and reduced antioxidant reserve is possibly an early pathogenic mechanism of inflammatory demyelinization in MS patients.

0038

THE COMPARISION OF METHODS DETECTING ANTIBODIES TO NUCLEAR ANTIGENS SPECIFIC TO PRIMARY BILIARY CIRRHOSIS

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Background. In addition to anti-mitochondrial antibodies (AMA) primary biliary cirrhosis (PBC) is characterized by the presence of antibodies to nuclear antigens (ANA), of which anti-Sp100, anti-PML and anti-gp210 are considered disease specific. Sp100 and PML show multiple nuclear dots (MND) and anti-gp210 shows as rim like membranous (RLM) pattern at indirect immunofluorescence. We aimed to estimate the relationship between PBC-specific ANA patterns and antibodies against specific target proteins.
Methods. We studied antibodies against PBC-specific target antigens in 77 MND, RLM or AMA positive cases. We used Liver Mosaic 8 for testing AMA and ANA patterns, Profile Autoimmune Liver Diseases EUROLINE for anti-Sp100, anti-PML and anti-gp210 (EUROIMMUN, Germany).
Results. From 20 ANA positive patients with RLM pattern 7 (35%) were anti-gp210 positive. 7 of 42 ANA positive patients without RLM pattern (16,7%) and 2 of 15 ANA negative patients (13,3%) were also anti-gp210 positive. From 14 ANA positive patients with MND pattern 8 (57%) were either anti-PML or anti-Sp100 positive. 7 of 48 ANA positive patients without MND pattern (14,6%) and 1 of 15 ANA negative patients (6,7%) were also either anti-PML or anti-Sp100 positive. 7 AMA negative patients who were either anti-PML, anti-Sp100 or anti-gp210 positive. None of them had PBC diagnosis at the moment.
Conclusions. The concordance between MND ANA pattern and anti-PML or anti-Sp100 was higher than between RLM ANA pattern and anti-gp210. Testing for these antibodies in PBC is useful only in the right clinical context.

Autoimmune disease

0039

SOLUBLE AND MEMBRANE EXPRESSION OF CD28 AND CTLA-4 IN EARLY AND CHRONIC RHEUMATOID ARTHRITIS

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Background. Rheumatoid arthritis (RA) is an autoimmune disease and T cells do not react normally. CD28 and CTLA-4 are the major costimulatory molecules whose signs influence the activation/inhibition of T lymphocytes, respectively. Objective is to analyze soluble and membrane expression of CD28 and CTLA-4 in early and chronic rheumatoid arthritis.
Methods. We included: 1)without_treatment_RA 2)early_RA and 3)chronic_RA and a control group. The membrane expression of CD28 and CTLA-4 were performed by flow cytometry. sCD28 and sCTLA-4 were measured by ELISA. Statistical analysis was performed using GraphPadPrism5Demo. A value of p<0.05 was considered statistically significant.
Results. We obtained significant differences in CD28 on CD8+ cells, where controls had a higher percentage with respect to the RA patients (62.5 *vs* 47.5 p=0.0446); there was higher IFM in controls compared with RA patients (122.9 *vs* 49.6 p=0.0049).We analyzed RA patients according to duration of symptoms, significant data were obtained only in the percentage of CD28 on CD8+ cells; controls showed the highest levels and patients with chronic RA had lowest levels (62.5 *vs* 39.5 p=0.0262). With regard to soluble levels, we observed that early RA patients had higher levels of sCD28 compared with chronic RA patients (1.06 *vs* 0.92 ng/mL, p=0.0363). sCD28 concentration were correlated with WBC levels (correlation, 0.4577; p=0.0110) and anti-CCP (correlation, 0.1150; p=0.0302).
Conclusions. The expression of CD28 in RA patients is lower in percentage and IMF on CD8+ cells than controls, whereas levels of sCD28 were higher in early RA than chronic RA. sCD28 concentrations correlated with WBC and anti-CCP levels in RA patients.

Autoimmune disease

0040

IS INCREASING AGE AT SYMPTOM ONSET IS ASSOCIATED WITH WORSE RADIOLOGICAL DAMAGE IN PATIENTS WITH EARLY RHEUMATOID ARTHRITIS?

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Background. It is well known that postmenopausal period associated with functional change in different systems, such as neuro-endocrine, immune and others; also we have change in process of bone remodeling. We postulated that this factors can influence on mechanism of focal bone erosion formation and radiological progression. Purpose is to evaluate radiological progression in patients with early rheumatoid arthritis (RA) after 1 year treated with disease modifying anti-rheumatic drugs (DMARDs).
Methods. 49 patients; female; age range, 51-79 years; disease duration 21,18 ±18,74 weeks with onset disease in postmenopausy were enrolled in this study. Disease activity was assessed with the Disease Activity Score 28 (DAS 28).Patients were treated DMARDs: 73,6%methotrexate 13,2% sulfasalazine and 13,2% leflunomide plus 5-10 mg/day prednisolone (3-6 months). Clinical remission identified in DAS 28<2,6. 34 young women(36,44±9,09 years) with early RA; disease durations 16,42±11, 75 weeks were considered as comparison group.Radiological progression estimated according tothe van der Heijde modified Sharp score. Erosion scores (ES), Joint Space Narrowing scores (JSNS) and modifications of Total Sharp scores (mTSS) were included.
Results. DAS 28 was lowerthen baseline level after 12 months of the treatment (p=0,036) and in the comparison group (p=0,046). Remission was achieved in 29,4% RA patients versus 46,6 % in comparison group. mTSS through 6 and 12 months in RA postmenopausal patients was 17,44 ±14,58-baseline ; 28,25 ±18,19 and 35,00 ±14,54 – in 6 and 12 months; p0;6=0,0006; p6;12=0,00095; and 9,57 ±7,44 – baseline. mTSS in RA postmenopausal patients was significantly higher comparison group patients through 6 and 12 months (p=0,028; p=0,047). We revealed JSNS progression through 6 and 12 months in RA postmenopausal patients (p0;6=0,0001; p6;12=0,00046) and in comparison group (p0;6=0,0031; p6;12=0,0138). ES was significantly higher through 6 and 12 months (p0;6=0,00167; p6;12=0,00079) and in comparison group (p0;6=0,049; p6;12=0,0148). Level of ES was significantly higher comparison group patients through 6 and 12 months(p=0,036 and p=0,041). We estimate significantly higher increase erosion rate/year in RA postmenopausal patients versus comparison group (1,36±0,85 versus 0,71 ±0,65; p=0,049)
Conclusions. All of the patients (in postmenopausal debut RA and young womenin comparison group) treated DMARDs during one year had radiological progression according to mTSS. Postmenopausal debut RA have more significantly higher increase erosion rate/year.

0041

THE ADVANTAGE OF DP IGA AND IGG GLIADIN IN CHILDHOOD COELIAC DISEASE: DO WE NEED FURTHER PROOFS?

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Background. Recently , a new generation of anti – gliadin (AGA) assays has been developed to detect antibodies to deamidated gliadin peptides (DPG). Antibodies against native gliadin have been considered to be of little diagnostic help. Serological test using DPG have been described as superior diagnostic parameters. In particular Deamidated Gliadin IgG is interesting in a diagnostic algoritm together with Tranglutaminase IgA. Aim is evaluate the clinical performance of EliA Gliadin DP IgA and IgG (Phadia) in comparison to the old EliA Gliadin assays based on purified wheat gliadin.
Methods. We detected 206 pediatric sera in those we had also the results of anti tTG IgA and EMA. Moreover , we could also stratify our data and antibodies anti – DPG were measured in sera of 40 samples from celiac disease patients with intestinal mucosa biopsy investigated, 50 samples from disease controls (gastrointestinal disorders different from CD and with infections), 100 patients with tTG IgA or EMA positives, 30 patients with Gliadin IgG positive, 50 patients with tTG or EMA or gliadin IgA, IgG negatives.
Results. The clinical performance in paediatric population showed a sensitivity of about 97 % . As expected conventional anti–gliadin test offer low specificity with positive not related to CD. The diagnostic performance of the anti DPG IgG assay is much superior in comparison with the old one.
Conclusions. The parallel determination of anti - DPG and anti tTG (IgA) achieves a good diagnostic accuracy. The performance of DPG assays is superior in comparison with old gliadin. The most reliable serological strategy for diagnosing CD has to be well evaluated.

Autoimmune disease

0042

BIOMARKERS OF ENDOTHELIAL DYSFUNCTION, CARDIOVASCULAR RISK FACTORS AND ATHEROSCLEROSIS IN RHEUMATOID ARTHRITIS

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Background. Rheumatoid arthritis (RA) is an inflammatory disease associated with premature atherosclerosis. We examined the hypothesis that mediators of endothelial dysfunction were increased and associated with the severity of coronary atherosclerosis in patients with RA.
Methods. 82 patients with RA aged ≤60 using metotrexat and 38 age and sex matched controls without diabetes mellitus or history of myocardial infarction, angina pectoris or stroke were included. Study subjects completed CVD risk factors, RA activity assessment and electron-beam computer tomography that measured coronary artery calcium (CAC). Blood was drawn for analysis of lipids, erythrocyte sedimentation rate (ESR), high sensitive C-reactive protein (hs-CRP), rheumatoid factor (RF), matrix metalloproteinase-9 (MMP-9), intercellular adhesion molecule-1 (ICAM-1).
Results. There were no significant differences in traditional CVD risk factors between RA patients and controls. Median CAC scores were higher in patients with RA than controls (0 (IQR: 0-25) and 0 (IQR: 0-2), $p < 0,05$). Patients with RA had higher levels of hs-CRP, ICAM-1 and MMP-9 compared to controls ($p<0,05$). Age (r_s- 0,34, $p<0,01$), gender ($p<0,05$), hypertension ($p<0,01$), smoking ($p< 0,05$), ICAM-1 ($r_s =0,38$, $p< 0,001$) were associated with higher amounts of CAC in patients with RA. Those in the highest quartile of ICAM-1were more likely to have any CAC (OR 14; 95% Cl: 1,66-117,99) and more extensive CAC (>100) (OR 11,3; 95% Cl: 1,95-65,93).
Conclusions. Patients with RA had increased coronary artery atherosclerosis when compared to age and sex matched controls, that may in part result from endothelial activation in these patients.

0043

INTRATHECAL IMMUNITY IN MULTIPLE SCLEROSIS: COMPARISON BETWEEN THREE METHODS OF ANALYSIS

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Background. Immunoglobulin intrathecal production is an important diagnostic parameter in patients with multiple sclerosis (MS), in which humoral immune system has been involved.
Methods. Pairs of cerebrospinal fluid (CSF) and serum (S) from 59 patients classified as definite MS were analysed. The performances of disc electrophoresis (DEP), isoelectric focusing (IEF) and IgG index were compared and the results were statistical established.
Results. Comparative analysis of detection of oligoclonal bands (OBs) showed fairly good correlation between the results of IEF and DEP. OBs were detected in 50 patients (85%) with both methods, 6 cases (10%) with IEF only and 1 patient (2%) only with DEP. Differences between the two methods were found only in 9 out of 59 patients (15%): in 6 patients (10%) OBs in CSF were detected by IEF, but not by DEP; in 1 patient (2%) OBs was obtained by DEP, but not by IEF. Sensitivity of IEF and DEP was estimated as 94,9% and 86,4% respectively. Elevated IgG index was found in 53 of 59 patients (90%) and all of them had OBs on IEF, as well as 3 patients (5%) with IgG index <0,7.
Conclusions. IEF seems to be superior in identifying OBs and aiding diagnosis of MS. IgG index calculation increase the diagnostic sensitivity of detection of the inrathecal IgG synthesis.

Autoimmune disease

0044

DEVELOPMENT OF A PROTOCOL FOR STUDYING PARANEOPLASTIC NEUROLOGICAL SYNDROMES (PNS) THROUGH ONCONEURAL ANTIBODIES IN OUR HOSPITAL

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Background. The paraneoplastic neurological syndromes (PNS) can be defined as remote effects of cancer, are seen < 1% of patients with cancer. The onset of neurological symptoms often precedes the cancer diagnosis. Clinical symptoms and signs besides onconeural antibodies, are the clues for diagnosis. These antibodies have high specificity, but one-third of the patients do not have detectable antibodies and 5-10% have an atypical antibody.
Methods. In order to make a diagnosis of SPN easier, Neurology and Laboratory Department have started up a protocol to study the onconeural antibodies. The diagnosis is organised in three levels:
- Neuro-Oncology Department: Clinical evaluation and request the autoantibodies. This Department classify patients in Classical Syndromes (high suspect) and Non Classical Syndromes.
- Laboratory Department: in Autoimmunity Section, study of the onconeural antibodies. The methodology used is an immunoblotting of the EUROLINE. This kit provides a qualitative assay for human autoantibodies of the IgG class to six antigens: amphiphysin, CV2.1, PNMA2 (Ma2/Ta), Ri, Yo and Hu.
- Reference Laboratory: positives and doubtful cases.
There is a database in which every case is compiled, it is shared between departments, showing clinical and laboratory details.
Results. We have studied 66 patients. We have found 3 positive Results. one was confirmed by Reference Laboratory, and 2 of them are pending of the confirmation. The first case is a PNS with prostate adenocarcinoma.
Conclusions. This protocol is making possible a faster and efficient diagnosis of PNS. The database is useful to follow the cases, and reevaluate and improve the protocol.

0045

ANALYSIS OF TNFR1 -383 A/C PROMOTER POLYMORPHISM IN PRIMARY SJÖGREN'S SYNDROME

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Background. Primary Sjögren's syndrome (pSS)is a chronic, systemic autoimmune disorder characterized by inflammation of exocrine glands and functional impairment of the salivary and lachrymal glands. Apoptosis of epithelial cells may play a role in the pathogenesis of this disease. TNFR1 is a transmembrane receptor which to activate the apoptosis pathway. A single nucleotide polymorphism at -383 position (A/C) has been proposed that affects the apoptotic death of epithelial cells and thus the pathogenesis of pSS.
Methods. We included 82 pSS patients, activity and damage indexes were applied. 84 healthy subjects (HS) were included as a control group. Genotypes were identified by PCR-RFLP technique. Soluble TNFR1 levels (sTNFR1) were measured by ELISA method. Statistical analysis was performed using SPSS v.17, and Epi Info v. 3.3.2.; $p<0.05$ was considered statistically significant.
Results. The genotype frequency for *TNFR1*-383A/C polymorphism in pSS was: 93% (A/A), 7% (A/C) and 0% (C/C) *vs* HS: 88% (A/A), 12% (A/C) and 0% (C/C) ($p=0.317$). The A allele frequency in pSS and HS was 91% and 94%; while the C allele: 9% and 6%, respectively ($p=0.329$). sTNFR1 levels was higher in pSS than in the HS (6234.2 pg/mL and 5536.9 pg/mL, respectively), however it was not statistically significant ($p=0.051$). Patients with the A/C genotype showed elevated rheumatoid factor levels ($p=0.040$ *vs* A/A genotype) and a highest score in the SSDAI index ($p= 0.045$ *vs* A/A genotype).
Conclusions. We suggest that polymorphism -383 A/C in the *TNFR1* gene is not associated with susceptibility in pSS.

Autoimmune disease

0046

ASSOCIATION OF *FAS*-670 A/G PROMOTER POLYMORPHISM IN THE FAS RECEPTOR GENE WITH PRIMARY SJÖGREN'S SYNDROME

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Background. Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease characterized by progressive lymphocytic infiltration of exocrine glands, mainly lachrymal and salivary, resulting in reduced secretory functions, and oral and ocular dryness. It has been proposed that A>G transition in the -670 position in *FAS* gene affects the receptor expression and therefore could modify the degree of glandular apoptosis, and thus the pathogenesis of pSS.
Methods. We included 82 pSS patientsand 84 healthy subjects (HS). Questionnaire for ocular and oral *sicca*, and SSDAI/SSDDI activity and damage indexes were applied. Genotypes were identified by PCR-RFLP technique. Soluble Fas levels (sFas) were quantified by ELISA test. Statistical analysis was performed using SPSS v.17; $p<0.05$ was considered statistically significant.
Results. The genotype frequency for *FAS*-670 A>G polymorphism was: in pSS 25% (A/A), 43% (A/G), 32% (G/G) and in HS: 24% (A/A), 50% (A/G), 26% (G/G). The G allele was more frequent in both groups: 53% in pSS patients and 51% in HS. Elevated sFas levels in pSS patients compared to HS was found ($p=0.037$). Patients with A/A genotype showed the highest sFas levels ($p=0.036$ *vs* G/G genotype), the highest number of lymphocytic infiltrate foci ($p=0.008$), the least amount of tear evaluated by Schirmer's test and a higher value for SSDDI index, including increased oral and neurological damage ($p=0.0034$ *vs* G allele) than patients who carried G/G or A/G genotypes.
Conclusions. Our findings suggest an association of the A/A genotype of *FAS*-670 A>G polymorphism with a severe form of the disease in pSS.

0047

PERFORMANCE OF ELIA ANA CTD SCREEN COMPARED TO HEP-2 AND SERAQUEST ANA SCREENING

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Background. To evaluate the diagnostic efficiency of a screening test EliA ANA CTD Screen.
Methods. We studied227patients (69 with known connective tissue disease, 94 with different autoimmune disease. All the samples were tested for ANAby ELISA(SeraQuest ScreeningANA) using acell extractenrichedwithHEP-2 DNA(dsDNA, nDNA), histones, SS-A/Ro, SS-B/La, Scl-70, Sm/RNP, Scl-70, Jo-1 andcentromericantigens, andEliaANACTDScreen(Phadia) to detectthefollowingspecific ANA(dsDNA, Sm, U1RNP (RNP70, A, C), SS-A/Ro(52 kDa, 60kDa), SS-B/La, CentromereB, Scl-70, Fibrillarin, PCNA, PM-Scl, RNAPolIII, Mi-2) and other antibodies(Jo-1, ribosomal-P proteins(P0, P1, P2)). Andalso we madeIFIonHEp-2 (Bio-Rad Laboratories). For statistical analysis we used the MedCalc program.
Results. Theareaunder thecurve(AUC) forANACTDScreenEliawas0.948(95% (from 0.910 to 0.973)), forIFI1 / 80was0.876(95% (from 0.826 to 0.916)), forIFI1 / 160was0.859 (95% CI (0.807 to 0.901) andANAScreeningSeraQuestwas0.830(95% (from 0.775 to 0.877)). Thecutoff obtainedbyROC curveanalysisforANACTDScreenEliawasaratioof0.5withsensitivity of89.86% (95% (from 80.2 to 95.8)) andspecificity of87.87% (95% (from 81.9 to 92.6)), LR+7.47, LR-0.12, PPV of 76, 5% andNPV of95.2%.
Conclusions. TheEliaCTDScreenshowed agoodsensitivityandspecificityandbetter diagnostic efficiency then SeraQuest Screening ANA.

Autoimmune disease

0048

KAPPA AND LAMBDA FREELITE* ASSAYS ON THE BECKMAN COULTER IMMAGE® 800: A NEW OPTIMISED PROCESS

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Background. Serum free light chains analysis in multiple myeloma and related disorders are becoming routine assays. However there are analytical concerns regarding high coefficients of variation, antigen excess risk, enhanced reactivity to polymer species and variable recovery with variation depending on the analytical platform used for analysis. Furthermore, the assays are time consuming to perform which increases laboratory costs. To improve the quality of results and analysis time for the Kappa, and Lambda Freelite* assays from The Binding Site (P/N LK016.IM lot 276264 and LK018.IM lot 273017), they were optimised for use on the Beckman Coulter IMMAGE® 800 nephelometric system.

Methods. Improvements were applied to the design of measuring ranges and to the sample dilution process. Apart to this change, the measurement protocol (i.e., timings, sample and reagents volumes) as defined in the original CE marked IFU of the reagent was maintained. The new process was compared to the analysis performed on the BN* nephelometric system from Siemens.

Results. There was good agreement between the two systems (N= 79, Kappa free r2=0.958 , Lambda free r2=0.9882). The new process for analysis of Kappa, Lambda Freelite* assays on the IMMAGE® 800 reduced the analysis time by 50%,, the manual handling steps by 44% and decreased the rerun tests from 62% to 38 %.

Conclusions. Optimization of the process on Immage 800 provides comparable results with another nephelemetric nephelometric system and simplifies the manual handling process thus saving significant time and cost.

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0049

PROFILING OF ANTIPHOSPHOLIPID ANTIBODIES – ASSOCIATION WITH CEREBROVASCULAR EVENTS IN ANTIPHOSPHOLIPID SYNDROME

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Background. Association of cerebrovascular events (CE) with antiphospholipid antibodies (aPL) in patients with antiphospholipid syndrome (APS) is reported controversially and depends on aPL reactivity and assay techniques.

Methods. A novel multi-line dot assay (MLDA) with phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL), and beta2-glycoproteinI (β2GPI) as phospholipid targets was used to detect aPL IgG and IgM in 85APS patients and65 disease controls (DC). Eight (9.4%) of the 85 APS patients APS had pregnancy morbidity, 62 (72.9%) arterial and/or venous thrombosis, 57 (67.1%) deep venous thrombosis, and 18 (21.2%) systemic lupus erythematosus. Thirteen (15.3%) APS patients demonstrated CE comprising cerebral transient ischemic attack (TIA) (10/65) and/or ischemic stroke (5/65).

Results. Anti-PI IgM (3/10), anti-PS IgM (5/10), and anti-CL IgM (7/10) antibodies detected by the MLDA demonstrated a significant higher prevalence in the APS patients suffering from TIAs compared with the remaining APS patients (0/75, $P < 0.05$; respectively). The detection of 3 or more aPL IgM by MLDA also revealed a significant higher prevalence in this APS patient cohort (5/10 vs 11/75; $P < 0.05$). Analyses of the 13 APS patients with cerebrovascular events revealed a significant higher prevalence of anti-PI IgM (3/13) and anti-CL IgM (9/13) compared with the remaining APS patients (0/72 vs 24/72; $P < 0.05$; respectively).

Conclusions. MLDA technique is an interesting alternative to ELISA for aPL antibody detection and profiling in APS patients. Multiplex detection of aPL IgM by MLDA revealed a significant association with cerebrovascular events in APS.

Autoimmune disease

0050

AUTOMATED INTERPRETATION OFANTINUCLEAR ANTIBODY ASSESSMENT ON HEP-2-CELLS BY STANDARDIZED PATTERN ALGORITHMS

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Background. Assessmnet of antinuclear antibodies (ANAs) by indirect immunofluorescence (IIF) on human epithelial–2 (HEp-2) cells is a basic tool for the serological diagnosis of autoimmune rheumatic disorders (ARD). Automation of ANA IIF interpretationcan improve assay reproducibility and intra- and inter-laboratory variability. Comparing automated and visual interpretation of ANA patterns, the usefulness for routine laboratory diagnostics was evaluated.
Methods. ANA detection on HEp-2 cells was performed in 1222 consecutive sera of patients with suspected ARD from a university routine laboratory (n=924) and a private referral laboratory (n=298). IIF interpretation findings obtained visually in routine diagnostics were compared with results by a novel automated interpretation system employing Aklides pattern recognition algorithms.
Results. Visual and automated interpretation of ANAdemonstrated a very good agreement regarding positive/negative discrimination (kappa=0.828). Only 98 (8.0%) of 1222 sera revealed discrepant results in the differentiation of positive from negative samples.The contingency coefficient of Chi-square statistics was 0.646 for the university laboratory cohort with an agreement of 93.0% and 0.695 for the private laboratory cohort with an agreement of 90.6%, p<0.0001, respectively. There was no significant difference in the university cohort (1.08%, p=0.25; McNemar test).
Comparing respective immunofluorescence patterns, 111 (15.3%) sera yielded differing results in both cohorts.
Conclusions. Automated Aklides interpretation of ANA is a reliable and robust method for positive/negative differentiation of IIF findings providing reproducible detection of specific patterns on HEp-2 cells. Automated interpretation can reduce drawbacks of ANA IIF detection in routine diagnostics providing more reliable data for clinicians.

0051

ASIALOGLYCOPROTEIN RECEPTOR (ASGPR) ANTIBODIES IN AUTOIMMUNE HEPATITIS – A REVIVAL

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Background. Liver-specific ASGPR is an autoantigenic target in autoimmune hepatitis (AIH). Apart from other AIH-specific antibodies, anti-ASGPR correlates with disease activity.
Methods. Affinity-purified rabbit ASGPR was used for standardised detection of anti-ASGPR by ELISA. Anti-ASGPR IgG was measured in patients with AIH (n=45), PBC (n=43), alcoholic liver disease(n=13), HBV infection (n=35), HCV infection (n=53), and118 blood donors.Anti-ASGPR was correlated with biochemical parameters of disease activity in 22 AIH patients with consecutive samples.
Results. Increase anti-ASGPR levels were demonstrated in 21/30 untreated (70%) and 5/15 treatedAIH patients (30%). Only one blood donorhad positiveanti-ASGPR.ALD and PBC patients were all negative.ROC curve analysis of AIH and disease-control patients revealed a sensitivity of 77.8% and a specificity of 99.4%. Furthermore, elevated anti-ASGPR was detected in 3/35 HBV (8.6%) and 7/ 53 HCV (13.2%) patients. In AIH patients, anti-ASPGR correlated with liver-transaminases levels. In 22 follow-up patients, elevation of anti-ASPGR preceded liver-transaminases increase.
Conclusions. The anti-ASGPR ELISA is a readily available and specificdiagnostic tool for anti-ASGPR assessment in AIH. Quantification of anti-ASGPR aidsin monitoring disease activity.

0052

PERFORMANCE OF A NEW ALGORITHM FOR CELIAC DISEASE DIAGNOSIS

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Background. Celiac disease affects at least 1% of the western population but still remains largely unrecognized. We use a novel algorithm offering both very high sensitivity and high specificity for pediatric celiac disease diagnosis in an outpatient setting. The aim of the present study was to challenge this algorithm and to test its performance in additional children and adults suspected for celiac disease.
Methods. Using the 3-assay algorithm (screening with tissue Transglutaminase (tTG) complexed with deamidated gliadin (DGP) neo-epitope IgA+IgG assay) and confirming with the specific two assays: tTG IgA and tTG IgA+IgG, we examined the serological results of 112 children aged 0-17 years old, and 60 adults in comparison to their respective biopsy Results. The algorithm performance was calculated by statistical analysis.
Results. The use of the new algorithm enabled us to provide celiac diagnosis with overall 98% sensitivity, 93% specificity and 95% accuracy in the children group and 94% sensitivity, 92% specificity and 93% accuracy in the total studied population. The false negative cases in the adults group were attributed to previous adherence to gluten free diet, and the single false negative result in a young child became true positive after six months. We have also monitored three celiac patients, before and after diagnosis and found that the algorithm may be suitable for disease monitoring.
Conclusions. The newly proposed 3-assay algorithm for celiac detection is very reliable in both children and adults. Due to its high performance, the further need for confirmatory intestinal biopsies will be reassessed.

0053

ROLE OF ANTI-CCP ANTIBODIES AND RHEUMATOID FACTOR IN DIAGNOSIS OF RHEUMATOID ARTHRITIS

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Background. Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, mainly characterized by inflammation of the synovial joints.It can lead to long-term joint damage, resulting in chronic pain. Until recently, laboratory diagnosis of rheumatoid arthritis has relied on the detection of rheumatoid factor.Identification of citruline as a target of a whole set of autoantibodies detected in the sera of RA patients has led to the development of anti-CCP assays that possess a high specifity for RA.
Methods. We analyzed anti-CCP antibodies (anti-CCP) and Rheumatoid factor (RF) in sera of 111 patients divided into two groups: the first group included 74 patients with diagnosed RA and the second group included 37 healthy individuals. Anti-CCP was measured by ECLIA on e411 Cobas analyzer (Roche Diagnostics). RF was determined by turbidimetry on ARCHITECT c8000 analyzer (Abbott Diagnostics, Germany).
Results. In control group of 37 patients anti-CCP and RF were both negative. In group of patients with RA 72 (97.3%) had elevated levels of anti-CCP. In the same group 62 (83.8%) patients had elevated levels of RF. 12 patients had elevated levels of anti-CCP with normal levels of RF, and 2 patients had elevated levels of RF with normal levels of anti-CCP.
Conclusions. Anti-CCP has a better diagnostic value than RF,which is reflected by a higher specificity and sensitivity. Additionally, anti-CCP antibodies are present early in the disease and also can predict future development of RA in patients with undifferentiated arthritis.

Autoimmune disease

0054

THE IMPORTANCE OF IMMUNOGLOBULINS LEVEL IN DIAGNOSYS OF PRIMARY IMMUNODEFICIENCIES

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Background. The primary immunodeficiency disorders (PIDs) reflect abnormalities in the development and maturation of cells of the immune system. They are uncommon, chronic and severe disorders of the immune system in which patients cannot mount a sufficiently protective immune response, leading to an increased susceptibility to infections. Aim of this study was to analyse the level of IgA,IgMand IgG in patients with immunodificiencies.

Methods. The sudy included 43 individuals grouped in two groups: experimental and control. Experimental group was composed of 33 individuals with abnormal Ig level and predispositions for immunodeficiency. Control group was composed of 10 healthy individuals. Serum Ig A, IgM and IgG were measured by immunoturbidometry method.

Results. Obtained results have shown significantly decrease in IgA concentration in experimental group for 82% (0,234±0,14 g/L), compared with control (1,28±0,49 g/L), which is for 67% less than minimal referent value (0,7-4,0 g/L). IgM concentration in experimental group was for 21% (6,355±3,41 g/L) less than control (8±2,55 g/L), and for 10% less than minimal referent value (7,0±16,0 g/L). And concentration of IgG in experimental group was 31% (0,72±0,42 g/L) less than control (1,04±0,56 g/L), but it was in the normal referent range (0,4±2,3 g/L).

Conclusions. Determination of IgA, IgM and IgG is very important for early recognition and diagnosis of PID, which can significantly alter the course of deficiency causing positive effect on patient outcome.

0055

EVALUATION OF ANTITHYROID ANTIBODIES IN CHILDHOOD IN AN AREA OF ADEQUATE IODINE INTAKE

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Aim. Investigation of antithyreoglobubin (anti-Tg) and antithyroid peroxidase (anti-TPO) antibodies (ATA) incidence and thyroid hormones values in children with evidence of thyroid disease.

Materials. In a two-year period (2008-09) 455 children (47,5% boys and 52,5 girls, aged 0-14 years), hospitalized (34%) or outgoing patients (66%) with symptoms of a dysfunctional thyroid gland, were examined for abnormal values of TSH, T3,T4 and the presence of ATA.

Methods. Samples were measured by microparticle enzyme-immunoassay AXSYM (Abbott).

Results. Positive ATA were found in 27,4% of the total 455 samples. More specifically, anti-Tg were detected in 65,6% followed by an 8,8% anti-TPO whereas both ATA were present in 25,6%. ATA detection was more frequent in female (64%) than male (36%) children. Most ATA positive values were found in ages 11-14 (44,8%) in contradiction to 8% when referring to ages 0-2 years. Autoimmune thyroiditis was discovered (anti-TG>1000 iu/ml, anti-TPO>300 iu/ml, TSH>6,8μu/ml, T3 and T4 normal values) in 4 cases and subclinical hypothyroidism (TSH >12μu/ml) coexisted in one. Furthermore, there was laboratory verification of 4 cases of hypothyroidism (TSH >12μu/ml) and 3 cases of hyperthyroidism (TSH<0,15μu/ml).

Conclusions. 1. Positive ATA were detected in 27,4% of children with clinical suspicion of thyroid dysfunction but the greater percentage were euthyroid. 2. Positive ATA titles are valuable serological markers in addition to pathological values of TSH and provide criteria for autoimmune thyroiditis diagnosis. 3. Lower titles of ATA mark early suspicion of autoimmune disease so thyroid hormone levels should be under laboratory monitoring.

Autoimmune disease

0056

PRODUCTION OF TH1 AND TH2-TYPE CYTOKINES BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background. Cytokines provide the network of effector molecules which link the cells of various target tissues into a complex conglomerate of responses and reactions. Many of the reactions in this network take place within the microenvironment of unique tissue-specific features, where identically functioning immune cells may exert different effects, especially in altered conditions such as their stimulation.
Methods. This study was performed in the supernatant of cultivated peripheral blood mononuclear cells (PBMC) obtained from the peripheral blood with cetrifugation on density gradient, stimulated or not with concavalin A and phorbol-myristat acetate (PMA). Samples from the patients with systemic SLE and healthy controls were used, and the concentration of parameters investigated, IFN-γ, TNF-α, IL4 and IL13, were determined by the ELISA Methods.
Results. TNF-α concentration in unstimulated PBMC supernatant was markedly elevated compared to controls (1035.50±138.00 pg/ml vs. 366.25±23.48 pg/ml, P<0.01), while IFN-γ, IL-4 and IL-13 were not significantly altered. In the conditions of concavalin A stimulation, IL-4 increased as an antiinflammatory cytokine (208.67±86.17 pg/ml vs. 18.83±10.24 pg/ml, P<0.01), while the PMA-PBMC stimulation elevated IFN-γ concentration 10-40 times, TNF-α concentration 2-55 times, I-L4 concentration 2-55 times, and IL-13 concentration 3-10 times.
Conclusions. Peripheral blood mononuclear cells stimulation results indicate that the outcome may be accompanied with the onset of positive feedback for secretion of certain cytokines. Closer determination of certain stimulated and supressed cytokines depends mainly on the disease phenotype and it opens the possibility for preventive and therapeutic interventions on the cytokines and their receptors.

Biobanking

0057

LONG TERM STABILITY OF SERUM CHEMISTRIES, PROTEINS AND HORMONES IN PEDIATRIC SAMPLES STORED AT -80°C

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Background. The Canadian Laboratory Initiative in Pediatric Reference (CALIPER) project aims to establish a comprehensive database of reference values from birth to late adolescence. The stability of analytes in the CALIPER bank of serum samples stored at -80°C is essential for ongoing establishment and future updating and validation of reference intervals.

Methods. Surplus serum samples collected from outpatient clinic children of 0-18 years of age were pooled into a single pool along with age-group specific pools. Following baseline measurement, each pool was aliquoted and kept frozen at -80°C until analysis. Samples were analyzed at monthly intervals over a 10-13-month period and each aliquot was subject to one freeze-thaw cycle before analysis. Sample analysis was performed on VITROS® Chemistry System, COBAS INTEGRA® 400 Plus, and IMMULITE® 2500 analyzers. Values obtained at monthly intervals were compared to baseline measurement and examined for trends over time.

Results. A majority of analytes measured in this study (e.g. electrolytes, glucose, enzymes, insulin, GH, IGF-1, vitamin B12) showed no significant change relative to baseline and no significant trend over time after up to 13 months of storage at -80°C. PTH showed a 27.2% decline after 10 months of storage with most of the decline evident after the first 2 months. Most analytes showed variability over time, which is thought to reflect method and instrument variability rather than the change in analyte stability.

Conclusions. Serum samples do not require immediate testing for reference intervals determination for most of the common analytes, with possible exception of PTH.

0058

SYSTEMATIC IDENTIFICATION OF ENDOGENOUS DECAY-MARKERS FOR QUALITY ASSESMENT OF SERUM SPECIMENS

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Background. High quality of serum specimens is crucial for any proteomics biomarker discovery approach but up to now, no appropriate analytical tools exist for quality assessment of complex proteinsamples. The proteolytic processing of proteins in serum specimens is a dynamic process and the peptide patterns are constantly changing. Here we identified multiple endogenous decay markers that allow the calculation of the preanalytical lapse of time for serum specimens.

Methods. Serum specimens of 6 volunteers were aged under controlled conditions and aliquots were taken at defined timepoints ranging from 0h to 45h. After peptide extraction the samples were analysed with nano-LC MS/MS and peptides were identified with PEAKS software. Peak intensities were calculated using SIEVE and Xcalibur software. In total, 210 peptides originating from 12 serum proteins (ALB, C4A, C3, F13, F2, FGA, FGB, ITIH4, KNG1, TMSB4X and FUK) were chosen for further validation.

Results. The identified peptides could be separated into four different categories according to the preanalytical lapse of time: Peptides with (I) decreasing, increasing (II), intermediate (III) and stable (IV) signal intensity. The multiparametric analysis of peptide patterns allowed the unsupervised classification of specimens according to their preanalytical status (fresh, medium, old).

Conclusions. The identification of endogenous decay markers in serum specimens will help to characterize the sample quality that is urgently needed to generate homogenous collectives for biomarker studies. The stability of peptide profiles that are related to sample quality should not be affected by different disease states and will have to be evaluated in prospective studies.

Biobanking

0059

CHEMOSENSITIVITY TESTING OF CIRCULATING EPITHELIAL TUMOR CELLS (CETC) DOES NOT CHANGE UNDER THE INFLUENCE OF CRYOPRESERVATION

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Background. Circulating epithelial tumor cells (CETC) can be identified in blood of patients with different solid tumors. In vitro sensitivity tests of CETC could rationalize and improve the choice of chemotherapy. Cryopreservation and prolonged storage of living CETC would enable multiple testing of chemosensitivity of patients' tumor cells and may be usable for several institutions interested in testing new chemotherapeutic agents.
The aim of the current study was to test CETC before and after freeze preservation for chemosensitivity.
Methods. CETC were isolated from blood of 20 patients suffering from breast cancer. Viability analysis of the cells with eutherapeutic concentration of four chemotherapeutic agents (Cisplatin, Doxorubicin, 5-Fluorouracil, Paclitaxel) was measured using the MAINTRAC method before and after cryopreservation.
Results. No significant differences in chemosensitivity were observed before and after cryopreservation using the method developed at our institution.In vitro-vitality reduction before and after cryopreservation compared for several agents were: 30,6% ± 18,3 vs. 29,8% ± 18,8 (Cisplatin), 92,7% ± 14,0 vs. 99,1% ± 1,2 (Doxorubicin), 34,4% ±17,5 vs. 34,0% ± 17,7 (5-Fluorouracil), 36,6% ± 22,5 vs. 34,1 ± 15,7 (Paclitaxel).
Viability of CETC after freezing-thawing procedure decreased on average by 14 % (239,7 ± 199,6 vs. 206,0 ± 162,1).
Conclusions. Circulating epithelial tumor cells can be succesfuly cryopreserved for further chemosensitivity tests.

Bioinformatics

0060

IMPLEMENTATION OF LABORATORY TEST MODULE AT ANDALUSIAN HOSPITAL OF LINARES (JAÉN)

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Background. The laboratory test module (LTM) manages the complete analytical process from test request to linking results inside Diraya, Andalusian electronic health record (HER). The LTM controls the traceability from the test request to the result query, at primary and specialized care.
Methods. Advantages evaluation by two indicators statistical analysis: percentage of correctly filled test request and demand impact. The evaluation period was of 6 moths and we evaluated only our principal Health Centre.
Results. Since the LTM start, we reach the 73% of electronic test request in 30 working days. The average percentage of correctly filled test request improved from 45.4% to 75.1% after LTM implementation. By request fields, date of bird improved from an average of 33.7% absent or incomplete dates to 15.7%. The diagnostic guidance improved from 19.7% absent to 8.6%. And the patient's ID number from 1.1% to 0.2% absent. About the demand impact, the number of test by request decreased from 13.7 to 13.3 after implementation. However, it was the same, compared with previous year same period. The number of test by request not included at primary care service portfolio decreased from an average of 1.03 to 0.32, during the previous and posterior LTM implementation months.
Conclusions. LTM implementation has supposed a perceived quality improvement. It's been improved correctly filled test requests and analytical results availability time. Spite of little variation at total demand impact, the number of test not included at service portfolio has been decreased, with the consequent Laboratory's economics cost reduction.

0061

MEASUREMENT OF IONISED CALCIUM IS INFLUENCED BY ALBUMIN CONCENTRATION – AN OLD BUT STILL RELEVANT ISSUE?

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Background. Several papers published in the 1980's described a clinically significant influence of albumin on the measurement of ionised calcium. The most likely cause was albumin directly influencing the reference electrode. We investigated whether this phenomenon is still a problem in patient samples.
Methods. We retrospectively examined serum ionised calcium (pH 7.4) (Ca^{2+}) and plasma albumin (albumin) analysed in samples from the same request to our laboratory in the period 1.1.2009 – 12.10.2010 (N=36,747), divided in outpatients (N=10,481) and inpatients (N=26,266). Ca^{2+} was measured with ion-selective electrodes using either KoneLab 30i (Thermo Scientific) or ABL700-series (Radiometer), while albumin was analysed using bromcresolgreen (Roche) on an UniCell DxC800 (Beckman-Coulter). Linear regression was used to assess the influence of albumin on measurements of Ca^{2+}.
Results. We found highly significant correlations between albumin and Ca^{2+} in the entire patient group (slope 0,0016, P<0,0001), as well as for both outpatients (slope 0.00093, P<0.0001) and inpatients (slope 0.001663, P<0.0001).
Conclusions. We confirmed a significant effect of reduced albumin concentrations on Ca^{2+}-results, however much smaller than previously reported of up to 0.066 mmol/l per 10 g/l albumin Thus, if it is found relevant to correct Ca^{2+}-results in case of reduced albumin, 0.015 mmol/l Ca^{2+} per 10 g/l albumin might be used. However, in most situations the small effect of changes in albumin on Ca^{2+}-results is probably of no clinical importance.

Bioinformatics

0062

MYCN ASSOCIATED REGULATIONS CONTRIBUTE TO THE METASTATIC POTENTIAL IN NEUROBLASTOMA

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Background. Neuroblastoma, the most common solid extracranial tumour in childhood, represents a heterogeneous group with different biological behaviour. In particular, metastatic ability varies amongst neuroblastomas and the presence of metastases generally determines survival of the patient. Several human neuroblastoma cell lines have been established and described over the last decades. However, essential molecular mechanisms determining the metastatic phenotype of neuroblastomas remain unclear so far.

Methods. Three human neuroblastoma cell lines (LS, LAN-1, IMR-32) were transplanted into SCID mice. The tumours exhibited clearly distinct, cell line dependent metastatic potential ranging from metastasis free progression (LS) over micrometastasis (LAN-1) to solid metastasis (IMR32). These tumours were analyzed on genomic and transcriptomic level using Affymetrix SNP 6.0 and Whole Genome Expression Arrays.

Results. The application of different approaches such as genotype-phenotype correlation and gene set enrichment revealed a number of potential genes and functional mechanisms, that seem to correlate with the observed tumour behaviour. While functional enrichments e.g. for transcriptional activity and multicelluar organismal development could be detected in IMR-32 induced tumour profiles, no significant enrichment for regulations contributing to the latter group could be observed in LAN-1 induced tumours. Instead a massive amplification and upregulation of MYCN and MYCN associated genes could be observed in this cell line. The identification of major candidates was highly assisted by strategies for correlation of genomic and transcriptomic data.

Conclusions. The reported results describe correlations between gene expression profiles and metastatic phenotype in a xenograft model that are supported by previous clinical findings.

Cancer/tumour markers

0063

THE DIAGNOSTIC ROLE OF REVERSE TRANSCRIPTASE-PCR (PT-PCR) IN PATIENTS WITH CHRONIC LEUCOCYTOSIS FROM SUDAN

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Background. Philadelphia chromosome, t(9;22)(q34;q11), in chronic myeloid leukaemia (CML) typically results in the two different BCR-ABL fusion transcripts b2a2 or b3a2. The type of the fusion gene in CML is thought to be related to the clinical course and outcome of the disease. There is limited information about the frequency of BCR-ABL transcripts in Africa, this study aimed to determine the frequency of BCR-ABL transcripts in relation to the clinical and haematolgical characteristics in patients with CML in Sudan.
Methods. Two mls of blood were collected from 151 consenting patients with a provisional diagnosis of CML.One hundredd and eleven patients were newly diagnosed and the remaining 40 were clinically diagnosed earlier and received hydroxyurea. Blood counts were routinely performed for all patients. Nested reverse transcriptase-PCR was performed using primers specific to M-bcr and m-bcr regions.
Results. One hunddred and forty seven patients (97.4%) were BCR-ABL positive. The frequency of BCR-ABL transcripts were 53.7% for b3a2; 27.3% for b2a2; 19% of patients showed other atypical and co-expressed transcripts. Platelets significantly higher in patients showing b3a2 compared to those with b2a2 transcripts (p=0.02). Other hematological parameters showed no significant differences between different transcripts.
Conclusions. The Philadelphia chromosome is seen in 97% of Sudanese patients with CML, making it an important diagnostic tool in chronic leucocytosis. The b3a2 transcript is associated with high platelets count.

0064

PROGNOSTIC SIGNIFICANCE OF APOPTOTIC MARKER IN BLADDER CANCER TREATED BY BACILLUS CALMETTE GUERIN THERAPY

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Background. Apoptosis and the genes regulating this process have recently become a focus of interest in the study of cancer development and progression. Although Bacillus Calmette-Guerin (BCG) intravesical therapy is a standard treatment for bladder cancer, eventual failure of response is a major problem. Treatments that can augment BCG therapy are urgently needed. The aim of the present study was to assess the prognostic significance of Bcl-2 expression in terms of recurrence after BCG immunotherapy in superficial bladder cancer.
Methods. The immunohistochemical evaluation of bladder tumor specimens that were obtained transurethrally for the expression of Bcl-2 was performed in 28 patients. All patients were treated with intravesical BCG-immunotherapy and followed up during 26 months. The prognostic significance of tumor stage, grade, loci number, tumor size, age and Bcl-2 in determining the risk for recurrence was studied with both univariate and multivariate methods of analysis.
Results. Univariate analysis showed a reduced recurrence-free survival (RFS) only for patients with pT1 stage (P=0.004) and high grade (P=0.004). However, multivariate Cox regression's analysis selected the model involving stage, age and Bcl-2 expression as the best independent variables of recurrence (p=0.005, p=0.020, p=0.020 respectively).
Conclusions. The evaluation of Bcl-2 expression in superficial bladder cancer could have a prognostic value in assessing the risk of disease recurrence after BCG immunotherapy. Further studies of other members of the Bcl-2 family are warranted, to define the roles and the interactions between them in order to ascertain new molecular markers of the response to BCG therapy.

Cancer/tumour markers

0065

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF HEMATOPOIETIC GROWTH FACTORS (HGFS) IN ENDOMETRIAL CANCER PATIENTS

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Background. HGFs play a role in the pathogenesis of cancer disease. We investigated the plasma levels of selected HGFs (SCF, stem cell factor; G- and M-CSF, granulocyte- and macrophage colony stimulating factor) in comparison to the tumor marker (CA125) in endometrial cancer patients and in relation to the control groups:uterine myoma patients and the healthy subjects.
Methods. Plasma levels of HGFswere determined using immunoenzyme assay (ELISA), CA125 concentrations by chemilumine-scent microparticle immunoassay (CMIA).
Results. Plasma levels of HGFs and CA125 were significantly higher inendometrial cancer patients as compared to the healthy control. Significantly different levels of SCF and CA125 were observed also between both control groups, but for M-CSF - between cancer and benign uterine tumor patients. The HGFs and CA 125 diagnostic specificities received high equal values. The diagnostic sensitivity, the positive and the negative predictive values were higher for M-CSF than for CA 125. The combined use of tested parameters resulted in the increase of the sensitivity range. The M-CSF area under the ROC curve (AUC) was the largest and slightly lower than the AUC of CA 125.
Conclusions. These results suggest a potential usefulness of M-CSF and SCF in diagnostic of endometrial cancer, especially in combined use with CA125.

0066

EVALUATION OF IL-17, TGF-B AND IL-6 TRANSCRIPTS IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH BLADDER CANCER

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Background. IL-17-producing CD4+ T helper (Th17) cells have recently been defined as a unique subset of proinflammatory helper cells are known by producing IL-17. IL-17 is an inflammatory cytokine with robust effect on many cells and it can play important roles in pathogenesis of a diverse group of immune-mediated diseases, including autoimmune diseases and cancers. Development of Th17 cells depends on signaling initiated by IL-6 and TGF-β and induction of RORγt. We, therefore, evaluated IL-6, TGF-β and IL-17 transcripts in the peripheral blood mononuclear cells patients with bladder cancer.
Methods. Blood samples from 37 patients and 37 healthy volunteers obtained. Then, abundance of them was determined by quantitative real-time PCR.
Results. Compared to the healthy individuals, IL-17 transcripts in the mononuclear cells in bladder cancer patients was significantly higher but TGF-β was lower. However, IL-6 in both groups didn't have any significant difference. Moreover there was not any significant difference between noted cytokines expression among patients with different stages and grades.
Conclusions. It is concluded that IL-17 as a prominent pro-inflammatory cytokine may play an important role in recruiting and infiltrating of anti-tumor immune responses in early stages of bladder cancer.

Cancer/tumour markers

0067

25(OH)D$_3$ AND SERUM PHOSPHOLIPID ARACHIDONIC ACID IN COLORECTAL CANCER PATIENTS IN RELATION TO DISEASE STAGE

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Background. Evidence suggests that some fatty acids and vitamin D play an important role in colorectal cancer etiology. In physiological state arachidonic acid (AA) is converted to prostaglandin (PG) which in certain condition may induce tumorigenesis. Because COX enzymatic activity and therefore PG synthesis is inhibited by vitamin D$_3$ metabolites it is reasonably to find out the relation between vitamin D$_3$ and AA in colorectal cancer patients.
Methods. Serum phospholipid AA was determined by gas chromatography and serum 25(OH)D$_3$ was analyzed by HPLC in 51 colorectal cancer patients before and one year after surgery. 26 patients were at 0, I or II stage of the disease (group I) and the remaining 25 patients were at III or IV disease stage (group II).
Results. For group I, the mean values of 25(OH)D$_3$ and AA level one year after surgery were significantly higher as compared to the mean values before surgery (p<0,01 - 0,04). For group II the mean value of AA was significantly higher (p<0,02) one year after surgery but no difference in vitamin D$_3$ level has been observed. Although there was no difference in the mean values of 25(OH)D$_3$ and AA concentrations before surgery between groups, one year after surgery the mean levels of vitamin D$_3$ did not differ between groups but there was significant decrease in the mean value of AA in group II as compared to group I (p<0,03).
Conclusions. The relation between AA and vitamin D$_3$ is confirmed only for earlier stage of colorectal cancer.

0068

DETECTION OF APOPTOSIS IN EXPERIMENTAL FIBROSARCOMA USING DNA FRAGMENTATION AND IMMUNOHYSTOCHEMICAL METHODS

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Background. Fibrosarcoma is driving from mesenchimal tissue. It is one of the maligntumors and usually seen in cats and dogs, however, it can be encountered in all animals.Apoptosis used for eliminating of cells which are functioning not properly. Recent studies on tumors showed that there is a connection between cancers and apoptosis.In this study, apoptosis in fibrosarcomas induced by 3-methylcholanthrene using immunuhistochemical and ELISA methods was investigated.
Methods. In order to induce fibrosarcoma in rats, at the beginning of the experimentanimals were injected subcutaneously on the neck with 0,2 mg 3- methylcholanthrenesolved in 0,25ml sesame oil. During the experiment which took between 150-210 days depending on the appearance of tumor tissue. At the end of the experiment animals werekilled under the ether anesthesia and necropsy is performed. DNA fragments of tumortissue cell were analized using ELISA, localisation of Bcl-2 and Bax was determined by immunohystochemical method.
Results. Immunohistochemically there was a lot of Bcl-2 and Bax positive cells. Contraryto this in control animals there was rarely. Furthermore, the absorbance of DNAfragmentation of experimental animals was 4 times higher than that of controls.
Conclusions. This data indicates that in the tumor tissues it is observed some cellsprogrammed to death by apoptosis but the others not.

Cancer/tumour markers

0069

_N_-GLYCOSYLATION OF SINGLE GLYCOPROTEINS FROM OVARIAN CANCER SERUM SAMPLES

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Background. Glycosylation is a post-translational modification occurring for instance in most serum proteins except from albumin. It is now accepted that glycosylation changes, occuring with various diseases, are often accompanied with cancerogenesis. The N-glycan analysis of human serum glycoproteins could be an interesting alternative technique to search for diagnostic biomarkers. The goal of our study is to detect tumor-associated alteration of *N*- and *O*-glycans in serum glycoproteins from patients affected with ovarian cancer.
Methods. The serum of 5 ovarian cancer patients and 5 age-matching controls was depleted of IgG and albumin then separated on two-dimensional (2D) gel electrophoresis. Single glycoprotein spots were analyzed from gel plugs of 1 mm diameter. The N-glycosylation of twenty spots was investigated in each 2D-gel. *N*-glycans were released enzymatically, desialylated and further characterized by MALDI-TOF-MS using an AnchorChip target and by capillary electrophoresis equipped with laser-induced fluorescence (CE-LIF).
Results. N-glycan quantification was achieved using CE-LIF. We observed an increase in fucosylation for the following glycoproteins: plasma protease C1 inhibitor, haptoglobin, apha-1 acid glycoprotein, alpha-1 antitrypsin and alpha-1 anti-chymotrypsin. For those mentioned proteins, statistically significant differences were observed for both core- and antennary fucosylation, for which marked increases were observed in ovarian cancer samples. Difucosylated tri- and tetraantennary structures were also observed but difucosylation did not occur in diantennary N-glycan structures.
Conclusions. Glycosylation pattern is glycoprotein-specific. Glycoprotein glycosylation is a promising tool for cancer biomarker discovery.

0070

GLYCOSYLATION STATUS OF ALPHA-1 ANTITRYPSIN IN OVARIAN CANCER

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Background. Protein glycosylation is altered with development of diseases such as cancer. Most human serum proteins, with the exception of albumin, are glycosylated. Alpha-1 antitrypsin (A1AT), which belongs to the family of serine-protease inhibitors, is involved in the protection of lungs from neutrophil elastase enzyme that drastically alter tissue functioning. A1AT is N-glycosylated at Asn-46, Asn-83, and Asn-247 and its concentration is modified in the serum of ovarian cancer patients. Aiming at biomarker discovery, we investigated the glycosylation status of A1AT in ovarian cancer.
Methods. Serum was collected from 15 patients as well as from 10 healthy age-matching women. A1AT was isolated from serum by immunoprecipitation. N-glycans were subsequently released using PNGase F and purified. Part of the samples was desialylated and analyzed by capillary electrophoresis using fluorescence detection (CE-LIF). The rest of the N-glycan pool was permethylated and analyzed by means of MALDI-TOF mass spectrometry.
Results. The CE-LIF analysis indicated an increase in monofucosylated di- and triantennary N-glycan structures in ovarian cancer patients. The mass spectrometric data revealed an increase in diantennary monofucosylated disialylated and triantennary monofucosylated trisialylated structures.
Conclusions. The glycan decoration of A1AT was significantly different in ovarian cancer serum from control serum. Fucosylation is an important N-glycan feature that could be used in biomarker discovery.

Cancer/tumour markers

0071

CHANGES OF SERUM GLYCOME IN PATIENTS SUFFERING FROM OVARIAN CANCER

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Background. Protein glycosylation plays an important role in many biological processes. Most human serum proteins, with the exception of albumin, are glycosylated. Glycosylation is known to be altered with development of diseases such as cancer. In the case of ovarian cancer, tumor markers among them CA-125 that are clinically used are known to have poor specificity. In addition, they fail to detect the disease at an early stage. Therefore, better biomarkers are needed. The aim of the present research work is to identify new potential glycan biomarkers by analyzing the serum N-glycome of patients suffering from ovarian cancer.
Methods. Serum was collected from 50 patients as well as from 25 healthy age-matching women. N-glycans were released from 10 ml serum by PNGase F digestion, permethylated and subsequently analyzed by means of MALDI-TOF mass spectrometry. The ClinProTools™ 2.2 software was used for all data interpretation steps.
Results. The N-glycome of patients was found to have more fucosylated structures, especially in tri- and tetraantennary sialylated glycans. The PCA analysis indicates that there are significant differences between the glycome of ovarian cancer patients and the glycome of healthy controls. We identified 14 potential N-glycan biomarkers. Six of them were tetraantennary sialylated glycans and five were fucosylated triantennary sialylated glycans. Other important deductions included the down-regulation of the high-mannose structure $Man_7GlcNAc_2$.
Conclusions. Our study has identified major differences between ovarian cancer sera and control sera, which could potentially be used in the future as biomarkers.

0072

A NEW SENSITIVITY METHOD TO ASSESSTHE GLOBAL METHYLATION IN FORMALIN-FIXED PARAFFIN-EMBEDDED DNA EXTRACTS

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Background. Archived formalin-fixed, paraffin-embedded (FFPE) specimens represent excellent resources for large scale studies on global DNA methylation degree alteration in tumor tissues. We developed the first capillary electrophoresis (CE) method able to measure global methylation in FFPE DNA extracts.
Methods. Genomic DNA was extracted from FFPE tumour tissue sections with a commercially available DNA extraction kit. Extracted DNA was hydrolyzed by means of formic acid and released cytosine (C) and 5-methylcytosine (mC) were measured by CE-UV detection using field amplified sample injection (FASI) technique
Results. C and mC were baseline resolved in less than 8 minutes using a running buffer Tris / phosphate 300mmol / L at pH 3.75. Using electrokinetic injection (10kV for 200s) the detection limit in real sample was 0.1 nmol/L, thus improving of about 400-fold the LOD of the previous described methods based on capillary electrophoresis. Sample extraction and purification have been optimized so that evaluation of the DNA methylation degree is possible starting from 0.5-1 μg of DNA with an intra- and inter-assay RSD for 5-methylcytosine/total cytosine ratio of 2.0% and 3.2%, respectively. The method application has been proved on a series of 17 cases of advanced colon-rectal carcinoma and 12 cases of lymphoma in which we found a mean of methylation degree of 4.23 ± 0.56 % and 4.53 ± 0.36 % respectively.
Conclusions. The elevated sensitivity and the relative simplicity make this method suitable for large-scale applications for studies on global DNA methylation degree alteration in extracted FFPE tumor tissues.

Cancer/tumour markers

0073

ANALYTICAL PERFORMANCE OF THE BECKMAN COULTER P2PSA ASSAY AND REPRODUCIBILITY OF THE PROSTATE HEALTH INDEX (PHI)

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Background. With the aim of improving the clinical performance of the prostate specific antigen (PSA) for the early detection of prostate cancer (PCa), Beckman Coulter recently developed a "prostate health index" (*phi)* which combines tPSA, fPSA and [-2] proPSA results (*phi*=[-2]proPSA/fPSA)x√tPSA). It is important to evaluate the variability of such index. The analytical performance of the p2PSA and the reproducibility of the *phi* index were evaluated in routine practice.
Methods. The serum concentrations of tPSA, fPSA, and [-2]proPSA were measured with Beckman Coulter immunoassays on DxC880i instruments. The study was performed with Beckman Coulter QC. Repeatability (intra-assay precision) of the p2PSA assay was assessed with 21 replicates of three different concentrations. To determine the *phi* index inter-assay precision, different concentrations of tPSA, fPSA and p2PSA QC were analysed in 40 separate analytical sessions. Various levels of *phi* index were then calculated using different combination of tPSA, fPSA and p2PSA concentrations.
Results. The Access tPSA and fPSA assays showed good repeatability with coefficient of variation (CV) below 7.7%. The p2P-SA assay demonstrated an even better intra-assay precision, with CV ranging from 2.6 to 5.8% depending on the concentration assessed. This excellent analytical performance was confirmed on pooled patient's serum samples (CV < 5%). The inter-assay precision of the phi index was also good with a maximum CV < 9%.
Conclusions. The results of this analytical study demonstrated an excellent repeatability of the p2PSA assay. The inter-assay variability of the calculated *phi* index was also very good with CV below 10%.

0074

TOTAL (TSA) AND LIPID-BOUND SIALIC ACID (LSA) IN THE SERA OF PATIENTS WITH PRIMARY PANCREATIC CANCERS

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Background. Serum concentration of sialic acid in sialoglycoproteins and sialoglycolipids have drawn considerable interest because of carbohydrate alterations in malignant cells exist. This findings have stimulated studies on total and lipid-bound sialic acid as a possible markers for the malignant transformation.
Methods. TSA and LSA concentration in the serum was measured according to enzymatic method (EnzyChrom Sialic Acid Assay Kit) using the colorimetric procedure for TSA and fluorimetric for LSA determination. LSA measurement was followed by the precipitation and extraction step (procedure of Katopodis and Stock'a).
Results. Mean total sialic acid (TSA) concentration in the sera of patients with pancreatic cancers (mean: 1.97 mM/L; range: 1.62 - 2.81; n=49) is significantly higher (P<0.001) than the mean level in the healthy controls (mean: 1.42 mM/L; range: 0.86 - 2.16; n=40). The level of LSA in cancer patients (66.74 mM/L; range: 31.48 - 123.56) is also significantly higher than that in the controls (24.68 mM/L; range: 14.02 - 53.13). There were no differences in TSA and LSA concentrations when cancer patients were analyzed according to the location of the tumours: head, body or tail, although the mean TSA level in tumour located in the tail of pancreas was lower (1.725; range: 0.887 – 2.813) than mean TSA level in total cancer group. In cancer patients, TSA correlated positively with LSA (R=0.299; P=0.037).
Conclusions. We conclude that both TSA and LSA may be used as a tumour markers and suggest that may be useful in the diagnosis of primary pancreatic cancers.

Cancer/tumour markers

0075

SERUM HER2-ECD DURING TRASTUZUMAB BASED THERAPY IN WOMEN WITH PRIMARY BREAST CANCER: PRELIMINARY RESULTS OF AN ITALIAN MULTICENTRIC STUDY (SHORT-HER).

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Background. In metastatic breast cancer serum HER2-ECD levels correlate with the clinical course of the disease. Aim of this study is to investigate the possible use of HER2-ECD measurement in Primary Breast Cancer (PBC).
Methods. SHORT-HER is a multicentric randomized study conducted in HER2 positive, surgically resected breast cancer patients in order to evaluate 2 different adjuvant Trastuzumab schedules (3 vs 12 months). We determined serum level of HER2-ECD at the beginning and at the end of therapy in 26 subjects randomized for the two treatments: long control arm (n= 10) in which Trastuzumab was administered for 12 months and short experimental arm (n=16) in which therapy was administered for 3 months. The assays were performed by ADVIA Centaur XP, Siemens; serum concentrations <15 ng/mL were considered normal.
Results. Intra assay variation (CV%) of HER2-ECD asdetermined from differences in duplicates (n=15) was 5,3% in the 6,4–13,6 ng/mL range. All patients showed HER2-ECD levels <15 ng/mL at the beginning and at the end of therapy. Three patients showed an increase >30% and one presented a decrease of 20% at the end of therapy compared to the baseline values.
Conclusions. Our data can contribute to define a possible use of serum HER2-ECD in PBC as a marker of response to Trastuzumab.The clinical significance of the observed variations will require an adequate period of follow up.

0076

N-ACETYL-L-CYSTEINE INHIBITS BLEOMYCIN INDUCED APOPTOSIS IN HUMAN TESTICULAR CANCER CELLS

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Background. Various anti-carcinogenic agents induce apoptosis by generating reactive oxygen species. In this study we investigated the susceptibility of the human testicular cancer cell line NCCIT to apoptotic cell death induced by Bleomycin and how this is effected by N-Acetyl-L-cysteine (NAC). Molecular mechanisms involved in the induction of cell death were determined by measuring the activities of caspase-8, -3, -9, and protein level of Bax.
Methods. The toxic concentration range of bleomycin was determined using methyl tetrazolium (MTT) assay test and caspase-8, -3, -9 proteolytic activities and Bax protein level were measured using colorimetric assay kits.
Results. Incubation of cells for 72 hours with Bleomycin at the concentration of 0.2 µM causing 20% loss of cell viability induced an increase in caspase-8, -3, -9 activities as well as an increase in Bax protein level. Incubation of cells with 10 mM NAC, an antioxidant that replenishes intracellular GSH, eliminated the effects of Bleomycin on caspase-3,-8,-9 activities and Bax protein level.
Conclusions. Our results indicated that NAC inhibits apoptotic cell death in NCCIT cells induced by bleomycin by preventing activation of caspases and bax level. Bleomycin mediated apoptosis was suppressed by NAC. Whether or not an antioxidant supplement would be helpful, harmful, or neutral depends on the specific antioxidant and its dose,the chemotherapy drugs being used,the type of cancer being treated, and the type of diet the patient is consuming.Well designed randomized controlled trials are needed to fully elucidate the impact of single antioxidants and antioxidant combinations in conventional cancer therapy.

Cancer/tumour markers

0077

THE PLASMA LEVEL OF MYELOPEROXIDASE (MPO), γ-GLUTAMYLTRANSFERASE (GGTP) AND TOTAL ANTIOXIDANT STATUS (TAS) IN GASTRIC CANCER PATIENTS AFTER SURGERY.

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Background. The human body is subject to constant effects of reactive oxygen species(ROS).Uncontrolled increase however is known as oxidative stress and may have serious consequences.The purpose of this investigation was to evaluate in the plasma of gastric carcinoma patients the level of MPO,γ-glutamyltransferase - sourceof ROS and TAS (total antioxidant *status).*
Methods. MPO concentration was measured using chemiluminescent immunoassay technology, activity of GGTP a spectrophotometric method and TAS colorimetric method.
Results. We tested 28 patients with III and IV stage of gastric carcinoma. Plasma samples were drawn before and 1 and 10 days after operation. In III stage,MPO concentration before (333,3 ng/ml) and after operation (342,8 ng/ml and 294,4 ng/ml) was higher in comparison to the control group(112,3 ng/ml). In IV stage before was 333,0 ng/ml, and after operation 321,0 ng/ml and 330,4 ng/ml. Activity of GGTP in III stage was 25 IU/L before operation, and after, was 31,5 IU/L and 149 IU/L. In IV stage was 36 IU/L before and 52 IU/L after 1, and 193 IU/l after 10 days. Activity of GGTP in control group was 42 IU/l.TAS concentration before operation in III stage was 1,71 mmol/ and after 1,74mmol/l and 1,72 mmol/l after operation. In group with IV stage was 1,77mmol/l before, and 1,68 mmol/l and 1,60mmol/l after operation.TAS in control group was 1,94 mmol/l.
Conclusions. These results suggest the presence of prolonged oxidative stress in malignant disease but it requires long time observationafter surgery.

0078

PROGASTRIN-RELEASING PEPTIDE (PROGRP) IN THE DIFFERENTIAL DIAGNOSTICS OF LUNG CARCINOMA

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Background. Our study was concerned with evaluation of the diagnostic potential of determination of the level of ProGRP as of a tumour marker for Small Cell Lung Cancer (SCLC).
Methods. Our cohort consisted of 117 patients. Out of this, there were 25 patients with SCLC, 61 patients with non-small cell lung carcinoma (NSCLC), and 31 patients with non-tumorous lung diseases (pneumonia, chronic bronchitis, sarcoidosis, tuberculosis, etc.). In the case of malignant tumours, they were newly diagnosed, as yet untreated cases. Besides the ProGRP level in the plasma, we determined neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and Cyfra 21-1 in the patients studied. We compared the ProGRP values established (median) in the three groups of our patients. Another criterion of comparison was represented by the value of Area under the ROC curve (AUC) for ProGRP, NSE, CEA, and Cyfra 21-1 in relation to SCLC diagnosis.
Results. The ProGRP values (median) in patients with non-tumorous lung diseases and in patients with NSCLC were 38.1 pg/ml and 40.7 pg/ml respectively. In patients with SCLC the ProGRP value established (median) 871 pg/ml. The Area under the ROC curve (AUC) in relation to SCLC diagnosis for ProGRP, NSE, CEA, and Cyfra 21-1 was 0.945, 0.652, 0.527, and 0.723, respectively.
Conclusions. The results of our study suggest that ProGRP is a useful and available tumour marker with sufficient sensitivity and specificity in relation to differential diagnostics of SCLC, both against non-tumorous lung diseases and against NSCLC.

Cancer/tumour markers

0079

COMPARISON OF THYROGLOBULIN (TG) AND TG AUTOANTIBODIES (ATG) MEASUREMENTS OBTAINED WITH TRACE AND ECLIA METHODS IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA (DTC)

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Background. Tg and aTg measurements are widely used in clinical practice for monitoring DTC patients. The aim of the study was to compare results of Tg and aTg determinations obtained with two immunometric technologies (TRACE and ECLIA) to support interpretation of serial measurements done by different methods during long- term observation of patients.
Methods. Tg and aTg were simultaneously measured in serum samples of 339 patients with DTC. TRACE technology (Kryptor analyzer- Brahms) is a immunofluorescent assay,ECLIA (Cobas e411 analyzer-Roche) is based on chemiluminescence.
We have compared intra and inter assay reproducibility (CV) for both Methods.
To evaluate the comparison between this two methods Pearson's correlation test and analysis of linear regression were used. To evaluate the concordance of aTg results Mc Nemar test was used.
Results. The range of Tg levels was 0,17-265200 ng/ml for TRACE and 0,10-568210 ng/ml for ECLIA (medians: 0,594 and 0,899 respectively). Both methods were significantly correlated r=0,99 for all Tg results; 0,88 for samples with Tg< 2 ng/ml; 0,92 for Tg 2-10 ng/ml and 0,88 for Tg10-30 ng/ml. The range of aTg levels was: 10- 4000 IU/ml, with significantly higher values for ECLIA than for TRACE with correlation between both methods r= 0,80
Conclusions. There was a linear correlation between TRACE and ECLIA Tg and aTg Results. Tg and aTg levels obtained by ECLIA are approximately twice higher, which have to be considered at the clinical interpretation.

0080

SERUM LEVEL OF CHITINASE-3-LIKE PROTEIN IN HEALTHY BULGARIAN PEOPLE

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Background. Chitinase-3 like-1 protein (CHI3L1) is a glycoprotein belonging to the family 18-glycosyl hydrolases that lack chitinolytic activity but retain chitin-binding ability. It is also known as YKL-40, breast regression protein – 39, human cartilage glycoprotein-39 (HC-gp39), 38-kDa heparin-binding glycoprotein (gp38k), chondrex. It is expressed and secreted by macrophages, chondrocytes, activated neutrophils, differentiated monocytes, vascular smooth muscle cell and cancer cells. Its biological functions in normal and pathological conditions are not fully understood. It is believed that CHI3L1 has a role in inflammation, cell proliferation and differentiation, protection against apoptosis, stimulation of angiogenesis, and regulation of extracellular tissue remodelling. The objective of the present study was to determine serum CHI3L1 levels in healthy Bulgarian subjects by a validatedreproducible enzyme-linked immunosorbent assay.
Methods. Serum CHI3L1 concentrations were determined by a two-site, sandwich-type, enzyme-linked immunosorbent assay (ELISA) in 14 healthy female and male volunteers aged 33-87.
Results. Our investigation showed a mean value $88{,}79 \pm 36.48$ ng/ml of serum glycoprotein in healthy subjects.Single-factor dispersion analysis found no age and sex dependency of CHI3L1 ($P > 0.05$) in the studied group. It coincides with results announced about Chinese and Japanese populations in the same age range.
Conclusions. Our study is the first in Bulgaria to measure serum CHI3L1 in healthy Bulgarian subjects. A possible function as a potential biomarker in inflammatory and malignant diseases is suggested.

Cancer/tumour markers

0081

CIRCULATING C-ERBB2, INSULIN LIKE GROWTH FACTOR 1 AND EPIDERMAL GROWTH FACTOR IN CANCER PATIENTS

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Background. Early detection of cancer, and/or recurrences is a cruical factor for a successful therapy and increased survival rate. Predictive potentials of c-erb-B2, insulin like growth factor 1(IGF-1) and epidermal growth factor (EGF) were examined in the present study.
Methods. Cancer patients (n=87) and healty volunteers (n=27) were included by the study. Serum levels of c-erb-B2, IGF-1 and EGF were measured by ELISA.
Results. Cancer group was constituted by patients with urinary bladder, breast and gastric cancer. In the cancer group serum level of c-erbB2 was found to be increased, serum level of IGF1 was found to be decreased when compared with the control group. No significant difference was determined between the study groups for serum level of EGF. Although c-erb-B2 level was higher in patients with metastase and in patients with high grade tumor as compared to their correspondings, these differences were not statistically significant. When data were evaluated in each cancer subgroup; serum level of c-erb-B2 was higher, serum levels of IGF-1 and EGF were lower in the patients with urinary bladder cancer as compared to control group; serum level of c-erb-B2 was higher in the patients with both breast and gastric cancer but serum levels of IGF-1 and EGF were not statistically different in these groups as compared to control group.
Conclusions. Data shows that reliable alterations occur in the serum levels of c-erb-B2 and IGF-1 in cancer patients. In order to reveal predictive/progressive potentials of these parameters further studies in larger groups should be performed.

0082

THE THYMIDYLATE SYNTHASE GENE ALLELE POLYMORPHISMS AMONG CANCER PATIENTS FROM UKRAINE

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Background. Thymidylate synthase (TYMS) plays a crucial role in themaintenance of deoxynucleotides pool for DNA synthesis. The polymorphisms of the variable number tandem repeats (VNTRs) sequence in the promoter enhancer region of the TYMS gene modify enzyme activity and interfere in normal cell division. The objective of the study was to analyse VNTRs alleles distribution of TYMSgene among patients with cancer from Ukraine.
Methods. Genotyping of 3R/2R and 3RG/3RCVNTR polymorphism of TYMS gene was performed in 72 patients with oncological diseases (colorectal cancer, breast cancer, leukemia) and in 30 healthy persons without cancer pathology in anamnesis. The molecular- genetic analysis was performed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism). Statistical analysis was conducted by Chi-square tests and odds ratio (OR)was calculated.
Results. The 3R allele frequency of TYMS gene was significant higher in patients with breast cancer (0.56) vs control (0.37). The increased risk of breast cancer development was associated withTYMS 3R/3R genotype (OR= 2.35) and colorectal cancer development with 2R/3R genotype (OR =1.71). The frequencyof 3RG and 3RC alleles was not differed among patients with cancer and control group. The 3RG/3RG genotype frequency of TYMS gene was higher in patients with leukemia (0.43) vs control (0.23) and the presence of the 3RG/3RG genotype was associated with 2.44 - fold increased risk of leukemia.
Conclusions. The results suggest that the polymorphic variant of VNTRs of TYMS gene play a role in the susceptibility to cancer development.

Cancer/tumour markers

0083

MOLECULAR DIAGNOSTIC OF PROSTATE CANCER USING A PANEL OF DNA METHYLATION BIOMARKERS

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Background. Prostate cancer is the most common malignancy of the urogenital tract. The pathogenesis of prostate cancer is attributable to multiple molecular changes, including gene mutations, changes in gene expression, protein functions and other cellular processes. Hypermethylation in the promoter regions of tumor suppressor genes is an early event which occurs during carcinogenesis.In our study we have investigated wheather a panel of DNA methyation biomarkers in plasma DNA samples is usesful to detect PCa.

Methods. We obtained plasma DNA samples from 236 patients with PCa and 282 patients with BPH. All patients presented elevated levels of PSA (>10ng/ml), but their prostate biopsy was negative.We analyzed the methylation status of 5 tumor suppressor genes including (GSTP1, RASSF1A, RARbeta2, APC and E-cadherine) by methylation-specific PCR (MSP) followed by methylation genotyping oligonucleotide analysis.The result was interpreted as being abnormal when hypermethylation was found in 2 or more genes.

Results. Plasma DNA samples from PCa patients presented high frequency of promoter hypermethylation for RASSF1A (81.5%) ,GSTP1(75%) and RARbeta2(69%).232 (98.3%) from 236 patients with PCa showed hypermethylation in at least one or more tumor suppressor genes, whereas only 20 (7%) from 282 patients with BPH showed hypermethylation.

Conclusions. Using a panel of DNA methylation biomarkers including RASSF1A, GSTP1 and RARbeta2 in plasma DNA samples may aid in differential diagnosiswith BPH and post-treatment follow-up of PCa.

0084

EPIGENETIC BIOMARKERS IN THE DETECTION OF PROSTATE CANCER

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Background. Prostate cancer (PCa) is the most commonly detected male cancer and the second leading cause of male cancer deaths in the U.S and Europe.Needle biopsies only contain small samples of tissue and often include only a few malignant glands among many benign glands. It is uncommon for many patients to be subjected to multiple biopsy examination, before a correct diagnosis is established.

In this study, we tested the ability of a panel of methylation biomarkers which are hypermethylated in PCa to improve the detection of PCa in sextant needle biopsies.

Methods. For our study we used sextant biopsies from 57 excised prostates, with negative biopsy results, and total PSA in a range of 3 ng/ ml to 10 ng/ ml.To detect the presence of PCa in the excised samples, we analyzed by quantitative real-time methylation-specific PCR the methylation status of three genes: APC, RARbeta2 and GSTP1.

Results. We detected 55 from 57 (96.5%) cases of PCa, using this panel of methylation biomarkers in combination with the histopathological result and total PSA.

Conclusions. Using a panel of methylation biomarkers (GSTP1, RARβ2 and APC), can aid to diagnose PCa, in cases with a negative biopsy result and a total PSA between 3 ng/ml and 10 ng/ml.

Cancer/tumour markers

0085

DETECTION OF ABERRANT PROMOTER HYPERMETHYLATION OF MULTIPLE GENES IN URINE SAMPLES FROM KIDNEY CANCER PATIENTS

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Background. Aberrant promoter hypermethylation of cancer-associated genes occurs early during carcinogenesis, and provides alternative pathway to gene deletion or mutation for the loss of tumor suppressor gene functions.This epigenetic change is regarded as a new biomarker in human cancers, including kidney cancer. In this study we have investigated wheather using a panel of tumor suppressor genes in urine sediments DNA is useful in early detection of kidney cancer.
Methods. For this study we analyzed matched kidney tissue and urine DNAs, from patients with kidney cancer and patients with no history of urinary diseases, as control subjects.
Using sensitive methylation-specific PCR (MSP), we analyzed matched tumor DNA and sediment DNA from preoperative urine specimens obtained from 67 patients with kidney tumors, for the hypermethylation status of a panel of three tumor suppressor genes: VHL, APC and RASSF1A.Hypermethylation of at least one of the three genes was found in all 67 tumor DNA and an identical pattern of gene hypermethylation was found in the matched urine from 64 of 67 patients (95.6%).
Hypermethylation of the three genes was not found in kidney tissue or urine sediment from control subjects.
Conclusions. Using an optimal panel of tumor suppressor genes could aid in early detection of kidney cancer by noninvasive **Methods.**

0086

MDR-1 AND APOPTOTIC GENES EXPRESSION IN ACUTE LEUKEMIA

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Background. Chemotherapy resistance is a major problem in the management of patients affected by acute leukemia. Overexpression or dysregulation of some oncogenes may have a crucial role in oncogenesis, by affecting intracellular growth controls, stimulating cytokine production and promoting or suppressing apoptosis.
Aim. The aim of the present study is to assess gene expressions of P-gp, P53 and Bcl-2 in acute leukemia (AL) patients in Mansoura Hospitals and to correlate these levels with other known prognostic factors and to patients' outcome.
The study comprised 48 patients with newly diagnosed AL and 20 healthy volunteers with matched age and sex were taken as a control. All patients received treatment for AL and were followed-up in an out patient department and observed over 24 months or until death. 17 patients died during the follow up time.
Methods. All patients (at diagnosis) and healthy controls were subjected to some laboratory investigations as CBC, ESR, serum LDH, creatinine, lipid profile, liver functions tests (ALT, AST, Albumin and Bilirubin). Assessment of gene expressions of P-gp, P53 and Bcl-2 using flow cytometric technique were carried out for patients (at diagnosis and at remission) and healthy controls. Our studies showed that P-gp, P53 and Bcl-2 expressions were significantly elevated in the AL patients compared to control group. Also there was a highly significant increase in P53 and Bcl-2 at diagnosis than at remission.
Results. Regarding P-gp expression, a highly significant increase in AL patients at remission compared to AL patients at diagnosis. The comparison between non-survived and survived AL patients show a highly significant increase in P-gp, P53 and Bcl-2 expressions in non-survived patients compared to survived patients at diagnosis.
Conclusions. From this study we conclude that proteins related to both apoptosis and multidrug resistance are among the most widely characterized drug resistance mechanisms leading to unsuccessful treatment of AL. The measurement of P-gp, P53 and Bcl-2 in acute leukemic patients at diagnosis deserves explanation in the prognostic evaluation of acute leukemia. Overexpression of both P-gp, P53 and Bcl-2 may reflect poor prognosis for which P-gp inhibitors and gene therapy is suggested to be used in future as adjuvant therapy to improve patient outcome.

Cancer/tumour markers

0087

CLINICAL EVALUATION OF PROSTATE HEALTH INDEXIN A GENERAL POPULATION IN ORDER TO DETECT PROSTATE CANCER

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Background. Because of the lack of specificity of the prostate-specific antigen (PSA) for prostate cancer (PCa) early detection, the [-2]proPSA, a proenzyme isoform of PSA has been introduced as a new biomarker. It has been integrated in a PHI index (Prostate health index) defined as $(p2pSA / fPSA) \times tPSA^{0.5}$. Its use is recommended in order to discriminate CaP patient from BPH in the total PSA grey zone of 2.5 ng/ml –10ng/ml. The goal is to reduce the number of prostate needle biopsies.
Methods. PSA, free PSA and [-2]proPSA were tested in 444 patients referred by GPs for blood sampling in order to calculate the clinical efficiency of PHI index. All the assays were run simultaneously on fresh serum samples on a UniCel® DxI 800 (Beckman Coulter).
Results. Among the 444 patients, 139 (31%) exhibited a total PSA in the target range from 2.5 ng/ml to 10 ng/ml. Among them, 95 were CaP free whereas 14 were diagnosed as CaP. 30 patients were excluded because of the lack of clinical data. If the PHI cut-off, set at 22.5 as recommended in the literature, is used, the sensibility and specificity of the PHI index were 100% and 12.6% respectively. At a cut-off of 40, suggested by Beckman Coulter data, the sensibility and specificity were 85.7 and 71.2%.
Conclusions. In order to reduce the number of unnecessary biopsies, the clinical cut-off of PHI index must be more precisely defined based on prospective studies.

0088

MASS SPECTROMETRY BASED FUNCTIONAL PROTEASE PROFILING OF SERUM SPECIMENS WITH REPORTER PEPTIDES FOR DIAGNOSIS OF MALIGNANT DISEASES

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Background. The progression of colorectal cancer (CRC) is characterized by the release of tumor-associated proteases like cancer procoagulant (CP), MMP2 and MMP7. The detection of tumor specific proteolytic activity in serum specimens is a new diagnostic tool in oncology.
Methods. Three reporter peptides (RP) were constructed that are specifically cleaved by tumorassociated proteases CP, MMP2 and MMP7 respectively. Accordingly, the concentration of proteolytic fragments is a surrogate marker of tumorspecific proteaseactivity. We developed a LC MS/MS method for the absolute quantification of RP-fragments with appropriate internal standards. Serum specimens of CRC-patients and non-malignant controls were spiked with RPs and incubated under standardized conditions prior to peptide extraction and LC-MS/MS analysis.
Results. The MS-assay for quantification of RP-fragments had a broad dynamic range with linearity over 3 log scales and excellent sensitivity with LOQ of 160 fmol and good reproducibility with CVs of <20%. Mean concentration of RP-fragments in serum specimens from tumor patients were 10.4, 11.4 and 7.6 [pmol] for RPs cleaved by CP, MMP2 and MMP7 respectively. In contrast, the mean concentration of these fragments in serum specimens from non-malignant controls were significantly lower ranging from 4.2, 5.2 to 4.0 [pmol] for RPs cleaved by CP, MMP2 and MMP7 respectively.
Conclusions. We established a multiplex LS-MS/MS assay for quantification of tumorassociated proteaseactivity in serum specimens. The peptide-quantification is robust and very similar to established laboratory methods (e.g. IFCC reference method for HbA1c). This new approach might lead to functional MS-based protease profiling for improved disease classification and monitoring.

Cancer/tumour markers

0089

TWO PSA ASSAYS OF THE SAME ANALYZER – ARE THE VALUES COMPATIBLE?

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Background. It is a fact that you can get different results for total prostate specific antigen (tPSA) obtained with different assays of different manufacturers and analyzers. We show that you can´t take identicalness of tPSA-values for granted, although obtained with the same immunoassay analyzer of the same manufacturer but with the two assays "PSA" and "Third GenerationPSA" (Immulite®).

Methods. The method comparison for the 2 immunoassays "PSA" (tPSA) and "Third Generation PSA" (tPSA,3rd Gen.) (Immulite®,Siemens) was performed using 340 serum samples. Free PSA (fPSA, Immulite®, Siemens) was determined if t-PSA was in the range 3,1 – 10 ng/ml. The ratios f/tPSA respectively f/tPSA,3rd Gen. were calculated. In 61 cases the prostate disease (31 prostate cancer,30 no evidence of cancer) was known and that allows to evaluate the diagnostic validity of the tests to differentiate between "cancer" and "no cancer".

Results. The tPSA,3rd Gen. - values maybe up to 20 % lower than the respective tPSA values. When using the cut-off PSA = 3,7 ng/ml there was a significant difference in regard to the diagnostic validity of the two assays. It follows a different classification of the patients to the groups "cancer" or "no cancer".

Conclusions. The two assays for the determination of tPSA - "PSA" and "Third GenerationPSA" (Immulite®) – are not compatible. Our results confirm that a change of these methods in the followup of a patient must not be done unchecked. Using the same cut-off the patients were different classified to the groups "prostate cancer" respectively "no cancer".

0090

SERUM AMYLOID A (SAA): A BIOMARKER FOR RENAL TUMOURS?

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Background. Kidney cancer patients have often elevated serum levels of C-reactive protein (CRP). Serum amyloid A (SAA) - an acute phase protein - responds faster and with more pronounced increases as CRP to an inflammatory stimulus. For this reason, SAA could be a more sensitive marker for advanced kidney cancer than CRP.

Methods. In 116 patients preoperative serum CRP, SAA and IL6 levels were determined. 51 tumours were small and localized (pT1), 65 patients had a higher tumour stage and / or positive lymph nodes and / or metastases (³ pT2). 12 patients with nonmalignant disease served as comparison group.

Results. The preoperative IL6, CRP and SAA levels in patients with advanced tumour stage (³pT2) are significantly higher than those of pT1 tumours. While 80% of advanced kidney cancer were associated with an increase in SAA were only 20% of the measured SAA levels in the pT1 group pathological. The corresponding results for CRP are: 70% (³pT2) and 23% (pT1), for IL6: 43% (³pT2) and 12% (pT1). In the comparison group, 17% of SAA, 15% of CRP and 11% of IL6 values were elevated. There is no difference to the pT1 group.In 10 patients with very high preoperative values, SAA concentration dropped below the baseline 5 days after surgery: an indicator that the tumor caused the markers increase.

Conclusions. Our results indicate that advanced kidney tumours are accompanied by increased acute-phase proteins such as CRP and SAA. SAA is more sensitive than CRP.

Cancer/tumour markers

0091

COMPARATIVE STUDY OF TWO IMMUNOASSAYS FOR PSA IN RADICAL PROSTATE SURGERY PATIENTS

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Background. PSA testing not only helps in the early diagnosis and screening of prostate cancer but also assists in assessing the response to therapy and determining tumor progression. The lower limit of detection is 0.01 ng/mL with most of the second generation Methods. In general, thePSA valuefromwhich to considerthepresence of biochemical recurrence,may bebetween 0.2 and 0.6ng/ml. The availabilityof ultrasensitive total PSA techniques, that allowdosing levels as low as 0.001 ng/ml, can indicate the appareance of recurrences faster than second generation tPSA tests.
Methods. Forty prostactemized patients (mean: 72 years) were enrolled in the study. We compared two Immunoassays of quantification of PSA:ADVIACentaur® PSA (second generation) and IMMULITE2000 uPSA (third generation).
Results. This comparison yielded a Spearman´s correlation coefficient of 0.829 (95%CI: 0,698 to 0,907),. Passing and Bablok linear regression equationobtained was: *Centaur®PSA= 0.933 Immulite2000 uPSA+0,0219.* (slope 95%CI:0.7463 to 1.2000, intercept 95%CI: 0.0128 to 0.0263). The 2,5 th,50thand 97.5th percentiles obtained by Centaur®PSA were 0.03, 0.055 and 0.160, and by IMMULITE2000 uPSA 0,005, 0,029 and 0.120respectively, which agreeswith the best functional sensitivity for the uPSA method
Conclusions. The uPSA assay is more sensitive and offers equimolar detection of PSA than the Centaur®PSA assay. The main disadvantage of this methodisits limitedlinearity, only up to 20 ng/mL. According to these results, theuseof uPSAshouldbe limited to monitoring of patients undergoingradical surgery,whichiscertainly moreusefulthan the othermethod studied.

0092

A NEW LC-MS/MS METHOD FOR THE ANALYSIS OF TYROSINE KINASE INHIBITORS IN HUMAN PLASMA

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Background. Uncontrolled cell proliferation of many tumor diseases result from overexpressed or overactive tyrosine kinases. Tyrosine kinase inhibitors (TKI) are synthetic drugs which inhibit the phosphorylation of intracellular or surface protein kinases.
Methods. While the measurement of various TKI in single assays is impracticable in the clinical laboratory a new LC-MS/MS-assay for the simultaneous determination of 7 registered tyrosine kinase inhibitors (dasatinib, erlotinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib) in human plasma was validated. The retention times are between 5 and 9 minutes and the intra- and inter day coefficients of variation were≤15%. The calibration range was 10-1000 ng/mL for sunitinib and 50-5000 ng/mL for the other TKI, covering the estimated plasma concentration range during standard therapy.
Results. For the treatment of CML a target imatinib plasma concentration of 1000 ng/mL was recommended in the literature. The measurement of patient samples with a dosage of 400 mg/d obtained a mean value of 1115 +/- 223 ng/mL (range 491-2570). Measurements of sunitinib (50 mg/d) in patients with renal cancer showed a mean plasma concentration of 35 +/- 7 ng/mL (range 11–92), while a recommended optimum of 50-100 ng/mL has been reported to inihibit tumor angiogenesis.
Conclusions. A new, fast and high sensitive multi-parameter assay was developed and applied to the analysis of TKI in patient plasma. First results show remarkable differences of plasma concentrations of patients treated with a routinely used drug dose. As TKI administration is accompanied with various adverse effects quantitative determination of TKI will optimize tumor therapy.

Cancer/tumour markers

0093

HE4: A NOVEL TUMOR MARKER IN DIFFERENTIAL DIAGNOSTICS OF OVARIAN CANCER

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Background. The prognosis of malignant ovarian tumors is dependent on their early diagnostics. HE4 is mentioned as a novel tumor marker with a higher specificity and sensitivity than CA125 for most types of malignant ovarian tumors.
Methods. 142 women enrolled: 57 (post- and premenopausal) benign gynecological diagnoses, 36 other benign diseases, both with normal findings in gynecological examination, 26 histologically verified ovarian carcinoma and 20 other tumors. CA125 and HE4 were measured on Architect (Abbott Lab.), compared in these groups and their diagnostics efficiency including ROMA index for ovarian cancer was established. Used cut-off values: CA125 - 35 kU/l for, HE4 - 70 pmol/l for pre- and 140 pmol/l for post-menopausal women , ROMA index - 7,4% for pre- and 25,3% for postmenopausal women, as recommended. For statistic used: ANOVA, ROC curves and kappa coefficient.
Results. Malignant ovarian tumors could not be differentiated from other tumors by using CA125, as opposed to HE4 in our patients (p < 0,05). Benign gynecological diseases (most cysts and endometriosis) and other benign diseases were differentiated well from malignant ovarian tumors (p < 0,05). Cut-off value 140 pmol/l for HE4 was calculated with SN 95,2% and SP 44%, what is the same as recommended by Abbott Lab. HE4 and ROMA index had better AUC than CA125 (HE4: 0,951, ROMA index: 0,916, CA125: 0,780). A moderate agreement between HE4 and CA125 for malignant ovarian tumors was observed (kappa 0,57).
Conclusions. a better diagnostics efficiency for malignant ovarian tumors was observed for HE4 than for CA125.

0094

BODY MASS INDEX ADJUSTED PROSTATE-SPECIFIC ANTIGEN AND HEMODILUCION. ITS APPLICATIONS FOR PROSTATE CANCER SCREENING

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Background. Obesity is considered a risk factor for prostate cancer (PCa) severity, although controversies exist in reference to its association with PCa development. Associations between increased body mass index (BMI) and decreased PSA have been previously reported and different methods to correct the hemodilution ofPSA in obesemen have been proposed.
Objetive. To perform a plasma volume correction of PSA, in order to evaluate the impact of hemodilution and to correct diagnostic criteria.
Methods. In 2009, 2906 men assisted to a voluntary screening for PCa (45-69 years). Plasma PSA was measured in 1905 by a chemoluminiscent method (Immulite, Siemens, LA, USA).Cvi and Cve were 3.2 % and 4.4% respectively. Men were divided according to their BMI (Kg/m2) in normal weight (n=452; N),overweight (n=958;OW) and obese (n=495;O).Total circulating PSA protein (PSA mass-μg) was calculated as PSA(ng/ml) multiplied by plasma volume(L) and in parallel plasma PSA was adjusted according to each individual BMI.
Results. Plasma volume was significantly increased in overweight and obese men (N: 2.99±0.01, OW: 3.21±0.01, O: 3.45±0.1; p<0.001) and PSA concentration was significantly decreased (N: 2.38±0.09; OW:2.08±0.06;O: 2.00±0.09; ng/ml p<0.001). No differences were found between groups in PSA mass (N: 7.10±0.30; OW: 6.66±0.21; O: 6.85±0.29) or BMI-adjusted PSA (O: 2.50±0.10; OW: 2.38±0.007N: 2.38±0.11)
Conclusions. Obese subjects show greater plasma volume and PSA differences are lost after adjustment.Hemodilution could beresponsible for the lower serum PSA concentration observed obese men.Obese patientsshouldbe biopsiedwith lowervalues of PSA thannormal weight men.

Cancer/tumour markers

0095

EVALUATION OF THE PERFORMANCES OF THE KRYPTOR CYFRA 21-1 ASSAY

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Background. Measurement of CYFRA 21-1 is used as complementary tumor marker for the diagnosis of non-small cell lung cancer. The aim of this study was to determine the analytic performances and reference values of the Kryptor™ CYFRA 21-1 assay (Thermoscientific).
Methods. Limit of quantification (LOQ) was determined through 10 measures of a 10% BSA solution and reproducibility with 5 measures of quality control materials. Method comparison was done with the established Roche CYFRA 21-1 assayusing 26 patients samples. CYFRA 21-1 levels were also measured for 29 healthy volunteers.
Results. LOQ was estimated as less than 0.16 ng/ml. Coefficients of variation were 3.36 and 2.51% for mean concentrations of 2.25 and 17 ng/ml, respectively. The correlation with the Roche assay was very good (r=0.99, p<0.0001, 95% CI: 0.978 to 0.996). Passing and Bablok regression analysis on log transformed data showed a slope of 1.35 (95% CI: 1.26 to 1.43), an intercept of –0.34 (95% CI: -0.39 to –0.31) and no significant deviation from linearity. Bland and Altman plot showed a slight trend of lower value on Kryptor (mean difference: 0.86 ng/ml, 95% CI : 1.92 – -0.21). For high levels of CYFRA 21-1, Kryptor assay values were higher. Healthy subjects had values less than 1.63 (mean: 0.34 ng/mL, 95% CI: 0.23 to 0.45).
Conclusions. Our preliminary study has demonstrated good analytic performances for the Kryptor CYFRA 21-1 assay and a significant correlation with the Roche assay. Our results are also in favor of the recommended cut point of 3.3 ng/mL.

0096

MOLECULAR ANALYSIS OF FUT3 AND FUT2 IN A CANCER PATIENT WITH LE (A-B-) AND SLIGHT CA19-9 ELEVATION

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Background. CA 19-9 (sialyl Lewis antigen) is a well-known tumor marker for pancreatic, colorectal and lung cancer. Its concentration in secretions including serum is regulated by Lewis enzyme (fucosyl transferase 3; FUT3) and Secretor enzyme (FUT2). Patients with a Lewis(a-b-) status have little or no CA 19-9 in their serum. Recently, we found a little but subtle elevation in serum CA19-9 level in a patient with end-stage rectal adenocarcinoma. This patient expressed erythrocyte Lewis-negative phenotypes. To reveal the reason why such Lewis negative patient showed an increase in CA19-9 by carcinogenesis, we investigated the molecular basis of this cancer patient by using immunohistochemical and molecular analyses.
Results. CEA and DUPAN-2 in the patient were also elevated. Lewis blood type was Le(a-b-). By immunohistochemistry le[a], le[b] and CA19-9 antigens were negatively stained in the normal mucosa and cancer tissues. By PCR and direct DNA sequencing analysis for *FUT3*, the patient was heterozygous for *le1* (Le59,508) and also heterozygous for 202T>C (W68R) and 314C>T (T105M). The *FUT3* genotype is so-called like null-type and the FUT3 activity can be very low or trace. Sequencing analysis for *FUT2* (secretor enzyme) revealed that the patient was homozygous for *sej* (Se357,385) and heterozygous for Se235 (novel missense mutation). This genotype means very low secretor enzyme activity, and also decreases CA19-9 generation.
Conclusions. We suggested that a small amount of CA19-9 was generated by trace FUT3 activity from a large amount of type 1 carbohydrate chain induced by carcinogenesis.

Cancer/tumour markers

0097

IDENTIFICATION OF MONOCLONAL IMMUNOGLOBULINS USING IG'K/IG'L NEPHELOMETRIC ASSAYS

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Background. Current screening algorithms to screen for monoclonal immunoglobulins utilise serum protein electrophoresis (SPE) and serum free light chain (FLC) analysis. Abnormal results are reflexed to immunofixation (IFE) to confirm and characterise the monoclonal immunoglobulin type. Novel antisera have been developed which recognise junctional, conformational epitopes between immunoglobulin light chains (κ and λ) and their heavy chain partners (γ,α,μ). Here we report the use of these antibodies in nephelometric assays (Heavy/light chain; HLC assays) as an alternative to IFE.
Methods. 1063 patient sera were screened using SPE and FLC. Sera showing monoclonal proteins or hypogammaglobulinemia (by SPE) or an abnormal FLC ratio were tested further by IFE and IgG, IgA, IgM HLC assays.
Results. 80/1063 patients were identified as having abnormal SPE or abnormal FLC results; 11/80 patients were positive for light chain only abnormalities. 31/80 patients had positive IFE results for intact immunoglobulins. 24/31 of these patients were positive by HLC. The 7/31 patients with a normal HLC ratio and positive IFE were all MGUS patients with a monoclonal protein load of less than 2g/L by SPE densitometry and a normal FLC ratio. Of the 38/80 patients with a normal IFE 9 had an abnormal HLC ratio, of which 3/9 had a confirmed haematological malignancy.
Conclusions. HLC analysis identified all symptomatic patients who were IFE positive and an additional 3 patients were with haematological malignances, which were missed by IFE. However, low risk MGUS may not be identified using HLC assays.

0098

CLINICAL UTILITY AND EXPERIMENTAL SENSITIVITY OF IG'K / IG'Λ MEASUREMENTS

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Background. International guidelines recommend the use of serum protein electrophoresis (SPE) to identify and quantify monoclonal immunoglobulins. However, monoclonal immunoglobulins can be difficult to quantify, for example when they co-migrate with other proteins. Specific polyclonal antibodies have been developed, which recognise conformational epitopes between immunoglobulin light chains (κ and λ) and their heavy chain partners (γ, α, μ; HLC antibodies).
Methods. Total IgG, IgA, IgM and IgGκ/IgGλ, IgAκ/IgAλ and IgMκ/IgMλ concentrations in sera were measured nephelometrically. Normal ranges were established by testing blood donor sera. Presentation sera from 530 IgG, 210 IgA multiple myeloma patients and 60 IgM Waldenstrom's Macroglobulinemia patients were retrospectively compared with normal ranges and historic SPE and immunofixation (IFE) data.
Results. IgG HLC ratio was abnormal when IFE was positive 526/530 (99%) sera. In 4 samples the IgG HLC ratio was normal when IFE was positive; in all cases the monoclonal protein levels were below the detection limit of SPE. There was concordance between IFE and IgA and IgM abnormal HLC ratio for all samples, 83/210 (40%) of IgA and 16/60 (27%) IgM samples were not accurately quantifiable by SPE, either because of their co-migration with other proteins or because of poor resolution.
Conclusions. There was good agreement between IFE and abnormal HLC ratios in the presentation samples tested. Furthermore IgA and IgM HLC assays were able to give a quantitative indication of monoclonality irrespective of their migration on SPE gels.

Cancer/tumour markers

0099

COMBINATION OF SERUM BIOMARKERS IN THE DIFFERENTIATION OF MALIGNANT VS BENIGN OVARIAN TUMORS

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Background. Differentiation between malignant and benign ovarian tumors is important for the appropriate and timely clinical management of patients with ovarian masses. The use of serum biomarkers is being investigated to improve diagnostic accuracy and triage.

Methods. 9 candidate serum biomarkers (CA-125, HE-4, IL-18, Leptin, MIF, FGF-2, IGF, Osteopotin, Prolactin) were evaluated prior to surgery in 52 patients with ovarian tumors (37 malignant and 15 benign based on pathologic diagnosis of the surgical specimen). ROC curves were built for each individual marker and for the logistic regression model using all 9 markers. In addition, ROC curves for risk management index I & II (RMI-I and II) using CA-125, menopausal status and imaging score were built and were compared to the ROC curve obtained for the 9 biomarkers regression model. Finally, ROC curves for logistic regression models combining biomarkers and RMI-I or II were established.

Results. Examining each biomarker individually, CA-125 was the best predictor of cancer (spec=93%; sens=68%). The model with all 9 biomarkers performed well with spec=93%, sens=84% and was better than RMI-I or RMI-II alone. A regression model combining RMI-II, CA-125, HE-4, IL-18, Leptin, MIF and FGF-2 (6 biomarkers) and the model combining RMI-II with all 9 markers perfectly predicted the cancer and the non-cancer cases in this pilot study.

Conclusions. The 9 biomarkers logistic regression model and the regression models using a combination of our markers in addition to the RMI-II scoring system yield some promise to differentiate malignant vs. benign ovarian tumors.

0100

PREDICTIVE AND PROGNOSTIC BIOMARKERS IN PATIENTS WITH PANCREATIC CANCER DURING CHEMOTHERAPY

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Background. In pancreatic cancer patients undergoing chemotherapy, biomarkers for predicting and early monitoring the response to therapy as well as for estimating the prognosis are highly needed.

Methods. We analyzed the weekly courses of nucleosomes by ELISA and cytokeratin-19 fragments (CYFRA 21-1), CEA and CA 19-9 by Elecsys 2010 in prospectively collected sera of 46 patients with pancreatic cancer during first-line systemic chemotherapy and correlated biomarker levels and kinetics with therapy response and overall survival.

Results. In radiologic staging investigations after two treatment cycles, 21 patients suffered from progressive disease while 25 had stable disease or partial remission. During the observation period, 39 patients have died. Therapy response correlated fairly well with overall survival (p=0.0166).

High pretherapeutic levels of CA 19-9 (p=0.0012), CEA (p=0.0062) and CYFRA 21-1 (p<0.0001) were associated with poor overall survival, while nucleosomes were not. Concerning response to therapy, high CA 19-9 and CYFRA 21-1 values before, as well as one, two, three and four weeks after start of the treatment and at time of staging investigations significantly indicated progressive disease. Kinetics of the markers during the early treatment phase were not as meaningful. However, the courses of CA 19-9 from therapy start to staging showed a more pronounced decrease in patients responsive to therapy than in progressive patients (median decrease 62% versus 17%; p= 0.0043).

Conclusions. CA 19-9 and CYFRA 21-1 are valuable biomarkers for the prediction and monitoring of the response to systemic chemotherapy as well as for estimating the prognosis in pancreatic cancer patients.

Cancer/tumour markers

0101

CYTOKERATINS IN PREDICTING THERAPY RESPONSE AND PROGNOSIS OF COLORECTAL CANCER PATIENTS

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Background. The role of cytokeratin markers was investigated for predicting therapy response and estimating prognosis in colorectal cancer (CRC) patients undergoing chemotherapy.
Methods. Courses of CYFRA 21-1, TPA, TPS, M30, CEA and CA 19-9 were analyzed in prospectively collected sera of 30 patients with colorectal cancer during adjuvant (N=15) and primary (N=15) chemotherapy and correlated with therapy response and overall survival (OS).
In addition, pretherapeutic levels of CYFRA 21-1, M30, CEA and CA 19-9 were measured in 42 CRC patients, 45 with benign colorectal diseases and 51 healthy controls.
Results. Median values of cytokeratins, CEA and CA 19-9 were not different in the adjuvant and primary setting. Concerning response of primary therapy, pretherapeutic (BV1) and values before cycle 2 (BV2) of CYFRA 21-1, TPA and TPS were lower in responding patients, and also M30 (BV1). Further, kinetics from cycle 1 to 2 (BV1-2) or 1 to 3 (BV1-3) discriminated between response groups. Favourable 3-year OS was indicated by low BV1 of CYFRA 21-1, TPA, TPS, CEA and CA 19-9 as well as by low BV1-3 of TPA, CEA and CA 19-9. In adjuvant therapies, a favourable 3-year OS was indicated by low BV1 of M30, CEA, CA 19-9 and by low BV1-3 of all cytokeratins. Similar to CEA and CA 19-9, CYFRA 21-1 was elevated in CRC patients as compared with benign diseases and healthy controls and showed stage-dependency, while M30 was not stage dependent.
Conclusions. Cytokeratins are valuable markers for predicting therapy response and estimating prognosis in CRC patients.

0102

EVALUATION OF PLASMA CYTOKINE AND GROWTH FACTOR LEVELS BY BIOCHIP ARRAY TECHNOLOGY IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background. Assessment of plasma levels of multiple cytokines and growth factors by biochip array technology in acute myeloid leukemia (AML) patients.
Methods. 15 AML patients (mean age 48.7±12.1 years, 8 males) treated with chemotherapy were studied. We evaluated plasma levels of the following cytokines: interleukin-1 alpha (IL-1alpha), interleukin-1 beta (IL-1beta), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML (active leukemia) and at 6 months after completion of chemotherapy (durable complete remission in all patients).
Results. Comparing cytokines levels in active leukemia and in durable complete remission, we found significant decrease in plasma IL-1beta (2.56±3.27ng/L vs. 1.63±2.17ng/L; p<0.05), IL-6 (46.24±83.14ng/L vs. 2.49±2.51ng/L; p<0.05), IL-8 (104.99±167.30ng/L vs. 11.72±4.34ng/L; p<0.05), IL-10 (7.58±14.15ng/L vs. 2.22±4.78ng/L; p<0.05) and TNF-alpha (4.65±4.27ng/L vs. 2.19±1.13ng/L; p<0.05). On the other hand, we found significant increase in VEGF (63.93±67.85ng/L vs. 114.39±54.90ng/L; p<0.01) and EGF (16.48±33.50ng/L vs. 64.42±35.33ng/L; p<0.001). Plasma levels of other cytokines were without significant differences.
Conclusions. Our results indicate that plasma levels of some cytokines and growth factors (EGF, VEGF, IL-1beta, IL-6, IL-10, TNF-alpha) could serve as useful diagnostic and prognostic parameters for AML patients, showing activity of the disease. Further studies will be needed to define the potential role of these and additional biomarkers in this context.

Cancer/tumour markers

0103

FAILURE TO REMOVE POLYMERISED IMMUNOGLOBULIN FREE LIGHT CHAINS BY HIGH CUT-OFF HAEMODIALYSIS

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Background. Serum free light chains (FLC) are responsible for the renal injury myeloma kidney. Recently the removal of FLCs by high cut-off haemodialysis (HCO-HD) has been described as an adjuvant therapy for these patients. This removal is dependent on the molecular-weight of the FLCs in relationship to the molecular-weight cut-off of the dialysis membrane. Here we describe two patients in whom HCO-HD did not effectively remove FLCs.
Methods. Patients' sera were fractionated by size exclusion chromatography on a Superdex S200 (1.6x70cm) column under native conditions. FLC concentrations in sample fractions were determined on a Siemens BN™II nephelometer. Fractions were also analysed by SDS-PAGE gels and were further characterised by Western blotting using isotype specific antisera.
Results. Patient one failed to clear lambda FLCs from serum following six sessions of HCO-HD (median 7% reduction). Size exclusion chromatography of the patient's serum demonstrated that the majority of lambda FLCs were high molecular weight polymers (>150kDa). Patient two received 5 sessions of HCO-HD with only limited reductions in serum kappa FLCs (median reduction 10.5%). Evaluation of the patient's serum demonstrated kappa FLCs at three molecular weights: ~25kDa (monomer), ~50kDa (dimer) and the majority at >100 kDa (polymer).
Conclusions. The presence of high FLC polymers prevents efficient removal of FLCs by HCO-HD.

0104

SERUM LEVELS OF MACROPHAGE COLONY STIMULATING FACTOR CORRELATE WITH THE EXTENT OF BONE DISEASE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Bone destruction is a hallmark of multiple myeloma (MM) and a major clinical problem in patients. Macrophage-Colony Stimulating Factor (M-CSF) is among the cytokines that accelerate osteoclastogenesis responsible for increased osteolysis. We evaluate the link between circulating levels of M-CSF and the extent of myeloma bone disease.
Methods. We studied 56 previously untreated patients with MM, 36 patients with Monoclonal Gammopathy of Undetermined Significance (MGUS) and 59 healthy age-matched subjects. Blood samples were collected before treatment and serum M-CSF was determined. For the evaluation of bone disease, conventional radiography was performed including X-Ray scans of the whole skeleton.
Results. Serum concentrations of M-CSF were increased in MM patients (928 pg/mL ±140) compared with MGUS (373 pg/mL ±28) and healthy subjects (493 pg/mL ± 16) (p = 0.0002). A significant difference was found in circulating M-CSF levels of MM patients with radiographically detectable osteolytic lesions in comparison to those without (P = 0.0018). M-CSF serum levels were significantly greater (P<0.05) in patients with high β2-microglobulin levels (≥3.5mg/L) than in those with lower levels.
Conclusions. Elevated levels of serum M-CSF is associated with bone disease in MM patients. Serum β2-microglobulin and M-CSF levels were significantly correlated indicating a relationship with tumour burden.

Cancer/tumour markers

0105

THE PROGNOSTIC RELEVANCE OF SERUM CA27.29 IN PRIMARY BREAST CANCER PATIENTS BEFORE ADJUVANT CHEMOTHERAPY-RESULTS OF THE GERMAN SUCCESS TRIAL

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Background. The German phase III, multicenter SUCCESS study compares the efficacy of gemcitabine in a taxane based chemotherapy of primary breast cancer patients. The prognostic value of MUC-1 marker CA27.29 in the adjuvant setting was prospectively evaluated within this trial.
Methods. Peripheral blood is drawn within the SUCCESS trial before and after adjuvant chemotherapy, as well as 2 and 5 years after completion of chemotherapy in patients with primary breast cancer, presenting node positive or high risk node negative disease.
CA27.29 is measured with the ST AIA-PACK CA27.29 reagent using MUC-1 for AIA-600II (Tosoh Bioscience, Tessenderlo, Belgium) in a total number of 3202 patients. The cutoff for positivity of CA27.29 is >31 U/ml.
Results. The mean CA27.29 serum level before adjuvant chemotherapy was 19.3 U/ml (SD +/- 15.5). About 8% of the patients had a marker of more than 31 U/ml.
Mean CA27.29 serum levels were significantly higher in patients with lobular carcinoma (p=0.001), patients with positive lymph nodes (p=0.02) and post-menopausal patients (p<0.001)
After the median follow-up period of 34 months 233 patients developed a recurrence of their disease and 108 patients died. CA27.29 before chemotherapy was a significant prognostic marker for disease-free survival (DFS) (p<0.0001) and overall survival (OAS) (p<0.0001).
Conclusions. These findings indicate the prognostic relevance of serum CA27.29 levels in primary breast cancer patients before adjuvant treatment in a large patient cohort. Further follow-up within the SUCCESS trial will show whether initial CA27.29 level could serve as a tool for adjuvant treatment monitoring.

0106

FIRST RESULTS FROM APPLYING HUMAN EPIDIDYMIS PROTEIN 4 (HE4)

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Background. From the beginning of 2011, we have the possibility in the Tokuda Hospital to evaluate the novel tumor marker for ovarian cancer HE4 in Bulgarian population.
Methods. A group of 88 healthy female Caucasian race controls divided in two subgroups - postmenopausal (FHS > 22 IU/ml and amenorrhea more than 1 year, mean age 57,5 years) and premenopausal (FSH < 11,5 IU/ml and mean age 34,5 years) were tested for HE4 using Architect HE4 Abbott test (CMIA method). Serum samples were acquired following a standard collection protocol. All samples were stored on -20°C until use. In other 4 patients with imaging data(CT or ultrasound) for pelvic mass HE4 was tested in combination with CA125 and Risk of Ovarian Malignancy Algorithm (ROMA) was calculated.
Results. Our preliminary results were for the postmenopausal group range 31.5-136.8 pmol/l (mean 50.2 ± 28.2 SD), and for the premenopausal group range 29.7-106.3 pmol/l (mean 53.4 ± 23.9 SD). In 3 patients with pelvic mass ROMA showed high risk of ovarian epithelial cancer: 71.7% and 98.9% in 2 postmenopausal patients and 9.3% in 1 premenopausal patient. The risk was real and the diagnosis ovarian cancer was histological confirmed after surgery.
Conclusions. We established that so far the cut off value 140 pmol/l proposed by Abbott is valid for Bulgarian population.

Cancer/tumour markers

0107

DIAGNOSTIC RELEVANCE FOR PROSTATIC ANTIGENSCREENING INVESTIGATION OF MALEPATIENTS WITH PROSTATE HYPERTROPHY INMINSK

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Background. 4160 patientswith prostate hypertrophy were observed in 2010on base of Minsk Consulting and Diagnostic Centre. Screening results were analyzed in the context of patients' age and prostatic antigen (PSA) content in the blood serum.
Methods. Patients under study were divided into the groups based on their age. The first group included patients from 40 to 49 years old, the second one – from 50 to 59, the third one – from 60 to 69 and the forth – from 70 to 79 y.o. Moreover, the patients' samples of the blood serum were divided into four zones based on the PSA content. The first zone was the region of physiological level of PSA up to 4 ng/ml. The second one was a boundary zone including PSA contentin the range from 4 to 10 ng/ml. The third one was the zone of moderate risk in which PSA content varied from 10 to 30 ng/ml.The content of PSA in the forth zone was more then 30 ng/ml.
Results. The PSA content exceeded physiological level in 57.2% of the observed patients. In the second zone the patients of the third age group (60-69 y.o.) dominated (about 58.7%). Whenever in the third and the forth zones the patients' percentage fromthe forth age group (70-79 y.o.) was 45.4% and 62.1% respectively.
Conclusions. The screening has clearly shown that the initialchanges in PSA content occured by 60-69 year old patients, whereas pathological changes that led to canceromatosis dominated among 70-79 year old patients.

0108

DEVELOPMENT OF A REAL-TIME QUANTITATIVE PCR METHOD FOR *PALB2* GENE AND APPLICATION IN SPORADIC BREAST CANCER

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Background. PALB2 protein (Partner and localizer of BRCA2) as its name implies co-localizes with BRCA2 (and BRCA1 also!) in nuclear structures and enables error-free homologous recombination. Its important contribution to DNA repair could be severely affected by DNA methylation in its promoter CpG island. Assessment of PALB2 transcript levels by a reliable quantitative PCR method could prove to be a prognostic biomarker with implication also in therapy (e.g. novel PARP inhibitors).
Methods.RNA was extracted from each of 15 frozen tissues of patients with sporadic breast cancer (RNAspin, GE) and converted to cDNA (Trascriptor, Roche). Then a real-time reverse-transcription quantitative PCR methodologywas developed and optimized in order to measure the expression of *PALB2* gene. A Taqman probe was specially designed and synthesized (TIB) to hybridize to the produced amplicon in the Light Cycler (Roche). The reverse primer was specific for *PALB2* cDNA since it spanned the boundary between exons 4 and 5. The external calibration curve was constructed from appropriately purified and quantitated amplicons.
Results. The method was evaluated for its sensitivity, specificity and precision with a PCR product from MCF-7 cells that express *PALB2*. The *PALB2*expression in our samples was $33.0\text{-}56.6\times10^5$copies/µg RNA. One of the samples was also weakly positive for methylation in the *PALB2* promoter.
Conclusions. In the future we plan to expand the number of samples measured for *PALB2* expression with our reliable method in order to be able to draw reliable conclusions for its prognostic and predictive value from data obtained from patient follow-up.

Cancer/tumour markers

0109

PROSTATE SPECIFIC ANTIGEN (PSA) IN SERUM IN RELATION TO AGE, BODY MASS INDEX (BMI), SMOKING AND TERM OF EMPLOYMENT IN BUS DRIVERS

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Background. To examine factors associated with PSA level in bus drivers.
Methods. Serum concentration of PSA was measured using a monoclonal two-site ELISA (DS-EIA-PSA-TOTAL). Spearman's Rank Correlation Coefficient was used for estimation of interrelationships.
Results. PSA level of 64 samples of serum from asymptomatic men working as bus drivers (Central Russia) have been measured. The age of the workers varied between 26 to 70 years (mean age 47,8±9,8 years). The term of employment was 25,4±9,7 years. Mean PSA level was 1,17±1,12 ng/ml (from 0,24 to 7,72 ng/ml). Out of 64 workers 35 (54,6%) had exceeded BMI (>25 kg/m^2), 54 (84,3%) were smokers. Smoking experience varied between 7 to 60 years (22,0±14,8 years) and amount of cigarettes was from 10 to 40 per a day. A positive correlation was found between serum PSA levels and age (R=0,27, p=0,032) and the term of employment (R=0,23, p=0,048). There was no correlation between PSA level and other factors: BMI and smoking experience (p>0,05).
Conclusions. We conclude that only age strongly correlates with PSA level. Effects of other factors (body composition, social habits, occupational hazards etc.) need to be investigated more closely.

0110

SIMULTANEOUS DETECTION OF *K-RAS/BRAF* MUTATIONS IN COLORECTAL TISSUE WITH BIOCHIP ARRAY TECHNOLOGY

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Background. Therapeutic agents targeting the epidermal growth factor receptor (EGFR) have improved outcomes for patients with advanced colorectal cancer but are only effective in a subset of patients. Mutations in the *K-ras* gene disrupt the EGFR pathway, rendering anti-EGFR therapy ineffective.*BRAF* mutations are associated with an additional 12-15% of patients who fail to respond to this treatment. To tailor patient care, it is advantageous to use analytical systems allowing multiplex detection of these mutations. We report a biochip array for simultaneous detection of the most frequent mutations in codons 12 and 13(*K-ras)* and codon 600(*BRAF*).
Methods. DNA from colorectal tissue samples(n=190) was analysed using the RanplexCRC Array. This assay combines probe hybridisation, ligation, probe-pairamplification and biochip array hybridisation, to generate a sensitive and specific mutation profile of the tissue. Chemiluminescent detection defines hybridisation, through analysis with the Evidence Investigator analyser. Results. 93 of the samples represented matched normal and tumour tissue. 22% of tumours exhibited K-ras mutations, 7% a BRAF mutation.71% were wild type (wt).3% of normal tissue also presented a K-ras mutation. Five different mutations within K-raswere observed. Multiple K-ras mutations were found in 3 samples. For comparison, K-ras positive(n=19) and wt samples(n=6) were screened for K-ras mutations using a commercially available assay and 100% correlation was observed. The commercial assay also confirmed K-ras mutations in 3 samples but failed to discriminate multiple mutations.
Conclusions. Data demonstrate applicability of this biochip assay to simultaneously detect specific K-*ras* and BRAF mutations in a single reaction in colorectal tissue.

Cancer/tumour markers

0111

A RAPID BACTERIAL-BASED BIOLUMINESCENT ASSAY FOR IN VITRO TESTING OF CHEMOTHERAPY SENSITIVITY

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Background. The main drug of chemotherapy for acute myeloid leukaemia(AML) is the nucleoside analogue cytosine-arabinoside(Ara-C). At present, there is no commercially available rapid pre-screening test to determine chemosensitivity to Ara-C. Here we report the use of a novel bacterial-based bioluminescent biosensor, which can determine a patient's chemosensitivity within 8 hours of receipt of peripheral blood or bone marrow aspirate.
Methods. This system consists of a microtitre plate assay in combination with a CCD camera-based image capture system complete with software. The assay is based on a genetically modified *E. coli,* which expresses the human deoxycytidine kinase gene responsible for the conversion of Ara-C to the active metabolite Ara-CTP *in vivo*(under the control of a T7 promoter). It also contains a lux-expression(luxCDABE) cassette, which produces an increase in bioluminescence in response to Ara-CTP.Isolated blast lysates from Ara-C sensitive patients will induce an increase in light from the bacterial reporter.
Results. Intracellular concentrations of phosphorylated Ara-CTP (0.025mM/L) were detected by significant increases in light output compared to control reactions(p<0.05). Results using AML cell lines with known response to Ara-C, showed correlation between this assay and a commercially available 3-day cytotoxicity test for Ara-C induced cell kill. 24 patient samples were evaluated and leukaemic cell response to Ara-C was successfully predicted within 8 hours.
Conclusions. The results show applicability of this assay system to the evaluation of patient sensitivity to the chemotherapy agent Ara-C.

0112

METABOLOME ALTERATIONS IN COLORECTAL CANCER

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Background. "Metabolomics" promise to comprise the complex metabolic alterations, which arise during tumorigenesis and may be valuable for the identification of new disease signatures and cancer-associated biomarkers. Hence, we performed serum metabolic profiling in colorectal cancer.
Methods. Highly standardized serum samples of patients suffering from colorectal cancer (n=60) and controls (n=60) were collected at the University Hospital Leipzig. We generated amino acid and acylcarnitine screening profiles using electrospray tandem-mass spectrometry. Metabolic profiles were evaluated using the Analyst 1.4.2 software. General and comparative statistics were performed by PASW 18.0.2 and R 2.11.1.
Results. Serum concentrations of 12 amino acids significantly differedbetween and correlated with the study groups exhibiting high discriminatory power as revealed by AUROC analysis.
Conclusions. Our serum metabolic investigations revealed multiple alterations in the metabolite profile with potential diagnostic power. Further prospective studies are necessary to prove the contribution of metabolite analysis for the development of comprehensive diagnostic pattern in cancer disease.

Cancer/tumour markers

0113

LABORATORY PROGNOSTIC FACTORS IN METASTATIC MELANOMA PATIENTS IMMUNIZED WITH HYPER-IL-6 GENE MODIFIED MELANOMA VACCINE (GMTV)

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Background. The incidence and mortality of melanomahave increased over the last several decades.The most problematic issue is the treatment of patients with advanced melanoma, due to poor clinical outcome of radio- and chemotherapy. New alternative methods of melanoma treatment based on activation of specific antimelanoma immune response need to be expanded. One of the most important issues in experimental therapy of cancer is to define the prognostic factors for monitoring treated patients. The aim of the study was to determine the laboratory indicators useful in the monitoring of melanoma patients immunized with Hyper-IL-6 gene modified melanoma vaccine.
Methods. Studies were performed in group of melanoma patients with stage 3 and 4 disease according to AJCC, included in the phase 2 clinical study aimed to assess toxicity and effectiveness of GMTV. Hematological factors such as: hemoglobin concentration, count of erythrocytes, leukocytes, neutrophils, lymphocytes, blood platelets, level of ESR were assessed. Biochemical factors such as: LDH activity, concentration of CRP, and iron were also studied.
Results. It was confirmed that concentration of CRP in patients with measurable metastasis and ESR in patients with removed metastasis can be useful factors in determining stage of clinical advancement of the melanoma. In patients with measurable disease increased CRP concentration, ESR level, count of leukocytes and neutrophils were unfavorable survival factors. It was shown that CRP, iron, and neutrophils are independent prognostic factors for overall survival.
Conclusions. Laboratory parameters should be useful as stratification factors in clinical trials.

0114
ANALYTICAL PERFORMANCES OF ARCHITECT CYFRA 21-1 ASSAY

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Background. CYFRA 21-1 is an immunoassay that determines the circulating level of cytokeratin 19fragments. Elevatedlevels of cytokeratin 19 fragments are seen in sera from patients with cancer, such as lungcancer.
Methods. The ARCHITECT CYFRA 21-1 assay utilized paramagnetic microparticles coated with the mAb KS 19.1, and acridinium-labeled mAb BM 19.21 conjugates on the ARCHITECT *i* System. Human seraand EDTA plasma were used as the samples.
Results. The analytical performances of the ARCHITECT CYFRA 21-1 assay were: (1) No more than 6% total imprecision (20-days, N = 80).(2) Linearity ranging from 0.50 to 100.00 ng/mL. (3) The Limits of Blank (LoB), Detection (LoD), and Quantitation (LoQ) at 0.02, 0.09 and 0.17 ng/mL respectively. (4) 2-6% mean cross reactivities with 10 – 100 fold of normal levels of tumor markers AFP, bHCG, CA 125, CA 15-3, CA 19-9 and CEA. (5) 8% differencesbetweenautomated dilutions versus neat samples. (6) 0-5% mean interferences when comparing the samples spiked with 14 therapeutic drugs individually to the control samples. (7) No more than 10% interferences from endogenous substances Bilirubin at 20 mg/dL, Hemoglobin at 5 mg/mL, Protein at 12 g/dL and Triglycerides at 3 g/dL.(8) No more than 12% and 6% interferences from Rheumatoid Factor and HAMArespectively. (9) No high dose hook effect, that is, extreme samples with high values (> 20-fold of the highest calibrator) exhibited greater RLU values than that of the highest calibrator (N = 3)). (10) Acceptable method comparison with Roche Elecsys CYFRA 21-1 assay by showing a linear correlation with a slope of 1.00 and a correlation coefficient of 0.99(N = 198).
Conclusions. The ARCHITECT CYFRA 21-1 assay is anautomated immunoassayfor the quantitative determination of human cytokeratin 19 fragments in human seraand EDTA plasma on the ARCHITECT *i* System.

Cancer/tumour markers

0115

DNA METHYLATION OF TUMOR SUPPRESSOR AND METASTASIS SUPPRESSOR GENES IN CIRCULATING TUMOR CELLS

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Background. Circulating tumor cells (CTCs) are associated with prognosis in a variety of human cancers and have been proposed as a liquid biopsy for follow up examinations. We show, that established tumor suppressor and metastasis suppressor genes (*CST6, BRMS1,* and *SOX17*) are epigenetically silenced in CTCs isolated from peripheral blood of breast cancer patients.
Methods. Peripheral blood was obtained from 56 patients with operable breast cancer, 27 patients with verified metastasis and 23 healthy individuals. Extraction of genomic DNA was performed in the EpCAM positive immunomagnetically selected CTC fraction of peripheral blood mononuclear cells (PBMC). DNA samples were subjected to sodium bisulphite conversion and tested for the presence of methylated and unmethylated gene promoter sequences by methylation specific PCR (MSP). All samples were also checked for *CK-19* expression by RT-qPCR.
Results. In CTCs of patients with operable breast cancer, *CST6* promoter methylationwas observed in 17.9%, *BRMS1* promoter methylation in 30.4%, and *SOX17* promotermethylation in 53.6%. In CTCs of patients with verified metastasis, *CST6* promoter methylation was observed in 37.0%, *BRMS1* promoter methylation in 44.4%, and *SOX17* promoter methylation in 74.1%.
Conclusions. Our results provide the first evidence that DNA methylation of tumorsuppressor and metastasis suppressor genes is a hallmark feature of CTCs and confirmtheir heterogeneity. Our findings add a new dimension on the malignant nature of CTCs and may underlie the acquisition of malignant properties, including their stemlike phenotype. Moreover, these epigenetic markers open new possibilities for the exploitation of CTCs in monitoring cancer metastasis.

0116

DEVELOPMENT OF A METHYLATION-SENSITIVE HIGH RESOLUTION MELTING ASSAY (MS-HRMA) FOR CYSTATIN C (*CST6*)

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Background. Methylation-Sensitive High Resolution Melting Analysis (MS-HRMA) enables highly sensitive, labor- and cost-efficient single locus methylation studies on the basis of DNA high-resolution melting technology. The aim of our study was to develop a closed-tube MS-HRMA methodology for the investigation of *CST6* methylation in breast tumors (Kioulafa et al, Int J Cancer, 2009).
Methods. We investigated117 breast formalin fixed paraffin-embedded tissues (FFPEs): 90 paraffin-embedded breast carcinomas from patients with operable breast cancer, 18 non cancerous breast tissues and 9 fibroadenomas. Our methodology was first optimized and evaluated by using certain mixtures of fully methylated to unmethylated bisulfite converted genomic DNA, as controls.
Results. The developed methodology can detect the presence of methylation within the studied region, and additionally provides a semi-quantitative estimation of *CST6* methylation level in terms of percentage. By using MS-HRMA for *CST6* methylation we observed 42/90 (47.7%) of breast cancer FFPEs samples analyzed to be methylated at various methylation levels. In non cancerous tissues *CST6* methylation was detected at very low levels in 2/18 (11.1%), while in benign breast tumors (fibroadenomas) *CST6* methylation was observed in 1/9 (11.1%). Our results for MS-HRMA when compared to Methylation-Specific PCR (MSP) for the same samples showed strong correlation (P<0.001).
Conclusions. The developed assay is highly sensitive, cost-effective, easy-to-perform, and can be used as a screening test prior to DNA sequencing for the exclusion of clearly unmethylated samples. MS-HRMA gives comparable results to MSP in less time, while can additionally provide an estimation of the level of methylation.

Cancer/tumour markers

0117

DEVELOPMENT AND EVALUATION OF A LIQUID BEAD ARRAY ASSAY FOR THE EXPRESSION OF VEGF$_{121}$, VEGF$_{165}$ AND VEGF$_{189}$SPLICE VARIANTS IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Background. Vascular endothelial growth factor (VEGF) is an endothelial cell–specific mitogen and a key regulator of angiogenesis. The aim of our study was to develop and validate a liquid bead array assay for the expression of VEGF$_{121}$, VEGF$_{165}$ and VEGF$_{189}$splice variants, and evaluate it in NSCLC.
Methods. The assay is based on liquid bead array LUMINEX technology. <u>Procedure:</u> total RNA isolation from samples, cDNA synthesis, multiplex PCR, treatment with ExoSAP-IT® reagent, target specific primer extension (TSPE), biotinylation of PCR products, hybridization of the biotinylated products to xMAP microspheres, addition of Streptavidin- phycoerythrin and measurement. An extensive optimization of every step was performed in terms of analytical sensitivity, specificity, repeatability and reproducibility. The clinical performance of the assay was evaluated in 20 pairs of fresh frozen cancerous and corresponding noncancerous adjacent tissue samples originating from patients with NSCLC. The results were compared with RT-qPCR using our previously developed methodology for VEGF splice variants, (Zygalaki et al, Clin Chem, 2007).
Results. The developed assay is highly specific and is characterized by satisfactory repeatability and reproducibility for each VEGF splice variant. Comparison with RT-qPCR has shown an accordance of 16/20 pairs (80%) for *PBGD*, 16/20 for VEGF$_{121}$ (80%), 18/20 for VEGF$_{165}$ (90%) and 17/20 for VEGF$_{189}$ (85%).
Conclusions. The developed assay can be used to detect VEGF splice variants simultaneously in clinical samples with high specificity and sensitivity. The method can be extended to analyze additional gene targets, while the use of an internal control could further enable quantification.

0118

PANNING AND SCREENING FOR A HIGH AFFINITY INTACT PSA SPECIFIC 4D4 ANTIBODY

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Background. Prostate specific antigen (PSA) is commonly used for the detection of prostate cancer. In blood, PSA can be either free or attached to inhibitors. Several subforms of free PSA have been found such as proPSA, intact PSA (PSA-I) and nicked PSA. The most general site for internal nicking lies between Lys145-Lys146. Intact PSA is a marker for prostate cancer whereas nicked PSA is associated with benign prostate hyperplasia (BPH). Measurements for intact, nicked and proPSA provide diagnostic enhancements discriminating benign prostate hyperplasia and prostate cancer. A monoclonal 4D4-Fab antibody, specific for intact PSA, has been developed at the Department of Biotechnology, University of Turku, Finland. In this study we aimed to increase the binding affinity (decrease off-rate dissociation) of 4D4-Fab to improve the sensitivity of assays for intact or nicked free PSA.
Methods. Phage display technology was used for the selection and enrichment of PSA-I specific antibodies. Single clone screening was performed for the isolation of high affinity clones. Isolated clones were sequenced.
Results. With each round of panning, output-to-input phage ratios were increased. After third round of panning, 930 clones were analyzed by immunoassay, and 26 high signal clones were selected for sequencing. Out of 26, 14 clones were found to be unique that have some mutations in different complementarity determining regions (CDRs).
Conclusions. The specificity and affinity of the 14 clones against PSA-I will be determined and finally, an assay based on the improved anti-PSA-I antibody for the intact and nicked PSA will be optimized.

Cancer/tumour markers

0119
HE4 AND CA125 IN BENIGNANDMALIGNANTDISEASES

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Background. Human epididymis protein 4 (HE4) is a novel biomarker expressed in high concentrations in malignant diseases. We evaluated the sensitivity and specificity of serum concentrations of HE4 in comparison to CA125 in patients with benign and malignant diseases.
Methods. Sera of 206 healthy individuals, 671 patients with various benign disorders, and 720 cancer patients before first treatment were analyzed retrospectively using the CA125 and HE4 assays on the ARCHITECT system (Abbott).
Results. The median (95th perc) of HE4 and CA125 in healthy men is 26 pM/10 U/ml (47/20), in women 40 pM/14 U/ml (74/31). Medians ofbenign diseases: 30 pM to 51 pM for HE4 (except benign urological diseases with 660 pM), 13 to 20 U/ml for CA125. For HE4 as well as CA125 the highest values are reached in ovarian cancer (n=125) with median values of 242 for HE4 and 391 for CA125, followed by lung cancer, oesophagus cancer and cervix uteri cancer for HE4 and cervix uteri, stomach, hepatocelluar and lung cancer for CA125. Highest sensitivity shows HE4 (68%) and CA125 (69%) at 95% specificity in ovarian cancer and Borderline Tumor (control group: benign gynaecological disorders) with AUCs 88.9% (HE4) and 91.9% (CA125). Both markers reach 100 specificity for discriminating ovarian cancer from all other diseases (HE4 7% sensitivity, CA125 18%) which increases to 23% at combined use.
Conclusions. HE4 and CA125 are released by various benign and malignantdiseases and show highest sensitivity in ovarian cancer and borderline tumors with increased sensitivity in combined use.

0120
COMPARISON OF THE NEW ARCHITECT CYFRA 21-1 ASSAY AGAINST EXISTING METHODS

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Background. CYFRA 21-1 is a relevant tumor marker for non-small cell lung cancer. A new automated assay that is available on the Architect Analyzer (Abbott) was compared to two established Methods.
Methods. The precision of the Architect CYFRA 21-1 assay was determined across the dynamic range using the assay controls in a 5 day CLSI setup. Correlation to the Roche and Brahms Kryptor CYFRA 21-1 assays was evaluated on a set of 166 selected patient samples (samples from 30 healthy individuals, 30 patients with benign lung disease, 84 patients with lung cancer and 22 patients with lung tumors from other or unknown primary).
Results. The inter-assay precision (%CV) for the Architect CYFRA 21-1 assay was 3.8%, 2.8% and 2.4% for the Low, Medium and High Control, respectively with a intra-assay precision (%CV) of 2.7%, 2.3% and 1.9%. The between run/between day precision was determined as 0.0%/2.6%, 0.0%, 1.6% and 0.5%/1.3%. Across all samples the Architect assay showed a proportional bias of 0.84 against the Roche assay and of 1.09 against the Kryptor assay at correlation factors of 0.94 and 0.91. The proportional bias increased to 0.81 and 1.20 if the analysis was limited to non-cancer samples (r = 0.99 for both assays). For lung cancer samples only, the proportional bias was 0.88 and 1.01 (r = 0.94 and 0.91). The ROC curves did not show significant differences.
Conclusions. The Architect CYFRA 21-1 assay exhibits an excellent precision and correlates well with the Roche and Brahms Kryptor CYFRA 21-1 assays.

Cancer/tumour markers

0121

DIAGNOSTIC CAPACITIY OF CYTOKERATIN 19 FRAGMENTS (CYFRA 21-1) IN LUNG CANCER: COMPARISON OF THE IMMUNOASSAYS ON THE ARCHITECT SYSTEM (ABBOTT DIAGNOSTICS) AND THE ELECSYS SYSTEM (ROCHE DIAGNOSTICS)

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Background. To compare CYFRA 21-1 measured on Architect (Abbott) and Elecsys (Roche).
Methods. We investigated retrospectively sera of 69 healthy individuals, 278 patients with benign lung diseases, 267 lung cancer patients (210 with non small-cell lung cancer (NSCLC), 57 with small-cell lung cancer (SCLC)). Architect (A) and Elecsys (E) CYFRA 21-1 immunoassays were measured in parallel.
Results. Our analyses indicated a strong concordance of CYFRA 21-1 values on Architect and Elecsys (particularly regarding values < 20 ng/ml): Spearman and Kendall Tau Correlation Coefficients were 0.98 and 0.92, respectively. The healthy individuals median (95th perc.) of CYFRA 21-1 was 0.9 ng/ml (1.7) for A and 1 ng/ml (2.1) for E. The median (95th perc) in benign lung diseases was 1.1 ng/ml (3.8) for A and 1.3 ng/ml (4.3) for E. In lung cancer (NSCLC; squamous cell) the median was 2.6 ng/ml (2.8; 3.5) for A and 2.8 ng/ml (3.1; 3.6) for E. At a specificity of 95% for healthy individuals (benign lung diseases) A was true positive in 63% (38%) (AUC 88.3% (78.6)), E in 64% (38%) (AUC 91% (77.7%). In NSCLC (specificity 95% for benign lung diseases) sensitivity was 39% (both assays) and 35% for SCLC (both assays), in squamous cell carcinomas 48% (A) and 46% (E) sensitivity was reached, in large cell cancer 51% (both).
Conclusions. Architect CYFRA 21-1 shows similar results concerning protein concentrations as the Elecsys. The known diagnostic capacity of CYFRA 21-1 in lung cancer can be confirmed with both assays to the same extent.

0122

HE 4 – A NEW MARKER FOR OVARIAN CANCER

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Background. HE4 is new more specific and earlier diagnostic marker in cases with ovarian cancer.
Ovarian cancer is the fourth most common cause of cancer-related death in women worldwide. In Europe, the mortality rate range is from 3.6 to 9.3 per 100 000 women. Human epididymis protein 4 (HE4) belongs to the family of whey acidic four-disulfide core (WFDC) proteins. HE4 was first identified in the epithelium of the distal epididymis and originally predicted to be a protease inhibitor involved in sperm maturation. HE4 is expressed in ovarian cancer tissue.
Methods. The HE4 is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique using monoclonal antibodies, directed against two epitopes in the C-WFDC domain of HE4, pre-coated onto a microplate. After the required incubation an enzyme-linked monoclonal antibody are added; followed by substrate solution, which develops color reaction in proportion to the amount of HE4 in the serum.
Results. In the past one year we measure 70 patients with ovarian cancer. The age was between 25 and 60 year old. In 30 cases the levels of HE4 and CA 125 were both elevated. In 10 cases we found no deviation from the normal ranges. In the rest 20 cases the levels of HE4 were high, in spite of low levels of CA 125. The made biopsies confirmed the diagnosis ovarian cancer.
Conclusions. HE4 is vastly more specific and early-phase diagnosed tumor marker in cases with ovarian cancer. Its common usual would reduce the mortality from this social disease.

Cancer/tumour markers

0123
PROGRP AND THE SMALL CELL LUNG CANCER

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Background. In industrial countries, lung cancer is the most frequently diagnosed cancer and the leading cause of death from cancer. Survival in lung cancer mainly depends on the histological type and tumor stage. Unfortunately, the disease is usually diagnosed late in most patients. Pro-Gastrin Releasing Peptide, originally isolated from porcine stomach, is secreted from Small Cell Lung Cancer cells. It is thought that it acts in the metastatic process via it's autocrine activity or through cell-to-cell interactions.
Methods. The assay is a solid-phase, one-step, non-competitive immunoassay based on antibodies specific for different epitopes specifically expressed in pro-gastrin releasing peptide. Calibrators, controls or unknown samples are incubated together with affinity purified biotinylated anti-ProGRP polyclonal antibody and horseradish peroxidase labeled anti-ProGRP monoclonal antibody E146 in streptavidin coated microtiter strips. During the enzyme reaction a color is developing, where the intensity is proportional to the amount of ProGRP present in the samples.
Results. For a period of 5 months we investigate 30 patients. 10 of them were women, 20 men; the age was between 40 and 65. 25 patients appeared with high Pro-GRP levels. After serum analysis they were histological diagnosed with small cell lung cancer. 2 patients were with renal failure, which seems to be the most important source of false positive Results.
Conclusions. Unfortunately, tumor markers are frequently not used in patients with lung cancer. This is why analyzing the serum levels of Pro-GRP is the most specific aim in early diagnosis of patients with small cell lung cancer.

0124
25-OH- VITAMIN D AND CANCER GLANDULE MAMMAE

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Background. Levels of vitamin D_3 are reduced in observed cases with cancer glandule mammae.
Vitamin D is fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight or it is supplied via dietary sources. The two most important forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). In the human body vitamin D_3 and D_2 are bound to vitamin D-binding protein in plasma and transported to the liver were both are hydroxylated.
Methods. The used method is competitive immunoassay. During the first incubation vitamin D_3 in the sample competes with the biotin labeled vitamin D in the complex (biotin-vitamin D/polyclonal 25-OH vitamin D_3-specific ruthenium labeled antibody). After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. Application of a voltage to the analyzer's electrode induces chemiluminescent emission which is measured by a photomultiplier.
Results. During a period of one year we measure 90 patients with cancer glandule mammae. The age was between 40 and 65. Diagnosis was established with the help of tumor marker CA 15-3 in serum, followed by mammography. The taking of the samples was consistent with biologic variation in the organisms.
In 81 patients were determined low levels of vitamin D3, combined with high levels of CA 15-3.
Conclusions. For correct diagnostics of vitamin D in human serum is important to measure 25-OH-Vitamin D3, because of the fact that it's the major preservation in the organisms.

Cancer/tumour markers

0125

CYTOLOGIC STUDY OF BODY FLUID BY USING VIRTUAL MICROSCOPY FOR DIAGNOSIS OF NEOPLASIA

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Background. Clinical data derived from analysis of body fluids (BF) are essential to make proper diagnosis to patients.Report of suspicious cells for malignancy is done from cytology laboratories,however,these findings can be identified during the first analysis,performed in emergency laboratories

Methods. We process all BF received in the emergency laboratory of our Hospital between January and June 2010 with the following workflow:

1. Cell-count on Sysmex-XE5000 analyzer equipped with a BF specific program that includes HF-BF parameter(high fluorescence cells: mesothelial cells,tumor cells,macrophages)
2. BF with HF-BF>5% are processed by virtual microscopy to detect atypical cells
3. By cytocentrifuge, we obtained sample cytospin and stained with May Grünwald-Giemsa.
4. BF are processed in Cellavision-DM96,which scans and photographs cell button and pre-classifies cells, with subsequent review by physicians
5. Results are compared with pathologist and patient diagnosis

Results. 121 BF(Pleural=51;Ascitic=41;CSF=20,Synovial=4;cyst-liver=1;Pericardial=3;Peritoneal=1)are positive for HF-BF and processed in DM96.26 BF were sent to pathologist for suspicion of neoplastic cells,19 of them were confirmed for neoplasia.59 BF were negative for neoplastic processes in both our laboratory and pathogenic anatomy and we never got a false negative

Conclusions. XE5000 and DM96 are tools to approach the patient diagnosis from laboratory, allowed to pass in time, stored images of cells for future reference and training of physicians,increasing results quality of BF analysis.

0126

IN VIVO BIOLOGICAL BEHAVIOR OF FIVE FIBROSARCOMA TUMOUR CLONES WITH DIFFERENT MHC CLASS I SURFACE EXPRESSION

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Background. We have generated a murine tumour system GR9, methylcholanthrene-induced fibrosarcoma. This system is composed of the primary tumor clones and metastatic cell lines obtained after injection of these clones in immunocompetent BALB/c mice.

Methods. We have compared five clones and analyzed local growth and spontaneous metastatic process. MHC class I expression was measured in metastatic cell lines by flow cytometry and compared with the injected cells.

Results. The clones have different expression of class I molecules, thus A7 and G2 clones show high levels of expression, the B7 clone shows intermediate levels and finally, B11 and C5 show lower levels of expression.We found no differences in the local, neither in used cell dose, of four clones included in this study. Only G2 clone didn't grow locally and the primary tumour was rejected. However, A7 clone which has lower expression of the molecule L^d on the surface did grow locally.The number of spontaneous metastasis obtained was significantly lower in clones with low expression of class I molecules. The clone A7was obtained a high number of metastatic nodules, unlike the assays with B11 metastatic nodules did not generated any dose. We have studied the MHC molecules expressionof all metastatic nodules.

Conclusions. Our results show a downregulation of the three class I molecules and total loss of the L^d molecule expression in some cases. This could be consequence of an important role played by the molecule L^d in the local growth tumour and its subsequent evolution to metastasis.

Cancer/tumour markers

0127

THE PROGNOSTIC IMPORTANCE OF PLATELET PARAMETERS IN NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH RADIOTHERAPY

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Background. Platelets play an important role in inflammatory processes, as well as the spread of metastases in certain cancers. This is related to their function, e.g. secretion of platelet-derived growth factor (PDGF) that stimulates angiogenesis and endothelial cell migration. Determination of platelets may therefore be a potential prognostic factor in these patients.
Methods. The study included 263 patients with locally advanced non-small cell lung cancer treated with radiotherapy. Determination of platelet parameters: platelet count (Plt), mean platelet volume (MPV) and large platelets (P-LCR) were performed using the fluorescence flow cytometry method (Sysmex XE-2100, Sysmex). To evaluate the prognostic value of the measured parameters the survival curves were plotted using Kaplan-Meier method and analyzed using Cox test.
Results. The analysis that assessed the impact of platelet parameters showed a statistically significant impact on overall survival of Plt (p= 0,003) and MPV (p= 0,04), whereas no statistical significance of P-LCR (p= 0,4). One-year overall survival was30% in a group of patients with Plt above the median of 269x10^3/μl, compared to 50% in patients with Plt below the median. Likewise, 1-year overall survival was30% in a group of patients with MPV above the median of 9,7fl, compared to55% in patients with MPV below the median.
Conclusions. High levels of platelets and high MPV are unfavorable prognostic factors in patients with non-small cell lung cancer treated with radiotherapy.

0128

HYPERMETHYLATION OF PHOSPHOLIPASE A2-RECEPTOR GENE AS A MOLECULAR BIOMARKER OF LEUKEMIA

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Background. Recently, it was shown that the phospholipase A2 receptor R1 (PLA2R1) plays a crucial role in senescence. In this study we analyzed the expression and methylation of *PLA2R1* gene in myeloid U937 cell line and blood leukocytes of patients with leukemia.
Methods. Expression of PLA2R1 in U937 was analyzed using RT-qPCR. To determine methylation levels of *PLA2R1*, bisulfite-modified DNA fragments were sequenced. After identifying CpG sites with different methylations between normal and leukemic patients, high resolution melting (HRM) analyses were performed to quantify *PLA2R1* methylation status.
Results. According to our data the expression of PLA2R1 is completely down-regulated in U937 cells. After exposure of cell to 5-aza-2´-deoxycytidine, however, PLA2R1 was re-expressed. Simultaneously, a 100% methylation of *PLA2R1* promoter was found in this cell line. By using HRM analyses we determined in 25 normal subjects methylation levels of *PLA2R1* below 10%, whereas in 27 patients with acute myeloid and acute lymphatic leukemia methylations averaged 37%± 18% (min. 15%, max. 80%). In one patient with AML blood leukocytes exhibited no elevated methylation levels. In a preliminary study of high-risk myelodysplastic syndrome (MDS) patients, 3 patients had increased *PLA2R1* methylations. In course of treatment with Vidaza (azacitidine) as methyltransferase inhibitor, 2 patients were responders, but 1 patient was a non-responder with increased methylation levels in blood leukocytes during treatment.
Conclusions. The study shows that (i) the *PLA2R1* promoter in leukemic cells is hypermethylated and (ii) the determination of *PLA2R1* methylation may be useful as biomarker in the therapeutic treatment of MDS patients.

Cancer/tumour markers

0129

CORRELATION OF HETEROGENEOUS EXPRESSION OF SIALYLTRANSFERASES AND MUC16 IN OVARIAN TUMOR TISSUES

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Background. A number of glycoproteins such CA125 are abnormally glycosylated in ovarian cancers. Most of aberrant glycosylations are a result of altered sialyltransferase (ST) expression.The aim of this study was to evaluate expressions of six sialyltransferases and MUC16 and their correlations in ovarian benignant and malignant tissues.
Methods. mRNA expression of six sialyltransferases and MUC16 was assessed from 16 human ovarian tumors (7 benignant tumors and 9 malignant tumors) by real time PCR.
Results. mRNA of ST6GAL I and ST3GAL I are not significantly up-regulated in ovarian cancer tissues, while ST6GAL II and ST3GAL IV were not significantly increased in benign tumors. There was no change between ST3GAL III, ST3GALVI expression and tumour sub-types. MUC16 was significantly increased in carcinoma tissue. Significant correlation was found betweenST3GAL III and ST3GAL IV. MUC16 correlated with ST3GALVI and ST6GALI. ST6GAL I is well correlated with ST3GALVI. ST6GAL II is significantly correlated to ST3GALIII and ST3GALIV, too.
Conclusions. The expression of the given sialyltransferases and MUC16 can be expressed at an heterogeneous level as a consequence of oncogenic transformation of the ovary. A strong correlation between MUC16 and sialyltransferases may be of specific impact on the glycosylation of the MUC16.

0130

HE4, CA 125 AND ROMA IN THE DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH GYNECOLOGICAL DISEASES

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Methods. CA125 and HE4 serum levels were determined and the Risk of Ovarian Malignancy Algorithm (ROMA) calculated in 66 healthy women, 285 patients with benign gynaecological diseases, and 143 with active gynaecological cancer (111 ovarian cancers). CA 125 and HE4 cut-offs were 35 U/mL and 150 pmol/L, respectively. ROMA algorithm cut-off was 13.1 and 27.7 for premenopausal or postmenopausal women, respectively.
Results. HE4, CA125 and ROMA results were abnormal in 1.1%, 13.6% and 25.8% of healthy women and in 1.1%, 30.2% and 12.3% of patients with benign diseases, respectively. Sensitivity in ovarian cancer was HE4 79.3%, CA 125 82.8% and ROMA 90.1%. HE4 and CA 125 were related to tumor stage and histological type, with the lowest concentrations in mucinous tumors. Significantly higher area under the ROC curve was obtained with ROMA and HE4 than with CA 125 in the differential diagnosis of benign gynaecological diseases versus ovarian cancer (0.952, 0.936 and 0.853, respectively). In our experience ROMA algorithm might be used only in patients with normal HE4 and abnormal CA 125 serum levels (cancer risk 44%). ROMA algorithm in HE4 positive had a similar sensitivity (80%) and only increases the specificity by 3.2% compared to HE4 alone.
Conclusions. HE4 has a significantly higher specificity and similar sensitivity than CA 125 in patients with gynaecological diseases. We found that using HE4 alone in patients with positive HE4 and ROMA algorithm in those with CA 125 abnormal and normal HE4, sensitivity was 90.1% (non-mucinous 94.9%) and the Positive Predictive Value 82.7%.

Cancer/tumour markers

0131

ARCHITECT CYFRA 21-1 COMPARED AGAINST ROCHE ELECSYS

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Background. To compare CYFRA 21-1 measured on the ARCHITECT (Abbott Diagnostics) and Elecsys (Roche).
Methods. The precision of the ARCHITECT CYFRA 21-1 assay was determined across the dynamic range using the assay controls in a 5 day CLSI setup. Correlation to the Roche CYFRA 21-1 assay was evaluated on a set of 150 patient samples (samples from 30 healthy individuals, 30 patients with benign lung disease, 90 patients with lung cancer [83 Small Cell Lung Cancer, 7 Small Cell Lung Cancer]).
Results. The total %CV for the ARCHITECT CYFRA 21-1 assay is 5.4%, 2.0% and 2.5% for the Low, Medium and High Control, respectively with a repeatability of 5.2%, 1.4% and 2.0%. The correlation analysis showed a high Passing-Bablok agreement of both methods (slope 0.86; Intercept -0.24; Pearson correlation 0.99), in particular for values between 3.3 and 10 ng/mL (slope 1.01; Intercept -0.91; Pearson correlation 0.88). The ARCHITECT assay reads slightly lower for values < 3.3 ng/mL (slope 0.79; Intercept -0.14; Pearson correlation 0.90). The CYFRA 21-1 Median levels for healthy, benign and malignant cases as well as per histological subtype are always higher on the Elecsys. The ROC curves are highly similar with insignificantly larger AUCs for the ARCHITECT assay. For benign vs. malignant cases the AUCs are 0.93/0.87 and for benign vs. malignant 0.77/0.76.
Conclusions. The ARCHITECT CYFRA 21-1 correlates well at high precision with the Roche CYFRA 21-1 assay, showing slightly lower concentrations. The ROC curves confirm the diagnostic utility of CYFRA 21-1 in Lung Cancer.

0132

EVALUATION OF SERUM HUMAN EPIDIDYMIS PROTEIN 4 (HE4) IN PATIENTS WITH MALIGNANT AND NON MALIGNANT DISEASES: COMPARISON WITH CA 125

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Background. The aim of this study is to evaluate HE4, in comparison with CA 125 in healthy subjects and in patients with benign and malignant diseases.
Methods. CA125 and HE4 serum levels were determined in 101 healthy individuals, 535 patients with benign pathologies (292 benign gynaecological diseases) and 423 malignant diseases (127 ovarian cancers). CA 125 and HE4 cut-offs were 35 kU/L and 150 pmol/L, respectively.
Results. HE4 and CA125 results were abnormal in 1.1% and 9.9% of healthy individuals and in 12.1% and 37% of patients with benign diseases, respectively. Renal failure was the most common cause of elevated HE4 levels in patients with benign disease with significantly higher HE4 concentrations (p=0.001) than in other benign diseases. HE4 showed a higher specificity than CA 125 in patients with benign gynaecological diseases with abnormal levels in 1.3% and 33.2% of the patients, respectively. HE-4 serum levels were abnormal mainly in gynaecological cancer and lung cancer. By contrast, CA 125 was elevated in a high number of different non-ovarian malignancies, including non-epithelial tumors. Significantly higher area under the curve was obtained with HE4 than with CA 125 in the differential diagnosis of benign versus malignant diseases (0.755 vs. 0.643) and in the differential diagnosis of gynaecological diseases (0.874 vs. 0.722).
Conclusions. HE4 has significantly higher specificity than CA 125 and the combination of CA 125 and HE4 improved the detection of ovarian cancer in all stages and histological types.

Cancer/tumour markers

0133

IMMUNOCYTOCHEMICAL DOUBLE-LABELING STAINING USED TO DISPLAY THE DISTRIBUTION OF BCL-2/KI-67 CELLS IN ENDOMETRIAL ADENOCARCINOMAS AS WELL AS NORMAL ENDOMETRIUM

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Background. Endometrial adenocarcinoma is the most common gynecologic malignancy and is the fourth most frequently diagnosed cancer in women in theUSA.
The aim of this study was to determine the expression of Bcl-2 and Ki-67 by double-labeling staining in type I and type II endometrial adenocarcinomas as well as normal endometrium.
Methods. During a 7-month period, 90 patients were evaluated with endometrial imprint cytology. Endometrial samples, freshly resected from women who underwent total abdominal hysterectomy were studied. 45 patients had endometrial carcinoma (21 cases were grade I, 18 grade II and 6 grade III). 45 cases were diagnosed as normal endometrium. Of these, 18 were of proliferative endometrium, 6 of secretory and 21 of atrophic endometrium. Bcl-2 immunostaining was localized in the cytoplasm (red fine granules), whereas ki-67 immunostaining was confined to the nucleus (distinct brown fine granules).
Results. Bcl-2 expression was strong and homogeneous in normal (proliferative, secretory and atrophic) endometrium and more frequent in low-grade endometrioid carcinomas. Completely negative staining of Bcl-2 was found to be strictly related to high-grade endometrioid carcinomas. Ki-67 expression was higher in patients with high-grade endometrioid carcinomas. Proliferative endometrium showed inconclusive Ki-67 expression levels and in the secretory endometrium, Ki-67 positive cells were markedly diminished and even disappeared. Completely negative staining of Ki-67 was found to be strictly related to atrophic endometrium.
Conclusions. Immunocytochemical double-labeling staining can be used to display the distribution of Bcl-2/ki-67 cells in endometrioid carcinomas as well as normal endometrium, in addition to cytomorphologic features and appeared to be useful for the more accurate diagnosis of endometrial carcinoma in endometrial cytology with imprint smears.

0134

DETERMINATION OF CUT-OFF VALUE FOR QUANTITATIVE IMMUNOCHEMICAL FECAL OCCULT BLOOD TEST IN ONCOLOGY PATIENTS

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Background. In our laboratory, we used qualitative fecal occult blood test to detect bleeding from gastrointestinal tract in oncology patients treated with chemotherapy. The aim of our study was to determine the cut-off value for quantitative automated test for detecting occult blood in feces of our patients.
Methods. We have tested 70 stool samples with two different tests. The first is qualitative immunochromatographic test HEM-CHECK-2 (Veda.lab) and is regularly used in our laboratory. The second is quantitative immunoturbidimetric latex test FOB Gold (Sentinel) which was performed on an analyzer Modular (Roche). For the statistical evaluation of the obtained data and calculation of the cut-off value, the MedCalc program was used.
Results. The results of the qualitative HEM-CHECK-2 test for fecal occult blood in feces samples were negative in 37 cases and positive in 33 cases. The results obtained by quantitative automated test were in the range from 0 to19520 ng/mL, with the arithmetic mean of 730 ng/mL and median 32 ng/mL. The receiver operating characteristics (ROC) curve analysis was performed to assess the cut-off value of 38 ng/mL for HEM-CHECK-2 test. The specificity at that cut-off value was 91.9%, sensitivity 81.8% and the area under ROC curve was 0.902. Kappa analysis showed a good agreement of results (Kappa=0.763).
Conclusions. FOB Gold method performed on an analyzer Modular is simple and precise and shows a good agreement of the obtained Results.

Cancer/tumour markers

0135

THE EMERGING VALUE OF HE4 IN THE DISCRIMINATION OF OVARIAN CANCER FROM OTHER MALIGNANT AND BENIGN PELVIC MASSES

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Background. CA-125 is the standard cancer marker in the laboratory-based screening of pelvic masses in women. However, the clinical utility of this biomarker has been limited due to its low sensitivity especially in the early stages of ovarian cancers and falsely elevated values in different non-malignant diseases.

Methods. We measured the serum levels of a novel tumor marker HE4 with CA-125 to increase the sensitivity in the screening of malignant ovarian tumors and to better differentiate these cases from other diseases with elevated CA-125 levels. Serum samples were analyzed by using ABBOTT Architect® chemiluminescent microparticle immunoassay.

Results. Sixty patients with different gynecological disorders (ovarian cancer, ovarian cysts, endometriosis, myoma, borderline ovarian tumor, other adnexal neoplasm) were analyzed and compared to healthy controls. The serum levels of HE4 were significantly (p<0.005) increased in patients with ovarian (150.7pM in I/II.; 1354.2pM in III/IV. stages) or endometrial (286.1pM) cancer versus normal subjects (39.6pM; n=10). The combined analysis of HE4 and CA-125 supplied a greater level of discrimination of malignant ovarian cancers compared to those cases when these markers were determined individually. This testing also provided useful information to the clinicians in the patients' eligibility for surgery. HE4 remained negative and CA-125 alone showed increased levels in patients with ovarian cysts, endometriosis or uterine myoma. Neither biomarker identified the presence of borderline ovarian tumors, however, radiological investigation predicted malignant forms of the ovarian tumor.

Conclusions. Simultaneous measurement of HE4 and CA-125 levels provides a better discrimination of malignant ovarian tumors from benign masses.

0136

GANGLIOSIDE SERUM LEVELS- PROGNOSTIC SIGNIFICANCE IN MELANOMA PATIENTS SURVIVAL

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Background. The transition of melanoma from radial to vertical growth phase is accompanied by several molecular changes leading to uncontrolled growth and an increase in invasive potential. A particular attention is paid to the contribution of gangliosides in melanoma metastases development.

Purpose. In this study, it was explored seric gangliosides status in relation with sex, age, site of primary tumor, histological type, Clark's level invasion, Breslow sites of metastases, different therapeutic strategies, relapse free interval and overall survival of melanoma patients.

Methods. The present study included a series of 761 patients with melanoma (353 with primary tumor, 408 advanced melanoma stage), monitored in Dermatovenereological Center Research, Bucharest, Romania(1991-2010). This study was performed to determine the possible significance of amount and composition of gangliosides in survival of metastatic melanoma patients treated with biological agents, chemotherapy, immunochemotherapy, radiotherapy. Before the therapy initiation, all patients were stratified by systemic therapy and a threshold of 40 mg/dl gangliosides.

All samples were obtained and processed by a well established protocol.

Results. Augmented seric gangliosides (more then 40 mg/dL) were predictive for decreased overall survival, whereas reduced total serum ganglioside levels were predictive of improved overall survival.

The response rate to therapeutical agents is better in patients with less then 40 mg/dL serum gangliosides. The dose, rhythm and time of administration of therapeutical agents affect the total ganglioside serum level. The mechanisms responsible for these actions include: seric antigangliosides antibodies presence, resistance and escape at drugs, apoptosis resistance, neoangiogenesis.

Cancer/tumour markers

0137

PRELIMINARY CHARACTERIZATION OF A NOVEL HUMAN GENE, UBE2Q1, OVER- EXPRESSED IN BREAST CANCER TISSUES

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Background. The ubiquitin-proteasome pathway facilitates the degradation of damaged proteins and regulates growth and stress response. This pathway is activated in various cancers, including breast cancer. We have previously reported that the novel human gene, UBE2Q2, is overexpressed in tumor mass and invasive epithelium in head and neck squamous-cell carcinoma and in breast cancer tissues against corresponding normal tissues.
In this study the expression pattern of the novel human gene, UBE2Q1, in breast cancer tissues and normal counterparts were investigated for the first time. UBE2Q1 is one of the homologs of UBE2Q2 gene and belongs to E2 enzyme family. There is no publication about UBE2Q1 gene and this study is a preliminary study for its characterization.
Methods. Real-time polymerase chain reaction was used to investigate the expression levels of UBE2Q1 gene in a collection of 21 breast cancer tissues. Immunohistochemistry and Western blot testing were also performed by using an antibody that we generated against an amino acid sequence predicted from the DNA sequence of UBE2Q1 gene.
Results. According to real-time PCR data in the 21 investigated cases, 14 patients (66.7%) showed up-regulation of UBE2Q1 gene when compared with corresponding normal tissues. These results were in agreement with the data from which showed higher levels of immunoreactivity in cancerous tissues in 68.75% of the cases for UBE2Q1.
Conclusions. Our data suggest that the novel human gene UBE2Q1 may have implications for pathogenesis of breast cancer and could be used in molecular diagnosis purposes in the future.

0138

THE ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) IN THE SERA OF PATIENTS WITH MYOMA UTERI AND ENDOMETRIAL CANCER

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Background. Changes in ADH and ALDH activity can lead to some metabolic disorders which can be associated with the pathomechanism of carcinogenesis. In previous experiments, we have found a increased level of class I ADH and total ADH activity in endometrial cancer tissues. Changes in this enzyme in cancer cells may be reflected by ADH activity in the serum. In this study we measured the activity of ADH isoenzymes and ALDH in the sera of patients with endometrial cancer.
Methods. Serum samples were taken from 40 women with endometrial cancer, 52 with myoma uteri and 52 as control group. Class I and II ADH isoenzyme activity and ALDH were measured by fluorometric method with class-specific fluorogenic substrates. For measurment of total ADH, class III and IV activity we emloyed photometric method.
Results. The total activity of ADH was significantly higher in the serum of patients with endometrial cancer (0.813 +/- 0.483 mU/l) than in patients with myoma uteri (0..571 +/- 0.461 mU/l) and healthy subjects (0.542 +/- 0.302 mU/l). The activity of the class I ADH isoenzyme was also significantly higher in endometrial cancer (1.740 +/- 0.960 mU/l) as compared to myoma (1..246 +/- 0.646 mU/l) and the control group (1..211 +/- 0.751 mU/l). The analysis of ALDH activity did not indicate significant differences between all tested groups.
Conclusions. The increased activity of total ADH only in the sera of patients with endometrial cancer, especially class I, appears to be caused by isoenzymes being released from cancerous cells.

Cancer/tumour markers

0139

OXIDATIVE STRESS AND ANTIOXIDANTS IN CANCER

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Background. There are conflicting views on the use of antioxidants in cancer patients, and their potential interactions with chemotherapy.Some chemotherapeutic agents induce oxidative stress by generation of oxygen free radicals (ROS) which might be an alternative mechanism for their cytotoxic effect via inducing apoptosis. A question has logically developed as to whether antioxidants taken concurrently during chemotherapy could reduce the beneficial effect of chemotherapy on malignant cells by inhibiting ROS and preventing apoptosis of cancer cells.
Methods. In order to clarify the roles of antioxidants in chemotherapy, we investigated Quercetin (3,3',4',5,7-pentahydroxyflavone) and N-acetylcysteine (NAC) in different cell types treated with anticancer drugs. We studied cytotoxic activity of Topotecan alone and/or in combination with Quercetin in two human breast cancer cell lines, MCF-7 and MDA-MB-231. We also investigated the effect of NAC on MRP1-mediated doxorubicin and vincristine cytotoxicity in Human Embryonic Kidney (HEK293) and its MRP1 transfected (293MRP) cells.
Results. Our data indicated increased oxidative status in MCF-7 and MDA-MB-231cells exposed to Topotecan. Treatment with Quercetin did not inhibit ROS generation, and enhanced cytotoxicity of Topotecan in both cells.In contrast, NAC enhanced resistance against doxorubicine and vincristine in MRP1 overexpressing cells. Our study demonstrate that Quercetin and NAC have diverse effects in the cytotoxicity of chemotheurapeutic drugs.
Conclusions. We conclude that whether an antioxidant supplement would be helpful, harmful or neutral depends in part on the specific antioxidant (and its dose), the chemotherapy drugs being used, the type and stage of cancer being treated.

0140

CYSTATIN C AS A BIOMARKER FOR GLOMERULAR FILTRATION RATE IN CANCER PATIENTS

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Background. Monitoring of kidney function is of critical importance during treatment with chemotherapeutic agents. Although the Glomerular Filtration Rate (GFR) is considered the best measure of overall renal function, its determination has too many methodological problems for the routine practice. So the estimation of GFR using endogenous filtration markers or prediction formulas presents a common alternative. Cystatin C is a molecule that is under intense investigation for its usefulness in this context.
Objective. The objective of the current study was the evaluation of cystatin C as a marker of renal impairment for cancer patients on chemotherapy.
Methods. One hundred and three blood samples from patients with solid tumors or hematological malignancies were analysed for creatinine and cystatin C. Creatinine clearance (Crcl) was calculated using 24-h urine collections.
Results. The Pearson correlation coefficient (r) of cystatin C serum concentration with Crcl was -0,56 (p<0,0001), while the r value for the correlation of serum creatinine with Crcl was only -0,45 (p<0,0001). In the group of patients with Crcl values below 90 ml/min, the association of both cystatin C and creatinine with Crcl became stronger and the correlation was significantly better for cystatin C (r was -0,69 , p<0,0001 and -0,60 , p<0,0001, for cystatin C and creatinine, respectively). Cystatin C showed better sensitivity than creatinine in detecting Crcl<70mL/min, a value critical for chemotherapy.
Conclusions. Serum cystatin C could be used as a screening test for the estimation of GFR, in place of Crcl, for cancer patients.

Cancer/tumour markers

0141

HORMONE RECEPTOR STATUS (ER, PR), HER-2 AND P53 IN SOUTH INDIAN BREAST CANCER PATIENTS

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Background. Breast cancer is the most common malignancy among women in the western world and also in India. Presence of Estrogen receptor (ER), Progesterone receptor (PR) and Human Epidermal Growth Factor -2 (HER-2/neu) status in invasive carcinoma is now-a-days routinely estimated as the markers of important prognostic significance. A tumor suppressor gene, p53 is also present in most breast cancers. Literature suggests that over-expression of HER2 and p53 may have adverse effect in breast cancer.
Methods. In this retrospective study of 7 years ER, PR analysis was done in 276 invasive breast cancer cases. In 230 cases additional markers like HER-2/neu and p53 was done. Immunostain for ER, PR, HER-2, p53 was done on three micron paraffin sections on 3-amino propyl ethoxy silane (APES) coated slides, with known positive controls by Polymer Horse Radish Peroxidase (HRP) IHC detection system. Primary antibodies used were monoclonal mouse anti- human antibody (ER- clone 1D5, PR-clone1A6, HER-2/neu-clone CB11, p53- clone D07). Antigen retrieval was done by pressure cooking for 5-10 minutes in Tris EDTA buffer, pH 9.0. Nuclear staining was assessed for ER, PR, p53 and membrane staining was assessed for Her 2.
Results. Maximum numbers of cases were in the 41 –50 yr age group. Our data showed overall 48.1% ER, 55.0% PR,69.2% % HER-2 and 62.8% p53 positivity respectively.
Conclusions. In this study ER, PR positivity is low and Her-2, p53 expression is high when compared to that described in western literature.

0142

LOSS OF FHIT TUMOUR SUPPRESSOR GENE EXPRESSION PROMOTES DOWNREGULATION OF MHC CLASS I SURFACE EXPRESSION IN MURINE METASTATIC CELL LINE

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Background. Loss of the fragile histidine triad (Fhit) gene expression is often associated with the development of epithelial tumours. Additionally, MHC class I downregulation is a very frequent escape mechanism develop by cancer cells to escape from immunosurveillance. Previously, we found that MHC class I surface expression and Fhit expression presented a direct relation.
Methods. The MN4.5 murine metastatic cells was derived from a lung metastasis originated by a fibrosarcoma tumour in BALB/c mice. The expression of Fhit was inhibited by transfection of specific si-RNA. MHC class I expression was analysed after 48 h, by flow cytometry with monoclonal antibodies against each one of the three H-2 class I molecules. Real-time RT-PCR was performed to measure of transcriptional expression of heavy chains and APM components.
Results. MN4-5 cells present expression of H-2 Kd, Dd and Ld molecules. These cells also present expression of Fhit gene. The transcriptional expression of Fhit decreased more than a 70 % after si-RNA transfection of MN4.5 cells. The inhibition of Fhit promoted a decrease in MHC class I expression in MN4.5 cells. The expression fell in the three H-2 class I molecules over a 56%. Transfection with an unspecific si-RNA did not produce changes in Fhit or MHC class I expression. Analysis of transcriptional expression by Real-time-RT-PCR showed a decrease in all H-2 class I heavy chains and in several APM components (LMPs, TAPs and Tapasin).
Conclusions. These results indicate that the Fhit gene might be a key regulator of MHC class I expression.

Cancer/tumour markers

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MHC CLASS I NEGATIVE METASTATIC CELLS ARE CONTROLLED IN DORMANCY STATE BY ADAPTIVE IMMUNITY

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Background. we have developed a murine tumour system composed by several clones of a fibrosarcoma induced by methylcholantrene and metastases derived from these fibrosarcoma clones in Balb/c mice. Several of these clones generated spontaneous metastases in immunocompetent mice, but one of them, GR9-B11 clone, did not generate spontaneous metastases in neither of the assays. We performed spontaneous metastasis assays with this clone in nude mice and in immunocompetent mice depleted of T lymphocytes.
Methods. spontaneous metastasis assays were performed in nude mice and in immunocompetent mice depleted of T lymphocytes immediately after extirpation of primary tumour or six months later. Depletion of T lymphocytes was performed by weekly injection of anti-CD4+ and anti-CD8+ specific monoclonal antibodies.
Results. GR9-B11 presents very weakly MHC class I surface expression in basal conditions, expressing only slightly H-2 Kd molecule. After treatment with IFN, H-2 K and D molecule were induced and slightly H-2 L molecule. In immunocompetent mice this clone did no generate spontaneous metastases, and the mice remained free of disease after one year. In contrast, when nude mice were used in spontaneous metastasis assays, this clone generated lung metastases. Immunocompetent mice were depleted of T lymphocytes immediately after extirpation of primary tumour and results were similar to found in nude mice. Surprisingly, when immunocompetent mice were depleted of T lymphocytes, 180 days later of extirpation of primary tumour, metastases appeared in the lungs.
Conclusions. these results indicate that metastases remained in latency state in immunocompetent mice, totally controlled by T lymphocytes.

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TUMOR SUPRESSOR GENES INVOLVED IN COORDINATED DOWNREGULATION OF ANTIGEN PROCESSING MACHINERY COMPONENTS AND MHC CLASS I MOLECULES

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Background. We have developed a murine tumoral model composed by spontaneous metastasis derived from a tumour clone B9, a methylcholanthrene- induced fibrosarcoma. These metastases show a different MHC class I phenotype depending on the immune system of the host: MHC class I-negative in inmunocompetent mice and MHCclass I-positive in nude mice. Previous studies have shown that the lack of expression of MHC class I was due to a coordinated downregulation at transcriptional level of several components of the antigenic processing machinery (APM): TAP-1, TAP- 2, LMP2, LMP-7, LMP-10, Calnexin and Tapasin.
Methods. To determine the molecular mechanisms implicated in coordinated down-regulation of these genes, we have performed a cDNAsubtraction libraries to compare mRNAs derived from the different metastatic variants: MHC class I-positives and -negatives. The different fragments obtained were sequenced and compared with the NCBI and SANGER databases.
Results. The assays were performed comparing MHC class I-positive metastatic cell lines (MN4.5 and MN4.1) with two metastatic cell lines with lack expression of MHC class I molecules (MP5 and MP12). We found twelve differentially expressed genes, some of them related to already known genes and others to new ones. Among them, we must mention the following genes as possibly implicated: FHIT (fragile histidine triad gene), Glis1, c-Myc and Ap-2alpha.
 For these genes have been described different implications in cancer.
Conclusions. Studies are being developed with these genes, at level of quantitative RT-PCR expression, blockage of the expression, to determine their direct implication in AMP component and MHC class I molecule expression.

Cancer/tumour markers

0145

β_2-MICROGLOBULIN IN MULTIPLE MYELOMA

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Background. β_2-microglobulin (B2m) determinations in serum have been introduced as a method of stratifying patients with multiple myeloma (MM). Conflicting results from several studies prompted us to study retrospectively the correlation of B2m with disease stage, as well as the prognostic value of B2m.Serum protein electrophoresis is used routinely for research the presence of abnormalities in different protein fractions, as well as monitoring of monoclonal gammopathies.B2m, used as a tumour marker, offers a diagnostic value in proliferative syndromes.
Methods. We analyzed retrospectively 129 patients (60 men, 69 women; age: 76 [65-82] years) with previous analytical presenting monoclonal peaks,by capillary electrophoresis for the evaluation of proteinograms and B2mdeterminations were performed by immunoassay. The correlations between B2m and gamma-serum protein fraction, and medium values obtained by diagnosis of confirmed MM were evaluated.
Results. According to diagnosis, 67.4% were confirmed ofMM, (B2m: 3417 [2677 – 4637] ug/mL, gamma fraction (γF): 29.4±11.9% and total proteins (TP): 7.9 [7.1 – 9.3]). These data were compared with patients no MM, and we obtained significances of B2m p=0.126; TP p =0.035 andγF p<0.001.Thecorrelation was significant between B2m and γF(r= 0.27, p=0.017). On confirmed MM we separated the population according to the staging cut-off: 4 ug/mL for B2m, p<0.001.
Conclusions. Both TP and γF correlated with the presence of the disease. Therefore,γF is an useful tool in the diagnosis of MM but nowadays is obsolete for prognosis. B2m would be a marker with significant clinical application in monitoring of MM patients.

0146

PRO-GRP LEVELS IN PATIENTS WITH LUNG CANCER: A PROSPECTIVE STUDY

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Background. Survival and therapy for lung cancer mainly depend on the histology and stage at diagnosis. We aimed to evaluate the diagnostic utility of ProGRP in patients with different lung malignancies.
Methods. We studied 274 consecutive patients, admitted to the European Institute of Oncology with suspicion or recent diagnosis of lung cancer. Patients with impaired renal function, a potential cause of elevation of ProGRP, have been excluded. Plasma ProGRP has been determined by a fully automated immunoassay on the ARCHITECT analyzer (Abbott Laboratories, Wiesbaden, Germany). The statistical evaluation was performed by the Mann-Whitney test.
Results. ProGRP values (median and ranges) in the different histological types were as follows: small cell lung cancer (SCLC-39 patients) 77.6 pg/ml (20.3-10572.5); adenocarcinoma (ADK-124 patients) 40.5 pg/ml (12.8-105.3); squamous cell carcinoma (SCC-56 patients) 40.9 pg/ml (20.9-101.4) and non malignant lung disease (NMLD-43 patients) 35.8 pg/ml (18.4 -66.9). ProGRP values in SCLC were significantly higher than in non small cell lung cancer (NSCLC) (p<0.0001) and in non malignant diseases (p<0.0001).
The percentage of samples distribution by ProGRP values (from 0 to 37.7, from 37.8 to 110, from 110.1 to 1000 and above 1000 pg/ml) in SCLC, ADK, SCC, and NMLD indicates that only two patient with SCLC showed values within the reference rangeand no patient with NSCLC showed values >110 pg/ml.
Conclusions. In patients with lung cancer, ProGRP values are strongly associated with histological diagnosis of SCLC and may be useful to discriminate among the different histological types.

Cancer/tumour markers

0147

SIDE-BY SIDE COMPARISON OF TWO AUTOMATED ASSAYS FOR CA 19-9 IN AN ONCOLOGY REFERENCE CENTER

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Background. Although most of the automated methods for CA19.9 determination use the same MoAb, there are difficulties in the comparison of different systems. This may be due both to the complexity of the CA19.9 molecule and to the lack of an international standard toward which calibrate the different systems. We evaluated the correlation and agreement of two automated immunoassays in routine practice.
Methods. Five hundred consecutive samples with clinicians' request for CA 19-9 were assayed by the Abbott ARCHITECT and Roche Cobas 411 assays. Clinical and histological data were used to resolve the discrepant Results. Pearson's correlation and Bland-Altman analysis were used for statistical evaluations.
Results. The correlation between methods was good (Pearsons's r= 0.930); significant differences (Bland-Altman) were found throughout the dynamic range (median: -47.3%) and in the range between 20 and 200 U/mL, were the majority of positive results by one or both methods were found (median: +20.6%). Forty-six samples gave discordant Results. only 5 were negative with one method and highly positive (>100 U/L) with the other. At standard cut-off, sensitivity, specificity and diagnostic accuracy were 24.7%, 94.1% and 46.6% for the Abbott method and 27.3%, 90.3% and 47.2% for the Cobas system.
Conclusions. The two CA 19.9 methods are not interchangeable but their diagnostic accuracy is substantially the same and the correlation is fairly good, with highly discordant samples in 1% of the cases. Our suggestion is that the patients should be monitored with the same assay and the method should be indicated in the report.

0148

SERUM LEVELS OF VEGF IN CUTANEOUS MALIGNANT MELANOMA

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Background. Angiogenesis is an important feature and a potential prognostic factor in various tumors, as it may function as a promoter for tumor growth and metastasis. The main growth factor involved in angiogenesis is vascular endothelial factor (VEGF).
Methods. We used ELISA test to measure VEGF levels in serum from patients with melanoma (n=45, 30 females: 28-86 years and 15 males: 28-80 years), nevus (n=16, 9 females: 30-75 years and 7 males: 29-77 years) and healthy volunteers (n=16, 9 females: 28-81 years and 7 males: 28-80 years). Serum lactate dehydrogenase (LDH) was measured on a biochemistry analyzer.
Results. Serum VEGF levels were significantly higher in melanoma patients compared to control group (414,11 ± 318,72 pg/ml vs. 147.18± 80.3 pg/ml, p<0.01). LDH is increased in melanoma patients compared to control group (419.72 ± 310,18 U/L vs. 306.21 ± 125,37 U/L). We analysed VEGF status depending on sex, age, the site of primary tumor, histological type, Clark level of invasion, Breslow index, and sites of metastasis. None of these parameters were correlated with VEGF levels. Instead, we found a significant correlation between serum VEGF levels and tumor progression (primary tumor without metastases or advanced melanoma with metastases) (p=0.0003), with the highest levels in advanced melanoma, and between VEGF and LDH levels in melanoma patients (p=0.0001). It was observed an important correlation between VEGF levels and the phase of tumor growth (superficial or in depth) (p=0.0474).
Conclusions. The obtained results suggest that VEGF is involved in melanoma angiogenesis and progression.

Cancer/tumour markers

0149

PERFORMANCE OF THE EUROPEAN RANDOMIZED STUDY OF SCREENING FOR PROSTATE CANCER (ERSPC) RISK CALCULATOR FOR HIGH GRADE PC IN A CLINICAL SETTING AND THE ADDITIONAL VALUE OF THE PROSTATE HEALTH INDEX (*PHI*)

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Background. Risk stratification to identify men at increased risk of having a prostate cancer (PC) is often done with risk calculators (RC). We report the performance of the ERSPC RC for high grade PC (Gleason score >= 7, (HG) in a clinical cohort (University Hospital Munster) and evaluate the additional value of the Prostate Health Index (phi) which combines total PSA, free PSA and [-2]proPSA test Results.
Methods. The clinical cohort consists of 205 biopsied men (PSA 2.0-10.0ng/ml), with 100 PC cases (48.8%) detected, 66 were HG PC (32.2%). Logistic regression analyses were used to assess the value in predicting HG PC of PSA alone, PSA plus DRE, *phi* plus DRE and the ERSPC HG RC (with and without *phi*). The calculated probabilities were compared with ROC analyses.
Results. PSA alone had lowest predictive capability (AUC: 0.61). *phi* and DRE (0.77) outperform PSA plus DRE (0.65). The ERSPC RC for HG PC (0.77), in combination with *phi* has the highest AUC (0.80). The addition of *phi* results in an increase in specificity from 40% to 58% (at 95% sensitivity).
Conclusions. The ERSPC RC performs well in a clinical setting. *phi* plus DRE outperform the classical approach for risk assessment (PSA plus DRE outcome). There is additional value of *phi* to the ERSPC RC (not significant), however specificity increases considerably. The combination of *phi* and DRE, results in equal performance (expressed as AUC) with the ERSPC RC for HG PC but circumvents the need of prostate volume assessment.

0150

[-2]PROPSA IMPROVES PREDICTION OF REPEAT PROSTATE BIOPSY RESULTS

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Background. The use of prostate specific antigen (tPSA) for the detection of prostate cancer (PCa) is being criticized because of a relatively low specificity. In repeat prostate biopsies the predictive value of tPSA is very low. A molecular isoform of free PSA (fPSA), [-2]proPSA, showed a higher accuracy for the detection of PCa in initial biopsies as compared with tPSA or f/tPSA. The clinical performance of [-2]proPSA for the detection of PCa in initial versus repeat prostate biopsies was evaluated.
Methods. A total of 528 patients with tPSA values between 1.6–8.0ng/mL (WHO-calibrated) underwent 10 core biopsies. 272 patients underwent their first biopsy session, 256 patients underwent repeat biopsies. The serum concentrations of tPSA, fPSA, and [-2]proPSA were measured with the Beckman Coulter immunoassays on Access2 instruments.
Results. ROC curve analysis showed that [-2]proPSA/fPSA (AUC=0.69) provided significantly (p<0.04) better clinical performance as compared to tPSA (AUC=0.59) but was not significantly better than f/tPSA (AUC=0.66) in predicting PCa in the first biopsy cohort. In the repeat biopsy cohort, [-2]proPSA/fPSA (AUC=0.77) provided significantly better prediction of biopsy outcome than f/tPSA (AUC=0.62) or tPSA (AUC=0.53), differences between AUCs p<0.001. The diagnostic accuracy of [-2]proPSA/fPSA improves with increasing prostate volume.
Conclusions. [-2]proPSA/fPSA shows a superior clinical performance in detecting PCa as compared to tPSA or f/tPSA. In pts. undergoing repeat prostate biopsy, [-2]proPSA/fPSA shows the highest AUC outperforming all other parameters investigated statistically significant thus possibly providing important information for the group of patients in which a decision concerning repeat biopsies is most difficult.

Cancer/tumour markers

0151

PROSTATE HEALTH INDEX (*PHI*) USING [-2]PROPSA IMPROVES DETECTION OF PROSTATE CANCER PREFERENTIALLY IDENTIFYING AGGRESSIVE CANCERS

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Background. The use of total prostate specific antigen (tPSA) for the early detection of prostate cancer (PCa) is being discussed due to limited clinical performance in low end serum concentrations. Beckman Coulter recently developed a "prostate health index" (*phi)* which combines tPSA, fPSA and [-2]proPSA. The clinical performance of *phi* for the detection of PCa was evaluated in a multicenter study.

Methods. A total of 905 patients with tPSA values between 1.6 – 8.0 ng/mL (WHO-calibrated), 448 with, 457 without PCa, underwent ≥10 core biopsies in four different sites. The serum concentrations of tPSA, fPSA, and [-2]proPSA were measured with Beckman Coulter immunoassays on Access2 or DxI800 instruments.

Results. Combined evaluation of the four cohorts using area under ROC curve (AUC) analysis showed that *phi* (AUC=0.74) provided significantly (p<0.001) better clinical performance relative to f/tPSA (AUC=0.62) or tPSA (AUC=0.55) in predicting PCa. Significantly higher median values of *phi* were observed for patients with Gleason score ≥ 7 (*phi*=55.0) as compared with patients with Gleason score < 7 (*phi*=45.4, p<0.001). The proportion of aggressive PCa (Gleason score ≥ 7) detected was increasing with the *phi* score. 68% of PCa detected in patients with a *phi* score > 80 had a Gleason score of ≥ 7.

Conclusions. The results of this multicenter study demonstrate that *phi* shows a superior clinical performance in detecting PCa in the tPSA range of 1.6 – 8.0 ng/mL as compared to tPSA or f/tPSA. *phi* tends to preferentially detect aggressive PCa and PCa missed are mainly Gleason < 7.

Cancer/tumour markers

0152

THE BECKMAN COULTER PROSTATE HEALTH INDEX (*PHI*) INCREASES THE SPECIFICITY OF DETECTION OF PROSTATE CANCER AND REDUCES THE NUMBER OF NEGATIVE BIOPSIES

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Background. Des études récentes indiquent que le [-2]proPSA, une isoforme du PSA, améliorent la détection du CaP.Recent studies indicate that [-2]proPSA, an isoform of PSA, improves the detection of prostate cancer (PCa).La société Beckman Coulter commercialise un test automatisé pour la détection du [-2]proPSA et a développé l'index phi («Prostate Health Index») qui permet la combinaison les résultats de PSA total, PSA libre et de [-2] proPSA.Beckman Coulter offers an automated assay called p2PSA for the detection of [-2]proPSA, and developed the Prostate Health Index (*phi*)‡ which combines the results of tPSA, free PSA and [-2]proPSA.Les performances cliniques du [-2]proPSA et de cet l'index phi ont été analysées du point de vue de la spécificité de détection du CaP et de la réduction associée du nombre de biopsies négatives.The clinical performance of the *phi* index was evaluated in a multicentric study. The improvement in specificity of detection of PCa of the pphi index was assessed in comparison to tPSA and %fPSA.

Methods. The 905 patients (448 with, 457 without PCa) from three different sites were recruited based on tPSA valuesAprès 6 mois de recrutement, 250 hommes (107 sans CaP et 143 avec CaP) avec un niveau de PSA total entre 1.8 – 8.0 ng/mL et des touchés rectaux (TR) non-suspects ont été inclus dans l'étude.The The between 1.8 - 8.0 ng/mL (WHO calibrated).Tous les CaP ont été confirmés par la réalisation de biopsies à 10 carottes ou plus.All PCa cases were confirmed by biopsies with 10 cores or more.Les concentrations sériques de PSA total, PSA libre et [-2] proPSA ont été mesurées sur un automate d'immunoanalyse Beckman Coulter DxI800.The serum total PSA, free PSA and [-2]proPSA were measured on a Beckman Coulter DxI800 automated immunoassay analyzer.

Results. The clinical specificity for tPSA, %fPSA and the phi index was determined at 90% sensitivity.A cette sensibilité, le %[-2]proPSA et l'index phi présentent une augmentation de spécificité d'un facteur 2 à 3 par rapport au PSA total et au PSA libre avec la meilleure spécificité pour le phi (26%).At this sensitivity, *phi* showed an increase of specificity by a factor 2 to 2.5 compared to tPSA and %fPSA, with the best specificity for *phi* (33%).L'augmentation de spécificité de l'index phi permet de limiter le nombre de biopsies négatives de 15 à 18% tout en détectant un nombre équivalent de CaP.The increased specificity of *phi* can reduce the number of negative biopsies by 16-17% while detecting a similar number of PCa.

Conclusions. Les résultats de cette évaluation démontre le bénéfice clinique %[-2]proPSA et de l'index phi en terme de spécificité.The results of this evaluation demonstrated the clinical benefit of the *phi* for the detection of PCa with an improved clinical specificity. The *phi* index can be an aid for prostate biopsy decisions, improve patient care and save healthcare resources by limiting the number of unnecessary biopsies.

Cancer/tumour markers

0153

MEASUREMENT OF STRESS MARKERS IN SALIVA FOR EVALUATION OF STRESS RELIEF OF PATIENTS WITH CANCER CHEMOTHERAPY, FOLLOWED BY AROMATHERAPY

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Background. Cancer chemotherapy can produce many side effects, such as anorexia, nausea and vomiting, and people with side effects may suffer inconvenience, discomfort and stress. Aromatherapy may produce positive benefits as a complementary treatment in reducing stress and anxiety. In this study, the levels of stress markers, i.e., amylase,chromogranin A and cortisol, were measured using patients'saliva with cancer chemotherapy, and then the effectiveness of aromatherapy stress relief in cancer chemotherapy has been estimated.
Methods. The stress load was given by the Kraepelin Test for healthy volunteers. Amylase in saliva was measured by a commercially available dry chemistry, and chromogranin A and cortisol in saliva were measured by EIA method. Citrus fruits aroma oil was used for aromatherapy.The visual analog scale (VAS) was used to measure the degree of stress.The levels of stress markers in saliva were compared to VAS for the patientswith cancer chemotherapy.
Results. The relationship between the levels of stress markers and the stress load under the presence of aroma oilfor healthy volunteers was not clear, whereas the relationship between the levels of stress markers and VAS for patients' saliva with aromatherapy was inclined to be significant.
Conclusions. The measurement of stress markers can be usefulfor the evaluation of the effectiveness of aromatherapy.
The clinical utility of aromatherapy for patients' stress relief was not well-defined at present.

0154

DIAGNOSTIC VALUE OF DETERMINATION OF SOLUBLE RECEPTOR OF TUMOR NECROSIS FACTOR P55 IN URINE AT THE BLADDER CANCER

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Background. The aim of the study was the search of noninvasive and relatively simple in methodical execution mark, heightened level of which is associated with bladder cancer (BC).
Methods. 60 patients with the primary BC and 20 healthy volunteers (control group) were analyzed for soluble receptor of tumor necrosis factor (sTNF-RI) p55 in voided and previously frozen urine samples. For this purpose ELISA (Enzyme-linked immunosorbent assay) kit and the automatic microplates immunoenzymatic analyzer were used.
Results. The results showed the significant increase of p55 in BC patients group in comparison with control group (p=0.0008). The median concentration of p55 in urine patients exceeded control group level practically in 5 times. There was established a significant increase of p55 concentration at patients with invasive (n=25) BC in comparison with noninvasive (n=35) BC (p=0.002). Correlation analysis detected the correlation dependence of tumor stage (R=0.46; p=0.0002) and degrees of tumor differentiation (R=0.37; p=0.0046) with urine p55 level. Significant differences of p55 concentration depending on quantity of tumors and the primary tumor size were not detected. However urine p55 level was significantly higher in group with solid tumors, than in group with papillary tumors (p=0.038).
Conclusions. The conducted research confirms the potential of the p55 determination as the additional test for laboratory diagnostics of BC and estimation of tumor differentiation degree of.

Cancer/tumour markers

0155

PERFORMANCE OF TURBIDIMETRIC IMMUNOASSAY MEASUREMENTS OF IG'κ AND IG'λ ON THE SPA PLUS

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Methods. Polyclonal antibodies recognising junctional epitopes between light chains (κ and λ) and their heavy chain partners (γ, α, μ; HLC antibodies) have been developed. Here we describe and evaluate the performance of this turbidimetric assay on the SPA PLUS.
Results. The measuring ranges and (sensitivities) for the assays were: IgGκ 1.9-40.0g/L (0.094g/L), IgGλ 0.92-29.5g/L (0.046g/L), IgAκ 0.18-11.2g/L (0.018g/L), IgAλ 0.16-10.4g/L (0.016g/L), IgMκ 0.2-5.0g/L (0.02g/L), and IgMλ 0.18-4.50g/L (0.018g/L). Intra (and inter) assay CV's measured at 3 different analyte concentrations (high, medium, low) were: IgGκ intra 1.2%, 1.2%, 2.3% (inter 2.0%, 2.6%, 4.1%); IgGλ 0.7%, 1.4%, 2.1% (2.5%, 2.6%, 4.7%); IgAκ 1.2%, 2.4%, 3.1% (2.7%, 2.3%, 4.1%); IgAλ 1.4%, 2.1%, 3.2% (2.3%, 2.4%, 2.2%); IgMκ 1.8%, 1.5%, 2.4% (1.8%, 1.3%, 3.3%); and IgMλ 1.7%, 2.0%, 2.0% (1.5%, 0.5%, 2.1%). Normal ranges established using blood donor sera (median: range) were IgGκ (6.75g/L; 3.84-12.07g/L), IgGλ (3.90g/L; 1.91-6.74g/L), IgGκ/IgGλ (1.74; 1.12-3.21), IgAκ (1.37g/L; 0.57-2.08g/L), IgAλ (1.25g/L; 0.44-2.04g/L), IgAκ/IgAλ (1.18; 0.78-1.94), IgMκ (0.63g/L; 0.19-1.63g/L), IgMλ (0.35g/L; 0.12-1.01g/L), IgMκ/IgMλ (1.81; 1.18-2.74). Interference was within ±10% when bilirubin (200mg/L), haemoglobin (4.56g/L) or Chyle (1540 FTU) were added to a pool of normal serum. Specificity was tested using a panel of monoclonal sera. In all cases the summated HLC values were within ±10% of the appropriate total immunoglobulin measurements. Additionally, when compared to the normal range, abnormal Ig'κ/Ig'λ ratios correctly identified clonality.
Conclusions. Measurement of Ig'κ and Ig'λ on the SPA PLUS provides a rapid and precise method of quantifying HLC specific immunoglobulins in serum.

0156

A HIGH CARBOHYDRATE ANTIGEN 19-9 (CA 19-9) SERUM LEVEL ACCOMPANIED BY A TRUE SPLENIC CYST

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Background. Cystic lesions of the spleen are an uncommon pathology. Cysts can be either primary (true) or secondary (pseudo cysts) This classification is based on the presence or absence of cellular lining on the cystic wall. The majority of primary cysts follow parasitic infection, especially that of Echinococcusgranulosus. They appear more often in the second and third decade of life and, in many cases, high serum values of tumour biomarkers are expressed. The objective is to report a case of elevated tumour biomarker serum values in the intercourse of an abdominal mass.
Methods. Case report: Female, 17 years old. During a gynaecological examination, a mass image in the abdomen was incidentally found. A CT scan was performed and showed a 9 cm-diameter cyst in the spleen. The lesion was excised by surgery. In addition, complementary tests were requested for tumour biomarkers both in serum and in the cystic fluid along with Echinococcus serology.
Results. The Echinococcal serology was negative. The tumour biomarkers analysis showed a relevant serum CA-19.9 of 134.5 UI/ml; whereas in cyst fluid, CA-19.9 of 2085 UI/ml, CA-125 of 5320 UI/ml and C.E.A of 361.9 UI/ml. The histopathology results concluded the presence of a Primary epithelial splenic cyst.
Conclusions. The presence of a cyst, accompanied by elevated tumour biomarkers, does not always suggest a bad prognosis. Ten months after surgical removal, the CA-19.9 control returned to a normal range (11.2 UI/ml).

Cancer/tumour markers

0157

COMPARATIVE ANALYSIS OF THE *CELLSEARCH* VS *IMAGESTREAM* SYSTEMS FOR CIRCULATING TUMOR CELLS COUNTING

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Background. Circulating tumor cell enumeration has important clinical value as prognostic and predictive factors in patients with solid cancers. The standardized *CellSearch* device *(Veridex)* is an immunomagnetic circulating tumor cell selection and enumeration system broadly used in clinical practice. *ImageStream (Amnis)* combines the strengths of flow cytometry and fluorescent microscopy in a single platform, offering an attractive potential to develop the application for circulating tumor cell counting.
Methods. The performance for circulating tumor cell enumeration of the *ImageStream* platform was compared to the *CellSearch* system. Previously to the cell analysis by *ImageStream,* tumor cell enrichment by immunomagnetic positive selection with anti-EPCAM was performed. Anti-CD45 and anti-CK markers were used to discriminate between tumor cells and leukocytes. Different number of PANC-1 tumor cells (1; 10; 100; and 1000 cells) was spiked into 7.5 ml of peripheral blood. Ratios of tumor cell recovered from each dilution were calculated for both Methods. Euclidean distances from the cell recovery curve to the ideal curve, were calculated. Wilcoxon rank test was applied to determine significant differences between both methodological approaches and with the perfect curve.
Results. Euclidean distances from ImageStream and CellSearch curves to the ideal one were 8.92 and 4.88 respectively. Significant differences were found between both methodological approaches and the perfect curve (p=0.012 for ImageStream and p=0.028 for CellSearch). On the contrary, no significant differences were found between both systems (p=0.40; Wilcoxon rank test).
Conclusion. The performance of ImageStream platform for circulating tumor cell enumeration is comparable to the CellSearch system.

0158

CEA, CA19.9, IL-6, AND SELECTED INFLAMMATORY PARAMETERS IN PATIENTS WITH COLORECTAL CANCER

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Background. Outcome in cancer patients is determined not only by the characteristics of the tumour but also by the host-response inflammatory factors. The prognostic value of the systemic inflammatory response markers such as cytokines and acute-phase proteins have been described in a variety of tumours.
Methods. The determinations of CEA, CA 19.9, IL-6, prealbumin, albumin, alpha-1 acid glycoprotein, haptoglobin and CRP were performed, before treatment, in a group of 60 patients with colorectal cancer and in the reference group. For each person, Glasgow Prognostic Score (GPS) was calculated.
Results. Significantly higher concentrations of CEA, CA 19.9, IL-6, CRP, AAG, HAP and significantly lower levels of prealbumin and albumin were found in colorectal cancer patients, in comparison to the reference group. Significantly higher AAG, HAP and significantly lower PRE and ALB concentrations were found in the group of patients with higher level of CEA (>5 μg/L). In the group with higher CEA level, percentage of patients with normal GPS was significantly lower than in the remaining patients (64.9% vs 30.3%). When analysing concentrations of the determined factors in respect to GPS (GPS 0 vs GPS 1 plus 2), significantly higher CEA, IL-6, AAG, HAP and significantly lower PRE concentrations were found in the group of patients with GPS of (1+2).
Conclusions. Significantly lower percentage of patients with normal GPS in the group with CEA concentration higher than 5.0 μg/L, which is generally accepted as an independent, unfavourable prognostic factor, seems to confirm the prognostic value of GPS in patients with colorectal cancer.

Cancer/tumour markers

0159

DATA BASED PREDICTION OF CLASSIFICATION OF TUMOR MARKERS AFP, CA-125, CA15-3, CEA, CYFRA, AND PSA

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Background. Data collected at AKH Linz (2005-2008) have been used as basis for identifying mathematical models for estimating tumor marker values. These models are created purely using empirical data and are used for estimating tumor marker values of patients using information about their standard blood parameters.
Methods. Data of 20,819 patients (stored in48,580 measurement samples) were preprocessed (linearly scaled and merged, missing values were replaced); tumor marker values are classified as 'normal' or 'elevated', this classification shall be modeled. The following machine learning techniques for training classifiers have beenused in this research project: Linear regression (LR), neural networks (ANN),the k-nearest-neighbor method (kNN), support vector machines (SVM),and genetic programming (GP). All these machine learningmethods have been implemented using the HeuristicLabframework (http://dev.heuristiclab.com).
Results. The following maximum test accuracies could be achieved: AFP 85.5% using ANN (baseline: 77.9%), CA125 68.1% using GP (baseline: 50.5%), CA 15-3 73.0% using GP (baseline: 64.2%),CEA 68.3% using GP (baseline: 56.3%),CYFRA 73.0% using ANN (baseline: 70.6%),PSA 67.5% using GP (baseline: 79.2%).
Conclusions. Tumor markers except PSA could be classified with accuracies significantly above the baseline accuracy. None of the methods used here produced the best results for all modelingtasks; in two cases (AFP and CYFRA) ANNs producedmodels that perform best on test data, in all remaining fourcases (CA-125, CA15-3, CEA, and PSA) extended geneticprogramming has produced best Results.

0160

PREDICTION OF LUNG CANCER ON THE BASIS OF BLOOD DATA

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Background. Data based prediction is a discipline in the field of machine learning and computational intelligence. Due to the increasing computational power even complex correlations in large datasets can be detected and described.
Methods. In this contribution genetic programming is used for generating the cancer prediction models. Genetic programming is a computational intelligence method, which is capable to identify complex nonlinear relations without having to take pre-assumptions about the structure and the used variables. Furthermore, the resulting models are human interpretable mathematical models which
Results. The results steam from a joint research cooperation of a research group specialized in the field of meta-heuristic optimization and machine learning with the central blood laboratory of the central hospital of Linz. General patient data like gender or age, standard blood parameters (like ALT, AST,…) and tumor marker values (like CEA, NSE, SCC) have been kinked with the patients ICD codes with a cancer diagnosis using the patients id as unique identifier. Several thousand benchmark date with positive and negative diagnosis have been used for data based modelling. The accuracy resulting of a 5-fold cross validation of the genetic programming models is about 90%. The prediction models generated by genetic programming are rather complicated mathematical formulas also including nonlinear terms and conditionals.
Conclusions. We conclude that general blood and tumor marker parameters can be used for data based generation of cancer prediction models whereby a sufficient number of training data is essential for the proposed approach.

Cancer/tumour markers

0161

ALPHA-FETOPROTEIN AND DES-GAMMA-CARBOXY PROTHROMBIN ASSERUM MARKERS FOR HEPATOCELLULAR CARCINOMA

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Background. *Lens culinaris* agglutinin–reactive fraction of alpha-fetoprotein (AFP-L3), des-gamma-carboxy prothrombin (DCP) and osteopontin (OPN) levels have been suggested as novel biomarkers for hepatocellular carcinoma (HCC). In the present study we evaluated the diagnostic accuracy of these tumor markers prospectively in a cohort of HCC patients with various tumor stages and cirrhotic controls and compared it with that of alpha-fetoprotein (AFP).
Methods. Consecutive treatment-naïve patients with HCC enrolled between 2007 and 2010 (n=58) and age- and sex-matched cirrhotic controls (n=25) were investigated. AFP, AFP-L3 and DCP were measured in serum samples using a new micro-total analysis system (Wako Chemicals GmbH, Neuss, Germany). OPN plasma levels were determined by ELISA (R&D Systems, Biomedica Medizinprodukte, Vienna, Austria). Tumor stage was classified by the Barcelona Clinic Liver Cancer (BCLC) or the UICC staging system. Diagnostic accuracy of tumor markers was assessed by ROC analysis and group means were compared by ANOVA.
Results. Compared to cirrhotic controls, AFP, AFP-L3 and DCP were elevated in both early and advanced HCC whereas OPN was elevated only in advanced HCC. ROC analysis revealed superior diagnostic accuracy of AFP (AUROC 0.92) and DCP (AUROC 0.90) as compared to AFP-L3 (AUROC 0.73) and OPN (AUROC 0.75) for diagnosis of HCC.
Conclusions. AFP and DCP showed superior diagnostic accuracy as compared to AFP-L3 and OPN for the distinction between HCC and cirrhosis.

0162

DEVELOPMENT OF NEW METHODS FOR DETECTING CIRCULATING NUCLEIC ACIDS AS CANCER BIOMARKERS IN LUNG CANCER PATIENTS

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Background. Circulating cell-free nucleic acids are non-invasive diagnostic tools for cancer detection. Overexpression of hnRNP B1has been found in the early stage of lung cancer, and *hnRNP B1* mRNA was detected in patients' plasma. T790M EGFR mutation has been recognized as a gatekeeper mutation for resistance to tyrosine kinase inhibitors (TKI). We applied novel methods for detection of plasma RNA by transcription-reverse transcription concerted reaction(TRC) and for T790M EGFR mutation by mutation-biased PCR and quenching probe (MBP-QP) method in plasma DNA.
Methods. Plasma mRNA was obtained from 97 healthy volunteers and from 44 lung cancer patients, and assessed by real-time RT-PCR or TRC for *hnRNP B1* mRNA. Sixty-seven plasma DNAs were obtained from 49 lung adenocarcinoma patients and 30 healthy volunteers and analyzed by MBP-QP Methods.
Results. We detected *hnRNP B1* mRNA in 39.1% (9/23) of lung cancer patients by TRC methods with levels ranging from 1.9 to 19045.5 copies/100ng RNA, and in 5.2% (5/97) of healthy volunteers. The T790M mutation was detected in plasma DNA from ten of nineteen patients (53%) who acquired resistance, but not in non-responders, patients responding to treatment, or those not treated with EGFR-TKI.
Conclusion. These results indicate that plasma *hnRNP B1* mRNA is a useful non-invasive markers for detection of lung cancer. In addition, the MBP-QP method is a non-invasive monitoring system for detecting T790M in plasma samples.

Cancer/tumour markers

0163

IS THE METABOLIC SYNDROME A RISK FACTOR FOR PROSTATE CANCER?

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Background. Metabolic syndrome (MetS) is one of the four major obesity-related risk factors for cardiovascular and other diseases. Prostate cancer (PCa) is one of the most common type of cancer and cause of death in men in the USA and in Europe. The aim of the authors' study is to establish the correlation between the MetS and the PCa. Besides the authors determined a new reference range of prostate-specific antigen (PSA) in the sera and the urine.
Methods. Between 1997 and 2010 we tested 683 patients with PCa (age: 48-91 years) and 13,523 controls (age: 30-98 years). The authors established the reference range based on age and showed the correlation of the level of serum and urine PSA with Roche reagents (Roche Ltd, Mannheim, FRG). Criteria of the MetS of WHO, the International Diabetes Federation and the National Cholesterol Education Programs Third Adult Treatment Panel were used.
Results. Significant correlation was found between the PSA levels in the sera and the age, the overweight/obesity and the PCa. According to the correlation between the different criteria of the MetS, distinct differences were found in the three versions and PCa.
Conclusions. The MetS based on any criteria increases the risk of PCa considerably. PSA, the basic diagnostical method of evaluation of the PCa, is largely influenced by the age of the patients. The MetS may mean a substantial public health problem for the PCa. Incidence of MetS and PCa increases rapidly in the elderly men.

0164

A NEW WAY IN THE EARLY DETECTION OF PROSTATE CANCER: THE [-2]PRO PROSTATE SPECIFIC ANTIGEN

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Background. Due to the limited specificity of prostate specific antigen (PSA), the prostate cancer (PCa) diagnosis searches for new biomearkers, especially at lower PSA levels (2.0-10.0 ng/ml). The aim of this study was to analise an isoform of proenzyme PSA called [-2]pro PSA (p2PSA) in serum and to determine the diagnostic accuracy of this subform for early diagnosis of PCa in comparison with total PSA (tPSA), free PSA (fPSA), the ratio of fPSA to tPSA (%fPSA) and ratio p2PSA to fPSA (%p2PSA).
Material. Sera from 163 subjects with suspected PCa (n=83) and controlls (n=80) were studied respectively. PCa diagnosed histologically using Gleason scores from prostate biopsies. Serum samples were collected before biopsies. tPSA, fPSA and p2PSA concentrations were analyzed with Beckman Coulter ACCESS 2 immunoassay system, chemiluminescent automated Hybritech Tandem assays (Beckman Coulter, Inc., San Diego, CA, USA). The Beackman Coulter Prostate Health Index (phi) was calculated mathematically. Diagnostic usefulness was assassed using ROC analysis, and areas under the curve determined in addition to clinical sensitivity and specificity.
Results. Interassay precision was from 1,2% to 4,8% for p2PSA. Comparing the tPSA, fPSA, p2PSA, the isoform of fPSA is increased significantly in men with PCa and with Gleason score ≥7, if the serum tPSA levels are between 2.0-10.0 ng/ml. The phi and the %p2PSA are significantly elevated in PCa.
Conclusions. The study demonstrate that %p2PSA is more reasonable in the diagnosis of PCa, and it is a better indicator of an aggressive pathological stage and grade.

Cancer/tumour markers

0165

THE ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) IN THE CERVICAL CANCER

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Background. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) catalyze the reactionwith a wide spectrum of substrates and play a significant role in metabolism of many biological substances (retinol, toxic aldehydes - products of lipid peroxidation).In this study we compared the metabolism in cervical cancer cells and healthy cervical tissue by measurement ADH isoenzymes and ALDH activities in these tissues.

Methods. The study material consisted of canc erous cervical tissues obtained from 40 patients. Class I and II ADH isoenzymes and ALDH were measured by fluorometric method with specific substrates. For measurement of class III, IV and total ADH activity we employed photometric method.

Results. The activity of the class I ADH isoenzyme was significantly higher in cervical cancer tissue (0.194± 0.160 nmol/min/mg protein) as compared to healthy tissue (0.138± 0.098 nmol/min/mg protein). The other classes of ADH tested isoenzymes did not show significant differences between the activity of cancer and healthy cervical tissues. The activity of total ADH was significantly higher in cervical cancer vs. healthy tissue (0.919± 0.385 vs. 0.800± 0.345 nmol/min/mg protein). Class I of ADH and total ADH activity were significantly higher in every stage (from I to III) of cancer as compared to the control. There are no significant differences between adenocarcinoma and planoepitheliale cancer.

Conclusions. The increased activity of total ADH in cervical cancer (especially class I) may be a cause of disorders in metabolism of biologically important substances and therefore might be a factor of metabolic changes in low mature cancer cells.

0166

THE PLASMA LEVELS AND DIAGNOSTIC UTILITY OF SELECTED HEMATOPOIETIC GROWTH FACTORS (HGFS) IN PATIENTS WITH OVARIAN CANCER

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Background. HGFs play a role in the pathogenesis of cancer disease. In this study, we investigated the plasma levels of selected HGFs (SCF, stem cell factor; G- and M-CSF, granulocyte- and macrophage colony stimulating factor) in comparison to the classical tumor marker (CA125) in ovarian cancer patients and in relation to the control groups:benign ovarian tumor patients and the healthy subjects.

Methods. Plasma levels of HGFswere determined using immunoenzyme assay (ELISA), while CA125 concentrations by chemiluminescent microparticle immunoassay (CMIA).

Results. Plasma levels of M-CSF and CA125 were significantly higher inovarian cancer patients as compared to the healthy control or benign ovarian tumor patients. The HGFs and CA 125 diagnostic specificities received high values. The diagnostic sensitivity, the positive and the negative predictive values were higher for M-CSF than for CA 125 in ovarian cancer group. The combined use of tested parameters resulted in the increase of the sensitivity range. The higher area under the ROC curve (AUC) was observed for M-CSF and was slightly lower than the AUC of CA 125.

Conclusions. These results suggest a potential usefulness of M-CSF in diagnostic of ovarian cancer, especially in combined use with CA125.

Cancer/tumour markers

0167

MACROPHAGE – COLONY STIMULATING FACTOR (M-CSF) IN THE DIAGNOSIS OF PATIENTS WITH ADENOCARCINOMA OF ESOPHAGUS

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Background. Macrophage – colony stimulating factor (M-CSF) is a hematopoietic growth factor (HGF), that may be produced in many malignancies, including esophageal cancer. The aim of the study was to compare clinical significance of serum M-CSF with classic tumor marker – carcinoembryonic antigen (CEA) in the diagnosis of patients with esophageal adenocarcinoma.

Methods. The study included 26 patients with adenocarcinoma of esophagus and 30 healthy subjects. The serum concentrations of M-CSF and carcinoembryonic antigen (CEA) were determined using immunoenzyme assays. The diagnostic criteria, such as diagnostic sensitivity and specificity, predictive value for positive (PV_{+ve}) and negative (PV_{-ve}) results, accuracy as well as the areas under the ROC curves (AUC) of both proteins tested were calculated.

Results. The levels of M-CSF in esophageal adenocarcinoma patients were significantly higher than in healthy subjects. The percentage of elevated results for M-CSF (73%) was higher than for CEA (15%), and increased in combined use of M-CSF with CEA (81%), similarly as predictive value for negative results and accuracy. The M-CSF area under ROC curve (0.73) was larger than AUC for CEA (0.52).

Conclusions. Our findings indicate higher usefulness of M-CSF than classic tumor marker (CEA) in the diagnosis of patients with esophageal adenocarcinoma.

0168

MONOCLONAL GAMMOPATHY SCREENING ARRAY IMMUNOASSAY, PROTOTYPE PERFORMANCE

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Background. Studies using specific antibodies for heavy chain/light chain (HLC) pairs have indicated that HLC ratios (IgA Kappa/IgA Lambda, IgM Kappa/IgM Lambda, IgG Kappa/IgG Lambda) provide a sensitive indication of monoclonal immunoglobulin production. Incorporating these antibodies with free light chain (FLC) antibodies in a bead array immunoassay, has allowed the development of a sensitive and reproducible screening assay for monoclonal gammopathy, with the additional measurement of cystatin-C and beta-2-microglobulin.

Methods. A monoclonal gammopathy screening assay based on a bead array system from Dynex Technologies Inc. incorporating the Binding Site Group Ltd. specific antibodies has been developed. Performance characteristics have been demonstrated in this study.

Results. The individual within-assay mean reproducibility data derived from 16 replicates of each of 3 normal blood donor sera (NBDS) was 4.1%, 3.9%, 6.2% 7.6%, 6.1%, 6.3%, 6.5%, 9.1%, 10.8% and 7.6% for the, IgA Kappa, IgA Lambda, IgM Kappa, IgM Lambda, IgG Kappa, IgG Lambda, Kappa FLC and Lambda FLC, beta-2-microglobulin and cystatin-C respectively.

The assay was tested using 96 sera from patients with known monoclonal gammopathy and 48 NBDS. The assay correctly confirmed normal ratios for all 48/48 normal sera and abnormal ratios in 95/96 (99%) of the monoclonal gammopathy samples, the exception was a patient with polyclonal hypergammaglobulinemia, but no indication of monoclonality by IFE.

Conclusions. This study concludes, that this array assay offers a suitable alternative for the screening and confirmation of monoclonal gammopathy. This could eliminate the need for gel electrophoresis, which in particular cases is subjective and requires experienced interpretation.

Cancer/tumour markers

0169

MONOTOTAL - PROGNOSIS AND THERAPY CONTROL IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Background. The aim of the study was to confirm the use of MonoTotal (MT) test for prognosis estimation and therapy effect control in patients with non-small cell lung cancer (NSCLC).
Methods. MonoTotal assay (IDL, Sweden) was measured using IRMA technology in the serum of patients with NSCLC and a control group of 80 people of corresponding age, with no history or evidence of cancer or lung disease.The results were compared with other soluble cytokeratin fragments:TPA (Dia Sorin, Italy), TPS (IDL, Sweden) and CYFRA 21.1 (Brahms, Germany) and CEA (Beckman, U.S.A.)
Results. We found statistically significant differences between the control group and patients with NSCLC in all cytokeratins:TPA ($p<0.0001$), TPS ($p<0.0002$), CYFRA 21.1 ($p<0.0001$), and MonoTotal ($p<0.0001$). Sensitivity during the preoperative period, at 95% specificity for cytokeratins, was as follows:MT 71%, TPA 53%, CYFRA 21.1 51% and TPS 25%.Serum levels of MonoTotal correlated in the group of patients treated with chemotherapy with therapy effect, independently with histological type, TPA, Cyfra particularly in patients with adenocarcinoma, less significant relationship was found for TPS.
Conclusions. MonoTotal is a more sensitive tumor marker for NSCLC than the cytokeratin markers currently used in routine clinical practice, especially in patients with squamous cell carcinoma.MonoTotal is also important for prognosis (disease free interval and overall survival) and it is also important for therapy effect control as well.

0170

DETERMINATION OF BIOLOGICAL VARIATION OF S100 IN DISEASE-FREE PATIENTS WITH MALIGNANT MELANOMA

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Background. The knowledge of the biological variation (BV) is important for determining analytical goals and for establishing the magnitude of change between two consecutive measurements which indicate a change in patient's health status. The aim of this work is to determine the BV for S100 in patients diagnosed of malignant melanoma with no evidence of recurrence of disease.
Methods. We have estimated biological variation from a mean of four consecutive measurements in 32 patients without evidence of recurrence of disease diagnosed of malignant melanoma, three months after tumor resection or four months after to finish adjuvant treatment. The mean sampling interval has been 3 months. We have determined S100 by an electrochemoluminiscentassay (Elecsys 2010).
Results. the mean concentration of S100 was $0.0557\mu g/L$. Between-run analytical variation (AV) was 3.5% at 0.0353 $\mu g/L$. Biological variation obtained for S100 was 14.2%. The analytical goals defined as a half of biological variation was 7.1%, and the reference change value defined as $2.77*((BV)^2+(AV)^2)^{1/2}$ was 39.3%.
Conclusions. The estimation of BV allows us to calculate analytical goals and reference change values, necessary tools for the correct interpretation of serial measurements in the follow up of patients.

Cancer/tumour markers

0171

IS HDL-CHOLESTEROL LEVEL A NEW PREDICTOR OF RISK CANCER?

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Background. Circulating total cholesterol has been inversely associated with cancer risk, but recently has been reported a significant inverse association between high-density lipoprotein cholesterol (HDLc) and the risk of incident cancer that was independent of low-density lipoprotein cholesterol (LDLc), age or sex. The aim was to evaluate the behavior of HDLc in healthy population and in a population with high probability of developing early cancer.
Methods. We examined retrospectively 315 patients (44.13% men and 55.87% women), age 62.7±14.0. We evaluated the relationship between HDLc and carcinoembryonic antigen (CEA) as the main non-specifically tumor marker implicated in several cancers. For that, two groups were established, group 1 (G1): CEA between 5-20 ng/ml and any value of HDLc on patients with or without cancer, and group 2 (G2): CEA>20ng/ml and HDL_c<40 mg/dl previously diagnosed with cancer. We studied the levels of HDLc in both groups.
Results. G1 showed highest levels of HDLc (51(39-62) mg/dl) and CEA (6.9(5.7-8.9) ng/ml) comparing with G2: HDLc (23(16.5-27.5) mg/dl) and the highest CEA (125 (33.4-286.6) ng/ml), both of them p<0.001. Within G1 we reanalyzed the values separating patients with cancer: HDLc (47(34.3-58.0) mg/dl) or without cancer: HDLc (53(42-65) mg/dl); p<0.001. HDLc and CEA showed signification correlation between them (r=-0.22;p<0.001).
Conclusions. Our findings confirms the inverse association of HDLc between healthy population and diagnosed with advanced cancer. Moreover within G1, we found higher HDLc levels in healthy individuals compared with those who has possible incipient cancer, demonstrating the possible negative predictive value of HDLc.

0172

TUMOUR MARKER HE4 IN PATIENTS WITH OVARIAN CANCER

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Background. Human epididymis protein (HE4) has been started to be used alongside with CA125 as a marker for the diagnosis of ovarian cancer. The aim of the study is to analyze the utility of HE4 for this purposes.
Methods. During the period September - December 2010 we analyzed concentration of serum tumour markers HE4 and CA125 in samples of total 244 women (101 premenopausal, 144 postmenopausal), including women with diagnosis of ovarian cancer, postoperative ovarian cancer, benign pelvic mass, benign gynecologic disease and healthy women. Markers were measured by chemiluminescent microparticle immunoassay (CMIA) on Architect ci8200 (Abbott Diagnostics) according to the manufacturer's instructions.
Results. The studied tumour markers were significantly higher in serum of patients with ovarian cancer (HE4 mean was 322±387 pM, CA125 mean 274±423 pM) as compared to healthy subjects and benign gynecologic disease, postoperative ovarian cancer cases or benign pelvic mass cases (p<0.0001 and p<0.0003 accordingly). HE4 had sensitivity as a single marker in detecting ovarian malignancy 75% (95% CI 0.41-0.93), specificity 94% (95% CI 0.9-0.96), CA125 had sensitivity 87.5% (95% CI 0.53-0.98) and specificity 70% (95%CI 0.64-0.76).
Conclusions. As a single marker, HE4 had the highest specificity for detecting ovarian cancer. On the contrary, CA125 sensitivity was higher than that of HE4. Combination of HE4 and CA125 may increase the accuracy of ovarian cancer diagnosis and provide valuable information allowing differenting ovarian cancer from other tumours and ovarian cysts.

Cancer/tumour markers

0173

BIOCHEMICAL INDICATORS OF SYSTEMIC INFLAMMATORY RESPONSE IN PREDICTING SCLC PATIENTS SURVIVAL

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Background. Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, proliferation, survival, invasion and metastasis. Apart from performance status (PS) and weight loss, changes of platelet and lymphocyte counts and albumin, prealbumin, CRP or alfa-1 acid glycoprotein levels as well as calculated on their basis Nutritional Risk Index (NRI), Prognostic Inflammatory and Nutritional Index (PINI), Cancer Serum Index (CSI), Platelet Lymphocyte Ratio (PLR) and Glasgow Prognostic Score (GPS) were also analyzed.
Methods. The study of albumin, prealbumin, C-reactive protein, alfa-1 acid glycoprotein levels, and platelet and lymphocyte counts were performed before treatment in 164 of SCLC patients. Values of NRI, PINI, CSI, GPS, PLR indicators of nutrition and inflammation status were calculated for each patients.
Results. In the SCLC group under study, weight loss over 10%, PS below 70 in Karnofsky scale, NRI below 100.1, and pathological results of PLR, GPS, PINI, CSI: were found in 25%, 37%, 50.6%, 28% 53.7%, 58.5% 67.1%. of patients respectively. Univariate analysis revealed that apart from extent of disease, poorer PS, male sex, NRI, PLR PINI, CSI as well as GPS were significantly associated with poorer prognosis. Relative risk of death of SCLC patients with extensive disease was 4.4 times higher than in those with limited disease. For PINI > 1.5, PLR > 195, CSI > 6.5, GPS > 0 those RRs were as follows: 1.86, 1.77, 1.55 and 1.48.
Conclusions. In prediction of prognosis of SCLC patients, the results of calculated systemic inflammatory response indicators seem to have similar values.

0174

KLK3 AND STEROID 5-ALPHA REDUCTASE TYPE II (SRD5A2) GENE POLYMORPHISMS MIGHT AFFECT CLINICAL RELIABILITY OF SERUM PSA MEASUREMENT

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Background. Serum levels of fPSA, tPSA and of their ratio are used for prostate cancer (PC) screening. PSA synthesis depends on pathology, but also on *KLK3* gene polymorphisms and on androgen transcriptional effects. The V89 isoform of Steroid 5-α reductase type II (*SRD5A2*) by enhancing diihydrotestosterone, might also enhance PSA.
Methods. we ascertained whether -5429T>G, -5412T>C, -4643A>G *KLK3* SNPs and their haplotypes, and V89L polymorphisms of *SRD5A2* gene might affect the serum levels of fPSA, tPSA and f/tPSA. We studied 711 men consecutively subjected to prostate biopsy: 291 PC, 351 benign prostatic hyperplasia (BPH), 69 controls.
Results. six *KLK3* haplotypes were derived by Arlequin software; nine genotypes were inferred, with an overall frequency of: TTA/TTA (62,03%), GCG/TTA (28.56%), GCA/TTA (3.37%), GCG/GCG (2.95%), TTA/TTG (1.55%), GCA/GCG (0.56%), GCG/TTG (0.42%), GTA/TTA (0.42%), TCA/TTA (0.14%). Among BPH patients, not PC or controls, fPSA (p=0.08) and tPSA (p<0.05) were decreased in TTA/TTA with respect to GCG/GCG and GCG/TTA (chi-square for trend: p=0.02 and p=0.01). Among PC, not BPH or controls, fPSA (p=0.03) and tPSA (p=0.01) were increased in SRD5A2 V/V homozygotes (chi-square for trend: p=0.01 and p=0.005). The NPV of tPSA <2.5 ug/L was higher (85%) among TTA/TTA than among GCG/TTA (67%). The PPV of tPSA >2.5 <= 10 ug/L and f/tPSA <=10% was higher among GCG/TTA (75%) than among TTA/TTA (64%).
Conclusions. when evaluating serum fPSA and tPSA to obtain a differential diagnosis between PC and BPH, individual genetic background might affect serum levels and reduce diagnostic reliability of the test result.

Cancer/tumour markers

0175

HE4AS SPECIFIC MARKER FOR OVARIAN CANCER: COMPARISON WITH CA125 IN DIFFERENTIAL DIAGNOSIS OF OVARIAN CANCER AND GYNECOLOGICAL PELVIC BENIGN DISEASES

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Background. Human epididymis protein 4(HE4), a recently found serum biomarker with improved sensitivity and specificity over cancer antigen 125(CA125) in detecting ovarian cancer, is postulated to play a discriminating role in differential diagnosis between benign pelvic diseases and malignant ovarian tumors in women with pelvic mass.
Methods. Serum were collected from 31 patients with ovarian cancer, 44 patients with adenomyosis, 32 patients with ovarian cysts, 30 patients with endometriosis and 65 healthy volunteers. Serum HE4 concentration were measured by Enzyme-linked Immunosorbent Assay (ELISA); Serum CA125 concentration were measured by Chemiluminesent Microparticle Immunoassay.
Results. The concentration of HE4 in ovarian cancer were markedly higher than those in gynecological benign groups and healthy controls (312.4±513.8) (P<0.01). In addition, the concentration of HE4in gynecological pelvic benign diseases with high CA125 have no significant difference compared with the healthy controls (P>0.05).
Conclusions. HE4 is a novel promising tumor marker. It can differentiate malignant ovarian tumors from benign disease. Asthe further study, HE4 may eventually be proven as an important marker in the diagnosis of ovarian cancer, and is better than CA125 in distinguishing patients with malignant ovarian disease from those with benign ovarian disease at high specificity.

Cardiovascular disease

0176

IMPLEMENTATION OF HS-TROPONIN ASSAYS IN NORWAY – ARE NATIONAL RECOMMENDATIONS FOLLOWED?

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Background. With the introduction of high sensitive troponin assays (hs-Tn), a joint letter of recommended diagnostic cut-off values for acute myocardial infarction (AMI) was distributed from National Societies of Cardiology and Clinical Biochemistry to all 53 hospitals treating AMI in Norway.
Methods. After 22 months a follow-up questionnaire was distributed to all 53 hospitals treating AMI and another questionnaire were sent to all 63 laboratories participating in the Norwegian EQA on troponins. The questionnaires evaluated implementation and clinical experience with hs-Tn.
Results. The response rate was 66% (medical departments) and 98% (laboratories). 64% analysed TnT (95% hs-TnT) and 92% of these used the nationally recommended cut-off (30 ng/L). About half of TnI laboratories followed the recommendations to use the 99 percentile as cut-off for AMI. Most TnT laboratories controlled CVa at a concentration similar to the cut-off for AMI, and values below 10% were usually obtained. For TnI methods, CVas were satisfactory, but at higher concentrations. Clinicians anticipated that the analytical variation (CVa) for the local assay at cut-off concentration was 12% (both TnT and TnI). About half had knowledge about the 99 percentile for the local Tn method. 70% of clinicians had experienced that more patients with AMI were diagnosed correctly (increased sensitivity), but also that more patients without ischemic heart disease incorrectly were diagnosed (decreased specificity), after implementation of hs-Tn.
Conclusions. National recommendations for diagnostic cut-offs and analytical CV were to a large extent followed in Norway. Clinicians experienced increased sensitivity and decreased specificity after implementation of hs-Tn.

0177

ENOS (G894T) GENE POLYMORPHISM IN A RANDOM SAMPLE OF EGYPTIAN POPULATION: LACK OF CORRELATION TO SERUM NO LEVELS

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Background. The endothelial nitric oxide synthase (eNOS) is responsible for the production of nitric oxide (NO) in endothelial cells. The produced NO is involved in signalling for vasorelaxation, platelet aggregation, proliferation of vascular smooth muscle cells among other mechanisms of cardiovascular homeostasis. eNOS gene has been extensively screened for variation. The only "coding" polymorphism that was identified is the G894T (guanine/thymine substitution) at exon 7 that leads to glutamate to aspartate substitution at position 298 in the mature protein. The aim of this study was to detect eNOS gene polymorphisms in Egyptian population to be used as a reference for future studies and to explore functional correlations of eNOS genotypes with serum NO.
Methods. Random unrelated 101 healthy subjects were recruited for the study from the healthy volunteers attending children cancer hospital, Cairo, Egypt. eNOS genotypes were determined by polymerase chain reaction – restriction fragment length polymorphism(PCR-RFLP). Serum NO was determined spectrophotometrically by Griess method.
Results. Genotype distribution of eNOS Glu298Asp polymorphism in random Egyptian population sample was 58.4% GG (wild type), 33.7% GT and 7.9% TT genotypes while the allele frequencies were 75.2 % and 24.8 % for the G and T alleles, respectively. No significant association was found between serum NO and specific eNOS genotype.
Conclusions. The present study demonstrated the predominance of the homozygous genotype GG over the heterozygous GT and homozygous TT. It also showed the lack of association between eNOS genotypes and serum levels of NO in this Egyptian population sample.

Cardiovascular disease

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EVALUATION OF THE RELATIONSHIP BETWEEN SERUM NEOPTERIN, CYSTATIN C AND CORONARY ARTERY DISEASE

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Background. Neopterin is produced by activated macrophages as a response to inflamation. Cystatin C is a sensitive marker of kidney function.The purpose of this study is to investigate the relationship of serum cystatin C and neopterin with CAD and cardiovascular risk factors in the patients with CAD established by coronary angiography. The results of 125 individuals (75 patient, 50 control) who attended to I. Cardiology department for angiography were included in the study. The results of these patients were taken from the labarotory information system. Gensini score system was used for luminal stenosis.
Methods. Turbidimetry for CRP and cystatin C and ELISA for neopterin
Results. Serum cystatin C concentrations was not statistically significant in patient group but serum neopterin concentrations were statistically higher in CAD group (respectively p=0.18, p=0.0001). The relationship between serum cystatin C, neopterin and the other biochemical parameters was evaluated by Pearson correlation analysis. There was a positive correlation between BUN, creatinine and cystatin C (p=0.0001). There was a positive correlation between TG and neopterin (p=0.017) and an inverse correlation with HDL and neopterin (p=0.05) and no correlation between glucose, total cholesterol, BUN, LDL-C,creatinine and CRP.
Conclusıons. Our results showed a significant association between cystatin C, neopterin and CAD.

0179

PERIOPERATIVE CORTISOL RELEASE IN PATIENTS WHO UNDERWENT CAROTID ENDAARTEREKTOMY. THE IMPACT OF ANESTHETIC MODALITY

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Background. Surgical trauma cause a necessary biological response known as hyper metabolic stress response. Our aim was to determinate the degree of surgical trauma caused by carotid endaarterektomy (CEA) by evaluating the levels of plasma cortisol, and to define differences related to the use of lokoregional (LA) or general anesthesia (GA).
Methods. We prospectively studied twenty consecutive CEA performed patients admitted at Clinic for Vascular Surgery. Patients were stratified for demographics and risk factors and operated under LA or GA depending on both the surgeon preference and patients compliance. Marker of stress response, serum cortisol, was measured preoperatively (before premedication), intraoperatively (before and after carotid artery cross clamping (CACC), and postoperatively (after admitting in postoperative care unit, 6 and 24 hours after surgery). Statistics were run by means of nonparametric ANOVA tests.
Results. CEA was preformed under GA in eight patients (40%), and under LA in twelve patients (60%). The two groups were comparable in terms of demographics and risk factors. Intraoperatively serum cortisol levels were higher (p<0.01) in the LA group for 29.5% in comparison to the GA group. Carotid artery cross clamping increased intraoperative cortisol levels in both GA and LA groups by 22.0% and 92.4% respectively, compared to the level before anesthesia application.
Conclusions. The stress response to CEA regardless of the type of anesthesia was abolished within 24 hours after operation. Intraoperative stress response, namely hypercortisolemia directly correlated with sub clinical and clinical cerebral hypo perfusion during CACC.

Cardiovascular disease

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VASCULAR ENDOTHELIAL GROWTH FACTOR PROTECTS THE VASCULAR ENDOTHELIUM FROM APOPTOSIS BY INHIBITING PRO-APOPTOTIC ENDONUCLEASES

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Background. Vascular endothelial growth factor (VEGF) is the most potent anti-apoptotic effector for endothelial cells (EC). Apoptosis is executed by endonucleases like caspase-activated deoxyribonuclease (CAD), but the influence of VEGF on CAD and inhibitor of caspase-activated deoxyribonuclease (ICAD) has not been investigated previously. Therefore, the effects of VEGF on CAD and ICAD were investigated in EC, an organ culture model and human arteriosclerosis.
Methods. EC (HUVEC, EC-lines) were incubated with VEGF-A(165) 100pg/ml-1µg/ml for 24h-6d in the presence or absence of ICAD-siRNA 10nM. Apoptosis was induced by cRGDfV 5µg/ml and measured by annexin-V flow cytometry, caspase-3 and endonuclease activity. ICAD was analyzed by Western blot and real-time PCR. VEGF receptors VEGFR-1, -2 and neuropilin-1 were investigated by immunofluorescence microscopy and incubation with specific inhibitors. Human arteriosclerotic versus normal arteries and a vascular organ culture model were analyzed by immunohistochemistry for expression of ICAD, CAD, VEGF and VEGF-receptors.
Results. Incubation of EC with VEGF reduced the sensitivity to apoptosis and increased ICAD expression leading to reduced CAD activity. The VEGF effect was demonstrated to be ICAD specific by mRNA-knockdown experiments. VEGF signaling involved VEGFR-2 and neuropilin-1. The relevance of *in-vitro* results was demonstrated in an organ culture model and in human arteriosclerosis.
Conclusions. VEGF exerts part of its anti-apoptotic effect by regulation of the endonuclease inhibitor ICAD. VEGF influences endothelial survival by interfering with the terminal stage of apoptosis execution through the inhibition of endonuclease activity. This offers a new potent target for the modulation of VEGF-driven vascular processes such as arteriosclerosis.

0181

ORTHO CLINICAL DIAGNOSTICS VITROS® WIDE RANGE CRP (WRCRP) ASSAY

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Background. C-reactive protein is an acute-phase reactant that can be used in the detection and evaluation of infection, tissue injury, and inflammatory disorders. The current MicroSlide VITROS Chemistry Products CRP assay has an upper reporting range limit of 90 mg/L. We have developed the VITROS Chemistry Products wrCRP Reagent that can be used to detect C-reactive protein levels up to 300 mg/L without dilution.
Methods. The performance of the VITROS Chemistry Products wrCRP Assay was assessed on the VITROS 5600 Integrated System using CLSI EP6 for linearity and 10 day CLSI EP5 for precision. Method comparison studies followed CLSI EP9 and used both the VITROS Chemistry Products MicroSlide assay and the Siemens Behring CRP assay run on the Siemens Behring ProSpec II as comparative Methods.
Results. The VITROS Chemistry Products wrCRP assay produced acceptable linearity between 1mg/L and 300 mg/L. The assay is precise across its reportable range.

Conc. (mg/L)	1.7	10.1	17.1	73.3	230
Within run %CV	2.5	2.6	2.1	2.0	3.2
Within lab %CV	4.2	3.2	2.4	2.2	3.5

Method comparison studies demonstrate acceptable agreement with both the Behring ProSpec CRP Assay and the VITROS Chemistry Products CRP Assay (MicroSlide CRP):
VITROS wrCRP (V5600) = 0.996 (ProSpec CRP) + 2.36 with a correlation coefficient of 0.974.
VITROS wrCRP (V5600) = 0.98 (MicroSlide CRP) – 2.76 with a correlation coefficient of 0.966
Conclusions. The VITROS Chemistry Products wrCRP Assay exhibits acceptable precision and accuracy across the reportable range of 1 mg/L to 300 mg/L.
*In development

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BIOMARKERS FOR CARDIOVASCULAR RISK ASSESSMENT IN POLYCYSTIC OVARY SYNDROME

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The metabolic and endocrine dysfunctions that may occur with polycystic ovary syndrome (PCOS) can be associated with future comorbidities such as diabetes, cardiovascular disease, and endometrial cancer. Although a definitive link between PCOS and these chronic illnesses has not been demonstrated, there is significant overlap in the clinical characteristics of these disorders. Consequently, the issue of identifying and measuring potential conditions that may be associated with PCOS is a priority and should be the standard of practice in its management. Hiperhomocysteinemia has been shown as independent predictor of cardiovascular events in patients with atherosclerosis. The aim of our study was to determinate levels of homocysteine in woman with polycystic ovary syndrome compared with healthy woman. Thirty patients (age, 23, 5 ± 5.5) with PCOS and twenty four (age, 25.5± 4.3) healthy woman were involved in the study. Blood samples were collected in early follicular phase. Total homocysteine was measured using fluorescent immunoassay. Statistically significant differences in serum concentration of homocysteine were observed between groups. Mean homocysteine level we found as (10.2± 2.9 vs. 7.0±1.5) in PCOS and normal group respectively (p< 0.05). For Macedonian population we found statistically significant increased homocysteine levels in woman with PCOS. Although the mean homocysteine levels are within normal limits, there are significant higher mean homocysteine concentrations between these two groups. Because an increased concentration of tHcy has been shown as and independent risk factor for cardiovascular alterations, it is essential in this group of woman to be taken measures for early prevention.

0183

HYPOLIMIDEMIC MEDICATION AND LEVELS OF COENZYME Q10 IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Background. From the literature, the supplementation with coenzyme Q10 may be positive for suppression of progress of atherosclerosis. Statins will decrease not only endogenously produced levels of cholesterol but coenzyme Q10 too, because both molecules share a common biosynthetic pathway. Fibrates increase amount of coenzyme Q10 in blood, but an exact mechanism has not been explained so far. We have studied the influence of hypolipidemic medication on coenzyme Q10 levels.
Methods. Levels of coenzyme Q10 were measured using HPLC method with coulometric detection and concentrations of parameters of lipid spectrum were determined with enzymatic in vitro assays (Roche and Dade Behring).
Results. Concentrations of coenzyme Q10 were not significantly differentbetween groups of patients with (n = 36) and without (n = 35) statins therapy (627.2 ± 330.5 and 541.9 ± 236.9 nmol/L,p = 0.215).We have found out significant difference between levels of coenzyme Q10 in the group of patients who were treated with fibrates (n = 10) and the group without hypolipidemic medication (893.6 ± 369.7 and 541.7 ± 236.9 nmol/L, p = 0.032).
Conclusions. These results support the hypothesis that fibrates increase levels of coenzyme Q10. However, the limitation of this finding is a relatively small number of patients who taking fibrates only. In contrast to expectation, we have found out any significant difference between levels of coenzyme Q10 in patients who were treated with statins and patients without hypolipidemic medication.

0184

RELATIONSHIP BETWEEN OXIDIZED LOW DENSITY LIPOPROTEINS AND APOLIPOPROTEIN B

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Background. The aim of the study was to investigate oxidized low density lipoproteins(ox-LDL) levels in plasma samples of the clinically healthy individuals with ultrasound assessed atherosclerosis and to detect the relationship between ox-LDL and apolipoprotein B (apo B)
Methods. This study included 57 (45±7 year old) men with clinically silent but ultrasound assessed atherosclerosis (intima-media thickness and/or plaque occurrence in carotid and femoral arteries were identified) and healthy control group (n=39). Ox-LDL were measured by competitive ELISA (Mercodia, Sweden) and apo B were detected by immunonephelometric assay (Siemens, Germany).
Results. Ox-LDL levels were higher among the individuals with ultrasound assessed atherosclerosis, than among control group, but not statistically significant. Statistically significant correlation was estimated between circulating ox-LDL and apo B (r=0.4, p<0.05) in the group of clinically silent atherosclerosis.
Conclusions. Significant association was estimated between circulating ox-LDL and apo B. This relation support the concept, that modified ox-LDL may play a major role in atherosclerosis development, so ox-LDL and apo B may be assessed together as markers of atherosclerosis progression.

0185

RISK FACTOR PROFILE IN ADOLESCENT OFFSPRINGS OF PARENTS WITH CORONARY ARTERY DISEASE

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Background. Coronary artery disease (CAD) is rapidly escalating health issue all over the world. Factors such as hyperlipidemia, hypertension, DM, sedentary life style and dietary pattern contribute to increased risk of atherosclerosis in adolescent offsprings of parents with CAD.
Methods. 100 adolescent children (aged 10-18 years) whose parents had family history of CAD were screened in detail and subjected to biochemical investigation (Total Cholesterol & Random Blood Sugar) and compared with control group with no family history of CAD.
Results. The dietary and exercise patterns were similar in both groups. Study group showed 62% vegetarianism, increase incidence of obesity 26%, pre-hypertension 28%, total cholesterol > 200 mg% in 11% as compared to control group which showed 89% vegetarianism, 7% incidence of obesity, pre-hypertension 7% and acceptable <170mg% of cholesterol levels. There was significant increase (p value<0.05) in risk factors in adolescent offsprings of parents with CAD.
Conclusions. Both unmodifiable (heredity, age, sex) and modifiable (serum lipids, obesity, physical inactivity) risk factors contribute to onset of CAD in adolescents.

Cardiovascular disease

0186

BIOMARKERS AND PROGNOSTIC IMPACT OF PERI-PROCEDURAL BLEEDING PATIENTS UNDERGOING PRIMARY PERICUTANEUS CORONARY INTERVENTION

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Background. Major bleeding is one of the most frequent procedural-related complications of primary percutaneous coronary intervention (PCI). The aim of this study was to determinate biomarkers and prognostic impact of peri-procedural bleeding in STEMI patients undergoing contemporary primary PCI.
Methods. All consecutive STEMI patients (643) who underwent primary PCI between 11/2006 and 7/2009 were studied. Major bleeding was defined according to the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) study criteria. Biomarkers were determined by standard laboratory Methods.
Results. Major bleeding occurred in 30 of 643 STEMI patients with primary PCI (4.7%). Patients with major bleeding were older, more frequently female and more often had renal failure. Multivariate logistic regression analysis showed that significant predictors of major bleeding were: gender (female) (OR=2.93; 95% CI for OR 1.19-7.22; p=0.019), advanced age (≥65 years) (OR=3.01; 95% CI for OR 1.19-7.62; p=0.020), hemoglobin (Hb) at admission (<120 g/L for female and <130 g/L for male) (OR=2.68; 95% CI for OR 1.07-6.73; p=0.035) and white blood cell (WBC) at admission (>15x10^9/L) (OR=2.52; 95% CI for OR 1.03-6.12; p=0.042).
Conclusions. Female gender, advanced age, low hemoglobin and high WBC at admission are main factors of major peri-procedural bleeding.

0187

RELATIONSHIP BETWEEN *APOA5*, *APOC3* AND *APOE* GENE VARIANTS AND SERUM TRIGLYCERIDES CONCENTRATION

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Background. Atherosclerosis is a major reason for developing cardiovascular events. Serum triglyceride concentration (sTGc) is a controversial independent cardiovascular risk factor (CRF). However, some works support that high sTGc causes endothelial damage in an independent way. This study aims to know the genetic contribution of *APOA5*, *APOC3* and *APOE* gene variants in sTGc.
Methods. Serum TGc was measured and variables potentially influencing sTGc were collected from 498 patients attending the Prevention Unity for the first time. The genetic variants studied were: -1131T>C and c.56C>G from *APOA5*, c˙40G>C from *APOC3*, and c.609C>T (E2) and c.471T>C (E4) from *APOE*. TaqMan assays were used for c.56C>G and c˙40G>C and PCR-RFLP for the rest. The influence of each variable in cTGs was studied by simple regression. Significant non-genetic variables were included in multiple regression to know the cTGs variation explained by them. Finally, genetic variants were introduced independently to know each one's effect on cTGs.
Results. Significant variables in simple regression were: cardiovascular history, blood presure, smoking, exercise and genetic variants, mainly c˙40G>C from *APOC3* (p=1,7·10^{-151}) andc.56C>G from *APOA5* (p=1,89·10^{-93}).
Multiple regression with non-genetic variables showed a R^2=0,253. When including c˙40G>C the rise of R^2 was highly significant (B=15,12, R^2=0,776, p=9,47·10^{-123}) and the same happened to c.56C>G (B=14,53, R^2=0,628, p=1,13·10^{-71}). When both were introduced simultaneously, c.56C>G lost its significance.
Conclusions. Minor alelles of c*40G>C and c.56C>G explain more than 50% of cTGs variation in this population. The c˙40G>C *APOC3* gene variant is enough to predict the genetic predisposition to develop hypertrygliceridemia.

Cardiovascular disease

0188

BIOCHEMICAL MARKERS OF LEFT VENTRICULAR REMODELING

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Background. Left ventricular (LV) remodeling is a process by which growing plaque get an eccentric and crested shape. Biochemical markers, which directly reflect or indirectly impact the remodeling process, could be used along with the clinical characteristics for the risk of LV remodeling stratification.The aim of our study was to determine the biochemical markers that have the highest impact on LV remodeling process and to compare those markers with clinical characteristics of LV remodeling.
Methods. Twenty-seven patient with LV normal geometry (age 60±5.4 years) and twenty six patients with eccentric hypertrophy (age 64±9.0 years) were observed and various inflammatory, lipid and cardiac markers were measured in their blood.Remodeling patterns (RP) were defined by relative wall thickness (RWT), LV mass index (LVMI), LV enddiastolic volume (LVEDV/BSA) and LV endsystolic volume (LVESV/BSA) according to Recommendations for chamber quantification.
Results. Observed groups showed difference in CRP ($p=0.0073$), RBC-Sedimentation rate ($p=0.009$) and BNP levels ($p=0.0185$). In group with eccentric geometry there was a significant correlation between logCRP and LVMI ($\rho=0.55$, $p=0.01$), fibrinogen and LVMI ($\rho=0.53$, $p=0.001$), RBC-SE and LVMI ($\rho=0.52$, $p=0.006$) and BNP and LVMI ($\rho=0.51$, $p=0.02$), but no correlation between lipid parameters and LVMI were found.
Conclusions. These results suggest that inflammatory markers are potentionaly useful factors for predicting LV remodeling. Increased levels of BNP were closely correlated with increased LVMI which are determinants of eccentric LV remodeling.

0189

COMPARISON OF APOLIPOPROTEIN B AND AI MEASUREMENTS WITH TRADITIONAL LIPID PROFILE IN PATIENTS WITH DIAGNOSED ACUTE CORNONARY SYNDROMES

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Background. Results from recent studies suggest that apolipoproteins measurement and apoB:apoAI ratio are superior to traditional lipids in the estimation of coronary risk. We compared apolipoprotein concentrations and apoB:apoAI index with traditional lipid measures in patients presenting with chest pain.
Methods. Study group consisted of 94 women and 133 men diagnosed with acute coronary syndromes (ACS; STEMI=59, NSTE-MI=64 and UA=104), 22 non-ACS and 55 clinically healthy controls. Measurements of serum TnI, lipid profile, high sensitivity C-reactive protein, apolipoprotein AI and apoB100 concentrations were performed; apoB:apoAI and TC:HDL-C ratios were calculated.
Results. ApoB was elevated (>90 mg/dL) in 33% of ACS cases, 9% of non-ACS and 15% of controls. ApoB concentration was the only feature that distinguished both, ACS females and males, from the non-ACS and controls. In females, apoB:apoAI and TC:HDL-C ratios were significantly higher in ACS than in controls ($p<0.05$) but in males this was observed only for TC:HDL-C. ApoB:apoAI ratio was of good diagnostic utility for discrimination between ACS females and controls (AUC=0,715) however, similar AUC value was found for TC:HDL-C (0,705). On the contrary, in males atherogenic indexes were of moderate diagnostic utility. No significant differences between apolipoproteins neither lipid measures according to clinical diagnosis of ACS was observed but the highest apoB:apoAI was found predominantly in STEMI whereas the lowest in UA and NSTEMI cases.
Conclusions. The clinical utility of apolipoproteins measurement as a potent predictive factor in assessing risk of acute coronary syndromes seems to be gender-dependent and needs further investigation.

Cardiovascular disease

0190

GENE POLYMORPHISM RS20455 KINESIN FAMILY-6 AS A RISK FAKTOR OF MYOCARDIAL INFARCTION. PRELIMINARY REPORT

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Background. Coronary heart disease is the cause of almost half (48%) of deaths of Europeans. The search for new markers associated with atherosclerosis and its complications is still main research interest in many laboratories. In recent years, kinesin family member 6 (KIF6) gene polymorphism rs20455 (Trp719Arg) was suggested as the factor of genetic predisposition of cardiovascular diseases (CVD). It was found that the risk of CVD in carriers of KIF6 gene variants increases by about 50%. The aim of our study was to evaluate the relation of this mutation with premature myocardial infarction (MI).
Methods. We studied 11 male patients (age 43.3±4.1 yrs) after myocardial infarction within last two years and 9 healthy subjects (male, age 42.0±5.8 yrs) without symptoms and a family history of coronary heart disease. Genotyping was performed by real-time PCR and melting curve analysis.
Results. In the control group, the frequency of allele 719 Arg/Arg in 11%, allele 719Arg/Trp in 44% and allele 719Trp/Trp in 45% was found. In the MI group, however, the allele 719Arg/Arg was stated only in 9% whereas allele 719Arg/Trp occur in this group in 64% and allele 719Trp/Trp in 27% of subjects. The risk ratio in the KIF6 Arg carriers was calculated as 2.13.
Conclusions. The carriers of 719Arg (Arg/Arg and Arg/Trp) more frequently were stated in patients group (73%) as compared to those in the control group (55%). It may be suggested, therefore, that these gene variants is associated with increase risk to develop myocardial infarction in young population.

0191

CHOLESTERYL ESTER TRANSFER PROTEIN TAQIB, I405V, R451Q AND A373P POLYMORPHISMS, LIPID PROFILE AND CORONARY STENOSIS IN A TUNISIAN POPULATION

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Background. The cholesteryl ester transfer protein (CETP) is involved in the reverse cholesterol transport and is therefore a candidate gene for atherosclerosis.
Methods. Four CETP polymorphisms: TaqIB, I405V, R451Q and A373P were studied in 316 coronaries Tunisian patients. Subjects were clinically examined and lipid profile was estimated. Genotyping was performed by PCR-RFLP.
Results. 451Q allele, associated with lower HDL-C and higher TC and ApoB concentrations, was also significantly associated with an increased risk of significant stenosis (OR 1.74, 95% CI 1.15–2.61, p= 0.007). B2 allele of Taq1B polymorphism had an increase in HDL-C concentration and was associated with a reduction in the prevalence of coronary stenosis, as described earlier. It was also associated with low risk of hypoHDLaemia (OR 0.615, 95% CI 0.377-1.002, p= 0.035). No significant effect of different A373P and I405V alleles was found on lipid profiles and on coronary stenosis. When ABCA1 polymorphisms were combined in haplotypes possessing possessing R451Q, A373P, I405V, Taq1B polymorphisms, 1112 haplotype seems to be the most protective against significant stenosis (OR=0.71, 95% CI 0.188-0.983; p=0.035) whereas 2111 was probably the most atherogenic with OR=2.17, 95%CI=1.06-5.88; p=0.039.
Conclusions. Q allele of R451Q polymorphism was associated with decreased HDL-C, increased apoB concentrations and increased risk of coronary stenosis. However, B2 allele of Taq1B polymorphism had protective effect. In haplotype analysis, we found that 1112 seems to be a protective haplotype whereas 2111 have atherogenic effect in a Tunisian population.

Cardiovascular disease

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EIGHT ADIPONECTIN POLYMORPHISMS WERE ASSOCIATED WITH OBESITY AND INSULIN RESISTANCE IN A TUNISIAN POPULATION

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Background. Adiponectin is a plasma protein produced by the adipose tissue, with insulin sensibility, anti-inflammatory and anti-atherogenic properties. Many adiponectin gene polymorphisms were described and their implication in obesity, insulino resistance (IR) and cardiovascular diseases was controversial.
Our aim was to study the relationship between genetic adiponectin variability and the risk of obesity in Tunisian volunteers.
Methods. We have recruited 169 non obese (mean age 43.54±3.56 years; mean BMI 24.7±3.2 Kg/ m^2) and 160 obese (IMC≥ 30Kg/m^2, mean age 50.52±11.25 years; mean BMI 37±4.9 Kg/ m^2). Genotyping was performed using PCR-RFLP. Glucose, insulin and lipid profile were measured. BMI and HOMA-IR were calculated.
Results. +276GT, 11391GA, 11374CG and +2019delA contribute in obesity whereas 45TG, 4522CT, 639CT and 395 GA polymorphisms seem to be not implicated. In fact adjusted ORs of obesity associated to mutated genotype of each polymorphism were respectively (OR=0.64, p= 0,043; OR=3.05, p= 0,049; OR=01.77, p= 0.049 and OR= 1.94, p=0,010).
Mutated genotypes at 276G/T were associated with lower serum insulin concentration, lower systolic and diastolic blood pressure. Mutated genotypes at 639T/C were associated with higher waist circumference, BMI, systolic and diastolic blood pressure. In addition, 11391AA genotype was associated with increased BMI. Concerning 2019delA, delAdelA genotype was associated with increased HOMA-IR and BMI suggesting a possible effect of these SNPs on IR parameters.
Conclusions. +276GT, 11391GA, 11374CG and +2019delA were associated with obesity and insulin resistance parameters.

0193

MYELOPEROXIDASE LEVEL AND OTHER MARKERS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background. Myeloperoxidase (MPO), hs-CRP, IL-6 and other markers have shown considerable utility in the patients with acute coronary syndrome (ACS) and congestive heart failure (CHF). Atherosclerosis is a chronic inflammatory process and myeloperoxidase seems to contribute directly to the pathogenesis of acute coronary syndrome.
Methods. We examined 85 patients with coronary artery disease after coronary stenting, mean age 64±7,85 years, body mass index (BMI) 27,35±4,28 kg/m^2 and 50 healthy patients with similar characteristics with normal coronary angiograms. All biochemical parameters are determined on Abbott's Architect C 8000, MPO on Architect i 2000, IL-6 on DPC Immulite 2000 from EDTA plasme, and hs-CRP is determined on nephelometer BN II Dade Behring from serum. All the samples were processed fresh.
Results. The values of MPO for patients with coronary artery disease (CAD) were 112,96±75,27 pmol/L, versus 73±29 pmol/L to control group (p<0,01); IL-6 is 6,39±3,95 pg/ml (p<0,05);
hs-CRP values were significantly higher in patients 2,5±2,47mg/L compared to control group 1,04±0,17mg/L (p<0,001).
Conclusions. The level of MPO is elevated in patients with CAD. The chronic inflammatory process can develop to an acute clinical event by the induction of plaque rupture and therefore cause acute coronary syndrome. C- reactive protein is a strong predictor of clinical outcome in coronary artery disease, inflammation has been implicated in the process. Plasma concentration cytokine IL-6 reflects the intensity of plaque vulnerability to rupture and restenosis following percutaneous coronary intervention. The results indicates the importance of determinating MPO and other risk factors for developing coronary artery disease.

Cardiovascular disease

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IMPACT OF THE HIGHLY SENSITIVE CARDIAC TROPONIN T (HSTNT) ASSAYON THE TRIAGE OF EMERGENCY DEPARTMENT (ED) PATIENTS

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Background. Troponin is recognised as the gold standard for diagnosing myocardial infarction. In our 600-beds university hospital, the 4th generation TnT assay was recently replaced by the hsTnT that improvesthe analytical sensitivity lowering the cut-off to 15 ng/L.
Methods. During the three-month audit evaluating the impact of hsTnT implementation, we focused on patients presenting to the ED by comparing triage of hsTnT positive patients with that of TnT positive patients examined in the same period one year before.
Results. In the two evaluated periods, the same number of tests (1472 vs. 1465 for TnT and hsTnT, respectively) was spread on a slightly different number of patients (from 1019 to 958, -6.0%).TnT and hsTnT gave 18.5% and 45.3% positive results (P<0.0001), with a 145% relative increase after hsTnT implementation. This corresponded to 196 and 434 positive examinations, respectively. After hsTnT implementation, the number of hospitalizedpatientswith positive troponin increased from 158 to 292 (+84.8%), even if the rate of admission in intensive and non intensive care departments was unchanged (P=0.108). 16 (8.5%) and 109 (26.6%) positive patients were discharged in the twoperiods.In the follow-up, only one discharged TnT patient returned to the ED, while 13 (12.9%) hsTnT patientswere readmitted, mainly for signs of cardiac involvement.
Conclusions. The introduction of hsTnT markedly increased the number of positive examinations and following hospital admissions in ED population. However, about ¼ of hsTnT positive patients were discharged showing that triage decisions are not only based on the biomarker Results.

0195

IMPACT OF IMPLEMENTATION OF THE NEW HIGHLY SENSITIVE CARDIAC TROPONIN T (HSTNT) ASSAY IN A UNIVERSITYHOSPITAL SETTING

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Background. Troponin is recognized as the gold standard for diagnosisof myocardial infarction. The new hsTnT (Roche Diagnostics) has improved both analytical sensitivity and imprecision, lowering the diagnostic cut-off to 15 ng/L, corresponding to 99th percentile limit of our reference population.
Methods. Recently, we replaced the 4th generation TnT assay (cut-off 0.03 μg/L) with hsTnT. Three months after the implementation, we performed an audit on the impact of hsTnT by comparing datawith the same period one year before.
Results. After hsTnT implementation, a 5.4% increase of troponin tests was recorded.A positive result was found in 31.7% of TnT and in 58.7% of hsTnT (+85%), corresponding to 22.2% and 47.0% positive patients, respectively (P<0.0001).64% of hsTnT positive resultsfell in the 16-65 ng/L range, determined as negative with TnT.The number of tests per examination averaged 1.54±1.0 before and 1.67±1.1 after hsTnT implementation (P<0.0001). By auditingtroponin curves (i.e. at least two results during patient examination), 39.1% for TnT and 69.0% for hsTnT had at least one result positive (P<0.0001). However, when the positive curves were classified as typical/atypical according to the established reference change value (+46/-32%), the difference in percentage of positive curves displaying a typical marker release became not significant (17.2% for TnT vs. 20.5% for hsTnT, P=0.32).
Conclusions. The introduction of hsTnT markedly increases the number of positive tests. Interestingly, our data show that in interpreting the almost doubled positive results, the evaluation of marker release may keep specificity at the same level of TnT.

Cardiovascular disease

0196

EVALUATION OF THE SENSITIVITY OF TWO HIGHLY SENSITIVE TROPONIN ASSAYS FOR EARLY DETECTION OF NON ST-ELEVATION MYOCARDIAL INFARCTION (NSTEMI)

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Background. The major clinical role for the cardiac troponin in the MI diagnosis is in patients with nondiagnostic initial ECG (suspected NSTEMI). The aim of this study was to evaluate the clinical sensitivity of two highly sensitive last generation troponin assays (Siemens Advia TnI-ULTRA and Roche Diagnostics Cobas e411 hsTnT) in the diagnosis of patients presenting with recent NSTEMI in comparison to a conventional troponin assay.
Methods. The study group consisted of 150 patients admitted to the Emergency Department with suspected acute coronary syndrome, to whom serial blood samples were taken every 3 h throughout the first 6 h after hospital admission. After evaluation of clinical history, ECG, and standard troponin results, obtained blinded to the results of tested troponin assays, 31 patients were diagnosed as NSTEMI. Positive results were considered valueshigher than 40 ng/L for TnI-ULTRA and 15 ng/L for hsTnT, respectively.
Results. Sensitivities for NSTEMI were 81%, 87%, and 58% at admission and 100%, 100%, and 94% after 6h for TnI-ULTRA, hsTnT, andstandard assay, respectively. Interestingly, withboth highly sensitive assays the maximal 100% sensitivity was already reached 3h after admission.
Conclusions. New highly sensitive troponin assays may significantly shorten the time to rule out NSTEMI. Although preliminary, our data suggest that the observation period of patients with suspected myocardial damage can be reduced from the conventional 12 h from hospital admission to only 3h. Accordingly, a new protocol, sampling at presentation and 3 h after, could be recommended.

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COMPARISON OF PROGNOSTIC VALUE OF HIGH-SENSITIVITY AND CONVENTIONAL TROPONIN T IN PATIENTS WITH NON-ST-SEGMENT ELEVATION ACUTE CORONARY SYNDROMES

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Background. The impact of high-sensitivity troponin (hsTnT) assays on the risk stratification of patients with non-ST-segment elevation acute coronary syndromes (NSTE-ACS) is unknown. We investigated the diagnostic shift from unstable angina to non-ST-segment myocardial infarction (NSTEMI) using hsTnT and sought to characterize the re-classified patients in terms of long-term prognosis.
Methods. The study included 447 patients with NSTE-ACS. The cardiac troponin T was measured prior to cardiac catheterization with conventional (cTnT 4th generation, Roche Diagnostics) and high-sensitivity assays (hsTnT, Roche Diagnostics) in parallel, using the same plasma sample. The primary end point was 4-year mortality.
Results. The use of the cut-off of 0.014 μg/L of the hsTnT assay instead of 0.03 μg/L of the cTnT assay increased the number of NSTEMI patients by 63% (from 168 to 266 patients). Re-classified patients from unstable angina to NSTEMI based on the use of the hsTnT assay had similar 4-year mortality compared to patients first diagnosed with NSTEMI with both assays (23 deaths among 98 re-classified patients (24.4%) versus 40 deaths in patients diagnosed with NSTEMI with both assays (24.0%), odds ratio 1.02, P=0.95). The Cox model identified hsTnT (hazard ratio [HR]=2.59, P=0.013 for hsTnT >0.014 μg/L versus hsTnT ≤0.014 μg/L) but not cTnT (HR=1.19; P=0.512 for cTnT >0.03μg/L versus cTnT ≤0.03 μg/L) as an independent correlate of 4-year mortality.
Conclusions. The use of the hsTnT instead of cTnT substantially increased the proportion of patients with NSTEMI among patients with NSTE-ACS and significantly improved risk stratification regarding long-term mortality.

Cardiovascular disease

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STAT MULTIPLEX DETERMINATION OF CARDIAC BIOMARKERS WITH EVIDENCE BIOCHIP ARRAYS

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Background. To facilitate the correct diagnosis and treatment of patients exhibiting symptoms of early stage acute coronary syndrome leading to improved patient prognosis, clinicians require rapid, sensitive, specific and reliable assays for the measurement of cardiac biomarkers. Biochip array technology enables the measurement of multiple cardiac markers from a single sample, increasing the test result output as a patient profile is generated. To bring this technology into the emergency room the STAT analyser Evidence Multistat provides rapid laboratory standard Results. We report here the applicability of this system to the simultaneous determination of CK-MB, H-FABP, cTnI.
Methods. The three simultaneous immunoassays define discrete test sites on the biochip surface, the biochip is also the vessel for the immunoreactions. With this system, two individual biochips and all the required reagents are provided in a cartridge. Sample is manually added to the sample well on the cartridge, which is then inserted onto the analyser, the rest of the process isfully automated.
Results. Six test results are produced within 30 minutes of sampling. The limit of quantification is less than the 99th percentile of the normal range. Total assay imprecision is typically<10% throughout the assay range with good agreement(>90%) with other immunoassay systems.
Conclusions. Data indicate applicability of this STAT system to the simultaneous measurement of CK-MB, H-FABP, cTnI. The system provides the means to input reference ranges for each analyte. The rapid results in less than 30 minutes in conjuction with clinical symptoms will help to make an immediate clinical decision.

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GLOBAL DNA METHYLATION IN ACUTE MYOCARDIAL INFARCTION

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Background. Cardiovascular diseases are the main cause of mortality and morbidity worldwide and it is reaching epidemic proportions in developing countries. We measured DNA methylation in young infarction and we compared the results with young controls. In addition, we quantified some analytes involved in the methylation cycle to verify the association between them.
Methods. We used capillary electrophoresis to measure DNA methylation, Cysteine (Cys), Homocysteine (Hcy) and asymmetrical dimethylarginine (ADMA) in 25 young infarction patients and 39 healthy volunteers.
Results. Epigenetic modifications, especially alterations in DNA methylation, are increasingly becoming a key factor in the pathogenesis of complex disorders, including atherosclerosis and cardiovascular diseases. We found that the levels of DNA methylation in patients are higher than in controls (median 4.14 vs 3.57, p=0.0002). These results could be influenced by methyonine cycle, so we measured the other analytes such as Cys, Hcy and ADMA and we found that there were not any differences between patients and controls. Then, we investigated on the correlation between plasma homocysteine levels and DNA methylation and we observed a significant positive correlation of global DNA methylation and homocysteine levels in patients (p=0.0083) but not in controls (p=0.0919).
Conclusions. It is well know that DNA hypermethylation is correlated with systemic inflammation. And since we found a positive correlation between hypermethylation and hyperhomocysteinemia in young heart attack, we can assume that this epigenetic modification may be linked to an increased cardiovascular disease risk.

0200

LOW-DENSITY LIPOPROTEIN S-HOMOCYSTEINYLATION DEGREE IN PROTEINURIC CHRONIC NEPHROPATHY DISEASE

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Background. It has been recently reported that low-density lipoprotein (LDL) atherogenicity could be enhanced by the modification of its S-homocysteiylation degree. We have investigated the levels of homocysteine (Hcy) linked to LDL in chronic proteinuric patients in which lipid abnormalities highly contribute to the excess of morbidity and mortality.
Methods. We used capillaryelectrophoresis to measure LDL-bound thiol Hcy, cysteine(Cys), cysteinylglycine (Cys-Gly), glutathione (GSH), andglutamylcysteine (Glu-Cys) in 30 chronic kidney disease(CKD) individuals and 60 healthy volunteers. Lipid profile and total plasma thiols were also measured.
Results. CKD has significantly low levels of HDL and increased levels of plasma triglycerides and LDL. Besides, more elevated levels of total plasma Hcy, Cys, GSH and Glu-Cys were found in patients than in controls and LDL-S-Hcy was significantly more increased in nephropathic subjects than in controls (median 23.9 vs 12.0 nmol/μmol apoB, p<0.001) like also LDL-S-Cys (median 386 vs 301 nmol/μmol apoB, p<0.01).By multiple linear regression, we found that in healthy people, total Hcy was the most important determinant of LDL-bound Hcy (t=4.78, p<0.0001) and Cys-Gly was negatively associated with apoB-Hcy concentrations (t=-2.28, p<0.03). In CKD the most important determinant of homocysteinylation was creatinine(t=-2.25, p<0.03),while total plasma Hcy was weakly associated with apoB-Hcy (t=3.69, p<0.001).
Conclusions. The increased levels in Hcy-LDL observed in CKD patients might account, at least in part, for the excess of cardiovascular risk; thus LDL S-homocysteinylation can be considered a key marker of risk for cardiovascular disease in these individuals.

0201

PHOSPHOLIPIDS MEDIATED HDL REMODELING GENERATES SMALL NASCENT HDL-LIKE PARTICLES CONTAINING APO A-II

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Background. We demonstrated that interaction between ultracentrifuged HDLs and lecithin liposomes generates a new pre-b mobility fraction containing apo A-I and apo A-II, in a phospholipids (PL) dose-dependent manner.
Methods. We used non-denaturing polyacrylamide gradient (2-27%) gel electrophoresis and immunoblotting for the analysis of the pre-b mobility fraction generated at liposomal PL (L-PL) to HDL-PL ratios 1:1, 3:1 and 5:1, after 1 hour incubation of HDLs and liposomes at 37°C.
Results. The pre-b mobility fraction generated at three L-PL/HDL-PL investigated ratios consisted of two major subpopulations of apo A-I containing HDL-like particles with average diameters of 9.7±0.3 and 7.6±0.1 nm. At L-PL/HDL-PL ratios 3:1 and 5:1 the fraction with average diameter of 9.7 nm was more pronounced. On the other hand at L-PL to HDL-PL ratio 1:1 pre-b fraction consisted of three major subpopulation of apo A-II containing HDL-like particles with average diameters of 12.5±1.3, 8.4±0.5 and 7.6±0.1 nm. At higher L-PL/HDL-PL ratios additional subpopulations of apo A-II containing particles with diameters of 9.2±0.5 and 8.2±0.3 nm appeared. At every of the investigated L-PL/HDL-PL ratios the subpopulation of particles with the average diameter of 7.6 nm was the largest.
Conclusions. Apo A-I dissociating from HDLs during their remodelling by PL created HDL-like pre-b mobility particles of defined PL/apo A-I ratios, irrespectively of the L-PL/HDL-PL ratio in incubation mixture. Apo A-II formed more heterogeneous subpopulations, depending on the amount of available PL. The most important observation is ability of apo A-II releasing from mature HDL to generation of small particles similar to nascent HDLs.

Cardiovascular disease

0202

OXIDATIVE STRESS AND GENE EXPRESSION OF ANTIOXIDANTENZYMES IN PATIENTS WITH CHAGAS DISEASE

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Background. The pathogenesisofchronicchagasiccardiomyopathy(CCM) iscontroversial; there are nodefinitiveproofsofwhich are the necessaryfactors to reach thedeterminatestage. Each hostgeneticfactorscould actively participate inthe evolutionofChagasdisease. Whereasthe variabilityofphenotypic expression ofthe CCMcould be because ofgenetic componentsof the patient, we decided todo a descriptive studyof genotype frequencies(GF) of SOD-Mn Ala-9-Val and the enzyme activitiesofsuperoxidedismutase(SOD),glutathioneperoxidase(GPx) and catalase (KAT) inchagasic patients with cardiomyopathy(CcC) andwithoutcardiomyopathy(CsC) comparedwithhealthy controls(CN).

Methods. Themolecularcharacterizationwas performedbyPCR-RFLP. Enzyme activitieswere determinedbyspectrophotometrictechniques. Thehypothesistestundernormal theoryproportionsandKruskalWallis testwere carried out.

Results. TheSOD-MnFG(IC 95%) were CN: Ala/Ala0.54(0.40-0.67), Ala/Val0.33(0.20-0.45), Val/Val0.13(0.04-0.21); CsinC: Ala/Ala0.36(0.07-0.64), Ala/Val0.46(0.16-0.75), Val/Val0.18(0.00-0.40); CconC: Ala/Ala0.35(0.14-0.56), Ala/Val0.30(0.10 - 0.50), Val/Val0.35(0.14 - 0.56). The enzime activitieswere: KAT(K/gHb): CconC316±68, CsinC332±41, CN185±28; GPx(U/gHb): CconC98±17, CsinC102±20, CN 61±11; SOD(USOD/gHb): CconC3270±833, CsinC 2590±188,CN895±314.The studyofSOD-Mn GF ofchagasic patients and CN showed significant differences(p <0.01) between them. TheactivitiesofKAT, SODandGPxshowed significant differences (p <0.01) betweenchagasic patientsand CN.

Conclusions. The datasuggestthatpolymorphismsinvolvedinoxidativestressmayhaveimplicationsinthepathogenesisofCCM, modifyingindividual riskin thedevelopmentof cardiomyopathies.

0203

A COMPARISON OF NT-PROBNP AND BNP IN THE EVALUATION OF HEART FAILURE IN CLINICAL PRACTICE

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Background. Natriuretic peptides such as BNP or NT-proBNP are established markers of heart failure (HF), however both are not specific and results may differ from each other and from the definitive clinical diagnosis.

Methods. 199 consecutive patients of an internal medicine department with suspected HF were examined clinically by standard procedures and attributed to NYHA stages I-IV. NT-proBNP (Roche) and BNP (Abbott) were measured in serum or EDTA-plasma, respectively. Following assay specific cut-off values biochemical diagnoses of HF were categorized as "HF unlikely", "HF possible" and "HF likley" and compared with the final clinical diagnosis.

Results. In 144 patients (72%) BNP and NT-proBNP provided concordant results with regard to HF, in 127 patients (63%) these were in accordance with the final clinical diagnosis, half of the reminder were associated to impaired renal function (median GFR 29.4 ml/Min). In 55 patients (28%) BNP- and NT-proBNP-defined HF catagories were discrepant, in 42 patients (21%) the NT-proBNP category was more severe than the BNP result. Compared with the final clinical diagnosis either NT-pro-BNP or BNP results were misleading in 11 and in 9 cases ("HF likely" in NYHA I, "HF possible" in NYHA IV), irrespective of renal function.

Conclusions. Only 63% of cases showed concordance of NT-proBNP and BNP HF categories and the final clinical diagnosis of HF. In 9% of patients biochemical and clinical diagnosis were different, partly due to impaired renal function. In 28% of patients NT-proBNP and BNP were discrepant with about equal shares in clinically misleading HF categorizations.

Cardiovascular disease

0204

RELATION BETWEEN ADHESION MOLECULES AND HOMOCYSTEINE LEVELS IN FAMILIAL HYPERCHOLESTEROLEMIA

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Background. Elevated plasma homocysteine (tHcys) is considered to be an independent risk factor for atherosclerosis. Oxidative stress and activation of proinflamatory factors have been proposed to explain the atherogenic effects of homocysteine. The aim is to investigate the relation between tHcys and markers of endothelial cell activation in hyperholesterolemia.
Methods. Twenty first-grade relatives of families withfamilial fypercholesterolemia (FH) and 22 age-and sex-matched controls were included in the study. The patients with hypercholesterolemia (LDL-cholesterol values were above3,36 nmol/l) had been categorized by Simon-Broome Register group criteria for a clinical definite and probable diagnosis of FH, with no prior history of cardiovascular disease. The concentrations of tHcys) were assessed by HPLC-FD, adhesion molecules (sICAM-1, sVCAM-1, P- and E-selectins) were measured using ELISA analysis.
Results. The group with hypercholesterolemia had significantly higher levels of sICAM-1 (457,5 vs. 249,2 ng/ml in controls, p<0.001), sVCAM-1 (780.7 vs.358.6 ng/ml, p<0.001) and tHcys (11.19 vs. 9.42 umol/l, p<0.05), while P- and E selectins show no significant difference in compared groups. Plasma tHcys was correlated with sICAM-1 (r=0.348, p<0.05) and LDL-cholesterol (r=0.337, p<0.05). ICAM-1 shows significant association (p<0.001) with total cholesterol (r=0.704), LDL-cholesterol (r=0.766) and sVCAM-1 (r=0.641).
Conclusions. Thesedata support the notion that plasma tHcys andadhesion molecules interact with the established cardiovascular risk factor – hypercholesterolemia in the pathogenesis of vascular disease and can be considered as silent markers of preclinical atherosclerosis.

0205

PLASMA ASYMMETRIC DIMETHYLARGININE LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Asymmetric dimethylarginine (ADMA) is an natural, competitive inhibitor, and one of the primary factors controling nitric oxide synthase activity. Elevated plasma ADMA levels are associated with many human diseases including ischemic heart disease. To assess ADMA diagnostic validity in patients with different stages of ischemic heart disease we studied this marker in patients having stable angina pectoris (SAP), with unstable angina (USAP), and acute myocardial infarction (AMI). The results were compared with the values of healthy individuals. Plasma ADMA levels were measured by high-performance liquid chromatography. In all patient groups ADMA levels were significantly elevated in comparison with control ones (p<0.001). In SAP patients the mean ADMA value was 0.98±0.57 µmol/L, in USAP patients 1.13±0.63 µmol/L, in the AMI group 1.10±0.16 µmol/L, while in healthy subjects it was 0.33±0.16 µmol/L. A testing between patient groups showed a significant difference only between the SAP and the AMI group (p<0.05). Diagnostic accuracy of ADMA was determined by ROC curve analysis. The highest area under the ROC (AUC) obtained in AMI patients was 0.976, similar AUC (0.972) was obtained in USAP patients, and some lower in SAP patients 0.937. There was no any significant difference between these three AUCs. The greatest sensitivity (95.65) and specificity (96.30) were found in the USAP group, in the AMI group these characteristics were 90.91 and 96.30, but in the SAP group 95.24 and 81.48. Considering these results ADMA is a valid marker in assessing ischemic heart disease but fail to identify the stage of this disease.

Cardiovascular disease

0206

CARDIOMARKERS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION RECEIVING SYSTEMIC THROMBOLYTIC THERAPY

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Background. The aim of this study was to define the role of quantitative detection of cardiac troponin I (TnI) in evaluation of efficiency of systemic thrombolysis in patients with acute transmural myocardial infarction (Q-infarction).
Methods. Blood sampling in 30 patients was done at the beginning of thrombolysys and every hour within next 10 hours. Detection of TnI, CK-MB mass was performed using analyzer "Architekt i2000" (Abbott)and "AQT 90 Flex"; ultrasensitive CRP, CK and CK-MB activity using "Hitachi 902".
Results. Thrombolysis was not effective in 26% of patients. Concentration of TnI at the beginning of thrombolysis varied from 0.1 to 21.5 ng/ml, in 13% of patients concentration of TnI was below upper reference limit, CK-MB mass within normal range was observed in 20% of patients.The peak of TnI during effective thrombolysis was observed between 4 and 6 hours and reached 2400-fold increase of upper reference limit. During inefficient thrombolysis values of TnI were not so high and maximum was observed later. Concentration of CRP at the beginning of thrombolysys varied from 0.4 to 25.5mg/l and did not exceed 3 mg/l in 18%.
Conclusions. For reliable diagnostics of Q-infarction with concentration of TnI at the admission below upper reference limit it is necessary to repeat the test in 8 hours from beginning of pain. Detection of CK-MB mass has significant advantages compared to detection of CK-MB activity and has no advantages compared to TnI. The predictive value of CRP-test is not high enough.

0207

COMPARATIVE ESTIMATION OF MARKERS OF AN EXCHANGE OF AN OSTEAL TISSUE AND CORONARY RISK AT SICK OF AN ISCHEMIC HEART DISEASE IN A COMBINATION WITH OSTEOPENIC SYNDROME

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Background. to compare level of biochemical markers of an osteal exchange: OC, OPG, fetuine and PTG and degree of coronary risk (a calcium index - CI)
Methods. 30 ischemic heart disease patients with osteopenia (1 group) and 60 ischemic heart disease patients of control group (control). The mineral density of an osteal tissue in the field of a backbone and a hip at patients of 1 group has been lowered (by T-criterion <−1,0). Coronary risk defined according to multispiral computer tomography of a thorax with definition CI by «Calcium scoring» program.
Results. At patients of 1 group authentic rising of coronary risk (CI>100 and CI>400 at 68 %, control at 40 % of patients) and rising of levels OC (25,71±1,31 ng/ml, control 9,18±1,98 ng/ml, p<0,05), OPG and PTG (4,92±1,07 pmol/l, To – 2,73±1,10 pmol/l, p<0,05 is taped; 48,16±0,96 ng/ml, To – 22,30±0,31 ng/ml, p<0,01, accordingly).
Conclusions. Thus, taped osteopenia at patients with an ischemic heart disease associates with the expressed calcification of coronary arteries and high coronary risk and with acceleration of an osteal exchange that specifies in balance disturbance between processes of osteal formation and resorption.

Cardiovascular disease

0208

ESTIMATION OF CORONARY RISK AND CALCIFICATION OF CORONARY ARTERIES AT PATIENTS WITH OSTEOPENIA, INTERRELATION WITH LABORATORY INDICATORS

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Background. to Estimate the general frequency and expression of a calcification of coronary arteries and degree of coronary risk at patients from an ischemic heart disease, to define influence osteopenic conditions on expression of a coronary calcification and to establish interrelation with osteoprotegerin and fetuine levels.
Methods. 70 male patients at the age from 42 till 78 years (middle age 62,8±8,6 years) from an ischemic heart disease, are surveyed by a stenocardia of II functional class. By results of the spent osteodensitometry with use T and Z-criteria and definition of mineral density of a bone at 25 patients it is taped osteosinging
Results. At patients with osteopenia high and highest degree of a calcification of coronary arteries is taped at 68 % of patients that is appreciable above than at patietns in group without osteopenia (40 %). Interrelation CI with the levels of fetuine, and T-criterion depression – with osteoprotegerin is shown.
Conclusions. the Calcium index reflects gravity of a coronary atherosclerosis and degree of an obstructive lesion of coronary arteries and is in a close connection with changes of a homeostasis and resorbtion of osteal tissue. Fetuine reflects activity of an inflammation, and osteoprotegerin serves as a measure of osteal tissue resorbtion.

0209

HOW DIFFERENT CAN DIRECT AND CALCULATED LOW-DENSITY LIPOPROTEIN CHOLESTEROL BE?

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Background. Although direct measurement of low-density lipoprotein cholesterol (D-LDL) is commonly used, clinical guidelines recommend that calculated LDL (C-LDL) should be used to guide therapy. Our aim was to determine the equivalence of D-LDL to C-LDL.
Methods. From May 2009 to April 2010, 6613 D-LDL measurements were made to different inpatients in our central laboratory. Of these, 6530 had a triglyceride concentration lower than 400 mg/dL. Direct LDL was determined with a direct homogenous assay on an Olympus AU5400 and C-LDL was determined using the Friedewald formula. Total cholesterol and triglycerides were measured by enzymatic assays and HDL- cholesterol directly by a homogenous assay. *P* values were obtained from Student *t*-tests for means. Pearson correlation coefficients and Bland–Altman graphs were used to compare D-LDL and C-LDL concentrations.
Results. 6530 determinations (3104 females-47, 5%; 3426 males- 52, 5%) were used for the comparison of D-LDL and C-LDL. Values (mean±standard deviation; 95% confidence interval) were for D-LDL (in mg/dL) (109,4±39,7; 108,5:110,4) and for C-LDL (also in mg/dL) (102, 6±43, 4;102, 6:103, 7). There was significant correlation between D-LDL and C-LDL (r^2= 0, 912, p< 0.001), and the difference (C-LDL-D-LDL) was (mean±sem) 6, 8±0, 2 mg/dL (p< 0,001), with a 95%CI 6,5 - 7,1 (p< 0,001). The differences between measurements decrease 4, 4 mg/dL for each 100 mg/dL of increase in D-LDL value.
Conclusions. As already shown by other authors D-LDL is higher then C-LDL. In our study, the difference between methods decreases for higher D-LDL values.

Cardiovascular disease

0210

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS AND INSULIN RESISTANCE ARE MODIFIED IN WOMEN WITH A PAST HISTORY OF SEVERE PREECLAMPSIA

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Background. Preeclampsia (PE), associated with insulin resistance(IR) and endothelial dysfunction, may signal increased risk of cardiovascular disease later in life.Angiogenic factor levelsare modified in both diabetics and women with PE, possibly caused by IR. We hypothesised that levels of angiogenic factors may be altered in women with a past history of PE.
Methods. From a cohort of 3799 nulliparous women prospectively recruited, we recalled 336 women (with a history of gestational hypertension(105) or PE(63), and controls matched for age and year of delivery) on average 7.8 years after delivery to evaluate their cardiovascular risk. We measured angiogenic factors (VEGF and sFlt-1) using ELISA. Insulin was measured by RIA on blood collected before and 2 hours after a 75 g OGTT. We calculated the area under the curve (AUC) of insulin concentrations.
Results. No significant sFlt-1 differences were observed. There was a trend for decreased VEGF concentrations in women with prior PE (p=0.06); the difference becoming significant (199 vs 246 pg/mL; p<0.05) in women with a history of severe PE. We found an increased insulin AUC in women with a history of PE (864 pmol/L/h) and severe PE (976 pmol/L/h) compared to controls (751 pmol/L/h) (p<0.01). IR prevalence is increased using the homeostasis model assessment in women with a history of PE (p<0.01).
Conclusions. VEGF levels are lowered and IR is increased in women with a history of PE, particularly severe PE, which may contribute to increased risk of cardiovascular disease later in life.

0211

COMPARISON OF THREE ROUTINE HIGH SENSITIVITY TROPONIN ASSAYS

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Background. Recently a number of high sensitivity immunoassays for cardiac troponin T and I (cTnT/cTnI) with claims of superior imprecision and a definable 99th percentile in a healthy population have been produced. Methods of calculating the 10% CV and selection criteria of patients for 99th percentile calculations vary, furthermore Standardisation of cTnI methods, and so comparison of assays is difficult. To overcome this scorecard criteria have previously been proposed.
Methods. We compared three high sensitivity Troponin assays enhanced AccuTnI (Beckman -Coulter), hsTnT (Roche) and TnI-Ultra (Siemens Healthcare Diagnostics) a single protocol for the assessment of the 10% CV and calculation of the 99th percentile and using a well defined population free from cardiovascular disease and.
Results. 10% CV values were 26, 17 and 45ng/L for the AccuTnI, hsTnT and TnI-Ultra respectively. The 99th percentile were 42, 15.5 and 39 g/L respectively with 97.7, 57.7 and 68.4% of subjects having a definable troponin below the 99th percentile.
Conclusions. All assays were guideline acceptable according independent scorecard criteria with the hs-TnT and TnI-Ultra assays being designated Level 2(first generation, hs) and the enhanced AccuTnI assay Level 4 (third generation, hs).

Cardiovascular disease

0212

ASSOCIATION OF SERUM FERRITIN AND SOLUBLE TRANSFERRIN RECEPTOR WITH CORONARY ATHEROSCLEROSIS

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Background. We examined whether there is an association between serum ferritin or soluble transferrin receptor (sTfR) concentration and coronary artery disease (CAD) assessed with coronary angiography.
Methods. This study included 83 patients (37 women and 46 men) with angiographically proven CAD and 52 healthy controls without CAD (24 women and 28 men). Blood samples were obtained on the day before angiographic procedure and ferritin, sTfR and C-reactive protein (CRP) were measured by nephelometry (BNII, DadeBehring). CRP concentrations were determined with high sensitive assay, sTfR index was calculated (sTfR/log ferritin).
Results. The values (median, 25th and 75th percentiles) of ferritin were significantly higher in male (148.5, 123 and 276.8 **μ**g/l versus 139, 84.4 and 157.0 **μ**g/l, $p<0.05$) but not in female (52.7, 27.2 and 87.8 μg/l versus 48.1, 33.8 and 65.6 μg/l, $p=0.27$) patients compared to controls. Concentrations of sTfR (1.19, 1.03 and 1.40 mg/l versus 1.17, 1.06 and 1.23, $p=0.43$) and sTfR index (0.63, 0.50 and 0.78 versus 0.63, 0.53 and 0.70, $p=0.49$) did not differ significantly between cases and controls. CRP values were significantly higher (1.48, 0.84 and 3.06 versus 0.57, 0.34 and 1.09 mg/l, $p=<0.001$) in patients compared to controls.
Conclusions. Our study supports the hypothesis that higher body iron stores, measured by serum feritin, especially in male population, are associated with risk for CAD. But, in view of higher CRP levels in cases, we concluded that inflammation was present that might have been responsible for elevated ferritin levels. sTfR concentrations were not related with development of CAD.

0213

ACUTE MYOCARDIAL INFARCTION: SIGNIFICANCE OF THE DETERMINATION OF SERUM COPEPTIN

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Background. It 's essential to quickly rule out the diagnosis of AMI in the ER. Currently, the ECG and determination of Troponin T are the first diagnostic approach for patients with chest pain or other warning signs of heart attack. The determination of Copeptin, c-terminal portion of pro-hormone vasopressin, a new endogenous marker of stress may be useful for a faster and reliable diagnosis.
Methods. 191 patients were selected from the Emercency DepartmentSan Paolo Hospital with diagnostic doubt for myocardial infarction and hospitalized in Critical Observation and Emergency Medicine. The settlement provided for research protocol at the time O' the determination of Copeptin (chemiluminescent immunoassay method of the company Brahms), Troponin T (Roche Diagnostics), ECG and Ecocardiography and the repetition of Troponin T and ECG after 3 and 6 hours.
Results. 93/191 (48.6%) of Copeptin measured were lower than the threshold value of 14.1 pmol / L; 88/93 (94.6%) had a Troponin T <0010 microgr / L with a nonspecific ECG. 100% did not develop AMI. Conversely the remaining 98 (51.3%) with a value> 14.1 pmol / L, 11 patients (11.2%) showed an increase in subsequent determinations of Troponin T and a pathogical ECG.
Conclusions. These preliminary data indicate that the association of negative Troponin T and negativeCopeptin, at the first blood assay, has a high negative predictive value in the exclusion of AMI.

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Cardiovascular disease

0214

GALECTIN-3, A CARDIAC REMODELING BIOMARKER, IS RELATED TO HEART FAILURE SEVERITY

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Background. Congestive heart failure (CHF) is associated with the activation of several neurohormonal systems and the release of cytokines. Galectin-3 is a carbohydrate-binding lectin emerging as an important mediator for the development of fibrosis and cardiac remodeling. We therefore determine circulating Galectin-3 concentrations in severe CHF patients as well as its relationship with other well-established biomarkers of CHF.
Methods. Eighty one fully treated CHF patients (mean age: 68 ± 13 years; ejection fraction $23 \pm 6\%$, NYHA II-IV) were included. Galectin-3 levels were determined using an optimized enzyme-linked immunosorbent assay (ELISA) (Galectin-3 assay; BG Medicine, Waltham, MA, USA). Reference values for Galectin-3 were confirmed with 25 healthy controls. Circulating levels of Nt-proANP, BNP, Nt-proBNP and Big-Endothelin 1 (Big-ET1) were also measured.
Results. In comparison to controls, Galectin-3 levels were significantly increased in CHF patients (geometric mean [95% CI]): 18.4 ng/mL [7.9-49.6] vs 10.3 [4.7-18.9]; $p<0.0001$). CHF patients classified as NYHA class II have Galectin-3 values (16.0 [7.9-42.3]) lower than those classified as NYHA class III (19.8 [8.0-45.5]; $p=0.01$) and than NYHA class IV (23.1 [14.3-49.6]; $p=0.01$). In our study cohort, Galectin-3 was significantly correlated with age ($r=0.35$), creatinin ($r=0.71$), ejection fraction ($r=0.71$), Nt-proANP ($r=0.53$), BNP ($r=0.36$), Nt-proBNP ($r=0.50$) and Big-ET1 ($r=0.36$).
Conclusions. Galectin-3 levels are increased in CHF, are related to severity parameters and are significantly correlated with cardiac biomarkers involved in the neurohormonal activation of CHF. Therefore, elevated Galectin-3 circulating concentrations, reflecing ongoing cardiac fibrosis and remodeling, may express the progression of CHF.

0215

EVALUATION OF UROCORTIN AND COPEPTIN, TWO STRESS RELATED PEPTIDES, IN PATIENTS ADMITTED TO EMERGENCY DEPARTMENT

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Background. Urocortin (UCN) and Copeptin (COP) are two peptides related to cardiovascular stress with circulating concentrations increased in cardiovascular disorders. The aim of our study was to determine the circulating levels of UCN and COP in patients admitted to the emergency department (ED) with suspected cardiac diseases and to evaluate the potential relationship between UCN, COP and established cardiac biomarkers.
Methods. Circulating levels of UCN and COP were measured in 77 ED patients (35 women, 42 men; mean age: 67 yrs) through specific immunoassays. Levels of Troponin I (TPNI), hsCRP, BNP and Nt-proBNP were also determined.
Results. Forty three patients were classified as cardiac. In the whole cohort, 19 patients (25%) had UCN values higher than the cut-point of 12.5 pg/mL and 32 patients (42%) had COP values higher than the cut-point of 20 pM. UCN mean values were not different between cardiac patients (18.2 pg/mL) and non-cardiac patients (17.1 pg/mL). COP values were significantly increased in cardiac patients (27.7 pM) in comparison to non-cardiac patients (13.3 pM; $p<0.05$). No significant correlations were observed between UCN, COP and other established cardiac biomarkers.
Conclusions. UCN levels are reported to be increased in myocardial infarction and congestive heart failure but no difference was observed in our study between cardiac and non cardiac patients. In contrast, COP levels were significantly higher in cardiac patients, as previously described in the literature. Nevertheless, the clinical value of Copeptine testing remains to be confirmed in larger studies and its cost-effectiveness should also be determined.

Cardiovascular disease

0216

CONTRIBUTION OF NAD(P)H OXIDASE P22 PHOX AND ENOS GENE POLYMORPHISM IN THE PREDISPOSITION OF EARLY ONSET ACUTE MYOCARDIAL INFARCTION IN EGYPTIAN POPULATION

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Background. C242T polymorphism of the p22 phox gene, an essential component of NAD(P)H oxidase in vasculature, and Glu298Asp polymorphism of endothelial nitric oxide synthase (eNOS) gene have been recently implicated as genetic markers for acute myocardial infarction (AMI) in many studies. The aim of this study is to collect information about the prevalence of these two polymorphisms and their relationship with occurrence of early onset AMI in Egyptian populations.
Methods. The study subjects consisted of 104 AMI patients and 101 aged matched volunteers. Genotyping of both genes was done by the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method.
Results. The genotype distribution for NAD(P)H oxidase *p22 phox* gene was significantly different between AMI patients (CC;41.3%, CT;58.7%, TT;0%) and control subjects (CC;27%, CT;72%, TT;1%) (P=0.026), whereas eNOS gene distribution was not significantly different between AMI patients (GG;49%, GT;45.2%, TT;5.8%) and control subjects (GG;58.4%, GT;33.6%, TT;7.9%) (P= 0.367).
Conclusions. The prevalenceof the CT+TT genotype of the C242T polymorphism was significantly more frequent in control subjects than in AMI patients. Our observations suggest that C242T polymorphism of the *p22 phox* gene of NAD(P)H oxidase may reduce susceptibility to AMI and is a novel genetic marker that has a protective effect on coronary riskwhile no significant association was observed between the Glu298Asp polymorphism of eNOS gene and incidence of AMI in this Egyptian population group.

0217

IMMUNOTURBIDIMETRIC ASSAY KIT FOR THE RAPID MEASUREMENT OF HEART-TYPE FATTY ACID BINDING PROTEIN

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Background. Heart-type fatty acid-binding protein (H-FABP) is a small cytoplasmic protein, involved in lipid homeostasis. It is abundant in heart muscle and is an early, highly sensitive marker of myocardial injury. As such, its measurement provides early diagnostic information, which is relevant for patient management. In this context, the availability of analytical tools enabling the rapid detection of H-FABP represents an advantage in clinical settings where a short time span for assessment is critical.We report an immunoturbidimetric assay kit applicable to automated analysersfor the quantitative determination of H-FABP in serum.
Methods. The principle of the assay is immunoturbidimetric. A latex agglutination complex (read at 700nm) is formed between H-FABP and antibody coated latex particles. Initial evaluation of analytical parameters was performed on the automated analyser RX Daytona. This system requires 8μl of neat sample, generates the first result after 14 min and includes dedicated software for data management.
Results. Sensitivity of the assay was <2.5ng/ml with linearity up to 120.0ng/ml. The within-run precision and total precision, expressed as %CV, were typically <3% and <5% respectively. Correlation of the immunoturbidimetric assay with a commercially available biochip based immunoassay was performed on 59 serum samples. Linear regression on the resulting data generated an r^2 value of 0.944.
Conclusions. The data generated on the RX Daytona indicates that this immunoturbidimetric assay kit for the measurement of H-FABP is applicable to automated analyzers and facilitates the rapid assessment of early myocardial ischemia in clinical settings.

Cardiovascular disease

0218

GENERATION OF RECOMBINANT ANTIBODIES TO NITROTYROSINE TO FACILITATE THE IDENTIFICATION OF NOVEL PROTEIN BIOMARKERS OF ATHEROSCLEROSIS

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Background. Today, several risk factors, like diabetes and smoking, help to identify individuals at high risk of developing coronary artery disease. However, there is still a high demand for the identification of novel biomarkers to improve the early diagnosis of acute coronary events and to identify pre-symptomatic patients at high risk for coronary events. Inflammatory processes play a pivotal role in the progression of atherosclerotic plaques, and we hypothesize that proteins in the plaque are modified and released into the blood stream. Possible posttranslational modifications include proteolytic cleavage and oxidation of amino acids, such as the formation of nitrotyrosine.
Methods. Antibodies against nitrotyrosine – in a protein context – were isolated from large antibody phage display libraries.
Results. Six different antibody specificities were generated, but recognition of the modified amino acids depended on the composition of the neighbouring amino acids. Using affinity maturation by error prone PCR of the recombinant antibody gene, followed by novel selections, we have improved the specificities and affinities of one of these recombinant antibodies, which resulted in the two antibodies 47A7 and 47B1.
Conclusions. Recombinant antibodies were generated that specifically react with nitrotyrosine in protein contexts. We will now apply these anti-nitrotyrosine antibodies to detect nitrotyrosine-containing proteins that are specifically present in plasma samples from patients with atherosclerotic cardiovascular disease and acute coronary syndromes. Potential targets will be isolated, using the antibodies as baits, and identified by mass spectrometry. Furthermore, the anti-nitrotyrosine antibodies will be used to study and quantify the nitrotyrosine content of known plasma proteins.

0219

IDENTIFICATION OF P81 AS A POTENTIAL NOVEL BIOMARKER OF CORONARY ARTERY DISEASE

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Background. In previous decades, considerable progress has been made with clinical laboratory diagnostics and prognostics of heart disease. Nevertheless, there is a great medical need of cardiovascular biomarkers for 1) diagnosing acute vascular events (ACS, stroke, and aneurysm), 2) identifying presymptomatic patients at increased risk, and 3) monitoring the progression and regression of atherosclerosis.
Methods. Subtractive antibody phage display and mass spectrometry were applied to identify protein biomarkers that are released from tissue cultures of atherosclerotic lesions, so-called secretomes.
Results. Recombinant antibodies were isolated that recognize proteins that are specifically released by atherosclerotic tissue. Nine of the most promising antibodies were used as baits to isolate their target antigens from secretomes and these targets were identified by mass spectrometry. One of the antibodies recognized protein p81 and immunoblotting confirmed that p81 is present in atherosclerotic secretomes, but not in control secretomes. The plasma concentration of protein p81 was then analyzed by immunoblotting in a small cohort of ACS patients (n=12), stable CAD patients (n=15) and angiographically confirmed CAD-free controls (n=15). The median plasma concentration of p81 was 2-fold elevated in the stable CAD group in comparison with the control group. Furthermore, the median plasma p81 concentration was 6- and 14-fold elevated in the ACS group in comparison with the stable CAD and the control groups, respectively.
Conclusions. Protein p81 is a potential novel biomarker of ACS and stable CAD, and will be further elevated in larger patient cohorts.

Cardiovascular disease

0220

MULTIPLE BIOMARKERS OF CARDIAC INJURY IN DETECTION OF CARDIOTOXICITY ASSOCIATED WITH HEMATOPOIETIC CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCIES

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Background. Assessment of cardiotoxicity during hematopoietic cell transplantation (HCT) with multiple biomarkers of cardiac injury – myoglobin, creatine kinase MB (CK-MB mass), cardiac troponin I (cTnI), heart-type fatty acid binding protein (H-FABP), glycogen phosphorylase BB (GPBB). Experience with new perspective cardiac biomarkers (GPBB, H-FABP) in this setting is very limited.
Methods. 53 patients (mean age 49.9±12.3 years, 33 males) transplanted for various hematological malignancies were studied. Cardiac biomarkers were measured the day after completion of preparative regimen (PR) and the day after HCT. Values above the reference range recommended by the manufacturer (Randox) were considered elevated.
Results. We found significant elevations in GPBB (above 7.30μg/L) in 8 (15.1%) patients after PR and in 9 (17.0%) after HCT. H-FABP increased slightly above the cut-off (4.50μg/L) after HCT in 1 (1.9%) patient. Other cardiac biomarkers remained within the reference range in all patients. We found a significant correlation between elevation in GPBB and diastolic left ventricular (LV) dysfunction on echocardiography (r=0.603; p<0.0001). No patient manifested clinical cardiotoxicity in the peritransplant period.
Conclusions. Our results suggest that administration of PR followed by HCT could be associated with myocardial injury manifested by increased release of GPBB from cardiomyocytes, which could correlate with diastolic LV dysfunction on echocardiography. Whether these acute changes will have predictive value for development of cardiomyopathy in the future is not clear and will be evaluated during a prospective follow-up. Further studies in a larger number of patients will be needed.

0221

CHELATE- AND NANOPARTICLE-BASED IMMUNOASSAYS FOR D-DIMER

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Background. D-dimer is a degradation product of fibrin that is secreted into circulation in pathological conditions like venous thromboembolism. Our objective was to develop fluorescent nanoparticle- and chelate-based immunoassays for D-dimer and to compare their performance against a commercial immunoturbidimetric assay.
Methods. The developed immunoassays exploited D-dimer specific antibodies either labeled with fluorescent europium(III)-chelate or attached onto internally dyed europium(III)-chelate polystyrene nanoparticles. The assays were performed in streptavidin microtitration wells coated with a biotinylated monoclonal capture antibody. Both assays were performed as sandwich-type format: the chelate-based assay as a one-step assay and the nanoparticle-based assay as a one-step assay with preincubation of the nanoparticles and the sample. A sample panel (n=65) of hospital leftover citrated plasma samples was assayed with both methods and the signal from the assays was measured with a plate fluorometer.
Results. The analytical and functional detection limits for the nanoparticle-based assay were 0.148 ng/ml and 11 ng/ml and for the chelate-based assay 0.027 ng/ml and 4 ng/ml. Both assays showed linear assay response to at least 5000 ng/ml. The concentrations obtained from the plasma samples correlated well with the immunoturbidimetric comparison assay. Correction factors were used for the results to be reported as fibrinogen equivalent units (FEU). Correlation coefficient and correction factor for the nanoparticle-based assay were 0.90 and 19 and 0.94 and 11 for the chelate-based assay.
Conclusions. The developed immunoassays enable rapid measurement of D-dimer and demonstrate good and equivalent performance characteristics compared to each other and a commercial reference assay.

Cardiovascular disease

0222

PROGNOSTIC VALUE OF HIGHLY SENSITIVE CARDIAC TROPONIN T MEASUREMENTS IN PATIENTS WITH ACUTE DYSPNEA

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Background. Recent studies have shown that previously undetectable cTnT levels, which can now be measured with the hs-cTnT assay, proved to be of prognostic value in stable coronary disease and heart failure. The highly sensitive assay may also improve prognostic power of cTnT in acute dyspnea.The purpose of this study was to investigate whether highly sensitive cardiac troponin T (hs-cTnT) improves risk stratification in patients presenting to the emergency department with dyspnea.
Methods. We prospectively studied theprognostic value of hs-cTnT for both early and long-term mortalityin 678 consecutive patients presenting to the emergency department with dyspnea.
Results. cTnT levels were measurable in 648 patients (95.6%) with the hs-cTnT assay, whereas only 331 patients (48.8%, P<0.001) had detectable cTnT levels with the conventional assay. Hs-cTnT was strongly associated with both early and long-term mortality, independent of other known clinical risk factors including NT-proBNP. Importantly, in patients with undetectable cTnT, hs-cTnT still had significant prognostic accuracy. The cut-off defining cardiac troponin T elevations for hs-cTnT of 0.016 μg/L showed excellent sensitivity (96%) and negative predictive value (98%), which could not be achieved with the conventional assay cut-off of 0.03 μg/L (65% and 93%, respectively).
Conclusions. Our data show that cardiac troponin T levels measured by the hs-cTnT assay have important prognostic value in patients presenting to the emergency department with acute dyspnea. Moreover, for clinical decision making, the hs-cTnT assay enables a better identification of subjects with a very low risk of early and long-term mortality compared to the conventional assay.

0223

TACHYPACING AND ANOXIA INDUCE THE RELEASE OF DIFFERENT FORMS OF CARDIAC TROPONIN

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Background. The Cardiac troponins (cTnI and cTnT) are important markers for the detection of myocardial damage, but it is unclear if cTn elevations represent necrosis or leakage from reversibly damaged cardiomyocytes. We hypothesize that cTn release can occur outside of cell-death and that the release patterns of various molecular forms of cTn differ in response to varying biological and chemical stimuli.
Methods. Cultured HL-1 cardiomyocytes were subjected to ischemia or mechanical stretch. Ischemia was modelled by anoxia and glucose deprivation and strenuous exercise by rapid electrical stimulation (tachypacing) at 3 Hz. After treatment, cTnT and cTnI concentrations were measured in the cell lysates and culture media. Different molecular forms of cTn are visualized using Western blotting.
Results. Ischemia and tachypacing induced a gradual decrease in the cTn content of the cells and this decrease exceeded that of the total protein content. After 8 hours of anoxia treatment the relative cTnT and cTnI content had dropped by 71% and 94%, respectively. After 8 hours of tachypacing the relative cTnT and cTnI content was decreased by 38% and 85%, respectively. Western blots of the tachypaced cells only showed intact cTnT (37 kDa) and cTnI (27 kDa), whereas anoxia treatment resulted in additional 28 kDa and 17 kDa degradation products for cTnT and cTnI, respectively.
Conclusions. Ischemic damage induces the formation and release of cleaved forms of cTn, whereas cTn remains intact upon Tachypacing (at 3 Hz). These findings suggest that different molecular forms of cTn are generated after different forms of cardiac damage.

Cardiovascular disease

0224

METALLOPROTEINASE PAPP-A AND OUTCOMES IN PATIENTS WITH ACS: INITIAL OBSERVATIONS FROM THE MERLIN-TIMI 36 TRIAL

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Background. Pregnancy associated plasma protein-A (PAPP-A) is a high molecular weight, zinc-binding metalloproteinase. Initial studies have suggested that PAPP-A is associated with vulnerable coronary plaque and may be a predictor of cardiovascular disease (CVD) and mortality. We investigated whether PAPP-A would be useful for risk assessment in patients presenting with NSTE-ACS.

Methods. We measured cardiac PAPP-A (Beckman Coulter DSL ELISA) at baseline in a randomly selected subset of 543 pts with NSTE-ACS randomized to ranolazine or placebo in the MERLIN-TIMI 36 trial. Patients were followed for an average of one year. The primary endpoint for this analysis was CV death, MI, or severe recurrent ischemia (RI).

Results. PAPP-A > 5.5 uIU/mL at presentation was associated with higher rates of the primary endpoint at 30 days (RR 3.3; 95% CI 1.6 – 7.7, p=0.001). Moreover, when stratified by baseline cTnI, PAPP-A remained associated with adverse CV outcomes at 30 days. At one year, PAPP-A was associated with higher rates of cardiovascular events including CVD/MI/SRI (HR 2.17; 95% CI 1.43 – 3.30), CVD or MI (HR 2.05; 95% CI 1.21 – 3.47), and severe RI (HR 2.16; 95% CI 1.17 – 3.96). After adjustment for cTnI, ST deviation, age, gender, diabetes, smoking, HTN, and history of CAD, PAPP-A remained independently associated with the risk of the primary endpoint at one year (adjusted HR 1.98; 95% CI 1.3 – 3.0, p=0.001).

Conclusions. PAPP-A was independently associated with short and long-term risk of recurrent cardiovascular events in patients with NSTE-ACS, adding to clinical predictors and cTnI.

0225

REDUCING AUTOANTIBODY INTERFERENCE IN CARDIAC TROPONIN I IMMUNOASSAY BASED ON FLUORESCENT NANOPARTICLES

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Background. Circulating autoantibodies (aabs) bound to the mid-fragment of cardiac troponin I (cTnI) have been shown to interfere with immunometric determination of cTnI. Our purpose was to investigate whether this kind of interference could be reduced in nanoparticle-based immunoassay by utilizing different combinations of capture antibodies and nanoparticle diameters.

Methods. Ternary troponin complex corresponding to final cTnI concentration of 0.5 μg/L was added to lithium-heparin plasma samples from volunteers previously determined to have high amounts of cTnI specific aabs in circulation. The recovery of cTnI was measured with a one-step, two-site immunoassay conducted in microtiter wells. The assay utilized two or three monoclonal antibodies as captures and a monoclonal antibody covalently conjugated to 68 nm or 107 nm Eu(III)-nanoparticles as a detector. The observed recoveries were normalized relative to the value obtained from aab-negative plasma.

Results. The analytical detection limits of the assays were below 2 ng/L (background + 3SD). The normalized cTnI recovery with two capture antibodies ranged between 15–40% with 107 nm, and 20–48% with 68 nm nanoparticles. With three capture antibodies the recoveries increased 2–14% and 10–38%, respectively. The maximum increase in the recovery values between the four assays was 14 percentage units on average.

Conclusions. Less interference from aabs was observed with smaller nanoparticles in combination with an additional capture antibody recognizing an epitope outside the mid-fragment. This was likely because of reduced steric hindrances relating to particulate label and aabs bound to cTnI.

Cardiovascular disease

0226

RELATION OF ADIPONECTIN AND RESISTIN TO HYPERTENSION AND OBESITY IN EGYPTIAN MALE PATIENTS

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Background. Hypertension affects more than 26% of adult Egyptians. Studies reported association of insulin resistance with hypertension. Adipose tissue is implicated in modulating insulin sensitivity by secreting leptin, adiponectin and resistin. This work aimed to evaluate the relation of adiponectin and resistin to hypertension and obesity in a cohort of Egyptian male patients.
Methods. The study included 50 male subjects subdivided into 4 groups: controls, non-obese hypertensives, obese hypertensives and obese normotensives. Clinical examination and anthropometric measurements were done. Serum glucose and lipid profile were evaluated using Konelab analyzer. Serum insulin, leptin, adiponectin and resistin were estimated using ELISA technique.
Results. Adiponectin was lower while resistin was higher in obese when compared to non-obese subjects (mean +/- SD :7.1+/-1.7 vs 16.4+/-4.8ug/ml for adiponectin and 40.6 +/- 4.2 vs 17.9 +/- 4.4 ng/ml for resistin in obese and non obese subjects respectively). Adiponectin was also lower in hypertensives(8.2 +/-4.9 ug/ml) when compared with normotensives of matched body adiposity (15.2+/-7.1ug/ml). Adiponectin negatively correlated with blood pressure, waste/hip ratio, insulin, insulin/glucose ratio, dyslipidaemia, resistin and leptin. Multivariate analysis and Odds ratio predicted hypoadiponectinaemia as an independent risk factor to develop hypertension with 4.57 folds risk more than normal adiponectin levels.
Conclusions. Hypoadiponectinaemia showed to be a prominent feature in obese subjects and in insulin resistant patients whether obese or not. Hyper-resistinaemia was also related to obesity. Hypoadiponectinaemia could be involved in the pathogenesis of essential hypertension by its significant correlations with risk factors as dyslipidaemia, obesity specially visceral type and hyperleptinaemia.

0227

25-HYDROXYVITAMIN D IN PATIENTS WITH CARDIOVASCULAR DISEASE AND ITS RISK FACTORS

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Background. Many recent studies reported that vitamin D deficiency was associated with cardiovascular disease (CVD) and also with several CVD risk factors such as HTN, diabetes, etc. This study compared the 25-hydroxyvitamin D (25(OH)D) between CVD group, several CVD risk groups, and a healthy control group.
Methods. A total of 150 patients selected by their diagnosis were grouped into 5 groups (CVD, dyslipidemia, hypertension, hypertension+diabetes, and control groups) and the demographic and clinical data were reviewed. 25(OH)D and other laboratory markers were measured and analyzed for the association with CVD and CVD risk factors.
Results. There was a significant difference of 25(OH)D (p=0.045) between groups, but adequate discriminatory function in the comparative study was not found between specific groups. In addition 25(OH)D did not have a meaningful effect on CVD development by regression analysis. Among the CVD risk factor such as sex, age, smoking, BMI, DM, HTN, dyslipidemia, and laboratory markers currently being used (lipid profile, homocysteine, hs-CRP, and carotid IMT), only in female, smoker, and dyslipidemia showed low levels of 25(OH)D.
Conclusions. There were no definite association between 25(OH)D level and CVD and between 25(OH)D and CVD risk factor such as DM and HTN unlike initial authors' hypothesis based on previous studies. But 25(OH)D levels have a meaningful correlation to smoking and dyslipidemia, showing a close relationship toward CVD risk factors and it seems that 25(OH)D is indirectly related to CVD by influencing dyslipidemia, rather than being directly related CVD or indirectly related to DM or HTN.

Cardiovascular disease

0228

HIGH CHOLESTEROL, HIGH TRIGLYCERIDES: INDICATORS OF HYPERTENSION-A POPULATION SURVEY

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Background. Hypertension is one of the major risk factors for cardiovascular mortality. The objective of the present study was to find out hypertension status and its associated risk factors among individuals aged 18 years and above in the study area including the hypertensive on treatment and excluding pregnant females.

Methods. A cross sectional population survey was conducted in Chandigarh using two stage stratified sampling technique in which a total of 1326 individuals participated. Fasting blood samples were collected and analysed for plasma glucose and lipid profile using standard protocols on Hitachi-902.

Results. The prevalence was 34.4% in urban areas and 38.2% in rural areas. Mean serum cholesterol was significantly higher (184.4±39.74 mg/dL) among the hypertensive group than the non-hypertensive group (161.90±33.34 mg/dL). Serum HDL was significantly lower (40.29±6.94 mg/dL) among the hypertensive than non-hypertensive (41.44 ±7.25 mg/dL), mean serum LDL was significantly higher (112.34 ± 38.54 mg/dL) among the hypertensive group than non-hypertensive group (96.90 ±31.63 mg/dL) respectively. High cholesterol levels (82.5%), high LDL levels (81.6%) and low HDL(57.3%) came out to be the major risk categories indicating hypertension. Also mean triglycerides 175.66± 82.56 mg/dL in the hypertensive was statistically significant(p<0.001).

Conclusions. Biochemical parameters including serum lipids and fasting blood glucose levels showed significant association with hypertension. Based on logistic regression analysis; age above 35 years, male population, middle/high socioeconomic status, alcohol consumption , BMI and family history of diabetes were found significant risk factors. Detection of subjects with pre-hypertensive stage itself may be source of motivation for them to lead a healthy life style.

0229

CHANGES IN ADMA AND TAFI AFTER STENTING IN CORONARY ARTERY DISEASE PATIENTS

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Background. This study aimed to examine the contribution of Asymmetric Dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase and a novel marker of vascular endothelial dysfunction and atherosclerosis, and Thrombin-Activatable Fibrinolysis Inhibitor (TAFI), a risk factor for venous thrombosis, in the predisposition of coronary restenosis after stent implantation in coronary artery disease (CAD) patients.

Methods. Thirty seven patients with CAD were recruited from the department of cardiology at Kobry El Obba Military Hospital, Cairo, Egypt. The patients were hospitalized for coronary angiography and coronary stenting (CS). Overnight fasting blood samples were collected from the patients before CS and four months later for the determination of plasma ADMA and TAFI levels by ELISA technique. The patients underwent follow up coronary angiography to reveal in-stent restenosis.

Results. Mean plasma levels of ADMA were shown to be significantly higher in CAD patients as compared with that reported for healthy subjects in previous studies. In addition, ADMA levels were shown to be significantly higher (p=0.01) by 30% in CAD patients four months after CS as compared to those before stenting. CAD patients who developed in-stent restenosis during angiographic follow-up (n=22) had 35% increase in ADMA levels (p=0.01) after CS. In non-diabetic CAD patients who developed in-stent restenosis (n=17), ADMA levels were higher by 19% (p=0.049) after CS. On the other hand, TAFI levels were not significantly changed after CS in CAD patients or any of the above subgroups.

Conclusions. ADMA, but not TAFI, is linked to the predisposition of in-stent restenosis after CS.

Cardiovascular disease

0230

DIFFERENTIAL ARACHIDONIC ACID METABOLISM IN HEALTHY SUBJECTS AFTER LPS WHOLE BLOOD ACTIVATION

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Background. Eicosanoids as metabolites of arachidonic acid are known to play key roles in promotion and inhibition of central inflammatory processes. Thus, the aim of our study was to investigate the individual eicosanoid response of healthy subjects on mediator and gene expression level as a potential marker of susceptibility for inflammatory diseases.

Methods. In preliminary experiments, human whole blood (lithium-heparin) from healthy subjects (n=10) was incubated with lipo-polysaccharide (LPS; 100ng) for 1, 4 and 24 hours. RNA was isolated and 6 target genes (*cyclooxygenase 1* (*COX-1*), *cyclooxygenase 2* (*COX-2*), *tromboxane synthase* (*TBXS*), *prostaglandin f synthase* (*PGFS*), *12-lipoxygenase* (*12-LOX*), *5-lipoxygenase* (*5-LOX*)) of arachidonic acid metabolism were analyzed by quantitative fluorogenic RT-PCR. Eicosanoids (11-hydroxy eicosatetraenoic acid (11-HETE), thromboxane b2 (TXB_2), prostaglandin e2 (PGE_2), prostaglandin f2α (PGF2α), 12-hydroxy eicosatetraenoic acid (12-HETE), 5-hydroxy eicosatetraenoic acid (5-HETE)) were analyzed in supernatants by LC-MS/MS (API 5500 QTrap, AB SCIEX).

Results. We found a time-dependent eicosanoid response on mediator and gene expression levels for all investigated pathways. Gene expression levels range from down-regulation of *TXS* (-80%, P<0.001) to a 30fold induction of *COX-2* (P<0.001). For COX-1 and COX-2 pathways, we observed a parallel increase in gene expression levels and mediator release. In contrast, eicosanoid response and gene expression of the TX and 5-LOX pathways were not coordinated suggesting regulatory mechanisms independent of regulation of gene expression.

Conclusions. Further work is necessary to investigate potential relations of gene expression, eicosanoid response and predisposition to inflammatory diseases such as coronary artery disease.

0231

CARDIAC TROPONIN IS DETECTABLE IN CARDIO-HEALTHY CHILDREN

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Background. There have been limited studies generating reference intervals from paediatric populations The lifestyle of our kids (LOOK) study investigates how physical activity contributes to health and development in a cohort of 830 school children. We have studied 727 cardio-healthy healthy children in this cohort on up to 3 separate occasions at ages 8, 10 and 12 years commencing in 2005.

Methods. Cardio health was defined by physical examination, echocardiography and high sensitivity troponin (hsTnT) measurement. hsTnT assays were performed on 548, 455 and 491 samples from the 2005, 2007 and 2009 cohorts respectively using the Roche E411analyser.

Results. Echocardiography was undertaken on these children and no abnormalities were noted. Troponin T was detected (>3 ng/L) in 80/548 (14.6%) in the 2005 cohort, 91/455 (20.0%) in the 2007 cohort and 65/491 (13.2%) in the 2009 cohort. 3/548 (0.5%), 4/455 (0.9%) and 4/491 (0.8%) in these respective cohorts had troponin concentrations greater than the 99th percentile of the manufacturer quoted reference interval. Troponin T was detected in 209/727 (28.7%) children on at least one occasion but only 3 children had detectable troponin on all three occasions.

Conclusions. Cardiac troponin has been demonstrated to be detectable in the cardio-healthy adult population. This study demonstrated that cardiac troponin is also detectable in cardio-healthy children. These data support the concept that troponin release is not necessarily associated with myocardial necrosis.

Cardiovascular disease

0232

CHANGES IN ADMA AND TAFI AFTER STENTING IN CORONARY ARTERY DISEASE PATIENTS

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Background. Pregnancy-associated plasma protein-A (PAPP-A) is a high-molecular-weight, zincbinding matrix metalloproteinase (MMP) belonging to the metzincin superfamily of MMP. It has been suggested a PAPP-A as a marker of atherosclerosis because circulating levels of PAPP-A were significantly elevated in patients with myocardial infarction. Framingham risk score (FRS) is widely being used in the early prediction of coronary artery disease (CAD). Autoantibodies (abs) to PAPP-A have never detected in patients with high FRS. Our objective was to examine the association between FRS and anti-PAPP-A abs in patients with lipidemic disorder.
Methods. 60 patients (35 male and 25 female)aged 46-78 were included in our study. The patients were divided into three groups: Group 1: patients with high FRS >20%, Group 2: intermediate FRS 10-20% and finally, group 3: low FRS <10%. Anti-PAPP-A IgG antibodies were determined by semi-quantitative ELISA (Immunculus, Moscow, Russia).
Results. From 60 patients 18 (30%) were positive for anti PAPP-A abs. 16 (88,9%) patients had high FRS (group 1). Two patients were positive from group 2 (11.1%) and finally, have never detected patiendts with anti PAPP-A abs positive in group 3 with low FRS.
Conclusions. Our results may indicate that in would be useful to determine anti PAPP-A abs in patients with lipidemic disorder. We are still investigating the issue with larger number of patients. We suggest further studies to determine the diagnostic accuracy of anti PAPP-A abs in cardiovascular diseases.

0233

INVESTIGATION OF THE PRESENCE AND PROGRESSION OF CORONARY HEART DISEASE BY ^{1}H-NMR-BASED LIPIDOMIC PROFILING OF PLASMA LIPOPROTEINS

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Background. The pathogenicity of coronary heart disease (CHD) is centered on lipid metabolism and its metabolic pathways. ^{1}H-NMR-based lipidomic analysis is a non-invasive approach that provides information on the presence of lipid molecules and gives insight into possible mechanisms of the onset and progression of CHD.
Methods. Serum samples from 159 patients with angiographically determined coronary heart disease [30 patients with one (mild), 29 with two (moderate) and 40 with triple (severe) vessel disease and 60 patients with normal coronary arteries (NCA)] were collected after an overnight fast. Lipid content of the HDL and non-HDL lipoproteins was extracted according to a standard procedure. In addition to the conventional biochemical analysis, pattern recognition analysis was applied on the ^{1}H-NMR HDL and non-HDL lipidomic data recorded on a Bruker DRX-600 Spectrometer.
Results. The ^{1}H NMR-based analysis revealed significant different HDL and non-HDL lipidomic profiles for patients with CHD and those with NCA. Moreover, distinct pair-wise profiles were observed among patients at any disease state (mild, moderate and severe). The onset and the progression of CHD are mainly characterized by gradually increased levels of saturated fatty acids, number of fatty acids and decreased levels of unsaturated fatty acids, degree of unsaturation, total omega-3 fatty acids and phospholipids (especially phosphatidylcholine and sphingomyelin) in plasma lipoproteins.
Conclusions. ^{1}H NMR-based lipidomic profiling of atheroprotective and atherogenic lipoproteins could provide non-invasive lipid biomarkers that characterize not only thepresence but also the severity ofdisease with a possible important prognostic significance.

Cardiovascular disease

0234

EXPORT OF GLUTATHIONE-S-CONJUGATES BY ERYTHROCYTES OF PATIENTS WITH ISCHEMIC HEART DISEASE

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Background. Search of blood cells markers that may reflect pathological changes of myocardium is very actual task of modern cardiology. In the present work we examined the export glutathione-S-conjugates from erythrocytes of patients with ischemic heart disease (IHD) by using 1-chloro-2,4-dinitrobenzol (CDNB) as xenobiotic.
Methods. Investigations were performed on donor and IHD erythrocytes. In this study the export of glutathione-S-conjugates from erythrocytes was evaluated by Board method using spectrophotometry at 340 nm.
Results. IHD patients were divided into two groups. First group (n=10) was included patients (mean age 53,7±8,2; all male) with preserved contractile function of myocardium (left ventricular ejection fraction – LVEF > 45%). Second group (n=12) was included patients (mean age 56,4±8,9; all male) with pronounced systolic myocardial dysfunction (LVEF < 45%). Yield of glutathione-S-conjugates was shown to occur differently in erythrocytes from IHD patients of two groups under study. Export of glutathione-S-conjugates of IHD erythrocytes from first group was lower on 15% as against donor erythrocytes. Export of glutathione-S-conjugates of IHD erythrocytes from second group was higher on 20% when compared with donor cells. Obtained results show that in erythrocyte of IHD patients the yield of glutathione-S-conjugates from erythrocytes depends from the extent of myocardium dysfunction.
Conclusions. Data about different yield of glutathione-S-conjugates from erythrocytes of IHD patients have significance in development of clinical methods for determination of the extent of myocardium dysfunction.

0235

USE OF A NOVEL HIGH-SENSITIVITY TROPONIN T, I AND MPO, NT-PROBNP ASSAYS TO DETECT MYOCARDIAL INJURY IN PATIENTS WITH ATRIAL FIBRILLATION TREATED BY DIRECT-CURRENT CARDIOVERSION

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Background. Novel high-sensitive cardiac troponin T (hsTnT) and I (TnI II) assays have the potential to detect myocardial injury with a higher sensitivity. The aim of the study was to assess the level of hsTnT and TnI II in patients with atrial fibrillation (AF) as compared to control and following direct current cardioversion. Levels of NT-proBNP, myeloperoxydase (MPO) and hs-CRP were concomitantly measured.
Methods. HsTnT, NT-proBNP, hs-CRP and TnI II determinations were realized on heparin plasma of 27 patients with AF successfully treated by cardioversion and 64 control subjects. MPO quantification was performed on the EDTA plasma samples. All assays were performed before (T0) and 4 hours (T+4h) after cardioversion.
Results. The levels of hsTnT and TnI II were increased in patients with AF compared to controls (p<0.005). Between T0 and T+4h, we observed an increased of TnI II (p=0.36) but not for hsTnT (p=0.5). Cardioversion was not associated with any statistically change in hsTnT and TnII levels. AF patients also had higher NT-proBNP level than controls (p<0.001) and increased level of hs-CRP (p=0.08). MPO level was not increased compared controlled nor between T0 and T=4h.
Conclusions. Our results showed that patients with persistent AF had increased plasmatic concentration of hsTnT and TnI II reflecting the presence of myocardial damage that was not further modified by cardioversion. In our population, AF was associated with increased level of NT-proBNP and sign of inflammation as reflected by elevated hs-CRP plasmatic concentration. MPO assays cannot be used in this case.

Cardiovascular disease

0236

ELEVATION OF CARDIAC AND OXIDATIVE STRESS BIOMARKERS AFTER A RUNNING ACTIVITY IN SEDENTARY SUBJECTS

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Background. We studied the kinetic of serum cardiac troponin and oxidative stress biomarkers [highly sensitive troponin T(hsTnT), highly sensitive troponin I (TNI II), NT-proBNP, myeloperoxydase(MPO), reduced glutathione (GSH), oxidized glutathione(GOX) and lipid peroxide (POXL)] in 15 sedentary subjects submitted to a running activity.
Methods. Venous blood samples of 15 young men were collected just before (T1) and after (T2) exercise, 3 hours (T3) and 24 hours (T4) after exercise for the determination of cardiac and oxidative stress biomarkers. The exercise consisted in a1-hour race on a treadmill at 75%VO2max. The VO2max of each participant was determined one week before the test.
Results. After the race, NT-proBNP, hsTNTand MPO increased during three hours post aerobic effort. NT-proBNP significantly increasedfromT2 to T4 compared to T1 (p<0.01).Seventy percent of subjects significantly overtook (p<0.05) the cut-off forhsTnT atT3 compared to other times.MPOslight increased at T2 but not statistically. POXLshowed moderate (not significant) increased values at T4. GOX significantly increased(p<0.05) at T2 compared to T1. There was no variation for GSHyet the ratio GSH/GOX-wasincreased (p=0.07) at T4 compared T2.
Conclusions. This work enabled us to deduce that the aerobic exercise produces an oxidative stress which continues in the 24 hours following the effort. No antioxidant adaptation was observed. Cardiac markers showed acardiac stress in the 3 hours following exercise.

0237
FATTY ACIDS AND CARDIOVASCULAR RISK

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Background. A fatty acid (FA) is a carboxylic acid with a long aliphaticchain, which is either saturated or unsaturated. Recently, the role of FA and particularly omega-3 and -6 has emerged as cardiovascular risk factor in the literature. The aim of our study was to establish reference value for these FA and to compare these results with data obtained in acute myocardial infarction (AMI) patients.
Methods. Fifty four healthy subjects were selected as reference population. We also evaluated FA in 33 patients admitted in emergency department for AMI. The fasting whole blood was drawn in vacutainer containing EDTA. Before the analysis, the samples were washed and transmethylated. We performed the quantification of different FA by gas chromatography associated with flame ionization detector (GC/FID).
Results. For the AMI group, the level of omega-6 were significantly higher (p<0.01) for C18:2n6 and C18:3n6. The level of omega-3 was significantly decreased (p<0.01) in comparison with reference value for C22:6n3. The omega-3 index was significantly lower (p<0.01) in AMI group compared reference value and the ratio omega-6/omega-3 was significantly higher (p<0.01) in AMI than reference patient.
Conclusions. We have established reference value for FA and compare them with the FA determination in AMI group. It is a new tool we are able to use and to process in our laboratory which can help clinician to highlight patients with the most cardiovascular risks seen the protector roleof polyunsaturated FAparticularly, omega3, and the implication of satured FA in the development of atherosclerosis.

Cardiovascular disease

0238

ROBUST DETERMINATION OF REFERENCE RANGES FOR MYELOPEROXIDASE AND TROPONIN-I ON THE ARCHITECT SYSTEM

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Background. Large, healthy populations are needed to accurately define the upper reference limits (URLs) for cardiac biomarkers. It is often difficult for individual laboratories to establish their own URLs due to the limitation of collecting a statistically significant number of healthy volunteers.

Methods. EDTA samples (n=1406, 700 female and 706 males, age >50) from the SAPALDIA cohort study (Swiss Study on Air Pollution and Lung Diseases in Adults), were collected in 2002 and stored at -80°C until biomarker assessment in 2010. ARCHITECT *STAT* Troponin I (TnI) and Myeloperoxidase (MPO) assays were used to determine Swiss population specific URLs, as well as stratification by age and gender.

Results. The overall mean and 99th percentile were 68 pmol/L and 236 pmol/L for MPO and 0.0013 ng/mL and 0.015 ng/mL for TnI. The MPO values were well within the measurement range of the ARCHITECT assay (LoD <20.0 pmol/L and the functional sensitivity at 20% CV <50.0 pmol/L). The values for TnI were within the measurable range but with greater uncertainty (LoD= 0.009 ng/mL, functional sensitivity at 10% CV= 0.032 ng/mL and 99th percentile at 0.028 ng/mL). There was no dependency on either age or gender for both biomarkers.

Conclusions. The URLs were robustly determined for both MPO and TnI in a large (>1400), well-defined cohort of the Swiss general population aged older than 50 years which is representative of the population typically evaluated for cardiac disease. This study contributes to provide a better estimate of the normal population reference range.

0239

EVALUATION OF THREE CARDIOVASCULAR BIOMARKERS USING THE B·R·A·H·M·S KRYPTOR SYSTEM

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Background. Midregional pro-atrial natriuretic peptide (MR-proANP), midregional pro-adrenomedullin (MR-proADM) and C-terminal pro Arginine Vasopressin (Copeptin) have been shown to improve the diagnostic and prognostic accuracy in patients with cardiovascular or respiratory pathology. These biomarkers were evaluated using the B•R•A•H•M•S Kryptor system.

Methods. Excess EDTA patient plasma were pooled into 4 different concentrations to measure the precision of the assays over 10 days; the highest concentrations were also used to determine any carry-over. Linearity spanning the direct measuring ranges were evaluated. The median and upper referece limits were established with excess blood donor EDTA samples. All tests were carried out according to manufacturer's instructions.

Results. The inter-assay (intra-assay) CV of MR-proADM ranged from 6.9% (12.7%) at 0.2 nmol/L to 2.3% (1.6%) at 5 noml/L; MR-proANP ranged from 3.3% (4.9%) at 18 pmol/L to 1.1% (2.3%) at 620 pmol/L; Copeptin ranged from 13.6% (7.4%) at 11.7 pmol/L to 2.2% (1.2%) at 220 pmol/L. No carry-over was observed at these concentrations. The recoveries of the assays were between 95% and 104% in the ranges of 0.3-4.7 nmol/L (MR-proADM), 25-450 pmol/L (MR-proANP) and 7-580 pmol/L (Copeptin). The median values, derived from 40 blood donors, for MR-proADM, MR-proANP, and Copeptin were: 0.37 nmol/L (0.55 nmol/L, 95th percentile), 29.4 pmol/L (54.9 pmol/L, 97.5th percentile), and 6.6 pmol/L (18.9 pmol/L, 97.5th percentile), respectively.

Conclusions. The performance of the assays in this study appear to meet the manufacturer's claim. The upper reference limits derived from our blood donors also concurred with those provided by the manufacturer.

Cardiovascular disease

0240

VASCULAR DAMAGE'S MARKERS IN GLAUCOMA PATIENTS

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Background. Recently, vascular risk factors show an increasing significance due to its association with glaucoma pathogenesis. For this reason, two blood markers of vascular damage, homocysteine and endotheline-1 (ET-1), have been determined in 48 patients affected with primary open-angle glaucoma (POAG) and in 13 patients affected with normal tension glaucoma (NTG). Results from glaucoma patients were compared with the data obtained in 78 healthy controls (C).
Methods. Patients were selected and evaluated in the Ophthalmology Department. Cold EDTA plasma and serum samples were collected. Homocysteine levels were determined by solid-phase competitive chemoluminiscence enzyme assay (Architect, Abbott). Enzyme immunoassay (Biomedica) was used to determine ET-1 levels. ANOVA and Pearson tests were used for statistical treatment of data.
Results. The levels of the two markers in the different groups of study were as follows: Homocysteine (POAG: 7.56+/-2.05 umol/L; NTG: 6.40+/-2.47 umol/L; C: 5.94+/-2.57 umol/L) (p=0.002) and ET-1 (POAG: 5.4+/-5.3 fmol/mL; NTG: 4.1+/-2.9 fmol/mL; C: 2.9+/-2.4 fmol/mL) (p=0.002). In the NTG group, a statistically significant correlation between both markers was found (r=0.928; p=0.028). However, this correlation was not found in the POAG and control groups.
Conclusions. An increase in the levels of both markers, homocysteine and ET-1, is found in patients affected either with open-angle glaucoma and normal tension glaucoma, indicating vascular damage in both pathologies. However, correlation studies suggest different mechanisms implicated in the appearance and evolution of each type of glaucoma.

0241

LEFT VENTRICULAR MASS INDEX AND COLLAGEN METABOLISM BIOMARKERS IN ESSENTIAL HYPERTENSION

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Background. This study was designed to evaluate the association between circulating biomarkers of collagen metabolism and fibrosis in serum and elevated left ventricular mass index (LVMI) in patients with essential hypertension.
Patients. Fifty-two patients with essential hypertension were included and compared with twenty-four healthy individuals. Left ventricular mass was measured, and LVMI was calculated using a validated formula: LVMI= 1.04 x 0.8 [(left ventricular wall thicknesses + internal dimension) – (internaldimension)] + 0.6 g. The biomarkers of collagen synthesis (carboxy-terminal propeptide of procollagen type I; PICP), degradation (matrix metalloproteinase 1; MMP-1) and fibrosis (transforming growth factor beta1; TGFβ1) were analyzed by ELISA
Methods. The biomarker of collagen degradation (C-terminal telopeptide of collagen type-I, ICTP) was analyzed by electrochemiluminescence immunoassay (ROCHE diagnostic).
Results. Compared with controls the levels of PICP was higher and lower levels of ICTP and MMP-1 when LVMI was elevated in the 2 groups of hypertensive patients (p<0.005). No statistically significant was found in levels of TGFß1 (p=0.160).
Conclusions. These findings suggest that, in this group of hypertensive patients, collagen synthesis type I predominates over degradation because of elevated PICP levels and reduced ICTP and MMP-1 levels, basically in patients with the highest LVMI. However we don't find elevated TGFβ1levels.

Cardiovascular disease

0242

CORRELATION BETWEEN BASIC LIPID PARAMETERS AND SOME LABORATORY MARKERS OF ENDOTHELIAL DYSFUNCTION IN ASYMPTOMATIC HYPERCHOLESTEROLEMIA

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Background. Atherosclerosis, also known as arteriosclerotic vascular diseaseis the leading cause of death in the developed world. Endothelial dysfunction is the earliest functional abnormally of the vascular wall in the development of the atherosclerosis. Finding additional, reliable markers for the detection of endothelial dysfunction in asymptomatic hypercholesterolemia patients is an important element of modern diagnostic strategy.

Methods. Plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and asymmetric dimethylarginine (ADMA) were detected by immunological assays (ELISA) in 70 patients with asymptomatic hypercholesterolemia without any cardiovascular risk factors and 70 gender- and age matched controls. For evaluation of biochemical parameters were used routine Methods.

Results. Comparison between asymptomatic hypercholesterolemia patients and control group of sVCAM-1 and ADMA plasma levels demonstrate statistically significant differences respectively 768.08 ng/ml ± 25.5 ng/ml toward 346.71 ng/ml ± 80.38 ng/ml for sVCAM-1 and 1.66 μmol/l ± 0.44 μmol/l toward 0.51 μmol/l ± 0.10 μmol/l for ADMA. We established statistically significant ($p < 0.01$) linear correlation of serum levels of sVCAM-1 and ADMA versus total cholesterol and LDL-cholesterol and statistically significant reverse correlation ($r = -0.378$; $p < 0.05$) between HDL-cholesterol and sVCAM-1 levels. Positive linear correlation between sVCAM-1 and ADMA serum levels ($r = 0.812$; $p < 0.01$) was found. The multiple stepwise regression analysis shows the remarkable influence of total Cholesterol and LDL-Cholesterol on the ADMA levels ($r_{xy} = 0.887$; $F = 252.48$; $p < 0.01$).

Conclusions. ADMA and sVCAM-1 are the most proper markers for evaluation of the endothelial dysfunction in asymptomatic hypercholesterolemia.

0243

PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE EVOLUTION OF HEMOGLOBIN IN PATIENTS WITH HEART FAILURE

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Background. In patients with chronic heart failure (CHF), decreases in hemoglobin concentrations and anemia are frequent and are associated with a worse prognosis. The objectives were to study the laboratory parameters which best reflect the evolutive changes in hemoglobin concentration in patients with CHF.

Methods. 59 outpatients in stable condition (54±14 years, 83% male) with CHF (ejection fraction of left ventricle of 28±10%), without anemia (WHO definition) and without previous blood transfusions were studied. Blood samples were obtained on inclusion and after 12 months follow up. Changes in haematological parameters were studied together with their correlation with the following parameters: iron metabolism (ferritin, iron, transferrin, soluble transferrin receptor, hepcidin), inflammation (C-reactive protein, soluble receptor tumor necrosis factor I (TNFRI), interleukin 6), cardiac function (B-type natriuretic peptide N-terminal (NT proBNP), troponin T), kidney function (creatinine) and lipid metabolism (cholesterol, triglycerides, high density lipoprotein cholesterol,*low density lipoprotein* cholesterol).

Results. At first hemoglobin concentrations were 14.7±1.5 g/dl and past 12 months were reduced significantly by a median of -0.4(-0.7 to -0.06) g/dl, p= 0.02 (interquertile range). This decrease in hemoglobin was significantly correlated with increased concentrations of TNFRI (r=-0.39, p=0.002) and hepcidin (r=-0.35, p=0.006,) but did not show any correlation with the evolution of the parameters of cardiac or kidney function.

Conclusions. In CHF patients without anemia, the evolution of hemoglobin is mainly determined by inflammatory parameters and iron metabolism but not by cardiac or kidney function.

Cardiovascular disease

0244

PREDICTORS OF ANEMIA EMERGENCE IN PATIENTS WITH CHRONIC HEART FAILURE

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Background. Anemia is very common in heart failure and is associated with greater morbidity and mortality in the follow-up period. The objectives were to evaluate the baseline laboratory parameters and changes in the monitoring involved in the development of anemia in patients with chronic heart failure (CHF).

Methods. 59 outpatients in stable condition (54±14 years, 83% male) with CHF (ejection fraction of left ventricle of 28±10%) and without previous blood transfusions were studied. Of the 59 patients, 8 (13%) had anemia (WHO definition). Blood samples were obtained at time of inclusion and after 12 months follow up. We studied parameters of iron metabolism (ferritin, iron, transferrin, soluble transferrin receptor, hepcidin), inflammation (C-reactive protein, soluble receptor tumor necrosis factor I (TNFRI), interleukin 6), cardiac function (B-type natriuretic peptide N-terminal (NT proBNP), troponin T) and kidney function (creatinine).

Results. On comparing non-anemic and anemic patients, we did not find any statistically significant differences regarding parameters of cardiac and kidney function. However, for iron metabolism and inflammatory parameters we found significant differences were detected: hepcidin values (-3.1±43.6 vs 59.2±53.5 (p=0.003)), ferritin (11.1±229.6 vs 128.6± 212.8 (p=0034)) and TNFRI (0.6±2.8 vs 1.9±2.5 (p = 0.008)) in the trace. Regarding the baseline parameters studied were not statistically significant differences in any of them.

Conclusions. In CHF patients with anemia, we observed an increased inflammatory state as evidenced by increased values of hepcidin and TNFRI in monitoring, but did not find on basal parameters compared to non-anemic patients.

0245

EVOLUTION OF FERROKINETICS AND INFLAMMATORY PARAMETERS IN PATIENTS WITH CHRONIC HEART FAILURE

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Background. To study the evolutionary changes of ferrokinetics and inflammatory parameters and observe their correlation with cardiac parameters in patients with chronic heart failure (CHF) for one year.

Methods. 59 outpatients in stable condition (54±14 years, 83% male) with CHF (ejection fraction of left ventricle of 28±10%) were studied. Blood samples were obtained at the study baseline and after 12 months of follow up. We studied the changes in parameters of iron metabolism (ferritin, iron, transferrin, soluble transferrin receptor, hepcidin), inflammation (C-reactive protein (CRP), soluble receptor tumor necrosis factor I (TNFRI), interleukin 6) and function heart (B-type natriuretic peptide N-terminal (NT proBNP), growth differentiation factor 15 (GDF15)).

Results. No statistically significant differences in ferrokinetics parameters and cardiac function. However we also found a significant increase in inflammatory markers such as TNFRI (p = 0,007) and CRP (p = 0.025). This increased inflammatory state correlated significantly with GDF15 (r = 0.35, p = 0.006,) but not with NT proBNP (r=0.10, p=0.424).

Conclusions. In patients with CHF, evolutionary changes during the monitoring year, were caused by inflammatory parameters, mainly the TNFRI and not by ferrokinetics parameters. We showed a correlation between inflammatory condition and cardiac parameters such as GDF15.

Cardiovascular disease

0246

CHARACTERIZATION OF INDIVIDUALIZED PROTEOMIC PROFILES IN ST-SEGMENT ELEVATION AND NON ST-SEGMENT ELEVATION ACUTE CORONARY SYNDROME

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Background. Acute Coronary Syndrome (ACS) is one of the main causes of morbidity and mortality in developed countries. Despite the research advances in recent years, ACS prevention and treatment strategies still suffer from significant limitations. The application of proteomics to ACS research constitutes an invaluable tool for the understanding of disease mechanisms and novel biomarkers and therapeutic targets identification. In this work, we focused in the establishment of an individualized proteomic profile in non ST-segment elevation ACS (NSTE-ACS) and ST-segment elevation ACS (STE-ACS) using two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MALDI-TOF/TOF).
Methods. 20 patients with NSTE-ACS, 20 patients with STE-ACS and 20 healthy controls were collected for this study. 2D-DIGE experiments were carefully designed for STES-ACS vs healthy controls (n=6) and for NSTE-ACS vs healthy controls (n=5). Previously, all plasma samples were depleted using a Multi Affinity Removal column (MARS Hu-14, Agilent Technologies).
Results. 24 differentially expressed spots were found in NSTE-ACS patients (12 upregulated, 12 downregulated) and 47 in STE-ACS patients (13 upregulated, 34 downregulated). At the moment, 28 proteins have already been identified and are being validated by inmunoblotting and/or selected reaction monitoring (SRM).
Conclusions. Our proteomic 2D-DIGE experiments demonstrate that STE-ACS and NSTE-ACS can be defined by different and individualized proteomic profiles. These results could illuminate the understanding of changes implicated in the atherosclerotic process in both cases. We also expect to identify interesting proteins, which could be used as novel potential biomarkers for the prognosis and/or treatment of ACS in clinical practice.

0247

CHARACTERIZATION OF THE VEGF RECEPTOR SYSTEM IN ARTERIOSCLEROTIC VESSELS AND IN A VASCULAR ORGAN CULTURE MODEL

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Background. Vascular endothelial growth factor (VEGF) acts anti-apoptotic via Akt and JNK-dependent signaling pathways and the VEGF-receptors. This study investigates the expression and distribution of the receptors VEGF-R1, VEGF-R2 and its coreceptor neuropilin-1 (NP-1) in arteriosclerosis and in a vascular organ culture model.
Methods. 25 human arteriosclerotic (carotid, femoral) and 10 normal arteries, as well as preparations of internal thoracic artery (IMA) andsaphenous vein (SV) organ culture models were studied. For the organ culture models, segments of IMA and SV were incubated with or without VEGF 10 ng/ml after surgical removal. VEGF-R1, VEGF-R2, NP-1 and von Willebrand factor (vWF) were demonstrated in cryosections by immunohistochemical and immunofluorescence microscopy. Human endothelial cells (EC) were cultured in presence of VEGF 10ng/ml and VEGF-receptors were detected by immunofluorescence microscopy.
Results. The expression of VEGF-R2 and NP-1 was increased in VEGF-supplemented IMA and in arteriosclerotic arteries. The cultured SV showed a higher expression of VEGF-R2, but neither NP-1 nor VEGF-R1 could be demonstrated. In EC, VEGF induced VEGF-R2 clustering and endocytosis was demonstrated by immunofluorescence, whereas VEGF-R1 was not detectable.
Conclusions. VEGF is the strongest stimulator of proliferation and a natural apoptosis inhibitor for EC. In arteriosclerosis, VEGF plasma levels and local production were described to be enhanced. IMA and arteriosclerotic arteries showed an increase in expression of VEGFR-2 and NP-1. The results suggest the involvement of the endothelial VEGF-receptors VEGFR-2 and NP-1 in arteriosclerosis which may provide a therapeutical target for vascular disease.

Cardiovascular disease

0248

DINAMICS OF REDUSED GLUTATHIONE AND HS-CRP IN VASCULAR SURGICAL PATIENTS

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Background. Oxidative stress and inflammation are important factors in the pathogenesis of vascular diseases as actively contributing endothelium dysfunction. Glutathione, as a major antioxidant, provide protection in state oxidative demage. CRP is the acute phase reactant of inflammation which activates complement and participate in intimal damage of blood veasels.It is believed that the level of hs-CRP is an independent risk factor for cardiovascular disease, and recent studies indicated that the level of glutathione (ie the ratio of reduced/oxigenised glutathione) may also be a good predictive factor for this disease.
Methods. We evaluated the change in levels of glutathione as a marker of redox status and concentrations of hs-CRP, as marker of vascular inflammation in patients on admission, two days after vascular surgery (carotid surgery), and after a month. Examined patients were both men and women, mean age 58±7 years.
Results. Preoperative mean value of hs-CRP, 8.895mg/L, was expected and placed them at high risk group.Posotoperative values 48,754 were significantly elevated, which is expected as a result of operation attact.Control values after 30 days (mean 13,794) were more than on the date of admission, still indicating inflammatory calming but it further control measurement of hs-CRP is necessary. Reduced glutathione two days after surgery has a 9% lower values than the date of admission when the control values (mean value 92,080 nM / ml), indicating that the balance between reduced and oxidized forms of glutathione shift to the oxidized side of the control value. GSH values, after 30 days were 12% more compared to preoperative values.
Conclusions. The results indicate that compensatory mechanisms of the whole organism are activated to overcome the state of increased inflammation and lipid peroxidation, as the basis of oxidative stress, and provide a reduced atmosphere for the cells.

0249

WHAT YOU SEE IS NOT WHAT YOU GET: CARDIAC TROPONIN T AND I ARE MAINLY DEGRADED IN ACUTE MYOCARDIAL INFARCTION SERUM

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Background. Cardiac troponins (cTn) are the recommended biomarkers for diagnosis and monitoring of an acute myocardial infarction (AMI). However, structural characteristics of cTnT and cTnI in serum/plasma are still unclear and are expected to be of importance for clinical interpretation and assay harmonization.
Methods. Non-covalent complexation of cTnT-cTnI-cTnC in serum was investigated using gel filtration chromatography (GFC). Fractions were subjected to immunoprecipitation and Western Blot (WB) analysis using cTnT and cTnI antibodies similar to the Roche 4th generation and Abbott Axsym-cTnI immunoassays, respectively. CTn in serum from an AMI patient (female, 84 y) was studied 3, 14, 22, and 46 hours after admission to the emergency department.
Results. GFC elution profiles illustrate that cTnT and cTnI conformation in serum of the AMI patient was dependent on the time from admission. Except for the first 3 hours, cTnT (MW estimated, 40 kD) as detected with the Roche antibodies was completely degraded (100%) into fragments of around 29, 17, 16, and 15 kD. In addition, cTnI (MW estimated, 28 kD) in serum as detected with the Axsym-cTnI antibodies was in complex with TnC and degraded for >50% into 25, 18, and 15 kD fragments.
Conclusions. Using the Roche catcher and detector antibodies, we were able to proof that cTnT in AMI serum is present as cTnT degradation products. In addition, we confirm that cTnI is in complex with TnC both in the intact and degraded form. Future research has to point out whether cTn structures in AMI subjects deviate from non-AMI subjects with elevated cTn concentrations.

Cardiovascular disease

0250

HIGH SENSITIVE CARDIAC TROPONIN T IN THE CLINICAL WORK-UP OF PATIENTS WITH STABLE CHEST PAIN

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Background. Numerous studies have demonstrated the association between increased concentrations of highly sensitive cardiac troponin (hs-cTn) and the incidence of cardiovascular disease. Unclear remains what the possible role of hs-cTnT is in the clinical work-up of patients with stable chest pain at the cardiology outpatient department.
Methods. We studied 1,088 chest pain patients (585 men, age 56 ± 11 years) with suspected coronary artery disease who underwent coronary computed tomographic angiography (CCTA) including coronary calcium scoring (CCS). Patients were followed up for the occurrence of revascularization, acute coronary syndrome, or all-cause mortality.
Results. During a mean follow-up of 1.5 ± 0.6 years, 84 events occurred in which hs-cTnT showed to be a significant predictor (HR 2.31; 95% CI 1.66-3.21; P<0.001). Moreover, adverse survival was seen for hs-cTnT concentrations in the highest quartile on top of Framingham risk scoring (FRS) >20% (HR 2.31, 95% CI 1.43-3.72; P=0.001), on top of CCS>400 (HR 1.78, 95% CI 1.05-3.01; P=0.033) and on top of >70% luminal stenosis on CCTA (HR 1.57, 95% CI 0.99-2.49; P=0.056). This was not the case for hsCRP and NT-pro-BNP. Furthermore, reclassification by adding hs-cTnT to FRS resulted in an IDI of 0.029 (P=0.002) and a NRI of 0.16 (P<0.001).
Conclusions. A significant adverse survival in cardiovascular events was found for hs-cTnT concentrations in the fourth quartile on top of FRS>20%, CCS>400 or >70% luminal stenosis on CCTA. This suggests that hs-cTnT has incremental value on top of currently used tools in the clinical work-up of patients with chest pain.

0251

PREVIOUS AND CURRENT GENERATION OF CARDIAC TROPONINS ASSAYS IN A REAL CLINICAL SETTING

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Background. The early diagnosis of acute coronary syndrome (ACS) is essential for a better patients (pts) outcome. The diagnostic performance of previous (n=1) and current generation (n=3) of cardiac troponin (cTn) assays were compared in an emergency department (ED).
Methods. Previous cTn generation: Troponin T (cTnT-R, Roche Diagnostics); current cTn generation: cTnI assays applied to the Dimension Vista (cTnI-V) and to the Advia Centaur analyzers (cTnI-A) (Siemens Healthcare Diagnostics), High Sensitivity Troponin T (hs-cTnT-R, Roche Diagnostics). cTn concentrations were determined on the first blood sample collected immediately at patients's arrival from n=452 pts (n=282 males, n=170 females; age: mean, range: 63, 15-100 years) presenting to ED for chest pain. cTn assays diagnostic performance were evaluated calculating the AUC (ROC curve analysis).
Results. Time from chest pain to blood withdrawal: < 3hour (A), n= 150 (33%); > 3hour (B), n=302 (67%). Discharge diagnosis: n=60 ACS, n=392 no-ACS. AUC, 95% CI: (A)=cTnT-R (0.76, 0.64-0.87), cTnI-V (0.82, 0.69-0.94), cTnI-A (0.73, 0.59-0.87), hs-cTnT-R (0.86, 0.75-0.97); (B)=cTnT-R (0.83, 0.75-0.90), cTnI-V (0.82, 0.74-0.90), cTnI-A (0.77, 0.68-0.86), hs-cTnT-R (0.87, 0.80-0.94). In (A) pts, current generation cTns assays show higher AUC than previous generation with a statistically significant difference (p<0.05) observed comparing hs-cTnT-R vs cTnT-R (A). In (B) pts the diagnostic advantage of current vs previous cTns assays is mitigated.
Coclusions. Current generation cTns assays are, overall, more effective than previous generation in making diagnosis of ACS, particularly in patients presenting early to ED, allowing a more rapid diagnostic workflow without a significant loss of clinical specificity.

Cardiovascular disease

0252

ASSESSMENT OF SERUM NITRITE/NITRATE (NOX) IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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Background. Hypercholesterolemia is widely accepted as one of the major risk factors for the development of cardiovascular diseases, and decreased bioavailability of nitric oxide (NO) and increased endothelium exposure to leukocytes are associated with early events in the atherogenic process. Therefore, the aim of this study was to assess the levels of nitrite/nitrate (NOx),the major stable metabolites of endogenous NO, in patients with hypercholesterolemia.
Methods. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and NOxlevels were assessed in 38 patients with hypercholesterolemia (LDL cholesterol levels ≥ 4.15 mmol/L) and 20 healthy controls (LDL cholesterol levels ≤ 3.37 mmol/L). NOx levels were measured by modified Griess method using the Cobas Mira clinical chemistry analyzer.
Results. Total cholesterol, LDL cholesterol and triglycerides were significantly higher in subjects with hypercholesterolemia, and no significant differences were observed for HDL cholesterol levels. The serum levels of NOx were 137.8 ± 37.7 µmol/L for healthy subjects and 109.9 ± 35.2 µmol/L for patients with hypercholesterolemia (P<0.05). In addition, a significant correlation was observed for LDL cholesterol and NOx (r=-0.314, P<0.05).
Conclusions. We conclude that serum levels of NOx were lower in patients with hypercholesterolemia, which promotes a decreased bioavailability of NO during the atherogenic process.

0253

ADVANCED OXIDATION PROTEIN PRODUCTS AS A MARKER OF OXIDATIVE STRESS IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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Background. Hypercholesterolemia is widely accepted as one of the major risk factors for the development of cardiovascular diseases. Atherosclerosis is an inflammatory disease associated with endothelial cell activation, oxidative stress, and the accumulation of leukocytes in the walls of arteries. Oxidative stress is associated with the damage of biological structures by reactive oxygen species, and overproduction of free radicals may also produce chemical modification of human serum albumin and other proteins. Therefore, the aim of this study was to evaluate the levels of advanced oxidation protein products (AOPP) as a marker of oxidative stress in patients with hypercholesterolemia.
Methods. A case-control study was performedin 38 patients with hypercholesterolemia (LDL cholesterol levels ≥ 4.15 mmol/L) and 20 healthy controls (LDL cholesterol levels ≤ 3.37 mmol/L). Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and plasma AOPP levels were measured.
Results. Total cholesterol, LDL cholesterol and triglycerides were significantly higher in subjects with hypercholesterolemia, and no significant differences were observed for HDL cholesterol levels. The plasma levels of AOPP were 39.9 ± 17.0 µmol/L for healthy subjects and 58.9 ± 27.2 µmol/L for patients with hypercholesterolemia (P<0.05).
Conclusions. We concluded that plasma levels of AOPP were higher in patients with hypercholesterolemia, and AOPP is a reliable measure of highly oxidized proteins during the atherogenic process.

Cardiovascular disease

0254

APOLIPOPROTEINA1 -75 (G/A) AND +83(C/T) POLYMORPHISMS, LIPID PROFILE AND CORONARY STENOSIS IN A TUNISIAN POPULATION

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Background. ApolipoproteinA1 (apoA1) is the major apoprotein of high-density Lipoprotein (HDL). Restriction site polymorphisms have been identified at -75 bp in the promoter region and +83 bp in intron 1 of the apo(A1) gene.
We studied the association of two polymorphisms (apo AI -75 (G/A) and apo AI +83(C/T)) in the ApoAI gene with lipid profile and significant coronary stenosis (SCS). Significant Coronary Stenosis (SCS) was defined as a luminal narrowing of ≥ 50% in at least one major coronary artery
Methods. 316 Tunisian patients underwent coronary angiography were recruited from the Cardiology Department of SahloulUniversityHospital, Sousse, Tunisia.Genotyping was performed using PCR-RFLP method. Lipids and apolipoproteins concentrations were measured.
Results. There were no significant differences in genotype frequencies of the -78 bp and the +83 bp polymorphisms between groups with SCS and those without SCS (p=0.296 and 0.265, respectively). The +83 bp polymorphisms seems to be associated with an increased risk of SCS (OR=2.20; CI [1.178-5.55]; p=0.039), and of hypoApoAI (OR=3.20; CI [1.087-9.43]; p=0.035). However, no significant effect was observed for -75pb (G/A) polymorphism.
In haplotype analysis, the haplogenotype (GA+AA) /(CT+TT) seems to be associated with an increased risk of SCS (OR=5.05; CI [1.45-6.3]; p= 0.003) and of hypoApoAI (OR=3.13; CI [1.33-7.53]; p=0.009) and of hypoHDLemia(OR=1.71; CI [1.06-4.76];p=0.048)
Conclusions. The +83 bp polymorphism seems to be associated with an increased risk of SCS and of hypoApoAI. In haplotype analysis, we found that (GA+AA) /(CT+TT) haplotype seems to have an atherogenic effect in a Tunisian population.

0255

CORRELATIONS OF CIRCULATING OSTEOPROTEGERIN WITH VON WILLEBRAND FACTOR COLLAGEN BINDING ACTIVITY AND ANTIGEN LEVELS IN PERIPHERAL ARTERIAL DISEASE

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Background. Von Willebrand factor (VWF), a glycoprotein stored in the Weibel-Palade bodies of endothelial cells is physically associated with osteoprotegerin (OPG), a cytokine marker of acute cardiovascular events. Addressing the cumulative risk evaluation focused on these parameters might be of interest in atherosclerosis.
Methods. 107 patients suffering of peripheral arterial disease (85 male, 22 female, age 64,38 ± 0,98 yrs) have been investigated by arterial Doppler measurement. Serum and plasma OPG, VWF antigen and collagen binding activity (CBA), CRP, plasma fibrinogen, serum lipids and ABO blood groups have been determined in all patients, in parallel with a healthy control group consisting of 70 individuals.
Results. Serum, plasma OPG, VWF antigen levels and CBA were all significantly higher in patients than in controls (p=0,014; p=0,05; p=0,003 and p=0,03, respectively). We could highlight a significant positive correlation between serum OPG, VWF ag (R=0,25, p=0,009) and VWF CBA (R=0,29, p=0,003). Both OPG and VWF levels proved to be dependent on ABO blood groups, the lowest values of both being measured at patients with group 0, while the highest at group AB (OPG) and B(VWF). However, a lack of correlation has been observed with an important functional measure of disease severity: the ankle-brachial index (ABI).
Conclusions. our observations reveal that the commonly stored OPG and VWF show increased circulating levels in atherosclerosis of the limbs. Moreover, it is interesting the mutual dependence of these proteins on ABO blood groups, a fact that should not be neglected in patient's biological risk evaluation.

Cardiovascular disease

0256

SERUM LEVELS OF CERULOPLASMIN AND MYELOPEROXIDASE IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE.

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Background. Ceruloplasmin(CP) was reporter to be an independent risk factor for cardiovascular disease. It has been identified as an acute phase protein and its carries about 95% of plasma copper. Myeloperoxidase(MPO), an abundant leukocyte enzyme, has been listed as a potentially useful risk marker in acute coronarysyndrome(ACS). Endothelial dysfunction and increased oxidative stress are commonly observed in patients with chronic heart failure andACS. More recently, it has been suggested that CP could be an physiological inhibitor of MPO. Given that its clinical utility in patients with stable coronary artery disease(CAD) is not yet defined, the aim of this study was to evaluate CP activity and MPO concentration in CAD patients and analize the association with other inflammatory parameter.
Methods. We studied 22 patients with CAD and 22 healthy subjects. CP activity was determined evaluating ferroxidase activity and MPO concentration was defined by enzyme immunoassay. High sensitive C-reactive protein(hs- CRP) were measured by standardized Methods.
Results. Cp activity and MPO concentration were significantly higher in CAD patients than in healthy subjects (891±179 vs 630 ±115 IU/L, $p< 0.0001$);(417±295 vs 179±145 ng/ml, $p= 0.0018$), respectively. Significant association were found between MPO levels and CP activity($r = 0.47$, $p= 0.0272$). There were no significant differences in CRP values in any of de groups(1,93± 1.43 vs 2.04± 2.94 mg/l , $p=0.8732$).
Conclusions. ElevatedMPO levels suggest that systemic release of MPO is characteristic feature of asymptomatic CAD. High CP activity could reflect subclinical atherosclerosis burden in patients who eventually developed symptomatic CAD.

0257

REGULATION OF THE OSTEOCLAST-ASSOCIATED RECEPTOR IN THE VASCULAR SYSTEM BY CYTOKINES AND GROWTH FACTORS

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Background. The osteoclast-associated receptor (OSCAR) is a member of the leukocyte receptor complex (LRC)-encoded family of surface receptors. So far, OSCAR was identified and characterized on immune cells and osteoclasts. We confirmed the expression of OSCAR on endothelial cells (EC) and vascular smooth muscle cells (VSMC). The aim of this study was to characterize the regulation of OSCAR by endothelial growth factors and inflammatory mediators in EC and VSMC.
Methods. OSCAR was detected on human EC and VSMC by immunofluorescence and Western blot. OSCAR-expression of the membrane, cytosol and nucleus of HUVEC was examined.Human EC (EA.hy926,HUVEC) were stimulated with VEGF, bFGF and TGF-ß and the proinflammatory cytokines TNF-α, INF-α and IFN-γ. VSMC were incubated with PDGF, TGF-ß and bGF. OSCAR-expression was quantified by Western Blot and Real-Time- PCR.
Results. OSCAR is localized on the cell membrane of EC and VSMC. VEGF, bFGF and TGF-ß did not significantly change OSCAR- expression on EC, but on VSMC OSCAR- levels were significantly enhanced by growth factors. IFN-γ caused a significant reduction of OSCAR-m-RNA and protein levels in EC, whereas the other used cytokines had no effect.
Conclusions. OSCAR isa surface receptor of EC and VSMC. In contrast to its role in EC, OSCAR seems to play a role in proliferation and maturation in VSMC. The down-regulation of OSCAR by IFN-γ could indicate an immunomodulatory role of OSCAR in EC. OSCAR could counteract the adhesion or diapedesis of immune cells and would thus be down-regulated for efficient immune response.

Cardiovascular disease

0258

KRP-206, A SELECTIVE S1P(1) AGONIST INHIBITS DEVELOPMENT OF ATHEROSCLEROSIS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT MICE

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Background. Sphingosine 1-phosphate (S1P), a bioactive lysosphingolipid associated with high density lipoprotein (HDL), at least partly accounts for anti-atherogenic properties of this lipoprotein. We previously demonstrated that FTY720 - a synthetic S1P analog targeting all S1P receptors but S1P(2) inhibits development of atherosclerosis in a murine model of disease. The present study addressed the identity of S1P receptor mediating atheroprotective effects of S1P.

Methods and Results. LDL receptor-deficient mice on a cholesterol-rich diet were given KRP-203, a selective S1P(1) agonist, at a dose of 3.0 mg/kg/day for 14 weeks. KRP-203 substantially reduced atherosclerotic lesion formation both in aortic root and arteria thoracica. Plasma lipids remained unchanged in course of KRP-203 treatment. However, KRP-203 induced marked peripheral blood lymphopenia, reduced total (CD4+, CD8+) and activated (CD69+/CD8+, CD69+/CD4+) T-cells in peripheral lymphoid organs, and interfered with lymphocyte function, as evidenced by decreased splenocyte proliferation and IL-2 and IFN-gamma production in response concanavalin A or phytohaemagglutinin as well as reduced RANTES levels in plasma. Plasma concentrations of macrophage-derived cytokines TNF-alpha and IL-6 were reduced by KRP-206 administration. Moreover, peritoneal macrophages from KRP-206 treated mice showed reduced surface expression of activation markers MCH-II and CD86 as well as LPS-elicited production of TNF-a and IL-6. In vitro experiments demonstrated reduced production of TNF-alpha and IL-6 in LPS-stimulated and IP-10 in INF-gamma-stimulated bone marrow macrophages.

Conclusions. Present results demonstrate that activation of S1P signaling pathways inhibit atherosclerosis by modulating lymphocyte and macrophage function and suggest that S1P(1) at least partly mediates anti-atherogenic effects of S1P.

0259

PLACENTA GROWTH FACTOR AS A PREDICTOR OF CARDIAC ALLOGRAFT VASCULOPATHY IN HEART TRANSPLANT RECIPIENTS

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Background. Long-term survival following heart transplant (HTx) is limited by cardiac allograft vasculopathy (CAV). Placenta growth factor (PlGF) has been shown to be an independent biomarker of adverse outcome in patients with coronary artery disease. Aim: to evaluate prognostic value of preoperative PlGF plasma levels for CAV development prediction.

Methods. Seventy heart transplant recipients, 61 men and 9 women, aged 18-67 years were followed for 6.5 (1-18) years after HTx performed for dilated or ischemic cardiomyopathy. Plasma levels of PlGF were measured by ELISA.

Results. CAV was diagnosed in 25 patients, which had significantly higher PlGF levels when compared to the patients without CAV (22.5 ± 5.0 and 12.3 ± 4.6 pg/ml resp., p=0.0012). There was no significant correlation for PlGF with age, gender, or plasma levels of C-reactive protein, interleukin-6, and homocysteine, but a positive correlation was found between PlGF and pregnancy-associated plasma protein A (r=0.55, p<0.05), neopterin (r=0.57, p<0.01) and soluble CD40 ligand (r=0.55, p<0.05). During the first 3 years after HTx CAV developed in 57.1% recepients with pretransplant PlGF level above median (12 pg/ml) and only in 4.7% patients with low PlGF. Event-free survival analysis showed significant difference in outcomes among patients with elevated and low levels of pretransplant PlGF (p<0.001).

Conclusions. A significant association between PLGF level and CAV was found in patients after HTx. Pretransplant PlGF was also found to be a risk factor for CAV and a measurement of PlGF concentration may be useful to identify patients at high risk of early development of CAV.

Cardiovascular disease

0260

BIOMARKERS OF THROMBOSIS INHEART TRANSPLANT RECIPIENTS

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Background. Elevated plasma levels of homocystein (Hcy), anticardiolipin antibodies (aCL) and soluble CD40 ligand (sCD40L) are independent risk factors for coronary artery disease and thrombosis. Aim: to evaluate the role of Hcy, aCL and sCD40L in development of cardiac allograft vasculopathy (CAV) in heart transplant recipients.

Methods. 70 patients, aged 45.5±10.1 years were followed for 1-18 years after heart transplantation (HTx). Plasma levels of Hcy, aCL, and sCD40L were measured by ELISA.

Results. Hcy and aCL levels in heart transplant recipient were significantly higher when in patients awaiting HTx (20.6+11.5 µmol/l, 28.7+10.7 U/ml vs. 14.8+15.3 µmol/l, 23.5+10.5 U/ml resp., p=0.0016 and p=0.009). sCD40L level did not differ among patients before and after HTx (1.83±0.75 and 2.0±1.1ng/ml, resp.).

No difference was seen between sCD40L levels in recipients with CAV (2.3±1.2 ng/ml, n=25) and those without CAV (1.82±0.98 ng/ml, n=45), but elevated levels of Hcy (22.9+9.8 µmol/l) and aCL (34.8+15.5 U/ml) were associated with CAV.

There was no significant correlation for Hcy, aCL, and sCD40L with age, gender, total cholesterol, triglycerides, plasma levels of CRP, IL-6, TNF-α.

Major study events defined as CAV or rejection occurred in 52% recipients with pretransplant sCD40Ls above median (1.6 ng/ml) and/or aCL levels >23 U/ml and only in 9.5% patients with low sCD40L and aCL levels.

Conclusions. In heart transplant recipients a combination of hyperhomocysteinemia and elevated aCL level seems to be a risk factor for CAV. Pretransplant sCD40L and aCL plasma levels are independent predictors for the development of CAV after HTx.

0261

CONTRIBUTION OF GLOBULINS TO HUMAN BLOOD PLASMA/SERUM COBALT BINDING CAPACITY USED AS BASIS OF THE ISCHEMIA-MODIFIED ALBUMIN TEST

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Background. The indirect ischemia-modified albumin (IMA) test based on hypothesis that N-terminal region of serum albumin can loose ability to bind cobalt ions during cardiac ischemia outset was developed at the beginning of the 2000s. Use of the IMA test in recent years showed up contradictions in diagnostic significance of the test Results. We examined whether some globulins influence on IMA test Results.

Methods. We used non-proprietary colourimetric protocol based on interaction between Co^{2+} and dithiotreitol to determ Co^{2+}-binding capacity (CoBC) of the proteins. In biological samples we quantified total proteins (TP), albumin(ALB), and total immuno-globulins (TIG).

Results. In vitro experiments showed that both whole immunoglobulin fraction and fibrinogen bound Co^{2+}. Specific CoBS of serum albumin was similar to CoBS of immunoglobulins and lower than fibrinogen. Significant difference in CoBS between serum and heparinized plasma samples from same persons (n=34) were observed (p=0,0003, Wilcoxon test). CoBS in sera/plasma of health volunteers (n=189) had no powerful correlations to TP, ALB, IG or ALB+IG (module of Spearman R <0,25 in all cases). On the other hand, CoBS in plasma samples from patient (n=15) with autoimmune diseases in acute phase has higher correlation with ALB+TIG (R=0,721) then to TIG (R=0,600) or ALB (R=0,346) alone. It was surprised correlation between CoBS and TP (R=0,406) then between CoBS and ALB.

Conclusions. We conclude that globulins make contribution to CoBS value in human serum/plasma and this contribution should be quantified to estimate analytical specificity of the IMA test.

Cardiovascular disease

0262

DETECTION OF SERUM PROTEIN PROFILE CHANGES IN ATHEROSCLEROSIS

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Background. Atherosclerosis and its complications are the major cause of morbidity and mortality in the world. Since atherosclerosis is a chronic disease and its development takes several years, it is advantageous to perform atherosclerosis studies in apolipoprotein E knockout (Apo E$^{-/-}$) mice models which develop atherosclerosis very fast in comparison to humans. The aim of this study was to identify serum protein profiles in the early stages of atherosclerosis in Apo E$^{-/-}$ mice and compare with the serum protein profiles of control C57BL/6 mice.
Methods. Proteomic analysis was performed in the serum samples obtained from atherosclerotic and control mice groups at the end of 20 weeks of age using Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS). The proteomic profiles obtained using three different chips, CM-10 (weak cation exchange), H50 (Reversed-phase) and IMAC-30 (immobilized metal affinity capture) were analysed with ProteinChip data manager 3.0 program.
Results. A total of 742 protein/peptide clustering peaks, >5 kDa, were found to be different between the atherosclerotic and control mice groups, and the differences reached statistical significance for 107 serum protein/peptide clusters (p<0.05).
Conclusions. Our study contributes to understanding the changes in serum protein/peptide profiles during atherosclerosis development, and to discover new protein biomarkers for early diagnosis of atherosclerosis.

0263

ISCHAEMIA MODIFIED ALBUMIN LEVELS DURING TREATMENT WITH FUROSEMIDE AND LEVOSIMENDAN IN MALE PATIENTS WITH ACUTE DECOMPENSATED HEART FAILURE AND PAST ACUTE MYOCARDIAL INFARCT

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Background. We have aimed at investigatingIschaemia Mmodified Albumin (IMA) alterations in the serum of male patients with chronic heart failure and past acute myocardial infarct with clinic symptoms of acute decompensated heart failure but without signs of acute myocardial infarct.
Methods. We enrolled 19 male patients (age 70.7 +/- 9.4 years) with the characteristics mentioned above. As a control sample we used15 healthy men of the same age. All patients were treated initially with intravenous infusion of furosemide and after their stabilization followed a 24-hour continuous infusion of levosimendan. We checked a) at the emergency department b) after the diuretic treatment c) 24 hours after the end of the levosimendan administration, the following measurements: left ventricular ejection fraction (LVEF), systolic (SBP) and diastolic blood pressure as well as the blood levels of IMA, creatinine, urea, cardiac fraction of creatinine kinase (CPK-MB), Troponin T, electrolytes Na+ and K+.
Results. 10 out of 19 patients presented increased levels of IMA (>85 U/ml) at the emergency department and 17 out of 19 presented at least one pathologic increase of IMA levels. We observed that the average value of IMA levels was significantly lower in the healthy patients compared to the first (p=0.002), the second (p<0.001) and the third (p<0.001) measurements.
Conclusions. The evaluationof these data leads to the conclusion that IMA can form a reliable marker of the surveillance of the treatment and its influence in the systemic and/or myocardial ischemia in patients with acute decompensated heart failure.

Cardiovascular disease

0264

CARDIOVASCULAR RISK ASSESSMENT : PREVENTIVE STRATEGIES AND GUIDELINES

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Background. Cardiovascular risk factors are lipoprotein subspecies, homocysteine, abnormal blood coagulation characteristics and emerging markers : MPO, PAPPA, hsCRP, protein S 100B, choline CD 40L, ADMA ,copeptin ,vitamin D and etc.
Methods. The aim of the study is to estimate lipid status in student's population from risk family and comparative analysis of students from family without risk to established novel targets for cardiovascular prevention. Fifty students from risk family (CHD, AMI, hypertension, obesity, smoking, diabetes mellitus, HLP) and 50 students from family without risk; both sexes were selected for study. The following determinations are performed: TCH, TG, HDL-c, LDL-c, VLDL-c, IA, RF , BMI.
Results. A value of TCH was significantly higher in risk group. While LDL-hol. TG , IA and RF was found to be significantly higher in students from risk family (p<0.01), HDL-hol was significantly lower (p<0.01) in students from risk group. The results also show-ed that between BMI and TCH,LDL-c.and TG statistically significant difference was found (p<0,05), but between BMI and HDL-c statistically significant difference wasn't found (p>0,05).
Conclusions. These data suggest that screening of lipid status is necessary in students from risk family, primary prevention is very important and can achieved through lifestyle changes, promotions of health way of life, as well as modifications of risk fac-tors in aim to prevention of atherosclerosis, coronary heart disease, and in some students therapeutic intervention of established clinical cases.

0265

LIPID CONTROL IN DIABETIC PATIENS FROM NORTHERN GREECE

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Background. The aim of this study was to assess the prevalence of dyslipemia in diabetic and non-diabetic patients living in urban areas from northern Greece.
Methods. A total of 220 patients (120 patients had type 2 diabetes mellitus-T2DM, 100 patients without this disease used as con-trol), followed up in primary care settings (Health Centre of Soxos) in northern Greece, were avalaible for analysis of dyslipemia. An over-night fasting blood sample was taken for total cholesterol (CHOL), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) determination. The majority (61%) of the T2DM were taking statins.
Results. CHOL and LDL were slightly higher in the control group (61,6% / 52,1%, respectively) compared to T2DM group (57,4% / 48,7%, respectively). TG was higher in the T2DM group (59.7%) compared to the control group (44.2%). HDL was lower in T2DM patients (54.8%) compared to non-diabetic subjects (42.6%). Among T2DM patients who were taking statins well controlled were only 36.8%.
Conclusions. The study shows a relatively poor control of dyslipemia in diabetics patients when compared to lower risk popula-tion, despite the frequent use of statins

Cardiovascular disease

0266

PLATELET FUNCTION TESTING WITH MULTIPLE ELECTRODE IMPEDANCE AGGREGOMETRYAND INDIVIDUAL TREATMENT IN PATIENTS WITH INADEQUATE RESPONSE TO CLOPIDOGREL

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Background. Insufficient inhibition of ADP dependent platelet aggregation by clopidogrel is associated with an increased risk for stent thrombosis (ST), suggesting
the need for higher doses or better optimizing the chois of the the antiplatelet drug.
Methods. Platelet function was assessed with multiple electrode impedance aggregometry (MEA) in whole blood on Multiplate analyzer (Dynabyte). 603 patients had undergone percutaneous coronary intervention (PCI) and were treated with standard aspirin (100 mg/day) and clopidogrel (300 mg +75 mg/day) therapy. Blood samples were drawn at least 14 h after the loading dose of clopidogrel.
Results. The optimal cut-off value according to ROC analysis to predict the occurrence of definite ST within 30 days was 45.5 AUC. The area under the ROC curve was 0.864 (95% CI 0.772–0.957, P<0.0001). When applying this cut-off value, 109 patients (18 %) had MEA values above this cut-off. The incidence of definite ST within 30 days was significantly higher in low responders [13% vs. 0.6%; OR 24.3; P<0.001]. With this cut-off value, MEA had 79% sensitivity and 84% specificity.By fifty three patients we subsequently increased the dose of clopidogrel to 150 mg/d, but 21 of them did not yet achieve sufficient inhibition of ADP dependent aggregation (ADP test 72±11 U) and the thienopyridine treatment was switched to prasugrel 10 mg/d (ADP test 30 ±13 U), p< 0.001. Another two patients were switched to 15 mg/d prasugrel.
Conclusions. High platelet residual activity require more aggressive inhibition of the P2Y12 receptor to overcome the early 30-th days ST.

0267

USE OF PREOPERATIVE PLASMA BNP LEVELS IN PREDICTING NEW- ONSET ATRIAL FIBRILLATION AFTER CORONARY ARTERY BYPASS GRAFTING

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Background. Atrial fibrillation (AF) occurs in up to 40% of patientsundergoing coronary artery bypass grafting (CABG) and isassociated with increased comorbidity. The aim of this studywas to evaluate whether preoperative higher plasma BNP levels correlate with occurrence of postoperative AF.
Methods. Twenty five patients (20 men and 5 women), age 64±13, with no history of atrial arrhythmia, undergoing cardiac surgery (valve repair – 46%, CABG – 33%, or both – 21%) were included. Plasma (EDTA) BNP levels were measured preoperative and on 1st postoperative dayat IUC. The measurement was performed with Biosite BNT tests on ACCESS -2 immunoassay systems (Beckman Coulter). The coefficient of variation was 6,11 – 9,23% and the 99th percentile for health population was 100 pg/ml.
Results. The preoperative plasma BNP level was higher in patients who developed postoperative AF (570 pg/ml vs 290 pg/ml, p< 0.05). Postoperative AF was documented in 7 pateints (30%). More patients in the group that developed postoperative AF (43% vs 23%) had a BNP level in the upper 75th percentile (575 pg/ml). The interquartile range was 84 – 1146 pg/ml vs 46 – 572 pg/ml. More patients in the AF group had undergone valve surgery (71% vs 29%). No significant difference in age, left ventricular function, hypertension, history of CAD, Tn I, CRP was found in the groups with or without postoperative AF. We did not observed any case of death or IM or stroke at 28th postoperative day.
Conclusions. High plasma BNP levels are associated with increased risk of postoperative AF.

Cardiovascular disease

0268

THE INFLUENCE OF SOME FACTORS ON THE TURNAROUND TIME OF TROPONIN

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Background. There is a consensus that the optimal turnaround time (TAT) for cardiac markers should be 60 minutes or less. Few reports concerning this topic have shown substantial difficulties to achieve this idea in the routine hospital conditions. In our communication we try to estimate the influence of two factors on the efficiency of a central laboratory in this area.
Methods. For 4 weeks we analyzed TATs of cardiac troponin I (cTnI) ordered by physicians in emergency department. Analyses were carried out in heparin plasmas using Vitros 5600 system (Ortho Clinical Diagnostics). Group A (n=330, the former two weeks) – medical/laboratory personnel didn't knew about investigation ("retrospective" analysis), group B (n=375, the latter two weeks) – medical/laboratory personnel was informed about the.undertaking ("prospective" analysis). Group I: physicians ordered tests between hours: 0.01 and 6.00 (n=65); group II: 6.01 - 12.00 (n=189); group III: 12.01 – 18.00 (n=257); group IV: 18.01 – 24.00 (n=194). Statistical evaluation: Kolmogorov-Smirnov test.
Results. TAT median for whole group (n=705) was 51 minutes (min.); A: 52 min., B: 50 min. (p<0,05); I: 37 min; II: 59 min.; III: 54 min; IV: 47 min (statistically insignificant only between II and III groups). TAT for 90 percentile was 76 min. The percentages of TAT > 60 min were: whole group: 32%; A: 33%; B: 31%; I: 5%; II: 46%; III: 36%; IV: 20%.
Conclusions. Both the time of day (substantially) and an information about the research (slightly) influenced TAT of cTnI ordered to the central laboratory by emergency department personnel.

0269

SHORT-TERM STABILITY OF CARDIAC TROPONIN I AT THE 99TH PERCENTILE VALUE FOR MYOCARDIAL NECROSIS

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Background. Current consensus guidelines for the diagnosis of myocardial infarction (MI) refer to a rise and/or fall in troponin concentration with one value above the 99th percentile of the upper reference limit. There has been general acceptance of a 20% change in serial troponin measurements as being diagnostically significant. The practice of retrospective addition of troponin measurement to samples several hours post-venepuncture is widespread in many laboratories. We determined the stability of troponin I (cTnI) at concentrations near the 99th percentile value for myocardial necrosis.
Methods. Samples (n=81) with baseline cTnI concentrations in the range 0.04–0.15 ng/ml were analysed routinely on arrival (median time from venepuncture; 2 h 15 min) and stored at room temperature. At 6 hours, samples were re-analysed and split, with aliquots stored at room temperature and 4°C. Samples at both storage conditions were re-analysed at 9, 12, 24 and 48 hours.
Results. The results demonstrated a decreasing trend in cTnI concentration with storage at both temperatures at each time point compared with baseline ($p < 0.001$). There was a 6% median decrease in cTnI between the baseline concentration and 6 hours post-venepuncture. Of the samples with baseline cTnI = 0.040 ng/ml, 80% had a cTnI concentration below this value on re-assessment at 6 hours.
Conclusions. Measurement of cTnI >3 hours post venepuncture in individuals with cTnI = 0.040 ng/ml may provide an erroneously negative result. In addition, the retrospective analysis of cTnI on stored samples to help determine a 20% change may be clinically misleading.

Cardiovascular disease

0270

CORRELATION BETWEEN PRESCRIPTION FOR STATINS AND THE EVOLUTION OF LIPID LEVELS IN A POPULATION OF BUENOS AIRES CITY

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Background. In Argentina, from 1999 to 2009 the prescription for statins grew more than 170%; the lipid levels decreased in both genders; and the number of people aged 40 and over who have prescription for statins has grown.
Methods. We conducted an epidemiological study of ecological type to assess the correlation between the prescription for statins and the evolution of the total cholesterol (TC) concentration in 450,936 urban people (41.1% men; 58.9% women) with high level of consumption of health care services, attended from 1999 to 2009 at an outpatient medical center. Average TC values and the prevalence of TC borderline high (200-239 mg/dL) and high (>240 mg/dL) were correlated with the data related to the prescription for statins in units published by IMS Health, from 1999 to 2009. The population was segmented in older and younger than 40 years. Spearman's rank correlation coefficient was calculated. The significance was established with the p-values adjusted by Bonferroni (<0.05).
Results. In the group younger than 40 we did not find significant association between prescription for statins and average TC levels (Rho = -0.64; p=0.07) and prevalence of TC >200 mg/dL (Rho = -0.66; p=0.06). However, we found a highly significant negative association in the group older than 40 for both parameters (Rho = -0.91; p<0.001).
Conclusions. The increase in the proportion of people aged 40 and over using lipid-lowering medication, likely contributed to the decreases in TC levels. However, a wide range of factors can also influence the observed TC levels.

0271

ACUTE MULTI THROMBOTIC STROKE AS CLINICAL MANIFESTATION OF HEPARIN INDUCED THROMBOCYTOPENIA (HIT) IN A PATIENT FOLLOWING CARDIAC SURGERY

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Background. HIT is an immune mediated response to heparinadministration with platelet (PLT) activation. It is associated with complications involving venous and more rarely arterial thromboembolism. Patients undergoing cardiac surgery are at risk of developing HIT. The incidence of postoperative antibody seroconversion in cardiac surgery patients ranges between 25-50% with 1-2% demonstrating vascular complications. Of these only 3-5 % show acute thrombotic arterial events.
We present a case of a patient who had rapid HIT onset and multiple apoplectic stroke following coronary artery bypass grafting (CABG).
Results. Urgent CABG was performed in a 63-year old male with 3-vessel disease and unstable angina under heparin treatment without intraoperative complications. Prolonged post anesthetic recovery and moderate coma were initially observed after operation. CT-scan and MRI showed multiple, disseminated zones of ischemia bilaterally in all areas of the cerebral arteries. On the 4th postoperative day PLT count decreased from 155 to 75/nl. HIT was suspected and alternative anticoagulation treatment with argatroban was initiated immediately. In a HIPA test, aggregation of thrombocytes was verified in the presence of heparin. The patient was discharged from cardiosurgical unit on the 34th day after operation and transfered to a neurologic center for rehabilitation.
Conclusions. HIT after cardiac surgery is often associated with increased length of hospital stay and poor outcome. Hit should also be suspected in cardiosurgical patients with neurological disturbances and prolonged post anesthetic recovery. In order to reduce morbidity and mortality early initiation of laboratory diagnostics and alternative anticoagulation strategies need to be implemented.

Cardiovascular disease

0272

SERUM CYSTATIN C CONCENTRATION AND ARTERIAL STIFFNESS IN PATIENTS WITH NONTREATED HYPERTENSION

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Background. Increased arterial stiffness measured as pulse walve velocity –PWV and kidney function impairment have been recognized as important predictors of cardiovascular mortality in patients with hypertension. Cystatin C regarded predominantly as a marker of renal function has also been recognized as a marker of atherosclerosis. The aim of study was to evaluate the relationship between kidney function expressed as eGFR or cystatin C and PWV in patients with newly recognized hypertension.
Methods. We examined 49 ambulatory patients aged 45-65 years with nontreated hypertension. Control group included 49 healthy volunteers matched by age and gender. Blood pressure was measured by ABPM; SBP≥130 and DBP≥80 mmHg were accepted abnormal. Cystatin C was measured by immunonephelometric assay. GFR was estimated with simplified MDRD formula. Carotid-femoral PWV was measured with Complior apparatus. Intima-media thickness (IMT) of the carotid artery was examined by ultrasound.
Results. IMT was significantly higher in hypertensives compared to controls [0,8 mm (0,66;0,90) *vs*0,65 mm (0,60; 0,75) respectively; p<0,001] as well as concentration of cystatin C (0,89±0,15 *vs* 0,78±0,10 mg/L respectively; p<0,001) and PWV (11,1±2,1 m/s *vs* 8,7±1,5 m/s respectively; p<0,001). No significant differences were found in serum creatinine level and eGFR. We have found a positive correlation between cystatin C and PWV (r=0,35;p<0,05) and cystatin C and IMT (r=0,46;p<0,05), but no relationship between eGFR and PWV in patients with hypertension (r=-0,24; p=NS).
Conclusions. Cystatin C may be regarded as an early marker of subclinical organ damage, including increased arterial stiffness, in patients with hypertension.

0273

OXLDL AND HS-CRP: BIOMARKERS FOR RISK ASSESSMENT OF CVD

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Background. Atherosclerosis is a chronic inflammatory process and the main cause of Cardiovascular Disease. Development of atherosclerosis is owed primarily to the oxidized LDL (OxLDL), which accumulates in atherosclerotic plaques, promotes inflammatory responses and plays an important role in atherogenesis. In this study we examined the association of serum OxLDL with the levels of hs-CRP and the lipid profile.
Methods. The sample of our study consists of 125 patients with increased LDL and Cholesterol levels and it was compared with 100 samples of healthy individuals. All samples were tested for hs-CRP and oxLDL serum levels. Elecsys 2010 was used for measuring LDL, Cholesterol and HDL concentration. OxLDL and hs-CRP levels were determined by ELISA and Nephelometry, respectively. The SPSS 16.0 was used for the interpretation of Results. Differences at p<0.05 were considered as statistically significant.
Results. SignificantpositivecorrelationswerefoundbetweenoxLDL andLDL (R=0,686, p<0,001), hs-CRP (R=0,778, p<0,001) and Cholesterol (R=0,780, p<0,001). Hs-CRPwas found to have significantly positive correlations with high levels of LDL (R=0,715, p<0,001) and high levels of cholesterol (R=0,807, p<0,001). Hs-CRP showed a negative correlation with low levels of cholesterol (R=-0.473, p<0.001) and low levels of LDL (R=-0.635, p<0.001).
Conclusions. This study suggests that hs-CRP in addition to lipid profile is very useful to evaluate the risk for CVD. OxLDL measuring oxLDL indicate that high levels could be a useful biomarker to assess CVD risk and to provide important information for risk assessment.

Cardiovascular disease

0274

ASSOCIATION OF LOW LEVELS OF SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS WITH CARDIAC TROPONIN I IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background. Interaction of advanced glycation end products (AGEs) with receptor for AGEs (RAGE) increases expression of cytokines, oxygen radicals and matrix metalloproteinases (MMPs). Oxygen radicals and MMPs have been implicated in plaque rupture and thromboembolism resulting in acute coronary syndrome (ACS) and cardiac cell death. Oxygen radicals directly damage the cardiac cells. This results in release of cardiac troponin I(cTnI) and other biochemical markers. Soluble receptor of AGEs (sRAGE) competes with RAGE for binding with AGEs, and thus exerting cytoprotective effect. Low levels of serum sRAGE would increase the levels of AGEs to interact with RAGE resulting in increase generation of oxygen radicals, cytokines and MMPs with adverse consequences.
Hypothesis. Low levels of serum sRAGE is associated with high serum levels of cTnI in patients with acute coronary syndrome.
Objective. To determine if there is an inverse relationship between serum sRAGE and cTnI in patients with ACS.
Methods. Serum levels of sRAGE and cTnI were measured in 36 patients with ACS and 30 control subjects.
Results. Levels of sRAGE were lower in ACS patients as compared to control subjects (802.56 ± 39.32 pg/ml vs. 1311.43 ± 66.92 pg/ml). Levels of cTnI were higher in ACS patients as compared to control subjects (2.18 ± 0.33 ug/ml vs. 0.012 ± 0.001 ug/ml). Levels of serum sRAGE were negatively correlated with the levels of cTnI.
Conclusions. Low levels of serum sRAGE are associated with high serum levels of cTnI. Levels of serum sRAGE are inversely related to the levels of serum cTnI.

0275

HIGH SENSITIVE C - REACTIVE PROTEIN AND OTHER MARKERS OF INFLAMMATION IN CORONARY ARTERY DISEASE: OUR EXPERIENCE

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Background. In an attempt to improve global cardiovascular risk assessment, considerable research has focused on markers of inflammation. Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events. High sensitivity C-reactive protein (hs-CRP) is an index of inflammation that is now believed to promote directly all stages of atherosclerosis, including plaque rupture.
Methods. The study included 110 CAD patients and 67 healthy individuals. Cases were included in the study to assess the risk of cardiovascular events associated with base-line levels of markers of inflammation. Parameters studied were estimated in Hitachi 917 and Bio-Rad coda EIA analyzer using commercial kits.
Results. Mean Hs-CRP levels in diabetic (DM-CAD) and non-diabetic (ND-CAD) group with heart disease were found to be 1.20 ± 1.39 (0.04 - 4.55) and 0.82 ± 0.86 (0.04 – 3.71) respectively as compared to healthy control group 0.17 ± 0.23 (0.01 – 1.29). IL-6 levels were 1.68 ± 1.17 (0.082 – 4.18) in DM-CAD group and 1.27 ± 0.62 (0.15 – 2.60) in ND-CAD group where as in control group it was 0.77 ± 0.45 (0.08-1.92). TNF-α. Levels in DM-CAD group were 2.47 ± 0.79 (1.02 - 4.72), in ND-CAD group 1.89 ± 0.48 (1.18-2.85) and 1.60 ± 0.28 (0.80 – 1.99) in healthy controls. The mean levels of Hs-CRP, TNF- α and IL-6 were statistically significant in both DM-CAD and ND-CAD group.
Conclusions. hs-CRP testing with other inflammatory markers enhances information provided by lipid screening or global risk assessment. The additions of the measurement of C-reactive protein IL-6 and TNF- α along with lipid levels provide an improved method of identifying persons at risk for future cardiovascular events.

Cardiovascular disease

0276

DIAGNOSTIC SENSITIVITY OF HIGH SENSITIVITY TROPONIN T IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background. Cardiac troponins are an inevitable part of acute myocardial infarction (AMI) diagnostics. Recently, several assays with increased analytical sensitivity (high sensitivity assays) were developed. However, the role of high sensitivity troponins in AMI diagnosis is still not clear. The aim of this study was to compare diagnostic sensitivity of Abbott Architect STAT Troponin I (cTnI) and Roche Troponin T hs STAT (hsTnT) in AMI diagnostics.
Methods. In the study, we have included 62 patients (median [IQR] of age: 62.5 [54 to 75] years; 14 females) presented to the Cardiac Intensive Care Unit (cICU) with chest pain and cTnI at admission < 0.1 μg/l. We measured cTnI (chemiluminiscent microparticle immunoassay by Abbott, Architect analyser), hsTnT (electrochemiluminiscence immunoassay by Roche, Cobas E analyser) and myoglobin (immunoturbidimetric test by Beckman-Coulter, AU 5400 analyser) at the time of patient admission. The diagnosis of AMI was evaluated by skilled clinician (according to "universal definition of AMI"). Cut-off values for AMI for cTnI and hsTnT were 0.04 μg/l and 14 ng/l resp.
Results. 5 of presented patients (8 %) didn´t fulfill criteria for AMI. The diagnostic sensitivities of admission cTnI and hsTnT values were 61.4 % and 77.2 % resp. The correlation between cTnI and hsTnT was 0.66 (Pearson correlation coefficient). Sensitivity of myoglobin values at the time of admission for AMI was 70.2 %.
Conclusions. We conclude that the sensitivity of hsTnT is better than cTnI and myoglobin for diagnosis of AMI in the subpopulation of patients with chest pain and admission cTnI<0.1 μg/l.

0277

SERUM FAS/FASL LEVELS IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Background. Ishemic heart disease is mostly a consequence of atherosclerosis. The Fas/Fas ligand (FasL)/caspase death pathway is activated in atherosclerotic lesions. The goal of this study is to evaluate the diagnostic values of soluble forms of Fas and FasL in patients with stable angina pectoris (SAP), unstable angina pectoris (USAP) and acute ST-elevation miocardial infarction (STEMI) patients.
Methods. We studied 30 patients with SAP, 27 with USAP, 39 with STEMI and 27 age-matched healthy volunteers (Control group). Serum Fas/APO1 and FasL concentrations were determined using a commercially available immunoassays (ELISA).
Results. Fas/APO1 levels in STEMI patients (6.981±2.689 ng/ml) were significantly higher than Fas levels in controls (5.092±1.252 ng/ml, p<0.01), but not significantly higher than Fas values in SAP (5.952± 2.069 ng/ml) and USAP patients (5.627±2.270 ng/ml). Levels of FasL did not show any significant difference between studied groups. In SAP patients Fas/APO1 showed a significant positive correlation with hsCRP (p<0.05) and a negative correlation with HDL-C (p<0.05), while FasL showed a significant positive correlation with LDL-C (p<0.05). Fas levels between the patients having cholesterol within normal range and those whose cholesterol was above normal range showed a significant difference (p<0.05) only in USAP patients. Fas and FasL levels between the patients with hsCRP lower than 3.0 mg/L and those with hsCRP higher than 3.0 mg/L of SAP group showed a significant differences (p<0.001, p<0.05, respectively).
Conclusions. The apoptotic process is disregulated in patients with ischemic heart disease. Fas and FasL showed interdependence with inflammatory and lipid markers.

Cardiovascular disease

0278

SIGNIFICANCE OF FAS/FASL LEVELS DETERMINATION IN SERA IN PATIENTS WITH ISCHEMIC HEART DISEASE

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Background. Ishemic heart disease is mostly a consequence of atherosclerosis. The Fas/Fas ligand (FasL)/caspase death pathway is activated in atherosclerotic lesions. The goal of this study is to evaluate the diagnostic values of soluble forms of Fas and FasL in patients with stable angina pectoris (SAP), unstable angina pectoris (USAP) and acute ST-elevation miocardial infarction (STEMI) patients.
Methods. We studied 30 patients with SAP, 27 with USAP, 39 with STEMI and 27 age-matched healthy volunteers (Control group). Serum Fas/APO1 and FasL concentrations were determined using a commercially available immunoassays (ELISA).
Results. Fas/APO1 levels in STEMI patients (6.981±2.689 ng/ml) were significantly higher than Fas levels in controls (5.092±1.252 ng/ml, p<0.01), but not significantly higher than Fas values in SAP (5.952± 2.069 ng/ml) and USAP patients (5.627±2.270 ng/ml). Levels of FasL did not show any significant difference between studied groups. In SAP patients Fas/APO1 showed a significant positive correlation with hsCRP (p<0.05) and a negative correlation with HDL-C (p<0.05), while FasL showed a significant positive correlation with LDL-C (p<0.05). Fas levels between the patients having cholesterol within normal range and those whose cholesterol was above normal range showed a significant difference (p<0.05) only in USAP patients. Fas and FasL levels between the patients with hsCRP lower than 3.0 mg/L and those with hsCRP higher than 3.0 mg/L of SAP group showed a significant differences (p<0.001, p<0.05, respectively).
Conclusions. The apoptotic process is disregulated in patients with ischemic heart disease. Fas and FasL showed interdependence with inflammatory and lipid markers.

0279

SIGNIFICANCE OF BIOMARKERS OF APOPTOSIS DETERMINATION IN ISCHEMIC HEART DISEASE PATIENTS

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Background. Apoptotic cell death may play a critical role in a variety of cardiovascular diseases, especially in those developing on the basis of atherosclerosis. The goal of this study was to compare the activity of caspase-3, the values of soluble forms of Fas/APO1 and FasL and Bcl-2 protein in sera in patients with various forms of ischemic heart disease, and to correlate these markers with inflammatory and lipid parameters.
Methods. We studied 30 patients with chronic stable angina pectoris (SAP), 27 with unstable angina pectoris (USAP), 39 with acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (Control group). Caspase-3 activity was determined by a colorimetric commercially available method, while serum Fas/APO1, FasL and Bcl-2 concentrations were determined using commercially available immunoassays (ELISA).
Results. Caspase-3 was significantly higher only in the USAP group (0.122±0.062 µmol/mg protein, p<0.05) in comparison with the control group (0.092±0.022 µmol/mg protein). Fas/APO-1 values were significantly higher in the STEMI group (6.981±2.689 ng/mL, p<0.01) than in USAP (5.627±2.270 ng/mL) and healthy (5.092±1.252 ng/mL). Concentrations of Bcl-2 were significantly higher in patients with SAP (0.310±0.075ng/mL) and USAP (0.329±0.102ng/mL) compared to healthy (0.250±0.069ng/mL, p<0.01) and the STEMI (0.266±0.041ng/mL, p<0.01) groups. ROC curves analysis showed that Bcl-2 was the best marker an atherosclerotic plaque acivity in SAP and USAP patients.
Conclusions. The studied markers of apoptosis present valuable parameters in evaluation of atherosclerotic plaque acivity and a new targets for therapy.

Cardiovascular disease

0280

IMMUNOASSAY FOR CARDIAC TROPONIN I BASED ON FLUORESCENT NANOPARTICLES AND A RECOMBINANT ANTIBODY FRAGMENT

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Background. Cardiac troponin I (cTnI) concentrations below reference levels may indicate minor myocardial damage; measurement of these low levels could assist in identification of individuals at higher risk for myocardial infarction and other cardiac conditions. For immunoassays to be reliable in measuring femtomolar concentrations of cTnI, nonspecific interferences need to be efficiently eliminated. This study aimed to develop a highly sensitive immunofluorometric assay for cTnI utilizing fluorescent nanoparticle labels.

Methods. A two-site immunoassay based on the use of europium(III) chelate-dyed nanoparticles was used. Four versions of a cTnI specific capture antibody were compared: native mouse monoclonal antibody, an enzymatically cleaved $F(ab')_2$ fragment, a recombinant Fab fragment and a modified Fab replacing the mouse with human constant regions. In addition to this binder, the assay used another chemically biotinylated capture antibody and a detection antibody covalently conjugated to nanoparticles. Nonspecific binding interactions caused by the sample matrix were studied using plasma samples obtained from individuals without known cardiac diseases.

Results. The best combination of capture antibodies regarding sample interference was the one with the partially humanized Fab fragment. The assay enabled measurement of cTnI in plasma samples in less than 15 minutes with an analytical sensitivity (background + 2SD) of 2 pg/mL. The 99th percentile of the assay estimated from 97 plasma samples was 10 pg/mL.

Conclusions. The use of a partially humanized recombinant antibody fragment in an assay utilizing antibody-coated particles with high specific activity resulted in elimination of sample interference and a potentially highly sensitive cTnI assay.

0281

RELATIONSHIP BETWEEN PARAOXONASE 1 ACTIVITY AND HIGH SENSITIVE C-REACTIVE PROTEIN AND THE DEGREE OF SEVERITY OF CORONARY ARTERY DISEASE IN PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

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Background. The antioxidant effect of HDL-C seems to be mediated through its enzymes in particular; paraoxonase-1 (PON-1). The mechanistic link between the activity of PON1 and the level of hs-CRP,was assessed in atherosclerotic patients with and without T2DM.

Methods. All selected individuals were subjected to full history taking, complete clinical examination, routine laboratory investigations in addition to hs-CRP and PON1 activity assays, ECG and the CAD patients groups were subjected to coronary angiography. Paroxonase activity is assessed in serum using Paraoxon (o,o-diethyl-o-p-nitrophenylphosphate

Results. CAD diabetic patients (n = 40) had statistically significant lower mean values of PON1 activity (255.9 ± 103.8 U/L) compared to the control subjects (395.1 ± 91.8 U/L) (p<0.05) . The mean PON activity in CAD diabetic and non diabetic groups decreased with increased severity of coronary vessels affection. On the other hand, in the non diabetic CAD group, the mean hs-CRP (8.1±4.7 mg/L) increased with increased severity of coronary vessels affection . The correlation study showed that PON activity was inversely correlated with diabetes duration in the CAD diabetic group. Also, it has been found that there was significant positive correlation between PON activity and HDL-C in the 3 groups of the study.

Conclusions. The significant decrease in PON activity level and significant increase in hs-CRP level in both the CAD groups proves the presence of a mechanistic association between low PON1 and inflammation in patients susceptible to the development of CAD, and may be the mechanism by which low PON1 levels promote atherosclerosis.

Cardiovascular disease

0282

CHARACTERIZATION OF IGG SUBCLASSES OF AUTOANTIBODIES TO CARDIAC TROPONIN

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Background. Autoantibodies (AAbs) to cardiac troponin are found in a high proportion of individuals with or without cardiac diseases. The aim of this study was to explore the IgG subclass frequency of these AAbs.
Methods. We analyzed admission and 3-months follow-up serums from 28 chest pain patients using an IgG-specific reference assay measuring total IgG and four subclass-specific assays. AAbs were first bound to troponin complex captured on a microtiter well by troponin I and C antibodies. Finally, the AAbs were detected with Europium-labelled anti-human antibody. We regarded net signals ≥100 counts per second positive if the T-test gave a P value ≤0.05 when the signals with and without added troponin were compared.
Results. The reference assay wasused to categorize the patients into two groups: the first included 14 AAb-negative patients and the second six patients who were AAb-positive already at admission and eight who reached the positivity at 3-months follow up.None of the samples in the first group were tested IgG1 or IgG2-positive but some were weakly positive for IgG3 and/or IgG4. All subclasses were found in the second group. Many samples were positive for multiple subclasses, the net signals were generally higher at the follow up and IgG4 was the most prominent subclass: eight patients were IgG4-positive at admission and 11 at the follow up.
Conclusions. Troponin leakage during acute coronary syndrome may induce an immune response leading to AAb formation. Also longer-term leakage may occur, because repeated antigenic stimulation usually leads to IgG4-response.

0283

PLASMA MYELOPEROXIDASE AND C-REACTIVE PROTEIN IN STABLE CORONARY ARTERY DISEASE PATIENTS

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Background. It was reported earlier that myeloperoxidase (MPO) substantially facilitates the early diagnosis of acute coronary syndromes and may have a prognostic role in patients with stable coronary artery disease (CAD). We aimed to assess whether MPO measurement may be of clinical value in CAD patients in relation to CRP concentration.
Subjects and Methods. The study included 100 patients with stable coronary artery disease (CAD) and 62 clinically healthy volunteers which served as controls. MPO concentration was measured in K2-EDTA plasma and cTnI in serum (Architect ci8200 Abbott Diagnostics), C-reactive protein was measured with high sensitivity method (BN II, Siemens Diagnostics).
Results. Median MPO concentration was 215 pmol/L (120-594) in CAD patients and 143 pmol/L (88-258) in controls whereas median hsCRP was only slightly higher in CAD cases 1,58 mg/L (0,73-3,33) than in controls 1,02 mg/L (0,36-2,77). Concentration of MPO in patients with CAD was related to hsCRP level. When hsCRP 3 mg/L was accepted as cut-off median MPO concentration in CAD patients with hsCRP< 3 mg/L was significantly higher than in controls (215 pmol/L vs 115 pmol/L; p=0,003). ROC analysis performed for MPO and hsCRP revealed not enough satisfactory Results. The discrimination power of both, MPO and hsCRP in this setting was only moderate (AUC= 0,658 and 0,624).
Conclusions. From these preliminary data it may be concluded that clinical utility of myeloperoxidase measurement for estimating cardiovascular risk in patients with stable coronary artery disease has a limited value.

Cardiovascular disease

0284

I-PRESERVE SUB-STUDY: PLASMA COLLAGEN MARKERS IN THE PREDICTION OF DEATH AND HOSPITALISATION IN PATIENTS WITH HEART FAILURE AND PRESERVED EJECTION FRACTION

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Background. Heart failure with preserved ejection fraction (HFPEF) is an increasing public health problem. Myocardial fibrosis is a pathological feature. Peripheral collagen markers may reflect this excess fibrosis. We measured collagen markers in a predefined cohort of patients of the i-PRESERVE study.

Methods. Plasma levels of procollagen type I amino-terminal peptide (PINP), PIIINP and osteopontin were measured in 313 samples of i-PRESERVE patients at baseline and six months following randomisation to placebo or irbesartan 300mg/day. The relation of baseline collagen markers to the I-PRESERVE primary endpoint (all-cause death and cardiovascular hospitalisation) was evaluated by univariate and multivariate analysis.

Results. Increased plasma levels of collagen markers at baseline were associated with increased risk of death and cardiovascular hospitalisation. For each 10mg/L increase in PINP, the hazard ratio (HR) for the primary endpoint was 1.09, 95% CI (1.052-1.13) $p<0.0001$; for 10mg/L increase in PIIINP the HR was 2.47 (0.97-6.33) $p=0.059$; for each 10 nmol/L increase in osteopontin, the HR was 1.084 (1.026-1.15), $p=0.004$. No parameter remained significant as independent predictor when introduced into a multivariate model. Both treatment groups tended to reduce collagen markers at 6 months.

Conclusions. Increased peripheral collagen turnover markers are associated with increased mortality and cardiovascular hospitalisation in a HFPEF population on univariate (but not multivariate) analysis. These findings suggest that pathological fibrosis in the heart could be contributory to adverse clinical outcomes in HFPEF patients.

0285

META-ANALYSIS OF THE EFFECT OF B-TYPE NATRIURETIC PEPTIDE TESTING ON CLINICAL OUTCOMES IN PATIENTS WITH ACUTE DYSPNEA

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Background. The accuracy of B-type Natriuretic peptide (BNP) testing in diagnosing acute decompensated heart failure (ADHF) has been extensively evaluated. However, the impact of this test on clinical outcomes remains controversial. We investigated the effect of BNP testing of Emergency Department (ED) patients presenting with acute dyspnea on clinical outcomes.

Methods. We identified randomized controlled trials (RCTs) of BNP testing compared with routine care, where clinical outcomes were reported. Estimates of effect size and 95% confidence intervals (CIs) were made by pooling data using Review Manager. Random effect models using inverse variance weighting were used for continuous outcomes.

Results. 5 RCTs with 2513 patients were included. The effect of BNP testing on all cause mortality was inconclusive (Odds Ratio 0.96, 95% Confidence Intervals 0.65, 1.41). A reduction in admission rates in the tested group is possible (OR 0.82; 95% CI 0.67, 1.01). Hospital length of stay was modestly reduced in the tested group compared with the control group (-1.22 days, 95% CI: -2.31, -0.14). Critical Care stay was reduced (-0.56 days, 95% CI: -1.06, -0.05). Limitations were a relatively small number of studies (n=5) with significant heterogeneity in patients, clinical setting and wide confidence intervals in outcome findings.

Conclusions. BNP testing in the ED for patients presenting with acute dyspnoea reduced hospital length of stay by about one day. The effect is better seen with longer hospital stay. Testing does not appear to reduce hospital mortality rates. A reduction in admission rates following BNP testing cannot be excluded.

Cardiovascular disease

0286

POLYMORPHISMS OF *CETP* AND THEIR ASSOCIATION WITH SEVERITY OF CORONARY ATHEROSCLEROSIS IN THAIS WITH CORONARY HEART DISEASE

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Background. Coronary atherosclerosis is the major cause of myocardial ischemia in coronary heart disease. A number of risk factors including dyslipidemia, hypertension, smoking, and genetic predisposition contribute to the pathogenesis. Polymorphisms of cholesteryl ester transfer protein gene(*CETP*) have been associated with atherogenic lipid profile implicated in coronary artery disease risk.

Background. To evaluate the correlation between *CETP* polymorphisms and severity of coronary atherosclerosis in Thai patients with coronary heart disease.

Methods. The *CETP* polymorphisms *-629C>A, Taq1B*, and *I405V* were genotyped in patients with coronary atherosclerosis and control subjects of equivalent age. Serum lipid levels were determined. The severity of coronary atherosclerosis was evaluated by quantitative coronary angiography and by Gensini score.

Results. All lipid levels, except HDL-C, were significantly higher in CAD cases than in control group. *CETP* polymorphisms of both *-629C>A* and *I405V* were significantly associated with high serum level of triglyceride (TG) (P= 0.032)and TG/HDL-C ratio (P=0.038). Vessel scores (number of vessels with ³ 50% stenosis) were also evaluated against lipid profile and CETP polymor-phisms. High vessel scores as well as Gensini score were significantly associated with high levels of TG and TG/HDL-C. However, only modest association of *CETP -629C>A* and *I405V* polymorphisms with vessel and Gensini scores were observed. There was no association of *Taq1B* polymorphism with vessel scores.

Conclusions. *CETP* polymorphisms may contribute to the severity of coronary atherosclerosis by increasing atherogenic lipid level.

0287

SERUM LIPIDS, HIGH SENSITIVITY C-REACTIVE PROTEIN, ANTI-OXIDIZED LDL ANTIBODY, AND URINE ALBUMIN IN NEPALESE SUBJECTS WITH HYPERTENSION, DIABETES AND BOTH

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Background. The identification of individuals who are at high risk for development of cardiovascular disease is an important aspect in primary prevention. The present study was designed to determine association of traditional risk factors including total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and emerging risk factors including high sensitivity C-reac-tive protein (hsCRP), anti-oxidized LDL antibody (oxLDL Ab) and urine albumin among Nepalese individuals with hypertension, diabetes and both

Methods. This study comprises 50 hypertensive, 50 diabetic, 50 individuals with both hypertension and diabetes, and 100 non-hypertensive non-diabetic controls. Fasting blood sample was analyzed for lipid profile- TC, HDL-C, LDL-C, triglyceride (Tg), hsCRP, oxLDL Ab (IMTEC HUMAN, Germany). Urine sample was collected to determine urine albumin creatinine ratio (UACR). One way Anova tool was used to determine difference in mean between these groups (SPSS, 11.5)

Results. TC and LDL-C was significantly elevated in diabetic compared to hypertensive and hypertensive-diabetic group. There was no significant difference in HDL-C among these groups. Serum Tg, oxLDL Ab and UACR was significantly elevated in hyper-tensive-diabetic compared to hypertensive and diabetic group. There was significant elevation of hsCRP in hypertensive-diabetic compared to hypertensive, diabetic and control groups with mean of 8.72 vs 4.26, 4.05 & 1.59 mg/L respectively (p<0.01). 70% of hypertensive-diabetic had FRS more than 20%, in contrast only 28% of diabetic and 23% of hypertensive had FRS more than 20%.

Conclusions. Concentration of hsCRP, oxLDL Ab and urine albumin significantly increased in Nepalese hypertensive-diabetic subjects compared to isolated hypertensive and diabetic individuals.

Cardiovascular disease

0288

NOVEL POSSIBLE WAYS OF MODULATION OF SYSTEMIC INFLAMMATION

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Background. Systemic inflammation is underlying mechanism of various diseases, including atherosclerosis. It can be modulated by different ways.
Methods. Studied parameters were determined in patients with advanced coronary artery (CAD) disease (at least 50% stenosis of the left main coronary artery according to coronarographic examination; S, n=91) and control group (n=49).
Plasma levels of beta-carotene, alpha-tocopherol and malondialdehyde were determined by HPLC, free radical concentration by direct method. Concentrations of hsCRP, interleukin-6 (IL-6), HDL-cholesterol, fibrinogen, were determined by standard procedures. Between-group differences in continuous variables were analyzed with the Hotelling T^2-test (software NCSS2000), analyses of correlation matrix using the software STATISTICA.
Results. In patients with coronary stenosis (S) higher level of inflammation coincided with lower levels of beta-carotene (BC) and alpha-tocopherol (AT). BC: S: 0.07±0.10 mmol/L vs C: 0.14±0.08 mmol/L (p<0.05); AT: S: 20.76±6.52 mmol/L vs C: 23.59±5.95 mmol/L (p>0.05); IL-6: S: 4.85±3.55 ng/L vs C: 2.90±2.76 ng/L (p<0.001). The inverse correlation between beta-carotene and IL-6 (correlation coefficient -0.28, p<0.05) was found in patients with CAD.
Conclusions. Advanced CAD coincided with significantly lower plasma concentrations of HDL-cholesterol and beta-carotene as well as with elevated levels of all inflammatory markers, but only mild increase of oxidative stress. Beta-carotene significantly inversely correlated with interleukin-6. This inverse correlation could suggest potential protective effect of beta-carotene on atherosclerosis due to the inhibition of inflammatory processes.

0289

ATHEROGENIC INDEX OF PLASMA [(LOG$_{10}$ TRIGLYCERIDES/HDL-CHOLESTEROL)] IN HEMODIALYSED PATIENTS

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Background. Cardiovascular diseases are the major cause of mortality in hemodialysed patients. The new atherogenic index of plasma [$\log_{10}$(TG/HDL-C)] is a sensitive predictor of cardiovascular risk and it also accurately reflects the presence of atherogenic small LDL and small HDL particles. The aim of this study was to determine if chronic hemodialysis treatment affects the atherogenic index of plasma (AIP) together with ratio apolipoprotein A-1/apolipoprotein B and total cholesterol/HDL-cholesterol.
Methods. This study included 48 hemodialysed patients. Total cholesterol, triglycerides, HDL-cholesterol, apolipoproteins A-1 (apo A1) and apolipoprotein B (apo B) were measured at the beginning of the hemodialysis session: first at the start of the study and then after 6, 12 and 18 months of regular hemodialysis treatment. Atherogenic index of plasma (AIP), apo B/apo A-1 and total cholesterol/HDL-cholesterol ratios were calculated. A sign test and ANOVA with the additional Tukey post hoc test was used to evaluate the significance of changes.
Results. After 18 months of regular hemodialysis, the total cholesterol/HDL-cholesterol ratio (4.45±1.72 vs 4.10±1.28; p=0.076) and apo B/apo A1 ratio (0.72±0.29 vs 0.69±0.24; p=0.301) remained unchanged. However, the AIP increased significantly (0.10±0.16 vs 0.22± 0.19; p=0.046).
Conclusions. The increased AIP after 18 months of regular hemodialysis treatment demonstrates that hemodialysis-induced dyslipidemia has a proatherogenic nature, suggesting that it may increase risk of cardiovascular diseases, even though the total cholesterol/HDL-cholesterol and apo B/apo A1 ratios remain unchanged.

Cardiovascular disease

0290

HIGH SENSITIVITY TROPONIN FOR DETECTION OF MYOCARDIAL INFARCTION: COMPARISON OF TROPONIN T HIGH SENSITIVE (TNT HS) AND PATHFAST CTNI

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Background. A new generation of troponin assays has been introduced recently. We intended to investigate wether the PATHFAST cTNI assay is comparable to the new high sensitive assays. Therefore we determined the diagnostic validity for detecting NSTEMI in comparison to TnT hs in patients with ischemic symptoms suggestive of acute coronary syndromes.
Methods. Troponin was measured using both assays in serum samples of consecutive patients admitted to the chest pain unit at presentation,3 and 6 hours after admission The results of TnT hs and PATHFAST cTNIwere compared to the discharge diagnosesSTEMI, NSTEMI, uAP, non cardiac chest pain, and others.
Results. To evaluate the diagnostic validity for detection of NSTEMI the results of TnT hs and PATHFAST cTNIwere compared by ROC analysis.Using the cut-offs based on the 99th percentile of normal the ROC analysis revealed sensitivities and specificities of85%/83% and 77%/96% at admission, 94%/81% and 92%/96% after 3 hours, 95%/79% and 90%/93% after 6 hours, respectively. The ROC analysis revealed AUCs for TnT hs and PATHFAST cTnI of 0.962 and 0.910 at presentation, 0.964 and 0.958 after 3 hours,0.958 and 0.949 after 6 hours, respectively.
Conclusions. The PATHFAST cTNI was comparable to TnT hs and highly sensitive for detection of NSTEMI, with increasing sensitivity already at admission and after 3 hours, not going along with decreased specificity. The PATHFAST cTNI assay is high sensitive and useful for early diagnostis of NSTEMI in patients with symptoms of acute coronary syndrome admitted to the emergency room.

0291

INCREMENTAL PROGNOSTIC VALUE OF TROPONIN T HIGH SENSITIVE (TNT HS) IN RISK STRATIFICATION OF STABLE CORONARY ARTERY DISEASE

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Background. Cardiac troponin T is an established prognostic marker in patients with acute coronary syndromes but not in stable coronary artery disease (CAD) like N-terminal pro-B-type natriuretic peptide (NT-proBNP). We now examined the prognostic value of a new high-sensitivity troponin T assay (TnT hs) in CAD.
Methods. 256 patients with stable CAD were included into a retrospective nested case-control analysis: 128 cases who died from cardiovascular causes during a median follow-up of 7.5 years and 128 survivors matched for age and sex. TnT hs and NT-proBNP were determined in baseline samples. using immunoassays (Roche Diagnostics, Germany).
Results. 62% of the 256 subjects exhibited TnT hs $\geq$ 14 ng/L, the 99th percentile cut-off for AMI. Logistic regression found TnT hs and NT-proBNP as independent risk markers. ROC analysis identified optimal cut-offs at 15 ng/L and 352 μg/L for hsTnT and NT-proBNP, respectively. Odds ratios of 6.68 (95 % CI 3.22-13.86), 6.94 (95 % CI 2.44-19.73) and 14.85 (95 % CI 7.03-31.32) were calculated for mortality risk in patients with elevated NT-proBNP, TnT hs or both markers. Patients with one or two positive markers exhibited 5-year cardiovascular mortality of 40% and 60% respectively. The addition of TnT hs to NT-proBNP significantly increased c-statistics of proportional hazards calculated from survival times as well as net reclassification indexes.
Conclusions. Many patients with stable CAD exhibited elevated concentrations of TnT hs which were strongly associated with mortality. The combined determination of hsTnT and NT-proBNP was superior for risk stratification compared to determining either marker alone.

Cardiovascular disease

0292

PERFORMANCE CHARACTERISTICS OF FOUR SOURCES OF CHOLESTEROL ESTERASE FOR TOTAL SERUM CHOLESTEROL ASSAY BY THE ENZYMATIC KINETIC METHOD

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Background. The first analytical reaction of enzymatic cholesterol method is hydrolysis of cholesterol ester to free cholesterolby the cholesterol esterase (CE). Using kinetic measurement, this reaction rate must be fast and complete before time measurement begins.This study compared performances of *Pseudomonas fluorescens, Candida cylindracea*, bovine pancreas and porcine pancreasCE for serum cholesterol determination by enzymatic kinetic method.
Methods. We studied the optimization and effect of sodium cholate concentration on each CE.The minimal enzyme activity necessary to produce the optimal linearity were selected.We evaluated and comparedlinearity, precision, recovery, interference, stability, and comparison with the standardized endpoint method.
Results. The optimization of *P. fluorescens, C.cylindracea*, bovine pancreas and porcine pancreasCE was 200,100, 100, and 100 U/L with sodium cholate concentration at 3, 5, 15 and 12 mmol/L, respectively. Because of using high enzyme concentration, *P. fluorescens* decided not to continue the evaluation. Linearity for *C.cylindracea*, bovine and porcine pancreasenzymes was up to 16.3 mmol/L. The average within-run and between-day CVs ranged from 0.7% to 1.8% and 2.5% to 4.6%, respectively. All assays compared favorably with the standardized endpoint method(r = 0.995 to 0.999). Effect of interfering substances was less for porcine pancreas than those for *C.cylindracea* and bovine pancreas. Reagents were stable up to 4, 3 and 5 weeks when using *C. cylindracea*, bovine and porcine CE, respectively.
Conclusions. One can use *C. cylindracea*, bovine pancreas and porcine pancreas as the sources of CE for serum cholesterol determined by the enzymatic kinetic method.

0293

ESTIMATION OF PLASMA SMALL DENSE LDL-CHOLESTEROL FROM CLASSIC LIPID MEASURES

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Background. Small, dense low density lipoprotein (sdLDL) particles are a powerful predictor of atherogenesis. However, most sdLDL methodologies are expensive, time consuming and technically demanding, making them too laborious for routine clinical practice. Calculated low-density lipoprotein cholesterol (cLDL-C) may differ from direct measurement (dLDL-C), and this difference may depend on presence of sdLDLparticles in addition to variation in triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) concentrations. The presence of such dependence would offer a simple means to estimate sdLDL. We studied dependence of sdLDL on cLDL-C, dLDL-C and other variables.
Methods. We measured glucose, creatinine, total cholesterol (TC), TG, HDL-C and dLDL-C using standardized methods (n=297). For sdLDL-cholesterol, a novel homogenous assay was used. The cLDL-C was calculated using the Friedewald formula: cLDL-C = TC – HDL-C – TG/2.2, for 220 subjects after excluding for liver or renal disease. We used stepwise regression to analyze the significance of these variables.
Results. Usingstepwise regression analysis identified non-HDL cholesterol, cLDL-C and dLDL-C as significant variables (p <0.001), R^2 =0.88 and the standard error of the estimate of 0.238 mmol/L. The regression equation was sdLDL-C(mmol/L) = 0.580 (non-HDL cholesterol) +0.407 (dLDL-C) -0.719 (cLDL-C) -0.312
Conclusions. The sdLDL-C concentration can be estimated from the non-HDL cholesterol, cLDL-C and dLDL-C, providing a cost-effective method for screening patients for the risk of cardiovascular disease. Moreover, the identification of a simple inexpensive marker for sdLDL particles may pre-select patients who would most benefit from a more definitive subfraction workup.

Cardiovascular disease

0294

COMPARATIVE EVALUATION OF B-TYPE NATRIURETIC PEPTIDE AND MID-REGIONAL PRO-A-TYPE NATRIURETIC PEPTIDE CHANGES FROM ADMISSION TO DISCHARGE IN PROGNOSIS OF HOSPITALIZED PATIENTS FOR ACUTE HEART FAILURE

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Background. BNP changes (deltaBNP) from admission to discharge are used for therapeutic monitoring and prognosis of patients (pts) with acute heart failure (HF) but the wide intraindividual biological variability (CVi), the analytical imprecision and the presence of other co-morbidity (i.e renal failure) have been led to discordant Results. Mid-regional pro-A-type natriuretic peptide (MRproANP) was demonstrated to be non-inferior with respect to BNP for HF diagnosis and prognosis and could show a lower CVi. Consequently deltaMRproANP could be a more useful prognostic marker compared to deltaBNP; we evaluated this hypothesis.

Methods. Retrospective study. 44 consecutive pts (mean age 68±11 years, 75% males, LVEF: 36±15%) with BNP (Access2-Beckman Coulter) and MRproANP (Kryptor-Dasit) paired data (admission-pre discharge) hospitalised for acute HF (class NYHA III-IV: 98%pts) during 12 months. End points: cardiovascular death/heart transplantation/readmission for HF. ROC analysis for prognostic evaluation.
Follow up: median 5 (range: 2-14) months.

Results. Aetiology: ischemic heart disease (48%), primary cardiomyopathies (27%), ventricular dysfunction secondary to hypertension (16%), valve heart disease (29%). 26 (59%) patients had renal failure (MDRD<60 ml/min). Adverse outcome: median deltaBNP: -34 (-53;-10), median deltaMRproANP 1 (-13; 11). Event free: median deltaBNP: -55 (-71;-33), median deltaMRproANP -21 (-40; -4). ROC curve analysis showed that the areas under curve were similar for deltaBNP (0.71; 95%CI 0.55-0.87, p=0.025) and deltaMRproANP (0.75; 95%CI 0.60-0.90, p=0.008).

Conclusions. DeltaMRproANP seems more convenient compared to BNP due to the rise/small fall in adverse outcome and marked fall in event free patients, although the accuracy is similar.

0295

EVALUATION OF THE RELATION BETWEEN CHANGES OF MDRD AND B-TYPE NATRIURETIC PEPTIDE FROM ADMISSION TO DISCHARGE IN PROGNOSIS OF HOSPITALIZED PATIENTS FOR ACUTE HEART FAILURE

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Background. B-type natriuretic peptide (BNP) correlates with outcomes in patients (pts) with heart failure (HF). The lower BNP levels, the better event-free survival. However, elevated baseline BNP values unlikely decrease to normal range after treatment and relative changes in BNP (cBNP) could be a better option for prognostic purpose. BNP is increased in pts with renal failure; pts who had improvement in renal function could have a decrease in BNP irrespective to the HF status. We evaluate if MDRD positive-negative changes (cMDRD) during hospitalization can influence the predictive value of cBNP.

Methods. Retrospective study. 63 consecutive pts (mean age 69±11 years, 75% males, LVEF:35±15%) with BNP (Access2-Beckman Coulter) paired data (admission-pre discharge) hospitalised for acute HF (class NYHA III-IV: 98%) during 12 months. A linear regression model was estimated to evaluate the cMDRD-induced cBNP variations and residuals from this model were computed (cBNP-free from cMDRD). A multivariable logistic regression model was used to estimate the prognostic impact of cBNP taking into account the cMDRD effect.

Results. 39 (62%) had renal failure. Event-free pts: median cBNP -60 [-68; -25]; adverse outcome: median cBNP -34 [-52; 11]. cMDRD partially determined cBNP (regression slope=0.2, p=0.04). cBNP values significantly correlated with cardiovascular death/heart transplantation/readmission for HF (every unit increase: HR 1.03, 95% CI: 1.02-1.04, p=0.03, follow up: 12±9 months). cMDRD was not correlated with outcome. The same results were obtained by using cBNP-free from cMDRD.

Conclusions. our preliminary results suggest that cMDRD does not skew cBNP prognostic value.

Cardiovascular disease

0296

HOMOGENEOUS MRI FSAP ASSAY BASED ON LOCI® REAGENT TECHNOLOGY* AND ITS CORRELATION TO PCR

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Background. The Marburg I (MRI) single nucleotide polymorphism (SNP) of the factor VII-activating protease (FSAP) gene has been associated with venous thromboembolism, atherosclerosis, incidence and progression of carotid stenosis and evolution of progressive liver disease in chronic hepatitis C infection. At present, MRI SNP testing is performed by costly nucleic acid testing (NAT).
Methods. Here, we describe a homogeneous assay based on Luminescent Oxygen Channeling Immunoassay (LOCI) technology to detect the MRI FSAP alloenzyme. The assay is based on a monoclonal antibody that can discriminate the MRI from wild type FSAP.
Results. Turn-around-time of the method is 10 minutes. Analysis of 131 specimens resulted in a diagnostic sensitivity of the assay of 100% (95% confidence interval (CI): 84.5 - 100%) and a diagnostic specificity of 100% (95% CI: 96.6 100%) compared to PCR.Coefficients of variation ranged from 1.6% to 4.9%. No cross-reactivity with homologues of the MRI FSAP alloenzyme was observed. Test performance was not impaired by typical interfering compounds.
Conclusions. We conclude that the preliminary research assay prototype accurately detects the MRI FSAP alloenzyme in human plasma, and therefore represents an attractive alternative to NAT. The described prototype assay will simplify MRI FSAP testing.

0297

DIRECT CHROMOGENIC SUBSTRATE IMMUNO-ACTIVITY ASSAY FOR TESTING OF FACTOR VII-ACTIVATING PROTEASE

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Background. The Marburg I (MRI) single nucleotide polymorphism (SNP) of the factor VII-activating protease (FSAP) gene has been associated with thrombophilia and atherosclerotic diseases. PCR is used to detect the SNP. The specific FSAP activity to cleave single chain urokinase-type plasminogen activator (scu-PA) serves as a surrogate for PCR testing. Development of further assays is indicated to increase testing opportunities for future studies. In this study we present a research prototype of a direct chromogenic substrate immuno-activity assay for FSAP (FSAP dcs assay).
Methods. Antibody captured FSAP cleaves a chromogenic substrate, measured absorption changes are proportional to the FSAP activity. Performance characteristics of the FSAP dcs assay were compared to the FSAP scu-PA assay.
Results. The FSAP dcs activity assay determines the presence of the MRI FSAP alloenzyme with a diagnostic sensitivity and specificity of 100% (95% confidence interval [CI]: 89.6 - 100%) and 95.8% (95% CI: 93.2 - 97.4%), respectively. The specific FSAP dcs activity (ratio of FSAP activity to antigen concentration) increased diagnostic specificity to 98.9% (95% CI: 97.2 - 99.6%).
Conclusions. The specific FSAP dcs assay could represent a reliable method for the detection of the FSAP MRI alloenzyme. Due to the limited correlation between the FSAP dcs and scu-PA assays these different measurands may exhibit different utility in research and clinical applications. Thus, the FSAP dcs assay may represent a valuable alternative for FSAP testing in future.

Cardiovascular disease

0298

ISCHEMIA MODIFIED ALBUMIN AFTER ELECTIVE PERCUTANEOUS CORONARY INTERVENTION

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Background. Ischemia modified albumin (IMA) has been suggested as a new biochemical marker of transient myocardial ischemia, which may be useful in monitoring of myocardial damage induced by percutaneous coronary intervention (PCI). Our goal was to investigate the effect of elective PCI on the IMA levels in patients with stable angina.
Methods. We studied 38 patients (mean age 60.1±8.6 yrs, 24 male) underwent elective PCI for the management of stable angina pectoris. The results were compared with two clinically different groups: 1) 43 patients presenting with acute myocardial infarction (AMI) but normal cardiac troponin I (cTnI) concentration and 2) 45 healthy subjects. Both IMA and cTnI in patients qualified for elective PCI were measured before, immediately after and 6 hours after PCI.
Results. Mean IMA levels (U/mL) were higher in patients with stable angina (120.1±33.2) as compared to the control group (96.76±15.3, $p<0.001$); and to the AMI group (111.61±29.7, $p<0.32$). In all these patients increase of IMA concentration immediately after PCI was observed to 132.1±25.6, $p<0.05$) and returned to baseline by 6 hours after PCI. In contrast, mean cTnI (μmol/L) values were not elevated immediately after PCI, but were significantly elevated above baseline 6 hours after the procedure (0.015±0.008 vs 0.074±0.054, $p<0.05$).
Conclusions. IMA levels were significantly higher in patients with stable angina compared with those without symptoms of ischemia. IMA levels rise in patients who develop ischemia during PCI. The results of our study suggest that IMA may be a marker of transient myocardial injury after PCI.

0299

PROTEIN-ENERGETICAL STATUS ASSESSMENT IN CHRONIC HEART FAILURE: FOCUS ON TOTAL LYMPHOCYTE COUNT

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Background. Challenge in the management of patients with chronic heart failure (CHF) lies in the integration of the multiple facets of diseases. Protein is the principal compound upon which body structure and function is based; it is not stored to any degree in a non-functional form awaiting use; in this context, a gain or loss of function, and thus evaluation of patient's protein nutriture/"protein-energetical status" (PES) can be very important, particularly in CHF. Our objective was to study PES assessment some clinical-laboratory indexes in CHF patients of different etiology and severity of disease.
Methods. 95 patients with CHF were investigated. PES assessment indexes; some other biochemical data; serum lipid markers including ApoA; ApoB; TNF-α; IL-6 level; serum T3, T4, IMA (T3/T4); also left ventricular ejection fraction (LVEF%) were investigated in groups divided by diseases etiology, severity (NYHA FC) and immune status (IS), assessed by total lymphocyte count (TLC).
Results. We identified, that in normal nutritional status group (TLC ›1800) were got CHF patients from all FC and their distribution, according the FC (II/III/V) were 57%/28%/15%; in mild nutritional depletion (TLC = 1200-1800) – 16%/60%/24%; in moderate depletion (TLC – 800-1199) – 29%/52%/19% and in severe group (TLC ‹ 800) – 0%/40%/60%. Investigation also represents, that PES assessment some and other studied markers are differed between the FC groups and between IS study groups. Between PES assessment indexes IS was not significantly differed according the disease severity.
Conclusions. Total lymphocyte count may have independent clinical value in the assessment of CHF patients.

Cardiovascular disease

0300

INFLUENCE OF EXTREME HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C) VALUES ON THE CALCULATION OF LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C) USING FRIEDEWALD FORMULA

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Background. Limitations on the use of Friedewald Formula (FF) for estimating LDL-C with triglycerides (TG)> 400 mg/dL or in several pathologic states are well described. However, there is no previous information about the influence of extreme HDL-C levels in the results given by FF.
Methods. HDL-C, LDL-C, TG and total cholesterol were measured using a Hitachi Modular D/P/ISE and Roche Diagnostics reagents. Samples with HDL-C 20 mg/dL or lower and over 120 mg/dL were collected during 2008 and 2009. LDL-C were measured by a homogeneous direct method (DM) and subsequently calculated with FF. LDL values were compared using Passing-Bablok regression and Bland-Altman plot that were performed with Method Validator Freeware V1.19.
Results. 2838 samples had HDL-C≤ 20 mg/dL. Intercept and slope (95% CI) for Passing-Bablok were (1.00; 1.04); (9.3; 12.0) respectively, and mean difference (95% CI) was (19.3; 22.4), r=0.61. 208 samples had HDL-C> 20 mg/dL. Intercept and slope (95% CI) for Passing-Bablok were (0.93; 1.03); (-5.4; 4.7) respectively, and mean difference (95% CI) was (-3.1; 0.7), r=0.94.
Conclusions. Results show that it is not possible to exchange LDL-C calculated with FF and LDL-C measured with a direct method when HDL-C is 20 mg/dL or lower. Consequently, it is necessary to add this limitation to the FF and we suggest measuring LDL-C concentration with a direct method or the beta-quantification procedure when HDL-C level is very low.

0301

THE IMPACT OF HEMOLYSIS ON THE RESULTS OF CARDIAC TROPONIN

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Background. Occasionally, hemolysis may occur because of a prehemolytic disease state, but it is more often due to improper preanalytical sampling or handling error. The specificity is an important requirement for cardiac troponin (cTn) measurement, for this reason it is essential to know how different immunoassay systems can be affected by hemolysis. Exact data from manufacturers in this respect are lacking. This study examines the effect of hemolysis on one cTn-T (Elecsys2010, Roche) and two cTn-I (Architect, Abbott and Vidas, BioMerieux) immunoassays.
Methods. The osmotic shock method was utilized to prepare hemolyzates. Nonhemolyzed pooled sera were collected from patients with five different cTn concentrations (from near to the diagnostic cut-off values) to produce hemolyzed specimens to varying degrees (Hgb: 0-12 g/l). The bias was calculated from cTn concentration without and with hemolysis.
Results. The bias of cTn-T levels was the highest (minus 20-40 %) in the low cTn-T range (0.035-0.172 ng/ml). The bias correlates with haemoglobin concentrations [% = -0.27*lnHgb(g/l)]. In the case of the cTn-I methods we observed smaller hemolysis interference (Architect: +4-minus10; Vidas: +13-minus22 %).
Conclusions. Hemolysis interference occurred in all three methods, however different degrees and can potentially lead to false-positive (both cTn-I) or to more dangerous false-negative (cTn-T) Results. We suggest in the case of cTn-T the correction of the results for hemolysis by use of mathematical model, but in the case of cTn-I it is not recommended. Laboratories should consider the effect of factors such as hemolysis when selecting methods and setting cut-off values.

Cardiovascular disease

0302

NATRIURETIC PEPTIDES ARE ASSOCIATED WITH SOCIAL DEPRIVATION AND SUBCLINICAL ATHEROSCLEROSIS

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Background. The association between social deprivation and cardiovascular risk is well recognised, but not adequately explained by classical cardiovascular risk factors. Evidence suggests that elevated concentrations of natriuretic peptides are associated with increased cardiovascular risk in the general population. The contribution of natriuretic peptides to the socioeconomic gradient in cardiovascular risk has not been previously studied.
Methods. B-type natriuretic peptide (BNP) and N-terminal proBNP (NTpro-BNP) were measured on plasma samples (n=490) from the Psychological, Social and Biological Determinants of Ill Health (pSoBid) study. BNP was measured on Siemens ADVIA Centaur and NTpro-BNP on Siemens Immulite 2500.
Results. BNP and NTpro-BNP were both higher in the most deprived (MD) compared to the least deprived (LD) participants: BNP (median (IQR)) was 15.5 (8.3, 30.3) pg/mL in MD and 11.1 (7.1, 21.2) pg/mL in LD (p=0.0008); NTpro-BNP was 51 (23, 96) pg/mL in MD and 26 (10, 54) pg/mL in LD (p<0.0001). On univariate analysis, both markers were associated with ultrasound indicators of subclinical atherosclerosis (common carotid intima-media thickness (cIMT) and plaque presence) (p<0.04 in all cases). On multivariate analysis, adjusting for age, sex and BNP or NTpro-BNP removed the significance of area level deprivation as a predictor of cIMT. However, deprivation remained a significant predictor of plaque presence after adjusting for age, sex and BNP or NTpro-BNP.
Conclusions. The associations of the natriuretic peptides with both social deprivation and cIMT suggest potential utility for these biomarkers in unravelling the factors underlying the socioeconomic gradient in cardiovascular risk.

0303

CLINICAL SIGNIFICANCE OF CK-MB ISOENZYME IN ACUTE MYOCARDIAL INFARCTION

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Background. Acute myocardial infarction (AMI), is a major cause of morbidity and mortality worldwide and continues to pose significant therapeutic challenges. Aim of this study was to analyze the serum concentration of creatine kinase MB isoenzyme (CK-MB) in patients, in different period after AMI.
Methods. The study included 66 individuals grouped in four groups. 1st - patients 24 hours after AMI(n=14; 9 male and 5 female, age 50-75); 2nd group - patients 5 days after AMI(n=14; 9 male and 5 female, age 50-75); 3th group (n=14; 9 male and 5 female, age 50-75) – patients 10 days after AMI, and 4th group (n=24, 15 male and 9 female, age 45-60 years) was control. Serum concentration of CK-MB was determined with spectrophotometric method.
Results. Obtained results have show very high level of isoenzyme CK-MB in first few hours after AMI (male: 27,66±5,52 U/L and female 13,4±2.57 U/L) – 1st group. After that the concentration of CK-MB continuously decrease (male: 15,22±5,5 U/L, and female 13,4±2.57 U/L) – 2nd group. In 3th group CK-MB values were 7,88±4,5 U/L –male patients, and 9,88±2,93 U/L in female patients. In control group CK-MB was 5,00±2,18 in male and 3,6±1,63 in female individuals. According results of this study AMI is two times more frequent in male than in female.
Conclusions. CK-MBas a reflection of myocardialcell damage, plays the most importantpart in establishing the early diagnosis of myocardial infarction.

Cardiovascular disease

0304

EVALUATION OF COLLAGEN PEPTIDES ON THE MYOCARDICAL TURNOVER IN HYPERTROPHIC CARDIOMYOPATHY

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Background. Hypertrophic cardiomyopathy (HCM) is characterised by inappropriate hypertrophy, small-vessel coronary artery disease, myocyte disarray and increased interstitial fibrosis. This study evaluates the collagen turnover in HCM.
Methods. We included 95 HCM patients, 72 males, aged 45.7±14.2 years and 45 healthy similar controls. A complete history and clinical examination was performed, including electrocardiogram, echocardiography, 24 hours ECG-Holter monitoring. Blinded cardiac MRI was performed with late myocardial-enhanced study with Gadolinium. Serum levels of a byoproduct of collagen III synthesis (PIIINP) were measured by radioimmunoassay and 3 peptides resulting from collagen I synthesis (PICP and PINP) and degradation (ICTP), by commercial ELISA.
Results. Patients had higher levels of ICTP than controls (2.35 (1.15-3.94) vs 1.78 (1.27-2.78), p=0.041) and higher levels of PICP/ICTP ratio (48.0(31-96.3) vs 46.8(32.2-70.0), p=0.026). PICP raised in patients with hypertension (p:0.021) and sudden familiar death (p:0.047). PINP raised in patients with maximal LV wall thickness >30 mm, (p=0.001). PIIINP raised on patients with history of syncope (p:0.007). We did not find any significant association with non sustainded ventricular tachycardia, abnormal blood pressure response to effort test, severe functional class, atrial fibrillation, significant obstruction, or late Gadolinum enhancement in MRI.
Conclusions. We propose the use of PICP/ICTP as apossible synthesis-degradation markerof type I collagen turnover, to estimate the degree of myocardial fibrosis with a simple serum test in HCM. We cannot confirm a potential clinical use for collagen type I/IIIpeptides because of only occasional associations were found.

0305

SCREENING FALSE POSITIVE CARDIAC TROPONIN-I WITH 2-FOLD DILUTION METHOD

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Background. It has been shown that measuringthe Cardiac TroponinI (cTnI) concentration can be used to distinguishmyocaridial injury. However, heterophilic antibodies and rheumatoidfactor havebeen published to interferewith one or more cTnI assays.This study demonstrated dilution samples could be screened the suspected presence of analyticalinterference.
Methods. A total of 216 samples with cTnI values >0.5μg/L were investigated. Laboratoryprotocol as follows:(a) diluted (1:1)with normal plasm and retested using the Beckman Coulter AccessAccuTnI system; (b) thesampleswere analyzed for TOSOH AIA Assays; (c) thesamples were tested forpolyethylene glycol (PEG)treatment and Heterophilic Blocking Tube(HBT)
Results. 212 samples were displayed a 10% difference betweenthe original and the dilution. The other 4 samples increased by more than 80% after 2-folddilution.Using TOSOH AIA Assay, HBT and PEG treatment were normal. Clinical data demonstrated that the 4 positive sampleswere suspected Results.
Conclusions. We conclude that a sample which cTnI value increased by more than 80% after 2-folddilutions was considered heterophile interference.Our study demonstrated that the 2-folddilution method was very simple, effective in screening falsepositive cTnI..

Cardiovascular disease

0306

***PPAR* POLYMORPHISMS AND RISK OF CORONARY ATHEROSCLEROSIS IN THAIS**

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Background. Peroxisome proliferator activated receptors (PPARs) are subfamily of nuclear hormone receptors which play important roles in metabolic disorders and vascular inflammation related to atherosclerosis. Both of the *PPARa*and *PPARg* gene polymorphisms have been implicated in the metabolic syndrome, type 2 diabetes (T2DM) and coronary artery disease (CAD).
Aim. To evaluate the association between *PPARs* polymorphisms and risk factors of coronary atherosclerosis in Thais.
Methods. The polymorphisms of *PPARa*(*V227A) and PPARg (P12A)* were genotyped in subjects with dyslipidemia and control group, using allele-specific polymerase chain reaction (AS-PCR) technique. They were evaluated for the association with lipid profiles, fasting blood glucose and diabetic condition.
Results. The frequencies of *PPARa227A* and *PPARg12A* alleles in the studied population were 0.020 and 0.025, respectively. The carriers of *227A* were associated with lower total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and non-high density lipoprotein cholesterol (non-HDL-C) as compared with *227V*. By contrast, the *12A* carriers of *PPARgP12A* were associated with higher level of TG and TG/HDL than subjects with *12P* allele ($P < 0.05$). The percentage of *12A* in non-diabetic group (6.015%) is higher than in diabetic group (2.13%). Furthermore, carriers of *12A* allele revealed lower level of fasting blood sugar than that of *12P*.
Conclusions. The PPARs polymorphisms *V227A* and *P12A* are low in Thais. The *227A* may associated with low risk of atherogenic lipid levels, whereas *12A* allele may correlate with low risk of diabetic development.

0307

MIRNA AS A NOVEL BIOMARKER OF MYOCARDIAL INJURY: STUDIES ON CABG PATIENTS

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Background. MicroRNAs (miRNA) are non-coding, small (20-25 nucleotides), endogenous, single-stranded RNAs. MicroRNAs modulate gene expression by binding to complementary sequences in the 3' untranslated regions in mRNA resulting in translation inhibition or mRNA degradation. There is increasing evidence that miRNAs play a critical role in many pathological processes such as cancer and cardiovascular disease. Moreover, recent studies indicate changes in miRNA expression in heart diseases. The studies on rat model show that plasma miRNA-208 may be a useful biomarker of myocardial infarction. The aim of this study was to investigate expression of miRNA-208 in plasma of coronary artery bypass graft (CABG) patients and its correlation with the myocardial infarction biomarkers and the clinical state of the patients.
Methods. The study included analysis of miRNA-208 expression in plasma samples from 30 patients undergoingthe CABG surgery. The samples were collected before, 3h, 6h and 12h after the surgery. Total RNA was isolated from plasma with *mir*Vana PARIS Kit.MicroRNA expression was analysed using TaqMan-based real-time PCR.
Results. Preliminary data indicate that miRNA-208 is present only in the plasma collected after CABG surgery (n=30). The expression of miRNA-208 increases to a detectable level already 3h after surgery (n=21) and decreases to undetectable level (Ct>40) in 12h after operation (n=14). Additionally, expression of miRNA-208 in plasma corresponded to plasma level of Troponin I and the clinical state of patients.
Conclusions. The plasma miRNA-208 might be considered as an early and specific biomarker of myocardial injury with a direct importance to the treatment.

Critical care/emergency medicine

0308

PROCALCITONIN AND C- REACTIVE PROTEIN AS PROGNOSTIC MARKERS IN A PEDIATRIC INTENSIVE CARE UNIT

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Background. Procalcitonin (PCT) and C-Reactive Protein (CRP) are often used to guide therapy in different kinds of infection. Their role as markers of severity is less studied. The aim of this study was to determine if PCT and CRP values could be used as risk markers in critically ill children.
Methods. PCT, measured with ADVIA Centaur® BRAHMS PCT assay, was analyzed in Pediatric Intensive Care Unit (PICU) patients during a period of seven moths (from May 2010 to December 2010), registering the value at admission and the maximum value of this parameter. We also registered the CRP value at admission, measured with Siemens Dimension® System. Intra-hospital mortality from these patients was also analyzed. Descriptive and comparative statistical analyses were performed using IBM SPSS Statistics 17.0.
Results. During a period of seven months, 124 patients (mean age: 79,6 ± 71,6 months; 71% males) had PCT and CRP measured during their PICU stay. Mean PCT and CRP at admission were 6,17±18.64 ng/ml and 7.38±8.28 mg/dL, respectively. Average PCT peak was 3.17±4.34 ng/mL. We found a significant positive correlation between PCT and CRP with mortality rate at admission ($p=0,005$; $\rho=0.29$). In patients that died during PICU stay PCT at admission was also significantly higher than survivals ($p=0.002$). CRP at admission was non-discriminatory ($p=0.23$).
Conclusions. PCT at admission and peak PCT were significantly higher in patients that died during PICU stay. This was not true for CRP at admission. There was a positive correlation between PCT and CRP at admission.

0309

USE OF NEUTROPHILS CD64 IN DIAGNOSIS OF INFECTION AND SEPSIS

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Background. Infection is still a common health problem in hospitals, especially in intensive care unit (ICU). CD64 is a high affinity Fc-gamma receptor expressed by activated neutrophils. It is sensitive and specific laboratory indicator of infection and sepsis. The aim of the study is to verify this statement.
Methods. Peripheral blood samples were taken from 75 ICU patients, who were divided into three groups (Gr.1, Gr 2 and Gr 3). Gr 1: 34 patients with no signs of systemic inflammatory response syndrome (SIRS negative). Gr. 2: 22 SIRS positive patients. Gr. 3: 19 patients with clinical diagnosis of sepsis. Neutrophil CD64 was measured with Leuko64kit, on hematology analyzer CELL-DYN Sapphire using the flow cytometry method, and compared to interleukin 6, WBC count, neutrophils, left shift and immature granulocytes. Interleukin 6 was tested on Siemens Immulite 2000 and hematology parameters were determined on Siemens ADVIA 120.
Results. There is a significant statistical difference amongCD64 values from these three groups, with the lowest CD64 mean level in Gr.1 and the highest in Gr.3. CD64 and IL6 showed correlation in Gr. 3 ($r= 0.49$, $p <0.05$) with weak correlation in Gr.1 and Gr.2. WBC count, neutrophils, left shift and immature granulocytes demonstrate weak correlation compared to CD64.
Conclusions. This studyshows that CD64 is a useful tool to identify infection and sepsis. The test is reliable and easy to perform, which makes it valuable in a critical care medicine.

Critical care/emergency medicine

0310

EVALUATION OF C-REACTIVE PROTEIN, FERRITIN AND NT-PROBNP LEVELS IN PATIENTS HOSPITALIZED IN INTENSIVE CARE UNITS

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Background. C-reactive protein (CRP) and ferritin (FE) are acute phase proteins present in the blood stream duringinflammatory process. The amino ending end of brain natriuretic peptide (NT-proBNP) is secreted mainly by heart ventricles due to their dilatation but also during bacterial infection and sepsis. Aim of our study was to evaluate CRP, FE and NT-proBNP serum levels in male infected patients that were hospitalized in ICUs and their correlation with the isolated microorganism and the site of the infection.
Methods. We evaluated, CRP, FE and NT-proBNP serum levels in 30 hospitalized male patients in ICUs . Patients presented 31 infection episodes. CRP levels were determined with nephelometry (Normal Values 0,0-0,8 mg/dl) FE levels with electroluminescence (Normal Values < 150 ng/ml) and NT-proBNP levels with electroluminescence (Normal Values <100 pg/ml),
Results. Increased levels of NT-proBNP and CRP were observed in all episodes while increased FE values were found only in 20 (64%) episodes. The highest levels were observed for NT-proBNP in bacteremias, for FE in bacteremias and for CRP in bacteremias and venous catheter infections. Statistically, significant correlation was observed between NT-proBNP and CRP (p<0,01), and between NT-proBNP and FE (p<0,01), while no correlation was observed between CRP and FE. Also, no significant correlation was observed between the isolated bacteria and NT-proBNP, FE or CRP.
Conclusions. 1)NT-proBNP levels are significantly correlated with CRP and FE levels in severe infections.2)NT-proBNP in combination with CRP and FE might be useful indicators for the evaluation of bacteremias and its prognosis.

0311

CORRELATION OF ERYTHROBLAST CONTENT IN PERIPHERAL WHOLE BLOOD WITH SURVIVAL IN SURGICAL INTENSIVE CARE PATIENTS

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Background. Erythroblasts are absent in the peripheral blood of adults but can be observed in various diseases. Recently published studies have linked the presence of erythroblasts and mortality in surgical intensive care patients.
Methods. Correlation of erythroblast number in peripheral blood from adults with various diseases and the Simplified Acute Physiology Score (SAP II Score). Also, the prognostic value of erythroblast measurements was examined with regard to hospital mortality.
Results. From May 2010 to September 2010, 555 surgical intensive patients were included in the analysis. In 62 patients, the erythroblast content in peripheral blood was determined. The mean age of all patients was 64.0 ± 0.76 years (range, 17-100 years), and the mean SAP II value was 40.0 ± 0.8. The in-hospital mortality rate of patients in whom erythroblasts were present in the peripheral blood was 73.3% (11 of 15) and 5.41% (30 of 555) in the entire study group. Erythroblast concentration and SAP II- value correlated positively (Spearman $r = 0.355$; $p < 0.0001$).
Conclusions. Erythroblast content in the peripheral blood of adult surgical intensive care patients is associated with increased mortality. There is a significant correlation between erythroblast concentration and SAP II-value. Therefore, erythroblast number is a rapid and inexpensive prognostic marker for mortality.

Critical care/emergency medicine

0312

INTEREST OF CHOLESTEROL AND HDL-CHOLESTEROL FOLLOW-UP AS PROGNOSTIC MARKER OF SURVIVAL OF PATIENTS IN INTENSIVE CARE UNIT (ICU)

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Backgroundd. Although a relative low incidence in general population (2-5%), hypocholesterolemia increases considerably (up to 50%) among hospitalized patients particularly when they present septic or post surgical complications.

Methods. During a 6 months laboratory survey, we examined all patients from ICU with at least one HDL-Cholesterol value < 0.51 mmol/L (19.3 mg/dL). These patients were classified in 3 groups according to disease evolution: death, survival with unfavourable evolution, and survival with favourable evolution. All cholesterol and HDL-cholesterol measurements made during the hospitalization were collected retrospectively. We calculated the difference between the nadir and the last ICU cholesterol or HDL-cholesterol (D).

Results. Among the 48 patients examined, 40 (83.3%) presented an infectious complication. Of those 10% survived with favourable evolution, 58 % presented an unfavourable evolution and 32% died. No significant difference in total cholesterol was observed between groups, but we observed a significant difference in HDL-cholesterol between groups who survived and those who died (p = 0.0054), the discriminating HDL-cholesterol value was 0.09 mmol/L (14.1 mg/dL) with a sensitivity of 100%. We observed also a negative correlation between intensity of HDL-hypocholestrolemia and length of stay in ICU as well as a significant difference in D HDL-cholesterol between the group of patients who died and those who survived (favourable and unfavourable evolution).

Conclusions. The follow-up of cholesterolemia and HDL-cholesterolemia is probably interesting but needs to be further investigated to determine the most informative data (kinetic evolution, intensity of decrease, cut-off value), and at what time the assay must be done.

0313

PLASMA DIMETHYLARGININES DURING THE ACUTE INFLAMMATORY RESPONSE

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Background. The endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) is increased in critical illness and associated with mortality. However, its concentrations during the acute inflammatory response have not been documented previously.

Methods. 38 patients undergoing elective total knee arthroplasty were studied pre-operatively and during the post-operative course until day 5 for dimethylarginines in plasma, urinary dimethylamine (DMA) as a marker of ADMA catabolism by dimethylarginine dimethylaminohydrolase (DDAH), and urinary nitrate as a marker of total NOS activity. Samples were collected in the fasting state. The Friedman test was used to assess serial changes.

Results. CRP peaked at 191 mg/L on day 3 post-op. ADMA decreased rapidly, with a nadir on day 2 (0.43 vs 0.62 mmol/L, p < 0.0001), recovering to baseline by the end of the study. Symmetric dimethylarginine (SDMA) showed no significant changes (p = 0.64). Urine DMA excretion did not change until day 5, whereupon its excretion doubled (105 vs 53 mmol/mmol creatinine, p = 0.001). The DMA:ADMA ratio in urine was unchanged throughout (p = 0.68). Urine nitrate showed a trend to decrease at day 2, but this failed to reach statistical significance (p = 0.089).

Conclusions. Plasma ADMA decreases rapidly and transiently during acute inflammation. This does not appear to be due to increased DDAH catabolism, as the excretion of DMA does not increase until the plasma concentration has recovered. These results suggest increased cellular partitioning of ADMA, which might represent a physiological response to regulate NOS in acute illness.

Critical care/emergency medicine

0314

SIGNIFICANTLY HIGHER PROCALCITONIN LEVELS COULD DIFFERENTIATE GRAM NEGATIVE BACTEREMIA FROM GRAM POSITIVE AND FUNGAEMIA

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Background. Procalcitonin (PCT) levels can distinguish between bacteremia and non-infectious inflammatory states in critically ill patients. However, there are some differences between Gram negative (G-), Gram positive (G+) and fungal bloodstream infections, particularly in different cytokine profiles, severity and mortality. Can PCT levels be a distinguishing mark between G+, G- bacteremia and fungaemia as well?
Methods. We have enrolled 275 samples from 180 septic patients with positive haemocultures. Sera from the date of blood-culture evaluation were examined on C-reactive protein (CRP) and PCT concentrations. The median (IQR) of CRP and PCT in G+, G- and fungal cohorts, and a comparison of measured values between groups was made using the Kruskal-Wallis test, with $p < 0.05$.
Results. In 178/275 (65%) of haemocultures, G+ microbes were detected: 92/275 (33%) were G-rods and 5/275 (2%) were fungi. PCT concentrations were significantly higher in G- compared to other cohorts: 8.90 (1.88; 32.60) in G-, 0.81 (0.32; 3.50) in G+, and 0.58 (0.35; 0.73) in fungi ($p < 0.00001$). CRP concentrations did not differ significantly in groups. In patients with PCT<10 ng/mL, G+ haemocultures predominated (68%); whereas a group with PCT>10 ng/mL was formed mainly (79%) from G- microbes. The highest PCT levels were found in patients with E. coli, Klebsiella and Pseudomonas in blood-cultures; whereas Candida, Streptococcus and Staphylococcus were linked with a mild PCT elevation.
Conclusions. Significantly higher PCT levels could differentiate G- bacteremia from G+ and fungaemia. In contrast to CRP, PCT is a good discriminative biomarker in different bloodstream infections.

0315

PERIOPERATIVE BEHAVIOR OF URINARY NGAL IN CARDIAC SURGERY

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Background. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is the most promising among all emerging biomarkers for acute kidney injury(AKI) in cardiac surgical patients. The aim of our study is evaluate uNGAL perioperative kinetics to find the best timing of this test.
Methods. Perioperative seriate uNGAL samples were collected in eight low risk patients with good ventricular function undergoing mitral valve repair and six high risk patients with poor ventricular function undergoing combined cardiac surgery. All patients underwent surgery with cardiopulmonary bypass (CPB) and custodial cardioplegia. Exclusion criteria were age < 18 years, chronic renal failure and emergent surgery.
Results. In low risk patients uNGAL never increases in a significant way. It peaked immediately after general anaesthesia induction (16.0 [range 8.0–24.7] ng/mL), then decreased during CPB and increased for the second time 24 hours after surgery (14.5 [range 0–28.6] ng/mL). In high risk patients uNGAL peaked for the first time at the end of surgery (59.3 [range 4.9–187.4] ng/mL) and had the higher values 24 hours after surgery (70.8 [range 18.7-147] ng/mL). uNGAL 24 hours after surgery was higher in the patient that needed renal replacement therapy (147 ng/mL) and in the two patients that died (147 ng/mL and 112.8 ng/mL).
Conclusions. These preliminary data indicates that, in the specific setting of high risk cardiac surgical patients, urinary NGAL can be an early and specific biomarker of AKI.

Critical care/emergency medicine

0316

CLINICAL EVALUATION OF NOVA BIOMEDICAL STATSENSOR LACTATE POCT SYSTEM IN THE ASSESSMENT OF THE ACUTELY UNWELL ADULT

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Background. Lactate has prognostic use in critically ill medical and trauma patients, and is a core component in identification of early sepsis .Elevated lactate levels in these patients prior to ICU admission e.g. in an A&E setting or pre hospital setting identify patients at risk of death and can trigger an earlier optimization of triage decisions and earlier targeted treatment.The aim of this study was to assess the performance and functionality of Stat Sensor Lactate.
Methods. Venous samples from 100 adult patients admitted to A&E analysed using Nova Biomedical StatSensor and Roche Diagnostics cobas b221 as per routine clinical assessment. Precision assessed using donated whole blood and spiked with a concentrated lactate solution. (Results were classified into risk categories (Low = <2.0 mmol/L, Intermediate = 2.0 – 5.0 mmol/L and high >5.0 mmol/L) and percentage concordance calculated.)
Results. Precision was acceptable at all levels tested. Low level: Mean 1.5 mmol/L CV % 6.85, three other levels: Mean 5.8, 10.4, 23.9 mmol/L CV% <4%. LactateRange 0.6 – 11.1 mmol/l Roche cobas b221, 0.8 – 10.6 mmol/l Nova StatSensor Lactate. Mean b221 3.59± 2.36 mmol/l, Nova Stat Sensor ± 3.52 ± 2.26 mmol/l. Regression analysis: r2=-0.99, slope=0.95, intercept 0.11. Bland Altman Analysis: Mean bias -0.06± 0.25, limits of agreement -0.49 – 0.43 mmol/l.Good concordance > 95% obtained across risk categories with Stat Sensor.
Conclusions. Stat Sensor Lactate demonstrated a good correlation to the reference method. Bland Altman analysis demonstrated minimal variation across the working range.Stat Sensor Lactateis ideally suited to triage at risk patients.

0317

THE USE OF THE ISTAT1 POINT-OF-CARE ANALYZER IN BONE MARROW ASPIRATE ANALYSIS

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Background. Intraosseous access is a rapid simple way of obtaining vascular access in an emergency. Often laboratories are reluctant to analyse bone marrow samples, as they may block autoanalyzers. This study examines the reliability of the disposable cartridge based iSTAT1 point-of-care (POC) analyzer in bone marrow aspirate analysis.
Methods. All children presenting for scheduled diagnostic bone marrow aspiration with venous blood sampling under general anaesthesia were included prospectively. Bone marrow aspirates and venous samples were taken simultaneously and analysed using the iSTAT1 analyzer. Statistical analysis was performed using SPSS (version 17.0) Approval of Medical Ethical Committee of the University Medical Centre was obtained.
Results. Data of 16 children were available. POC testing on bone marrow aspirate was feasible in all children. Linear regression analysis showed a significant relationship between venous blood and bone marrow for bicarbonate (p= 0.014; 95% CI 0.103 - 0.753), base excess (p= 0.000; 95% CI 0.707 - 1.547), sodium (p= 0.002; 95% CI 0.417 -1.418), ionized calcium (p= 0.027; 95% CI 0.143 - 2.070) and glucose (p= 0.00; 95% CI 0.444 - 1.299).
Conclusions. Checking for the presence of hypoglycaemia, metabolic acidosis and hypocalciemia is very important on presentation in the emergency department, as these metabolic disarrangements are frequently present and prompt treatment has immediate effect on outcome. This study provides preliminary evidence that iSTAT1 analysis of bone marrow aspirate is reliable and feasible and can be useful to guide management in the emergency department.

Critical care/emergency medicine

0318

P-SELECTIN, MARKER FOR PROGNOSIS AND FOR MONITORING TRAUMA PATIENTS WITH HEMORRHAGIC SHOCK

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Background. P-selectin, cell adhesion molecule is present on the surfaces of activated platelets and endothelial cells. P-Selectin orchestrates interaction between leukocyte recruitment to inflammation site and thrombosis by acting on platelets and neutrophils co-localize at sites of vessel injury, hemorrhage and thrombosis. This role leds us to investigate whether P-Selectin could be used as a prognositic marker for monitoring resuscitation of the patients with hemorrhagic shock. We sought to determine the relationship between serum levels of P-selectin and clinical outcomes in trauma patients with hemorrhagic shock.
Methods. Serum was collected from a convenience sample of twenty subjects at admission and three hours later, and P-Selectin levels were measured with ELISA by individuals blinded to subject identity and clinical course. Corrected P-Selectin levels were calculated as the ratio of P -Selectin to total protein (ng P-Selectin/mg protein) and it was used throughout. Clinical data regarding hospital course and outcomes were collected on each subject.
Results. Subjects who died (N=5) had significantly higher initial P-selectin levels than those who did not (median [IQR]: 45.7 [24.9,56.7]ng/mg vs. 21.9 [14.3,29.8]ng/mg, p=0.003). Subjects who developed multiple organ dysfunction syndrome (MODS; N=2), had significantly higher P-Selectin levels at three hours than those who did not (median [IQR]: 46.1 ng/mg vs. 24.4 ng/mg, p=0.026).
Conclusions. Elevated P-Selectin in trauma patients with hemorrhagic shock, were strongly predictive of the subsequent development of organ dysfunction and death. Further investigation is needed to determine if these findings apply to long-term outcomes, and to potential therapy based on the P-Selectin level.

0319

NGAL IN INTENSIVE CARE UNIT THREE HOURS AFTER CARDIAC SURGERY

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Background. Neutrophil gelatinase-associated lipocalin (NGAL) is an early marker of acute kidney injury (AKI). Aim of this study is to define the usefulness of plasma NGAL (pNGAL) and urine NGAL (uNGAL) 3 hours after cardiac surgery in adults.
Method. Triage kit (Alere) for pNGAL, ARCHITECT (Abbott) for uNGAL, Jaffé kinetic (Roche). 50 patients (53-84 years) were evaluated.
Results. 17 patients developped an AKI, according to the acute kidney injury network criteria. Among these patients, 6 reached a stage 3. Two patients died from sepsis and multisystemic failure. pNGAL was >149 ng/ml in 8 AKI patients including the dead ones and 8 patients without AKI. Positive likelihood ratio (LR+) was 1.9, negative likelihood ratio (LR-), 0.7 and area under ROC curve, 0.58 (0.41-0.75). Only one uNGAL was >132 ng/ml in the 15 AKI patients and one in the 28 non AKI patients in witch urine sample could be collected. uNGAL (ng/ml) ratio to ucreatinine (mg/dl) seems to improve uNGAL performances. For a 0.62 ratio, the LR+ is 3.0, LR-, 0.42 and area under ROC curve, 0.62 (0.44 à 0.81).
Conclusions. The LR+ of these markers are too low and their LR- too high to be useful 3hours after cardiac surgery at bedside.

Critical care/emergency medicine

0320

BIOMARKER S100B AND GLASGOW COMA SCALE (GCS): PROGNOSTIC INDICATORS IN TRAUMATIC BRAIN INJURY (TBI)

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Background. The main clinical indicator of Traumatic Brain Injury (TBI) is theGlasgow Coma Scale (GCS). We study and calculate the predictive capacity of S100Band compare it with (GCS) in (TBIs).
Methods. A prospective, observational study. Patients with (TBI), attended within six hours of lesion.
Variables measured a) clinical: exitus yes/no; age and gender, (GCS). b) Radiological:CT scan. c) Biochemical: serum S100B 6 hours after (TBI) and drug abuse in urine. S100B analysis by induced chemiluminescence reaction. (LIASON Sangtec 100).SPSS 16.0
Results. Over 34 months we included 149 patients (119 males and 30 females), average 42.85 years old (15-84) with average (GCS) of 9.3 (3-15). S100B 6 hours Mean (CI 95 %), median, range: 2.11 μg/L (1.6-2.6), 1.1, 26.6. S100B mean Deaths/ Survivals 3.5 / 1.6 μg/L (N=38/111) P=0.001
Predictive capacity of S100B for Survival/lethality, bivariate analysis, negative association significance between S100B and survival, odds ratio (OR):1.2. (ROC) Curve S100B, cut-off: 1.5 ug/L, with (AUC) of 80.2 % (75.8-90.3 %), sensibility 73 %, specificity 75.5 %.
Calculated predictive value (GCS),logistic regression revealed a significant positive association between (GCS) and survival, correct classification 78%, sensibility 50 % , specificity 87.6 %.
Conclusions. S100B is a predictive survival marker after (TBI). S100B levels are significantly higher in fatalities. Each unit increase in S100B increases the death risk 1.2 times.S100B 6 hours would complement the GCS in the prognosis of (TBI) improving sensitivity and ability to classify.

0321

PLASMA AND SERUM CELL-FREE DNA ARE MARKERS FOR DISEASE SEVERITY AND PROGNOSIS IN TRAUMA PATIENTS

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Background. Trauma is still a major causes for mortality of people <50 years. Biomarkers are needed to estimate the severity of the condition and the patient outcome.
Methods. Cell-free (cf) DNA was determined in plasma and serum of 164 patients at time of admission. Among them were 64 patients with multiple trauma (Injury Severity Score (ISS) ≥16), 51 patients with minor trauma (ISS<16) and 49 patients with single fractures (24 femur neck and 25 ankle fractures). Disease severity was objectified by ISS and Glasgow Coma Scale (GCS).
Results. Cf-DNA levels in plasma and serum were significantly higher in patients with multiple trauma than in those with minor trauma (each p=0.002) and with single fractures (each p<0.001). Cf-DNA in plasma and serum correlated very strongly with each other (R=0.89; p<0.001) as well as with levels of alanine-aminotransferase, creatine-kinase, glucose and inversely with hemoglobin (for all R≥0.30 and p<0.05). AUC in ROC curves for identification of multiple trauma patients was 76.5% and 74.3% for cfDNA in plasma and serum, respectively. Within the group of multiple trauma patients, cf-DNA levels were higher in more severely injured patients (GCS≤8 versus GCS>8). 13 of the multiple injured patients died during the first week after trauma. Levels of cfDNA were significantly higher in non-surviving patients than in survivers (p<0.001). AUC in ROC curves for identification of non-surviving patients was 82.3% and 80.8% for cfDNA in plasma and serum, respectively
Conclusions. Cf-DNA is valuable for estimation of trauma severity and prognosis of trauma patients.

Critical care/emergency medicine

0322

HELICOBACTER (HP) PYLORI INCIDENCE IN SOME ABDOMINAL SURGERIES

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Background. HP infection is the most frequent infection affecting humans predominantly in the countries with low socio-economic condition.The aim of this study was to explore the relationship and prevalence of Helicobacter pylori (HP) infection in different 9 surgical diseases.
Methods. We analyzed 81 patients who underwent surgical treatment at the Department of Emergency Surgery of Clinical Center of Serbia, over eight months. Patient data consisted of age gender, diagnosis and HP status by serology. The most commonly diagnostics approach Commercial test Enzignost Anti-Helivobacter pylori II/IgA(IgG) (DADE-Behring) was used for quantitative determination of human IgA and IgG antibodies.
Results. 42 males (51.8%) and 39 females (48.2%)were included in the study. 61 patients were <65 years old and 20 patients >65 years old.
The patients, who were operated on, were divided into two groups with respect to diagnosis. The first group consisted of 36 patients with appendicitis, subocclusion endileus. Statistically significant inverse association was found between seropositive HP and acute appendicitis. In the second group, consiting of 45 patients with malignity (gastric, pancreatic, hepatocellular, intestinal, colon and rectal cancer) positive HP was confirmed in all 45 patients.
Conclusions. This study have shown that HP positive may be indicated in high-risk population in different surgical diseases.

0323

HYPONATREMIA DUE TO NON-OSMOTIC STIMULI AS A CONSEQUENCE OF INCREASED COPEPTIN AND MYELOPEROXIDASE IN IMMUNOSUPPRESSANT THERAPY

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Background. Hyponatremia is side effect of therapy with tacrolimus (TAC) and cyclosporine (CYA) in transplant recipients. TAC induced more pronounced hyponatremia than CYA. Arginine-vasopressin (AVP) is secreted in hyperosmolal situations and suppressed during hypoosmolality (hyponatremia). The purpose of our study was to evaluate possible mechanism for non-osmotic stimuli for AVP secretion in relation to oxidative stress.
Methods. A set of 59 samples from 47 hyponatremic transplant recipients (PNa < 137 mmol/l) was selected consecutively during the monitoring of immunosupressant therapy. PNa, myeloperoxidase (MPO), TAC and CYA were measured on Architect ci8200 (Abbott), copeptin (cut-off 14 pmol/l) on Kryptor (B.R.A.H.M.S.). eGFR was calculated according to MDRD formula.
Results. Median (25 - 75 percentile) of age was 56 years (48,5 – 63,8), eGFR (MDRD) 0,765 ml/s (0,645 – 0,980), plasma sodium 134,8 mmol/l (133,4 – 135,8; only patients with PNa < 137 mmol/l, range 118,9 – 136,9 mmol/l). Median of plasma copeptin in CYA group was 12,8 pmol/l (25 - 75 percentil: 6,10 – 23,9), in TAC group 18,3 pmol/l (13,2 – 45,2), p = 0,0314. MPO increased significantly (p < 0,005) with concentration of TAC (TAC 2,2 – 7,0 μg/l, mean of MPO: 168,0 pmol/l, TAC 7,1 – 12,9 μg/l, MPO: 228,3 pmol/l, TAC 13 – 19,2 μg/l, MPO: 325 pmol/l).
Conclusions. Significant increase of copeptin in TAC treated patients is comparable to septic or ACS patients. Upregulation of vasopressin-neurophysin II-copeptin gene during immunosuppressant therapy might be an explanation for hyponatremia. We speculate about increased MPO and oxidative stress as a background of changes in hormonal regulation in immunosuppressant therapy.

Critical care/emergency medicine

0324

A 56-YEAR OLD MAN WITH CARBON MONOXIDE INTOXICATION, AN APPARENTLY NORMAL OXYGEN SATURATION AND A DEAD CANARY

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Background. Based on a case of a 56-year old man who was found unconscious in his houseboat, we illustrate that reported oxygen saturation parameters, SaO_2, O_2-sat and SpO_2 for pulse oximetry, can be misleading in diagnosing CO intoxication.
Methods. Blood gas analysis was performed by pulse oximetry and on a Rapidlab 1265 (Siemens).
Results. On admission to the emergency department, the patient was intubated and subsequently transferred to the Intensive Care. Blood gas analysis revealed a respiratory compensated metabolic acidosis and SaO_2 of 99%. Chest x-ray and cerebral CTA were normal and hypoxia was not recognized. Medical history showed an autointoxication, but toxicological screening was negative. On the second day he was extubated but still awaiting a diagnosis. At that moment the discovery of a dead canary on the patient's houseboat, suggested CO poisoning. Our blood gas analyzers are equipped with a CO-oximetry module and non-functional hemoglobin variants are determined in each sample, however only reported when requested. The initial bloodgas analysis could be reevaluated and revealed 25.4% COHb.
Conclusions. The diagnosis CO poisoning was initially overlooked, because SaO_2 and SpO_2 do not account for dyshemoglobins. To prevent future misdiagnoses, we decided to automatically report COHb and MetHb results if they exceed the reference value, whether or not they are requested. Since in many hospitals pulse oxymetry and point of care analyzers without co-oximetry, that are not capable to determine FO_2Hb and dyshemoglobins, are interchangeably used, knowledge of the different reported saturation parameters is essential for correct and fast diagnosis of CO intoxication.

0325

PLATELET AND ENDOTHELIAL RECEPTOR DENSITY CHANGES IN SEVERE SEPTIC PATIENTS

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Background. Microparticles (MP) are known to have a diverse role in inflammatory diseases including severe sepsis. We observed the quantitative and receptor density changes of platelet (PMP) and endothelial microparticles in severe sepsis. We also assessed the mortality predictive value of MP measurements and the influence of platelet count on microparticle production. A novel approach to microparticle surface receptor density was also studied.
Methods. Twenty-seven septic patients and 20 healthy volunteers were included as age-adopted controls. Platelet and endothelial microparticles were measured on one occasion in healthy controls by flow cytometry and on the 1st, 3rd and 5th study days in septic patients.
Results. Total MP number along with CD41^{+} and CD61^{+} MPs were elevated in severe sepsis compared to healthy volunteers throughout our study period (p<0.01). CD42a was increased significantly in all measurements (p<0.05). The PAC1 marker was elevated significantly on the 1st and 5th day (p < 0.05). The survivor and the non-survivor group presented insignificantly different microparticle characteristics. Platelet count showed no correlation with microparticle number. In septic patients receptor density measurements presented the significant decrease of phosphatidylserine and constitutive platelet markers but an increase of activated platelet markers.
Conclusions. The pathophysiological mechanisms behind the development of sepsis and related organ failures are not fully understood. We found that PMPs does not have an outcome predictive value or correlation with platelet count. The receptor density changes were significant in severe sepsis and may help the differentiation between apoptotic and activated MPs.

Critical care/emergency medicine

0326

INTRODUCTION OF A FLOW CYTOMETRIC METHOD FOR MICROPARTICLE MEASUREMENTS

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Background. Cell-derived microparticles (MPs) are irregularly shaped vesicles in circulation with diameter between 0,1 and 1,0 μm, that arise by shedding from the plasma membrane after cell activation, apoptosis or exposure to shear stress in physiological or pathological conditions. Their defining characteristic is the exposure of the anionic phosphatidylserine on their surfaces. They express a broad array of proteins that reflect their cellular origin. Much of the confusion associated with understanding MP biology results from the lack of standard methods for their isolation and analysis.

Methods. A method of choice for quantifying and immunophenotyping MPs is flow cytometry. We aimed to introduce a reproducible and stabile method for isolation and measurement of MP by a Beckman-Coulter FC500 flow cytometer.

Results. We used citrated blood (0,129 M) samples. Within 2h after collection, samples were centrifuged to obtain platelet rich plasma, platelet poor plasma and cell free plasma. At the end MP was pelleted. For cell-specific identification we used different CD markers and Annexin V for generic MP detection. MP was safely gated and separated by size from the background noise (range 0,5 - 1,0 μm). Concentration of Annexin V and each CD antibodies were titrated to rich the optimal separation from the unspecific staining of the background. The absolute MP number was counted by using a known concentration 1 um diameter fluorescent labeled beads. We also tested the reproducibility of our test.

Conclusions. Our test is a stabile and adequate method for MP measurement, useful in determining the outcome ofseptic patients under critical care surveillance.

0327

MINIMIZING IN VITRO HAEMOLYSIS IN STAT BLOOD SAMPLES

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Background. During a project aimed at increasing the throughput in the emergency wards, we found that the frequent occurrence of haemolytic samples was an important factor causing delayed test Results. Initially, approx. 10 % of all venous blood samples where potassium analysis was ordered were rejected due to high haemolytic index.

Methods. The incidence as well as the different reasons contributing to in vitro haemolysis in venous blood samples were studied in two separate emergency wards. Both used the same type of sampling tubes (lithium heparin plasma) for general biochemistry tests, but they differed as to transport system (two different pneumatic tube systems) and analytical platform (Beckman-Coulter and Roche Modular, respectively). All samples were collected by staff in the emergency ward. During the study, repeated training in sampling technique was provided.

Results. The most important factor in reducing the number of haemolytic samples was to establish a routine where venous blood samples were collected by primary venipuncture rather than via a peripheral venous catheter. The use of 21 Gauge needles (0,8 mm) instead of 22 Gauge needles (0,7 mm) was also encouraged. After a two-year intervention period the incidence of venous samples collected in the emergency wards where potassium results could not be delivered due to haemolysis was reduced to <3%.

Conclusions. Repeated training in an area expected to be elementary is sometimes needed. Motivation has to be created by the use of real-life examples and continuous presentation of actual incidence data.

Critical care/emergency medicine

0328

IMPACT OF HAEMOLYSIS ON EMERGENCY PATIENTS

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Background. Patient Safety is a priority objective at both National and International levels as promoted by WHO. The majority of laboratory errors are in the pre-test phase (71%). Correct extraction and handling of the sample guarantees reliable results and shortens the length of the patient's stay in accident and emergencies. Haemolysisis one of the reasons why tests have to be repeated.
Objective. To study the impact of haemolysison patient waiting time in the hospital accident and emergency department.
Methods. Descriptive study. Period: year 2009. Population: 17,074 samples from the Emergency Services. 20% haemolized. Detection: Siemens Dimension RXL HIL System. Laboratory measurement turnaround time.
Results. 256 (71,3%) patients had just one extraction. 71 (19.7%) had 2 extractions with an average waiting time of 3 hours. 25 (7.2%) had 3 extractions, waiting for 6 hours, and for 6 (1.7%) 4-5 extractions were necessary, with them waiting 13 hours. 70.2% of the patients with haemolized samples were discharged without repeating the blood test.
Conclusions. Correct venous extraction and handling of the tube contributes to decreased haemolysis. Haemolysis implies: repetition of tests, lengthy stays and diagnostic delay. It is necessary to confirm the utility of urgently requested tests. It is necessary to check whether staff education and training (in the extraction and handling of the sample and in identifying the need for the test), could be one of the main causes, as has been described in a causal analysis of sentinel events at international level .

0329

THIOPENTAL DETERMINATION IN INTENSIVE CARE PATIENTS USING AN AUTOMATED BARBITURATE IMMUNOASSAY

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Background. Thiopental sodium is a barbiturate used in the management of intracranial hypertension in intensive care. Usually measured by HPLC, the feasibility of plasma thiopental determination using an automated liquid barbiturate immunoassay was here investigated.
Methods. Barbiturate level was measured with the CEDIA® barbiturate immunoassay on a CDX90® system (Thermo Fisher Scientific). Calibration was realized with n=5 levels of secobarbital (manufacturer calibrators). This assay was applied to n=17 intensive care patients without barbiturate (n=15) or treated with thiopental (n=2). Statistics were performed with Method validator software.
Results. Within- and between run precision (n=15) was <5.2 and 4.9% for low (225 ng/mL) and high barbiturate control (375 ng/mL); accuracy was 84% and 92%, respectively. When reagent was stored at +4°C capped, calibration stability was 3 weeks. Limit of quantification determined by serial dilution of the 200 ng/mL calibrator was 100 ng/mL. Using thiopental (Pentothal®, Hospira France) spiked plasma, mean cross-reactivity for thiopental was 8.8% (n=6 levels) over the analytical linear range of 125 to 2500 ng/mL of barbiturate thus corresponding to 1.4-28.4 mg/L of thiopental (Passing Bablok; r=0.965; n=6). All 15 patients without barbiturate were found negative with the CEDIA® barbiturate immunoassay; treated patients had detectable levels of thiopental (2 plasma samples per patient) with 95%, 81%, 119%, and 57% recovery *vs.* HPLC.
Conclusions. The CEDIA barbiturate immunoassay (Thermo Fisher Scientific) could allow easy and rapid measurement of plasma thiopental in the therapeutic range required in intensive care patients.

Critical care/emergency medicine

0330
ARTERIAL BLOOD GAS SAMPLES VERSUS CAPILLARY GAS SAMPLES

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Background. The objective of this work was to determine the correlation of values of blood gases taken from arterial and capillary blood. Comparison of blood gas values of pO2, pCO2, SO2 capillary blood collected in plastic and glass tubes
Methods. The used method was the potentiometric method on the analyzer – Radiometar Kopenhagen Type ABL555.
Results. The values of pO2 and sO2 in arterial blood were higher than in capillary blood. There is highly statistically significant difference between the average values of pO2 and sO2 taken from the arterial and the average values taken from the capillary blood. Values of pCO2 in arterial blood were slightly higher then the same in capillary blood. There is no statistically significant difference between the average values of pCO2 in aretrial blood and the average values of pCO2 from capillary blood. Average values of pO2 and sO2 were statistically significantly lower in the sample taken from the glass tubes in relation to the sample taken in plastic tubes, while the values of pCO2 were higher but statistically insignificant.
Conclusions. Capillary blood samples taken in plastic tubes to be analyzed immediately

0331
INFLUENCE OF THE TIME ON PARAMETER VALUES OF GAS ANALYSIS

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Background. Determinewhether the time or way of taking samples for analysis of respiratory gases influence the results of arterial and capillary gas analysis, determine the values of gases in samples which had certain mistakes (such as bubble of air, inhomogeneous sample, clot) and their influence on final results of blood gases.
Methods. Potentiometric method at analyser „Radiometer Copenhagen" type ABL555
Results. The comparison between results of arterial blood samples taken immideately (0-15) to 60 minutes, are showing statistically significant difference between values of pO2 and sO2, and don't show the same for pCO2.The comparison between results of arterial and capillary blood samples taken in period of time from 0 to 15 minutes, shows statisticaly significant difference between values of pO2 and sO2, and don't show the same for pCO2.
The values of pO2 were higher statisticaly significant difference in samples with air bubbles comparing to values of sO2 which were higher but no statisticaly significant difference.Average values of pCO2 were lower but with no statisticaly significant difference.The correlation between arterial and capillary blood pCO2 shows that there is statistically significant positive correlation.
Conclusions. The time and way of taking samples influance the values of blood gases. The time particulary affects the values of pO2 and sO2, in samples taken from arterial and capillary blood comparing to values of pCO2 which are not significantly different.

Critical care/emergency medicine

0332

COMPARISON OF VALUES OF SO2 FROM ARTERIAL AND CAPILLARY BLOOD VERSUS SAME VALUES WITH PULS OXIMETER

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Background. Comparison of values of sO2 from arterial and capillary blood versus same values with puls oximeter.
Methods. Puls oximeter method include two basic physical principles :
• the presence of pulsed signals of arterial blood which is quite independent compared to venous, capillary blood and other tissues
• the fact that oxihemoglobin and reduced hemoglobin have different absorption spectra.
Results. The average values of sO2 mesured with pulsoximeter were higher comparison to the same values in capillary blood, but with no statisticaly significant difference. The average values of sO2 measured with puls oxmeter were lower comparison to the same value in arterial blood but with no statisticaly significant difference. The correlation between results of sO2 in arterial blood and results of sO2 with pulsoximeter are in statisticaly positive correlation.
Conclusions. Comparation of values of sO2 with puls oximeter method versus arterial and capillary blood samples shows difference, but no statisticaly significant difference.

0333

DIAGNOSTIC AND PROGNOSTIC VALUE OF SOLUBLE CD14 SUBTYPE (SCD14-ST) IN EMERGENCY PATIENTS WITH EARLY SEPSIS USING THE NEW ASSAY PATHFAST PRESEPSIN

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Background. CD14 is expressed on the membrane of monocytes/macrophages and activates the TLR4-specific inflammatory reaction against infectious agents whereby soluble CD14 is released yielding a subtype (sCD14-ST) by proteolysis. First evidence suggests that sCD14-ST may be an advantageous indicator of sepsis. The validity of the newly developed sCD14-ST- assay PATHFAST Presepsin should be examined in patients presenting with clinical signs for sepsis at the emergency department.
Methods. sCD14-ST was compared to clinical scores and biomarkers currently used in sepsis management. Primary endpoint was death within 30 days. Secondary endpoints were admissionto the intensive care unit, mechanical ventilation, and renal replacement.
Results. The clinical scores exhibited significant differences (p≤0.0005) between patients with SIRS/initial sepsis (n=91) and severe sepsis/septic shock (n=55). The diagnostic cutoffs for PCT and sCD14-ST to differentiate between the patients groups derived from ROC analysis were 3.81 ng/ml and 981 pg/ml with sensitivies and specificities of 47%/76% and 65%/67 %, and AUCs of 0.635 and 0.694, respectively. Theincrease of BNP, creatinine, IL-6, CRP, lactate, and PCT (p≤0.01) was topped by sCD14-ST (p≤0.0001). sCD14-ST but not PCT differed between survivors and non-survivors (p≤0.0001). The highest quartile of sCD14-ST was strongly associated with mortality risk (p≤0.0005). ROC analysis revealed an AUC of 0,929 for sCD14-ST compared to 0.521 for PCT.
Conclusions. The diagnostic efficacy of sCD14-ST is comparable to PCT, but the prognostic power of sCD14-ST is significantly better. In contrast to PCT, sCD14-ST allows risk stratification in sepsis already at admission using the PATHFAST Presepsin assay at POC.

Critical care/emergency medicine

0334

HIGH SENSITIVE (HS) TROPONIN T STAT ASSAY DIAGNOSTIC VALUE IN PATIENTS WITH CHEST PAIN AND UNDERLYING CHRONIC DISEASE

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Background. Increased analytical sensitivity of hs cTnT assay leads to increased number of "false positive" results in patients with chronical cardiovascular diseases, renal insufficiency (especially those with advanced age). Our results indicate higher baseline concentrations of cTnT in mentioned patients (considering that 99th percentile URL is 14 pg/mL, AMI cut-off ≥ 50 pg/mL). Goal of this study was to determine diagnostic value of hs cTnT for AMI in patients with chest pain and underlying chronic disease.
Methods. hs-cTnT was measured by ECLIA method, on Cobas e411 analyser (Roche Diagnostics).
Comparison of cTnT concentrations of two groups male patients with chest pain without ST segment elevation (c.p.p non ST) (1st group- 30 c.p.p. non ST, age 45 ± 5 years; creatinin , urea, glucose, CRP in reference ranges; 2nd group- 28 c.p.p. non ST; age 60 ± 5 years; one of the aforementioned biochemical parameters ≥ 30% compared to normal ranges). cTntT concentrations were measured 3-4 hours after chest pain onset.
Results. Statistically significant differences in cTnT concentrations between observed 2 groups of c.p.p. ($p > 0.05$)
1st group: cTnT conc. 45 ± 20 pg/mL
2nd group: cTnT conc. 220 ± 70 pg/mL
Conclusions. Among relatively young and mostly healthy individuals hs TnT assay is definitively good diagnostic tool for AMI (perfect correlation with EKG and other cardiomarkers elevations). Due to increased baseline concentrations in elderly patients with other chronic diseases cTnT-hs is only an indicator of higher risk for an adverse outcome, and has no diagnostic but only prognostic value.

0335

USEFULNESS OF URINARY BIOMARKER NGAL IN THE MANAGEMENT OF RENAL REPLACEMENT THERAPY (RRT, HEMOFILTRATION)

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Background. Acute kidney injury (AKI) requiring initiation of hemofiltration (HF) is seen in approximately 6-10% of patients after cardiac surgery and is associated with high morbidity and mortality. There are currently no guidelines for initial management of HF therapy. Neutrophil Gelatinase Associated Lipocalin (NGAL) is a novel urinary biomarker suggested to be released by renal tubules very early in response to ischemic damage. A new algorithm including NGAL was used to improve management criteria for early HF treatment.
Methods. Urinary NGAL was tested routinely in all consecutive patients undergoing cardiac surgery at our institution in 2010 before and 4, 24 and 48 hours postoperatively. In contrast to previous years, HF therapy was only initiated when NGAL at 4 hours was markedly increased versus baseline and/or urine production decreased within 4 days (<0.5 mL/kg/h, >4h) independent of creatinine and urea. Exclusion criteria was HF treatment for hemodynamic reasons. Number of HF therapies (2010) was compared to previous years.
Results. HF therapy in 2006-2009 was initiated at day 3 after surgery in 7.5% to 9.0% of patients with average length of therapy of 7.7 days. Using the new algorithm, number of HF therapies was 4,9%. Preliminary data suggests no difference in extrarenal complications and permanent dialysis requirement.
Conclusions. Urinary NGAL can be used safely as an early predictor for HF requirement in patients after cardiac surgery. Inclusion of NGAL can help in the RRT management and may reduce cost by decreasing the number of necessary HF treatments and length of therapy.

Diabetes mellitus and metabolic syndrome

0336

TO WHAT EXTENT ARE CURRENT RECOMMENDATIONS ABOUT SELF MONITORING OF BLOOD GLUCOSE IN NON INSULIN DIABETES PATIENTS USING THE PRINCIPLES OF EVIDENCE-BASED MEDICINE?

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Background. To evaluate if Clinical Practice Guidelines (CPG) recommendations on self monitoring of blood glucose (SMBG) in non insulin diabetes patients are using the principles of Evidence Based Medicine (EBM).
Methods. Comparisons were made between CPG recommendations and the conclusions of systematic reviews (SRs) cited by the CPGs; graded on a four-point scale (1: evidence of negative effect of SMBG; 2: effect unknown; 3: partial efficacy; 4: efficacy in all patients).
Results. 15 CPGs were included, and their recommendations regarding evidence for or against the use of SMBG in non insulin diabetes patients had an average grade of 3,33; higher grading were seen for CPGs that stated that their recommendation were based on consensus. The CPGs cited 11 SRs, 11 RCTs, 35 non–RCTs and 17 other CPG/reports. From 0 to 55% of SRs that were published the year before the release of the CPG were cited. SRs with low grading were less cited. The conclusions of the 11 cited SRs had a mean grading of 2,38; higher values were seen for SRs that included non-RCT articles or were funded by industry. In total 18 RCTs were included in the different SRs. A large variation was seen in the inclusion or exclusion of RCTs, and about half of RCTs included in the SRs added an educational intervention which made it difficult to isolate the effect of SMBG.
Conclusions. CPGs were more positive towards SMBG than the SRs. CPGs currently in use are not based on the principles of EBM.

0337

METABOLIC SYNDROME – A PHYSIOLOGICAL ADAPTAION TO LIFESTYLE DISEASES

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Background. Unfavourable pattern of lifestyle diseases among the local population at an younger age.
Methods. A 2yr follow up study was conducted among 300 school going teens. The selected institutions were having children of both sexes from different geographical area and social backgrounds in the country. Anthropometry and biochemical risk factors were measured and calculated at the start and at intervals of one year.
Results. boys: Girls ratio 9:3.31% of the children had family history of DM, 11% had hypertension and 3% CAD.35% of student population showed HOMAIR>2.5,but no significant difference between sex. Anthropometric parameters showed maximum sensitivity and specificity were skinfold thickness and Fat Mass Index>85percentile and 90 percentile respectively. Similarly glucose>100mg/dl and TG>130mg/dl had strong association with IR. Though HDL had a higher sensitivity but lower specificity as a marker. But α- lipoprotein is a better marker in this regard. While repeating the study after 1 year, 12% reduction was observed in the number of IR subjects who adopted recommended lifestyles changes-diet/exercise or both. Again repeating the study after 2 years. there was a reversal ,i.e. 7% increase in the number of students with HOMAIR>2.5.among those who failed to continue the regime of lifestyle adopted.
Conclusions. It can be hypothetically stated that body has temporary and permanent mechanism for adaptation towards handling lifestyle changes and such adaptations may be influenced right from gut which warrant further study.

Diabetes mellitus and metabolic syndrome

0338

ASSOCIATION BETWEEN GAMMA-GLUTAMYLTRANSFERASE AND INSULIN RESISTANCE MARKERS IN ADULT TURKISH INDIVIDUALS

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Background. Serum gamma-glutamyl transferase (GGT) has been used as a marker of alcohol induced liver disease. Recent studies have shown that serum GGT levels are strongly associated with the metabolic syndrome. The aim of this study was to evaluate the possible relationship of GGT with insulin resistance and beta-cell function in adult Turkish individuals with various statuses of glucose metabolism.
Methods. A total of 295 subjects including with impaired fasting glucose (IFG) (n = 68), newly diagnosed type 2 diabetes (NT-2DM) (n = 46) and normal glucose tolerance (NGT) (n = 181) were recruited. GGT and glucose analyses were carried out with an autoanalyzer using a colorimetric method (Modular PPP Analyzer; Roche Diagnostics GmbH, Mannheim). Insulin levels were determined by electrochemiluminescence immunoassay using autoanalyzer (Roche E170 Analyzer; Roche Diagnostics GmbH, Mannheim).Group classification was done according to ADA (American Diabetes Association) 1997 criteria. Homeostasis Model Assessment of-insulin resistance (HOMA-IR) levels and beta-cell function were determined.
Results. Serum GGT levels were significantly lower in NGT group compared with IFG and NT2DM groups (20.75 ± 15.65 U/L, 42.60 ± 47.36 U/L, 41.91 ± 47.37 U/L, respectively, $p < 0.05$). HOMA-IR levels were significantly increased in IFG and NT2DM groups compared with NGT (6.12 ± 5.17, 7.48 ± 6.84, 2.73 ± 1.58, respectively, $p < 0.05$), while the beta-cell index significantly decreased (181.72 ± 146.01, 63.35 ± 62.18, 310.27 ± 376.91, respectively, $p < 0.05$).
Conclusions. Serum gamma-glutamyltransferase levels are associated with glucose tolerance abnormalities, insulin resistance and reduced beta-cell function. Our results indicates that monitoring GGT levels might serve to help prevent the development of metabolic syndrome.

0339

HYPERCHOLESTEROLEMIA AND LDLR GENE MUTATION AMONG TYPEII DIABETES MELLITUS PATIENTS IN JORDAN

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Genomic DNA was extracted from white blood cells of 99 healthy, 44 diabetic, 57 Hypercholesterolemic and 101 hypercholesterolemic diabetic patients from Jordan. Part of the low–density lipoprotein receptor gene in exon 12 was amplified by Polymerase chain reaction (PCR) and digested with HincII restriction enzyme. LDL, TC, TG, HDL and FBS levels were measured in all subjects. RFLP analysis was Conducted to identify allele variation of LDLR in patients with hypercholesterolemia and type 2 diabetes mellitus .The results showed that no significant correlation existed between this RFLP locus and TYPEII diabetes mellitus (D.M) while it is significant in hypercholesterolemia .Marked differences were found between the genotype distributions of LDL and TC levels subgroups of hypercholesterolemic, hypercholesterolemic diabetic and normal controls. It was inferred that the H1 allele might be associated with high blood cholesterol levels, and the H2 allele with low cholesterol levels. Disturbances of lipid metabolism occur frequently in diabetes mellitus. This study suggested that the differences in LDLR genotypes might affect the phenotype of lipid metabolism.

Diabetes mellitus and metabolic syndrome

0340

ESTIMATION OF SERUM CALCIUM AND PARATHYROID HORMONE (PTH) LEVELS IN DIABETIC PATIENTS IN CORRELATION WITH AGE AND DURATION OF DISEASE

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Background. A potentially important role for calcium in the development of diabetes has been implicated. This study document the assessment of serum calcium and parathyroid hormone (PTH) levels in diabetic patients and the correlation between calcium and PTH with the duration of the disease and age of the patients.
Material & Methods. This study included 30 patients had diabetes were submitted to the out patients clinic of Baghdad teaching hospital. Those patients were varying in their ages (30-70) and duration of disease (1-22 years) compared with 20 apparently healthy controls (non diabetic), with the same range of age.
Ca estimation was done by atomic absorption method, while PTH level was estimated by ELISA kit.
Results. In this study there is a significant decrease in both serum calcium and serum PTH ($p < 1.91E-05$), ($p < 6.57E-05$) respectively compared with the control. Strong direct correlation between patients' Ca & PTH levels ($r=0.3$), while strong indirect correlation was found between patients' age and their calcium level ($r=-0.46$)
Conclusions. Monitoring of serum calcium and serum PTH are very important in the follow up and treatment of diabetic patients.

0341

COMPARISON OF TWO IMMUNOTURBIDIMETRIC METHODS FOR THE MEASUREMENT OF GLYCATED HEMOGLOBIN A1C

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Background. Hemoblobin A1c is the most important parameter for monitoring the metabolic control of patients with Diabetes Mellitus. To optimize the measurement of HBA1c working with a biosafe method, an immunoturbidimetric assay in whole blood was compared with the currently used in our laboratory.
Aim. To verify that both methods are statistically comparable without affecting the clinical interpretation of the results
Methods. Two methods were compared : Tina-quant A1c Gen 2(â Roche) on a Cobas 6000 c501(whole blood samples) and Tina-quant A1c (â Roche) on Modular P (hemolized whole blood) across all the measurement range
Accuracy and veracity were ascertained on a previous study through EP15-A2 protocol, CLSI. Deming linear regression (Alternate Comparison Method –EP Evaluator) was used to compare both Methods. The criteria applied to verify that two methods are statistically comparable were: correlation coefficient(R) > 0.975 ; the confidence interval (CI) of 95% of the slope must include number one, the CI of the intercept must include number zero and there must not be any significant difference in the medical decision levels.
Results. Slope: 0.992 (CI 95% :0.981 to 1.003) ; Intercept: 0,058 (CI 95%: -0,023 to 0,138) ; R: 0,9989.Medical decision levels :5.9 and 7.0 are included in the 95% CI
Conclusions. Both methods are comparable and there were no significant differences in the medical decision levels. Taking into account the optimization of the process, a decrease in the analytical error and the improvement in biosafety, the implementation of the method was accepted

Diabetes mellitus and metabolic syndrome

0342

SERUM FATTY ACID BINDING PROTEIN 4 AND ADIPONECTIN RATIO IS ASSOCIATED WITH GLYCEMIC CONTROL IN DIABETES

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Background. Adipocyte fatty acid binding protein (FABP4) has been shown to be closely associated with diabetes as well as metabolic syndrome (MetS), obesity, and development of atherosclerosis. Our purpose was to evaluate serum FABP4 levels in type 2 diabetics (T2Ds) in association with the markers of poor glycemic control and inflammation.
Materials and Methods. We studied 262 T2Ds, 29 pre-diabetics (PreDs) and 57 controls categorized according to obesity and pharmacological treatments. Serum FABP4, adiponectin levels, and the glycemic control markers were measured.
Results. FABP4/adiponectin ratio was significantly different between three study groups (p=0.017), and in poor controlled T2Ds (>7% HbA1c) from good controlled (≤7% HbA1c) (p=0.01); and in insulin (p=0.001), antihypertensive (0.033) and antihyperlipidemic (p=0.046) drugs treated groups from untreated groups. Gender, MetS, exercise and BMI are strong independent predictors of FABP4 levels where MetS is of FABP4/Adiponectin ratio after adjustment of the other markers.
Conclusions. Serum FABP4/Adiponectin ratio could be a good predictor of poor controlled type 2 diabetes mellitus.

0343

URINARY 8-HYDROXYDEOXYGUANOSINE AS A BIOMARKER OF MICROANGIOPATHIC COMPLICATIONS IN TYPE 2 DIABETIC PATIENTS

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Background. Reactive oxygen species (ROS) produced either endogenously or exogenously can attack lipid, protein and nucleic acid simultaneously in the living cells. Increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications. Urinary 8-hydroxydeoxyguanosine (8-OHdG) has been reported to serve as a sensitive biomarker of oxidative DNA damage.
Objective. To evaluate urinary 8-hydroxydeoxyguanosine (8-OHdG) as a marker for diabetic microangiophatic complications and to correlate its levels with the severity of diabetic nephropathy and retinopathy.
Methods. The study included 50 patients with type 2 diabetes mellitus and 30 non-diabetic age and sex matched control subjects. Urinary 8-hydorxydeoxyguanosine (8-OHdG), urine creatinine and urinary albumin excretion (UAE) rate were measured in all patients and control subjects. Both 8-OHdG and UAE rate were assayed by immunoassays. Assessment of glycemic control in patients was achieved by measurement of HbA1c. All of the patients underwent direct opthalmoscopy and photography with pupils dilated.
Results. There was a highly significant difference between different groups of type 2 diabetic patients classified according to retinopathy, and controls as regards 8-OHdG (50.4 ± 12.8 vs 19.2 ± 8.4 respectively, F = 5.6 (p<0.01), and albumin/creatinine (alb/creat) ratio (257 ± 29.3 vs 16 ± 6.4 respectively) (F = 5.2) (p<0.01). Statistical comparison between groups of patients classified according to alb/creat, ratio using ANOVA test revealed a highly significant difference regarding 8-OHdG, (F = 5.2, p <0.01) Also there was a significant difference between patients with microalbuminuria as regard 8-OHdG excretion (71.3 ± 11.8 vs 53.0 ± 18.5 respectively) (p<0.05). Similarly a significant difference between patients with microalbuminuria regarding 8-OHdG excretion(71.3 ± 11.8 vs 26.1 ± 8.1 respectively) (p<0.01). There was also a significant difference regarding 8-OHdG between patients without retinopathy and those with simple retinopathy (30.6 ± 11.5 vs 56.5 ± 12.8 respectively) (p<0.05), and a highly significant difference regarding the same marker between patients without retinopathy and those with proliferative retinopathy (30.6 ± 11.5 vs 64.0 ± 14.1 respectively) (p<0.01). Using ROC curve, the diagnostic utility of urinary 8-OH-dG in discrimination of diabetic patients with retinopathy from those without retinopathy at a cutofflevel of 34.4 ug/g creatinine had a diagnostic sensitivity of 92.9 % specificity of 86.4 % and efficacy of 90 %%.
Conclusion. Measuring Urinary 8-hydroxydeoxyguanosine (8-OHdG) is a novel convenient method for evaluating oxidative DNA damage. Diabetic patients, especially those with advanced nephropathy and retinopathy had significantly higher that such changes may contribute to the development of microvascular complications of diabetes.

Diabetes mellitus and metabolic syndrome

0344

CHITOTRIOSIDASE ENZYME ACTIVITY IN PATIENTS WITH METABOLIC SYNDROME

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Background. Chitotriosidase, a protein synthesized exclusively by activated macrophages, has been proposed as a biochemical marker of macrophage accumulation in several lysosomal diseases, especially in Gaucher's disease. Chitotriosidase activity was found to be elevated in atherosclerotic tissue, showing a clear connection between chitotriosidase and lipid-laden macrophages inside human atherosclerotic vessel wall. This study aimed to analyzethe plasma chitotriosidase activity in patients with metabolic syndrome (MetS) and if chitotriosidase activity could be related to the predisposition of atherosclerosis in patients with MetS.
Methods. 95 patients with MetS and 37 healthy controls were enrolled in the study.The MetS was defined according to NCEP/ATPIII definition. Plasma chitotriosidase enzyme activity was measured by the method described by Hollak et al with minor modifications.Chitotriosidase activity was expressed as micromoles of substrate hydrolyzed per hour per liter of incubated plasma.
Results. The mean±SD age of MetS patients and control group were 45±12.6 and 41±20.1, respectively. 36.8% of the MetS group and 40.5% of the control group were smokers. There were not any statistically significances in terms of age (P=0.096), sex (P=0.254) and cigarette smoking (P=0.363) between MetS patients and control group. Chitotriosidase activity in patients with MetS was 69.03±15.85 μmol/L·h (mean±SD) and in control group was 55.07±23.24 μmol/L·h. The difference was statistically significant (P=0.009).
Conclusions. Plasma chitotriosidase activity is significantly increased in patients with MetS. This result shows that chitotriosidase could play a key role in determining the predisposition of atherosclerosis in patients with MetS.

0345

FIBROBLAST GROWTH FACTOR-19 LEVELS IN TYPE 2 DIABETIC PATIENTS WITH METABOLIC SYNDROME

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Background. This study aimed to investigate serum FGF-19 levels in type 2 diabetic (T2DM) patients with metabolic syndrome (MetS) and to evaluate the relationship between FGF-19 and other cardiovascular risk factors such as atherogenic index of plasma (AIP) and hsCRP.
Methods. 26 T2DM patients with MetS and 12 healthy controls were enrolled in the study. Serum FGF-19 levels were measured by sandwich ELISA and compared with the other cardiovascular risk factors; lipid profile, AIP, hsCRP and HbA1c. AIP was calculated as log (Triglyceride/HDL-c).
Results. The median (1st-3rd quartile) FGF-19 levels in T2DM patients with MetS were lower than healthy controls, 122.9 (108.63-237.60) pg/ml and 293.45 (153.64-370.31) pg/ml, respectively (P=0.003). When patients were grouped as BMI<30 kg/m^2 (n=13) and ≥30 kg/m^2 (n=13), the median (1st-3rd quartile) values were 168.70 (113.54-275.77) pg/mL and 115.89 (97.94-200.40) pg/mL, respectively (P=0.007). Significant negative correlations were found between FGF-19 and triglyceride, log (Triglyceride/HDL-c), hsCRP and HbA1c (r=-0.327, P=0.050; r=-0.312, P=0.050; r=-0.435, P=0.006; r=-0.357, P=0.028, respectively). In T2DM patients with MetS, the AUC (95% CI) of FGF-19, log (Triglyceride/HDL-c) and hsCRP were calculated with ROC analysis and were 0.798 (0.655-0.941), 0.845 (0.720-0.969) and 0.780 (0.635-0.926), respectively.
Conclusions. We showed that FGF-19 levels are low in T2DM patients with MetS. The negative relationship between FGF-19 and other cardiovascular risk factors suggest that FGF-19 is a new useful marker in predicting cardiovascular disease risk in diabetics with MetS.

Diabetes mellitus and metabolic syndrome

0346

ADIPONECTIN AND FREE FATTY ACIDS IN TUNISIAN PATIENTS WITH METABOLIC SYNDROME

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Background. The metabolic syndrome (MS) is a cluster of interrelated common clinical disorders, including hypertension, insulin resistance, glucose intolerance, dyslipidaemia, and obesity. Adiponectin is an adipokine secreted specifically from the adipose tissue. Serum adiponectimia was associated withthe risk of MS.
 The aim of our study was to evaluate adiponectin and free fatty acid (FFA) in patients with MS.
Methods. Our study involved 53 patients with MS (mean age 49 ± 14 years) and 55 healthy subjects constituted the control group (mean age 41 ± 10 years).
 Serum adiponectin were measured with ELISA method (Abcys). FFA was determined by enzymatic colorimetric method at 550 nm (Randox, Antrim, UK).
Results. Serum adiponectin were significantly lower in patients with MS than control groups (adiponectin: 7. 35±2.20 μg/ml vs 11.12±3.46 μg/ml, p $<10^{-3}$).
While, serum FFA was significantly higher in patients with MS than control groups (FFA: 0.85±0. 51 mmol/l vs 0.42±0. 17 mmol/l, p $<10^{-3}$). We found that adiponectin was correlated negatively with FFA (r:-0.322; P$<10^{-3}$)
Conclusions. Our data conclude that hypoadiponectemia associated to higher level of FFA were involved in the development of metabolic syndrome in patients.

0347

ANTIOXIDANT STATUS IN TUNISIA PATIENTS WITH METABOLIC SYNDROME

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Background. The metabolic syndrome (MS) is a complex disorder combining obesity, dyslipidemia, hypertension, and insulin resistance. Oxidative stress plays a critical role in the pathogenesis of metabolic syndrome.
The aim of study was to evaluate antioxidant status (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase, total antioxidant status (TAS) in patient with MS.
Methods. 53 patients (mean age 49±14years) with MS and 55 controls (mean age 41±10years) were recruted in the study.
The TAS, SOD and Catalase activity were determined by colorimetric Methods. The GR and GPx activities were determined at 340 nm.
Results. The serum activities of antioxidant enzyme were significantly lower in the patients than the control group (SOD: 1808 ±585 U/gHb vs 2334 ±415 U/gHb, p $<10^{-3}$, GPx: 94.39 ±15.24 U/gHb vs 119.31 ±71.01 U/gHb, GR: 10.30 ±2.22 U/gHb vs 13.55 ±4.07 U/gHb, p $<10^{-3}$, Catalase: 311.88 ±90.64 kU/gd'Hb vs 551.55 ±191.65 kU/gd'Hb, p $<10^{-3}$).
Serum TAS was significantly lower (TAS: 1.12 ±0.37 mmol/l vs 1.33 ±0.53 mmol/l, p $<10^{-3}$)) in the patients.
Conclusions. Our founding demonstrates that patients with MS have a deficient antioxidant protection that increase their vulnerability to oxidative damage and promote the development of cardiovascular complications.

Diabetes mellitus and metabolic syndrome

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GLUCOSE, GLYCATED HAEMOGLOBINAND ADVANCED GLYCATION END-PRODUCT IN TUNISIAN DIABETIC PATIENTS

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Background. Glycated haemoglobin (HbA1c) and advanced glycation end-products products (AGE) accelerated chemical modification of proteins by glucose during hyperglycemia and contribute to the pathogenesis of diabetic complications including micro and macrocomplications.
The aim of this study was to determine the levels of glycemia, HbA1c and AGE in patients with diabetes mellitus type 2.
Methods. Prospective study, G1: 70 patients with diabetes mellitustype 2 (age 53±11 years). G2:45 healthy subjects (age 54±11 years). Glycemia was determined by enzymatic colorimetric method using the glucose oxydase (Randox, Antrim, UK); glycated haemoglobin was determined by immuniturbimitric method (Cobas integra) .AGE were determined by spectrofluorimetric method according to Henle and al.
Results. Patients with diabetes mellitus had significantly higher values of glycemia(10. 35±3.11mmol/l vs 5. 74±2.56mmol/l,P<10^{-3}) than healthy subjects. The level of HbA1c had significantly higher in patient group than controls (9. 84±1.65 % vs 5. 96±0.65 %, P<10^{-3}). The diabetes mellitus patients had significantly higher values of AGE (9.81±2. 44 10^{-3} UA /g vs 5. 06±1. 40 10^{-3} UA /g, P<10^{-3}) than controls.
Conclusions. We conclude that chronic hyperglycemia objectived by high level of HbA1c (Amadori product) and AGE constitutes a risk factors for the development of micro- and macrovascular complications in this patients with diabetes mellitus.

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INTEREST OF ALBUMINEMIA MEASURMENT IN THE INTERPRETATION OF SERUM FRUCTOSAMINE

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Background. In cases where HbA1c is not measurable or the result not interpretable (hemoglobinopathy, decrease of erythrocyte life cycle….) serum fructosamine is measured. Fructosamine allows to estimate glycemic balance over a short period, 3 weeks vs 8 weeks for HbA1c. Normal fructosamine values are given with the reserve of a normal albuminemia value. Here we tried to define a standard interpretation of fructosamine results in case of hypoalbuminemia.
Methods. The study was performed on 178 blood samples. We measured at the same time HbA1c by HPLC, fructosamine, albumin and total protein (TP) by colorimetric technique. The correlations between HbA1c and fructosamine, fructosamine/albumin or fructosamine/TP were analyzed.
Results. The correlation between HbA_{1c} and fructosamine/albumin ratio was better (y=0.6314x + 2.4655, r=0.85) than the correlation between HbA_{1c} and fructosamine (y=0.0165x + 3.3124, r=0.76). Using HbA_{1c} values >6% or fructosamine values >280 μmol.L^{-1} as markers defining bad glycemic balance, 60% of patients were misclassified by fructosamine compared to HbA_{1c}. Using the equation established from experimental data, we determined that a threshold of 5.6 for fructosamine/albumin ratio allowed correct classification of patients with good or bad glycemic balance. There was no advantage in using the fructosamine/TP ratio.
Conclusions. Some diabetics patients cannot be followed by HbA1c. In such cases, fructosamine is interesting providing that the interpretation of fructosamine results is correct. We recommend to measure albumin in parallel and to use the fructosamine/albumin ratio in case of hypoalbuminemia: a ratio >5.6 can be considered as marker of bad glycemic balance.

Diabetes mellitus and metabolic syndrome

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THE PREVALENCE OF BIOMARKERS AS CLINICAL PREDICTORS OF DIABETIC NEPHROPATHY

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Background. About 35% of all Insulin dependent Diabetes Mellitus (IDDM) patients developed diabetic nephropathy characterised by partial albuminuria,changes in glomerular filtration speed, blood pressure and tubular dysfunction.
Methods. Serumcreatinine, Cystatin Cconcentration(DAKO) test, NAG activity (standardised kinetically) and albuminuria(DAKO test) was measured in group of patients(N=198) enables the identification of early risk to developed diabetic nephropathy(Type1= 96,Type2=102). Albumin creatinine ratio was measured to gane the level of functioning of the kidneys.
Results. We confirmed a significant increase of NAG activity and albuminuria in the group of patients (NIDDM,N=102), compared to the control group(N=50):NAG x=2.54+/-0.87,v.s. 0.73+/-0.36U/mmol creatinine,albuminuria-x=193+/-36.5,v.s20 mg/L. In this group no correlation was confirmed between the examined parameters and the urine proteins. Concentration of serum CysC was slowlyincreased, but not significant. In the group of patients dependent of insulin(N=96) we confirmed a very significant increase of NAG-activity-x=4.57+/-1.5U/mmol,albuminuria-x=385.5+/-98.0. In this group a positive correlation between the NAG-activity, albuminuria and the concentration of urinary proteins has been founded. Cystatin C concentration was significantly changed -2.35+/- 0.56;v.c.g. 0.70 +/-0.50 mg/L,p<0.01.
Conclusions. Determination of the NAG activity (tubular dysfunction), albuminuria , creatinine and Cystatin C concentration (Glomerular dysfunction) are more sensitive tests for monitoring patients with Diabetes mellitus and developed Diabetic nephropathy

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EFFECTS OF PPARγ, APOE, ACE AND AT1R GENE VARIANTS ON DEVELOPMENT OF METABOLIC SYNDROME

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Background. Metabolic syndrome (MS) is a cluster of risk factors including hypertension, abdominal obesity, dyslipidemia and hyperglycemia. The contribution of genetic factors to the development of MS has been widely recognized, but the contribution of genes has not yet been fully clarified. We investigated the possible role of gene polymorphisms of PPARγ (Pro12Ala), ApoE, ACE (I/D) and AT1R (1166A>C) in MS.
Methods. Genotyping of PPARγ, AT1R and ACE was performed by PCR-RFLP, APOE by real-time PCR in a group of 281 patients and 119 controls. Associations of alleles and genotypes with biological and clinical variables were performed using independent t-tests or χ^2 where appropriate and UNPHASED-3.0.10.
Results. In comparison to females, males were older, had higher BMI and waist circumference, higher triglycerides and glucose levels and lower HDL. Males had significantly more often high blood pressure. Age accounted for the differences in glucose levels and HDL. We found significant association of AT1R variants and the development of MS (p=0.03), with the C allele being associated with lower risk. We found association of waist circumference with: ACE variants (p=0.03), with D allele carrying the higher risk; AT1R with A as the risk allele (p=0.05). APOE4 variant was associated with the waist circumference (p=0.05) and the levels of HDL (p=0.03), and ACE genotype was associated with glucose levels (p=0.02), with II genotype carrying the lowest risk.
Conclusions. Gene variants of AT1R, ACE and ApoE could be susceptibility factors of lipid status, obesity and glucose intolerance contributing to the development of metabolic syndrome.

Diabetes mellitus and metabolic syndrome

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IMPLEMENTATION OF NEW RECOMMENDATIONS FOR THE DIAGNOSIS OF GESTATIONAL DIABETES (GDM): A FIVE-MONTH AUDIT

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Background. Early diagnosis of GDM has an important role to prevent adverse outcomes. Recent international recommendations, according to the HAPO study and IADPSG Consensus Panel (Diabetes Care 2010;33:676), were implemented in our university hospital. After five months, we aimed to audit the impact and the appropriateness of use of the new diagnostic approach.
Methods. Two five-month (June-October) periods, one before [2009, using the two OGTTs standard approach (S1)] and one after the implementation of the new criteria [2010, (S2)], were compared.
Results. In the two periods, 256 (S1) and 245 (S2) pregnant women were examined. 298 OGTTs (50g, n=195; 100g, n=103) and 252 OGTTs (75g) were executed in S1 and S2, respectively. In S1, 54 (27.7%) 50gOGTTs resulted positive and 36 (66.7%) of those performed the 100gOGTT. In addition, 3 (1.5% of total) 50gOGTT negative women were submitted to 100gOGTT. 63 women did 100gOGTT only. In total, 14 (13.6%) 100gOGTTs were positive. In S2, 38 (15.1%) OGTTs resulted positive. In women who did the whole diagnostic evaluation in our hospital, 92.3% in S1 and 77.0% in S2 performed the correct protocol. The rate of incomplete OGTTs was low (0.7% in S1 and 2.4% in S2).
Conclusions. Our data show that in our hospital new recommendations for GDM diagnosis are not correctly applied in about ¼ of cases. The main issue seems to be the lack of consideration of the new threshold of fasting glycemia (92 mg/dL) as main decisional driver for performing OGTT. Further education of ordering physicians is advisable.

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REVALUATION OF BIOLOGICAL VARIATION OF HBA$_{1c}$ USING AN ACCURATELY DESIGNED PROTOCOL

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Background. HbA$_{1c}$ has a key role for diagnosing diabetes and monitoring glycemic state. Knowledge of its biological variation (BV) is critical for result interpretation. As recently published results showed a marked heterogeneity of available BV data, we revaluated HbA$_{1c}$ BV using a well designed protocol.
Methods. We took five blood specimens from 18 apparently healthy subjects (9 men and 9 women, ages 26-52 years) on the same day every two weeks for two months. Samples were stored at -80°C until analysis and assayed in duplicate in a single run by Roche Tina-quant Gen.2 immunoassay on the Integra 400 platform. Data were analyzed by the ANOVA. To assess the assay traceability to the IFCC reference method, we preliminarily carried out a correlation experiment on four blood samples.
Results. The regression equation (Tina-quant = 1.05 IFCC reference method – 1.6 mmol/mol) confirmed the alignment of the employed assay. There were no differences in HbA$_{1c}$ values between men and women (global mean, 36 mmol/mol). Within- (CV$_I$) and between-(CV$_G$) subject BV were 2.5% and 7.1%, respectively. Desirable analytical goals derived from BV for imprecision (0.50 CV$_I$), bias [0.25 (CV$_I^2$ + CV$_G^2$)$^{1/2}$] and total error [1.65 (0.5 CV$_I$) + 0.25 (CV$_I^2$ + CV$_G^2$)$^{1/2}$] were 1.25%, 1.88%, and 3.94%, respectively. HbA$_{1c}$ had marked individuality, therefore the use of population-based reference limits could be inadequate for test interpretation. The reference change value, useful to interpret changes in serial results, was ~9%.
Conclusions. Our data show that strict analytical goals are needed for the clinical application of HbA$_{1c}$ measurements.

Diabetes mellitus and metabolic syndrome

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IN OBESE WOMEN THE RELATIONSHIP OF PARAOXONASE I AND ARYLESTERASE ACTIVITIES WITH LIPID METABOLISM

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Background. Obesity is a serious disease with increasing importance all around the world; it increases the risk for Type 2 Diabetes Mellitus, cardiovascular diseases and cancer. The aim of this study was to identify the effects of serum lipids, Apolipoprotein A-I (Apo A-I), Apolipoprotein B (Apo B) and Paraoxanase 1 and Arylesterase (PON1/ARE) activities on obesity in women.
Methods. The study was conducted on 90 subjects categorized in three groups, there were 30 women in each group with an age range of 18-48 years. Group I had 30 abdominal obese patients, Group II had 30 gynoid obeses and Group III included 30 healthy control subjects. For the patient and control groups, the following measurements were performed: serum PON1 and ARE activities, total cholesterol, triglyceride (TG), HDL cholesterol (HDL-C), Apo A-I, Apo B, direct LDL cholesterol (D-LDL-C), high sensitive C-reactive protein (hsCRP), insulin and plasma glucose levels. The HOMA-IR index was calculated. Variance analyses were carried out in accordance with the distribution of the groups. The correlation among the measured parameters was studied.
Results. There was a statistically significant difference between TG, HDL-C, Apo B, D-LDL-C, hsCRP, HOMA-IR levels of Group I and II (p=0,001, p=0.000, p=0.016, p=0.001, p=0.000, p=0.000 respectively). For HDL-C, D-LDL-C, hsCRP, HOMA-IR levels, there were statistically significant difference between Group I and III (p=0,023, p=0.023, p=0,000, p=0,001, respectively). There was no such difference between TG, HDL-C, Apo B, D-LDL-C, hsCRP, HOMA-IR of Group II-III (p=0.294, p=0.250, p=0.322, p=0.627, p=0.080, p=0.496). There was no such difference among the groups for glucose, total cholesterol, Apo A-I, PON1, ARE parameters (p=0,135, p=0,235, p=0,150, p=0,886, p=0.288, respectively). There was significant positive correlation between total cholesterol and TG (p=0,000), Apo A-I (p=0.001), Apo B (p=0.000), D-LDL-C (p=0,000); between TG and Apo B (p=0.000), HOMA-IR (p=0.001), D-LDL-C (p=0,000), BMI (p=0.001); between HDL-C and Apo A-I (p=0.000); between Apo B and D-LDL-C (p=0.000), BMI (p=0.007); between HOMA-IR and BMI (p=0.000); between D-LDL-C and BMI (p=0.000), hsCRP (p=0.013); finally between BMI and hsCRP (p=0,000). There was a significant negative correlation between triglyceride and HDL-C (p=0,007) and between HDL-C and BMI (p=0.000), hsCRP (p=0,025).
Conclusions. TG, Apo B, HDL-C, D-LDL-C parameters differed significantly in abdominal obesity, whereas there was no difference among the groups for PON1/ARE activities. Having similar PON1/ARE activity among the groups might be related to several factors like genetic, demographic and metabolic.

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GLYCATED HEMOGLOBIN VS. 75 GR. ORAL GLUCOSE TOLERANCE TEST IN DIAGNOSIS OF DIABETES MELLITUS TYPE 2

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Background. For decades, the diagnosis of diabetes has been based on plasma glucose (PG) criteria, either fasting PG (FPG) or 2-h 75-g oral glucose tolerance test (OGTT) values. The aim of the study was to compare the use of glycated hemoglobin (HbA1c) and the oral glucose tolerance test in the diagnosis of type 2 diabetes.
Methods. There were 150 patients (74 women, 41 men, mean age: 51.6±17.1 years) enrolled in this study aged between 18 and 80 years. Patients were recruited among those making their visit to the department of Clinical Biochemistry to perform 2-h 75-gr OGTT. Glycated hemoglobin was performed on Variant turbo HPLC (Biorad laboratories) using a method wich is NGSP certified and standardized to the DCCT assay.
Results. Forty two percent (9/21) of patients with DM diagnosed by OGTT had HbA1c>6.5, and 90% (19/21) HbA1c>6.0. ROC curve for HbA1c as a diagnostic indicator for a 2 hours plasma glucose <200 mgr/dl (7.7 mmol/L) showed an area under curve of 0.884 (p<0.0001) (0.806-0.939). ROC curve for HbA1c as a diagnostic indicator for impaired tolerance glucose showed an area under curve of 0.682 (p<0.0012) (0.590-0.765)
Conclusions. These values allow us to consider HbA1c as a diagnostic initial test for diabetes type 2 instead 75 gr OGTT, since we obtain a negative predictive value of 95% when prevalence of diabetes in our sample is 20% . A value of HbA1c 6% showed maximum combined sensivity (80.9%) and specificity (85.3%), because we recommend this value such as decision point.

Diabetes mellitus and metabolic syndrome

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DIAGNOSTIC RELEVANCE OF URINE AND SERUM URIC ACID IN PROGRESSION OF TYPE 2 DIABETES MELLITUS – THERAPY AND GENDER DIFFERENCES

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Background. Studies implicating the role of uric acid(UA) in progression of prediabetes to diabetes are highly contraversial and deserve further analysis.Recently, correlation between glucose concentration and serum uric acid was reported up to glucose concentration of 8 mmol/L, leaving doubt about possible correlations at higher glucose levels and possible gender differences.

Methods. In this study, uric acid as a potential biomarker for an impaired glucose metabolism and diabetes progression, was analysed in serum and urine samples collected from 40 T2DM male and 40 female patients classified according to type of therapy(insulin or metformin) by endocrinologist in Sarajevo General hospital „Prim.dr Abdulah Nakaš" and 40 age-matched controls with normal glucose tolerance and no obesity. Patients were further subclassified in those with well controlled diabetes and poorly controlled diabetes. UA and glucose analyses were performed on fresh samples of urine and serum on Dade Behring analyser.

Results. Our results demonstrated a profound increase in both urine and serum uric acid levels in type 2 diabetic patients with a trend of their positive correlation. This increase was more evident in insulin treated patients.Serum uric acid levels remained within reference limits which was not the case for urine UA levels. Gender differences were evident at the level of urine uric acid. Well controlled diabetic patients had lower levels of urine uric acid levels when compared to poorly controlled diabetic patients.

Conclusions. This study justifies the use of both serum and urine uric acid as possible markers in progression od DM 2.

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IMPAIRED GLUCOSE METABOLISM AND INSULIN RESISTANCE IN PATIENTS WITH ASYMPTOMATIC HYPERURICEMIA

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Background. Hyperuricemia has been considered an important association of metabolic syndrome and insulin resistance. Asymptomatic hyperuricemia is the term applied to settings in which the serum urate concentration is elevated, but neither symptoms nor signs of urate crystal deposition (gout) have occurred. There are few studies demonstrating alteration in glucose metabolism in patients with asymptomatic hyperuricemia.

Methods. This case control study was done to evaluate the insulin resistance status and glucose metabolism in subjects having asymptomatic hyperuricemia. Homeostasis model assessment of insulin resistance (HOMA IR) status and HbA1c levels were estimated in 50 subjects found to have asymptomatic hyperuricemia in routine health checkup. The same biochemical parameters were assayed in 30 age, sex and BMI matched healthy controls.HOMA IR was calculated from fasting plasma glucose and fasting serum insulin levels.HbA1c was assayed using HPLC.

Results. There was a significantly higher level of HOMA IR and HbA1c level in the hyperuricemia group as compared with controls. Although the mean levels of HbA1c in both groups were in the non diabetic level (less than 6%), asymptomatic hyperuricemia subjects had higher HbA1c than control population. The mean levels of HOMA IR in hyperuricemia subjects was 1.78 as compared to 0.72 in control group(p<0.001).Mean HbA1c was 5.68 and 5.05 in the above mentioned groups respectively(p<0.005).

Conclusions. In conclusion it can be hypothesized that asymptomatic hyperuricemia might be a silent and early indicator of future occurrence of diabetes or impaired glucose tolerance. Hence such subject should undergo thorough investigations and treated accordingly if required.

Diabetes mellitus and metabolic syndrome

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HIGH SENSITIVITY C-REACTIVE PROTEIN CONCENTRATION IN NON-DIABETIC AND DIABETIC POPULATION

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Background. Inflammation marker, high sensitivity C-reactive protein (hsCRP), is an independent cardiovascular risk factor and many studies have shown that higher hsCRP concentrations are found in patients with diabetes mellitus (DM). The purpose of this study is to examine hsCRP levels in Thai population age ranged 35 to 74 years old with and without DM.
Methods. Serum hsCRP was determined in 3,676 sera obtained from participants of the International Collaborative Study on Atherosclerosis and Stroke in Asia (InterASIA) in 2000 using nephelometric method on BN 100 analyzer. Triglyceride, cholesterol, HDL-C and plasma glucose were determined on the Dimension RxL analyzer and direct LDL-C was determined on the COBAS Integra 400 analyzer.
Results. We found 10.06% of participants were classified in diabetic group and a relation between hsCRP concentrations and DM was statistical significant ($p<0.001$).The geometric mean with 95% CI of hsCRP was significantly higher in diabetic participants (2.44 mg/L; 2.22 to 2.68 mg/L) than those non-diabetic participants (1.31 mg/L; 1.27 to 1.36 mg/L), $p<0.05$. HsCRP levels were positively related to glucose, triglyceride, cholesterol and LDL-C while negatively related to HDL-C levels, respectively.
Conclusions. HsCRP was elevated in diabetic group in Thai population. According to its ability on prediction of future cardiovascular disease, hsCRP levels are useful for providing additional information to guide health planners to develop strategies plans for reducing cardiovascular risk in diabetic population.

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BASIC BIOCHEMICAL PHENOTYPE ANALYSIS OFETHNIC LITHUANIAN'S

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Background. Investigation of basic biochemical phenotype of ethnic Lithuanian's was found to be interesting in setting-up health recommendations adapted for the Lithuanian population. Main goal of analysis –create preconditions for wide scale complex investigation of human phenotypic expression in different groups of pathology of Lithuanian population.
Methods. Blood samples from relatively healthy volunteers were collected at six main ethnolinguistic regions of Lithuania. Glucose, cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and hsCRP were investigated on Architect ci8200 (Abbott) system.
Results. 269 volunteers were enrolled in the study, 80 males (age range 2-74) and 189 females (age range 6-79). No statistically significant difference was observed between male and female results of investigated analytes, except of HDL cholesterol results (p<0.0006) which was found to be higher in females. 61.25% of total cholesterol and 57.50% of LDL cholesterol results in males and 72.78% and 59.17% of results respectively in females were above normal ranges. No clinically and statistically significant difference of investigated analytes concentrations was found between males from towns (population >30,000) and villages (population <16000).In contrast statistically significant difference of total cholesterol (p<0.01), HDL-Chol (p<0.02), LDL-Chol (p<0.002) and hsCRP (p<0.006) was found in females.
Conclusions. Need forsetting-up different health recommendations for ethnic Lithuanian females from towns and villages were found.Only minor differences of total and LDL cholesterol were observed with the tendency to be higher in males from villages. Male's population requires bigger number of participants to obtain more reliable Results.

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HBA1C MEASUREMENT FOR THE DIAGNOSIS OF DIABETES: IS IT ENOUGH?

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Background. New diagnostic criteria have been recently proposed by American Diabetes Association (ADA) focusing on A1C for diagnosis of diabetes as well as for identification of the subjects at increased risk, being values ³ 6,5% (48 mmol/mol) diagnostic for diabetes and values between 5,7 - 6,4% , (39- 47 mmol/mol) suggestive of a pre-diabete condition.
Methods. The study, started on April 2010, includes all subjects presenting to outpatients department with request of OGTT in which the diagnostic performance of new proposed criteria and standard 75-g oral glucose tolerance test (OGTT) has been compared. Until now 146 subjects (male n=64, females n=82 and a age from to 26 to 74) have been recruited..
Glucose concentrations have been measured using hexokinase method (Modular D, Roche Diagnostics) and A1C with HPLC procedure (Adams HA-8160 Arkray, Kyoto, Japan). Analytical performance were monitored using IQC and participating to a national EA scheme.
Results. OGTTs identified a pre-diabetic condition in 20.54% of subjects (n=30) while the new diagnostic criteria in 41.09% (n=60); the diagnosis of diabetes occurred in 17,12 % of subjects (n=25) versus 5,4% (n=8) according to OGTT and new proposed criteria respectively. Discordant classification has been observed in 1,36% of cases (2 out of 146) showing A1C ³ 6,5% (A1C= 6,6% and A1C= 6,7% respectively) and normal OGTTs.
Conclusions. The data obtained in our study evidence that the proposed new diagnostic criteria are questionable because 13,46% of diabetes's cases are misclassified.

0361

METABOLIC DISTURBANCES AND INFLAMMATORY MARKERS IN OBESITY

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Background. Obesity and dyslipidemia might be related to a proinflammatory state as inflammatory cytokines play a role in lipid's and carbohydrate's metabolism and energy expenditure.
Methods. In this study, we have examined the presence of metabolic abnormalities in relation to IL-6, TNF and CRP serum level in 102 obese patients (71 men (M), 24 women (W) (BMI ≥25) and 77 non-obese controls (39 M and 38 W) BMI <25. In obese group diabetics and non-diabetics were included. Plasma total cholesterol and triglyceride were determined enzymatically on Hitachi 912 analyzer (Roche Diagnostics). HDL-cholesterol (HDL) was measured using a homogenous method with polyethylene glycol-modified enzymes and alpha-cyclodextrin. LDL-cholesterol (LDL) was calculated by the Friedewald equation. Cytokines level was determined by ELISA method.
Results. The mean IL-6 concentration in non-obese subjects was lower than in obese patients but significant difference was found only in the group of W (p=0,001).The IL-6 concentration was higher in obese patients with type 2 diabetes when compared to obese, non-diabetic patients. The difference was significant in the group of W (p=0,01). The level of TNF in obese patients was significantly higher than in non-obese group (p<0,001). TNF level was significantly higher in obese patients with diabetes than in the non-diabetic, obese subjects. In all subgroups results were statistically significant (whole cohort: p<0,0001; M: p=0,0002; W: p<0,0001). The correlation between BMI, IL6 and C-reactive protein was positive (p<0,01).
Conclusions. This study supports evidence that metabolic syndrome is characterized by chronic activation of proinflammatory pathways connected to the development dyslipidemia and diabetes.

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USE OF PMN ELASTASE COMPLEX IN PREDICTION OF OUTCOME AT THE PATIENTS WITH DIABETES MELLITUS

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Background. Recording of some parameters at patients with Diabetes Mellitus, in function of insulin treatment, through the changes in glucose level regulation as the eventually changes in elastase–complex concentration.
Methods. Diabetic patients (N=96) under the insulin therapy were investigated. Therapy was set according to patient's state and needs. Glucose level (Gly), glucosilated hemoglobin (HbA_1c) were determinate spectrophotometricly, and elastase-complex concentration with turbidimetric method.
Results. Higher level of glucose (104, 1%) andHbA_1c (61, 8%) was found at diabetic patients from our study, as well as significant increase of elastase-complex concentration (162,3%).
Increased elastase-complex concentration is due to changes in lipoprotein layer of cell membrane as a result of lipoprotein metabolism disbalans, so common in diabetes. This situation can be base for more complicated patient's condition.
Conclusions. Our opinion and recommendation is that estimated results should be considered in individual therapy definition in achieving the positive effects of treatment and avoiding additional complications as possibility of unexpected and difficult for treatment infection as a result of disturb cell integrity.

0363

ASSOCIATION BETWEEN PRO12ALA POLYMORPHISM OF THE *PPARG* GENE AND METABOLIC SYNDROME IN BOSNIAN POPULATION

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Background. Peroxisome proliferator-activated receptor gamma (PPARgamma) is a key transcription factor in adipogenesis and also regulates various lipid metabolism genes. The common variant Pro12Ala (rs1801282) of *PPARG* gene has been associated with type 2 diabetes. In this study, we investigated association of Pro12Ala polymorphism with metabolic syndrome (MetS) and its characteristics in Bosnian population.
Methods. The study included 42 patients diagnosed with MetS and 43 healthy controls. Subjects were genotyped for Pro12Ala polymorphism by classic PCR followed by restriction fragment length polymorphism analysis. Influence of genotypes on clinical and metabolic parameters was assessed.
Results. There was no significant difference in mutated allele frequency between MetS patients and controls. The Pro12Ala variant was significantly associated with lower body mass index (p=0.012) and lower waist circumference (p=0.045) in the control group. It also showed trend of association with lower low-density lipoprotein and total cholesterol, although this was not statistically significant (p=0.070 and p=0.060, respectively).
Conclusions. Our results indicated that acommon variant of *PPARG* gene, Pro12Ala might have a protective effect against obesity.

Diabetes mellitus and metabolic syndrome

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EVALUATION OF A NEW GENERATION HEMOGLOBIN A1C ASSAY ON BECKMAN COULTER UNICEL® DXC SYNCHRON® CLINICAL CHEMISTRY SYSTEMS*

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Background. Hemoglobin A1c (HbA1c) is used to monitor long-term glucose control in patients with diabetes mellitus. The objective of this study was to evaluate the performance of a next generation HbA1c assay on UniCel DxC systems.
Methods. HbA1c and total hemoglobin (THb) concentrations are measured in human whole blood by immunoturbidimetric inhibition and colorimetric assays, respectively. HbA1c concentration is reported as a ratio of HbA1c to THb concentration. The assay is standardized to the IFCC reference method; results are converted to National Glycohemoglobin Standardization Program (NGSP) %HbA1c units using the master equation (NGSP=0.09148 IFCC+2.152).
Results. In development studies, within-run imprecision was 1.4% and 1.3% CV at 5.2% and 9.5% HbA1c, respectively. Between-run imprecision was 2.9% and 2.7% CV at 5.3% and 9.5% HbA1c, respectively. Linearity was demonstrated between 0.4–2.4 g/dL (0.248–1.49 mmol/L) HbA1c and 6–24 g/dL (3.72–14.9 mmol/L) THb (sample recovery 100±6%). Methods comparison (Deming regression analysis) against 1) NGSP HPLC values yielded y=1.01x–0.09, r=0.995, n=54 and 2) against the existing HbA1c assay yielded y=1.05x–0.35, r=0.997, n=75. HbA1c reagent calibration stability was 14 days and on-board stability was 30 days (control recovery 100±6%).
Conclusions. In development studies, the next generation HbA1c assay on UniCel DxC systems demonstrated improved correlation to HPLC method and acceptable precision, linearity and reagent stability.
Pending clearance for use in EU and USA

0365

HEMEOXYGENASE-1 AND ITS METABOLIC ROLE IN THE LIVER

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Background. Obesity and diabetes are the new epidemics of the 21st century. Given the dramatic rise in number of obese patients, it is imperative to identify novel treatment avenues. Hemeoxygenase-1 (HO-1) has gained great importance as a key protective enzyme with potent anti-inflammatory properties. Interestingly, in vivo studies of various designs demonstrate that systemic activation of the HO-1 system by chemical means ameliorates obesity and diabetes in various rodent models.
Methods. We measured HO-1 mRNA expression levels in livers of C57BL/6J mice fed a low (LFD) or high fat diet (HFD). In the next step we generated a hepatocyte-specific conditional HO1 knockout mouse model by crossing HO-1fl/fl mice and Albumin-Cre mice (LiHoko). These mice were fed a high fat diet (HFD) for a period of 16 weeks. Oral glucose tolerance tests (oGTT) and intraperitoneal insulin tolerance tests (ITT) were performed to test for metabolic changes.
Results. HO-1 mRNA levels were higher in livers of obese, insulin resistant HFD fed mice (P=0.0152).
After 16 weeks on HFD LiHoko mice weighted significantly less than their control littermates. Fasted blood glucose levels were increased. In oGTT blood glucose levels of LiHoko mice were higher whereas in ITT they were lower compared to their controls.
Conclusions. We successfully generated a hepatocyte-specific conditional HO1 knockout mouse model which will allow us for the first time to address and dissect the metabolic function of HO-1 in hepatocytes in vivo. Our first findungs demonstrate significant differences in their metabolic response to HFD.

Diabetes mellitus and metabolic syndrome

0366

INFLAMMATION AND DYSLIPIDEMIA IN MOROCCAN OBESE PATIENTS WITH OR WITHOUT METABOLIC SYNDROME

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Background. Obesity, independent predictor of incident cardiovascular events, predispose to numerous risk factors for cardiovascular disease, including hypertension, dyslipidemia, and diabetes, leading to the metabolic syndrome (MetS). Inflammation, reported as a link between obesity and cardiovascular disease, contribute to the development of atherosclerosis.
The main goals of the study were to determine the incidence of the metabolic syndrome on lipid and inflammatory profiles, in one part, and on the cardiovascular risk, in another part, among Moroccan obese patients.
Methods. Our study concerned 88 obese patients, mean-aged 52.01 ± 9.13 years-old, with or without MetS enrolled at Pasteur Institute of Morocco. Obesity and MetS have been identified according to the BMI measure and NCEP definition, respectively. Our data included anthropometric measurements, lipoprotein profiles and C-Reactive Protein levels.
Results. Highly significant differences in age and blood pressure have been noticed in obese patients with MetS. In patients with MetS, lipoprotein profiles alterations included high levels of TG, Apo B, TC/HDL ratio, Apo B/HDL and Non-HDL-C. Our population showed high risk of cardiovascular disease. Otherwise, they presented significant higher levels of hsCRP compared to patients without MetS. Furthermore, diabetes, followed by hypertriglyceridemia and hypertension, were four times higher in obese with MetS than in those without MetS. Hypo-HDL-emia was the least prevalent MetS component.
Conclusions. The present study shows that the metabolic syndrome leads to lipoprotein metabolism alteration, inflammation, as well as increased cardiovascular risk in obese patients. Diabetes, hypertriglyceridemia and hypertension were the most frequent cardiovascular risk factors associated with obesity.

0367

LEPTIN CONCENTRATIONS IN INDIVIDUALS WITH METABOLIC SYNDROME AND TYPE 2 DIABETES MELLITUS

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Background. Metabolic syndrome describes a cluster of cardiovascular risk factors including obesity (central), dyslipidaemia, insulin resistance, and hypertension. Leptin, an adipocyte derived hormone involved in weight regulation and energy balance has been implicated in the pathogenesis of metabolic syndrome and type 2 diabetes mellitus. This study aims at evaluating the concentrations of leptin in metabolic syndrome, type 2 diabetes mellitus and apparently healthy Nigerians.
Subjects and Methods. One hundred and thirty six subjects (47 type 2 diabetes mellitus, 45 metabolic syndrome and 44 apparently healthy individuals as controls) were recruited from the University College Hospital, Ibadan , Oyo state, Nigeria and environs. Demographic, anthropometric characteristics and blood pressure were obtained using standard methods and questionnaires. Five milliliter (5ml) of blood samples were obtained for determination of leptin by enzyme linked immunosorbent assay (Diagnostic Automation, Inc,USA). Data obtained was analysed statistically with SPSS software version 16.0.
Results. This study showed significantly higher concentrations of leptin in metabolic syndrome group compared with controls(p<0.05). Leptin was higher in diabetics but not significantly different when compared with controls (p >0.05). It positively correlated with body mass index in all groups tested, hip circumference and percentage body fat in metabolic syndrome group and controls, and waist circumference in diabetics. Circulating leptin concentrations negatively correlated with blood pressure in metabolic syndrome group only.
Conclusions. Leptin is associated with indices of metabolic syndrome and its assessment may be useful in early diagnosis and prevention of metabolic syndrome and associated chronic diseases in the Nigerian population.

Diabetes mellitus and metabolic syndrome

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OXIDATIVE STRESS PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES

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Background. Persistent hyperglycemia has been reported as a cause of increased production of reactive oxygen species. Enhanced oxidative stress observed in diabetes is thought to be important etiological factor of chronic diabetic complications.
Methods. Measurements of oxidative stress parameters have been performed in plasma, serum and hemolysate obtained from 80 type 2 diabetes patients and 80 apparently healthy, gender and age matched control subjects. The plasma total antioxidant capacity (FRAP), reduced glutathione concentration (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR), γ-glutamyltranspeptydase (GGT) activities and uric acid concentrations were measured spectrophotometrically. Albuminuria and hsCRP concentrations were determined using immunonephelometry and immunoturbidimetry, respectively.
Results. FRAP, uric acid, hsCRP levels and GGT activities were significantly higher in the diabetes patients as compared with the control group. The activity of GPx measured in plasma and hemolysate was higher in diabetic patients than in healthy subjects and GR activity was significantly higher in the control group. Significant correlation between FRAP and uric acid concentrations was observed in both groups. No significant correlations between oxidative stress parameters and glycemic control assessed by HbA1c and mean daily glycemia were found. There were no significant differences in oxidative stress parameters between diabetes patients with and without long-term complications except of microalbuminuric subjects who had higher FRAP, GPx and GR levels.
Conclusions. Noticeable differences in pro-oxidative/antioxidative balance in diabetic patients as compared to healthy subjects were found. These differences are associated with inflammation, increased albuminuria and changes in uric acid levels observed in type 2 diabetes patients.

0369
USEFULNESS OF SCREENING FOR GESTATIONAL DIABETES

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Background. To detect gestational diabetes (GD) a simplified glucose tolerance test (GTT50g) is performed. If blood glucose exceeds 140 mg/dl the test is positive and we use a confirmatory diagnosis with another test (GTT100g). Our objective is to evaluate the diagnostic efficiency of GTT50g and review the cut-off.
Methods. We retrospectively reviewed the GTT50g done over three months in the La Ribera Hospital.Those tested positive were reviewed GTT100g. Blood glucose was measured in a Modular analyzer (Roche ®). Sensitivity (S), specificity (E), positive predictive value (PPV), negative (NPV), positive likelihood ratio (LRP), negative (LRN), and the area under receiver operating characteristic (ROC) curve were calculated. For statistical analysis using Excel 2003, Epidat 3.1 and SPSS 12.0.
Results. 564 GTT50g samples were reviewal, 122 were positive, and 14 of those confirmed the diagnosis. The results of S, E, PPV, NPV, LRP and RVN, were 77.8%, 80.2%, 11.5%, 99.1%, 3.93 and 0.28, respectively. The area under the ROC curve (AUC) was 0.863 (95%:0.799-0.926). In considering other cut-offs, we found that 136 mg/dL, improved sensitivity (88.9%) maintaining the specificity (76.2%).
Conclusions. The results obtained for the different indicators together with the AUC showed that the test has a good efficiency. However the low PPV suggests that we should carry out a selective screening in order to improve the diagnostic performance. We must reconsider the cut-off for the GTT50g and improve sensitivity, which is expected in a screening test. But a greater number of women will have to be subjected to the GTT100g.

Diabetes mellitus and metabolic syndrome

0370

INSULIN RESISTANCE IN BULGARIAN PREGNANT WOMEN WITH RISK FOR GESTATIONAL DIABETES MELLITUS, DETERMINED BY HOMEOSTASIS MODEL ASSESSMENT (HOMA)

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Background. Patientswith gestational diabetes mellitus (GDM) are insulin resistant (IR). The homeostasis model assessment (HOMA), has been widely validated and applied for quantifying IR. The HOMA of insulin resistance (HOMA-IR) index is regarded as a simple and reliable surrogate measure of IR.

Methods. 87 women from whom 75pregnant and 12 post part, gestational weeks (24 Vs 25 ± 4). Based on the OGTT, participants were stratified into 4 groups (IADPSG criteria): I-st. group healthy pregnant women with normal glucose tolerance (NGT) (n_1= 7; 9.4%), II-nd group pregnant with impair glucose tolerance (IGT) and high levels of insulin (n_2 = 56; 64%), III-rd group pregnant women with GDM (n_3= 13;14.9%), and IV-th group - 6 weeks post part with GDM (n_3= 13; 12.6%). Venous blood was taken to determine the levels of insulin and glucose from 0,60,120 min. The analysis was done by GM 9 Analyzer "Analox Instruments" for glucose and "Elecsys 2010" – Roshe for insulin. HOMA-IR is calculated.

Results. The pregnant with NGT had significantly lower IR (n_1 = 1.2 ± 0.6). The pregnant with IGT (n_2 = 2.4 ± 1.6 , P = 0.027) and GDM (n_3 = 5.3 ± 4.7 , P< 0.0001) had significantly higher HOMA-IR values compared to pregnant with NGT.

Conclusions. Pathological IR, common for GDM, is a manifestation of a substantial loss of insulin sensitivity with constant character and does not disappear completely after birth. HOMA-IR after delivery is higher without statistically significant difference (p=0.733).

0371

CORRELATION BETWEEN BMI AND WAIST SIZE WITH LEPTIN, ADIPONECTIN AND RESISTIN SERUM LEVEL IN OBESE PATIENTS

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Background. Adipose tissue secretes bioactive peptides (leptin, resistin, and adiponectin) which play an important role in the development of numerous metabolic diseases. Adiponectin has antiatherogenic and antidiabetic effect, whereas leptin and resistin show a strong proinflammatory, proatherogenous and prodiabetic effect.

Background. To bind the level of leptin, resistin and adiponectin with BMI and waist size in the obese.

Methods. The study were carried out in 59 obese patients. All study subjects were conducted and anthropometric measurements were determined by serum concentration of leptin, resistin and adiponectin.

Results. The mean age was 36 ± 8.3 years, of whom 42 (71.2%) women and 17 (28.8%) men. The average BMI among women was 33.2 ± 7.5 kg / m², and for men 40.3 ± 8.8 kg / m². The average waist size for women was 96 ± 3.2 cm and 111 ± 7.45 cm in men.

Spearman correlation showed a statistically significant correlation (r = 0.62, p <0.001) between leptin with BMI. The values of resistin with BMI and waist size are statistically significantly correlated (r = 0.51, p <0.001 and r = 0.52, p <0.001), and the values of adiponectin were negatively correlated with BMI and waist circumference (r =- 0.55, p <0.001 and r =- 0.62, p <0.001)

Conclusions. In obese patients serum concentration of leptin and resistin positively (leptin no) and adiponectin negatively correlated with BMI and waist size. Therefore, their determination is completed by evaluating the risk of typical disease (diabetes mellitus, cardiovascular disease, dyslipidemia,...) for obese people.

Diabetes mellitus and metabolic syndrome

0372

ANTIOXIDANT CAPACITY AND LOW GRADE INFLAMMATION STATE IN PREGNANCY COMPLICATED BY DIABETES

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Background. Antioxidant capacity measured with FRAP, two suggested serum antioxidants: uric acid (UA) and GGT (gamma-glutamyl transpeptidase) and inflammation marker CRP were measured in diabetic and non diabetic pregnant women.
Methods. Tests were performed in two groups of pregnant women: with gestational diabetes mellitus (GDM, 36 patents) and type 1 diabetes (DM1, 17 patents), in healthy pregnant (HP, 26 subjects) and non-pregnant women (NP, 22 subjects) in age 18 – 35. hsCRP, GGTP, and UA were measured in serum, and FRAP in plasma.
Results. FRAP, GGTP and UA were elevated in diabetic groups as compared to HP with significantly higher only UA in GDM (3,22 vs. 2.31 mg/dL p=0,014). In NP group FRAP was the highest , amounting to 0,807 mmol/L, the mean GGTP and UA levels were the same as in HP group 3 U/L, 2,30 mg/dl, respectively. Significantly higher CRP concentrations in HP as compared to NP (1,78 and 0,44 mg/L, respectively, p<0,001). CRP concentration were increased in diabetic women.
Significant correlations between UA and remaining parameters (UA/FRAP, R=0,4994 , p<0,001; UA/GGTP R=0,3190 , p=0,002; UA/CRP, R=0,2749 , p=0,007) were found. CRP correlated significantly also with GGTP (R=0,2201 , p=0,033). No significant correlation were found between FRAP and CRP (R=-0,0061,p=0,954).
Conclusions. Our findings indicated elevated of total antioxidant capacity and low grade inflammation in pregnancy complicated by diabetes. UA was strongly associated with antioxidant capacity and inflammation that suggests its role in antioxidant defence. GGTP is not an antioxidant marker.

0373

PERFORMANCE OF HBA1C IN DIAGNOSIS OF DIABETES IN AN ASIAN POPULATION

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Background. The International Expert Committee has recommended diabetes should be diagnosed when HbA1c is ≥6.5%.This study assesses the performance of HbA1c compared to oral glucose tolerance testing (OGTT) for the diagnosis of diabetes in a mixed Asian outpatient population.
Methods. Details of OGTT with paired HbA1c samples from 2005-10 were extracted from the laboratory database for statistical analysis. OGTT (75 glucose) was performed and interpreted according to WHO recommendations with 0 and 120 min sampling. All HbA1c (turbidometric immunoinhibition method) and glucose measurements were performed on Beckman Coulter LX20 PRO analysers
Results. There were 137 records available with average age 57y (30-95); 84 men, 103 Chinese, 22 Indian, 12 Malay. The prevalence of diabetes by OGTT was 66/137. The AUC for HbA1c to predict diabetes by OGTT was 0.8 (0.72-0.88). At the suggested cut-off of HbA1c 6.5, sensitivity was 60.6% and specificity was 84.5%. 100% sensitivity was at HbA1c 4.6% (specificity 0%) while 100% specificity was at HbA1c 7.3% (sensitivity 22.7%). Comparing diabetes diagnosis using OGTT and HbA1c >=6.5, concordance was 73% and kappa 0.45.
Conclusions. Despite moderate agreement of diabetes status using HbA1c >=6.5 and OGTT, 39% of OGTT diabetics would be considered non-diabetic using HbA1c alone while 15% of OGTT non-diabetics would be considered diabetic using HbA1c alone. Diabetes prevalence in this population would drop from 48% to 37% with use of HbA1c alone. The 2 approaches are not interchangeable and identify different patient groups. Prospective studies are needed to discover which approach better predicts diabetic complications.

Diabetes mellitus and metabolic syndrome

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S-LDL AND PARAOXONASE ACTIVITY IN DIABETIC PATIENTS

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Background. The objective was to assess the interest of s-LDL and paraoxonase (PON) activity for prediction of complications in diabetic patients.
Methods. This study included 83 patients with type 2 diabetes from endocrinology department of the University Hospital of Monastir (23 males and 60 females, mean age = 58.86 ± 11.74 years).
Total cholesterol, HDLc/LDLc and triglycerides were determined by enzymatic method and apolipoprotein A and B by immunoturbidimetric technic, on Cobas 6000™analyser (Roche Diagnostics).
Small, dense LDL cholesterol (s-LDL) and paraoxonase activity were determined on Konelab 30 ™ analyser (Thermo Electron Corporation).
Results. We noted a significant positive correlation between total cholesterol, LDLc, triglycerides, ApoB and s-LDL (r were respectively = 0.703; 0.680; 0.674 and 0.731). A significant negative correlation (r= -0.364) was also noted betweenHDLc and s-LDL. For a cut-off of 0.9 mmol/L, s-LDL was associated with the development of macrovascular disease with a sensitivity of 0.68 and specificity of 0.63.
No significant correlation between paraoxonase activity and lipid parameters was noted.
A negative correlation between paraoxonase activity and the different stages of renal failure in our population was observed (p = 0.063).
Conclusions. In diabetic patients, increased sLDL seems to predispose to macrovascular disease. The decrease in paraoxonase activity is rather related to the stage of renal failure.

0375

INAPPROPRIATELY LOW HBA$_{1c}$ VALUES IN HPLC CAUSED BY A NEW HAEMOGLOBIN VARIANT

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Background. Some structural variants of Hemoglobin (Hb) are known to cause analytical interference in the measurement of glicated haemoglobin by cation-exchange HPLC. In this study we have characterized the Hb species in the blood sample of 61 year-old Spanish male with a history of diabetes mellitus type II and a low value of Hb A$_{1c}$.
Methods. We performed hemoglobin protein analysis by cation-exchange HPLC, reverse HPLC and electrophoretic separation (alkaline and acidic conditions). Functional properties of Hb were determined by oxygen equilibrium studies. **β**-globin gene was amplified by PCR and the sequencing was performed on an ABI Prism 310 sequencer.
Results. A new structural variant of hemoglobin Hb (Hb Seville) was detected by cation-exchange HPLC and reverse HPLC. This Hb variant was clinically silent and had functional properties similar to those of normal Hb A. Sequencing of **β** gene reveled a single base mutation at codon 81 (C□T) resulting in a leucine-to–phenylalanine substitution in the position 5 of EF helix. This novel haemoglobin variant of the β-globin resulted in falsely low Hb A$_{1c}$ measurement with cation-exchange HPLC (6.2%) in disagreement with a high fasting glucose level (12,2 mmol/L) . However, glycohemoglobin as measured by boronate affinity chromatography was 8.4%, according to glucose level.
Conclusions. This case points out the necessity of careful inspection of the chromatograms and the use of additional methods to Hb A$_{1c}$ measurement when the presence of aberrant peaks is detected.

Diabetes mellitus and metabolic syndrome

0376

THE *APOB* GENOTYPE CC/GG IS ASSOCIATED WITH CORONARY ARTERY DISEASE IN THE PRESENCE OF TYPE 2 DIABETES MELLITUS

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Background. Single nucleotide polymorphisms from *APOB* have been associated with risk of coronary artery disease (CAD) and glucose concentrations. The purpose of this study was to investigate the association of the g.2488C>T and the g.4154G>A *APOB* polymorphisms in a well defined sample with CAD in the presence and absence of type 2 diabetes (T2D).
Methods. A sample of 140 unrelated Euro-Brazilian submitted to coronary angiography were classified as T2D–CAD+ (presence of CAD and absence of T2D) and T2D+CAD+ (presence of CAD and T2D), accordingto the presence of stenosis > 50% in any coronary artery and American Diabetes Association 2010 criteria. Genotyping was performed by PCR–RFLP using *Xba*I and *Eco*RI as restriction enzymes.This is the first Brazilian study involving g.2488C>T and g.4154G>A *APOB* polymorphisms that considered the presence of T2D as a criterion for the classification ofthe sample.
Results. There was no significant difference between the genotype and allele frequencies of the g.2488C>T and g.4154G>A APOB polymorphisms when the groups were compared with each other (P>0.05). The frequency of the *APOB* CC/GG genotype of the g.2488C>T/g.4154G>A compared to the other *APOB* genotypes showed significant difference between the T2D-CAD+ and T2D+CAD+ groups(P = 0.019, c2 = 6.43).
Conclusions. We believe that the presence of subjects with T2D combined with CAD could be responsible for the association of the CC/GG genotype with CAD in previous report and in our study. These findings should be of interest for population studies and for understanding the interaction of diabetes and CAD.

0377

EVALUATION OF CAPILLARYS 2 FLEX PIERCING® (SEBIA) AS A NEW ANALYZER FOR HBA$_{1c}$ ASSAY

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Background. HbA$_{1c}$ is a key biomarker for the monitoring of glycemic balance in diabetic patients. It may be measured by various methods, including high-pressure liquid chromatography (HPLC) and immunoassays. Here we report the evaluation of Capillarys 2 Flex Piercing®, a new analyzer using capillary electrophoresis for the separation and the quantification of HbA$_{1c}$ from whole blood in primary capped tubes. Eight capillaries are used in parallel, allowing high throughput.
Methods. The analytical performances of the assay have been tested and HbA$_{1c}$ values obtained were compared to those of a HPLC assay routinely used in the lab (Variant II® analyzer, Bio-Rad). The influence of the most frequent analytical interferences on HbA$_{1c}$ assay was also studied.
Results. Intra- and inter-assay CVs are respectively lower than 1.98% and 2.68%. The linearity is excellent for HbA$_{1c}$ values ranging from 3.9% (19 mmol/mol) to 16.9% (161 mmol/mol) (r=0.999). The results are well correlated with those obtained by the HPLC Methods. HbA$_{1c}$[Capillarys 2] = 0.941 x HbA$_{1c}$[Variant II] + 0.303 (r=0.993, n=500). Moreover, the use of external quality control samples indicated a good accuracy of the method, since the results are in agreement with IFCC targets. The presence of labile HbA$_{1c}$ or carbamoylated hemoglobin did not affect HbA$_{1c}$ measurement, as well as the presence of some of hemoglobin variants, such as hemoglobin S, E and D.
Conclusions. This evaluation showed that the analytical performances of Capillarys 2 Flex Piercing® analyzer for HbA$_{1c}$ assay allow to recommend its implementation in clinical chemistry laboratories for a routine practice.

Diabetes mellitus and metabolic syndrome

0378

EVALUATION OF ANTIPSYCHOTIC DRUGS IN RELATION TO THEIR EFFECT ON METABOLIC RISK FACTORS

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Background. The high incidence of metabolic abnormalities such as diabetes and hyperlipidemia, seen in schizophrenic patients are result of complex interaction of number of factors including lifestyle and antipsychotic medication. This study indicates the effect of various drugs which includes both conventional and atypical drugs in their effects in causation of metabolic abnormalities leading to metabolic syndrome.
Methods. Efforts were made to select 210 (divided in 30-30 each group)subjects who were given the antipsychotics i. e. Olanzapine, Clozapine ,Quietapine, Risperidone, Amisulphride , Aripriazole (as atypical antipsychotics)and Haloperidol (as conventional drug) in therapeutic flexible dose as per clinical regimen. Patients were observed for the changes in the anthropometric and biochemical parameters after 16 weeks of Anti psychotic treatment and were subjected to ATPIII defined criteria for metabolic syndrome.
Results. Highly significant(p<0.001) weight gain was observed in patients receiving Olanzapine(3.2 Kg) followed by Clozapine (2.8Kg). An average weight gain of1.9Kg,1.8Kg, 1.06 Kg ,was observed in patients with Quietapine,Risperidone and Haloperidol medication respectively. No significant weight gain(p>0.05) was observed in patients receiving Aripriazole or Amisulphride. Percentage of patients with metabolic syndrome ($\geq$ 3 risk factors) increased form 3.3% at baseline to 36.6% after Olanzapine medication. However,no increase in metabolic risk factors was observed in patients after receiving Haloperidol, Aripriazole or Amisulphride. A highly significant (p<0.001) increase in blood glucose levels from baseline(82.73$\pm$8.27 mg/dl) to after 16 weeks(103.17+ 12.12 mg/dl) of olanzapine medication was observed.
Conclusions. Regular monitoring of weight and metabolic risk factors is important in patients with antipsychotic medication.

0379

FAECAL ELASTASE 1 LEVELS AS A MARKER OF EXOCRINE PANCREATIC FUNCTION IN PATIENTS WITH DIABETES MELLITUS

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Background. The measurement of faecal elastase (FE1) is used widely to screen for pancreatic exocrine insufficiency. Due to the close anatomical position and functional relationships between the endocrine and the exocrine pancreas, pathological conditions of one of these parts may cause impairment of the other. The aim of our study was the FE1 determination in patients with diabetes mellitus and the evaluation of the relationship of FE1 with residual beta-cell secretion and metabolic control.
Methods. FE1 was determined by ELISA method, using monoclonal antibody (ScheBo Biotech, Giessen, Germany), while serum fasting C-peptide, amylase, lipase, triglycerides and HbA1c concentrations were assayed by routine laboratory tests.
Results. FE1 values in 40 diabetic patients were significantly lower (Student t-test) than in the 20 healthy volunteers (mean 435±358 vs. 742±369 μg/g stools; P=0.0041). In 11 of 40 patients, FE1 was lower than 200 μg/g indicating pancreatic functional insufficiency. Among patients, FE1 correlated positively (Pearson's correlation test) with C-peptide levels (P=0.0084), amylase (P=0.0481) and lipase (P=0.0283), but there were no significant correlations between FE1 levels and duration of diabetes (P=0.5820), HbA1c levels (P=0.5877) and triglycerides (P=0.7515).
Conclusions. Our result has demonstrated a strong association of diabetes mellitus with low FE1 levels. We propose that pancreatic function be evaluated in diabetics who exhibit a sudden deterioration in blood glucose control, weight loss or gastrointestinal complaints of unclear origin.

Diabetes mellitus and metabolic syndrome

0380

INSULIN SENSITIVITY IS INCREASED BY INHIBITION OF PROTEIN-TYROSINE-PHOSPHATASESIN OBESE INSULIN RESISTENT C57BLACK6J MICE

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Background. A post-insulin receptor defect has been proposed to impact on insulin resistance. The contribution of protein-tyrosine-phosphatases (PTPs), enzymes dephosphorylating tyrosine residues, in experimental insulin resistance has not yet fully been explored.
Methods. To induce obesity-associated insulin resistance *in vivo*, C57Black6J mice received a high-fat diet (HFD, 60% kcal from fat) over 10 weeks. Animals that were fed a low-fat diet (LFD, 10% kcal from fat) served as controls. The impact of PTPs on obesity, and glucose/insulin metabolism was analyzed by treatment with PTP-inhibitors for further 6 weeks.
Results. HFD-treated mice were characterized by significantly higher body weight, and a decrease in serum levels of the fat-tissue derived peptide-hormone adiponectin. Metabolic phenotyping by intraperitoneal insulin/glucose tolerance tests (ITT, GTT) demonstrated reduced glucose- and insulin tolerance in HFD-treated animals. Quantitative RT-PCR based gene expression profiling revealed PTPs, such as SHP-1, LAR, and PTP1B, being upregulated in insulin-sensitive organs. Analysis of the hypothesis that PTPs represent potential molecular targets in insulin resistance included administration of PTP-inhibitors (SHP-1 inhibitor Sodium Stibogluconate, broad PTP-inhibitor Bis(maltolato)oxovanadium(IV) (BMOV)) to HFD-treated mice. This resulted in reduced PTP-activity in insulin-sensitive organs by BMOV-treatment. While basal insulin levels were not altered by PTP-inhibition, GTT- and ITT revealed beneficial effects on glucose/insulin metabolism, which was accompanied by a loss of body weight.
Conclusions. We conclude that PTPs antagonize the insulin receptor, and PTP-inhibition is effective in mediating insulinomimetic effects. Thus, targeting PTPs leads to improvement of obesity/insulin resistance, which might represent a pharmacological tool in metabolic diseases.

0381

LEVELS OF APELIN-36 IN PREDIABETICS AND NEWLY DIAGNOSED DIABETES MELLITUS PATIENTS

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Background. Prediabetes is characterized with insulin resistance and β cell dysfunction. Therefore prediabetics have an increased risk for cardiovascular diseases and Type 2 DM. Apelin-36 levels are up-regulated by insulin resistance, obesity and hiperinsulinemia. In this study we aimed to investigate the levels of parameters for glucose metabolism and cardiovascular risk factors and apelin-36 inpatients grouped as having impaired fasting glucose(IFG), IFG and impaired glucose tolerance(IGT), newly diagnosed type 2 DM and the control group.
Methods. Fifty-three women and twenty-seven men, totally eighty subjects were enrolled in this study. The patients were classified into four groups according to their OGTT Results.Group 1:Normoglycemic controls(n:20), Group2:subjects with IFG(n:20), Group3:subjects with IFG and IGT(n:20), Group4:Newly diagnosed type 2 DM patients(n:20). Levels of glucose, lipids and HbA1c were analyzed by enzymatic methods and turbidimetric method, respectively. Hormone levels were detected using an chemiluminescent system and Apelin-36 levels were analyzed by enzyme linked immunosorbent assay (ELISA).
Results. There was a statistically significant difference regarding the levels of Apelin-36 between group 1 and the study groups 2,3 and 4, respectively(p=0.005,p< 0.0001, p<0.0001). Other parameters analyzed for glucose metabolism and cardiovascular risk factors such as fasting glucose, HbA1c, HOMA-IR, fibrinogen, insulin, cortisol, C-peptide, LDL-chol, HDL-chol, VLDL-chol, TG, total cholesterol were significantly higher in the study groups when compared to the control group (p<0.005).
Conclusions. As a conclusion, in the future apelin-36 can be used as an indicator for presenting the insulin resistance and impairment in glucose metabolism in the early periods.

Diabetes mellitus and metabolic syndrome

0382

RATE OF APOPTOSIS IN DIABETIC RATS BRAIN

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Background. The purposeofthisstudy was toinvestigate the effects ofstreptozotocin-induced diabeteson ratbraintissue.
Methods. Twenty rats were divided into control and experimental groups at random. In experimental animals diabetes was induced by intraperitoneal injection of a single 50 mg/kg dose of streptozotocin, while the animals in control group received sodium citrat buffer. Apoptosis rate was measured by detecting cytoplasmic histone-associated DNA fragmentation using ELISA test. Brain and serum levels of glucose, malondialdehyde, ascorbic acid andtotalantioxidantcapacity(TAS) weredetermined.
Results. Apoptosis rate in brain tissue, for healthy brain tissue was increased significantly in brain tissue from diabetic rats ($P < 0.05$). Compared to the control animals brain MDA levels in diabetic rats were remarkably, but not significantly ($P > 0.05$), higher. Besides, a statistically significant ($P < 0.05$) increase from 16.21 ± 2.85 µmol/L to 19.30 ± 3.46 µmol/L was detected in serum MDA levels of the diabetic rats. Compared to the brain ascorbic acid levels of healthy rats, diabetic rats showed a significant decrease ($P < 0.05$) in their brain ascorbic acid concentration. Likewise, serum ascorbic acid concentration of experimental animals was significantly ($P < 0.001$) lower than that of controls . As to brain and serum TAS, compared to the control rats a statistical significant ($P < 0.05$) decline was observed in diabetic rats.
Conclusions. As a resultof hyperglycemiain streptozotocin-induced diabetesin ratscaused byoxidativestressinduces. In addition toincreasedfree radicalsis thought toinduceapoptosis.

0383

RELATIONSHIP OF FIBRINOGEN LEVELS AND OTHER RISK FACTORS FOR CARDIOVASCULAR DISEASE IN THAI PATIENTS WITH DIABETES

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Background. Cardiovascular disease (CVD) is highly prevalent and is responsible for the major cause of mortality in diabetes patients which is unlikely to be explained by hyperglycemia per se. It has been well established that CVD is a multifactorial disease. Among the established biochemical markers for CVD, fibrinogen is regarded as the risk factor for the disease. This study therefore aims to evaluate the associations of fibrinogen levels with diabetes as well as the classical risk factors for CVD.
Methods. A total of 111 subjects attended at Sathingphra hospital during October to December 2009 were recruited in the study. Of those, 51 individuals were diabetic patients and 60 subjects were healthy volunteers with the age of 48.45 ± 6.65 and 46.10 ± 7.42 (mean+SD) years old accordingly. Prothrombin derived-fibrinogen method was applied to determine plasma fibrinogen levels.
Results. Diabetic patients showed significantly higher fibrinogen concentration as compared to control group ($411.37 + 102.24$ and $306.69 + 96.70$ mg/dL respectively; $p < 0.001$). Investigation of other classical risk factors of CVD in the subjects revealed significant elevation of BMI, waist to hip ratio, systolic blood pressure, triglyceride concentration and LDL-C/HDL-C ratio ($p < 0.05$). Furthermore, significant relationship between fibrinogen concentration and BMI was found in the patients ($r=0.374$, $p=0.007$).
Conclusions. This study suggested that elevated fibrinogen levels may interact with BMI and in turn increase risk of CVD in diabetes patients.

Diabetes mellitus and metabolic syndrome

0384

TOTAL ANTIOXIDANT CAPACITY, SUPEROXIDE DISMUTASE AND CATALASE IN DIABETIC POLYNEUROPATHY

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Background. Oxidative stress resulting from enhanced free-radical formation and/or a defect in antioxidant defenses has been implicated in the pathogenesis of experimental diabetic polyneuropathy (DP). The antioxidant capacity is always decreased in diabetic patients, but it seems necessary to measure all the components to ascertain the reasons. The aim of this study was to determine the plasma total antioxidant capacity (TAC) and changes in the activities of superoxide dismutase (SOD) and catalase (CAT) in patients with diabetic polyneuropathy.
Methods. We evaluatedTAC, SOD, CAT in samples obtained from 30 DP patients and 30 healthy sex and age matched subjects as control group. Laboratory analyses involved fasting blood glucose and glycated hemoglobin (HbA1c) levels. The activities of SOD and CAT were determined by standard spectrophotometric Methods. Total antioxidant status was measured using Randox kit.
Results. Serum glucose and HbA1c levels were significantly higher in DP patients versus the control group (p<0.001). The TAC was significantly depleted in the diabetic group than healthy donors (p<0.001). Average SOD and CAT activity were significantly lower in patients with DP than control group (p<0.01). There was significant negative correlation between the TAC and serum glucose level, TAC and HbA1c (p<0.001), but not between TAC and duration of diabetes. Significant correlation in the case of SOD and CAT was not marked.
Conclusions. Examinationof the data from diabetesand total antioxidant status, SOD and CAT strongly implicateshyperglycemia-induced oxidative stress in DP.We conclude that striving for superior antioxidative therapiesremains essential for prevention of neuropathy in diabeticpatients.

0385

MULTIPLEX DETERMINATION OF ANALYTES RELATED TO METABOLIC SYNDROME AFTER WEIGHT LOSS WITH EVIDENCE BIOCHIP ARRAYS

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Background. Variations in the levels of adiponectin, C-peptide, CRP, cystatin C, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNFα have been associated to metabolic dysfunctions. Biochip array technology enables their multiplex determination from a single sample, at a single point in time. This generates a patient's profile, which provides more information than single analyte determination. The aim of this study was to determine these analytes in plasma of obese subjects following weight loss.
Methods. Plasma samples from obese subjects (n=12, average IBM>30kg/m^2) were analysed. For weight reduction, 5 subjects followed dietary restriction and 7 subjects underwent bariatric surgery. Two different biochip arrays were used for the multiplex determination. Simultaneous chemiluminescent immunoassays applied to the Evidence Investigator analyser were used. Statistical significance between groups was calculated using the paired Student's t-test (two tailed).
Results. Following dietary restriction, significant reductions were observed in plasma insulin and CRP levels(p=0.045 and 0.030 respectively). C-peptide, IL-6, leptin and TNFα exhibited moderate but no statistically significant reduction. A marginal increase was observed in PAI-1 and resistin. Adiponectin and IL-1α remained unchanged. Following bariatric surgery, a highly significant reduction was observed in plasma leptin(p=0.005) and a significant increase was observed in plasma adiponectin(p=0.014). C-peptide and PAI-1 were reduced and IL-1α, resistin and TNFα increased but did not reach statistical significance. No significant changes were detected in CRP, IL-6 and insulin levels.
Conclusions. This multi-analytical approach using biochip arrays shows differences between the groups undergoing weight loss and represents a valuable analytical tool in research settings.

Diabetes mellitus and metabolic syndrome

0386

PREANALITICAL ISSUES AND PROPER DIAGNOSIS OF DIABETES

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Background. Complete sample protection for glucose stability is obligatory for laboratory medicine and medical diagnosis of glycaemia states.
Methods. Glucose concentration, determined with hexokinase Modular P Roche method, was compared for different type inhibitors of glycolysis: NaF, iodine acetate from Greiner and FC mix with NaF/citrate from Terumo. The loss of glucose in blood samples has been studied in time dependence. The average population glucose value was compared for two different period, the year before and the year after adapting in laboratory new inhibitor system of glycolysis (FC mix tubes).
Results. The base values of glucose were obtained in 15 minutes and compared with concentration of glucose in 2 and 6 hours after blood collection in tubes with NaF, iodine acetate and FC mix (n=44).
Mean loss of glucose was -7,5%*; -6,7%*; -1,1% after 2 hours and -8,2%*; -7,4%*; -0,5% after 6 hours in tested tubes, respectively (p<0,001*).
We decided to change our glucose collection system from NaF to FC mix in 2008. The monthly average population glucose value (in mg/dl) were from 98,4 to 100,2 in 2008, and from 104,7 to 108,0 in 2009. The medium year glucose value was 99,3 mg/dl in 2008 and 106,5 mg/dl in 2009.
Conclusions. We conclude, that using of FC mix tubes allows to achieve complete stability of glucose. On the other side, current diagnostics cutpoints for diagnosis of diabetes should be reevaluated.

0387

ALTERATIONS OF THE *ERYTHROCYTE SUPERFICIAL CHARGE* BY THE IN VITRO EFFECT OF THE GLUCOSE

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Background. The non-enzimatic glycosylation (GnoE) of proteins is considered one of the most important mechanisms in the genesis of the micro and macrovascular complications of the diabetes. The GnoE could modify the erythrocyte hemorrheologic parameters such as aggregation, deformability and the viscoelastic properties of the red blood cell membrane.
Methods. The aim of this task was to study the effect of the glucose solutions on the erythrocyte anionic charge, using as a tool the partitioning in the biphasic aqueous system (Dextran T500, Polyethylene Glycol 6000), the digital analysis of images, the Eritroagregameter and the Erythrodeformeter in samples of erythrocytes from healthy donors which were incubated in vitro along with glucose solutions at 0,2g/dL, 0,5g/dL and 1g/dL at PBS pH 7,4. In the partitioning, the erythrocytes are retained in the superior phase according to the erythrocyte anionic charge. As a result, the coefficient of partitioning (P) was calculated. Through the digital analysis of images, a shape parameter (ASP)of the aggregates was also calculated. By using the Eritroagregameter, the time needed for the cells to reach 50% of their maximum capacity of aggregation (t50%) was determined and by using the Erythrodeformeter, the possible alterations of the index of the erythrocyte deformability (ID) were analyzed.
Results. The significantly different P and ASP values (p< 0,001) in the samples incubated with glucose, as regards the non-treated samples, would show alterations of the erythrocyteanionic charge, which could be associated with a process of GnoE. No significant variations were detected in t50% or in ID.
Conclusions. From the results obtained, this methodology could contribute to the study and partial understanding of the mechanisms involved in the hemorrheologic alterations observed in diabetic patients under inadequate metabolic follow-up.

Diabetes mellitus and metabolic syndrome

0388

GLUCOSE MONITORING: COMPARATIVE STUDY BETWEEN *ACCU-CHEK®AVIVA* AND THE LABORATORY EQUIPMENTS *DIMENSION VISTA®1500*, *COBAS®6000* AND *SYNCHRON LX®20 PRO*

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Background. The self-monitoring equipments revolutionized the measurement of glucose levels, particularly, for diabetic patients. This study compares the capillary glucose values obtained by self-monitoring equipment (*ACCU-CHEK®Aviva*) with serum glucose values obtained by equipments from clinical laboratories (*Dimension Vista®1500*, *COBAS®6000* and *SYNCHRON LX®20PRO*).
Methods. After informed consent, glucose values of 50 patients from each laboratory were analysed by the *ACCU-CHEK®Aviva* and the laboratory equipment. Results were compared using Paired samples *t*-test, Pearson's correlation test and Bland and Altman analysis.
Results. The correlation between values obtained by *ACCU-CHEK®Aviva* and other equipments was strong (r=0.985; n=50; P= 0.000, *SYNCHRON LX®20 PRO;* r=0,981; n=50; P= 0.000, *COBAS®6000* and r=0,953; n=50; P= 0.000, *Dimension Vista®1500*). There was no differences between the values from *ACCU-CHEK®Aviva* and *COBAS®6000* (P=0.095), in opposition to *SYNCHRON LX®20 PRO* (P=0.001) and *Dimension Vista®1500* (P=0.000). The mean differences between ACCU-CHEK®Aviva and the laboratory equipments were -2.30±7.397; −11.42±9.31; 3.66±7.246 (mg/dL; mean ±sd), respectively for *COBAS®6000*, *Dimension Vista®1500* and *SYNCHRON LX ®20 PR.* Bland and Altman analysis show low agreement for values obtained for all equipments and the clinical differences were 4% (2) for *COBAS®6000* and 6% (3) for *SYNCHRON LX ®20 PR* and *Dimension Vista®1500*. The results of glucose >75 mg/dL were evaluated globally according to ISO 15197:2003, noting that 96% is within the range of ±20%. Assessing individually, only *Dimension Vista®1500* didn't achieved these criteria (88%).
Conclusions. *ACCU-CHEK®Aviva* is reliable in glycemic control, although there are limitations, such as concealment of hypoglycemia and hyperglycemia cases.

0389

VITAMIN D BINDING PROTEIN GENE POLYMORPHISMS AND RISK OFTYPE 1 DIABETES MELLITUS AMONG EGYPTIANS

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Background. Type 1 diabetes mellitus (T1D) is an autoimmune disease and environmental factors contribute to its development. Studies in vitro have shown that the biologically active form of vitamin Dis modulator of the immune system. Active vitamin D prevents T1D in animal models and its supplementation was associated with a decreased risk of the disease. The vitamin D binding protein (DBP), formerly known as group-specific component of serum (Gc-globulin) is the major plasma carrier protein of vitamin D. Two frequent polymorphisms, in exon 11 of the DBP gene, result in amino acid variants: GATàGAG substitution replaces aspartic acid by glutamic acid in codon 416; and ACG>AAG substitution in codon 420 leads to an exchange of threonine for lysine. These DBP variants lead to differences in the affinity for vitamin D. Few published studies, about the correlation between DBP alleles and T1D, yielded conflicting Results.Therefore,we investigated the association of these polymorphisms with T1D in Egyptian subjects.
Methods. Unrelated type 1 diabetice patients and healthy controls were examined for polymorphisms of DBP gene using polymerase chain reaction-restriction fragment length polymorphism(PCR-RFLP). Differences in distributions between the two groups were examined by chi square test.
Results. Allele frequencies at both codons did not differ in T1D patients and in control subjects (P> 0.05). Distributions of genotypes at both loci, and the common haplotypes constructed by them, were also very similar in both groups.
Conclusions. DNA polymorphisms in the DBP gene are not associated with T1D in Egyptian patients.

Diabetes mellitus and metabolic syndrome

0390

GENDER DIFFERENCES IN AST AND GGT ENZYME ACTIVITY IN DM 2 AND PREDIABETES PATIENTS WITH RESPECT TO DEFINED CUTT OFF VALUE OF HBA1C

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Background. Hemoglobin A1c is recognized as highly relevant diagnostic tool in defining patients with diabetes risk as well as in monitoring those already diagnosed with diabetes. Although, strong association of activity of liver enzymes, such as AST (aspartate amino transferase) and GGT (gamma glutamyl transpeptidase) and Type 2 diabetes mellitus (T2DM) and prediabetes is suggested in numerous studies, results related to relevance of continuous monitoring of those markers in progression of prediabetes to T2DM , especially wit respect to HbA1c values and gender are lacking. .
Methods. Activities of ,AST,GGT, glycosylated hemoglobin (HbA1c), and fasting plasma glucose (FPG) were determined in 40 T2DM patients, 40 prediabetes patients and 40 age-matched controls. Blood samples were collected from all participants in 3 regular 2-months intervals . All study participants were free of evidence of hepatitis, viral infection, or active liver and kidney damage. All biochemical analyses were performed on BT plus 2000 and Vitros 350 Chemistry systems.
Results. We observed a significant increase of AST activity (within reference limits) in patients wit diabetes mellitus type 2 in all examined periods . Interestingly, a significant correlation was obtained between AST activity and HbA1c levels in male, but not female diabetic patients.Gender differences were evident at the level of GGT,but not AST activity and HbA1c in prediabetic patients.
Conclusions. Close monitoring of AST activity is suggested for male diabetic population, while in male prediabetic population, monitoring of GGT activity is emphasized.

0391

RELATIONSHIP BETWEEN GAMMA-GLUTAMYLTRANSPEPTIDASE, URIC ACID, ANTHROPOMETRIC AND METABOLIC PARAMETERS IN YOUNG WOMEN WITH EXCESSIVE BODY WEIGHT

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Background. The mechanism of relationship between obesity, insulin resistance, gamma-glutamyltranspeptidase activity (GGTP) and uric acid (UA) concentration has not been fully clarified. We investigated the association between GGTP, UA, anthropometry and components of metabolic syndrome in young overweight and obese women.
Methods. GGTP, UA, fasting glucose, insulin and lipids were determined (ARCHITECT ci8200) in blood samples obtained from overweight and obese (n=98;BMI≥25kg/m^2) women aged 25-40 yrs and age-matched healthy controls (n=39; BMI<25kg/m^2). Anthropometric measurements and blood pressure (BP) were determined. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR). In 59 subjects metabolic syndrome (MetS) was diagnosed (IntlDiabFederation 2005).
Results. GGTP was elevated (>39 U/L) in 13% women with excessive BMI and 22% with MetS but UA was elevated (>6 mg/dL) in 10% of them. Median GGTP (17U/L) and UA (4,4 mg/dL) were higher in overweight and obese compared to controls (10 U/L and 3,8mg/dL; p<0,0002) whereas in women with MetS compared to these without only GGTP was higher (20U/L vs 13U/L; p<0,00001). In obese and women with MetS, GGTP positively correlated with waist circumference, systolic and diastolic BP, insulin and HOMA-IR. Moreover, GGTP was an independent predictor of diastolic BP and HOMA-IR (β=0,35; p= 0,002 and β= 0,23; p= 0,04) in obese and also an independent predictor of diastolic BP in MetS (β= 0,30; p= 0,02).
Conclusions. GGTP activity, but not UA concentration, seems to be related with insulin resistance and diastolic BP, essential components of metabolic syndrome in young women.

Diabetes mellitus and metabolic syndrome

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PROINSULIN IN NON-INSULIN DEPENDENT DIABETES MELLITUS

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Background. Proinsulin, a polypeptide of 86 amino acids is synthesized in the β cells of the Islets of Langerhans in the pancreas. This protein is processed to C-peptide and Insulin forms. Both are secreted in equimolar amounts into the blood. The level of proinsulin in serum can be a reflection of β cell status and a consequence of dysfunction of proinsulin processing and/or secretion. Proinsulin is the precursor of insulin, which is the principal hormone responsible for the control of glucose metabolism.
Methods. The assay employs the quantitative sandwich enzyme immunoassay technique. During the thirst incubation is added monoclonal anti-human proinsulin antibody specific for the epitope at the C-peptide/Insulin chain junction (which bides "des (31.32)"-proinsulin and "split (32.33)"-proinsulin, but not insulin. Then, a horseradish peroxidase labeled anti-human proinsulin antibody specific for the epitopes at insulin β chain/C-peptide junction is added into the well (which bides "des (64.65)"-proinsulin, but not insulin and C-peptide). The enzymatic reaction provides a color reaction which is proportional to the concentration of human proinsulin.
Results. For a period of 4 months we investigate 30 patients; 18 women and 12 men; the age was between 30 and 60. 26 of them were with non-insulin dependent diabetes with high levels of proinsulin. 1 patient was diagnosed with insulinoma. Normal ranges for non obese fasting patients are 1.28 – 3.84 pmol/l.
Conclusions. Serum levels of proinsulin provide useful valuable information for the diagnosis of insulinomas and in non-insulin dependent diabetics.

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CYTOKINES IL-2 AND IL-6 IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

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Background. Type 1 diabetes mellitus (DM) is a chronic autoimmune disease characterized by destruction of the pancreatic islets of Langerhans. Proinflammatory cytokines may be involved and play important role in the pathogenesis. The aim of this study was to determine serum concentrations of IL-2 and IL-6 in children with type 1 DM and healthy controls.
Methods. Thirty-five children with type 1 DM, and 22 healthy controls were recruited for this study. Diabetic children were divided into: Group 1 - diagnosed within the last 18 months, Group 2 - with long standing DM. Concentration of IL-2 was measured using commercially available Human IL-2 Instant enzyme-linked immunosorbent assay kit (Bender MedSystems GmbH, Austria). Concentration of IL-6 was measured using commercially available Elecsys IL-6 assay kit (Roche Diagnostics GmbH, Germany) with 7 pg/ml determined as a reference range.
Results. Mean age and age ranges were: group 1 (11.4±4.3 years) (4.1-17.8 years), group 2 (9.7±3.7) (2.9-16.7), and control group (10.4±4.5) (1.9-17.5). Mean diabetes duration was 0.7±0.4 years in group 1, and 4.5±2.6 in group 2. In all groups detected levels of IL-6 were within reference range. Mean IL-2 levels were 2.2±3.7 pg/ml in group 1, 0.9±1.9 in group 2, and 1.2±2.6 in control group. The difference was not statistically significant.
Conclusions. This study hasn't determined the significantly different levels of IL-2 and IL-6 among children with type 1 DM and healthy controls. Since the results of other studies are different and there is no unique conclusion, further studies with larger study groups are warranted.

Diabetes mellitus and metabolic syndrome

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URINE MICROALBUMIN, GLYCOSILATED HAEMOGLOBIN, ORAL ANTIDIABETICS AND OTHERS VARIABLES ON THE CONTROL OF TYPE 2 DIABETES MELLITUS

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Background. The control of diabetes is based on the use of appropriate analytical measurements andpharmacological treatment according to the pathways.
Hypothesis: Inappropriate use of resources can lead to poor control of the disease.
Aim: To establish the influence of the PAIDiabetes variables on the control of type 2 Diabetes Mellitus (DM2).
Methods. Cross-sectional descriptive study.
Population: Patients with DM2 in 2009 in a Health Area (N = 3267).
Variables: Dependent: Last glycosylated haemoglobin ((HbA1c) measurement. Independent: number of HbA1c in the last year, age, sex, treatment, use of reagent strips, number of urine microalbumin (UMA) determinations.
Analysis: Binary logistic regression (bivariate and multivariate). Linearity analysis, Wald (significance) and Hosmer-Lemeshow test (validity).
Results. Reference variable considered: poor blood glucose control. The non-measurement of UMA favoured poor control (OR=2.01 P<0.001). Every extra HbA1c measurement did not favour blood glucose control (OR=0.66 P<0.001)
Combined insulin+oral antidiabetic treatment (OR=13,47 P<0.001) led to better blood glucose control than only insulin (OR=10.69 P<0.001) or oral antidiabetics (OR=2.87 P<0.001). Self blood glucose measurement was associated with control (OR=1.23 P<0.048). Sex and age was not significant. Nagelkerke R2 = 0.227. Hosmer-Lemeshow Test P<0.894.
Conclusions. The model explained 22.7% of the established objective. The three factors that contributed to better diabetes control are, combined treatment, UMA determination, and the use of reagent strips. Despite its usefulness, the HbA1c data may be explained by the poorly controlled patients have more determinations; this being the result and not the cause of uncontrolled blood glucose.

0395

DEVELOPMENT AND EVALUATION OF THE PERFORMANCE CHARACTERISTICS OF A NEW IMMUNOTURBIDIMETRIC ASSAY FOR HBA$_{1c}$ ON BECKMAN COULTER AU® CLINICAL CHEMISTRY SYSTEMS*

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Background. HbA$_{1c}$ is used for long-term assessment of diabetic glycaemic control. In response to increasingly stringent international recommendations for standardisation and accuracy, Beckman Coulter is currently developing a next generation HbA$_{1c}$ assay. The performance of this assay was evaluated on AU Clinical Chemistry systems.   .
Methods. HbA$_{1c}$ concentration, relative to that of total haemoglobin(THb), was determined using an immunoturbidimetric inhibition assay for HbA$_{1c}$ and colorimetric measurement of THb. The assay is standardised to the IFCC reference method.
Results. In development studies, the HbA$_{1c}$ assay demonstrated within-run imprecision on the AU680 analyser of < 1.5 %CV at concentrations of 5.2%, 7.3% and 10.0% HbA$_{1c}$ respectively. Method comparison versus the current AU HbA$_{1c}$ assay, OSR6192, showed r = 0.998, slope = 1.02 and intercept = -0.55 mmol/mol HbA$_{1c}$ (NGSP samples; n=40; range 51 – 110 mmol/mol).
No significant interferences were observed from bilirubin, Intralipid**(Kabivitrum Inc.), and ascorbate up to concentrations of 30 mg/dL, 500 mg/dL, and 50 mg/dL, respectively. Calibration and on-board reagent stabilities were 14 days and > 14 days respectively. NGSP and CAP sample testing met the required criteria (NGSP: 95% CI of the differences between the test and SRL method within ±0.75%HbA$_{1c}$; CAP: recovery within ±6% of NGSP target).
Conclusions. In development studies, the next generation HbA$_{1c}$ assay provided a rapid, accurate and convenient means of measuring HbA$_{1c}$ in human whole blood on Beckman Coulter AU Clinical Chemistry Systems.
* Assay currently under development and not for clinical use
**All trademarks are property of their respective owners

Diabetes mellitus and metabolic syndrome

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HYPERHOMOCYSTEINEMIA AND LIPID PEROXYDATION IN HYPOTHYROIDISM

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Background. Hypothyroidism is a clinical entity resulting from the deficiency of thyroid hormone. It has been linked to an increase risk of atherosclerosis.
The purpose of this study was to determine Thiobarbituric Acid Reactive Substances (TBARS), and homocystein levels in hypothyroidism treated patients
Methods. The study population included 27 hypothyroidism patients treated with Levothyroxine® (mean age 42± 10 years) and 25 healthy controls subjects (mean age 44 ±11 years).
The homocystenemia (Hcy) was determined by fluorescent polarization immunoassay (Axsym - Abbott). The Thiobarbituric Acid Reactive Substances (TBARS) was determined by fluorimetric assay (Yagi method).
Results. Compared to healthy control subjects, treated patients had showed a decrease of TBARS, and the difference was statistically significant (1.44 ±0.74 μmol/L VS 1.99 ±0.41 μmol/L; p≤ 0.05).
Whereas Hcy levels were higher in hypothyroidism treated patients than in the control and the difference was statistically significant (14.76 ±1.19 μmol/L VS 10.08 ±1.87 μmol/L; p≤ 0.05)
Conclusions. Our finding suggests that the Thyroid hormone supplementation will protect against lipid peroxidation. However, hyperhomocysteinemia was not corrected and may have a role in the pathogenesis of atherosclerosis in hypothyroidism patients.

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THE EFFECTS OF CUMINUM CYMINUM L COMPARED TO SIBUTRAMINE ON THE WEIGHT, SERUM LEPTIN, GLUCOSE AND LIPIDS IN RAT

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Background. Diabetes is one of most popular metabolic disease in the world. It contains 6.6% of world population and about of 3 million individuals in Iran. Nowadays the chemical and herbal medicines prescribed to cure obesity. In Iran, Cumin (Cuminum Cyminum L.) is a plant used in traditional medicine to cure obesity, and some of the new studies suggested that Cumin has a role in diabetes treatment also reducing lipids level.
Objectives: In this study, we investigate the Cumin oil and sibutramine effect on the prevention of the weight gain and the level of serum leptin, glucose, and lipids in rat.
Materials and Methods. We divided 36 male rats of Wistar race into 3 hexadic groups: the control group with normal regiment , the Cumin oil group with normal regiment, the sibutramine group with normal regiment. The consumed dosages of Cumin oil and sibutramine were 400 μg/kg and 3mg/kg respectively which was given to the rats by Gavage (tube feeding). In this study, we took samples of the hungry rats during three various periods including the first day of the study, 20th day (the beginning of the medicine usage) and 55th day (the end of the medicine usage) in order to measure their glucose and serum lipids.
Results. The results of this study indicated that a significant decreasing in glucose cholesterol triglyceride (LDL (p<0.001) and a significant increasing in serum HDL (p=0.05). Both drug prevented weight gain at the end of study(P=0.05).
Conclusions. The findings indicate that cumin oil like sibutramine via consumption of Gavages can affect the serum glucose and lipids in rate and also prevent weight gain.

Diabetes mellitus and metabolic syndrome

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ASSOCIATION OF THE *HINDIII* AND S447X POLYMORPHISMS IN LPL GENE WITH HYPERTENSION AND DIABETES IN MEXICAN FAMILIES

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Background. Several studies in families have demonstrated that genetic component play a central role to the development of the comorbidities associated to obesity, such as dyslipidemias, hypertension, type 2 diabetes (T2DM) and cardiovascular disease. Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism and is associated with dyslipidemias. LPL gene polymorphisms can be related with the development of cardiovascular risk factors.
Aim. Analyze the association of the *HindIII* and S447X polymorphisms in LPL gene with comorbidities in families with obesity.
Methods. Ninety members of 30 Mexican families, in which an index case had obesity, were included in the study. The families were integrated by case-parents trios.We evaluated the body composition by bioelectrical impedance. Peripheral blood samples were collected to determine biochemical parameters. Screening for *HindIII* and S447X LPL polymorphisms was performed by PCR-RFLPs.
Results. The genotype frequencies of *HindIII* polymorphism were 57.8% TT, 40% TG, and 2.2% GG. For the S447X polymorphism, the frequencies were 80% CC, 20% CG and 0% GG. In the parents, both polymorphisms were in Hardy-Weinberg's equilibrium. The genotype TT of *HindIII* was associated with diastolic blood pressure ≥ 85 mmHg (OR=1.1; p=0.011), whereas the genotype CC of S447X was associated with systolic blood pressure ≥ 130 mmHg (OR=1.2; p<0.001), diastolic blood pressure ≥ 85 mmHg (OR=1.3; p<0.001), T2DM (OR=1.3; p<0.001) and with increase of total cholesterol (β=23.6 mg/mL; *p*=0.03).
Conclusions. The *HindIII* and S447X LPL gene polymorphisms can confer susceptibility for the development of hypertension and T2DM in Mexican families.

0399

THE RELATIONSHIP OF ADIPONECTIN, FIBROBLAST GROWTH FACTOR 21 AND ADIPOCYTE FATTY ACID BINDING PROTEIN LEVELS TO DYSLIPIDEMIC PHENOTYPES- PILOT STUDY

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Background. Adipose tissue is an important place of many metabolic and inflammatory processes. Adipokines are considered the mediators of these pathways. The aim of our study was to evaluate the relationship between adiponectin, FGF 21 and A-FABP levels and dyslipidemic phenotypes defined on the basis of concentrations of triglycerides and apolipoprotein B.
Methods. 119 dyslipidemic patients were divided to 4 groups according to Sniderman criteria (DLP1-DLP4). Serum biochemical parameters were measured on Modular analytical system (Roche). The levels of adipokines were analyzed by Elisa methods (Bio Vendor) .
Results. The highest levels of adiponectin were observed in DLP1 (10.6 ±6.0 mg/l, n.s.). On the contrary, FGF 21 and A-FABP were significantly increased in the groups with the most important atherogenic potecial, DLP2 and DLP4 (FGF 21: 333.3±359.5 ng/l and 384.4±347.7 ng/l, A-FABP: 33,9±29.0 µg/l and 29.2±18.4 µg/l), in comparison to DLP1 and DLP3 (p<0.01). These two parameters correlated with higher levels of triglycerides, fasting glucose, BMI and lower HDL cholesterol, both in DLP2 and DLP4.
Conclusions. In accordance with recent literature we observed higher levels of FGF 21 and A-FABP and their correlation with other risk factors of atherosclerosis in the groups with triglycerides above 1.5 mmol/l. The increase of FGF 21 concentrations are probably due to the compensatory response to higher A-FABP, that is considered the predictor of metabolic syndrome.

Diabetes mellitus and metabolic syndrome

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HBA1C AND BLOOD SUGAR LEVELS IN KNOWN DIABETICS

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Background. Interest in Glycated haemoglobin(HbA1c) as a monitoring and diagnostic tool in diabetes mellitus is rapid increase in theglobal prevalence of diabetes mellitus. This study aimed to determine the significance of the HbA1c in known diabetics (KD) and the correlation in the levels of HbA1c established to those of random blood sugar (RBS).
Methods. The sample from KD and the control group were analysed for HbA1c using the Micromat II®. RBS was determined using glucose meter and haemoglobin (Hb) estimation.
Results. The mean age for the KD group (n=91) was $44.3^{+/-11.7}$), median 47.0 with a range of 18-65 years. The mean HbA1c for KD was 8.6% $^{+/-2.5}$ (70 mmol/mol$^{+/- 15}$), median 8.2% (66 mmol/mol) with a range 5.0-16.0% (31-151mmol/mol). Correlation between HbA1c and RBS (r=0.64, p<0.001) in the KD and no correlation was observed between HbA1c and all the other studied parameters.The mean for the control group for HbA1c is 5.6$^{+/-0.4}$, the control group as 4.5-6.3% (26-45mmol/mol).Agreement of the mean HbA1c for the KD with an earlier study done at the same centre.
Conclusions. We conclude that there is a correlation significance between HbA1c and random blood sugar that there is poor management of diabetes mellitus. We recommend that HbA1c be done periodically in all KD and further research is recommended.

0401

INDICES OF METABOLIC SYNDROME IN 755 APPARENTLY HEALTHY INDIVIDUALS IN NIGERIA

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Background. Metabolic Syndrome (MS), recently observed as a pre-diabetic phase increases the risk of developing cardiovascular diseases, type-2 diabetes and hypogonadism. This study aims at identifying indices of MS in Nigerians in an attempt to provide novel approaches to preventing and managing non-communicable diseases.
Methods. 755 apparently healthy participants were recruited into this prospective cohort study of traders in a local market in Bodija, Ibadan, Nigeria. Demographic, anthropometric characteristics (weight, height, body mass index, %body fat, waist and hip circumferences and their ratio), blood pressure were obtained from questionnaires and use of standard Methods. Blood samples (5ml) were obtained for the determination of glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) by enzymatic methods while low density lipoprotein cholesterol (LDL-C) was calculated. SPSS software 16.0 was used for statistical analysis.
Results. Results show that more than half of the subjects were overweight/obese (n=564) while 17 and174 were underweight and normal weight respectively. Age, anthropometric indices and systolic blood pressure readings were significantly different between groups (p<0.05). Fasting blood glucose, total cholesterol, triglyceride but not diastolic blood pressure, HDL-C and LDL-C were significantly different between groups (p<0.05). All indices increased from underweight to obesity except fasting blood glucose that was highest in underweight subjects.
Conclusions. Non-communicable diseases like cardiovascular diseases, type-2 diabetes, sexual and reproductive dysfunctions will increase if lifestyles are not checked and may pose a challenge to the health system in Nigeria, Africa's most populous country which is still considered as a poor resource setting.

Diabetes mellitus and metabolic syndrome

0402

THE DIAZYME DIRECT ENZYMATIC HBA1C ASSAY MODIFIED FOR A MICROPLATE READER AT ROOM TEMPERATURE

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Background. Hemoglobin A1c (HbA1c) measurement is a key diagnosticcriterion and a key parameter for the follow-up of the treatment of diabetes mellitus. Typically, immunochemical assays of HbA1c are carried out in clinical chemistry analysers. In this study, we applied the HbA1c assay on a microplate reader at room temperature.

Methods. HbA1c samples were measured using the Direct Enzymatic HbA1c Assay™ of Diazyme Laboratories (Poway, CA, USA) by aPlate Chameleon™ V Microplate Reader (Hidex Co., Turku, Finland) according to the manufacturer's protocol at +37ºC (1) and with a modification of the method at room temperature (+22ºC). The Tosoh HLC®-723G7 HPLC method for HbA1c (Tosoh Co., Tokyo, Japan) was used as a comparative method. Both methods are traceable to the IFCC Reference Measurement Procedures. The comparisons between the results were performed by using the MedCalc® statistical software.

Results. There was **a** good correlationin HbA1c results when the assay was performed at room temperature (+22ºC) compared with that at +37ºC (r =0.987). The modified method was linear over the HbA1c range of 4 – 14 %. Analysis of HbA1c results from 50 blood samples by the modified method gave good agreement with HPLC method as %-results (r = 0.990) or as mmol/mol results (r = 0.970).

Conclusions. The modified Diazyme Direct Enzymatic HbA1c Assay™ appears to work as well as at +22°C than at 37C (performed according to manufacturer's protocol).

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0403

DIS-COORDINATED ACTIVATION OF *DE NOVO* LIPOGENESIS AND STEAROYL-COA DESATURASE BY MONOSACCHARIDES DETERMINES LIVER FAT ACCUMULATION IN HUMANS

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Background. Increased hepatic *de novo* lipogenesis of fatty acids has been implicated in the pathophysiology of fatty liver, potentially due to lipotoxic effects of the generated saturated fatty acids. Activation of fatty acid desaturation by stearoyl-CoA desaturase (SCD1) in parallel with lipogenesis may prevent lipotoxic effects and protect from hepatic steatosis.

Methods. 20 healthy subjects (female/male 8/12, age 30.5 ± 2.0 years, BMI 25.9 ± 0.5 kg/m²) received a 4-week hypercaloric diet supplemented with 150 g/day of monosaccharides. Hepatic SCD1-activity and de novo lipogenesis (DNL) was determined using established markers (C16:1/C16:0 and C16:0/C18:2-ratios) in VLDL-triglycerides. Liver fat content was measured by localized [1]HMR spectroscopy.

Results. Liver fat content (+33%, p=0.04) and DNL (+19% p=0.04) and hepatic SCD1-activity (+8% p=0.12) increased after the hypercaloric diet. Changes in liver fat content and DNL closely correlated (R=0.75; p=0.0001). No correlation was observed between SCD1-activity and DNL or changes thereof during the intervention. High hepatic SCD1-activity was associated with low liver fat content after the hypercaloric diet (R=-0.63, p=0.002). Interestingly, high SCD1-activity after the intervention correlated even closer with liver fat content at baseline (R=-0.79, p<0.0001). No associations of baseline SCD1 activity with liver fat could be observed.

Conclusions. Our results support the relevance of DNL for the development of hepatic steatosis. However, we have no evidence for a parallel activation of SCD1 and DNL on an individual level. The data rather suggest that the individual induction/inducebility of hepatic SCD1-activity may be a determinant of liver fat accumulation.

Diabetes mellitus and metabolic syndrome

0404

A TWO METHOD COMPARISON FOR THE DETERMINATION OF GLYCOSYLATED HEMOGLOBIN (HBA1C)

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Background. Besides the glucose determination during a standard glycemic check-up, glycosylated hemoglobin (HBA1c) is used as a marker, in order to evaluate the long-term regulation of the glucose levels in patients with diabetes. The aim of this study is to compare two methods; High Pressure Liquid Chromatography (HPLC) and Turbidimetry for the HBA1c determination.
Methods. 167 samples were collected and tested using the automatic analyzer HPLC VARIANT (Biorad), following the manufacturer's instructions and the laboratory's accredited methods, and the COBAS INTEGRA 800 analyzer (Roche) with the COBAS INTEGRA Tina-quant Hemoglobin 1Ac Gen.2 reagent system. Acetylated and carbamylated Hb are not known to interfere with the test Results.
The data were statistically analysed using linear regression, to evaluate the slope, the y axis intersection and the correlation factor.
Results. The statistic analysis of the results from both methods showed a very good linearity (y=0.9489x+0.5146, R^2=0.9864) and an excellent correlation (r=0.993, p<0.001).
HBA1c values ranged from 4.1% to 14.1% since we wanted to have a representative group of samples with wide range in order to include the majority of cases.
Conclusions. Comparing the two methods we conclude that they have almost identical results and therefore both can be used in clinical laboratory routine with the same reliability.

0405

RISK FACTORS FOR CORONARY HEART DISEASE IN NON-INSULIN-DEPENDENT-DIABETICS

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Background. Coronary heart disease is found to be more frequent in patient with non-insulin-dependent-diabetes mellitus. There are metabolic factors might contribute to onset of CHD in non insulin diabetics. We focused in level of insulin and the disturbances in lipoprotein metabolism.
Methods. We choose 20 patients with NIDDM and with CHD and 20 NIDDM patients without CHD, 20 nondiabetic patients with CHD and 20 healthy controls. We measured insulin levels, total cholesterol,HDL-ch,LDL-ch and triglicerdes.Insulin were determinate with chemiluminiscent immunoassay and cholesrerol, HDL-ch,LDL-ch and triglycerides with standard metods.
Results. The insulin level in NIDDM patients with CHD were significantly higher compared to diabetics without CHD(p<0,01) and nondiabetic patient with CHD compared to healthy controls(p<0,01).Insulin levels in both NIDDM patients groups with and without CHD were significantly higher than in healthy controls(p<0,001).The levels of total cholesterol and LDL-ch in NDDM patients with CHD were higher compared to NIDDM patients without CHD (p<0,01) and in nondiabetic patients with CHD compared to healthy controls(p<0,05).The HDL-ch levels in NDDM patients with CHD were significantly lower compared to NIDDM patient without CHD(p<0,001) and in nondiabetic patients with CHD compared to healthy controls(p<0,001).The triglycerides levels in both NIDDM patients groups with and without CHD and in nondiabetic patient with CHD were significantly higher than in healthy controls(p<0,01).
Conclusions. This data suggest thatmonitroring of plasma insulin levels,lipoprotein subfractions,especially HDL-ch is very important for detecting the individuals at high risk for development of CHD among NDDM patients.This mightbe reliable basis for implementation of therapeutic intervention trials in an attempt to prevent the appearance of CHD.

Diabetes mellitus and metabolic syndrome

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DIFFERENCES BETWEEN THE CKD-EPI AND THE MDRD EQUATIONS WHEN ESTIMATING THE GLOMERULAR FILTRATION RATE IN DIABETIC PATIENTS

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Background. The inadequacies and limitations of creatinine clearance as a marker to monitor kidney function are well described. We investigated GFR in diabetic patients with MDRD-IDMS and CKD-EPI equations.
Methods. Creatinine measurements were made on Olympus AU600, by the spectrophotometric Jaffé method, traceable to IDMS method with CRM 967 and 909b.
Results. Depending on the eGFR, patients (n=954; age: 19 – 92 (median age 59); male: n=502 (52,6%); both types of diabetes) were divided into 5 stages of chronic kidney disease (CKD) with the biggest difference in stage 1: MDRD (n=87; 9,1%) and CKD-EPI (n=195; 20,4%). Kappa index showed fair (0,546), good (0,643), very good (0,852) and excellent agreement (0,971) between MDRD and CKD-EPI for stages 1, 2, 3 and 4, respectively. Better agreement was observed when equations were compared in male patients (kappa indeks: stage 1 – 0,668, stage 2 – 0,728, stage 3 – 0,899, stage 4 – 1,000) and in patients older than 65, regardless of gender (kappa indeks: stage 2 – 0,913, stage 3 – 0,929, stage 4 – 0,951) . Reference change value was 14,0% both for MDRD and CKD-EPI eGFR. Individuality index was 0,24 for MDRD and 0,25 for CKD-EPI.
Conclusions. CKD-EPI equation estimatedhigher GFR in diabetic patients and reclassified 108 MDRD-classified stage 2 patients (11,3%) into stage 1 CKD. However, very good and excellent agreement was observed in more advanced stages of CKD. We conclude that both equations are suitable for longitudinal observation of kidney function in diabetic patients, but shouldn't be used interchangeably.

0407

DISTRIBUTION OF HSCRP AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTOR VARIABLES OF THE METABOLIC SYNDROME IN ADOLESCENT LEARNERS FROM THE WESTERN CAPE, SOUTH AFRICA

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Background. Metabolic syndrome (MetS) and its associated cardiovascular risk are on the increase in children. High-sensitivity C-reactive protein (hs-CRP) has emerged to be a useful marker for inflammation associated with atherosclerosis and cardiovascular disease. Our aim was to determine the distribution of hs-CRP and in an effort to identify the MetS variable that is critical in modulating plasma CRP levels in a population of South African adolescents.
Methods. Anthropometric variables, blood pressure, fasting blood glucose and lipids were performed on 324 consenting learners aged 15-18 year old from 3 different ethnic groups (black, white and mixed ancestry). The NCEP ATP III for ages 15-18 year olds was used to define the MetS.
Results. The prevalence of MetS and obesity was 3.7% and 7.1%, respectively. hs-CRP levels were significantly higher in subjects with a waist-circumference > 90th percentile (P <0.01) and obese learners with the MetS and was lower in those with MetS and normal weight. Median hs-CRP levels increased with increasing number of metabolic abnormalities and exceeded 3 mg/ l in 19 % of adolescents. Gender and ethnic differences were observed.
Conclusions. Our findings suggest that obesity and waist circumference appear to be major mediators of hs-CRP levels in South African adolescents.

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SENIORLABOR – LABORATORY ANALYSIS ON PERIPHERAL BLOOD OF THE ELDERLY EXEMPLIFIED BY PREDIABETES AND DIABETES

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Background. A study (www.seniorlabor.ch) initated in 2008 (KEK-Berne-Study No.166/08) will perform 82 different analyses to be compared to normal ranges provided by the automation industry.
Methods. Fasting glucose (FG): in fluoride plasma (FGP) by hexokinase method on Roche Integra 800; HbA1c: in EDTA whole blood by HPLC (Biorad). ADA january 2010 cut-offs for diagnosis of prediabetes (PreDM): HbA1c 5.7-6.4%, FGP 5.6-6.9 mmol/L, and diabetes mellitus (DM): HbA1c $\geq$6.4%, FGP $\geq$7.0 mmol/L, were applied.
Results. A total of 899 subjects (378 male/521 female, age-range 60-96) were studied. Crude prevalence of PreDM was 65.18% [95% CI 62.01, 68.23] (586/899), whereas prevalence of unknown DM was 7.9% [95% CI 6.31, 9.85]. Of the 638 individuals with normal FGP (71 % [95% CI 67.91,73,84]), 242 individuals (38%) had a normal HbA1c. 385 of the 638 (60% [95% CI 56.5, 64,07] had a PreDM HbA1c, and 11 (1.7%) had a DM HbA1c. Those with a PreDM FGP (n=233, 26%), 41 (18%) had a normal HbA1c, but 160 (69%) had a PreDM HbA1c. Of the elderlies with diabetic FGP (n=28, 3.11%), two had a normal HbA1c, 4 a PreDM HbA1c and 22 diabetes confirmatory levels of HbA1c. In the persons with DM (n=71), screening with FPG alone leaves 43 (61% [48.89, 71.12]) and screening with HbA1c alone leaves 6 (8.45% [4.0, 17.26]) undetected.
Conclusions. Thus, introducing HbA1c in addition to FPG as a diagnostic marker for DM here increased the disease prevalence among senior citizens by 253% (71 vs. 28 cases).

0409

ROLE OF ASYMMETRIC DIMETHYL ARGININE (ADMA) AS A MARKER FOR MICROVASCULAR COMPLICATIONS IN PATIENTS WITH TYPE II DIABETES MELLITUS

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Background. The Correlation of ADMA levels and the occurrence of microvascualr complications among patients with type II diabetes mellitus was suspected to be a result of hyperglycemia induced oxidative stress which impairs DDAH activity
Methods. ADMA in μmol/l was measured quantitatively by enzyme linked immunoassay technique.
Results. The present study was carried on 70 individuals classified into 3 groups :Group I included 45 patients of type II diabetes mellitus associated with one or more of the three main microvascular complications which are nephropathy, neuropathy and retinopathy. Group II included 15 patients of type II diabetes mellitus without microvascular complications .Group III was the Control group ,A statistically very highly significant difference of plasma ADMA level was found between the three groups (P value=0.00). Plasma ADMA showed statistically very highly significant higher mean value in diabetic patients with microvascular complications (mean:0.692µmol/l) when compared to both groups; diabetic patients without microvascular complications (mean=0.364µmol/l) and healthy control subjects(mean=0.108µmol/l),and also, very highly significant higher mean value in uncomplicated diabetics when compared to healthy controls, which means that ADMA level was highest in type II diabetic patients with microvascular complications, intermediate in those without complications, and lowest among healthy controls.
Conclusions. These results suggested that hyperglycemia induced oxidative stress impairs DDAH activity, leading to elevation of ADMA and inhibition of endothelium derived synthesis of NO with subsequent endothelial vasodilator dysfunction So, ADMA may play an important role as a marker of type II diabetes and its main three microvascular complications; retinopathy, neuropathy, and nephropathy.

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MULTICENTER EVALUATION OF A NEW HBA1C GEN. 3 ASSAY AND LIQUID HBA1CQUALITY CONTROLS ON ROCHE/ HITACHI, COBAS INTEGRA®AND COBAS C SYSTEMS

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Background. The present abstract describes the analytical performance of a new HbA1c assay (Tina-quant® [a] HbA1c Gen. 3, Roche Diagnostics GmbH, Mannheim, Germany) with applications on RD/MODULAR P, COBAS INTEGRA® 800 and *cobas c* 501 systems.
Methods. Total Hb is measured bichromatically during the pre-incubation phase of the immunological HbA1c determination without a separate colorimetric reagent. The method is based on the turbidimetric inhibition immunoassay principle and is standardized against the IFCC reference method.
Results. Recovery of assigned IFCC and CAP samples (n=10) was found within a range of 100 ± 9.8% HbA1c. Repeatability (%CV) ranged from 0.5 – 2.5 (Median 1.1) for concentrations between 5.1 – 10.9 % HbA1c using new liquid ready to use quality control materials PreciControl HbA1c norm and path. CV's calculated from daily routine simulation experiments (cobas c 501, n=20 days) using 2000 aliquots without weekly cleaning solution and 4000 aliquots with weekly cleaning solution gave comparable results (0.9 – 3.0 % and 1.1 – 1.7 % respectively). The primary measuring range, which was defined by linearity testing, was confirmed from 2.48 to 24.8 mmol/L Hb and 0.186 – 1.55 mmol/L HbA1c (recovery rate: 100 ± 10%). Statistical Passing/Bablok analysis of method comparison against Tina-quant® [a] HbA1c Gen. 2 and Biorad HbA1c yielded correlation coefficients > 0.96, slopes between 0.94 – 1.10 and intercepts from - 0.64 to + 0.48 % HbA1c using ≥ 122 whole blood samples.
Conclusions. The new HbA1c Gen. 3 assay demonstrated highly reliable analytical performance, good correlation with existing HbA1c methods and convenient reagent handling.

0411

METABOLIC SYNDROME, HIGH-SENSITIVITY C-REACTIVE PROTEIN AND CARDIOVASCULAR RISK

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Background. Metabolic syndrome is defined as clustering of multiple metabolic risk factors - obesity, dyslipidemia, hypertension, abnormal glucose, increased levels of C-reactive protein that increase the risk of cardiovascular diseases, diabetes mellitus and all-cause mortality.
Methods. 122 patients with metabolic syndrome were investigated. Following parameters were taken into consideration and discussed: fasting glycemia, postprandial glycemia, glycated hemoglobin, immunoreactive insulin, HOMA-index, high-sensitivity C-reactive protein, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, body mass index, blood pressure.
Results. The most common components of metabolic syndrome for women were abdominal obesity and elevated blood pressure, whereas for men there were elevated blood pressure and high triglyceride levels. A higher percentage of women had abdominal obesity and low HDL-cholesterol levels, whereas a higher percentage of men had high triglyceride levels and abnormal glucose metabolism. The odds of having elevated high-sensitivity C-reactive protein levels were ≈ 3 times higher in participants than in health controls. Mean C-reactive protein and HOMA-index increased as number of components of metabolic syndrome increased. Abdominal obesity was significantly associated with elevated high-sensitivity C-reactive protein.
Conclusions. Metabolic syndrome is associated with insulin resistance and elevated high-sensitivity C-reactive protein. Interrelationship between inflammation, high sensitivity C-reactive protein, insulin resistance, impair insulin signaling and contributes to atherothrombosis is discussed.

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THE EFFECTS OF THEAFLAVINS ON LOW DENSITY LIPOPROTEIN GLYCATION BY MODEL SYSTEM IN VITRO

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Background. Nonenzymatic glycation reaction of low density lipoprotein (LDL) is greatly accelerated and is important in the pathogenesis of diabetic complications. The aim of this study was to investigate the effects of theaflavins on LDL glycation as *in vitro*.
Methods. First, LDL was isolated by sequential density ultracentrifugation from normolipidemic human plasma. Then, glucose was added to LDL fraction and LDL glycation level was estimated without and with various concentrations of theaflavin, theaflavin monogallate and theaflavin digallate by sodium periodate assay.
Results. Results showed that theaflavins were decreased LDL glycation in a dose dependent manner. The effects of these compounds were as follows: theaflavin digallate > theaflavin monogallate > theaflavin.
Conclusions. The results of this study show that theaflavins probably with their antioxidant properties were inhibited LDL glycation and thus may have a role in ameliorating atherosclerotic risk of patients with diabetes mellitus.

0413

USEFULNESS OF GLYCATED HEMOGLOBIN IN PREDIABETES

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Background. HbA$_{1c}$, traditionally used as a marker for chronic glycemic exposure, has recently been proposed as a new criteria for the diagnosis of type 2 diabetes. The aim of this study was to compare the use of glycated hemoglobin (HbA1c) and the oral glucose tolerance test (OGTT) in the diagnosis of impaired glucose tolerance in individuals at high risk of developing type 2 diabetes.
Methods. A total of 791 patients with at least two risk factors for the development of type 2 diabetes (obesity, dyslipidemia, hypertension, previous history of IGT or family history of diabetes) were enrolled in this study. Fasting glucose and HbA1c were measured in all individuals. Patients whose fasting glucose concentrations were below 7.0 mmol/L underwent an OGTT.
Results. From the 791 patients, 262 were euglycemic, 218 had impaired fasting glucose, 129 presented impaired glucose tolerance and 182 met the diagnostic criteria for type 2 diabetes. OGTT was performed in a total of 640 patients (80.9%). Statistically significant differences were observed for HbA1c concentrations in all groups. Receiver operating characteristic curve analysis was performed to assess the capability of HbA1c to discriminate between normal glucose tolerance and impaired glucose tolerance. An HbA1c value of 36 mmol/mol (5.4%) gave an optimal sensitivity of 85% and a specificity of 73%, and a negative predictive value of 97% for identifying patients with impaired glucose tolerance.
Conclusions. HbA1c can be used to rule out patients at high risk of developing type 2 diabetes.

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COMPARISON OF URINARY 8-HYDROXY-2'-DEOXYGUANOSINE (8-OHDG) LEVELS WITH URINE ALBUMIN/CREATININE RATIO (UACR) AS A PREDICTOR OF DEVELOPMENT OF DIABETIC NEPHROPATHY BY USING MASS SPECTROMETER

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Background. Measurement of urinary 8-Hydroxy-2'-Deoxyguanosine (8-OhdG) has recently become more popular as a means of assessing oxidative stress in the human body. The aim of this study is to compare the levels of urinary 8-OHdG with and without albuminuria in patients with type 2 diabetes and to evaluate its role as biochemical marker for distinguishing these patients from noncomplicated and healthy people.

Methods. For this purpose, 52 patients with type 2 diabetes (32 albuminuri, 20 non albuminuri) and 20 healty control subjects were included in this study. The urinary concentrations of 8-OHdG were measured by modified LC-MS/MS method and compared with the urine albumin/creatinine ratio in the first morning voiding urine sample and HbA1c values of the same patients.

Results. The type 2 diabetic patients with albuminuria usually have higher urinary 8-OHdG concentrations than the control subjects and the patients without albuminuria and the difference is statistically significant(patients with and without proteinuria and control group respectively; 3.47±0.94, 2.92±1.73, 2.1±0.93 nmol/mol creatinine, p<0.01). There is significant corrrelation between urinary 8-OHdG and urine albumin/creatinine ratio (r=0.501, p<0.001). According to ROC analysis, the AUC value of HbA1c was higher than the value of the AUC of 8-OHdG (respectively, 0.882, 0.771)

Conclusions. This study provides evidence that although the results of 8-OHdG were higher in diabetic patients, 8-OHdG in urine is not a useful clinical marker as albuminuria to predict the development of diabetic nephropathy in diabetic patients.

0415

ACTIONS OF LONG TERM LOW INTENSITY PHYSICAL EXERCISE PROGRAMMES ON LABORATORY TESTS OF METABOLIC SYNDROME

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Background. Obesity is frequently associated with diabetes, hypertension and hyperlipaemia, these acts together as metabolic syndrome (MS). Lack of daily exercise is a good reason to evolve MS. Leptin and insulin are produced by the adipose tissue and the pancreas, and in addition the developed resistance to them play key role in the development of MS. We investigated the actions of recreational type of physical exercise on the levels of insulin and leptin in association with glucose- and lipid-metabolism parameters of MS.

Methods. 185 volunteers participated in a 5-month-long, low intensity, recreational type of aerobic physical activity program. We measured the levels of serum insulin, leptin, cholesterol, triglyceride, glucose and haemoglobin A1c (HBA1c) in4 BMI categories (20-25, 25-30, 30-35, 35<) at the beginning (day 0) in the middle (day 50) and at the end (day 150) of the program.

Results. We found decrease of body weight (88,6 kg+2,2 to 81,0+2,0 kg) in the high BMI groups (30<), where body fat content (from 42,7+1,0 to 38,5+1,0%), cholesterol (from 6,2+0,4 to 4,6+0,4 mmol/l), triglyceride (from 1,5+0,1 to 1,0+0,1 mmol/l), HBA1c (from 6,4+0,1 to 5,5+0,05%), leptin (from 35595+4400 to 17319+1816 pg/ml) and insulin (from 7,6+1,6 to 3,0+0,4 μIU/ml) levels were all decreased (at a minimum of P<0.05 level), while the decrease in glucose level was more moderate (from 5,7+0,4 to 4,9+0,1 mmol/l).

Conclusions. Long term recreational physical activity program decreases insulin and leptin resistance. Monitoring of these and other obesity associated hormones seems to be useful to follow up the efficacy of physical exercise programmes.

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EVALUATION OF THE INFLAMMATORY STATUS OF OBESE AND NONOBESE SUBJECTS USING PROCALCITONIN AND NEOPTERIN

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Background. Obesity, excess amount of fat in the body, is known as an independent risk factorby means of cardiovascular diseases.Generation of an inflammatory condition by obesity may be one ofthe reasons of increasing pathogenesis of these diseases. In this study weaimed to conduct a study on the overweight andnormal weight subjects grouped according to body mass index(BMI) and waist-to-hip ratio (WHR), to investigate the effects of inflammation as a risk factor for cardiovascular diseases, by comparing thelevels of highly-sensitive CRP(hs-CRP),neopterin(NP), and procalcitonin(PCT).
Methods. Sixty-seven healthy adults were included in this study who were classified according to BMI and WHR. Cases were divided into three groups according to BMI values; 21 normal weight(BMI ≤25), 22 over-weighted(BMI:25-29,9) and 24 obese(BMI≥30). Also, cases were divided into two groups according to their WHR values to determine central obesity (cut-off was 0.9). Among these groups, whether there were any differences on the levels of CRP,NP and PCT, which are inflammatory indicators have been investigated.
Results. While statistically significant differences(p<0.05) have been detected between CRP and PCT levels between the groups composed according to BMI, there was some difference among groups composed according to WHR values which was not statistically significant(p>0.05).
Conclusions. It has been shown in this study that obesity can cause an inflammatory condition generally. However, it has been concluded that, this difference can be closely related to the degree of obesity more than the distribution of fat. More studies with expandednumbers of cases are needed to evaluate this situation.

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ASSOCIATION OF METABOLIC RISK FACTORS WITH SUBCLINICAL CAROTID ATHEROSCLEROSIS

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Background. Metabolic Syndrome (MS) contributes to pathogenesis of Type2 Diabetes and Carotid Artery Disease (CAD).Insulin Resistance (IR) is the key factors of MS which is also implicated in the development of CAD.
Methods. The prevalence of Carotid Atherosclerosis in MS and contribution of Metabolic Risk Factors (MRF) in causation of atherosclerosis in non diabetic North Indian population was investigated. 500 male subjects ranging in age 30-65 years participated in the study. MS was defined by NCEP ATP III. Waist circumference (WC) and blood pressure (BP) were measured. Fasting serum samples were analyzed for Glucose, Triglyceride, Cholesterol and its fractions, Insulin & Free Fatty Acids (FFA). IR was estimated by HOMA and Insulin Sensitivity (IS) by Quicki's Index. Early atherosclerosis was assessed by Intima Media Thickness (IMT) using ultasonography and B score for extent of plaques of carotid arteries.
Results. The prevalence of Metabolic Syndrome was 22%. Carotid IMT Parameters were significantly higher (p<0.001) in subjects with MS (835.42±12.36 μm) as compared to those without MS (758.88±16.82 μm) and number of MetS components was significantly associated with plaque prevalence. Subjects with Increased carotid IMT showed Hyperinsulinemia, Insulin Resistance, Insulin insensitivity along with significantly elevated levels of WC(p<0.001), BP(p<0.01), TG(p<0.002), FFA(p<0.01) & decreased HDL-Cholesterol(p<0.001). Further studies are needed to understand the role of the MetS in the progression from subclinical to clinical atherosclerotic disease.
Conclusions. MetS is associated with increased atherosclerotic burden, and thus, increased cardiovascular risk. The results of present study uphold the importance of screening and early intervention in Indian population.

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HBA1C PERFORMANCE TO DIAGNOSE DIABETES IN SAUDI ARABIA-RETROSPECTIVE ANALYSIS OF GLYCEMIC DATA AS PER EXPERT COMMITTEE REPORT

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Background. Diabetes prevalence in the Saudi population is 16.7%. HBA1C test is recommended as a faster and easier to diagnose diabetes. We test if international expert committee's recommended HBA1C criterion helps to classify (Non Diabetic ≤5.6, Risk Group 5.6-6.4, Diabetic Group ≥6.5

Methods. HBA1C standardized to DCCT was measured by Roche Integra analyzer by immunoturbidimetric method, with internal controls levels of 5.8 with CV of 1.7% and 12.2 with CV of 1.6%. Retrospective observations from 2 years at the RiyadhMilitaryHospital of subjects were analyzed by SAS Statistical Software for Pearson's correlation between HA1BC and FBS and p-value less than 0.05 was considered as statistically significant.

Results. The study had 32947 females and 26104 males with their mean age 55.39 years and 54.62 respectively. For both the sexes the HA1BC mean values were slightly different (Female: 7.99, Male: 7.90) whereas fasting glucose mean values for female was moderately elevated with respect to the male subjects (Female: 8.27, Male: 8.10).For female subjects the Pearson correlation coefficient between HA1BC and FBS was 0.73 with p value less than 0.0001, whereas for male subject this Pearson correlation coefficient between HA1BC and FBS was 0.70 with p value less than 0.0001; for both the sexes the p values seems to be highly significant as this value is less than 0.05.

Conclusions. This observational study information is helpful in planning to use the proposed HBA1C criteria of the international expert committee to diagnose Diabetes.

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HAEMOGLOBIN VARIANTS AND THEIR INTERFERENCE IN HBA1C DETECTION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Background. The IFCC working group has defined HbA1c as a haemoglobin (Hb) which is irreversibly glycated at one or both N-terminal valines of beta chains. In diabetic patients it is advisable to check the HbA1c level to control the treatment and monitor the disease's progression. Since January 2010, this method has been included as a diagnostic marker showing the presence of diabetes when the value is higher than 6.5 %.

In this case report, we highlight how the haemoglobinopathies can interfere with HbA1c analysis when using the HPLC method.

Methods. Case report: Male, 40-years-old, native of Morocco, with no personal history of any disease. He was seen in the emergency department following the onset of diabetic decompensation. HbA1c analysis was performed using an HPLC system (HA 8160 Menarini®) and Hb electrophoresis was carried out on cellulose acetate at pH alkaline and at pH acidic.

Results. Our HPLC analysis showed a variant haemoglobin along with Hb F (38.1%) and Hb A2 (3.8%). It was not possible to detect HbA1c. Haemoglobin electrophoresis at pH 8.5 and at pH 6.0 demonstrated that the variant Hbwas Hb S (56.1%). This data supported the diagnosis of asymptomatic compound heterozygous for Hb S and hereditary persistence of fetal haemoglobin (HPFH).HbA 1c could not be detected.

Conclusions. People affected by diabetes where a haemoglobin variant is present, could not be either diagnosed or monitored using an HPLC technique to control the disease; instead, an alternative method measuring the glycaemic level has to be employed.

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IMPACT OF MAST CELLS ON MACROPHAGE PHENOTYPE IN ADIPOSE TISSUE

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Background. White adipose tissue (WAT) from obese humans and mice contain more mast cells than WAT from their lean counterparts. Adipose tissue macrophages consist of at least two different phenotypes (i.e., classically activated M1 and alternatively activated M2 macrophages). In this study we aimed to clarify the potential impact of mast cells on M1/M2 macrophage-polarisation in fat tissue.
Methods. Mast cell-deficient Kit$^{W-sh/W-sh}$ and C57BL/6J mice were fed high-fat (HF; 60% calories from fat) and low-fat (LF; 10% calories from fat) diet for 6 months. Mice were monitored by indirect calorimetry over a 72-hour period.Oral glucosetolerance and insulin tolerancetests wereperformed. Relative mRNA levels of selected genes from epididymal fat tissue were analyzed by real-time PCR.
Results. Kit$^{W-sh/W-sh}$ mice gained significantly less weight and showed higher insulin sensitivity than wild-type (WT) controls, both on the HF-diet. Indirect calorimetry analyses revealed that Kit$^{W-sh/W-sh}$ mice had significantly greater energy expenditure, O_2 consumption and CO_2 production than WT mice during the light but not during the dark phase. Gene expression of macrophage marker F4/80 was significantly higher in both Kit$^{W-sh/W-sh}$ and C57BL/6J on HF- comparing to the animals on LF-diet. Expression of M1-specific genes was significantly elevated in C57BL/6J but not in Kit$^{W-sh/W-sh}$ mice on HF- comparing to the littermates on LF-diet. HF-diet resulted in significant increase in expression of M2-specific genes in animals of both genotypes.
Conclusions. These findings suggest that mast cells play critical role in the determination of M1 versus M2 polarization of macrophages in WAT.

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ACUTE INFLAMMATORY PATTERN IN SERUM PROTEIN ELCTROPHORESIS OF UNCONTROLLED TYPE II DIABETES

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Background. Diabetes has now become a global pandemic affecting about 6.4% of the total adult population. Development of Type II diabetes has been associated with low grade systemic inflammation. Levels of different inflammatory markers like hsCRP, alpha1antitrypsin ,alpha1 acid glycoprotein, ceruloplasmin ,fibrinogen have been be estimated individually to prove the state of inflammation in diabetes. Most of the acute phase reactants fall in alpha 1 and alpha2 region of electropherogram in cellulose acetate electrophoresis of serum proteins. Hence, in our study, the pattern of electrophoresis of serum proteins in uncontrolled and controlled diabetes was considered for the holistic effect on the acute phase reactants and their correlation with glycemic control.
Methods. Serum protein electrophoresis was done on 50 diagnosed cases of Diabetes [controlled and uncontrolled] using cellulose acetate paper technique. The different bands were stained with PonseuS and then quantitated using densitometer. The density of alpha1 and alpha 2 bands were correlated with HbA1c level using SPSS version17.
Results. With the glycemic control below HbA1c 6.5%,the alpha 1 and alpha 2 serum proteins were within normal limit .However,a significant increase in percentage of alpha1 and alpha2 proteins [0.697and0.714(p=0.01)] were found with the increasing level of HbA1c .
Conclusions. The systemic inflammation can definitely be lowered by glycemic control in diabetes patients and serum protein Electrophoresis can also be used as a sensitive tool to screen the inflammation status in diabetes.

Diabetes mellitus and metabolic syndrome

0422

EVALUATION OF ADAMS™ A$_{1C}$ MENARINI HA-8180 HPLC ANALYSER FOR HBA$_{1C}$ DETERMINATION

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Background. ADAMS ™ A$_{1C}$ HA-8180 is a HPLC system for the measurement of HbA$_{1C}$. The analysis time is 48 seconds per sample. The analytical performance was evaluated to verify quality of analysis, according to the criteria established in the recently published documents of consensus on this analyte.

Methods. Precision and linearity studies were performed according to CLSI and manufacture´s guidelines. Recovery, the effect of Hemoglobin (Hb) concentration and the presence of coexistent interfering substances were evaluated.

Results. Precision: Intra assay,mean HbA$_{1C}$ 4.2%, CV 0.4%; mean HbA$_{1C}$ 10.5%, CV 0%

Inter-assay: mean HbA$_{1C}$ 6.0% intra run CV 0.52%; inter run CV 0%; between day CV 0.18%; Total CV 0.57 %

mean HbA$_{1C}$11.5%, intra run CV 0.24%; inter run 0.14%; between day CV 0.1%; Total CV 0.29 %.

Recovery (Passing Bablock) y = 1.00 x - 0.1, r = 0.999, analytical range 5.0-12.5%

Effect of the total concentration of Hb: a value of HbA$_{1C}$ 5.5% is not affected by a concentration of Hb in the range 95-223 g/L.

A concentration of HbA$_{1C}$ 5.0% is not affected by the presence of a fraction of labile A$_{1C}$ of 4.5%, nor 5.3% carbamylated Hb, nor by 6.3% acetylated Hb.

Conclusions. The drastic reduction of the analysis time does not impair the overall analytical quality of Results.

Given the short time of the analysis this is a suitable system for the control of diabetic patients in laboratories with high workflow.

0423

GLUCATED HEMOGLOBIN CONCENTRATION AT NEWLY DIAGNOSED PATIENTS WITH DIABETES MELLITUS

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Background. Diabetes mellitus is a complex disease which is caused by a lack of insulin, bringing disturbance in the metabolism of carbohydrates, fats and proteins. Nowadays, we determine the glucated haemoglobin (HbA1C) to follow and treat this disease. The aim of this study was to examine the HbA1C concentration in the blood of one hundred newly diagnosed patients (75 women and 25 men) with Diabetes mellitus. The patients were divided into three groups according to the concentration of glucose in the blood.

Methods. HbA1C (r.v. 4-6%) and glucose (r.v.4.2-6.4mmol/L) were determined using sets from ABBOTT by a biochemical analyser ALCYON – ABBOTT. All groups were compared to the control group of 30 healthy patients (20-40 years old).

Results. The gained values for the patients and the control group are the following: first group, 68 patients with glucose values → x=6.9±2.1mmol/L; p<0.01; HbA1C → x=5.9%±1.8%; p<0.01; second group, 20 patients → x=9.2±5.3mmol/L; p<0.01; HbA1C → x=7.9%±2.4%; p<0.01; third group, 12 patients → x=15.8±7.5mmol/L; HbA1C → x=11.1%±3.2%; p<0.01; control group → x=4.9±0.7mmol/L; HbA1C → x=4.3%±0.9%; p<0.01.

Conclusions. These results show increased concentrations of HbA1C in the serum of patients in the first and second group which means, these patients had increased values of glucose in the blood in the previous three months. The glucose managed to bind with the haemoglobin, producing increased HbA1C concentration in the blood, but the patients had subjective or objective reasons not to see their doctor to get an early diagnosis and start with treatment.

Diabetes mellitus and metabolic syndrome

0424

INVESTIGATION OF EXERCISE BIOMARKERS BY UHPLC-TRIPLE QUADRUPOLE MASS SPECTROMETRY, CAPILLARY ELECTROPHORESIS AND MOLECULAR BIOLOGICAL TECHNIQUES

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Background. In a non-targeted metabolomics approach we identified medium chain acylcarnitines (mcAC) as the dominating biomarkers in plasma under moderate exercise conditions. On the other hand increased levels of acylcarnitines in blood are well known biomarkers of inborn mitochondrial diseases and were recently also detected in obese and type 2 diabetic subjects. But, a potential physiological function of these extracellular biomarkers is currently unknown.
Methods. To investigate effects of extracellular acyl-carnitines in tissue specific cell culture the kinetics of acylcarnitine production and release is analyzed by UHPLC-triple quadrupole mass spectrometry, the effect of acylcarnitines on cellular energy charge is analyzed by capillary electrophoresis and the effects on skeletal muscle cell fusion and oxidative capacity is determined by qPCR of marker genes.
Results. Incubation of primary human skeletal muscle cells revealed a rapid turnover of ^{13}C-palmitate to palmitoylcarnitine and a delayed production of C14:0- and C12:0-carnitine in the cell lysates; all acylcarnitines were also released and detected in the supernatant after 24 h. The recently detected increase in the oxidation of palmitate can not be explained by altered mRNA expression of genes involved in fatty acid metabolism or mitochondrial biogenesis. No effect on the intramyocellular storage of lipids was detected. But a significant enhancement in the insulin-stimulated phosphorylation of Ser-473 of AKT exposed to mcAC for 60 min was demonstrated.
Conclusions. Medium chain acylcarnitines, representing dominating exercise biomarkers, are released from skeletal muscle cells, show relevant alteration of key processes of lipid metabolism, as well as effects on insulin signaling transduction.

0425

PLASMA E-SELECTIN CONCENTRATION IN PERSONS WITH INCREASED RISK FOR TYPE 2 DIABETES (PRE-DIABETES)

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Background. Specific adhesive molecules are involved in earliest stage in development of atherosclerotic plaque.
The aim of the study was to asses plasma Endothelial Leukocyte Adhesion Molecule (E-selectin) concentrations in persons with high risk for type 2 diabetes.
Methods. 25-74 years old centrally obese (IDF 2005 criteria) and elevated BMI ($\geq$ 25 kg/m^2) Caucasians apparently healthy underwent clinical examination. Subjects with neither acute nor chronic disease were qualified for oral glucose tolerance test (OGTT). Plasma lipids: T-C, HDL-C, TAG (bioMérieux), insulin (ELISA-BioSource) and E-selectin (ELISA-R&DSystems) were determined fasted. LDL-C (Friedewald formula) and insulin-resistance indiceswere calculated. Results of OGTT were used to select normal glucose tolerance, NGT (n=42; 18 male, 24 female; aged 51±14), impaired fasting glycemia, IFG (n=32; 14 male, 18 female; aged 60±10) and impaired glucose tolerance, IGT (n=32; 12 male, 20 female; aged 54±13) groups. Statistica 6.0 and MedCalc programs were used.
Results. Intra-assay coefficients of variation (CV) 4,0-5,2% and inter-assay CV 5,4-6,0% for E-selectin measurements were calculated. E-selectin values varied among NGT, IFG and IGT groups (median±SE: 21,8±1,4 vs 35,8±2,2 vs 46,5±3,0 ng/ml; p=0,0000) using Kruskal-Wallis ANOVA test. ROC curve analysis revealed 30,7 ng/ml of E-selectin in differentiation between NGT and IFG+IGT characterized with 86,54% sensitivity, 78,33% specificity and AUC of 0,895.No correlations between E-selectin and metabolic parameters were observed in the subgroups, while in whole study population positive correlations between E-selectin and glycemia and HOMA-IR were found.
Conclusions. In the studied subjects E-selectin appeared as an important proatherogenic factor in high risk for type 2 diabetes population.

Diabetes mellitus and metabolic syndrome

0426

ELEVATED SERUMLEVELS OF β$_2$-GPI-LP(A) COMPLEXES IN TYPE 2 DIABETES MELLITUS

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Background. Increased circulatingβ$_2$-glycoprotein-I-oxidized low density lipoprotein (β$_2$-GPI-ox-LDL) complexes have been found in patients with autoimmune disorders and chronic diseases including type 2 diabetes mellitus (T2DM) as a contributor to the development of atherosclerosis. Our recent study found the existence of circulating complexes ofβ$_2$-GPI I with lipoprotein (a) [Lp(a)] and the elevated complexes concentrations in autoimmune disorders. Here we examined the levels and relation ofβ$_2$-GPI-Lp(a) complexes with T2DM.
Methods. Forty T2DM patients (25 with complications and 15 free from complications) and 40 age/sex-matched healthy controls were studied. Serum concentrations ofβ$_2$-GPI-Lp(a) and ox-Lp(a) were measured by "sandwich" ELISAs. In addition, the lipid profile and glucose were investigated.
Results. Serumβ$_2$-GPI-Lp(a) (1.10 ± 0.32U/ml vs. 0.87 ± 0.20 U/ml, *P*<0.0001) and ox-Lp(a) concentrations (12.54 ± 11.95 mg/l vs. 5.49 ± 4.31 mg/l) in T2DM were both significantly higher that those of controls. There was a significant difference in the level of circulatingβ$_2$-GPI-Lp(a) (15.28 ± 11.62 U/ml vs. 10.90 ± 12.08 U/ml) between the subgroup of diabetic patients with nephropathy/retinopathy and patients free of complications. The area under the ROC curve (AUC) forβ$_2$-GPI-Lp(a) and ox-Lp(a) was 0.721 and 0.739, respectively. β$_2$-GPI-Lp(a) were positively correlated with Lp(a) (r=0.691, *P*<0.0001), ox-Lp(a) (r=0.780, *P*<0.0001)and T2DM complications (r=0.427, *P*<0.01) in T2DM.
Conclusions. β$_2$-GPI-Lp(a) complexes concentrations increased in T2DM, especially in patients with complications. Elevatedβ$_2$-GPI-Lp(a) complexes in circulation suggest the intriguing possibility that the complexes may also play a role in the development of atherosclerosis and/or cardiovascular complications in T2DM.

0427

THE DIFFERENCE IN THE INTERFERENCE OF FETAL HEMOGLOBIN (HBF) BETWEEN CATION-EXCHANGE HPLC AND TURBIDIMETRIC INHIBITION IMMUNOASSAY HBA1C METHODS

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Background. Studies have shown that significantly increased fetal hemoglobin (HbF) interferes with certain HbA1c assays, but the degrees of interference vary in different Methods. The objective of this study is to determine if there is a bias in HbA1c values in patients with elevated HbF between a cation-exchange (CE)-HPLC and an immunoassay and if the bias correlates with HbF and HbA1c levels.
Methods. Thirty-four EDTA whole blood samples with elevated HbF were tested with Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension turbidimetric inhibition immunoassay (TINIA) HbA1c Methods. HbF was quantified by Bio-Rad Classic Variant with Beta-thalassemia Short Program.
Results. The HbF levels in these 34 samples ranged from 7.8% to 35.7% with an average of 23.5%±7.3% (mean ± SD). The average HbA1c values with CE-HPLC and TINIA were 7.4%±2.2% and 6.4%±1.9% (mean±SD) with an average bias of 1.0% (P = 0.0005). Linear regression analysis showed a proportional relationship between the bias of HbA1c and the levels of HbF: y (HbA1c bias) = 0.06x (HbF) - 0.36 (R = 0.3, SEE = 1.4). Linearity regression analysis also showed a proportional relationship between the %bias of HbA1c and HbA1c levels with CE-HPLC: y (%Bias) = 3.5x A1c (CE-HPLC)-14.8 (R = 0.5, SEE = 15.3).
Conclusions. There is a significant bias in HbA1c levels between Bio-Rad Variant II TURBO Link CE-HPLC and Siemens Dimension TINIA HbA1c assays in patients with elevated HbF. The bias correlates with HbF values and the %bias correlates with HbA1c values at small and medium levels, respectively.

Education

0428

HUMAN ANTI-ANIMAL ANTIBODIES: EVEN TODAY, IT IS STILL A PROBLEM IN ROUTINE PRACTICE! ILLUSTRATION WITH 4 CLINCIAL CASES

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Background. Human anti-animal antibodies (HAAA) are known as a cause of analytical interferences. This knowledge, however, tends to be lost. This can be due to less informed new generation of Clinicians or Biologists or to the industrialization of the production of the results (with huge analytical platforms) where the clinical result of an immuno-assay for a single patient has less importance than the turnaround time. We present here 4 cases to illustrate the problem.
Methods. Case 1: 60 yo. woman with calcitonin (CT) levels at 63 pg/mL, not stimulable by pentagastrin. Underwent a thyroïdectomy, but CT remained elevated. Then underwent different expensive examinations (scanners, Pet-scans, scintigraphys,…). Six years later, she still presents high CT levels and is followed-up for a multiple endocrine neoplasia. Case 2: 29 yo woman with PTH levels >2000 pg/mL, without renal disease or phosphocalcic metabolism abnormalities. Case 3: 61 yo renal transplanted man with PTH levels increasing from 187 to >5000 pg/mL in 6 months. Case 4: 46 yo. stressed and tired woman which presented unexpected high levels of fasting insulin at three different occasions. She was thus hospitalized three days for exploration.
Results. All of these 4 patients presented an interference due to HAAA (anti-goat, anti-mouse or not species specific) or due to rheumatoid factor. These falsely elevated results have had different consequences, like expensive unnecessary extra-investigations and have stressed the patients.
Conclusions. HAAA still exist and cause every day medical errors. In front of an unexpected result, the dialogue Clinician-Biologist is absolutely essential.

0429

COMPARE OF THE TWO EDUCATIONAL METHODS IN GENERAL PATHOLOGY LEARNING OF THE MEDICAL STUDENTS

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Background. Nowadays various methods develop to improve medical education. In this study we aimed to improve the learning levels of the medical students.
Methods. In this study we enrolled 32 medical students in two similar groups based on the mean of the average score of students. We selected four titles of general basic pathology for lecture. According the teaching method we named the usual method as 1st group, and the new method as 2nd method. We prepared some papers which consisted of the title of the lecture, definitions, classifications of the subjects , tables, and algorithms as partially completed forms. These papers were available to students taking: the students were required to complete these papers during the lectures time.Finally the mean scores of these two groups were compared with each other.
Results. The mean of average score in the 1st group was 63 from 100, and in the 2nd group was 84 from 100. Statistical analysis of the data was performed by SPSS software and student t-tes, (p value=0.035)
Conclusions. use of partially completed forms which require to complete during lecture time by the students improve the educational and learning levels in medical students. The analysis of the above data let us to conclude that 2nd method because of active participation of the students to complete the forms required more attention so the students learn and remind the subject more easily.

Education

0430

INNOVATION STUDY PROGRAMS AIMED AT ANALYSIS OF BIOLOGICAL MATERIALS AT UNIVERSITY OF PARDUBICE

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Background. Operational program for education and competitivenessfundedby European Union sponsors also the project Innovation of study programs „Special Chemical and Biological Fields of Study at University of Pardubice". The main resolver is the Department of Biological and Biochemical sciences and duration time of the project is 3 years. This department at the Faculty of chemical technology, was established in 1996 to precise the education and practical preparation of students for analysis of biological materials. The department has committed to provide the specialists in diagnostic assays in medicine and is in a constant need for qualified personnel who shall oversee and assist in the construction and developing of diagnostic methods and instruments.
Methods. Project team forms our academics, specialists from clinical laboratories,external lecturers,partners from Hospital of Pardubice and laboratory company MeDiLa. Innovation is incorporated in laboratory education accordingnew trends in clinical laboratory diagnostics and analysis of biological materials, because the main aim is to provide interdisciplinary education with possible occupational chance of graduatesnot only in Czech Republic but even in other countries of European Union.
Conclusions. Graduatesacquiretheoretical and practical knowledge in clinical laboratory and diagnostic branches and know how to operate with the relevant analytical devices controlled by computing technology and use modern laboratory instrumentation, following up the actual trends as miniaturization and automatization.

0431

CORELATION OF HOMOCYSTEINEMIA WITH OCCURRENCE AND DEVELOPMENT OF CORONARY ARTERY DISEASE

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Objective. The aim of the study was to establish if there is correlation between total plasma homocysteine (tHcy) levels with occurrence and development of coronary artery disease (CAD).
Methods. Total number od 165 patients were examined which were divided into 3 groups based on 10 years risk for CAD established according ATP III and Framingham criteria: high risk group consist 60 patients with CAD risk above 20%; group of 49 patients with angiographyally proven CAD and 56 patients, control group, with CAD risk less than 10%. All patients were evaluated for the following risk factors and markers: sex, age, smoking status, hypertension, family history of CAD, lipids, lipoproteins, glucose, white blood cells, urea and creatine.
Results. Mean plasma tHcy levels in high risk group were 16.0 micromol/L (p<0.04), in the group with CAD, 15.3 micromol/L respectively (p<0.02) vs. control (13.0 micromol/L). There was correlation between tHcy and total CAD risk (p<0.04) and white blood cells count (0.02) in high risk group. In the group with CAD, tHcy correlated with the frequency of high grade of coronary artery stenosis, >95% of arterial lumen (0.04).
Conclusions. We concluded that elevated tHcy correlated with the total CAD risk and the stage of coronary artery disease.

Education

0432
WHAT ARE NOMINAL PROPERTIES AND EXAMINATIONS?

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Background. Metrology deals with quantities and their measurements. ClinicalLaboratory Sciencesalso concernsnominal properties, that arenot covered by the'International vocabulary of metrology – Basic and general concepts and associated terms (VIM)'. Therefore there is a need for a complementary vocabulary about examinations others than measurementse.g. classification of blood cells, blood typedetermination, and identification of bacteria.
Methods. The process to create this vocabulary is similar to thatof the VIM revision : a wide involvement of relevant organisations providing recommendations. The definitions and examples of the VIM are partly adapted to nominal properties and examinations. ISO terminology work rules are applied.
Results. The current document includes 73 entries, terms with definitions, notes and examples. It comprises three parts : Nominal properties, Nominal examinations, Nominal examination standards.This poster presents educational excerpts of the document including somesuperordinates, that are concepts covering bothquantities and nominal properties. A generic concept diagram shows some essential concepts. Discussion points are raised, aiming at opening exchanges between authors and readers : are nominal properties a sort of quantities? How to define 'nominal examination uncertainty'? Is it best to define 'nominal examination precision' or 'nominal examination imprecision'? What is the equivalent of a 'scale' for nominal examinations? What is a 'blank nominal property value'? Is the concept of 'calibration' applicable to nominal examinations?
Conclusions. This work helps clinical laboratory scientists, professional societies and manufacturers to discuss and understand the basic concepts for nominal properties dealt with, hitherto lacking a proper terminology.

0433
ASSESSMENT TOOLS IN HYBRID-PROBLEM BASED LEARNING (PBL) OPTED MEDICAL CURRICULUM: NURTURING LEARNING ENVIRONMENT

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Competence is not an achievement but rather a habit of lifelong learning. Assessment drives any curriculum, there is no one other component in an educational program that has such a powerful effect on the way students study and the way teachers teach. The move from discipline-base curriculum to hybrid- Problem-based learning (Hybrid-PBL) curriculum in medical schools, the continuous assessments of student are compulsory to evaluate their achievements.Current paper gives an overview of some of the vital student assessment strategies in PBL adopted medical schools and also provide information on how to create fair student assessments, that is, assessments that are both reliable and valid. Formative assessments are most valuable when they are separated from summative assessments, so that they are perceived to be low threat performance experiences. MEQ's, MCQ's SEQ's are defined as written exercise assessments and are play an important role in any type of education curriculums whether it is Problem based curriculum or Discipline-based curriculum. OSCE/OSPE and standardized patients, incognito standardized patients and High-technology simulations can strengths professionalism and self reflection of the medical graduates. Multisource ("360-degree") assessments also have a great impact on student performance and faculty performance. Faculty checklist rating, Tutor evaluation and Progress test tools also valuable in curriculum as well as institution evaluation. Furthermore, each fair assessment strategy should meet *Reliability, Validity,Feasibility,Acceptability* and *Educational Impact* factors. We strongly believe and conclude that an assessment system that strives towards these factors would also support a nurturing learning environment.

Education

0434

COMPETENCY IMPROVEMENT OF THAI CLINICAL LABORATORY PERSONNEL BY COOPERATION OF ACADEMIC STAFF AND PRIVATE SECTOR VIA CORPORATE SOCIAL RESPONSIBILITY

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Background. The economics crisis has been run for few years. Thai clinical laboratory service has been affected by budget and manpower shortages. Laboratory personnel are working under pressure of patient's expectation, the law issue, and the rocket high of science and technology development. However, their competency must be maintained. These problems were realized and concerned. The competency improvement of laboratory personnel was urgently needed.
Methods. The human resource development program had been established by a group of academic staff to offer the free of charge continuing education. Cooperation between academic staff and interested private sector on the basis of Corporate Social Response (CSR) has been occurred. Laboratory personnel development was going through the "THE STAR" program. Participating laboratory personnel of both government and private hospitals from all over Thailand was sprat interest to obtain their professional inspiration by the friendly environment.
Results. Within 5 years, a total of 54 projects created and presented in the "THE STAR" program by inspired laboratory personnel. These were divided into 4 categories: category A was the creative and novel production to enhance service efficiency, category B was the transform of routine to research, category C was the research for development, and category D was the problem solving by management; of 9,9,8 and28projects; respectively.
Conclusions. Competency of laboratory personnel improved which demonstrated through the project presented in "THE STAR" program and these successes were facilitated by cooperation between academic staff and private sector on the principle of CSR.

0435
THE PUSH BUTTON PROJECT

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Background. Biological and analytical variation significantly influence the interpretation of a laboratory result. However, physicians and clinical biochemists often do not reflect on this and rather tend to interpret the "naked" result. We, therefore, developed an educational tool to increase awareness about the above influences.
Methods. We constructed our educational tool, called the "Push button Project", as a Java application. It is freely available at www.stt-consulting.com for use. The Java applet makes use of Java SE 6, calculations are performed by Apache Commons Math 2.1 library, and graphs are generated by JFreeChart 1.0.13 library.
Results. The applet starts with the presentation of a "naked" result and its location relative to the reference interval for the concerned analyte. Consecutively, the influences of analytical error (within-run/total analytical variation, bias and sample-related effects) and the (within) biological variation on the assessment of a person's health status are taken into account. In addition, the influence of replication of measurements on analytical variation is shown. Each effect is displayed by way of a modification of the distribution of the patient's result with calculation of the percentage of that distribution outside the reference interval for the healthy (reference) population.
Conclusions. The "Push Button Project" can be used for self-education to investigate the relevance of the different influences on a single laboratory result encountered in daily laboratory work. If it finds sufficient interest in the laboratory community, it is the intention to expand the project to a Smart-phone application.

0436

THE CLINICAL USEFULNESS OF SENSITIVE COMMERCIAL IMMUNOASSAYS FOR DETERMINATION OF LOW 17B-ESTRADIOL CONCENTRATIONS IN CHILDREN

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Background. Specific and sensitive assays for 17b-estradiol measurements are needed in clinical practice for evaluation of pubertal disorders in children and low postmenopausal levels in women. Therefore, the assessment of analytical and functional sensitivity is important for method evaluation.
Methods. The lower limit of detection and clinical usefulness (functional sensitivity) of threecommercial estradiol immunoassays (Advia Centaur Estradiol-6III, Diasource, Immunotech) were validated by use of sera from children. The analytical sensitivity has been evaluated based on repeated measurements of the model which does not contain analyte and functional sensitivity was estimated based on relation between precision profile. The dilution test and recovery test has also been performed.
Results. Analytical sensitivity of the tested method of the Advia Centaur Estradiol- 6III was 8.49 pg/ml, DiaSource was 7.06 pg/ml, Immunotech was 2.2 pg/ml, however functional sensitivity was 30.0 pg/ml, 38.0 pg/ml and 10.3 pg/ml respectively. Based on the dilution test it was found that depending on sample used for dilution the underestimation of concentration was observed for Advia Centaur Estradiol- 6III kit and overestimation of concentration for DiaSource kit. The percentage of recovery ranged from 64.41 % to 176.52 % (average 112.61 %) for kit of Advia Centaur Estradiol- 6III, from 142.8 % to 588.2 % (average 388.3 %) for kit of the DiaSource and from 105.7 % to 137.5 % (average 121.6 %) for kit of the Immunotech.
Conclusions. The Immunotech kit is suitable for measurement of low 17b-estradiol concentrations in children.

0437

A CASE OF CONSISTENT DISCREPANCIES BETWEEN URINE- AND BLOOD HCG MEASUREMENTS

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Background. Our laboratory was confronted with two successive urine samples from a single patient which tested positive for human chorionic gonadotropin (hCG) in both qualitative- and quantitative assays, combined with no detectable hCG in corresponding plasma samples.
Methods. Serial dilution and recovery experiments were performedin order to investigate the presence of interfering substances or a high-dose hook effect. The ovarian cysts that were removed from this patient were immunohistochemically stained using polyclonal anti-human hCG antibodies. Furthermore, a urine sample was sent to the USA hCG Reference Service for hCG variant analysis.
Results. Dilution and recovery experiments in urine- and plasma samples were unremarkable. The biopsy stained negative for human hCG and free beta-subunit. hCG isoform analysis in the urine sample revealed that approximately 87.5% of the immunoreactive hCG lacked the beta-subunit C-terminal peptide.
Conclusions. We report a rare case in which two successive urine samples test positive for hCG whereas in corresponding plasma samples hCG is undetectable. The majority of the total hCG contained a degraded form of beta-subunit that lacks the C-terminal peptide. This hCG variant, possibly of pituitary origin, is thought to have an extreme fast clearance rate possibly explaining the discordance between the hCG results in urine- and plasma samples.

Endocrinology

0438

MEASUREMENT OF SALIVARY CORTISOL LEVELS BY AN AUTOMATED ELECTROCHEMILUMINESCENCE IMMUNOASSAY METHOD (ECLIA): EVALUATION OF ANALYTICAL PERFORMANCE

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Background. Diagnosis and treatment of Cushing`s Sindrome (hypercortisolism) represents a challenge in clinical endocrinology. The measurement of salivary cortisol is especially useful for the assessment of ambulatory patients and the pediatric population, given it is not an invasive method.
Aim. To evaluate the analytical performance of the ECLIA method for the dosage of salivary cortisol
Methods. Modular E170 (â Roche) equipment was used. Samples of saliva were obtained from healthy volunteers (n=20) between 26 and 45 years of age. BMI<25 kg/m² at 8:00, 15:00 and 23:00 hours.
Precision of two levels of concentration was evaluated with pools of saliva samples according to EP15-A2 CLSIPool1(P1):8.36 nmol/l, Pool 2 (P2):16.56 nmol/l .The quantification limit (LoQ) was determined based on Spenser et al. The reference interval (RI) of 8:00 am and 15:00 pm was ascertained following C28 A3 CLSI protocol, the 95 percentile was estimated for the 23:00 hour samples.
Results. Intra-assay precision: CV%: 4.0 (P1), 4.4(P2); Inter-assay precision: CV%: 6.7(P1), 5.6 (P2) .LoQ<2.2 nmol/l. Clinical linearity was determined in the concentration range 0.74-17.0 nmol/l. RI 8:00hs: 4.25-16.6 nmol/l, 15:00hs :2.05-10.5nmol/l , both included in the RI proposed by the manufacturer. RI 23:hs :up to 5.0 nmol/l
Conclusions. The Precision and RI specifications proposed by the manufacturer were verified .The LoQ obtained insures the precision of the results in the clinical decision levels. Confirming our preliminary results of the reference interval in the 23:00 hour sample will be of great value in the evaluation of the hypercortisolism disorders.

0439

THE PERCENTAGE OF MACROPROLACTIN IN RELATION TO TOTAL PROLACTIN

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Background. Complex of monomer prolactin andprolactinin serum, macroprolactindoes not possessgreatbiologicalactivity, but isdetectedusingimmunochemicaltestsof prolactin.
Methods. Macroprolactinfromserum sampleswasdepositedby precipitationwithpolyethylene glycoland theconcentration ofprolactinwas measuredbeforeandafterprecipitation. Theratio ofprolactinconcentrationafterprecipitationin relationto theconcentrationbefore thedepositionwas calculatedand expressed in percentages(percentage of macroprolactin). Measurementof serum prolactinbeforeandafterprecipitation of macroprolactin was conducted atCobas411analazyer using Rochereagents.
Results. Measured concentrations ofprolactinwereclassifiedintothreegroups: Group Iwith aconcentration ofup to700mIU/l, Group IIwith aconcentration700-1500mIU/l andgroup IIIwith theconcentrationof 1500mIU/l.In thefirstgroup,the percentage of macroprolactinwasfrom 8 to30% (median 17.7%), the secondgroupof11to51 (median 19%) in thethirdgroupof6to 22% (median 13%). There were nosignificantdifferencesinmedianvalues. Two sampleswiththe percentage of macroprolactinabove50%(9.5% of totalsample) were registered in the secondgroup.
Conclusions. Slightly higherpercentage of macroprolactinwas in thesamples with a concentrationof prolactin700-1500mIU/l, butitdoes notdiffersignificantlyfromthe percentageinthe othertwogroupsof samples tested. The percentage of macroprolactinabove50% at9.5% testedsamplesindicatesthatdetermination of macroprolactinin patientswithelevatedprolactinvalues should be required, especiallythosewhohave nosymptoms ofhyperprolactinemia.

Endocrinology

0440

MACROPROLACTIN: PEG RECOVERY RATE IS FIXED

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Background. Macromolecular isoforms of prolactin (PRL) are classified as Big-PRL and BigBig-PRL, known as macroPRL. MacroPRL complex seems to be born from the combination of monomeric PRL and circulating Immunoglobulins G. These forms can be present in both normoprolactinemic and hyperprolactinemic human sera, but they are not differentiated by current PRL immunometric assays.
The aim of this study was to detect PRL and macroPRL isoforms in hyperprolactinemic patients and to investigate the variation of the isoforms ratio over time.
Methods. Serum PRL levels were measured in 109 patients (22 M and 87 F) at basal (T1) and after 6-12 months (T2). In all serum samples PRL measurement was obtained before and after PEG 6000 (25% w/v) precipitation (PRL immunoassay Architect®, Abbott, USA).The recovery limits of ≤ 40% and ≥ 60% after PEG precipitation were adopted to classify the cases of macroPRL and true-hyperprolactinemia respectively; the cases ranging between 41 and 59% were classified as variable presence of macroPRL.
Results. Our data show statistically significant differences between: (i) PRL T1 and T2 values ($p<0.001$), (ii) PRL T1 and T2 values after PEG precipitation (true PRL) ($p<0.001$) and (iii) macroPRL T1 and T2 values ($p<0.001$). Comparison of recovery percentages T1 and T2 does not show significant differences ($p=0.1467$).
Conclusions. The fluctuation of serum PRL levels were not related to variations in recovery percentages. The ratio between the PRL isoforms is fixed in the time.

0441

MORNING AND LATE EVENING SALIVARY CORTISOL CONCENTRATION IN PREGNANCY

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Background. The clinical manifestation of Cushing's syndrome in pregnant women can be masked by physiological symptoms of pregnancy. Monitoring of late evening salivary cortisol could be a useful additional screening test for these patients, if we establish the reference values for the salivary cortisol in healthy pregnant women.
Methods. Saliva was collected from the controls and healthy pregnant women at 7-9 am and 10-11:30 pm using Salivette system (Sarstedt). The concentration of cortisol was analyzed on Elecsys 2010 automatic analyzer (Roche Diagnostics)
Results. In healthy controls the morning and late evening salivary cortisol concentration were 0.588+/-0.308 ug/dl (n=76) and 0.199+/-0.108 ug/dl (n=74) respectively and morning serum cortisol concentration was 14.34+/-5.14 ug/dl (n=31). In healthy pregnant women morning salivary cortisol concentrationsin I, II and III trimester of pregnancy, and 3-6 months postpartum were: 0.676+/-0.333 ug/dl (n=64), 0.776+/-0.339 ug/dl (n=74), 0.847+/-0.314 ug/dl (n=74) and 0.611l+/-0.283 ug/dl (n=35) and evening salivary cortisol concentration was:0.164+/-0.081 ug/dl (n=47), 0.187+/-0.100 ug/dl (n=67), 0.246+/-0.137 ug/dl (n=68) and 0.151+/-0.103 ug/dl (n=42) respectively.Comparedtononpregnant controls the durinal cycle of salivary cortisol during the pregnancy was preserved.
Conclusions. We conclude that during pregnancy the increase of morning salivary cortisol is much lower than of the serum cortisol. The reference values of late evening salivary cortisol for healthy pregnant women may be within the same range as for healthy controls.

Endocrinology

0442

THE VALUES OF THYROTROPIN AND FREE THYROXINE IN BLOOD SERUM OF POSTMENOPAUSAL WOMEN IN THE PREŠOV REGION

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Background. The problem of osteoporosis, its genesis, risk factors, therapy and prevention belong to the issues to which experts and general public pay attention. The most serious complication of osteoporosis is femoral neck fracture, which affects approximately three times more women than men. Menopause and oestrogen deficit are also important risk factor in genesis of postmenopausal osteoporosis. It is therefore necessary to pay attention not only to the therapy, but also to osteoporosis prevention. Secondary, hormonally conditioned osteoporosis can be caused by states of either deficit or, on the contrary, excess of hormones. From the point of view of osteoporosis, clinically important thyroid hormones, parathormon, corticoids, insulin, growth hormone, prolactin and others. Hyperthyroidism is one of the factors related to osteoporosis genesis.
Methods. In this study we focused on monitoring of thyrotropin (TSH) a free thyroxin (FT4) in blood serum of 100 postmenopausal women irrespective of thyroid gland diagnosis or preceding longtime thyroxin therapy.
Results. In the observed group, mean values of thyrotropin 2.997 mU/L (minimum 0.30 mU/L, maximum 16.89 mU/L). Average value of free thyroxine (FT4) was 15.501 ng/L (min. value 11.20 ng/L, max. value 26.47 ng/L).
Conclusions. An important factor that encourages the development of osteoporosis is a violation of thyroid function. The thyroid gland is involved in calcium metabolism.

0443

EXPRESSION OF A SUBSET OF MICRORNAS IN CLINICALLY NON-FUNCTIONING PITUITARY ADENOMAS CORRELATES WITH TUMOR SIZE

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Background. MicroRNAs (miRs) are small (16-29 nt), non-coding RNA molecules which regulate protein expression posttranscriptionally via RNA interference. Pituitary adenomas are the most common intracranial tumors, but the genetic background of their pathogenesis is poorly characterized. Several previous studies including ours demonstrated that altered miR expression profile detected in pituitary tumors compared to normal tissue may be involved in the pathogenic process.
Objective. To identify biological pathways potentially involved in growth of pituitary tumors altered by miRs.
Methods. Expression profile of miRs in 8 clinically non-functioning adenomas (NFPA) and in 4 normal pituitary tissues was determined using miR array based on quantitative real-time PCR. Pathway analysis was performed by the DIANA-mirPath tool using TargetScan v5. target prediction software followed by enrichment analysis of multiple miR target genes by Pearson's Chi-squared test.
Results. Of the 457 miRs detected in both NFPA and normal pituitary tissues, 162 were significantly under- or overexpressed in NFPA compared to normal pituitary tissues. Whole array based pathway analysis indicated several previously suggested pathway alterations. Expression of 18 miRs was found to be negatively correlated with tumor size. Of these 18 miRs, 6 were underexpressed in tumor tissue. Pathway analysis for these miRs revealed involvement of Wnt, PI3K/Akt and MAPK signaling pathways.
Conclusions. miR expression profiling is a suitable method for identification of novel biomarkers for pituitary tumor progression. Our results support and further validate that the previously suggested signaling pathways potentially involved in pituitary tumorigenesis may be influenced by miRs.

Endocrinology

0444

CORRELATIONS BETWEEN ENDOCRINE MARKERS AND ULTRASONOGRAPHY IN OVARIAN RESERVE ASSESSMENT

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Background. The assessment of the ovarian reserve is mandatory in women undergoing assisted reproduction.Growing evidence indicates that Anti-Mullerian Hormone (AMH) is the best biochemical marker of ovarian follicular status, as its decline through reproductive life appears to mirror the decay in the number of non-growing primordial follicles. We evaluated the correlation between serum AMH levels and other markers of functional follicular reserve.
Methods. 40 women aged 20-45 yrs, divided into 3 groups, were studied: control group (n=20), non-PCOS infertility (n=11) and polycystic ovary syndrome(PCOS) (n=9).
Each group wassubdivided into 4 age-related subgroups: 20-29yrs; 30-35yrs, 36-39yrs and 40-45 yrs.
AMH, gonadotrophs, sex hormones (E2, P) were measured in serum samples obtained in the early follicular phase of the menstrual cycle (days 3-5); PRL, TSH and FT4 were measured to eliminate hyperprolactinemia or thyroid diseases. Antral follicle count was performed by ultrasonography.
Results. AMH levels significantly decreased with chronological age (r=-0.96, p=0.003), in the whole lot.
There was a significant negative correlation between mean values of AMH and E2 (r=-0.47, p=0.001), AMH and FSH (r=-0.89, p=0.05). AMH level in PCOS patients is significant higher than in control group (p<0,01). A strong positive correlation between AMH and the number of antral follicles was found (p<0,05).
Conclusions. Currently available ovarian reserve tests do not provide sufficient evidence to be solely considered ideal. Serum-AMH measurements may be relevant in assessing ovarian reservein various clinical conditions. We sustain a gradual approach by differentiated treatment schemes for improving results in assisted reproduction.

0445

EVALUATION OF SALIVARY CORTISOL IN PATIENTS WITH ADRENAL CARCINOMA TREATED WITH MITOTANE: COMPARISON BETWEEN TWO METHODS

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Background. Mitotane treatment, an adrenal carcinoma(AC) therapeutic, may lead to corticoadrenal insufficiency(Addison's Disease-AD), requiring substitutive hydrocortisone therapy. Therapy efficacy may be evaluated by testing for cortisoluria and cortisolemia (invalidated by mitotane-induced increase of CBG and clearance levels) and plasma ACTH levels (indirect evaluation of corticosteroid). Salivary cortisol(SC) reflects a free fraction of plasma cortisol and may help improve patient monitoring during mitotane treatment.
The aim of the study is theSC evaluation as a follow-up tool for AC-affected patients byECLIA and HPLC coupled to tandem-mass spectometry(LC-MS/MS).
Methods. We enrolled 5 mitotane-treated patients and 1 control patient with AD, all undergoing hydrocortisone therapy. Samples were collected the same day: blood (n=60) and saliva (n=60) cortisol was assayed by ECLIA,using Roche ModularE170, while SC was measured also by LC-MS/MS.
Results. the comparison of SC values, obtained by ECLIA and LC-MS/MS, shows a correlation of $R^2=0,76$. Nonetheless, results obtained by ECLIA are significantly(P=0,0017) higher than those obtained by LC-MS/MS. Indeed, a patient's10.00am SC value was31,72nmol/L by ECLIA, whereas LC-MS/MS measured 13,52nmol/L. Furthermore, we observed secondary peaks at 10.01(cortisol peaks at 10.62r.t.) presumably due tosteroidogenesis intermediates. Since steroidogenesis in AD is absent, both methods yield the same results ($R^2=0,99$). SC concentration for this patient is the same for both assays (ECLIA 10,26nmol/L;LC-MS/MS 10,21nmol/L).
Conclusions. SC measured by LC-MS/MS, an interference-free method, permits specific quantifications of hydrocortisone, omitting steroidogenesis intermediates. For this reason, LC-MS/MS offers a sturdy system for substitutive therapy monitoring, and may be used as reference method.

Endocrinology

0446

ELEVATED ALDOSTERONE LEVELS IN PATIENTS TAKING DROSPIRENONE, A SYNTHETIC PROGESTIN WITH ANTIMINERALOCORTICOID EFFECTS FOUND IN COMBINATION WITH ETHINYLESTRADIOL IN CONTRACEPTIVE FORMULAS (YASMINE®): IMPACT ON THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM EVALUATION

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Background. We were surpised to observe very high levels of aldosterone (sometimes >1000 pg/mL) in young women who underwent a screening for primary aldosteronism. We found that most of these women were taking drospirenone (a progestatin presenting analogy of structure with aldosterone), a molecule found in some contraceptive formulas like Yasmine®. The antimineralocorticoid effect of drospirenone counteracts the estrogen stimulation action of the renin-angiotensin-aldosterone system, thus lowering water retention symptoms due to classical oral contraception. We aimed to evaluate the interference of drospirenone with plasma aldosterone concentration (PAC), plasma renin activity (PRA) and potassium levels in 25 healthy women taking oral contraception (11 with an ethinylestradiol-drospirenone association, 14 with another contraceptive formula).
Methods. PAC was evaluated by an immunoradiometric method (Aldo-CTK, DiaSorin, Stillwater, MN). PRA was calculated as the difference observed in angiotensin I levels after incubation of the samples 3 hours in parallel at +4°C and +37°C (REN-CTK, DiaSorin). Potassium was determined on Modular (Roche Diagnostics, Mannheim, Germany).
Results. Age was comparable in both group. Aldosterone was significantly (p<0.001) higher (510; 95%CI: 272-1127) vs. 119; 95%CI:90-239) pg/mL) in the drospirenone group while potassium and PRA were not different. As a consequence, ARR was significantly (p<0.01) increased in the drospirenone group (24.4 (95%CI:12.7-44.4) vs. 10.0 (95%CI: 7.4-15.2).
Conclusions. Drospirenone, with its antimineralocorticoid effect, lowers water retention symptoms. As a consequence, the patients present significantly higher aldosterone (and ARR) levels, not explained by an increase in renin activity. Another contraceptive methodology should be proposed before screening a patient for primary hyperaldosteronism.

0447

VALIDATION OF THE ABBOTT ARCHITECT 25OH-VITAMIN D ASSAY

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Background. To validate the Abbott Architect 25-OH vitamin D assay, a new method for 25(OH)D determination.
Methods. Determination of repeatability, reproducibility, functional sensitivity, recovery, linearity, accuracy profile and determination of 25(OH)-vitamin D2 recovery with native samples. Comparison with DiaSorin Liaison and RIA.
Results. Intra and inter-assay CVs: <6% at 10.1ng/mL and 2.1% at 92.6ng/mL. Detection limit: 2.0 ng/mL and functional sensitivity: 9 ng/mL. Mean recovery and linearity estimated according the CLSI Guidelines 96.4±5.2%.Between 9.3 and 92.6 ng/mL, the accuracy profile shows that the risk that one result falls out of the ±20% acceptance limits is <5%, indicating that the method is completely validated. The recovery of 25(OH)D2 is 75,8% (95% CI: 61,9 to 89,7%).
On 253samples, the regression equation with the DiaSorin RIA and Liaison were respectively Architect =1.02XDiaSorin_RIA+ 1.2 (r²=0.78)and Architect= 1.07X DiaSorin_Liaison+3.7 (r²=0.875). Bland-Altman plots show that for values >50 ng/mL, the Architect and the Liaison tend to systematically overestimate the 25(OH)D levels obtained with the DiaSorin RIA. On the samples distributed for the October DEQAS distribution, the Architect levels were in accordance with the mean of LCMSMS users, except an overestimation of 10% of the samples presenting the highest level (40 ng/mL).
Conclusions. Abbott Architect 25OH-vitamin D is a robust method, with good CVs, linearity, recovery and good agreement with DiaSorin RIA for values <50 ng/mL. The accuracy profile shows that the method is completely validated between 9.3 and 96.2 ng/mL. Even if not 100%, the recovery of the 25(OH)D2 remains acceptable.

Endocrinology

0448

DIAGNOSTIC ACCURACY OF PTH AND CALCIUM LEVELS IN HYPOCALCEMIA AFTER TOTAL THYROIDECTOMY

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Background. Hypocalcemia is the most frequent complication after total thyroidectomy. The aim of this study was to identify patients with low risk of hypocalcemia after total thyroidectomy, on the basis of PTH and Ca^{2+} level during the first postoperative day.
Methods. 142 patients undergoing total thyroidectomy were prospectively studied. The causes of total thryoidectomy were: multinodular goiter, Graves´s disease and thyroid nodule. PTH was quantified on Modular analytics E170 by electrochemiluminiscence immunoassay and Ca^{2+} on GEM Premier 3000 by electrical conductance. ROC curve analysis of PTH and calcium were performed to exclude patients who did not develop hypocalcemia. MedCalc.v9.0 was used.
Results. The areas under the ROC curves (AUC): AUC-PTH 0.987 [CI: 95%, 0.952 – 0.998], cut-off PTH: 14.89 pg/ml, S: 92.3 %; E: 97.4 %; PPV: 88.9 %; NPV: 98.3 %; positive +LR: 35.69; -LR: 0.08, AUC-Ca^{2+} 0.951 [CI: 95 %, 0.902 – 0.980] cut-off Ca^{2+}: 4.0 mg/ml, (S: 80.8 %; E: 96.6 %; PPV: 84.0 %; NPV: 95.7 %; +LR: 23.42; -LR: 0.2) and AUC-PTH_ Ca^{2+} 0.970 [CI: 95 %, 0.927 – 0.991] cut-off PTH_Ca^{2+}: Ca^{2+} ≤ 4.0 mg/ml and PTH≤ 14,89, (S: 100 %; E: 94.0 %; PPV: 78.8 %; NPV: 100 %; +LR: 16.57; -LR: 0.00).
Conclusions. PTH is better than Ca^{2+} levelto exclude hypocalcemia after total thyroidectomia. The combination of PTH and Ca^{2+} levels has better sensitivity and NPV than separately. This means that when PTH and Ca are above the cut off the probability of developing hypocalcemia is very low (NPV = 100).

0449

AMH AND FSH PREDICT OVARIAN RESERVE AND CHANCE OF SUCESSFUL PREGNANCY

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Background. Delayed child bearing has resulted in increase of infertility from 6% in women to > 30%.This has created a demand for method to monitor fertility.
After birth, AMH is produced by the ovarian granulosa cells and becomes undetectable after menopause, its concentration directly related to antral follicle count, indicating ovarian function.
In the present study we evaluated role of AMH, FSH, in assisted reproduction, their role in predicting the ovarian response and chance of successful pregnancy.
Methods. 46 Patients in the age group of 20-40 were selected, AMH (EIA method); FSH (ECLIA, Cobas e411), were measured, during the preovulatory phase and results were compared with outcome.
Results. The results were analyzed using SPSS v17. The result was followed up with Invitro fertilization outcome and patients were divided into 2 groups based on outcome, group I whom assisted reproduction did not help in conception or there was premature loss of conception. Group II patients whom the IVF resulted in conception and favorable outcome with a live birth. The mean serum AMH was more (p<0.001) in patients who conceived successfully, serum FSH concentration was less (p<0.003) in this group with AMH being more sensitive (83%) than FSH (79%) in predicting the pregnancy outcome
Conclusions. Based on our results AMH can be considered as ideal test to be performed before the initiation of cycle which was significantly higher in patients who achieved clinical pregnancy and AMH was far better than FSH in predicting the chance of successful pregnancy.

Endocrinology

0450

TESTOSTERONE BIOAVAILABLITY AND BODY BURDEN OF *P,P'*-DDE WITH NON-OCCUPATIONAL EXPOSURE TO THE PESTICIDE DDT

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Background. The DDT metabolite *para,para'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) bio-accumulates and purportedly has anti-androgenic properties. Impaired semen quality has been reported with DDT exposure.
Methods. A cross-sectional study was conducted in the Limpopo Province where DDT has been used for malaria vector control in designated areas since 1945. Cases lived in huts sprayed with DDT and controls lived in non-spraying areas. Total-, free- and bio-available serum testosterone (t-T, f-T and bio-T), luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG) were determined by RIA, serum albumin (S-Alb) by automated analyser and *p,p'*-DDE using GCMS.
Results. t-T, f-T, bio-T and S-Alb were significantly higher, and SHBG and FSH significantly lower in cases (n=298) compared with controls (n=232). A dose-dependent effect was observed in cases across *p,p'*-DDE quartiles for f-T and bio-T (linear trend p<0.008 and p<0.004 respectively). t-T concentrations above the upper reference limit for 18-40 year old males (27.8 nmol/L) were observed in 30(13%) controls and 83(28%) cases. t-T subgroups (≤ and >27.8 nmol/L) in cases were compared. Higher SHBG (58.17±5.64 vs 34.23±17.93), lower S-Alb concentrations (43.37±5.64 vs 46.84±5.69), and lower %f-T (1.85±0.60 vs 2.13±0.56) and %bio-T (44±6 vs 54±14) were associated with higher t-T (p<0.0001). LH and FSH of the subgroups were not significantly different. Higher *p,p'*-DDE concentrations and lower *p,p'*-DDE/ *p,p'*-DDT ratios were associated with higher t-T (p<0.0001), the lower ratios suggesting more recent exposure to DDT.
Conclusions. This study illustrates changes in testosterone homeostasis associated with a body burden of *p,p'*-DDE in young men.

0451

IS THERE A CORRELATION BETWEEN INITIAL PROLACTIN CONCENTRATION AND PERCENTAGE RECOVERY AFTER POLYETHYLENE GLYCOL PRECIPITATION?

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Background. Macro-prolactin (MPRL) is a common cause of interference in prolactin immunoassays that can lead to diagnostic confusion and mismanagement of hyperprolactinaemic patients. Prolactin methods differ in their response to MPRL, which is routinely screened for using polyethylene glycol (PEG) precipitation. Previous studies indicate a possible correlation between initial prolactin concentration and the percentage recovery after PEG precipitation.
In this study we examine this potential relationship. We also look at the incidence of MPRL in a large teaching hospital using the Beckman Access analyser.
Methods. 119 patient samples over a range of prolactin concentrations (37-9126 mIU/L) underwent PEG precipitation and the correlation between initial concentration and post-PEG values was determined.
839 samples from hyperprolactinaemic patients (screening cut off: 600 mIU/L) were identified using the laboratory information system. The post-PEG recovery values were identified for each sample and the values were compared with the monomeric prolactin reference range for the method (70-469 mIU/L)
Results. The overall mean % recovery after PEG precipitation was (100% SD 10%), comparison of results under and over 600mIU/L showed no significant difference (mean100%, SD 11% and mean 99%, SD 15%, p=1). This is different from published data using prolactin standards (mean recovery of 122%).
Audit data showed macroprolactinaemia in 22 patients giving an incidence rate of 2.6% using the Beckman assay.
Conclusions. This study has not shown a correlation between initial prolactin concentration and post-PEG recovery values. The study also shows a low but significant number of cases of MPRL are identified using the Beckman assay.

Endocrinology

0452

RECOGNISING MACRO-TSH: A RARE CAUSE OF INAPPROPRIATELY HIGH TSH VALUES

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Background. A 72 year old lady presented to the endocrinology clinic with lethargy and past medical history of osteoarthritis, iron deficiency anaemia, chronic gastric ulcer, diverticular disease and coeliac disease. She did not report symptoms of cold intolerance, weight gain, skin or hair changes and was clinically euthyroid with no palpable goitre. Thyroid function tests were requested but returned unexpected Results.
Methods. Thyroid function tests were performed on the Beckman Coulter Unicel® DxI 800 chemiluminescent immunoassay analyser and compared on the Roche E170* electrochemiluminescence immunoassay analyser.
Results. Repeated analysis showed persistent, grossly elevated TSH, normal Free T4 and Free T3 on both analysers. Routine biochemistry and MRI scan of the pituitary were normal. Samples were tested for familial dysalbuminaemic hyperthyroxinaemia and heterophilic antibodies to T4 and T3; which were all negative. Samples were analysed by gel filtration chromatography and the immunoreactivity profile revealed a heavy form of TSH (molecular weight approximately 200kDa) that dissociated after pretreatment with acidified glycine buffer pH 3.5, which dissociates immunocomplexes and disappeared after pretreatment with polyethylene glycol.
Conclusions. Elevated TSH levels were probably due to macro-TSH (possibly TSH bound to anti-TSH antibodies) that was causing assay interference. Macro-TSH may not be biologically active and that would explain the patient profile. This case highlights the importance of investigating biochemical results that are not consistent with the clinical findings. Macro-TSH should be considered in patients with unexpectedly high TSH values to prevent misdiagnosis of subclinical hypothyroidism.
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0453

AMH GEN II: A COMPARISION OF RESULTS VERSUS DSL AMH ELISA AND REFERENCE INTERVAL DATA

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Background. Anti-Müllerian Hormone (AMH) profiles in women decrease through reproductive life and mirror the decline in the number of primordial follicles – the ovarian reserve. Correspondingly, one of the main clinical utilities of the assay is in the assessment of ovarian potential. Until recently two ELISA assays have been available; from Diagnostics Systems Laboratories (DSL) and Immunotech. These are standardized differently and give different values of AMH. The two assays have recently been harmonized by replacing the DSL ELISA with AMH Gen II. The objective of this study was to assess the performance of the new assay versus the original assay and present reference intervals for women of childbearing age.
Methods. AMH was measured in serum using AMH Gen II and DSL ELISA assays.
Results. The assays were compared in samples from 196 females ($y = 1.43x – 0.14$, $R^2 = 0.9891$). Reference interval data for the AMH Gen II assay was obtained from 1607 women of childbearing age (24-53 years) in whom polycystic ovarian syndrome and premature ovarian failure were excluded. Statistical analysis was performed using SAS* 9.2 software and 5th and 95th percentiles calculated by age. The following AMH cut points were found for AMH Gen II assay: Fertile range 1.3 – 7. 0 ng/ml, reduced fertility <1.3 ng/ml.
Conclusions. This study provides AMH cut points as a guide to interpretation and demonstrates that results from AMH Gen II assay correlate well with the original DSL ELISA assay, although results are approximately 40% higher.
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Endocrinology

0454

RELATIONSHIP BETWEEN PLASMA LEVELS TNF-α RECEPTORS AND RESPIRATORY FUNCTION IN OBESE PATIENTS

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Background. A potential interaction between pulmonary function, abnormal adipose tissue activity, and systemic inflammation has been suggested. This study explores the relationship between circulating soluble TNF-α receptors (sTNF-R1 and sTNF-R2) and respiratory function parameters in obese subjects. Thirty-one non-diabetic morbidly obese women with a history of non-smoking and without prior cardiovascular or respiratory disease were prospectively recruited in the outpatient Obesity Unit of a Hospital Universitary Vall d'hebron, Barcelona.
Methods. Pulmonary function testincluded aforced spirometry, static pulmonary volume measurements and arterial gas blood sampling. Circulating levels of sTNFR-R1 and aTNF-R2 were determined using ELISA (BLK Diagnostic, Spain). Statistical analysis included a multivariate regression analysis taking into account the potential confounders.
Results. sTNF-R1 positively correlated with BMI (r=0.571, p=0.001) and arterial carbon dioxide pressure (PaCO$_2$, r=0.381, p=0.038), but negatively with forced expiratory volume in 1 s (FEV$_1$, r=-0.437, p=0.012), maximum midexpiratory flow (FEF$_{25-75}$, r=-0.370, p=0.040) and forced vital capacity (FVC, r=-0.483, p=0.005). However, no correlation between sTNF-R2 and BMI and either pulmonary function tests or arterial blood samples was observed. Multiple linear regression analysis showed that sTNF-R1 independently predicted FEV$_1$ (beta=-0.437, p=0.012) and FVC (beta=-0.483, p=0.005).
Conclusions. Circulating levels of sTNF-R1, but not sTNF-R2, are related to reduced lung volumes and airflow limitation in morbidly obese patients prior to the development of a clinically recognized respiratory disease. Therefore, studies addressed to evaluating the potential beneficial effect of anti- TNF-α agents on pulmonary function tests in obese subjects seem warranted.

0455

COMPARISON OF PTH MEASUREMENT IN CHRONIC RENAL DISEASE PATIENTS WITH 3 DIFFERENT ASSAYS: ARCHITECT® I2000 (ABBOTT), UNICEL® DXI 800 (BECKMAN COULTER) AND COBAS® E411 (ROCHE DIAGNOSTICS)

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Background. Parathyroid hormone (PTH) monitoring is required for the follow-up of the bone status of patients with chronic renal disease. The new Kidney Disease Improving Global Outcomes (KDIGO) recommend to adjust therapy to maintain PTH values between 2 and 9 fold the upper PTH limit used in the laboratory, taking into account, the change with time together with other biological parameters (Calcium, phosphorus, 25-OH Vitamin-D, bone alkaline phosphatase).
Methods. PTH (UniCel® DxI 800 (Beckman Coulter) was measured on serum in 77 hemodialysis patients [243 pg/mL (31 - 2199)] and 93 patients after renal transplantation [57 pg/mL (17 - 832)] (median; range). Assays were performed on the same day with: Architect® i2000 (Abbott), UniCel® DxI 800 (Beckman Coulter) and Cobas® e411 (Roche Diagnostics). Assays were compared with Passing-Bablok fit (Analyse-it® 2.05 for Microsoft Excel).
Results. In hemodialysis patients, comparisons were : Architect = 1.23*Roche (1.18-1.25) +18.01 (12.64-27.91), DxI 800= 0.88*Cobas (0.85-0.91) - 8.63 (-15.08- -2.47) ; Architect = 1.35*DxI 800(1.29-1.41) + 40.04 (27.42-52.24). Comparisons for renal transplanted patients were : Architect = 1.36*Cobas (1.33-1.41) - 8.09 (-11.09- -5.54); DxI 800 = 0.93*Cobas (0.90-0.98) - 6.07 (-9.58- -3.79); Architect = 1.44* DxI 800 (1.39-1.49) + 1.96 (-0.43-4.54) (95 % CI for all parameters). These results uncover significant differences between PTH assay in chronic renal diseases.
Conclusions. Significant differences between PTH assays in patients with chronic renal disease have to be taken into account in clinical practice.

Endocrinology

0456

VITAMIN-D RELATED PARATHYROID HORMONE REFERENCE RANGES AND THEIR IMPACT ON THE DIAGNOSIS OF MILD PRIMARY HYPERPARATHYROIDISM

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Background. Parathyroid hormone (PTH) reference ranges vary according to the 25-OH-Vitamine D (25(OH)D) status. The importance of this effect and its impact upon diagnosis of mild primary hyperparathyroidism (PHPT) remains a matter of debate.
Methods. Intact PTH (UniCel® DxI 800, Beckman Coulter) and 25(OH)D (Liaison®, Diasorin) were measured in 254 healthy subjects, (18 - 65 years), in Belgium (end of winter - summer). A 25(OH)D cut-off of 50 nmol/L constituted 25(0H)D-replete [7.0 (5.0 – 15.2 nmol/L); n = 127] and 25(OH)D-deficient [3.2 (0.7 – 5.0 nmol/L); n = 127] subgroups (median; range). 49 patients with surgically proven mild PHPT were evaluated. PTH diagnostic efficiency was evaluated by ROC analysis. Control PTH correlated with calcium. The related 95 % bivariate normality density ellipses werecompared to values of the PHPT patients(JMP®, SAS Institute).
Results. PTH confidence intervals (CI) were lower in 25(0H)D-replete than 25(0H)D-deficient controls: 3.4 (1.7 – 7.1) vs. 4.13 (2.1 – 8.6) (geometrical mean; 95 % CI) (P<0.001). PTH correlated with 25OHD (r = -0.3192; p<0.0001). AUCs for PHPT vs. replete or deficient 25(OH)D controls were 0.970 (SE: 0.0116) and 0.920 (SE: 0.0208), respectively (P = 0.0358). The 95 % bivariate normality density ellipses for replete and deficient 25(OH)D controls differed slightly, and none of the PHPT patients did overlap any of the ellipses.
Conclusions. Referring to 25(OH)D-replete healthy subjects improves diagnosis of mild PHPT, considering PTH alone. The 25(0H)D status of the reference population has no influence on the PHPT diagnosis when PTH and calcium are used in association.

0457

DIFFERENCES IN HEMATOLOGY AND BIOCHEMICAL PARAMETERS IN DIABETIC PATIENTS

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Background. This study related to investigated differences in hematology and biochemical parameters in patients with Diabetes Mellitus(type1 and Diabetic Retinopathy and type 2).A direct relation between platelet dysfunction and the development of diabetic complication has yet to be established.Some studies have suggested that the enhanced activation of circulating platelets is particularly apparent in diabetics with microvascular disease.
Methods. In This study was investigated hematological and biochemical parameters in differences between two type of diabetes(type1 N=160;type 1 and Diabetic Retinopathy,N=48 and type 2 N=180) in relation to a control population. Hematological parameters was analyzed In K_3EDTA blood used hematological analyzer Sysmex KX-21. The concentration of glucose, HbA1C,Total cholesterol, TG, HDL,VLDL and LDL was determined in Cobas Integra 700 analyzer.The Proptrombin time and fibrinogen concentration are measured in BFTII analyzer.
Results. Our results showed an increased in hematocrit, leucocyte,lymphocyte and monocyte number in diabetic patients,while MCV,MCH(affected of the glucose concentration) and neutrophyls number are decrease. Patients with type 1 diabetes showed an increase in erythrocytes,Hct, leucocytes, lymphocytes (<p,0.05) and glucose(p< 0.01) concentration compared with the control. Hematocrit and platelets index(PDW,MPV,P-LCP) are significantly increased (p<0.05) while neutrophils and monocytes are decreased (p<0.05) in patients with type 1 diabetes and AMI in relation with type 2 diabetes.Diabetic patients have significantly higher plasma fibrinogen concentration compared with control group(4.75+/-0.95 g/Lvs.c.g.3.15+/-1.1 g/L,p< 0.05). Significant elevation in total serum cholesterol and LDL-cholesterol levels is observed in IDDM cases. Increased serum Triglycerides and VLDL-cholesterol levels are not statistically significant. Serum HDL–cholesterol levels are significantly decreased
Conclusions. Our results suggest that platelet hyperfunction in diabetic patients may by implicated in the pathogenesis of diabetic retinopathy.These results established an altered in plateled volume indices in insulin dependent diabetics suggesting that platelets may involve in developing micro and macro vascular complication in patients.

Endocrinology

0458

GENETIC DETERMINANTS OF SERUM TESTOSTERONE CONCENTRATIONS IN MEN

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Background. Testosterone concentrations in men are associated with cardiovascular morbidity, osteoporosis, and mortality and affected by age, smoking, and obesity. Because of its high heritability, we investigated the genetic determinants of testosterone concentrations in men.

Methods. We performed a genome-wide association study of testosterone concentration among 14,429 Caucasian men from 10 cohorts. Seven cohorts were designated discovery cohorts (n = 8,938), one *in silico* replication cohort (n = 871), and two *de novo* replication cohorts (n = 4,620). Inverse variance weighted fixed-effect model meta-analysis of study-specific results was performed. Serum testosterone < 300 ng/dl was deemed low.

Results. Two independent variants at the sex hormone-binding globulin *(SHBG)* locus (17p13-p12) reached genome-wide significance in the discovery cohorts and were confirmed in the replication cohorts (combined p-value rs12150660, $p=1.2 \times 10^{-41}$; rs6258, $p=2.3 \times 10^{-22}$). Subjects with ≥3 risk alleles of these variants had 6.5-fold higher risk of having low serum testosterone than subjects with no risk allele. The rs5934505 polymorphism near *FAM9B* on the X chromosome was associated with total testosterone ($p=5.6 \times 10^{-16}$) and free testosterone ($p=6.7 \times 10^{-15}$) concentrations. The rs6258 polymorphism in exon 4 of *SHBG* affected SHBG's affinity fortestosterone binding and the free testosterone fraction ($p < 0.01$).

Conclusions. Genetic variants in the *SHBG* locus and on the X chromosome are associated with a substantial variation in testosterone concentrations and increased risk of low testosterone. rs6258 is the first reported *SHBG* polymorphism, which affects testosterone binding to SHBG and the free testosterone fraction and could influence the calculation of free testosterone using law-of-mass-action equation.

Endocrinology

0459

EIGHT COMMON GENETIC VARIANTS ASSOCIATED WITH SERUM DHEAS LEVELS SUGGESTS A KEY ROLE IN AGEING MECHANISMS

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Background. Dehydroepiandrosterone sulphate (DHEAS) is the most abundant circulating steroid secreted by adrenal glands - yet its function is unknown. Its serum concentration declines significantly with increasing age, which has led to speculation that a relative DHEAS deficiency may contribute to the development of common age-related diseases or longevity.

Methods. We conducted a meta-analysis of genome-wide association data with 14,846 individuals and identified eight independent common SNPs associated with serum DHEAS concentrations.

Results. Genes at or near the identified loci include *ZKSCAN5* (rs11761528; $p=3.15\times10^{-36}$), *SULT2A1* (rs2637125; $p=2.61\times10^{-19}$), *ARPC1A* (rs740160; $p=1.56\times10^{-16}$), *TRIM4* (rs17277546; $p=4.50\times10^{-11}$), *BMF* (rs7181230; $p=5.44\times10^{-11}$), *HHEX* (rs2497306; $p=4.64\times10^{-9}$), *BCL2L11* (rs6738028; $p=1.72\times10^{-8}$), *CYP2C9* (rs2185570; $p=2.29\times10^{-8}$). These genes are associated with type 2 diabetes, lymphoma, actin filament assembly, drug and xenobiotic metabolism, and zinc fingers. Several SNPs were associated with changes in gene expression levels and the related genes are connected to biological pathways linking DHEAS with ageing.

Conclusions. This study provides much needed insight into the function of DHEAS.

Endocrinology

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ANTI-MÜLLERIAN HORMONE (AMH) AS IMPORTANT MARKER OF OVARIAN RESERVE

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Background. The estimation of ovarian reserve is highly important for evaluation of woman fertility. AMH is produced from small primordial folicles of ovary and is proportional to the their number wich declines with increasing age according to apoptosis and ovulation. The concentration of AMH represent the promising marker for the size of the ovarian folicle pool and ovarian reserve.
Methods. The concentration of AMH was measured in serum samples of 82 women with different age (16-76). The enzyme linked immunosorbent assay (ELISA) was used for measuring AMH.
Results. We confirmed the higher concentration of AMH (ng/ml) with the widest value range in the youngest group (age: 16-26): 4,099± 2,150; in a group (age: 27-37) the average conc. was 1,583 ± 0,942 and in a group (age: 38-48): 0,395 ± 0,366. In the oldest group of women (age: 49 – 76) we have found the lowest concetration < 0,03 ng/ml).
Conclusions. Our data support that serum levels of AMH declines with age and can be used as a marker of ovarian aging. The wide range of concentration, especialy in the youngest group, confirm that ovarian reserve is very different among the homogene age group and indicates their different fertility. The lowest concentration of AMH in the oldest group indicates the menopause. The ability of AMH to measure ovarian reserve provide a vital indication of remaining fertility and help to predict the probable success of in vitro fertilisation as well.

0461

CONGENITAL ADRENAL HYPERPLASIA-LATE DIAGNOSES-CASE PRESENTATION

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Background. Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) presents a group of hereditary disorders occurring worldwide with an overall incidence of 1:13.000-1:15.000 live births. As a regional referent laboratory for CAH during the 2010 we had 3 more confirmed cases of CAH.
Aim. Evaluation of the clinical findings in children with congenital adrenal hyperplasia in our region regarding the late diagnoses.
Methods. The clinical phenotype and hormonal profile was evaluated in this case by a physician to categorize the patient with classical salt waiting, simple iridizing, or neoclassical forms of 21-OHD. Serum 17-OH-Progesterone (ng/dL or nmol/L) was tested by the Enzyme Immunoassay method (DRG-Diagnostics). We have also tested Androstendione, DHEAS, Cortisol and ACTH.
Results. 15 years old girl with nonclasssical form of CAH. Anamnesis: pubarcha has started when she was 6, but she was never referred to an endocrinologist. Currently she has clitoromegalia, deepening voice, excessive body hair, facial hair, severe acne and primary amenorrhea. Hormonal profile:17-OH Progesterone=160.0 ng/ml; Testosterone=6.60 nmol/L; Androstendione=7.37; DHEAS=6.23 μg/ml; ACTH 114.1 pg/ml; Aldosterone 481 pg/ml. Based on the mutation analysis: CYP-21 gene in her family, CAH disease is presented not only in our case but in her sister and brother. All of them have mutation in Egzon 1/Egzon 4 that clinically appears as NC-CAH.
Conclusions. Initiation of screening program for CAH in Kosovo and referral of children with NC-CAH as soon as possible is of great importance for early diagnoses in children, to avoid the life threading crisis, clinical and psychological complications.

Endocrinology

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ASSESSMENT OF PTH RESPONSE, USING NEW DIASORIN 1-84 PTH THIRD GENERATION ASSAY, TO ORAL 50000 UNITS VITAMIN D3 LOAD

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Background. Till present measurement of intact PTH serum level in clinical research and practice was subject to assay induced variability due to use of antibodies that detect PTH fragments. The new DiaSorin 1-84 PTH, is a third generation assay that measures only 1-84 PTH and is supposed to provide better precision in the assessment of this hormone level.
Methods. In order to test this hypothesis serum PTH was assed in 57 healthy volunteers that received oral vitamin D3 load of 50000 IU and in 31, that received placebo. Serum samples for 25OHD and PTH were collected before vitamin D administration and at day 1, 7, 14 and 28 thereafter. PTH was measured with Roche's 2nd generation assay and with new DiaSorin 1-84 PTH. Results were assessed using two way repeated measures multivariate model with Bonferroni correction.
Results. Serum 25OHDsignificantly increased at the treatment group from day 0 to 14 and 28. A weak significantnegative correlation was demonstratedbetween 25OHD and PTH serum levels: r -0.166 p=0.001 for Roche and r- 0.267 p=0.0001 for PTH Liason that displayed a superior correlation to the increase in serum 25OHD, p=0.01.
Conclusions. Correlation between Vitamin D and the DiaSorin new 1-84 PTH is better than the correlation with the intact PTH (Roche). This may indicate that measuring the 1-84 PTH might have a more accurate clinical value and might bring uniformity and a better tool for clinical and research evaluation

0463

EARLY BIOCHEMICAL MARKERS OF CARDIOVASCULAR RISK IN ADULT PATIENTS WITH DEFICIT ORGANIC GROWTH HORMONE WITHOUT CLINICAL MANIFESTATIONS OF METABOLIC SYNDROME

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Introduction. The deficit of GH / IGF-I in adults is recognized as a cause of clinically relevant cardiovascular disease, is associated with endothelial dysfunction and inflammation, but is not known whether this alteration is due to GH deficiency itself or is related to clinical manifestations of metabolic syndrome in these patients. This has particular relevance in patients with organic GH deficiency and may be important to assess the potential involvement of GH deficits "subclinical" or "functional" and increased cardiovascular risk.
Background. Assess the degree of endothelial dysfunction and inflammation in patients with adult GH deficiency in the absence of criteria for metabolic syndrome (MS).
Methods. We studied 47 patients with untreated GH deficiency and 33 healthy controls for age, sex and BMI equivalent. We have excluded both patients and controls with clinical or analytical criteria of MS. In all cases, laboratory tests were performed at baseline, metabolic profile , endothelial dysfunction in relation to cell adhesion, parameters of oxidative stress , and inflammation.
Results. No statistically significant differences either for BMI or metabolic parameters between the group of GH deficient patients and controls. The variables that showed significant differences P <0.05) among the group of patients with organic GH deficiency and controls were the variables of oxidative stress, SOD and total peroxides, molecular adhesion sCD40L, SP selectin and VCAM 1 and PCR-u flash and IL6.
Conclusions. Our results show that there are changes in the parameters of endothelial dysfunction in patients with GH deficiency who are not related to the presence of metabolic syndrome in these patients.

Endocrinology

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THE CLINICAL UTILITY OF MEASUREMENTS OF INSULIN-LIKE GROWTH FACTOR-I AND GROWTH HORMONE IN DIAGNOSIS AND MONITORING OF ACROMEGALY TREATMENT

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Background. Acromegaly is characterized by hypersecretion of growth hormone (GH). The diagnosis of acromegaly is based on both clinical and biochemical findings. The following biochemical tests may be used to supplement a diagnosis of acromegaly: random measurement of GH and mean 24-h GH serum concentrations, GH response to OGTT, and random measurement of insulin-like growth factor-I (IGF-I) concentrations. The aimof this study was the evaluation of clinical utility of GH and IGF-I measurements in patients group with suspected acromegaly.
Methods. Blood samples were collected into SST™ Plus tubes with separation gel (BD Vacutainer™). Serum GH test was performed on IMMULITE system (Siemens) by chemiluminescent detection technology. IGF-I was measured by radioimmunoassay (RIA), (DIAsource ImmunoAssays).
Results. 89 females (age range 28-80 years) and 55 males (age range 19-72 years) admitted with suspected acromegaly over three years (2008-2010). Diagnosis of acromegaly was proved for 39 females and 23 males. The median values for GH and IGF-I in female group were: 7.59 mU/l and 160.45 mkg/l and in male group – 2.36 mU/l and 193.15 mkg/l respectively. The weak positive correlation was observed between GH and IGF-I in both groups (r=0.24). GH levels demonstrated extensive variability ranging from <0.12 mU/l to 52.1mU/l in female group and from <0.12 mU/l to 98.4 mU/l in male group.
Conclusions. Results of GH and IGF-I measurements proved the clinical utility of both tests in diagnosis of acromegaly, treatment strategy and monitoring of patients after surgery or during pharmacotherapy.

0465

SERUM CYSTATIN C IN PATIENTS UNDERGOING THYROIDECTOMY

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Background. Cystatin C, as an inhibitor of cysteine proteinases, participates in the progress of tumour growth, invasion and metastasis. It has been evaluated as a marker for several neoplasms but not for thyroid carcinoma.It may be affected in different thyroid states. We evaluated the values of preoperative serum cystatin C in patients undergoing thyroidectomy and factors associated with it.
Methods. One hundred and seventypatients,without renal failure, undergoing thyroidectomy were included. Serum cystatin C, calcium and phosphate were measured on Architect c8000,T_3, T_4, FT_3, FT_4, TSH, anti-Tg,anti-TPO, parathormone (PTH) and 25(OH)VitD$_3$ on Architect i2000. Parameters evaluated for association with cystatin C were also demographics, diagnosis and thyroid weight. Separate analyses were performed regarding benign/malignant pathology and hyperthyroidism/hypothyroidism/euthyroidism.
Results. Mean patients' age was 52.7±3.8years (female:71.7%).Thyroid carcinoma was found in 48 (28.2%) whereas benign pathology in 122 (71.8%). Elevated cystatin C values were noticed in 57 (33.5%) (group A) while 113 (66.5%) had normal cystatin C (group B). Comparison between group A and B revealed that group A patients were older (57.7±2.1 vs. 50.8±1.2years, p=0.01), and had higher preoperative calcium (10.1±0.1 vs. 9.4±0.1mg/dl, p=0.005) and PTH (60.2±2.1 vs. 30.8±3.4pg/ml, p=0.04). Cystatin C had apositive correlationwith age (p=0.01), PTH (p=0.02) and calcium (p=0.04). In the multivariate analysis, cystatin C was independently correlated with age (p=0.001), sex (p=0.007) and PTH (p=0.001).
Conclusions. Cystatin C was not found to be associated with thyroid cancer. In contrast,it was independently correlated with age, sex and PTH.

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PREOPERATIVE EVALUATION OF SERUM CYSTATIN C IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM

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Background. Cystatin C has been suggested as a new sensitive marker of renal function. The aim of this prospective study was to evaluate the results of preoperative cystatin C measurement in primary hyperparathyroidism patients and to identify factors associated with cystatin C in these cases.

Methods. Sixty-two patients undergoing parathyroidectomy were included, during a 2-year period. Cases with renal failure or secondary hyperparathyroidismwere excluded. Serum cystatin C,24hour urinary calcium and phosphate,serum calcium and phosphate, creatinine and albumin were measured on Architect c8000,T_3, T_4, FT_3, FT_4, TSH, anti-Tg,anti-TPO, PTH and 25(OH)$VitD_3$on Architect i2000.We evaluated the association of cystatin C with these biochemical parameters and with demographics, parathyroid gland weight and diameter. Statistical analysis was performed with Spearman's correlation test and a multivariate analysis model with cystatin C as the independent variable.

Results. Mean age of the patients was 58.4±7.6years (female:74.2%). Mean cystatin C levels was 0.98±0.04 (range:0.44-0.93mg/L). Elevated cystatin C was noticed in 33 (53.2%) patients whereas creatinine in only 5 (8.1%). Cystatin C was significantpositively related withpreoperativePTH (p=0.04), serum calcium (p=0.04) and albumin (p=0.01). In the multivariate analysis, cystatin C was independently correlated with PTH (p=0.02) and albumin (p=0.02).

Conclusions. Cystatin C is elevated in primary hyperparathyroidism and seems to be an earlier and more accurate marker of renal function. The renal impairment that cystatin C seems to indicate is a possible mechanism but may also others be involved in primary hyperparathyroidism and further investigation is needed.

0467

ASSOCIATION BETWEEN FASTING PLASMA GLUCOSE AND SHORTENED ACTIVATED PARTIAL THROMBOPLASTIN TIME

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Background. A shortened activated partial thromboplastin time (APTT), either as a risk factor or as a marker for hipercoagulability, has gained interest recently.

Methods. We retrospectively analyzed the combined levels of APTT, prothrombin time (PT), fibrinogen and fasting blood glucose(FPG) for a 16 months period. APTT, PT and fibrinogen were assayed on the MDA II ,Trinity Biotech reagents (Platelin LS for APTT, TriniCLOT PT HTF for PT with an International Sensitivity Index of 1.22, MDA fibriquick for fibrinogen determinations). Hexokinase method has been used as glucose reference technique.

Results. Subjects were clustered in three classes according to their FPG value: euglycemia (<5.6 mmol/L, n:2913), impaired fasting glucose (IFG) (from 5.6 to 6.9 mmol/L, n:1394), and diabetes (>7 mmol/L, n:1295).Abnormal distribution of groups was detected by Kolmogorov Smirnov test and difference between APTT and fibrinogen was detected by Kruskal-Wallis. In our 1295 study cases with more than 7 mmol/L of glucose, 182 patients (14.1%) had APTT less than 22 s and 63 had (4.9%) had APTT values between 15.9-20.4 s. We excluded 52 cases who had APTT values less than 22 s with glucose levels more than 22 mmol/L. Comparison by Mann-Whitney test revealed shortened APTT in both IFG and diabetic groups compared to euglycemics (p<0.001).Statistically significant higher fibrinogen levels were also found for the same groups (p<0.001).

Conclusions. We conclude that shortened APTT may be an indicator of procoagulant disequilibrium in patients with IFG and diabetes. However, these results need to be confirmed with other commercial kits and retrospective analysis.

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THE CLINICAL LABORATORY IN THE DIAGNOSIS OF PANHYPOPITUITARISM

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Background. Panhypopituitarism is an endocronine system disease which produces a deficit of pituitary function. This deficit may have hypothalamic or pituitary origin.
Patient and Methods. 42 years old female patient, who smokes 5 cigarettes a day, presented secondary amenorrhea with hormone replacement therapy. She goes to the endocrinology service from primary care to assess a cervical node. We analyze thyroid hormones, FSH, LH, anti-TPO antibodies, prolactin and estradiol. Based on these results, we made astimulation test with TRH, insulin-induced hypoglycemia test, gonadotropin test stimulation with GnRH, IGF-1 test and GH levels. All these tests were made to evaluate the hypothalamic pituitary axis activity.
Results. The values obtained in the tests applied were as follows: TSH= 0,47 μU/mL (0,5-4,0), T4L: 0,8 ng/mL (0,8-2), FSH:1,3 U/L (3,4-21,6), LH: 0,6 U/L (2,9-21,9), anti-TPO antibody was negative, estradiol <18 pg/mL (35-169), prolactin 2,4 ng/mL (8,7-30), IGF-1: 17,6 ng/mL (83-320), GH<0,1 ng/mL (0,5-5).The stimulation test with TRH, the GnRH stimulation test and the stimulation test by insulin-induced hypoglycemia were negative. In healthy patients, these tests should produce a peak of hormone secretion. The final diagnosis was panhypopituitarism by pituitary hypoplasia.
Conclusions. The laboratory has an important role in the diagnosis of this disease, because in this case laboratory findings lead the clinician to the final diagnosis, which is confirmed with imaging techniques.

0469

THE ROCHE ELECSYS PROLACTIN II ASSAY IS LESS AFFECTED BY MACROPROLACTIN THAN THE ABBOTT ARCHITECT PROLACTIN ASSAY BUT CAN NOT REPLACE PEG PRECIPITATION TEST

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Background. All prolactin immunoassays used in clinical routine measure macroprolactin to varying degrees. The polyethylene glycol (PEG) precipitation test is recommended to detect pseudohyperprolactinemia caused by macroprolactin. The aim of the present study was to evaluated weather the Roche assay is less affected by macroprolactin than the Abbott assay.
Methods. Twenty consecutive samples with serum prolactin >50 μg/L with our routine method Abbott Architect were reanalysed with the Abbott assay after PEG precipitation according to the manufacturer. The serum samples were stored in -20 °C until analysed with the Roche Elecsys prolactin II immunoassay on the e411 analyser. The serum prolactin results from Abbott, post-PEG Abbott and Roche assays were compared.
Results. The correlation was higher between Roche and post-PEG Abbott than between Abbott and post-PEG Abbott: r^2 0.98 vs r^2 0.85. There was no significant difference between serum prolactin concentrations with Roche and post-PEG Abbott: median 71 (range 30-416) vs median 54 (range 9-395) (p=0.18) whereas prolactin concentrations with Abbott were higher before than after PEG precipitation: median 77 (range 59-430) vs median 54 (range 9-395) (p=0.002) In three samples where serum prolactin with post-PEG Abbott were <15 μg/L, the Roche assay showed 30, 25 and 39 μg/L respectively.
Conclusions. We conclude that although the Roche Elecsys prolactin II assay gave more accurate serum prolactin concentrations than the Abbott Architect assay, it still produced slightly elevated serum prolactin in some samples where the Abbott assay showed normal values after PEG precipitation.

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MORTALITY ASSOCIATED WITH PERTURBATIONS IN THE CALCIUM-PARATHYROID HORMONE-VITAMIN D AXIS - A COMPREHENSIVE PREVALENCE STUDY IN PRIMARY HEALTH CARE PATIENTS, DENMARK

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Background. To determine the prevalence of vitamin D deficiency and primary and secondary hyperparathyroidism (PHPT and SHPT) among patients in general practice in Copenhagen (population 1.1 mill.) and to investigate the association with mortality.
Methods. Test results from blood samples collected from 2004to 2010 were included. 3-years mortality associated with single parameters studied by Kaplan-Meier analysis. The association of age, gender, SHPT and PHPT with 3-years mortality was evaluated by step-wise Cox regression analysis.
Results. The prevalence of vitamin D deficiency (s-25OHD < 50 nmol/l) among 247.626 patients was 53 % (significantly higher among men than women (p< 0.0001)). Mean age was 53 (males) and 52 years (females). From February to April the average vitamin D level was significantly lower than from July to September (p < 0.0001), both among men (38 versus 61 nmol/l) and women (46 versus 63 nmol/l). 34.997 patients had also measurement of parathyroid hormone (s-PTH) and 29.943 had measurement of both s-PTH and total s-calcium. 14% suffered from SHPT and 1% from PHPT. 3-years mortality was 4,49% (males) and 3,6% (females). All single parameters (low s-25OHD, low s-calcium, high s-PTH, PHPT and SHPT)were associated with higher mortality. In the regression analysis age, gender, SHPT and PHPT was significantly associated with increased mortality.
Conclusions. Vitamin D deficiency and secondary hyperparathyroidism is prevalent. All parameters were associated with higher mortality (low s-vitamin D, low s-calcium, high s-PTH, PHPT and SHPT). Gender, PHPT and SHP T but not s-vitamin D, were risk factors of mortality.

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ADIPONECTIN AND METABOLITE SYNDROME

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Background. Adiponectin is an adipocyte-specific, secreted protein with potential roles in glucose and lipid homeostasis. Adiponectin is induced during adipocyte differentiation and its secretion is stimulated by insulin. High levels of Adiponectin lead to an insulin-independent decrease in glucose levels. This is likely due to insulin-sensitizing effects involving Adiponectin regulation to triglyceride metabolism. A negative correlation between obesity and circulating Adiponectin, such as concomitantly increased levels with weight loss. A class of insulin-sensitizing, anti-diabetic drugs elevates Adiponectin in insulin-resistant patients.
Methods. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the Adiponectin globular domain has been pre-coated onto a microplate. After the required incubation an enzyme-linked monoclonal antibody are added; followed by substrate solution, which develops color reaction in proportion to the amount of Adiponectin in the serum.
Results. During one year period we study 50 patients. 35 of them were women, 15 men; the age was between 30 and 55 years. 39 of them were on sulphanylureic medicines. 10 were diagnosed with type-2 diabetes; they were with extremely low Adiponectin levels. In 32 patients (from the group on drugs such as Tolbutamide) we determine significant high levels of Adiponectin corresponding to glucose and insulin homeostasis.
Conclusions. Lowering Adiponectin levels leads to insulin-resistance and high insulin levels in serum. Low levels discovers in patients with type-2 diabetes. Drugs from the group of sulphanylureic increase Adiponectin levels in patients with insulin-resistance. High Adiponectin levels leads to low risk of type-2 diabetes development.

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LEPTIN – THE APPETITE HORMONE

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Background. Leptin is best known for its role in regulating rodent appetite and energy expenditure. Leptin is released by fat cells as they increase in size, a consequence of calorie intake.
Human Leptin is a 16 kDa, 146 amino acid residues, non-glycosylated polypeptide that was originally identified using genetic mapping to locate a mutation that caused obesity. Named Leptin (from leptos, Greek for thin) because of its ability to reduce fat stores. Leptin is referred to as OB protein, the product of the obese gene. Lack of sleep increases Ghreline levels, stimulating appetite by lowering Leptin levels in human body.
Methods. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the Leptin has been pre-coated onto a microplate. After the required incubation an enzyme-linked monoclonal antibody are added; followed by substrate solution, which develops color reaction in proportion to the amount of Leptin in the serum.
Results. For a period of 12 months we study 35 patients with abnormal weight, who went under a strict diet. 25 of them were women, 10 men. The age was between 30 and 60. After 6 to 9 months in 33 of them we determine lower Leptin levels, parallel with weight loss
Conclusions. The circulating levels of Leptin are closely and positively correlated with body fat. It now appears that multiple factors both in the brain and periphery are involved in weight loss and metabolism. Leptin is also proposed to play a role in hematopoesis and renal cortex function.

0473
RESISTIN AND TYPE-2 DIABETES

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Background. Resistin acquired initial attention as a potential link between obesity and glucose regulation.
Resistin, also known as found in inflammatory zone 3 (FIZZ3) or adipocyte secreted factor (ADSF), is a member of protein family known as the Resistin-like molecules (RELMs). It is reported that Resistin is expressed by pre-adipocytes, placenta, pancreatic islets and primary leukemia cells. Resistin has been shown to activate endothelial cells, leading to the production of adhesion molecules, endothelin-1 and chemokines.
Methods. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the Resistin has been pre-coated onto a microplate. After the required incubation an enzyme-linked monoclonal antibody are added; followed by substrate solution, which develops color reaction in proportion to the amount of Resistin in the serum.
Results. For a period of 12 months we investigate 75 patients. 50 of them were women, 25 men; the age was between 30 and 60. 35 patients from the group went under a strict diet; other 35 were on sulphanylureic medicines (such as Tolbutamide). The second group shows significantly lower Resistin values, due to the drugs therapy. In the opposites, the group that went on a diet shows much higher Resistin levels compared to initial.
Conclusions. Serum levels were shown to increase in diet-induced and genetic forms of obesity. They decrease in response to insulin sensitizing drugs. In addition, function-blocking Resistin antibodies enhanced insulin actions while treatment with recombinant Resistin caused glucose intolerance and insulin resistance.

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0474

STUDY OF A TEENAGER PATIENT WITH HYPOGLYCEMIA: A CASE REPORT

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Background. Congenital adrenal hyperplasia for a deficiency of 21-hydroxylase can occurs in a non-classical form, where the symptoms of androgen excess are at the end of childhood or adulthood. The most common clinical manifestations consist of early appearance of pubic hair, accelerated growth and an advancement of bone age, acne, hirsutism, menstrual disorders and infertility. Hypoglycemia in these patients is caused by the absence of cortisol that significantly decreases hepatic glucose production.
Methods. A 18-years-old woman was derived to the Endocrinology service due to low levels of glucose and adrenergic symptomatology. Diabetes and the intake of hypoglycemic drugs were rapidly discarded. The Adrenal function was studied, the pituitary-adrenal axis were within the reference values, but show a slightly elevated androgen values. To discard an adrenal insufficiency, the patient underwent an ACTH test, suspecting a mild congenital adrenal hyperplasia secondary to late-onset 21-hydroxylase deficiency. There was a normal response of cortisol, whereas the 17-OH progesterone values were increased. Direct analysis of the gene steroid 21-hydroxylase by PCR (gene amplification and allele-specific hybridization) allowed us to demonstrate two mutations: Val281Leu (exon 7) and Pro453Ser (exon 10).Both mutations are associated with mild forms of deficiency.
Conclusions. That shows the importance of a comprehensive study in patients with hypoglycemia, which includes an assessment of adrenal reserve that can reveal a deficit in the adrenal steroidogenesis.

0475

EVALUATION OF THE TOSOH ST AIA-PACK DHEA-S

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Background. Dehydroepiandrosterone sulfate (DHEA-S) is a steroid hormone secreted from adrenal cortex. DHEA-S is used for the index of various disease of the adrenal cortex, and is especially useful for the differential diagnosis of the adrenal lesion of Cushing's syndrome. Moreover, it often becomes elevated in patients with virilism and polycystic ovary syndrome.
Methods. We evaluated the TOSOH ST AIA-PACK DHEA-S on the TOSOH AIA-2000 analyzer. The ST AIA-PACK DHEA-S is an enzyme immunoassay which is performed entirely in the test cups. The sample is automatically pipette into the test cup. DHEA-S present in the sample competes for the antibodies coated on the beads with enzyme-labeled DHEA-S. After 10 minutes incubation at 37°C, the magnetic beads are washed to remove unbound materials and are then incubated at 37°C with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled antibody that binds to the magnetic beads is indirectly proportional to the concentration of DHEA-S in the sample. The clinical validation included analysis of 200 samples with a routine request for DHEA-S and a comparison with Abbot DHEA-S test.
Results. Method correlation over the range of 0 to 800 μg/dl (undiluted serum samples)
resulted in the following Passing Bablok linear regression equation: Tosoh = 1.03 *Abbott +0.8 with a correlation coefficient of 0.99. The Tosoh test showed an inter and intra assay of 1.7 to 3.7 and of 1.9 to 3.2 respectively. The Tosoh test revealed a linearity from 600 to 6 μg/dl..
Conclusions. Our results demonstrate that Tosoh ST AIA-PACK D-DHEA-S correlates well with the Abbot DHEA-S test and showed an excellent analytical performances

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BONE MARKERS CTX AND OSTEOCALCIN ASSESSMENT IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS ON ALENDRONATE TREATMENT

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Background. Postmenopausal is characterized with reduced bone formation, increased bone resorption, consecutive bone loss and osteoporosis development.
Methods. Basal serum levels of bone formation marker osteocalcin (OC) and bone resorption marker CTX and their levels after 3, 6 and 12 months of 70mg alendronate treatment (AT) were determined with electrochemiluminescence method, automatic imunoanalyser Roche Elecsys 2010. Postmenopausal women (n=100) with osteoporosis, diagnosed with DEXA bone density measurement, with mean age (59.35±5yr.), menopause duration (11.65±6.3yr.) were studied.
Results. CTX values after 3 months AT (0.2±0.12ng/ml) are lower compared to the basal levels (0.48±0.12ng/ml)(p<0.0001), but higher compared to 6 months (0.12±0.06ng/ml)(p<0.003), and 12 months AT (0.09±0.07ng/ml)(p<0.0001). CTX levels after 6 month AT are lower compared to basal levels (p<0.0001), and 3 months AT (p<0.003), but were not significantly different compared to 12 months AT (p>0.05). OC levels after 3 months AT (18.71±5.95ng/ml) are lower compared to basal levels (26.52±8.63ng/ml)(p<0.001), but not significantly higher compared to 6 months (15.49±5.18ng/ml)(p<0.019), and 12 months AT (13.23±3.86ng/ml)(p<0.0001). OC levels after 6 months are lower than basal levels (p<0.0001), and compared to 3 months AT (p<0.019), but are not significantly different compared to 12 months AT (p>0.05).
Conclusions. AT enabled progressive and significant reduction of CTX and OC level after 3, 6 and 12 months, but after 6 to 12 months the reduction is not significant. Optimal antiresorptive effect is achieved after 6 months of AT and it is persistent after 12 months of AT.

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DETERMINATION OF SERUM CYSTATIN C AND CREATININE VALUES IN NEWLY DIAGNOSED HYPERTHYROID PATIENTS

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Background. It is recognized that thyroid dysfunction may alter creatinine and cystatin C (Cys C) blood levels. Cys C, a new marker for kidney function is superior then creatinine because it is less influenced by age, gender, race and lean muscle mass. Aim of this study was to evaluate serum Cys C and creatinine concentrations in newly diagnosed hyperthyroid patients and potential influence of thyroid dysfunction on these values.
Methods. 30 newly diagnosed hyperthyroid women were included in our study, before thyrosuppressive therapy administration. 30 healthy sex and aged matched persons were control group. Serum Cys C, creatinine, fT4, fT3 and TSH were estimated in all blood samples. TSH, fT3 and fT4 were performed at once by immunometric assays on ARCHITECT i2000SR and Cys C and creatinine on OLYMPUS. All data were processed by standard statistical analysis.
Results. There is statistical difference (p<0.001) between Cys C levels in hyperthyroid group (X=1.31mg/mL;SD=0.29) regarding the values of Cys C in control group (X=0.64mg/mL;SD=0.10). Mean value of serum Cys C were significantly higher in hyperthyroid patients, outside of the estimated reference range fot the method (0.5-1.03 mg/mL). Creatinine values in hyperthyroid patients (X=59.75µmol/L;SD=9.57) are significantly lower than creatinin values in control group (X=79.55µmol/L;SD=10.31), but inside the reference range in both groups.
Conclusions. Hyperthyroidism elevates Cys C concentrations, possibly influencing the production rate of the protein, but inversely lowering the serum creatinine concentrations. Due to major impact of hyperthyroidism on Cys C values, it is recommended that thyroid function should be considered when Cys C is increased.

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CORRELATION BETWEEN TOTAL ANTI-OXYDANT STATUS, URIC ACID AND GLUTATHIONE PEROXYDASE IN TUNISIAN PATIENTS WITH BREAST CANCER

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Background. Breast cancer, the third most common cancer worldwide, accounts for the highest morbidity and mortality. The etiology of breast cancer is multifactorial. Antioxidants status play an important role in breast cancer prognostic.
This study investigated the correlation between the anti-oxidant parameters in patients with breast cancer: Total anti-oxidant status (TAS), uric acid (UrAc) and Erythrocyte Glutathione Peroxidase (GPx).
Methods. Our study includes 31 treated women with breast cancer (mean age 50 ±13 years) recruted from gynecology department.
Determination of antioxidant parameters: TAS as well as UrAc was determined by a colorimetric method, while GPx activity was determined at 340nm (Randox, Antrim, UK).
Results. The results showed a statistically significant positive correlation between TAS and UrAc (r = 0. 04; p=0. 02) in patients. It is the same for GPx wich was also positively correlated with UrAc (r = 0. 38; p=0. 03) and TAS (r = 0. 49; p<10^{-3}).
Conclusions. The positive correlations found between TAS, UrAc and GPx, in patients with breast cancer, demonstrate the co-operative effects of antioxidant mechanism agains oxygen free radical.

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IMMUNOPOSITIVE GH CELLS IN LONG-TERM OVARIECTOMIZED FEMALE RATS TREATED WITH ESTRADIOL

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Background. Estradiol is important hormone which controls secretory activity of hormone producing cells in the rat female's pituitaries. Binding to the estrogen receptors represents the main mechanism of estradiol action in GH cells.In the present study, the influence of estradiol dipropionate(EDP)on the morphology and secretory activity of GH cells in ovarictomized(ovx)Wistar rat females was studied.
Methods. Female Wistar rats were ovxat 12 weeks of age. Animals were divided into two groups, each comprising seven females. The first group of long-term ovx femalesreceived i.p. 250mg EDPdaily for 4 weeks, while the second group,long-term ovx controls, received the adeqate volume of sterile olive oil. All females were sacrificed 24 h after the last injection. GH-producing cells were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Serum concentrations of GH in control and estradiol treated female rats were measured by the hGH-Delfia kit.
Results. The absolute and relative pituitary weight in EDP treated females were significantly increased (p<0.05) by 159.4% and 228.1% respectively, in comparison with the controls. Immunohistochemically labelled GH cells in the control rat pituitaries were ovoid to pyramidal in shape, usually located along the blood vessels. In the estradiol treted ovx females GH cells were longer, irregularly shaped, with more intensely stained cytoplasm. Concentration of GH hormone in the serum of estradiol treated ovx females was significantly increased (p<0.05) by 44.7%, compared to the controls.
Conclusions. Estradiol application has caused the change of morphofunctional parameters of GH cells in long-term ovx females.

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LABORATORY EVALUATION IN CLINICAL DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME (PCOS)

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Background. PCOS is a grave disorder affecting pubertal and reproductive age. Manifestations are clinical, radiological, biochemical and endocrinal. High prevalence (77.1%) in a tertiary hospital in urban India highlights the nature of its existence.
Methods. 35 consecutive subjects (age: 24.38±5.41 years) with irregular menstrual cycle enrolled on day two of menstrual cycle, follicular phase. (a)Blood Glucose,lipids,creatinine-Synchron Cx9, BeckmanCoulter. (b)Hormones-ELISA Testosterone, Dehydroeriandrosterone, 17-a-hydroxyprogesterone(EQUIPAR Diagnostici, Italy). (c)Follicle Stimulating Hormone(FSH), Luteinizing hormone(LH),Insulin,Thyroid Stimulating Hormone,Prolactin,HsCRP (AccuBind™,Monobind Inc).
Results. (a)ESHRE/ASRM criteria diagnosed PCOS in 77.1% (b)Increased androgens 25.9% (c)Clinical symptoms with (hirsutism/acne/alopecia) with elevated androgens 22.2% (d)Ultrasound findings of Polycystic Ovaries 70% (e)Increased levels of LH 37% (f)Altered FSH, LH ratio 33% (g)Dyslipidemia (h)Positive correlation between BMI and insulin levels
Conclusions. PCOS is high risk for developing Type II Diabetis Mellitus and Coronary Artery Disease. Opportunity to reverse associated risks, need lifestyle modification of weight reduction, exercise, environmental destress and early pharmacotherapy based on individualized needs of metabolic, gynecological and cosmetic concerns, thereby reducing the incidence of long term consequences of metabolic sequelae.

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IS SUBCLINICAL HYPOTHYROIDISM MORE COMMON WITH WORSENING KIDNEY FUNCTION?

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Background. The thyroid-kidney relationship is complex, and subclinical hypothyroidism is related to severity of chronic kidney disease in some studies, although the effects on mortality and morbidity are unclear. We look at the prevalence of subclinical hypothyroidism in abnormal kidney function.
Methods. 3691 thyroid-stimulating hormone(TSH) and free thyroxine(fT4) tests were done in the National University Hospital(NUH), Singapore in December 2010. Samples excluded were: estimated glomerular filtration rate(eGFR) above 59ml/min(calculated using MDRD equation with creatinine values within 1 week of thyroid testing), patients below 16 years old, pregnancy, positive thyroid antibodies, previous abnormal thyroid testing in NUH and fT4 < 10 pmol/L or >23mol/L. After exclusion, 209 samples were evaluated according to TSH and eGFR values. Subclinical hypothyroidism is defined here as TSH >4.5mIU/L with normal fT4.
Results. The median age is 73.5 years old (17-98 years old), and female to male ratio is 1.82.
6.7% had subclinical hypothyroidism, with 57% having eGFR 30-59ml/min, 29% having eGFR 15-29ml/min and 14% having eGFR <15ml/min. In comparison, 69.9% had normal thyroid testing (TSH 0.45-4.5mIU/L), with 70% having eGFR 30-59ml/min, 17% having eGFR 15-29ml/min and 13% having eGFR <15ml/min(P value >0.4 for all groups).
Conclusions. There was no statistical significance between subclinical hypothyroidism from stages 3 to 5 of chronic kidney disease compared to normal thyroid function, contrary to previous studies. This may be due to small sample size, and more data is needed to determine the prevalence and effect of hypothyroidism in kidney disease.

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THYROID HORMONE PROFILE IN PATIENTS WITH END STAGE RENAL DISEASE

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Background. Thyroid abnormalities are common in patients with end stage renal disease (ESRD) on hemodialysis, although signs and symptoms are rarely suggestive and often confused. The aim of this study was to investigate the prevalence of thyroid dysfunction in patients with ESRD on hemodialysis.
Methods. 130 patients with ESRD (78 men / 53 women) and median age 63,5 ± 7,8 were included. At the time of the study all patients were clinically euthyroid and noone had a history of recent infection or other illness or received any medication that could affect thyroid function. Serum samples were obtained before the first dialysis of the week and serum levels of TSH, FT3 and FT4 were measured (Roche, Modular E170). Subclinical hyperthyroidism was definied when TSH was abnormal low (<0,27 µIU/ml) and FT3 (3,1-6,8 pmol/lit) and FT4 (12-22,0 pmol/lit) were present at normal levels. Subclinical hyporthyroidism was definied when TSH was abnormal high (>4,20 µIU/ml) and FT3 and FT4 were present at normal levels.
Results. Subclinical hyperthyroidism was detected in 6/130 (4,6%) patients [4/78 men (5,1%) and 3/53 women (5,6%)]. Subclinical hypothyroidism was detected in 12/130 (9,2%) patients [3/78 men (3,8%) and 9/53 women (p<0,05) (16,9%)]. Hyperthyroidism was not detected and only 2/53 (3,7%) women presented with hypothyroidism.
Conclusions. Thyroid abnormalities were not unusual in patients with ESRD. Therefore serum levels of TSH, FT3 and FT4 should be considered in evaluation of every ESRD patient.

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LEVOTHYROXINE POISONING CONSUMPTION: A CLINICAL CASE

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Background. Levothyroxine is the drug of choice of Hypothyroidism's treatment. The poisoning (intoxication) with levothyroxine is rare, but, overcoat it can be accompanied of secondary cardiovascular complications.
Clinical case. We present the case of a 6-year-old girl who arrived at the Emergency Department due to suspicion of levothyroxine ingestion in unknown quantities. Physical Exam: Weight: 18.7 kg Temp: 37 ºC, HR: 125 bpm, BP: 118/65 mm /Hg. Laboratory tests: hemoglobin: 12.6 g/dl, hematocrit: 37%, platelets: 268x103/µL, leukocytes: 6790µ/L. Biochemistry. Glucose: 90 mg/dl, urea: 27 mg/dl, Na: 136 mEq/L, K: 4 mEq /L, Cl: 104 mEq /L. Concentrations of thyroid hormones during admittance: TSH: 0.232, 0.094, 0.07, 0.053, 0.046 μUI/ml, FT4: >7.7, >7.7,>7.7, 5.33, 4.42 ng/dl FT3: values for the last 3 days: 11.7, 9.8, 8.2 pg/ml.
FT3 and FT4 quickly rise after ingestion which is why their determination is recommended in the first few hours after intoxication. However there is a reservation: when concentrations exceed the upper limit using this technique, the sample should not be diluted given that a high proportion is joined to plasmatic proteins and dilution breaks the balance providing an erroneous result.
Conclusions. Quick action should be taken after intoxication (either by plasmapheresis or pharmacological action). FT3 should be determined as an early marker of evolution and treatment efficiency in cases of intoxication.

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DYSLIPIDEMIC DISORDERS ASSOCIATED WITH HYPOTHYROIDISM AND SUBCLINICAL HYPOTHYROIDISM

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Background. Several authors propose a relationship between hypothyroid disorders and the alteration of total cholesterol, HDL, LDL and triglycerides levels. Nevertheless, there is no consensus about this issue. This study explores this controversial topic.
Methods. We analyzed 6319 patients divided in three groups: euthyroid (TSH=0.5-4 mU/L; T4L=0.8-2 ng/dL; N=2034), hypothyroidism (TSH>4 mU/L; T4L<0.8 ng/dL; N=1887) and subclinical hypothyroidism (TSH>4 mU/L; T4L=0.8-2 ng/dL; N=2398). Thyroid hormones were quantified in Architect i2000 (Abbott diagnostics). Lipid parameters were determined in Modular Analytics (Roche Diagnostics). Data were statistically processed by the program SPSS 15.0.
Results. We observed a statistically significant increase in the hypothyroid group compared with the control group in total cholesterol levels (P <0.001), HDL (P <0.03), LDL (P <0.001) and triglycerides (P <0.001). Differences between means were respectively 20.23 (95%CI: 17.34-23.14), 1.07 (95%CI: 0.09-2.06), 6.87 (95%CI: 4.31-9.44) and 27.78 (95%:CI 21.23-34.33). By contrast, the subclinical hypothyroid group showed increase in lipid concentration which were only significant for total cholesterol and triglycerides (P <0.001 in both cases), with a difference between means of 12.12 (95%CI: 9.68-14.57) and 15.58 (95%CI:10.83 -20.32) respectively.
Conclusions. The results suggest that: 1.- The hypothyroid patients are more likely to develop dyslipidemia than euthyroid population. 2.- The severity and the proportion of patients with dyslipidemia were higher in the hypothyroid group than in the subclinical hypothyroidism group.

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THE OCCURENCE OF THYROID DYSFUNCTION IN THE REGION OF EAST SLOVAKIA

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Background. Thyroid dysfunction is one of the most common endocrine diseases occuring nowadays.
Methods. In work we focused on the most frequently occurring disorders of the thyroid in relation to the seriousness of the disease in two regions of eastern Slovakia within the period of eight years.Comparing the incidence of thyroid diseases in the north-eastern Slovakia - the increased incidence of thyrotoxicosis was revealed in the years 2004 - 2006 (143 - 152 patients) in comparison to the years 2000 (133) and 2008 (86). The increased incidence of hypothyroidism was observed in the years 2003 - 2006 (641 - 769) comparing to the years 2000 (479) and 2008 (618).
Results. The lowest incidence of thyroiditis occured in 2008 (739 patients), the highets in 2000 (1401). In the central part of the region the increasing occurence of all diseases was recorded (thyrotoxicosis in 52 - 154 patients in the years 2000 - 2008, hypothyroidism - from 345 to 844 patients in the same period and thyroiditis from the original 1208 cases to 1989 in 2008).
Conclusions. The results obtained show that in the north-eastern part of Prešov region the decrease of observed diseases of thyroid gland is recorded, while in the central region the increase of such diseases. We suggest that the medical examination of thyroid gland is included in the standard procedures provided during preventive examination.

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SOME HORMONES TESTED IN WOMEN WITH PRIMARY INFERTILITY(STERILITET)

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Fertility in women is ability to become pregnant.Today 8 – 10% couples experience some form of infertility problems.In primary infertility – sterilitet,concepton has not occured after one year of tryining. Blood hormones tests are using to help diagnosis and treatment of unfertility. FSH(Follicule-Stimulating Hormone)E2(Estradiol),LH(Luteinsing Hormone)and PRG(Progesterone)measures are tests of ovarium functionsEstradiol indicates the growth of follicules and quality of oocytes.LH and testosterone can be used to detect Polycystic Orarian Syndrome.TSH and prolactine help to determine cause of infertility –thyroid pituitary. The purpose of our study was to follow up levels of TSH,FSH,LH,E2,prolactin and testosterone in woman with primary infertility in follicular phase(3.day of menstruation).
Examined groups included 72 women aged from 21 to 42 old.All parametres were determinated in serum using the Advia Centaur XP immunoassay System,the princip of directly chemiluminescence.Concentrations included:
TSH: 0,16-11,68 mIU/L,lower values than normal reference rang have 4,higer have 4 women; FSH:<0,3-6,7 mIU/L,lower values have 4,higer have 10 women; LH: <0,1-104,9 mIU/L,lower values have 4,higer values have 9 women; E2: 14,6-285,6 pg/ml,lower values have 2 women,higer 1 woman; Prolactin 2,18,6 ng/ml values than reference have 11 women; Testosterone:15-115 ng/mil,higer values have 10 women; 10,3% of all results were not in reference rangs.
Measured hormone levels and fisical examinatione,transvaginal ultrasound,pelvic examinatione,hysterosalpinography,hysteroscopy,laparoscopy,diagnosis of uterine and other phisical abnormalities...All together discover the cause of female sterilitas and increase chance of having a child.Simple blood test of hormone levels are some of the first examinationes wich doctor will perform.

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DEFINITION OF A GROWTH HORMONE BY AN ENZYME IMMUNOASSAY METHOD

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Backgrounds. One of the challenging problems in endocrinology is early diagnostics and treatment of somatotropic insufficiency in children and improving diagnostics of decreased skeletal growth.
Methods. Quantitative measurement of a hormone of growth was made with the help of enzyme immunoassay set DSL-10-1900 by a principle of enzymatically strengthened two-phasic immunoanalysis of a "sandwich" type. Measurement was made on semi-automatic analyzer SunRise-Tecan.
Results. Research is made on 223 patients. Measurement of level of a hormone of growth was made with the use of clonidine and insulin tests on 5 points. 56 patients had a decrease in a hormone of growth of less than 10ng \ml, total deficiency of a hormone of growth was equaled to 7ng \ml, fractional deficiency of a hormone of growth-7-10ng\ml. For provocative tests we used tests with clonidine and insulin on 5 points (0,30, 60, 90,120 min).
Conclusions. Timely revealing of insufficiency of level of a hormone of growth prevents the development of the decreased skeletal growth.

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SERUM-HEPCIDIN-25 IN COMPARISON TO BIOCHEMICAL MARKERS AND HEMATOLOGICAL INDICES FOR THE DIFFERENTIATION OF IRON DEFICIENT ERYTHROPOIESIS

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Objective. The objective of this study was to evaluate the extent to which IDA can be differentiated from ACD and ACD/IDA based on hepcidin-25 alone or on its combination with other biochemical markers or hematological indices. The second objective was to investigate the association of hepcidin-25 with biochemical markers and hematological indices of iron status.
Materials and Methods. In 155 anemic patients (ACD 67, IDA 52, ACD/IDA 36) serum hepcidin-25 and a panel of biochemical markers and hematological indices of iron metabolism were determined. Hepcidin-25 was determined using an isotope-dilution micro-HPLC-tandem mass spectrometry method.
Results. Hepcidin-25 enabled differentiation of IDA from patients with ACD (AUC_{ROC}, 0.968; 95% CI 0.915- 0.992; sensitivity 98.1%, specificity 84.5%; p=0.0001) and IDA/ACD (AUC_{ROC} 0.995; 95% CI 0.948-1.000; sensitivity 98.1%, specificity 97.1%; p=0.0001). However, it was not possible to differentiate between ACD and ACD/IDA (AUC_{ROC} 0.569; 95% CI 0.461-0.671; sensitivity 42.3%, specificity 75.0%; p=0.2590) in patients with inflammation. As the ROC analysis indicated weak discriminatory power of hepcidin-25 alone to differentiate ACD from ACD/IDA , we described the relationship between hepcidin-25 and the CHr (hemoglobin content of reticulocytes) in a diagnostic plot divided into quadrants that corresponded to the four states ACD, ACD/IDA, IDA (classic) and ACD/IDA (latent state).
Conclusions. The results show that erythropoiesis can be iron deficient even in the presence of high hepcidin-25 levels. The combination of hepcidin-25 with CHr, a potent marker of iron-deficient erythropoiesis in a diagnostic plot, appears to be useful for the differentiation of ACD/IDA from ACD and IDA.

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THE EFFECT OF HORMONAL REPLACEMENT THERAPY (HRT) ON BLOOD LEVELS OF GASTRIC INHIBITORY PEPTIDE (GIP), GLUCAGONLIKE PEPTIDE-1 (GLP-1) AND INSULIN IN RELATION TO GESTAGEN USED IN POSTMENOPAUSAL WOMEN

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Background. It is known that HRT influences the plasma incretins (GIP, GLP-1) and insulin levels depending on route of estrogen administration but no data of concomitant use of gestagens on these hormones are available.
Methods. The study included 49 healthy postmenopausal women (mean age 56.0 ±1.8 yrs) on HRT. Transdermal 17-β estradiol (0.05 mg/d) with 5 mg/d dihydrogesteron orally were used in 26 patient (group A) and transdermal 17-β estradiol (0.05mg/d) with 5 mg/d medroxyprogesteron orally were applied in 23 women (group B). Basal and one hour meal-stimulated plasma estrogens (RIA), GIP, GLP-1 (EIA) and insulin (IRMA) concentrations were measured before and after six months of HRT treatment.
Results. The mean plasma estrogen level was significantly higher (p<0.01-0.001) after six months of HRT on both groups. Significant decrease of mean values of basal and mean values of meal-stimulated blood levels of GIP,GLP-1 and insulin (p< 0,05 - 0,01) was observed after six months of HRT treatment in group A as compared to the respective mean hormones values before treatment. For group B differences between the mean hormones levels before and after treatment, both in basal and meal-stimulated condition, have not been observed.
Conclusions. HRT has a positive effect on the enteroinsular axis in postmenopausal women but the effect of HRT on GIP,GLP-1 and insulin concentration depends not only on estrogens but also on gestagen used.

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THE PREVALENCE OF VITAMIN D DEFICIENCY AMONG FEMALES STUDENT AT QATAR UNIVERSITY

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Background. Vitamin D deficiency is a major worldwide problem with several health consequence such osteoporosis, hypertension, diabetes mellitus, autoimmune diseases and some cancers. The Prevalence of Vitamin D deficiency among female adult students with associated biochemical markers was not measured in the state of Qatar previously.
Aim. Measurement of vitamin D level and other clinical parameters related to vitamin D levels.
Methods. Randomly selected 71 female students were recruited in Health Science Department of Qatar University for this study. Blood was drawn for measurement of vitamin D, calcium, albumin, alkaline phopsphatase and creatinine. Time of exposure to sun, duration, size of body exposed and dietary intake of vitamin D were quantified based on questioner. Body mass index (BMI) and waist circumference were measured.
Results. High percentage of vitamin D deficiency was observed among female students, 97.2% showed severe deficiency and insufficiency in vitamin Dconcentration.100% of the subjects showed a serum calcium level below the optimal level. No significant difference was observed of vitamin D status by nationality. Calcium level and skin color were the significant predictors of vitamin D level in the study while other predictor variables were not significant. Overall, vitamin D deficiency has high prevalence among female student at Qatar University accompanied by hypocalcaemia.
Conclusions. Vitamin D deficiency accompanied with hypocalcaemia is highly remarkable among female students (17-30 years old) in Qatar University.

0491

BENEFITS AND DIFFICULTIES OF UNIVERSAL THYROID-DYSFUNCTION SCREENING IN PREGNANCY IN THE CZECH REPUBLIC

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Background. Thyroid dysfunction in pregnancy is known to cause minor or major problems not only for the outcome of pregnancy, but also for the development of the fetus or the child. The Czech Republic is a region with sufficient iodine supply. As the case finding attitude to the thyroid-screening diagnosed less than 20 - 50% of women with thyropathy, we have now carried out a pilot project (PP) for universal screening in 13 regions of the Czech Republic. The pilot project was performed during 2009-2010 with the financial support of the General Insurance Company.
Methods. Thyroid examination was offered to women in the 9-11th week of pregnancy. Thewomen with any positivitywere offered immediate endocrinological examination.
Results. Blood tests (TSH, FT4 and TPO-Ab) were carried out in 2877 asymptomatic women in the 9-11th week of pregnancy. A total of 197 (6.8%) screened women had TSH higher than 3.7 - 5.0 mU/l according to the claimed reference interval. FT4 levels under 11.5 pmol/l were found in 96 women (3.3%). TPO-Ab were increased in 9% women examined.
Conclusions. This project proved the usefulness of universal screening of thyroid disease in pregnancy. The occurrence of pathological results in laboratory tests was 556/2877. Determination of the specific reference intervals for TSH, FT4, and TPO-Ab in pregnancy is one of the basic requirements when implementing the general examination.
Cooperation with gynaecologists differed, the main stumbling block was the willingness of gynaecologists to inform pregnant women about the project.

Endocrinology

0492

SALIVARY CHROMOGRANIN A (CGA-LIS) COULD BE A SUBSTITUTE FOR PLASMA CHROMOGRANIN A (CGA) IN PHEOCHROMOCYTOMA DIAGNOSIS

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Background. Besides plasma metanephrines,plasma CgA is considered a good complementary marker in pheochromocytoma diagnosis.Salivary CgA-LIS could precise this diagnosis in a non-aggressive manner using saliva instead of plasma samples.

Methods. A group of 14 tumoral patients aged between 37-72 years and a matched group of 10 normal subjects were biochemically investigated. Both analytical and diagnosis performance of the salivary Elisa Cosmo-Bio CgA-LIS was compared to that of plasma Elisa LDN CgA using Passing & Bablok regression and Receiver Operating Curves (ROC analysis).

Results. In tumoral group, median for CgA was: 580.5ng/mL(95% CI for the median: 416.72 to 1485.58),median for CgA-LIS was:6.13 pmol/L(95% CI:3.76 to 31.85).In normal group, median for CgA was:77.5ng/mL(95%CI:53.33 to 92.22),median for CgA-LIS was:0.94pmol/L(95%CI:0.24 to 1.59).Passing & Bablok regression equation for all 24 subjects was:$Y=0.0797 + 0.0132X$. Cusum test for linearity revealed no significant deviation from linearity($P>0.10$).

Spearman's coefficient of rank correlation (rho) between CgA and CgA-LIS in all 24 subjects was $0.75,P=0.0003$ (95%CI:0.496 to 0.885).ROC curve for CgA showed an area under the ROC curve=1.000 (95%CI =0.856 to 1.000).

ROC curve for CgA-LIS calculated an area under the ROC curve=0.993(95%CI:0.843 to 1.000).Pairwise comparison of ROC curves for both markers showed no significant difference between areas:0.007(95%CI:-0.026 to 0.040),$P=0.671$.

Salivary chromogranin A-LI could be used successfully instead of plasma chromogranin A in biochemical diagnosis of endocrine tumors.

Conclusions. We can conclude that salivary chromogranin could be used as a non-stressfull marker for diagnosis purpose in pheochromocytoma.

0493

PLASMA CHROMOGRANIN A-LI (CGA-LI) COULD IDENTIFY MALIGNANT PHEOCHROMOCYTOMA

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Background. Sometimes, pheochromocytoma diagnosis is a challenge in the medical world. Malignant cases are difficult to manage because biochemical arsenal is not rich in specific and sensitive markers for this purpose.

Besides plasma metanephrines, plasma chromogranin A-LI(CgA-LI) could contributes to pheochromocytoma diagnosis with good sensitivity and specificity. This parameter seems to be more increased in malignant pheochromocytoma.

Methods. Our study implied a group of 10 pheochromocytoma cases (aged between 40-63years) diagnosed by overincreased plasma free normetanephrines/metanephrines(NMNp/MNp) by Elisa and a matched normal group of 10 subjects with normal plasma metanephrines. We tested also plasma EIA CgA-LI in both groups.

Descriptive statistics, correlation coefficients, receiver operating curves analysis(ROC) were used in this study.

Results. NMNp median in all 20 cases was 122.5 pg/mL(95%CI:58.68 to 421.32),MNp median was:33.0 pg/mL (95% CI:18.89 to 91.89) and CgA-LI median was:1.14pmol/L(95%CI:0.26 to 1.92).

Only in 3 tumoral cases(proved malignant after tumor resection) we tested overincreased values for NMNp between:1000 to 6000pg/mL and also for CgA-LI between 6.53 to 33.33pmol/L.We can speculate that CgA-LI could be a good marker for malignant cases.

Rank correlation between NMNp and CgA-LI was established :0.629(95%CI:0.258 to 0.838),$P=0.0061$

Comparison of ROC curves showed: area under ROC curve for NMNp=1.000,for MNp=0.675,for CgA-LI=0.910.

Pairwise comparison of ROC curves revealed no significant difference between areas NMNp/CgA-LI, MNp/CgA-LI.

Conclusions. Our study proved the importance of plasma CgA-LI as a good marker in pheochromocytoma diagnosis. We can speculate that CgA-LI could be an interest marker in malignant pheochromocytoma.

Endocrinology

0494

COMPARISON OF BRAHMS AND SIEMENS COMPETITIVE IMMUNOASSAYS FOR TSH AND FREE THYROXIN DETERMINATION IN PREGNANCY

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Background. Due to lack of good standardization and different method's formats interpretation of the results of TSH and free thyroxin (FT4)is immunoassay dependent. However, comparability of different immunoassay also depends on population used for methods comparison.
Methods. In 88 pregnant women (third trimester) and 34 healthy age- and sex-matched controls plasma concentration of FT4 and TSH were measured by two competitive Methods. RIA (BRAHMS) and CLIA (Centaur Siemens). In addition, the level of thyroxin binding globulin (TBG), total T4 (BRAHMS) and T_3Uptake (Centaur Siemens) were determined.
Results. Both immunochemical methods for FT4 measurement were correlated (r=0.725; p<0.001), but in pregnant women the mean FT4 concentration value obtained by CLIA method was significantly of higher as compared to the mean value obtained by RIA (p<0.003). In pregnant women with serum TGB concentration less than 39 mg/l (normal values 13.3-28.9 mg/l) and T_3Uptake value higher than 0.70 (normal value 0.75-1.25) as well as in controls no differences between two methods for FT4 measurement have been found. In pregnant women and in controls there were no differences between methods for TSH measurement/.
Conclusions. RIA (Brahms) and CLIA (Siemens) methods for TSH measurement are well harmonized. The results of FT4 concentration measured by BRAHMS and Siemens competitive methods cannot be used interchangeably if thyroid binding proteins are abnormal.

0495

ADIPOKINES ARE PRESENT IN SEMINAL PLASMA AND CORRELATED TO AN IMPAIRED SEMEN QUALITY IN OBESE MEN

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Background. An inverse relationship between adipose tissue and spermatozoa function has been connected to raising prevalence of obesity in the last decades. The molecular background for this male reproductive dysfunction has not been clarified. Adipokines play a major pathophysiological role in obesity-related diseases and might be a link between obesity and male subfertility.
Methods. Measurement of leptin, adiponectin, resistin, progranulin, chemerin, NAMPT and vaspin was validated in seminal plasma (SP) and concentration of these adipokines was analyzed from 130 sperm donors (mean±SD, age: 37.4±12.7years, BMI 27.8±6.24 kg/m²). The data were compared to corresponding serum values and correlated to standard semen parameters (WHO). Influence of BMI on semen adipokine concentrations and sperm parameters was compared in age-matched subgroups of donors (n=22) with and without overweight/obesity.
Results. In serum, mean concentrations of adiponectin, leptin and chemerin were significantly higher, whereas vaspin, progranulin and NAMPT were lower than in SP(P<0.05). Overweight/obese men had significantly less motile, normomorph spermatozoa and lower SP adiponectin concentrations, whereas concentrations of chemerin and vaspin in SP were higher than in normalweight men. Furthermore, SP concentrations of adiponectin and progranulin correlated positively and vaspin and chemerin negatively (P<0.05) with sperm parameters.
Conclusions. Our results indicate that adipokines are present in the male reproductive tract where they appear to be regulated independently from concentrations in peripheral blood. Adipokine concentrations in SP are potentially related to semen quality based on the individuals' body weight. The molecular pathogenesis of these findings is currently under investigation.

Endocrinology

0496

LOW RANGE ACCURACY OF TESTOSTERONE IMMUNOASSAYS AND POSSIBLE IMPROVEMENT THROUGH SAMPLE EXTRACTION

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Background. Immunoassays have been shown to overestimate testosterone concentrations in the low measuring range. We studied the accuracy of five in the year 2010 commercially available testosterone immunoassays and possible improvement through sample extraction, by comparison with liquid chromatography tandem-mass spectrometry (LC-MS/MS).
Methods. We measured testosterone concentrations before and after a diethyl ether sample extraction in 68 sera categorized by LC-MS/MS as ≤ 3.2 nmol/l. Immunoassays studied were Abbott Architect®, Beckman Coulter Access®, Siemens ADVIA Centaur®, Siemens Coat-a-Count® and the second generation assay Roche Cobas®.
Results. The Cobas® assay turned out to be the only immunoassay not significantly different compared with LC-MS/MS (slope 1,20; intercept -0,14; R 0,80). The results for the other assays were: Architect® (slope 1,79; intercept 0,75; R 0,71), Access® (slope 1,67; intercept -0,24; R 0,76), ADVIA Centaur® (slope 1,50; intercept 0,08; R 0,86) and Coat-a-Count® (slope 1,29; intercept -0,17; R 0,93). Sample extraction clearly improved the accuracy of the Architect® assay (slope 0,88; intercept 0,15; R 0,88) and the Coat-a-Count® assay (slope 1,12; intercept -0,12; R 0,91), as a result of which no significant differences compared with LC-MS/MS existed anymore.
Conclusions. The Cobas® assay was the only immunoassay tested, that is reliable for the investigation of testosterone concentrations ≤ 3.2 nmol/l in untreated samples. Sample extraction has to be performed in advance for accurate measurement with the Architect® and Coat-a-Count® assays. Results for the Siemens Immulite® 2000 and the second generation Architect® assays are in progress.

0497

CORRELATIONS AMONG THE TESTOSTERONE FRACTIONS IN HYPERANDROGENIC WOMEN

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Background. Large proportion of plasma total testosterone (TT) is bound to binding proteins, so it does not provide reliable information about the biologically active testosterone level. In females free testosterone (fT) is about 0.7-2.2% and bioaviable testosterone (BA-T) is 15-48% of the TT. The aim was to investigate the analytical and clinical correlations among measured TT and calculated free testosterone index (fTi), BA-T and fT levels.
Methods. sera from 61 women (26±10 years) were analysed: 12 healthy controls and 49 patients – the latter divided in two groups on the basis of clinical findings: having high (n=26, HRP), or low risk (n=23, LRP) for true hyperandrogenism. TT and SHBG were measured using electrochemiluminescence immunoassays (Roche) and albumin with colorimetric method. Then fT, fTi and BA-T were calculated.
Results. All sera had normal albumin levels (46±3 g/l). Significant (p<0.01), but weak correlations were between TT and fTi (r=0.68), between TT and BA-T (r=0.79) and between TT and fT (r=0.79), while correlations between fTi and BA-T (r=0.94) and between fTi and fT (r=0.89) were stronger. In HRP all fractions were higher (p<0.001) compared to the control and LRP. The sensitivities and specificities of testosterone fractions were as follows: fTi, 54% and 85%; BA-T, 54% and 88%; TT, 50% and 76%; fT, 46% and 74%.
Conclusions. only marginal differences are among sensitivities of fTi, BA-T, TT and fT with normal albumin levels. Our data confirm that only the SHBG should be measured to calculate the fTi when TT is out of the reference range.

Endocrinology

0498

THE INFLUENCE OF THE ORAL CONTRACEPITVES' GESTOGEN COMPONENT ON THE ALDOSTERONE/RENIN RATIO

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Background. It is well known that oral contraceptives (OC) can influence the aldosterone/renin ratio (ARR). Various gestogen components of the OC may affect plasma aldosterone (ALD), plasma renin activity (PRA) or direct renin concentration (DRC) differently. Aim was to analyze the effect of OC's gestogen (desogestrel, DSG; gestoden, GD; drospirenon, DRSP) component on ARRs (ALD/PRA vs. ALD/DRC).
Methods. PRA, DRC (DiaSorin) and ALD (Immunotech) were measured with RIA in 61 samples of healthy, normotensive volunteers: 22 women taking no OC were controls; 19 received DSG, 15 GD and 5 DRSP.
Results. moderate correlation (r=0.78; p<0.01) was between PRA (1.72±1.31 ng/ml/h) and DRC (12.3±10.1 ng/l). ALD was significantly (p< 0.01) higher (43.5±29.5 ng/dl) in DRSP group. DRC (7.96±6.56 ng/l) was significantly (p<0.05) lower in women receiving DSG than in control and DRSP (14.5±9.5 and 20.1±17.6 ng/l) groups. ALD/DRC, but not ALD/PRA was significantly (p<0.05) higher in women receiving DSG and GD (2.85±3.19 and 3.07±3.3 ng/dl/ng/l) than in controls (1.32±0.78 ng/dl/ng/l). Number of cases with ALD/PRA out of the cut-off value (>37.6 ng/dl/ng/ml/h) was low (2 in DSG and 1 GD groups), but much higher (5 in DSG, 3 in GD and 1 in DRSP groups) if determining ALD/REN (cuf-off >3.9 ng/dl/ng/l).
Conclusions. Week analytical and clinical correlation exists between PRA and DRC. ALD/PRA is usually more reliable in patients on OC. Our preliminary results indicate that in women receiving DRSP-containing OC not only higher ALD but also high PRA and DRC are present because of the antimineralocorticoid effect of these gestogens.

0499

SERUM RESISTIN AND INSULIN-LIKE GROWTH FACTOR-I LEVELS IN PATIENTS WITH HYPOTHYROIDISM AND HYPERTHYROIDISM

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Background. In this study we aimed to evaluate the serum levels of resistin and insulin like growth factor-I (IGF-I) and also the relationship between resistin and IGF-I in hypothyroid and hyperthyroid patients.
Methods. 15 cases of hypothyroid, 16 cases of subclinical hypothyroid, 15 cases of hyperthyroid, 15 cases of subclinical hyperthyroid and 17 healthy individuals, totally 78 subjects have been included to this study. We measured serum resistin levels using enzyme linked immunosorbent assay method, and serum IGF-I levels by chemiluminescence immunometric method.
Results. Serum resistin levels in hypothyroid total group were significantly higher than those in control group (12.66±6.04, 8.45±2.90 ng/mL, respectively, p<0.05), and in subclinical hyperthyroid group they were significantly higher than controls (14.88±7.73, 8.45±2.90 ng/mL, respectively, p<0.05). Serum IGF-I levels were significantly lower in hypothyroid total group compared to hyperthyroid total and control groups (117.22±52.03, 155.17±51.67, 184.00±49.73 ng/mL, respectively, p<0.05). Furthermore serum IGF-I levels in hypothyroid group were significantly lower compared to control group (123.70±44.03, 184±49.73 ng/mL, respectively, p<0.05), and in subclinical hypothyroid group they were significantly lower than those in control and subclinical hyperthyroid groups (111.11±59.35, 184.00±49.73, 166.60±47.87 ng/mL, respectively, p<0.05). No correlation was observed between serum resistin and IGF-I levels.
Conclusions. We concluded that increased resistin levels are directly related to thyroid dysfunction and serum IGF-I levels decrease in hypothyroid status. This decrease of IGF-I may be a risk factor for hypothyroidism.

Endocrinology

0500

THE IMPORTANCE OF HORMONAL STATUS IN PATIENTS WITH HYPERANDROGENISM

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Background. The most common clinical manifestations of hyperandrogenism in women are: hirsutism, polycystic ovary syndrome (PCOS), congenital adrenal hyperplasia (CAH), and androgen-secreting tumors. Aim of this study was to analyse the hormonal status in patients with: PCOS, late onset of CAH, and idiopatic hirsutism (IH).
Methods. The sudy included 100 individuals, gruped in four groups. 1st (n=62) - patients with PCOS (age 16-39 years), 2nd (n=8) - patients with late onset of CAH (age 18-35 years), 3th (n=19) - patients with IH (age 16-27 years), and 4th (n=11 healthy women, age 19-25 years) was control group. Serum FSH, LH, PRL, estradiol, progesterone, DHEA-S, and 17α OH-P were measured by electrochemiluminescence.
Results. The FSH level was: 1st group - 7,1±4,7 mIE/ml, 2nd – 7,03±5,02 mIE/ml, 3th – 6,5 ± 4,2 mIE/ml, and control -5,62±3,0 mIE/ml.LH level was: 1st group - 19,3±15,6 mIE/ml (90% higher than control – 10,01±14,7), 2nd - 10,55±4,5 mIE/ml, and 3th - 9,17±5,9mIE/ml. Testosterone value was 170% (1st), and 280% (2nd) higher than control. The values of DHEA-S, cortizol, and estradiolwere in the referent range in all groups, The values of prolactin was 50,9% (1st group), 15,3% (2ndgroup), and 23,1% (3th group) higher than control (p<0,05). The highest value of 17α OH-P has a 2nd group (99,21% higher than control, p<0,01).
Conclusions. Analysis of hormonal status in patients with hyperandrogenismis very important for determination of origin of excess production of androgens, which can be by the ovaries and/or the adrenal glands.

0501

EXPERIENCE OF PETROSAL SINUS CATHETERIZATION IN OUR HOSPITAL. AS EXAMPLE OF MULTIDISCIPLINARY COLLABORATION

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Background. The aim of this study is to present our experience on bilateral and simultaneaous inferior petrous sinus catheterization, on those patients with ACTH-dependent Cushing´s Syndrome. We describe the procedure and our Results.
Material and Methods. A retrospective study was held between January 2003 and September 2009, including nine patients (2 men ,7 women) presenting ACTH-dependent Cushing´s syndrome.
Simultaneous inferior petrosal sinus catheterization was performed in all of them,sampling basal ACTH and after CRH stimulation. The determination of ACTH is made by chemiluminescent immunoassay. ACTH leves gradient in different pituitary locations and peripheral blood levels was recorded. Diagnosis was suggested when inappropriate and maintained hypercortisolemia. High urinary free cortisol levels and no response to dexamethasone suppression were detected. Eight out of nine patient had a prior negative imaging test result.
Results. Inferior petrosal sinus bilateral catheterizacion was successfully performed in all cases, with no evidence of further complications. The results showed definitive diagnosis in all cases. In four patients ACTH levels gradient was lateralizad to the left., leading to a specific surgical approach. One patient presented pituitary ACTH-secreting adenoma. Two other patients showed ectopic ACTH production,one showed suprarenal adenoma secreting ACTH and other one showed response to pituitary stimulation without side lateralisation, presenting a historial dignosis of pituitary hyperplasia.
Conclusions. Petrosal sinus catheterization is shown to be efficient procedure to manage Cushing´s syndrome differential diagnosis and to obtain specific anatomical information.

Endocrinology

0502

PATIENTS WITH SIADH IN HOSPITAL FRYDEK-MISTEK

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Background. SIADH or Schwartz –Barter syndrom was firstly published in the end of the fifties in USA. Typically for this syndrom are mineral and water disorders with hyponatremia, hypochloremia together with normal urine concentration. ADH is pathologically released from neurohypophisis or cancer cells with the connection of nefron tubulus and re asorbtion of water to ECT, later ICT. The target of study: Inpatients with hyponatremia and suspected from SIADH, a few laboratory tests were carried out to document or eliminate the syndrome of abnormal secretion of ADH. The inpatients were hospitalized at the surgery ward, internal and neurology ward.
Methods and Results. The basic meaning for the diagnostic of SIADH has measurement of renal parameters, mainly EWC and CEI.
Conclusions. Syndrom SIADH is for hospatalized inpatients diagnosed more often than we would have expected. It is very important to have rapid cooperation between the doctors and the lab personnel. The diagnostic is built mainly on the biochemistry tests – it is very important to exclude endocrinology disorders and CSWS.

0503

LATE-NIGHT SALIVARY CORTISOL FOR DIAGNOSING CUSHING'S SYNDROME, AS MEASURED BY LC-MS/MS AND AUTOMATED IMMUNOASSAY

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Background. The objective of the study was to describe the diagnostic performance of late-night salivary cortisol (LNSC) for diagnosing Cushing Syndrome (CS).
Methods. Three groups of subjects were studied in whom at least one LNSC sample was collected: normal subjects (n=77), patients in whom Cushing syndrome (CS) was excluded or could not be confirmed (pseudo-Cushing; n=19), and patients with proven CS (n=14). Saliva was collected at 23:00 h using a Salivette with polyester swab (Sarstedt). LNSC was measured by both liquid chromatography tandem mass spectrometry (LC-MS/MS, TQD Waters) and automated electrochemiluminescence immunoassay (ECLIA, Roche).
Results. For both assays, LNSC levels (median (range)) were higher in patients with CS (7,3 (2,0-42,6) nmol/L for LC-MS/MS and 13,0 (6,0-121,7) nmol/L for ECLIA), compared with patients with pseudo-Cushing (1,4 (0,6-4,4) nmol/L for LC-MS/MS and 3,5 (0,6-13,9) nmol/L for ECLIA), and normal subjects (0,7 (0,5-9,0) nmol/L for LC-MS/MS and 2,5 (0,5-15,5) nmol/L for ECLIA), $P < 0.0001$. For LC-MS/MS, the highest combined sensitivity (86%) and specificity (90%) was achieved at a cut-off point of 3,05 nmol/L. For ECLIA, the highest combined sensitivity (79%) and specificity (79%) was achieved at a cut-off point of 7,40 nmol/L.
Conclusions. LNSC is a non-invasive and promising test for screening patients suspected to have CS. LC-MS/MS shows the highest diagnostic performance (sensitivity/specificity) when compared to ECLIA.

Endocrinology

0504

THE INFLUENCE OF CORTICOSTEROID THERAPY ON INSULIN AND C-PEPTIDE LEVEL IN PATIENTS WITH MULTIPLE SCLEROSIS

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Background. Multiple sclerosis is a neurodegenerative and autoimmune disease affecting the nervous system white matter. The cause of the disease is unknown and there is no right therapy except palliative treatment with drugs used to alleviate the symptoms of the disease including corticosteroids. Their long-term use is not recommended because of numerous complications (diabetes, osteoporosis, ulcers).
Methods. The goal of this study was to investigate the effect of corticosteroid therapy on insulin and C-peptide level in the blood, before and after therapy. We studied 30 patients of both sexes: 19 women and 11 men. All patients were in the process of relapse of disease and were hospitalized in the Department of Neurology, Military Medical Academy. They got 1g/day Methylprednisolone as an infusion over five days. The levels of insulin and C-peptide in the blood were measured one day before the therapy and after the therapy. Determination of the hormone levels has been performed using immunoassay-based methods of electrochemiluminiscence (Roche Diagnostics, Elecsys 2010).
Results. Observed mean values before treatment are: insulin 44.94 pmol/L, C-peptide 0.71 nmol/L, and after therapy: Insulin 180.59 pmol/L, C-peptide 1.14 nmol/L. Statistical analysis of data by applying Student's t-test was shown a significant difference before and after administration of the drug.
Conclusions. Based on these results we can conclude that, due to the great influence of corticosteroids on the level of these hormones, long-term use could lead to the development of diabetes as one of the many complications in patients with multiple sclerosis.

0505

THE STUDY OF LIPID PROFILE IN DIFFERENT GROUP OF HYPOTHYROIDISM PATIENTS ATTENDING REFERRAL HEALTH CARE CENTER (TU TEACHING HOSPITAL), KATHMANDU, NEPAL

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Background. Hypothyroidism is characterized by elevated thyroid-stimulating hormone (TSH) level in patients with decreased serum free thyroid hormone (FT3 and FT4). Nepal is one of the developing countries and most of the part of Nepal belongs to mountainous regions which are endemic for iodine deficiency. Thyroid disorder, along with a higher than average prevalence of goiter, is a major public health problem in that area. Hypothyroidism is well known causes of dyslipidaemia which in turn is associated with an increased risk of coronary artery disease. Thyroid hormones have significant effects on the synthesis, mobilization and metabolism of lipids. The aim of the present study was to evaluate the pattern of serum lipid alterations in patients with different degree hypothyroidism in comparison to age- as well as sex-matched euthyroid group.
Methods. Serum lipid parameters of 100 patients with hypothyroidism and 100 age- and sex-matched euthyroid (diagnosed clinically and laboratory report (FT3.FT4 and TSH) controls were evaluated in a cross-sectional study.
Results. The total cholesterol and LDL cholesterol of the patients with hypothyroidism were significantly increased in comparison of euthyroid controls (p=0.001 and p=0.000).The mean value of total cholesterol and LDL cholesterol were found to be increased with increase in TSH levels. The highest mean values of lipid were found in group-III hypothyroidism.
Conclusions. Hypothyroidism is significantly associated with hypercholesterolemia and increased level of LDL-cholesterol .Higher degree of hypothyroidism leads to higher degree of hypercholesterolemia.

Endocrinology

0506

INDIVIDUALS WITH POSITIVE THYROID PEROXIDASE ANTIBODIES: A 40 MONTH FOLLOW-UP OF THYROID FUNCTION

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Background. Thyroid peroxidase antibodies (TPOAb) above cut off values detect early Autoimmune Thyroid Disease (AITD) before changes in thyroid-stimulating hormone (TSH), allowing identification of risk groups, with an annual rate of progression to hypothyroidism of up to 5%.

The objective of this study was to diagnose thyroid dysfunction in clinically healthy people withpositive TPOAb (TPOAb(+)) followed for 40 months.

Materials and Methods. This study began in July 2007, with controls in January 2009 and November 2010, with approval of the Ethics and Academics Committee of the Maciel Hospital and with informed consent of the subjects.

Fourtheen healthy TPOAb(+) adults (12 females, mean age 36 years) and equal number of negative TPOAb (TPOAb(-)) controls (11 females, mean age 39 years) were included in the follow up.

Serum TSH third generation immunoassay (reference range 0,48 -3,56 mIU/L), serum TPOAb (cut off 12 IU/mL) and serum free Thyroxin (T$_4$L, reference range 0,71-1,85 ng/dL) were determined by the Microparticle Enzyme Immunoassay (MEIA) method in ABBOTT Axsym's equipment.

Results. Two TPOAb(+) subjects were lost before the first control. Six of the 12 remaining TPOAb(+) subjects presented hypothyroidism: 1 clinical case (at 40 months, high TSH, low T$_4$L) and 5 subclinical cases (high TSH, normal T$_4$L), one of which was transient. All TPOAb(-) controls had low TPOAb values and remained with normal thyroid function.

Conclusions. This study shows the usefulness of determining TPOAb as a risk marker for AITD.

Genetic disease

0507

54-YEAR-OLD DIABETIC MAN WITH UNEXPECTEDLY LOW SERUM CHOLESTEROL LEVELS

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Background. Familial hypobetalipoproteinemia (FHBL) is an autosomal co-dominant disorder, characterized by very low plasma levels of LDL-C and apolipoprotein B (apoB). FHBL may be linked or not to the *APOB* gene. We reported a case of type 2 diabetes mellitus (T2DM) with extremely low serum cholesterol levels.
Methods. An asymptomatic 54-year-old diabetic man was referred for further examination of extremely low serum cholesterol levels. The patient had no history of childhood illness, lipid malabsorption and any cardiovascular or neurological dysfunctions. The physical examination was within normal limits.
Results. Laboratory studies revealed decreased levels of total cholesterol of 1.81 mmol/L, triglycerides of 0.25 mmol/L, LDL-C of 0.26 mmol/L and apoB of <0.2 g/L. Liver, kidney and thyroid function tests were within the reference intervals. There was not any pathological finding in his complete blood count. The measurement of serum fat-soluble vitamin revealed reduced vitamin E and beta-carotene levels. Hepatic steatosis and mild hepatomegaly were observed in the abdominal ultrasonography. The clinical diagnosis of FHBL was confirmed through molecular diagnosis with identification of the mutation in the *APOB* gene. The sequence analysis of the *APOB* gene showed the presence of a single nucleotide substitution in exon 26 in heterozygous state. This substitution leads to the formation of a truncated apoB containing 2494 amino acids, which was designated as apoB-55.
Conclusions. We have identified an apoB-55 truncation mutation in a 54-year-old hypobetalipoproteinemic man with T2DM. The mutation specifying the truncated form of apoB seems to ameliorate the lipid abnormalities usually present in T2DM.

0508

ABERRANT SUBTELOMERIC REARRANGEMENT IN CHROMOSOME 2 IN PAEDIATRIC PATIENT WITH DEVELOPMENTAL/ MENTAL DELAY

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Background. Subtelomeric regions are usually enriched for genes, and are more susceptible to aberrant rearrangements than other chromosomal regions. Subtelomeric imbalance is widely accepted as leading to developmental/mental delay or multiple congenital anomalies, although the exact cause-and-effect relationship has not been well defined. The clinical consequences are probably determined by the location and kind of the rearrangement, such as deletions or duplications, as well as the size of the aberrations, including the numbers and function of the genes involved. Study of aberrations in gene-enriched subtelomeric regions provides essential clues for localizing critical regions, and provides a strategy for identifying new candidate genes.
Patient and Methods. The proband is a boy of four years old studied in Neuropediatric Service by mental/developmental delay. Boy presented microcephalia, psychomotor alterations, muscular hypotonia, facial dysmorfic features, flat feet and cryptorquidia. Karyotype, hormonal levels and blood analysis were normal. In order to detect genetic anomalies we obtained genomic DNA from peripheral blood and a MLPA analysis for mental/developmental delay was performed using P-036, P-070, p-106 and P-245 SALSA MLPA kits.
Results. Patient was carrier of alterations in subtelomeric regions of chromosome 2 detected with the P-036 SALSA MLPA kit. This kit contains one probe for each subtelomeric region and is designed to detect deletions/duplications. There were a deletion in the p arm (2p25) affecting one copy of ACP1 gene and a duplication in the q arm (2q37.2) with an increase in genetic dosage of CAPN10 gene. Parents were analyzed and were normal for these rearrangements.

Genetic disease

0509

SHOX GENE DELETION IN SHORT STATURE PAEDIATRIC PATIENT DETECTED BY MLPA ANALYSIS

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Background. The SHOX gene is part of a larger family of homeobox genes, which act during early embryonic development to control the formation of many body structures. Specifically, the SHOX protein is essential for the development of the skeleton. One copy of the SHOX gene is located on each of the sex chromosomes (the X and Y chromosomes) in an area called the pseudo-autosomal region or PAR1 (Xq22/Yp11.3).
Patient and Methods. The propositus was a boy with a developmental delay detected at 2 years old. Boy presented short stature, microcephalia, dorsal hypercyfosis, Attention-Deficit/Hiperactivity Disorder, epilepsy and frontal lobule dysfunction. MRI from central nervous system was normal. Karyotype, hormonal levels and blood analysis were normal. EEG showed a pathologic image with centroencephalic spikes discharges. In order to detect genetic anomalies we obtained genomic DNA from peripheral blood and a MLPA analysis for mental/developmental delay was performed using P-036, P-070, p-106 and P-245 SALSA MLPA kits.
Results. Patient was carrier of only one copy of SHOX gene, detected with the P-036 SALSA MLPA kit. This kit contains one probe for each subtelomeric region and is designed to detect deletions/duplications of each subtelomeric region, and contains one probe for each of the two X/Y PAR regions, as well as two small synthetic MLPA probes for non-telomeric Y-chromosome specific sequences. Parents and one sister of patient were analyzed too, and his mother was carrier of only one copy of SHOX gene, but she only presented short stature without any pathologic symptoms.

0510

THREE NOVEL CYP11B1 MUTATIONS IN MOROCCAN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA DUE TO STEROID 11BETA-HYDROXYLASE DEFICIENCY

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Steroid 11beta-hydroxylase deficiency (11OHD) is the second cause of congenital adrenal hyperplasia (CAH). It accounts only for 5% of all CAH. To date, only 51 different mutations of the *CYP11B1* gene have been reported with poor clinical and biological data and most of them could be considered as private mutations.
As one mutation, the p.R448H was identified especially in Moroccan Jews and two other *CYP11B1* mutations have a high incidence in Tunisian patients, we report from another Maghreb population the clinical, follow-up and molecular genetics of Moroccan patients with classical 11OHD.
Five patients belonging to 3 families were recruited on clinical data. The diagnosis was confirmed by 11-deoxycortisol measurement. Sequencing of the *CYP11B1* gene and molecular modeling were performed. Clinical, hormonal and follow-up data were consistent with a severe form of 11OHD. Gender reassignment and evolution of hypertension were discussed. We identified three novel mutations, p.Ala259Asp, p.Gly446Val and IVS5+2T>G. As each patient was homozygous for one mutation, we could deduce from their phenotype and our modeling studies that the p.Gly446Val mutation was more severe than p.Ala259Asp.
Our study shows a good correlation between phenotype and genotype. Each *CYP11B1* mutation is new and private, contrasting with the high incidence of p.R448H mutation in Moroccan Jews and the two Tunisian mutations.

Genetic disease

0511

ASSOCIATION BETWEEN PARAOXONASE ACTIVITY AND PON1 L55M IN BIPOLAR I PATIENTS

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Background. In the recent years, lower paraoxonase activity has been reported to represent a risk factor not only for atherosclerosis, but also for neuropsychiatric disorders.The information on association between bipolar disorder and this activity is scanty. This study aims to investigate the variations of paraoxonase activity in Tunisian bipolar I patients according toPON1 L55M polymorphism and to explore its relation to sociodemographic, clinical and therapeutic characteristics of this population. Patients and
Methods. Our study included 53 patients with bipolar I disorder diagnosed according to the DSM-IV, and 52 controls, aged 37.9 ± 13.5and 33.5 ± 16.0 years, respectively. Paraoxonase activity was determined by kinetic methods and PON1 L55M polymorphism by PCR-RFLP.
Results. Compared with controls, patients had significantly lower paraoxonase activity (1167 ± 116 vs. 255 ± 94, p < 0.001). This decrease was noted independently to PON1 L55M polymorphism; however, the subjects with MM genotype had the lowest activity. Moreover, there was no significant change in paraoxonase activity in patients in relation to illness episodes and treatment, whereas the lowest values of this activity were seen in manic patients and those taking lithium.
Conclusions. Bipolar patients had a significant decrease in paraoxonase activity independently to PON1 L55M. The decrease in this activity was not significantly associated with the clinical and therapeutic characteristics of this population. Further studies are required to clarify the implication of this polymorphism and paraoxonase activity in the pathophysiology of bipolar I disorder.

0512

ATP7B GENE MUTATIONS ASSOCIATED WITH INCIDENCE OF WILSON DISEASE IN CROATIAN POPULATION

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Background. Wilson disease (WD) is an autosomal recessive disorder of copper metabolism resulting from the absence or dysfunction of copper transporting P-type ATPase (*ATP7B*). More than 400 disease causing mutations of the *ATP7B* gene have been identified to date.
Methods. Genomic DNA was used to amplify 21 exons of the *ATP7B* gene. Sequencing analysis was performed by PCR and capillary electrophoresis with BigDye Terminator v3.1 kit on AB Genetic analyzer 3130xl.
Results. Here we report results of sequencing analysis of the *ATP7B* gene. We have analyzed coding region of the *ATP7B* gene of clinically diagnosed WD patients from Croatia, already screened for the most common His1069Gln mutation. It accounts for 54,4% of Wilson disease alleles in croatian population. Out of the total number of 71 tested patients with WD, molecular analysis has confirmed the clinical diagnosis in 44 patients (61,9%) so far. 17 (23,9%) patients are homozygous for the most common His1069Gln mutation. In 13 patients (18,4%) only one mutation has been identified. Mutations in croatian population are mostly distributed in exons 5, 8, 13, 14, 15 and 21 of the *ATP7B* gene.
Conclusions. Sequencing analysis of the *ATP7B* gene is the best method to establish the frequency of mutations in specific population so the screening test panel for most common mutations can be developed for this population.

Genetic disease

0513

GILBERT'S SYNDROME DIAGNOSIS AND HIGH RESOLUTION MELTING ANALYSIS (HRMA): HOW DO TA-REPEATS IN THE *UGT1A1* GENE PROMOTER AFFECT THE MELTING TEMPERATURE (T_M)?

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Background. Gilbert's Syndrome is a benign, and clinically inconsequential entity of mild unconjugated hyperbilirubinemia and it is present in approximately 10% of the Caucasians; in this population the basis of the disorder is the result of a homozygous TA insertion into the promoter of the gene (*UGT1A1*28 allele*). Recently, our group published a paper where we identified the most frequent polymorphisms of the *UGT1A1* gene promoter present in Caucasians by HRMA. Our results showed that the $(TA)_7$ amplicon presents a T_m reduction of about 0.4°C as compared with $(TA)_6$ amplicon.

Aim and Methods. Since our resultswere in disagreement with a paper where the Authors found that $(TA)_6$ amplicon shows a T_m lower than $(TA)_7$ amplicon using SYBR Green I methodology and suggesting that these results were consistent with the fact that the $(TA)_7$ amplicon is longer and melts after, we applied their protocol in HRMA, by monitoring the fluorescence both during amplicon renaturation phase and while the amplicon is denaturing.

Results. The wild type $(TA)_6$ amplicon presents a T_m higher than the $(TA)_7$ amplicon. The TA di-nucleotide insertion determines the same effect on T_m both in 70bps and in 132bps amplicon. This behavior doesn't depend on the denaturation or renaturation steps in which the fluorescence is acquired. Thus, in presence of TA repeats, the T_m seems to mainly be dependent on the nucleotide composition rather than on the amplicon length. However, we don't know if the $(TA)_5$ and $(TA)_8$ amplicons, rare in Caucasians, follow the same behavior.

Conclusions. The results obtained are in agreement with the findings showing that TA tandem repeats in DNA are intrinsically unstable and suggest that $(TA)_7$ amplicon has a T_m lower than the $(TA)_6$ amplicon, since a TA insertion may determine further instability in a region carrying TA repeats.We confirm the limitations of the SYBER Green methodology as tools to assign length of a specific amplicon based on the T_m. On the contrary, HRMA seems to be a screening technique with high genotyping power; the absolute T_m as well as the melting profiles and difference fluorescence plots, obtained by high-resolution, can help to assign the length to the specific amplicon studied.

0514

BIOCHEMICAL AND GENETIC TESTING IN A LARGE COHORT OF PATIENTS WITH SUSPICION OF MITOCHONDRIAL DISEASE

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The Center of Metabolic Disease, MunichMunicipalHospital has an over twenty year experience in the investigation of patients with suspicion of mitochondrial disease. We present conclusions from the statistical analysis of samples investigated within the last five years (1.1.2005-31.12.2009).

Out of 1940 samples 1430 were muscle biopsies. In 921 biopsies the activities of respiration chain complexes I-IV were measured. Genetic testing was performed in 701 samples. In 322 samples a defect of respiration chain could be measured (35.0%). Isolated complex I defect was detected most frequently (118 = 12.8%), followed by combined complex I and IV defect (66 = 7.2%).

In 139 out of the 701 genetically investigated samples a positive result was found. In 35 cases (5.0%) mtDNA deletions and in 29 cases (4.1%) a depletion of mtDNA was detected. Interestingly within the 20 deletion positive cases also measured for respiratory chain activity only in 7 cases a defect was detected. In contrast, within the 19 depletion positive samples measured for respiratory chain 16 cases a defect was found.

In 253 samples genetic testing was performed initially without investigation of the respiratory chain acticity. In as much as 72 (28.5%) of these cases a diagnosis could be established.

The analysis of samples sent to the Center of metabolic disease Munich with suspicion of mitochodriopathy shows the high value of the muscle biopsy in the elucidation of mitochondrial disease. However a normal respiratory chain activity in skeletal muscle can not rule out mitochondrial disease. Solely due to clinical symptoms a further genetic workup might be indicated. Analysis of mtDNA deletions or depletion should be the initial step.

Genetic disease

0515

HIGH PREVALENCE OF UNRECOGNIZED FAMILIAL CASES PRESENTING AS APPARENTLY SPORADIC MTCS THAT CARRY THE RARE G533C MUTATION IN EXON 8 OF THE RET GENE IN GREECE

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Background. Genetic screening for *ret* mutations is a routine practice in the evaluation of Medullary carcinoma of the thyroid (MTC). Roughly 25% of these tumours are familial, as components of the multiple endocrine neoplasia type 2 syndromes (MEN2A) or familial MTC (FMTC). In familial cases the majority of mutations lie in the hot spot region which includes exons 10 , 11, 13 and 14 mutations. A rare mutation at exon 8 (G533C) has been reported in apparently sporadic MTC patients in Greece. The aim of the present study was to evaluate a series of isolated, apparently sporadic MTCs, with negative family history for the exon 8 mutation.

Methods. Genomic DNA was extracted from peripheral lymphocytes. Samples were analysed in Applied Biosystems 7500 Real-Time PCR using a Taqman® SNP Genotyping assay, and comfirmed in Mega BACE sequencer analyzer of Amersham Biosciences using a sequencing kit of Amersham.

Results. The G533C exon 8 mutation was identified in 9 out of 126 unrelated patients. This raises the prevalence of hereditary disease due to this specific mutation in apparently sporadic tumors to 7.14% (9/126).

0516

DIAGNOSTIC TESTS FOR THE DEFECTS OF RENAL URATE TRANSPORTERS

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Backround. Primary hereditary renal hypouricemia is a genetic disorder affecting renal uric acid (UA) reabsorption with complications such as nephrolithiasis and exercise-induced acute renal failure. The known causes are: defects in the SLC22A12 gene, encoding the human urate transporter 1 (hURAT1), and also impairment of voltage urate transporter (URATv1), encoded by SLC2A9 (GLUT9) gene.Diagnosis is based on hypouricemia (< 119 μmol/l) and increased fractional excretion of UA (> 10%). To date more than one hundred Japanese patients with mutations in hURAT1 gene have been described and this number is unique worldwide. Hypouricemia is sometimes overlooked, therefore we have set up the flowchart for this disorder.

Methods. Serum and urinary UA and creatinine were determined. The sequence analysis of SLC22A12 and SLC2A9 genes were performed.The patients were selected for molecular analysis from 620 Czech hypouricemic patients. These cases were found in 3 600 blood and urine samples.

Results. Secondary causes of hyperuricosuric hypouricemia were excluded.The estimations of : 1) serum UA, 2) excretion fraction of UA, 3) and analysis of hURAT1 and URATv1 genes follow. We have found 3 transition, 4 deletions in SLC22A12 gene and one nucleotide insertion in SLC2A9 gene in overall 7 Czech patients. Three patients had acute renal failure and urate nephrolithiasis.

Conclusions. Our finding of the defect in URATv1 gene gives further evidence that SLC2A9 is a causative gene of primary renal hypouricemia. Hereditary renal hypouricemia is still unrecognized condition and probably not wide spread in East Asia only.

Genetic disease

0517

THE SANGER INSTITUTE MOUSE GENETICS PROJECT

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Background. The Sanger Institute Mouse Genetics Project (MGP) capitalizes on the mutant mouse embryonic stem cell resources generated by the EUCOMM and KOMP projects, by generating and phenotyping mutant mouse lines on a large scale. The project makes the mutant strains and the phenotypic data available to the scientific community. The aim is to galvanize deeper analysis to uncover the molecular mechanisms involved in the phenotypic alterations resulting from the mutant alleles.
Methods. The MGP has produced over 440 mutant strains, of which more than 250 have finished the phenotypic screen. Every line of mice is characterized using a standardised battery of phenotypic tests relevant to key disease areas including diabetes, obesity, immune and haematology disorders. Complementing this analysis, we identify the expression profile of each gene using the *lacZ* reporter gene.
Results. An overview of the phenotyping pipelines will be presented along with examples of the sort of phenotyping data generated by the project. This ranges from clinical pathology, CBCs and naïve immune profiling to growth curves, densitometry and glucose tolerance. These data can be obtained by visiting the Sanger Mouse Portal (http://www.sanger.ac.uk/mouseportal/). The website offers the opportunity to download a weekly updated summary heat map for all the strains being examined. Scientists are also encouraged to sign up to a phenotypic alert email list to receive early notification of interesting phenotypes.
Conclusions. The MGP is generating a biological resource of mutant mice and primary phenotyping data that is openly available to the scientific community.

0518

R182C MUTATION IN EXON 4 OF NOTCH3 GENE IN A SPANISH FAMILY WITH CADASIL

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Background. CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is the most common form of hereditary stroke disorder, and is thought to be caused by mutations of the NOTCH3 gene on chromosome 19. The disease belongs to a family of disorders called the Leukodystrophies. The most common clinical manifestations are migraine headaches and transient ischemic attacks or strokes, which usually occur between 40 and 50 years of age, although MRI is able to detect signs of the disease years prior to clinical manifestation of disease.
Patients and Methods. The propositus was a woman of 39 years old with the following symptoms: migraines preceded by aura and facial hypoesthesia area. MRI study showed bilateral demielynating subcortical lesions. Patient referred that her mother had been previously diagnosed as CADASIL. A sister of 34 years old suffered of migraines preceded by aura too. Genomic DNA was obtained from peripheral blood and all coding exons and intron boundaries of NOTCH3 gene were sequenced.
Results. Three members of the family were carriers of mutation R182C. This mutation consists in a CGC-to-TGC transition in exon N3 of the NOTCH3 gene that resulted in substitution of cys for arg (R182C) in the EGF-like domain. R182C mutation has been previously described in literature in several families with this syndrome. CADASIL-associated mutations are distributed throughout the 34 epidermal growth factor-like repeats (EGFRs) that comprise the extracellular domain of the NOTCH3 receptor and result in a loss or gain of a cysteine residue in one of these EGFRs.

Genetic disease

0519

S394L MUTATION OF SERPINC1 (AT-III MILANO) RESPONSIBLE OF AT-III DEFICIENCY IN A SPANISH FAMILY

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Background. Antithrombin III is the most important inhibitor of thrombin and other coagulation proteinases. It belongs to the serine proteinase inhibitor (serpin) superfamily of inhibitors, which contain reactive centers that have evolved to attract and entrap certain proteinases. Inherited antithrombin III deficiency (OMIM 613118) is a risk factor for the early development of venous thromboembolism. Up to 228 distinct mutations have been described in the SERPINC1 gene associated with two types of deficiency:I and II. Type II deficiency, with significant clinical heterogeneity, is further sub-classified on the basis of mutations that either alter the function of the reactive site, the heparin-binding site or have multiple or pleiotropic effects.

Patients and Methods. The proband, a 21-year old Caucasian male, developed a deep venous thrombosis (popliteal venous of the right leg) at the age of 14 after traumatism and immobilization. Since then, he has been under stable oral anticoagulation with coumarins without recurrence. Plasma and DNA from all family members were obtained and presence of mutations in SERPINC1 was investigated by automatic sequencing.

Results. The proband, his mother and a sister presented low levels of Antithrombin III (60%). Genetic analysis of 7 exons and intron boundaries of SERPINC1 showed that three patients were carriers of S394L mutation in exon 6 (AT-III Denver). This family was carrier of a variant of this mutation with a change of TCG-to-TTG in codon 394 predicting the same Ser394-to-Leu substitution, known as AT-III Milano. This mutation is defective in serpin activity but binds heparin normally.

Haematology

0520

BASOPHIL COUNT ON CELL-DYN SAPPHIRE, SYSMEX XE-2100 AND ADVIA 120 EVALUATED BY A FLOW CYTOMETRIC METHOD

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Background. The basophil count of several hematology analyzers has previously been shown to correlate poorly with each other, and with the manual reference method. Because of the low percentage of basophils the manual reference method is not suited for the basophil count. Our goal was to evaluate the performance of the basophil count of three commonly used hematology analyzers against a modified form of a previously published flow cytometric method (FCM).
Methods. Basophils were counted by Cell-Dyn Sapphire, Sysmex XE-2100 and Advia 120 in 112 patient samples. Results were compared to FCM using CD123 (IL-3R) and CD193 (CCR3) as basophil markers. Additional antibodies were included to exclude other leukocytes.
Results. When compared to FCM we found a relatively good correlation for Cell-Dyn Sapphire (r=0,81), an intermediate correlation for Sysmex XE-2100 (r=0,64) and a poor correlation for Advia 120 (r=0,24). A large proportion of samples with an elevated count by Sysmex and Advia were found to have a normal count by FCM and Cell-Dyn. Advia and Cell-Dyn underestimated the basophil count, the slopes of regression lines were 0,29 and 0,51,respectively.
Conclusions. The basophil count of the evaluated instruments has limited value. Sysmex XE-2100 and Advia 120 have a low specificity. Advia 120 and Cell-Dyn Sapphire underestimate the basophil count. Cell-Dyn Sapphire might be useful in identifying basophilia.

0521

FEASIBILITY OF INTRACYTOPLASMATIC FLOW CYTOMETRIC FREE LIGHT CHAIN ANALYSIS USING FITC-LABELED ANTIBODIES RECOGNIZING KAPPA AND LAMBDA LIGHT CHAINS (FREE LITE®, THE BINDING SITE)

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Background. The measurement of total serum light chains has been a common tool for the determination of plasma cell disorders and B-cell lymphomas for years. The development of sensitive and specific serum tests for free kappa and free lambda has opened the door to new applications and increased their clinical importance. In contrast, the detection and analysis of intracytoplasmatic free light chains is an unused method by the time. Therefore we have established a method for intracytoplasmatic detection of free light chains.
Methods. Sample preparation was performed with the INTRAPREP® kit from BECKMAN COULTER and FITC-labeled antibodies specific for the detection of free light chains (FREE LITE®, THE BINDING SITE).
Results. In a preliminary test series of 8 bone marrow samples from myeloma patients and 6 peripheral blood samples from healthy donors we could detect the intracytoplasmatic excess of clonal light chain production in the myeloma cells, corresponding to the serum paraprotein isotype. In peripheral B-cells from healthy donors we could not detect a signal specific for intracytoplasmatic free light chain expression.
Conclusions. Our preliminary series with bone marrow samples from myeloma patients shows that a flow cytometric detection of intracytoplasmatic free light chains is feasible in myeloma cells. An advantage of this approach could be the determination of free light chains, maybe also in nonsecretory myelomas, independent from factors such as tumor mass or renal function.

Haematology

0522

A METHOD COMPARISON FOR D-DIMER MEASUREMENT

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Background. D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. The determination of D-dimer concentration may to help diagnose thromboembolism. Its plazma values rises in pulmonary embolism, venous thrombosis and disseminated intravascular coagulation. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes.
Methods. In this study we compare immunoenzymatic Vidas D-Dimer Exclusion (MiniVidas - Bio Merieoux) to quantitative Roche Cardiac Reader D-Dimer(Roche Diagnostics).Dimer levels were measured in 72 citrated plasmas immediately and simultaneously. Statistical analysis was made by using OLP (Ordinary least products regression) analysis.
Results. According to the datas, the mean values for Vidas D-Dimer Exclusion and Roche Cardiac Reader are 1,25 ve 0,95 ng/ml and measurment ranges are 0,09-8,57 and 0.1-10.0 ng/ml, respectively. According to the regression analysis (y=0.105+1.42x, r= 0,933) a significant positive correlation was found between to methods (p<0,001).
Conclusions. The results of each method found to be compatible. However, the results of Vidas D-Dimer Exclusion seems to be higher than the results of Roche Cardiac Reader.

0523

REFERENCE VALUES OF THE EXTENDED AUTOMATED BLOOD CELL COUNT WITH A MODERN AUTOMATED HEMATOLOGY ANALYZER

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Background. Modern hematology analyzers as the Sysmex XE 5000 can measure many new parameters in addition to conventional hematological blood cell counts.
Methods. A Sysmex XE 5000 analyzer was used to differentiate blood cells by plotting the sideward scatter versus the fluorescent signal. Within the analyzer DNA and RNA are stained using polymethines. Conventional blood cell count alone is unable to detect the here described populations of robustly fluorescing cells—DNA- and/or RNA-rich cell populations. We believe that these cells are immature forms and/or active cell forms of the respective cells.
Results. Novel hematological parameters were measured in the blood of 270 healthy subjects (medical students), including the Delta-he, which is the difference in hemoglobin between reticulocytes and erythrocytes (delta-he), the coordinates of the means of the neutrophil granulocytes in the fluorescence-versus-side scatter plot (NEUT-X, NEUT-Y), the high-fluorescing lymphocytes (HFLC), the immature granulocytes (IG),the percentage of hypo hemoglobinized red cells (HypoHe),),the percentage of hyper hemoglobinized red cells (HyperHe), the percentage of microcytic red cells (MicroR) and the percentage of macrocytic red cells (MacroR)..Reference values determined as described are respectively 19.6 [20-27.5] [mmol/l], 134.5 [134-139] [channels] , 43.2 [43-46] [channels], 0.09%[0%-0.3%] , 0.02 [0.01-0.03] [10^9/L], 0.4% [0.2%-1%] , 0.93% [0.9%-1.2%], 1.87% [1.4%-5%] and 6.6% [6.4%-8.5%]. For all: mean [5%quantile-95%quantile].
Conclusions. With these new hematological parameters promising innovative parameters are provided. These values can be determined stably and reproducibly. Some, if not all, of these parameters are expected to be introduced shortly into routine diagnostic practice.

Haematology

0524

EVALUATION OF SYSMEX SIS MIDDLEWARE LONGTERM PERFORMANCE

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Background. Different middleware solutions are available to manage the process of automated haematological cell counting. A set of criteria for further follow-up actions are usually implemented in the form of rules, often based on the presence of certain analyzer flags. A slide review with or without microscopical differentiation is the most frequent follow-up action performed.
Methods. We evaluated all rule frequencies of the Sysmex Information System (SIS) middleware software, which contains 36 distinct rules.
Results. For 105156 samples during 1 year, 39964 rules were generated (3455 ± 299 rules/month). Rules were divided arbitrarily into 3 separate categories, depending upon their prevalence. Coefficients of variation (CV) of the monthly frequencies of rules of the first category (<2% prevalence: 23 rules) had a range from 15.8% to 156.0% (mean 47.6±16.3%). CV's of rules of the 2nd category (2%<prevalence <5%: 8 rules) varied from 7.3% to 16.4% (mean 11.9±2.8%) and finally, the CV's of rule frequencies of the 3rd category (>5% prevalence: 5 rules) were quite narrow and fluctuated between 4.0% and 9.3% (mean 7.0±2.8%). Moreover, one of the more frequent rules in particular (rule 35, involving the detection of nucleated red blood cells) showed a CV over the last 3 months of 0.1%, indicating excellent stability.
Conclusions. We have found a stable rule frequency-based sample flow in our SIS hematology middleware. Furthermore, long-term follow-up of rule frequencies, especially for high-frequency rules, seems promising and may serve as a monitoring tool for the cell count process and its automated steps.

0525

PROHEPCIDIN AND ANEMIA OF CHRONIC DISEASE

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Background. Anemia of chronic disease (ACD) results from 3 major processes: slightly shortened red cell survival, impaired reticuloendothelial system iron mobilization, and impaired erythropoiesis. Hepcidin is an acute-phase protein with specific iron regulatory properties, which, along with the anemia seen with increased hepcidin expression, have led many to consider it the major mediator of ACD. Hepcidin is a principal iron regulatory hormone and its expression is stimulated by cytokines. The aim of this study was to determine serum levels pro-hepcidinin in ACD anemia.
Methods. The study included 115 patients, 72 males and 46 females. Anemia was defined as hemoglobin below 12 g/dl in females and 13 g/dl in males. We have 68(57.6%) anemic patients, 37(31.4%) have ACD, 17(14.4% IDA), 11(9.3%) ACD= IDA and 50(42.4%) no anemic patients. TNFα, interleukin IL6 and levels were determined by Immulite 1000. DRG ELISA kits were used for prohepcidine determinations. Independent Sample Test, Anova test, Chi-Square Tests was used for statistical analysis.
Results. Serum prohepcidin, IL6, TNFα concentrations observed in ACD vs IDA is (329.42+263.22 vs102.63+38.63 p=0.000, 11.8+8.9 vs 4.4+5 p=0.000, 9.8+6.4 vs 8+5.3 p=0.012respectively). Serum prohepcidin concentration have a strong correlation with serum ferritin and IL6 concentration(r =.226, p=0.041 r =.309p=0.006 respectively) and IL6 have a strong correlation with serum ferritin and TNFα (r=.215 p=0.031, r=.507 p=0.000 respectively)
Conclusions. We suggestion that hepcidin is a principal iron regulatory hormone in ACD and its expression is stimulated by IL6 cytokine.Serum prohepcidin concentration is the best marker to diagnose ACD

Haematology

0526

EVALUATION OF IRON STATUS IN PATIENTS WITH CONGESTIVE HEART FAILURE (CHF)

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Background. Anemia is common in patients with congestive heart failure (CHF) and is an independent prognostic marker for mortality in community-based patients with congestive heart failure (CHF).
The aim of the study is to evaluate the diagnostic efficiency of laboratory tests including serum transferring receptor and prohepcidin measurements in the diagnosis and differentiation of anemia in CHF patients.
Methods. Blood samples were obtained from 54 CHF patients. The samples were analyzed for full blood count, iron, ferritin, transferrin, sTfR (serum soluble transferrin receptor) and prohepcidin. The TfR-F index (sTfR-log ferritin index) was calculated.
Results. Anemia was observed in 23 patients (42.7%), 15 of which had anemia of chronic disease (ACD), 3 patients had iron deficiency anemia (IDA) and 4 patients had ACD +IDA anemia. The mean MCV, MCH, iron and Hb level was significantly lower in anemia patients compared with no anemia patients (p<0.001). The prohepcidin level was significantly lower in IDA patients compared with ACD+IDA and ACDpatients (88.4ng/ml versus 204.5ng/ml and 553.9ng/ml; p=0.000).
 STfR levels in IDA group was significantly higher than the ACD patients (15±1.4μg/ml versus 1.0±0.7μg/ml p=0.000). We have found a high sTfR/ log ferritin index (3.0±3.7) in the IDA patients compared with ACD group (0.2±0.3). ACD+IDA patients had high sTfR and high sTfR- log ferritin index compared with ACD patients (p<0.001).
Conclusions. We conclude that the sTfR, TfR-F index and prohepcidin measurements represent efficient tests for the clinical evaluation and differentiation of anemia in CHF patients.

0527

ASSESSMENT OF THE PERFORMANCE OF SERUM HEAVY IMMUNOGLOBULIN CHAINS IMMUNOASSAY TO MONITOR RESPONSE TO TREATMENT AND ITS CORRELATION WITH OTHER TUMOR MEASUREMENTS

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Background. Currently available methods for the measurement of paraprotein in multiple myeloma includesimmunofixation, electrophoresis andtotal immunoglobulin analysis. The recent development of highly specific antibodies allowes the (specific) measurement of serum IgGκ, IgGλ, IgMκ, IgMλ, IgAκ and IgAλ concentrations(HLC; Hevylite™).We evaluated the correlation of serum HLC assays with conventional paraprotein analysis in immunfixation positive patients.
Methods. M-spike was evaluated by capillar zone electrophoresis (CZE),total immunoglobulins by immunonephelometry and HLCconcentrations were quantitatedon a Siemens BNII Analyzer.
Results. Of 53 patients with monoclonal IgA (IFE) 47 patients (89%) have a pathological HLC-IgA ratio (IgG: 126/188 (67%); IgM: 34/38 (89%)).
28/53 patients (53%) have pathologically increased total IgA (IgG: 30%; IgM: 63%). 18 of the 21 patients with normal total IgA have a pathological HLC ratio (86%) (IgG: 70%; IgM: 71%).
33 of the 53 (62%) IgA-positive patients show a visible M-spike in CZE (IgG: 65%;IgM: 63%). From 20 patients with unremarkable CZE 17 patients (85%) showa pathological HLC ratio(IgG: 28%; IgM: 71%).
Suppression of the uninvolved subtype (e.g. IgGκ in IgGλ myeloma) may be helpful for clinicians in the overall assessment of the patient immune function. 17/47 IgA-positive patients (36%) with a pathological HLC ratio show HL-pair suppression (IgG: 96%; IgM: 35%).
Conclusions. We conclude that the new test Hevylite™ is an interesting tool for disease monitoring in patients with paraproteinaemia. In patients with IgG paraprotein the suppression of the uninvolved IgG subtype might be an indication of immunosuppression in myeloma patients.

Haematology

0528

IRON STATUS AND HEMATOLOGICAL PARAMETERS IN POSTPARTUM MOTHERS COMPARED TO NON-PREGNANT AGEMATCHED CONTROLS

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Background. Pregnancy and lactation induce a strain on maternal iron stores. Iron status, as determined by s-ferritin, s-transferrin receptor (TfR), reticulocyte hemoglobin content (CHr) and percentage of hypochromic erythrocytes (%Hypo), and other hematological parameters were investigated in mothers during the first 11 months postpartum and compared to age-matched, nonpregnant women.
Methods. Healthy mothers were investigated at 6 weeks (n=104), 4 months (n=100) and 11 months (n=43) after giving birth to a term infant. Healthy age-matched, nonpregnant women (n=61) were investigated once as controls.
Results. Compared to nonpregnant controls, postpartum mothers had significantly higher hemoglobin level (Hb), red blood cells (RBC) and reticulocytes during the first 11 postpartum months. From 6 weeks to 11 months maternal CHr increased, while %Hypo and TfR decreased. At 6 weeks and 4 months postpartum mothers had higher TfR levels along the whole s-ferritin distribution. At 11 months this discrepancy had disappeared.
Conclusions. During the first postpartum months there is an increased erythropoiesis, which may affect certain parameters, particulary TfR, indicating a need for specific reference levels for certain haematological and iron parameters in the pregnancy and postpartum period.

0529

MEAN CORPUSCULAR VALUE (MCV) IN POLISH NONANAEMIC POPULATION

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Background. MCV is one of the most important haematological parameters in differential diagnosis of anaemia, nevertheless the reference range provided by several authors and used by various laboratories are different. That is why the aim of our study was to make the calculation on Polish nonanaemic outpatients population (n=7183)
Methods. Data from 1869 females < 50 years and 3176 >50 , 997 males <50 and 1141 >50 from outpatients department with haemoglobin within the normal range for sex were included. Haematological analyses were performed on Sysmex XT2000i analyzer.
Results. In both groups of women there is a prevalence of MCV 85-89.9 fl (44,7% and 41,8% respectively). In group >50 35,4% patients has MCV 90-94.9 fl. In male population >50 40,4% have MCV 90-94,9. MCV range (mean +/- 2 SD) for groups are as follow: females <50 79,7-96,9 >50 , 80,9-98,1 fl, males <50 79,7-96,5 fl ,>50 81,9-98,7 fl. MCV for females >50 is significantly higher than <50. Mean MCV males >50 is significantly higher than <50, but it doesn't differ from females group >50 . There is a difference between MCV in females and males >50.
Conclusions. MCV increase with age and both in females and males above 50 years of age are higher than in young people below 50. Values observed in men are < 50 are higher in males. It can be concluded that sex and age should included in calculation of reference ranges for MCV

Haematology

0530

BODY FLUID CYTOLOGY: COMPARISON BETWEEN AUTOMATED AND MICROSCOPIC ANALYSIS

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Background. Routinely, determination of cell counts in body fluids is performed by microscopic examination, which is a subjective and time-consuming procedure. The objective of this study was evaluate the performance of the automated global and differential cell counts compared to the microscopic analysis.
Methods. We studied 104 pleural and peritoneal fluid samples. All samples were sent in an anticoagulant-treated tube and analyzed up to 2 hours after collection. The laboratory routine included manual erythrocytes (RBC) and leukocytes total (WBC) and differential counts and automated total and differential cell counts (Sysmex XE-5000).
Results. The automated WBC and RBC counts were highly correlated with that of the microscopic reference method (r≥0.95 in both cases). A good agreement between both methods was also observed for mononuclear cells (r=0.86) and polimorphonuclear cells (r=0.88). Polimorphonuclear cells showed a significant reduction of the percentages obtained by the Sysmex XE-5000 compared to manual method. This reduction is probably due to changes of size and shape of these cells, which are frequently observed in these fluids. High fluorescence cells >2.0/100WBC suggest the presence of macrophages and/or mesothelial cells and/or neoplastic cells.
Conclusions. Automated RBC, WBC and differential leukocytes counts of body fluids show good correlation with the manual method. Considering that body fluids are generally sent for urgent analysis, its laboratory routine requires a skilled personal and microscopic analysis is not available 24h/day in most laboratories, the use of this automated analyzer has the potential of reducing the time to report a preliminary result to the clinician.

0531

HEMATOLOGICAL DISEASES, THERAPIES, ANA PATTERN : THEIR ASSOCIATION IN TWO CASE REPORTS

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Background. New therapies have been showing autoimmune phenomena and the appearance of autoantibodies. In particular fluoroscopic ANA pattern are associated with diseases and their treatment
Methods. we report two cases: 1: a male with LLA , allogenic transplant with tacrolimus therapy; 2: a female with LMA , allogenic transplant with tacrolimus therapy.
Results. In the first case , we observed an ANA pattern suggestive for ribosomal one, with fine, dense homogeneous cytoplasmic staining, associated with nucleolar staining but no general nuclear staining : During the several years follow-up of this patient, we have been always detecting this fluoroscopic image. In the second case , we observed a CENP-F/Na pattern , with staining of the cleavage furrow and either side of the midbody was seen in anaphase and telophase.
Conclusions. The high incidence and intensity of autoantibodies responses among haematological patients highlights the interest of clinicians: how treatment influence fluoroscopic ANA pattern, if a specific treatment is always associated to a particular one. It is conceivable that further selection of case finding and their autoantibody response could lead to a more comprehension of disease, molecular therapy and spontaneous occurrence immunological response.

Haematology

0532

EFFECTS OF ALTITUDE TO BLOOD PARAMETERS

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Breckground. Reducing of partial pressure of oxygen in the air leads to a reduced arterial oxygen s aturation and increased secretion of erythropoietin, wich stimulates erythropoiesis.
Methods. Study included 63 healthy children aged 7 years,divided into 3 groups. I group consists of 21 children from suburb of altitude of 370 m, II group of 22 children from the village on 822 m, III group of 21 children from the town on 411 m. Complete blood count was determined on a hematology analyzer HmX (Beckman Coulter).
Results. Statistical analysis of data showed that children from II group have a higher average values of erythrocytes than children from the I ($p<0.01$) and III ($p < 0.05$),and the higher values of hemoglobin than children from I and III ($p<0.01$). II and III group had lover average values of leukocytes,related to I group ($p <0.01$).
 The boys from II group had more erythrocytes and hemoglobin then boys from I ($p<0.01$), and more platelets than boys from the III group ($p<0,05$), but less leukocytes than boys from I and III group ($p < 0.01$). Girls from II group had higher values of erythrocytes, hemoglobin, hematocrit and platelets ($p<0.01$) than the girls from I group.
It is not found statistically significant variation of parameters between boys and girls withim the same group nor erythrocytes and hemoglobin of I and II and platelets of all group ($p>0.05$).
Conclusions. Results show that stay in the village is usefull for stimulation of erythropoiesis.

0533

THROMBOCYTOPENIA AND ITS RELATED FACTORS IN CHRONIC HEPATITIS C

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Background. Thrombocytopenia in patients with chronic hepatitis C may be the result of several factors: bone marrow inhabitation, the decrease of liver thrombopoetin production and an autoimmune mechanism. Clinical variables such as age, gender, severity of liver disease could influence the severity of platelet reduction.
The aim of this study was to explore the association between thrombocytopenia and its related factors.
Methods. Eighty patients (55,9% men and 44,1% women) with chronic hepatitis C and thrombocytopenia were included. The mean age was 49,4±12.3 years (range 20 to 79) Thrombocytopenia was definition as the platelet count below 150 X 10^9 /L; patients chronically infected with HCV defined as either HCV RNA positive or having two times the upper limit of alanin transaminase. Determinations were made by standard biochemical.
Methods. The t-test, chi-square test and multivariate logistic regression analysis were used.
Result: In 62.7% patients had moderate thrombocytopenia (platelet count between 100-125 X10 9/L) but 23.6% patients had severe thrombocytopenia (platelet count less than 100 X10^9 /L). Thrombocytopenia was significantly associated with ALT values. The factors significantly related to thrombocytopenia were increasing age (OR, 1.04; 95% CI, 1.001-1.005), liver cirrhosis (OR, 8.02;95% CI 2.03-19.07) and splenomegaly (OR, 11.03; 95% CI 5.21-43.36).
Conclusions. Our date demonstrates that chronic hepatitis C is associated with a variable degree of thrombocytopenia and above mention factors.

Haematology

0534

QUANTITATIVE CHANGES OF NK CELLS IN UMBILICAL CORD BLOOD OF NEONATE IN RELATION TO THE MODE OF DELIVERY

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Background. NK cytotoxic cells are the important element connecting innate and acquired resistance in human body. They can be detected in fetal liver in the 6th week of pregnancy, and in the second half of pregnancy, their number increases to the values observed in adults.

Aim. The main aim of the study was the assessment of the NK cells number, percentage and defining a relationship between the mode of delivery and NK cells quantitative changes in umbilical cord blood.

Methods. The study included 72 neonates born in the years 1998-2003 in the Department of Perinatology and Gynecology in Zabrze Medical University of Silesia in Katowice. Taking into consideration the time and mode of delivery, the children were divided into following groups. Group I included 40 full term neonates: 17 neonates born vaginally and 23 to cesarean section. Group II included 32 near-term neonates: 6 neonates born vaginally, 26 to cesarean section. The method of blood labeling with sequential red blood cell lysis was applied in the assessment of NK cells in umbilical cord blood.

Results. It was shown that the NK cells percentage in all full term neonates was statistically higher than in near–term ones. Higher mean percentage of those cells was also shown either in full term neonates born to cesarean section and in near-term ones. Mean number of NK cells lymphocytes in full term neonates born to elective cesarean section was statistically significantly lower than in neonates born to emergency cesarean section.

Conclusions. Cesarean section can be connected with significant quantitative changes in NK cells in the umbilical cord blood. Near-term neonates, regardless to the mode of delivery, show lower values of NK cells in umbilical blood. Elective cesarean section carried out at term can be a cause of an decrease of mean number of NK cells in neonate umbilical blood.

0535

ANALYTICAL VALIDATION OF THE HEVYLITE™-IGA ASSAY FOR THE DIAGNOSIS OF MONOCLONAL GAMMOPATHIES

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Background. Currently used detection methods in the diagnosis of monoclonal gammopathies are mainly based on electrophoretic techniques. Now there is the Hevylite™-IgA (HLC) assay, a newly developed immunoassay for the detection of monoclonal heavy chains IgAκ and IgAλ.

Methods. Intra- and interassay precision were determined using patient sera (n=8) containing IgA monoclonal protein in a range between 0.1-31.0 g/L. Accuracy was estimated by testing linearity after dilution of sera with high monoclonal protein levels (n=4) and by spiking of assay calibrators into healthy sera (n=3). Interference with rheumatoid factors (RF) and other monoclonal (MC) immunoglobulins was investigated by measurement of samples with different amounts of RFs or MC proteins.

A method comparison has been performed with sera from patients with monoclonal gammopathies. Comparison between summed concentration of HLC IgAκ and IgAλ pairs and total IgA was performed by measurement of patient sera containing different amounts of total IgA (n=44).

Results. Intra- and interassay precision ranged between 1.5 to 19.0 % for the coefficient of variation. Accuracy resulted in a linear recovery from 0.8 to 20,2 g/L for IgAκ/IgAλ. RF did not affect test Results. Cross-reactivity with other MC immunoglobins could not be detected. A high concordance between the immunoassay and the immunofixation electrophoresis could be shown in the detection of monoclonal protein.

Conclusions. Our test validation revealed high precision, accuracy and a high concordance between test results of IFE and the HLC assay. We are currently further investigating the diagnostic value of the Hevylite™-IgA assay in estimation of disease activity.

Haematology

0536

IDENTIFICATION OF THE MUTATION VAL617PHE IN JANUSKINASE 2 (JAK2):COMPARISON BETWEEN A QUALITATIVE AND A QUANTITATIVE METHOD

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Background. The myelodysplastic syndrome (MPD) contains a group of diseases with variable clinical expression which affect the hematopoetic system. This category includes polycythemia vera, essential trombocythemia and primary myelofibrosis. Patients with these diseases may develope a point mutation in exon 14 in the gene for Januskinase 2, a cytoplasmic tyrosine kinase, whereby the valine residue in position 617 is changed to phenylalanine. Our aim was to evaluate a qualitative method and a quantitative method for identifying the mutation Val617Phe.

Methods. The analysis by qualitative PCR followed by agarose electrophoresis required DNA samples from both whole blood and granulocytes. The quantitative assay, based on TaqMan probe based real-time PCR (Ipsogen MutaQuant Kit), required only DNA from whole blood.

Results. Of 71 analysed samples, both methods identified 22 samples to be positive for the Val617Phe mutation and 47 samples to be negative for the mutation. Two samples, which showed to be negative for the mutation with the qualitative method, were found to be weakly positive with the quantitative method. Genomic DNA prepared from whole blood and isolated granulocytes was analysed with both methods in 8 cases, whereby identical results were obtained.

Conclusions. The quantitative method has a higher degree of sensitivity. The quantitative method also offers more advantages since it is carried out on DNA from whole blood. Results from the qualitative method demands manual assessment, while the quantitative method enables automatized calculation that improves reliability.

0537

ENDOGENOUS COLONY GROWTH PREDICTS FOR A LESS PRONOUNCED MOLECULAR RESPONSE TO IMATINIB THERAPY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Endogenous erythroid colony (EEC) growth is a hallmark of polycythemia vera and also often observed in essential thrombocytopenia and primary myelofibrosis. Reports on the existence of EEC in chronic myeloid leukemia (CML) are rare and contradictory and nothing is known about the clinical significance of EEC in CML.

Methods. Blood mononuclear cells of 57 patients with newly diagnosed CML (53 patient in chronic phase, 2 in accelerated phase, 2 in blast crisis) were screened for EEC growth using the methylcellulose culture assay. Results were associated with clinical disease characteristics at presentation and the molecular response to imatinib therapy.

Results. 17 (30%) patients exhibited EEC growth at diagnosis. The presence of EEC growth was associated with higher hemoglobin levels (p=0.0421) and a better Sokal score (p=0.0159). The probability to achieve a major molecular response to imatinib (defined as a 3 log reduction of BCR-ABL levels) was similar for both groups (p=0.6926). However, the degree of reduction was more pronounced in patients without EEC growth. The probability to achieve a 4 log reduction of BCR-ABL levels after 3 years was 70% for patients without and 7% for patients with EEC growth (p=0,0157). 8 out of 36

Conclusions. Our data suggest that EEC growth in CML might identify patients with a less pronounced molecular response to imatinib.

Haematology

0538

MOLECULAR CHARACTERIZATION OF THALASSSEMIA IN A MEDITERRANEAN SPANISH HEALTH DEPARTMENT (2008-2010)

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Background. Thalassemia is an inherited autosomal recessive blood disease. In thalassemia the genetic defect, which could be either mutation or deletion, results in reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin. This can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of the thalassemias.

The aim of this study was to determine the molecular defects of talasemia and to ascertain their distribution in the Health Department of "La Ribera" in a Spanish Mediterranean region since 2008 to 2010.

Patients and Methods. We have studied 182 thalassemic patients diagnosed by haematological and haemoglobin electroforetic parameters. Genetic analysis was performed by different Methods. PCR-ASO for study of alpha-globin and delta-globin gene and sequencing for beta-globin gene.

Results and Conclusions. We confirmed thalassemic diagnostic in 62 (34.06%) patients, 38 (61.92%) alpha-thalassemic, 16 (25.8%) beta-thalassemic and 8 (12.9%) delta-beta thalassemic patients respectively. The most prevalent genetic defect found in alpha-globin gen was 3.7 deletion (35 cases, 92.1%; 7 cases with homozigotic status); followed by MED deletion (2 cases) and FIL deletion (1 case). In beta-globin gen, the most prevalent mutation was IVS-1-110 (4 cases) and CD6 (3 cases). Other beta-globin mutations found were: LYS8FS, IVS-1-6; IVS-1-1; CD39 and CD9. We have detected 8 cases of delta-beta thalassemia, all of them were heterozigotic carriers of Spanish deletion for delta-beta gene region. The distribution of the mutations is similar to that found in the Mediterranean population.

0539

CEREBROSPINAL FLUID (CSF): AUTOMATED ANALYSIS OF BLOOD CELLS

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Background. Elevated white blood cell (WBC) and/or red blood cell (RBC) counts in CSF are frequently observed in neurological disorders. Considering that cerebrospinal fluids (CSF) are generally sent for urgent analysis, its laboratory routine is labor-intensive and requires a skilled personal, the objective of this study was evaluate the performance of the automated global and differential cell counts compared to the microscopic analysis.

Methods. We studied 112 CSF samples. All samples were analyzed up to 2 hours after collection. The routine included manual erythrocytes (RBC) and leukocytes total (WBC) and differential counts and automated total and differential cell counts (Sysmex XE-5000).

Results. The automated WBC and RBC counts were highly correlated with that of the microscopic reference method (r>0.90). There is a high correlation for the entire range of WBC, although at the low end of the data spectrum (WBC≤5mm^3) a weak correlation was observed. In this range, 20% of the samples were misclassified as abnormal by the automated analyzer and polimorphonuclear (PMN) and mononuclear cell (MN) counts is of limited value. Despite the good correlation for WBC>5/mm^3 (r=0.91), PMN are overestimated and MN are underestimated by the automated analyzer (p<0.001).

Conclusions. Most patients were correctly classified as normal or abnormal, however a careful review of the results is still mandatory. The results observed at the low end of the data suggest that larger studies may be necessary to determine the need of a new reference range for automated CSF WBC counts.

Haematology

0540

THE RELATIONSHIP BETWEEN PLATELET PARAMETERS AND PLATELET SEROTONIN LEVEL IN HEALTHY POPULATION

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Background. The most frequently described platelet parameters are mean platelet volume (MPV), plateletcrit (Pct) and platelet distribution width (PDW). The issue of possible association of platelet anatomic parameters with serotonin level in the lysates of peripheral blood platelets has not been investigated. The aim of this study is to investigate this association in physiological conditions.

Methods. We determined platelet parameters on Beckman Coulter LH 750 haematology analyzer in healthy volunteers (n=143) of both sexes. Analysis has been done for all samples two hours after blood smapling.In addition, we prepared platelet rich plasma(PRP) and optimized conditions for determination of serotonin content in the lysates.The samples of PRP lysates were frozen at -20° C ;the measurement were performed within two weeks using ELISA.The content of circulatory serotonin in platelets is refered to 10^9 platelets.The correlations with serotonin concentration and platelet parameters were analyzed by ANOVA.

RESULTS :The criterias in our statistical model were: significance level (F=7.5; p<=0.001), significant negative correlationbetween platelet serotonin level and MPV(p=0,03), significant positive correlationbetweenplatelet serotonin level and Pct (p=0,001) and no correlation betweenplatelet serotonin and PDW (P=0,346).

Conclusions. Our results suggests that a serotonin level is higer in smaller platelets as a result of their aging, and it is not associated with cell size distribution. Under normal coditions , there is an inverse relationship between platelet size and number, so it is possible that serotonin level is higher if the total platelet mass is higher.

0541

IRON DEFICIECY ANAEMIA-HOW FREQUENT THIS REALLY IS?

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Background. Iron deficiencyanemia is a frequent diagnose for the hospital admittance of a child. Iron deficiencyanemia is considered to be the last stage of iron deficiency., after iron depletion and iron deficiency The diagnose of iron deficiency requires determination of haemoglobin, MCV and ferritin.

We took in our study all the children admitted in the Pediatric Hospital Sibiu during 3 months period, october 2010-december 2010, with the clinical diagnose of anaemia and made the lab tests for the diagnose: Haemoglobin, MCV, and ferritin..

Methods. Haemoglobin and MCV were made on the Sysmex XS-1000i analyzer, and the ferritin on a Vidas system

Results. From the total of 114 patients aged between 1 week-16 years of age (61 males, 53 females,81 patients came from urban medium,33 from rural .medium) we found iron depletion (normal values of Haemoglobin, MCV and low values of ferritin) in 15 patients=13,1% iron deficiency (low values of Haemoglobin, MCV, and normal values of ferritin) in 21 patients=18.2% and iron deficiency anaemia (low values of Hb, MCV , ferritin) in 20 patients=17.5%

Conclusions. Iron deficiency anaemia is present only in 17.5% of the admitted children. with the clinical suspicion of iron deficiency anaemia , so we consider there is an over diagnose of anaemia in the admitted children.

Haematology

0542

USABILITY OF ABSOLUTE NEUTROPHIL COUNT FROM HEMATOLOGY ANALYZERS IN PATIENTS TREATED WITH CHEMOTHERAPY

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Background. Absolute neutrophil counts (ANC) are used in monitoring the treatment of cancer patients. ANC obtained by analyzers are more accurate and precise. However, these analyzers cannot recognize immature cells. Microscopic examination is more time consuming and has poor reproducibility of results due to small number of cells counted. Our aim was to check in which cases automated analyzers can replace microscopic examination of ANC.

Methods. We analyzed 189 blood samples from patients receiving chemotherapy by using Beckman Coulter LH 750; 120 samples were also analyzed by Siemens ADVIA 120. Manual differential counts for all included blood samples were also performed. We compared ANC obtained by an analyzer and that obtained by a microscope by using Passing Bablock regression. ANC obtained by a microscope was used as reference for sensitivity, specificity, positive and negative predictive value for automated ANC at three cut-off points ($1.0*10^9$/L,$1.5*10^9$/L and $2.0*10^9$/L).

Results. Regressions for ANC obtained by hematologic analyzers: ANC(LH750)=1.042* ANC(microscope)-0.08, r=0.96 and ANC(ADVIA 120)=0.987*ANC(microscope)-0.05, r=096. The specificity of ANC obtained by LH750 and ADVIA 120 was at all cut-off points greater than 90%. Three false positive ANC results were obtained by LH750 at the cut-off point $1.0*10^9$/L, but the difference was higher than 30%only in two. The ANC performed by ADVIA 120 provided at the cut-off point $1.0*10^9$/L only one false positive result with the difference higher than 30%. These samples contain metamyelocytes and myelocytes or higher percentage of monocytes.

Conclusions. ANC obtained by LH 750 and ADVIA 120 is a reliable parameter in monitoring cancer patient treatment and can replace microscopic examination of blood smear in most of cases.

0543

PROTEIN OXIDATION IN PATIENTS WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) caused by uncontrolled proliferation of plasma cells is a malignant disease charecterized by lytic lesions, osteopenia and osteoporosis in skeletal system together with detoriations in renal functions, tendency to infections, hyperviscosity, anemia or thrombocytopenia. Althought an increase in oxidative stress in MM patients has been reported in several studies, most of these studies assessed only the oxidative stress and/or lipid peroxidation and there is limited information about the status of protein oxidation in MM patients. Therefore, in the present study, we investigated the status of serum protein oxidation in MM patients.

Serum Advanced Oxidation Protein Product (AOPP) , Total Oxidative Status (TOS), Total Antioxidative Capacity (TAS) and Oksidative Stress index (OSI) were determined in 36 patients with MM (m/f: 23/13, 64.4 ± 10.1 years) and 51 control subjects (m/f: 33/18, 61.22 ± 11.8 years).

Serum AOPP levels of MM patients (26.50 ± 18.66 umol/l) were significantly (p< 0.005) higher than those of control subjects (17.31 ± 10.73 umol/l). Although TOS value in MM patients (17.18 ± 4.14 umol/l) was higher than that in control subjects (16.14 ± 2.77 umol/l), the difference was not statistically significant. TAS decreased (2.52±0.30 vs. 2.65 ± 0.17 mmol/l, p<0.01) and OSI increased (7.13 ± 0.56 vs. 6.07 ± 0.17 AU, P<0.05) significantly in MM patients compared to control subjects.

In conclusion, the findings of increased AOPP leves together with increased OSI and decreased TAS values in the present study indicated to an increment in protein oxidation in patients with multiple myeloma.

Haematology

0544

COMPARISON OF TWO CRITERIA FOR ACCEPTABLE PERFORMANCE FOR COAGULATION IN CHINA EQAS

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Background. Several strategies have been proposed in laboratory medicine (including haematology) for setting the quality specifications associated with acceptance limits for laboratory performance in EQAS. Various strategies have their own advantages and disadvantages from the Stockholm consensus conference. The first choice logically should be the strategy at the top of the hierarchy. Through comparing two criteria for acceptable performance of Coagulation, we can choose a quality specifications which is more suitable for external quality assessment in haematology.
Methods. In a nationwide external quality assessment (EQA), data information of PT, APTT and FIB were analyzed with CLIA'88 criteria for acceptable performance and acceptability limits based on biology goals respectively, and the results were compared.
Results. The score of each analyte obtained from each criteria were different: thescore of PT based on the acceptable limit of CLIA'88 and those based on the minimum performance of biological variation are in agreement; the score of APTT based on the acceptable limit of CLIA'88 are hinger than the score based on the minimum performance of biological variation;
the score of FIB based on the acceptable limit of CLIA'88 agree with those based on the desirable performance of biological variation.
Conclusions. CLIA'88 criteria for acceptable performance is relatively wider, while biological variation is more theoretically and practically based, more relevant to medical needs, therefore more suitable for external quality assessment.

0545

COMPARISON OF TWO CRITERIA FOR ACCEPTABLE PERFORMANCE FOR COAGULATION IN CHINA EQAS

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the score of FIB based on the acceptable limit of CLIA'88 agree with those based on the desirable performance of biological variation.
Conclusions. CLIA'88 criteria for acceptable performance is relatively wider, while biological variation is more theoretically and practically based, more relevant to medical needs, therefore more suitable for external quality assessment in China.

Haematology

0546

JAK2 V617F PROMOTES EXPRESSION OF ONCOSTATIN M IN MYELOPROLIFERATIVE NEOPLASMS: A POTENTIAL LINK BETWEEN ABNORMAL JAK-SIGNALING AND BONE MARROW MICROENVIRONMENT ALTERATIONS

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Background. Polycythemia vera, idiopathic myelofibrosis, and essential thrombocythemia are a group of myeloproliferative neoplasms (MPN) characterized by the activating JAK2 point mutation V617F. It remains unclear how JAK2 V617F contributes to bone marrow microenvironment alterations, notably increased angiogenesis and fibrosis. OncostatinM (OSM), a cytokine of the IL-6 family has been shown to promote growth of endothelial cells and fibroblasts as well as megakaryocytic and erythropoietic differentiation.
Methods. Expression of OSM in the bone marrow of MPN patients was investigated by immunohistochemistry and real-time PCR. To investigate the role of JAK2 in OSM expression, Ba/F3 cells with doxycycline-inducible expression of wild type JAK2 or JAK2 V617F were generated and the JAK2 V617F+ cell lines HEL and SET2 were used.
Results. Megakaryocytes and myeloid progenitors expressed the OSM protein at high levels and MPN patients were found to express significantly higher OSM mRNA levels than normal bone marrow samples. In Ba/F3 cells, doxycycline-inducible expression of JAK2 V617F led to a substantial upregulation of OSM. Correspondingly, JAK2 V617F-positive cell lines were found to express significant levels of OSM and knockdown of JAK2 downregulated OSM. Finally, STAT5 was found to be involved in JAK2 V617F-dependent expression of OSM since RNAi knockdown of STAT5 substantially reduced OSM expression and a constitutively activated STAT5 mutant upregulated OSM.
Conclusions. Together, our data show that neoplastic cells in MPN express OSM in a JAK2 V617F- and STAT5-dependent manner. The role of OSM in growth and differentiation of neoplastic cells and/or remodelling of the microenvironment is currently under investigation.

0547

PLATELET MICROPARTICLES MEASUREMENT ON A HAEMATOLOGY ANALYSER

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Background. Platelet microparticles (PMP) are associated with coronary events and other thrombotic disorders. Flow cytometry is the preferred method for measuring PMP, and standardization of the assay is an absolute requirement. This communication reports a standardized method for measuring PMP using the flow cytometric capabilities of the automated CELL-DYN Sapphire haematology analyzer (Abbott Diagnostics, Santa Clara, USA).
Methods. Afterincubation with CD61 and CD41fluorescent labelled monoclonal antibodies, whole blood samples were processed with the CELL-DYN Sapphire PLT Flow mode and the resulting list mode data file (FCS 2.0) was analyzed using standard flow cytometry software. For standardization, Megamix calibration beads (Biocytex, Marseille, France) were used, in accordance with internationally accepted recommendations. Blood from 20 healthy volunteers was used for establishing reference ranges.
Results. The gating strategy distinguished platelets from other cells by their positivity for CD41 and CD6, while a plot of CD41 fluorescence versus 7° scatter was used in conjunction with Megamix beads for particle size calibration (7° scatter gave better signal/noise ratios than 0° scatter). PMP were then defined as CD41+ platelet events of $< 1.0 \mu m$ and numerically quantified relative to platelet count. Using this approach, we found $0.54 \pm 0.20\%$ (mean $\pm$ SD) PMP in healthy donors, equivalent to $1.32 \pm 0.57 \times 10^9$/L. The PMP data were normally distributed, providing a reference range of $0.15 – 0.94\%$ or $0.21 – 2.43 \times 10^9$/L.
Conclusions. We have demonstrated that it is feasible to measure PMP in a haematology analyzer and to standardize the assay using calibrated beads.

Haematology

0548

OPTIMIZATION OF LABORATORY WORKFLOW IN CLINICAL HEMATOLOGY LABORATORY WITH REDUCED MANUAL SLIDE REVIEW: COMPARISON BETWEEN SYSMEX XE-2100 AND ABX PENTRA DX120

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Background. Manual slide review (MSR) to validate the results from automated hematology analyzers is currently an inevitable work process in clinical hematology laboratories. Laboratory workload would be optimized, if the rates of MSR are reduced without compromising patient care. We wanted to know whether the slide-making rates would be different between the two hematology analyzers, which were paired with their own automated slide makers/stainers: Sysmex XE-2100 with SP-1000i (Sysmex, Kobe, Japan) and ABX Pentra DX120 with SPS evolution (ABX-Horiba, Montpellier, France).
Methods. A total of 943 samples were run in parallel between Sysmex XE-2100 and ABX Pentra DX120. Reflex slides were automatically made in each analyzer according to its own rules, which reflected the criteria of MSR in our laboratory. The slide-making rates were compared, and the results were further confirmed using the criteria of MSR.
Results. The slide-making rates in Sysmex XE-2100, ABX Pentra DX120, and manual review were 22.5% (212/943), 15.91% (150/943), and 11.5% (108/943), respectively. In 774 (82.1%) samples, the three methods showed concordant results, and all made slides in 82 samples. Using the manual method as a standard, the sensitivity and specificity were 86.1% and 85.8% in Sysmex XE-2100 and 89.8% and 93.7% in ABX Pentra DX120.
Conclusions. Our data shows that the slide-making rates are variable in different hematology analyzers. It also implies that although MSR cannot be fully substituted by the modern hematology analyzers, it can be effectively reduced to optimize laboratory workload.

0549

CHEDIAK-HIGASHI SYNDROME: A CASE STUDY

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Background. Chediak-Higashi Syndrome (SCH) is a primary immunodeficiency, autosomal recessive, in which exists a defect in an essential protein (CSH), involved in the formation of vacuoles and protein transport. The disease is defined by the presence of giant intracitoplasmatic granules in all hematopoietic lines, mainly in neutrophils and eosinophils and this cause cellular dysfunction and decreased bactericidal power.
Clinical case. The patient (four-years-old male) arrived at hospital with high fever and progressive decay, with clinical suspicion of infectious mononucleosis. The out-standing symptoms were his pallor mucocutaneous, silver-grey hair, and marked pancytopenia.
Bone marrow aspiration was performed, and after observation of atypical granulation, a visceral leishmaniasis was diagnosticated. When symptoms persist after treatment, a second bone marrow aspiration was performed. A microbiological culture and serological study were done, ruling out the parasitization by Leishmania. The detailed study of bone marrow detected abundant immature elements in the granulocytic line and revealed the presence in them of unusual large azurophilic granulation. The molecular study showed a mutation (G à A) cDNA in position 11348 (LYST gene) in homozygous. This mutation is confirmed in heterozygosity in both parents.
Conclusions. Patient`s clinical features, microscopic examination of both bone marrow and peripheral blood, and observation of the giant granulation in different cell types, oriented the diagnosis of SCH. The confirmation was made by sequencing the LYST gene, which encodes the protein CSH.

Haematology

0550

SERUM HEPCIDIN LEVELS AND IRON STATUS DURING LONG-TERM ENDURANCE EXERCISE

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Exercise-induced iron deficiency is a common disorder in endurance athletes. Recently, hepcidin received much attention in conjunction with exercise induced iron deficiency and inflammation. The aim of our study was to assess the influence of long-term endurance exercises on serum hepcidin levels and iron status.

Hepcidin, iron, ferritin, CRP, IL-6 levels and RBC were measured in 18 female runners preparing for long-distance run. Blood samples were taken at different time-points during the eight-weeks training programme. Mean serum hepcidin level (95% CI) before the training was 177.5 (140.2 – 214.9) μg/L. The concentration of hepcidin decreased at the first training load ($p<0.001$) and at the highest training point increased to 161.9 (134.7 – 187.5) μg/L. IL-6 levels were below 2 ng/L at all time points. The baseline CRP level 1.16 (0.52 – 1.8) mg/L did not change significantly. The initial serum iron level 21.5 (16.6 – 26.4) μmol/L decreased non-significantly ($p = 0.41$) during the training. Mean RBC varied at different time-points between 4.15 and 4.35 10^{12}/L depending on training load. Serum ferritin decreased from 30.9 (14.7 – 47.2) μg/L to 26.7 (11.8 – 41.7) μg/L during the study, the decrease was only marginally significant ($p = 0.07$).

The results provide valuable insight of the markers involved in the inflammation-hepcidin-hypoferremia axis. The decrease of hepcidin levels at the beginning of the training was probably induced by increased iron needs. Chronic training stimulus together with exercise-induced stress resulted in the increase of hepcidin levels during the training.

0551

ANALYTICAL PERFORMANCE EVALUATION OF THE ALLHEM ANALYZER USED FOR CAPILLARY BLOOD HEMOGLOBIN MEASUREMENTS IN BLOOD DONORS

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Background. The hemoglobin (Hb) concentration is the most often used parameter in screening of blood donors for anemia, and the value below 12.5 g/dl for females and 13.5 g/dl for males is a widely accepted donation cut-off point. The gold standard for assessing Hb concentration is the direct cyanmethemoglobin method.

Methods. A total of 120 females and 120 males unselected prospective blood donors (age 18-65 years) were included in the study. Hemoglobin was measured in capillary blood samples, using the ALLHEM analyzer. Venous blood hemoglobin in simultaneously collected samples was measured using the automated hematology analyzer Beckman Coulter LH 750.

Results. The mean Hb concentration in capillary blood measured using the ALLHEM analyzer was 13.8 (±2.68) g/dl (range 11.2-17.7). The mean venous blood hemoglobin measured using the Coulter LH 750 analyzer was 14.0 (±2.4) g/dl (range 11.9-16.7). The coefficients of variation (CV) for the two methods amounted to 2.5% and 2.1% at mean Hb concentrations of 6.4 and 7.5 g/dl (bias: 1.56%;2.66%); 2.8% and 2.5% at mean Hb concentrations 13.7 and 14.1 g/dl (bias: 0.73%;1.42%); 2.6% and 2.9% at mean Hb concentrations 18.3 and 18.5 g/dl (bias: 1.09%;1.62%), respectively. Statistically significant correlation between results obtained using both method was found: $r=0.911$ ($p<0.0001$), regression equation: $Hb_{ALLHEM}=0.128+ 0.98 \times Hb_{Coulter}$). The mean difference between ALLHEM and Coulter analyzer was -0.31 (95%CI: -1.62 to 1.0) g/dl.

Conclusions. The ALLHEM analyzer used for Hb measurement in capillary blood showed good imprecision, accuracy and agreement with the Coulter LH 750 hematology analyzer.

Haematology

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EMA (EOSIN-5'-MALEIMIDE) TEST MODIFICATIONS IN THE DIAGNOSTICS OF HEREDITARY SPHEROCYTOSIS (HS)

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Background. EMA test is a modern tool widely used to detect HS. The aim of the study was to evaluate if the storage time of stained sample can influence the results of EMA test.

Methods. 5 μl EDTA – anticoagulated blood samples from children, were washed and incubated for 1 hour with 25 μl of EMA dye (0.5mg/ml in PBS, Fluka) in dark, at room temperature with intermittent mixing. After incubation time RBC pellets were washed 3 times with PBS/FBS (0.5% fetal bovine serum in PBS). Washed cells were resuspended in 500 μl of FBS/PBS. For flow cytometric analysis 100μl of labeled cells were resuspended in 1.4ml FBS/PBS. Fluorescence of EMAwas determined in FL-1 channel for 100 000 events on Cytomics FC 500 flow cytometer (Beckman Coulter).

Results. 35 samples were stained immediately after blood collection. They were subsequently analyzed at time 0, after 1 hour and 24 hours of storage at 4˚C. Results are presented as percentages of decrease of EMA fluorescence comparing to reference samples. Fluorescence level at different time-points in the group of HS patients was 63.1 ± 5.2%, 62.83 ± 5.39% and 63.25 ± 5.38% respectively. In the group of non HS patients the fluorescence level was 98.89 ± 5.94%, 98.96 ± 6.01% and 99.34 ± 6.50% respectively. All the differences are non significant (p>0.05)

Conclusions. Our study indicates that EMA labeling test results are repeatable when performed 1 and 24 hours after staining.

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COMPARISON OF ERYTHROCYTE SEDIMENTATION RATE METHODOLOGIES: ALIFAX TEST 1 THL, VES-MATIC 20 AND WESTERGREN

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Background. The erythrocyte sedimentation rate (ESR) is a nonspecific reaction, been a measure of the present severity of pathological processes. The Westergren method is considered the reference method, although in recent years, other technologies have been developed.

Methods. We compared the ESR values obtained by the reference method and by the automatic systems Alifax TEST 1 THL (n= 208) and Ves-matic 20 (n= 189).

Samples from 212 hospitalized and external patients at the Centro Hospitalar do Barlavento Algarvio, EPE were analyzed. All samples hemolyzed, icteric, lipemic, coagulated and with insuficient volume were excluded, only blood samples within 4 hours, between collection and analysis, were studied. The age, sex, hemoglobin concentration and packed cell volume was considered. Results were compared using Paired samples *t*-test, Pearson's correlation test and Bland and Altman analysis.

Results. The correlation between values from Westergren and automatic systems was strong (Ves-matic $r = 0.869$;n =189; $P<0.001$; Alifax $r = 0.853$; n = 204; $P <0.001$). Bland & Altman analysis did not show a significant bias in either of the automatic equipments. All of the methods seem to be affected by hemoglobin concentration and packed cell volume, although the Alifax system seems to be the least affected.

Conclusions. We conclude that the Alifax system had the strongest agreement with the Westergren method and has numerous practical advantages.

Haematology

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LABORATORY VALIDATION OF THE MULTIDRUGQUANT™ (MDQ) ASSAY KIT

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Background. Multidrug resistance is the most frequent type of resistance to anticancer chemotherapy and disease-modifying antirheumatic drugs (DMARDs), whichusually results from the overexpression of efflux transporters, such as the MDR1, MRP1 and BCRP. The MDQ kit is a flow cytometric method to measure the functional activity of these transporters applying fluorescent dyes and inhibitors. The present study aimed to evaluate the performance of the MDQ kit.
Methods. Validation of the kit was carried out according to the standards of the Clinical Laboratory Standards Institute in three university centres. Mononuclear cells were separated using Ficoll gradient and tested at $2–5×10^6$/ml within 6 hours after specimen collection. Activities of the multidrug transporters were calculated from the difference between the mean fluorescent intensity of cells w/o the specific inhibitors, respectively. Inaccuracy and comparative measurements were carried out using cell lines with low and high activity of the transporters. Results on different flow cytometers were compared using CD45 CD19 or CD3 monoclonal antibodies for gating the population of interest.
Results. The assay proved to be specific and robust at various concentrations of the fluorescent dyes (10–100 % of the original) or inhibitors (50–150 % of the original). Both intraassay and interassay reproducibilities were <5 %. Multidrug resistance activity values determined on different flow cytometers were comparable and eligible.
Conclusions. The MDQ-kit provides quantitative results on the activity of the MDR1, MRP1 and BCRP in the target cells, which might be used to predict the resistance of these cells to particular cytotoxic agents.

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QUANTITATIVE ESTIMATION OF T AND B LYMPHOCYTESAND THEIRSUBPOPULATIONSINCORD BLOOD OF INFANTS BORN PREMATURELY

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Background. Defining to what degree time and a way of delivery cause changes in percentage and quantity of CD3 lymphocytes and their subpopulations CD4+, CD8+, CD25+, CD19+ lymphocytes and their subpopulations CD5+ and CD23+.
Material and Methods. The study included 32 healthy neonates born in the years 1998 – 2003 in the Perinatology and Gynecology Ward of Silesian Medical University in Zabrze. Considering the time of pregnancy and the method of delivery. Children were divided in the following groups: group 32 neonates born prematurely , which included (II a) 6 neonates born naturally (II b) 26 neonates born by cesarean section, which included: (II be) 18 with elective indications (II bn) 8with emergency indications. In the current study the cord blood leukocyte staining followed by red cell lysis method was applied.
Results. Statistically significant higher mean percentage of CD8+ lymphocytes in the neonates born prematurely naturally in comparison to the neonates born prematurely by cesarean section was found. Statistically significant lower mean number of CD3+, CD4+ and CD25+ lymphocytes in the neonates born with elective cesarean section in comparison with the neonates born prematurely by cesarean section with emergency indications was proved.
Conclusions. 1. In the neonates born prematurely the mean percentage of T cytotoxic and suppressor lymphocytes can express a relationship between natural delivery and cesarean section.
2. Cesarean section done in mother due to emergency indications can be connected with an increased mean number of T lymphocytes, auxiliary T lymphocytes, activated T lymphocytes in a prematurely born neonate.

Haematology

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RETICULOCYTE INDICES IN HEALTHY CHILDREN

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Background. Reticulocyte counts as well as reticulocyte qualitative characteristics (reticulocyte indices) are increasingly used in pediatric practice to diagnose for instance disturbances of erythropoiesis and iron metabolism. In order to correctly interpret the results of laboratory tests, it is necessary to know reference intervals, which may vary depending on the age and gender of a child.
Methods. Venous blood samples were taken from 65 healthy children (35 female, 30 male; age range 5 month – 17 years, median 6.3 years) at prophylactic medical examination. All samples were measured by a SysmexXT-2000i analyzer, and the following reticulocyte parameters were analyzed: absolute and relative reticulocyte number (Ret and Ret‰, respectively), mature reticulocyte fraction with low fluorescence ratio (LFR), immature reticulocyte fraction (IRF), reticulocyte hemoglobin equivalent (Ret-He), and difference between Ret-He and erythrocyte hemoglobin equivalent (deltaHe).
Results. By analysis of variance based on F-statistics we did not detect any effect of gender and age on the analyzed parameters, and combined all participants into one group. After removal of outliers, the median values (represented as Me (2.5-97.5 percentiles)) were as follows: Ret ($x10^9$/L) 36.4 (16.2-65.7), Ret (‰) 7.4 (3.5-13.4), LFR (%) 96.6 (91.7-99.3), IRF (%) 3.5 (0.7-8.3), Ret-He (pg) 32.5 (28.9-34.6), deltaHe (pg) 3.7 (1.4-5.1). The interindividual (between-subject) variation (CVi) for Ret number was 33.0%. In contrast, the CVi for Ret-He was much lower – 5.2%.
Conclusions. Our data will help to implement these new reticulocyte parameters in the clinical practice.

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COMPARATIVE STUDY OF PERIPHERAL BLOOD MORPHOLOGY BY CONVENTIONAL MICROSCOPY AND CELLAVISION DM96 IN HEMATOLOGICAL AND NON HEMATOLOGICAL DISEASES

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Background. We evaluated the CellaVision DM96 (*CellaVisionAB, Lund, Sweden*), an automated image analysis system for digital peripheral blood cell analysis, comparing the results with direct manual microscopy
Material and Methods. We analyzed 234 PB films from patients of the Hospital Clínic of Barcelona. Leukocyte values were from 1.12 to 282 x 10^9/L. 177 of the PB films were from patients with hematological diseases. WBC differentials were abnormal in 120 cases. Statistical analysis was performed using correlation (Pearson) and concordance (Lin) tests.
Results. Correlation coefficients between results obtained from the CellaVision DM96 preclassification and by conventional direct microscopy were excellent for segmented neutrophils, lymphocytes, monocytes and blasts (r>0.87<0.94 y p<0.0001) and good for band neutrophils, eosinophils, basophils and plasma cells (r>0.74<0.81 and p<0.0001). Pearson and Lin coefficients were higher when we compared DM96POST values. After the reclassification of the cells very good concordance coefficients were observed for promyelocytes and myelocytes (> 0.7), intermediates for reactive lymphocytes and erythroblasts (>0.5 and <0,7) and low (<0.5) for metamyelocytes.
Whatever the pathology and the number of blasts on the manual review films, all 97 patients were positive for blast detection on DM96. Digital images showed dysplastic features or inclusions in blood cells and morphologic alterations in red cells or platelets were easily identified.
Conclusions. Advantages of the Cellavision DM96 over direct microscopy include that requires less time than manual differentiation, is a good tool for educational purposes, improve the trazability of the results and can have an important role in a modern Hematology Laboratory.

Haematology

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FREE LIGHT IMMUNOGLOBULIN CHAINS AS THE PROGNOSTIC MOLECULAR MARKERS IN PLASMAPROLIFERATIVE DISORDERS

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Background. The quantitation of the monoclonal immunoglobulins (Ig_s) and its fragments are used for the monitoring of plasmaproliferative disease course and effect of therapy introduced. The aim of free light chains (FLC) examination was to evaluate significance of k/λ ratio as a prognostic factor for progression and survival in a different diseases groups.
Methods. The concentrations of Ig_s and FLC were measured by immunonephelometric method on a "SIEMENS" DADEBehring BN II analyser with reagents (Freelite, The Binding Site, UK).In this examination 151 patients from 3 different disease groups were investigate:1. Light Chain Disease (LCD, n=37),2. *Biclonal gammopathy (BG, n*=23) and 3. *Monoclonal Gammopathy*of *Undetermined Significance (MGUS, n*=91).According to the *ISS for Myeloma* as an abnormal serum ***k**/λ*ratio was taken (<0.03 or > 32).
Results. The patients with *LCD* and *BG*with abnormal k/λ ratio and a combination of adverse risk factors (76.7%) had median survival times of 22-30 months, versus patients with normal κ/λratio without adverse risk factors (23.3%) with an median survival times of 39-51 months.About 38% patiens which have lowered FLC values more than 50% under therapy, achieved the disease remission in theLCD and *BG*groups.In patients with *MGUS*, 66.0% had normal or slightly modified k/λ ratio which corresponding to low risk of disease progression, as opposed to 34.0% with abnormal k/λ ratio (<0.25 or >4) which corresponding to high risk.
Conclusions. Abnormal k/λratio could be an independent risk factor of progression and survival in examined groups.

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NEWS IN THE CODING *G6PD* GENE SEQUENCE: THREE NOVEL SINGLE NUCLEOTIDE SUBSTITUTIONS (SNS) FOUND IN THE ITALIAN POPULATION

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Background. Glucose 6-phosphate dehydrogenase (G6PD) deficiency is a common disease determined by a large number of genetic mutations. About 190 *G6PD* mutations have been described with distribution and frequency that differs among various populations depending on ethnicity and geographical areas. For example, in Italy, the mutations more frequent are *G6PD Mediterranean, G6PD Cassano, G6PD Seattle,G6PD Chatam and G6PD A*.
Aim and Methods. We report three families carrying novel *G6PD* SNS.Genetic tests were performed by whole *G6PD* gene sequencing and by a new primer's set.
Results. *Family 1:* The proband is an asymptomatic 41-year-old female of Umbria ancestry (Central Italy). The whole *G6PD* genetic study performed by direct sequencing, revealed the presence of a novel silent nucleotide substitution of G>A at position 733, without amino-acid change.
Family 2: The proband is an asymptomatic 11-year-old male, of Calabria ancestry (Southern Italy), referred to our hospital to confirm the *G6PD* deficiency; the sequencing of the whole *G6PD gene*, revealed the presence of a novel missense mutation of C>T at position 183, causing a change at position 60 of Leucine to Proline. We named this mutation as *G6PD Costanzo.*
Family 3: A 1-month-old female newborn was admitted at our Hospital Emergency Care Unit after an episode of lipotimia/fainting. The mother referred that the lipotimic event occurred 12-16 hours after she had eaten a large amount of fava beans and having subsequently breastfeeded her daughter, also supplementing baby's diet with Vitamin K. For this reason we performed on the mother the biochemical and genetic evaluation of the G6PD status. The whole gene sequencing analysis showed the presence of a novel missense mutation of G>A at nucleotide 170, with change at position 57 of Arginine to Glutamine confirming it on the her daughter. We named this mutation as *G6PD Palestrina.*
Discussion. Our study confirms the high heterogeneity of the *G6PD* mutation*s* in Italy. We suggest to sequence the whole *G6PD* gene, in symptomatic individuals resulted as negative to the routinely first genetic screening, especially in those patients coming from peculiar Italian regions.

References
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Haematology

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THE ROLE OF P-SELECTIN GLYCOPROTEIN LIGAND-1 (PSGL-1) DURING G-CSF TREATMENT IN A MOUSE MODEL

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Background. The effect of G-CSF (Neupogen) was investigated in wild-type (WT) and PSGL-1 knockout (KO) mice to establish the role of PSGL-1 in myeloid cell mobilization. G-CSF activates tissue proteases that cleave several adhesion molecules, thus enhances the mobilization of myeloid cells and haemopoietic stem cells.
Methods. WT and KO mice (12-16 week old mice, 20-25 gram body weight, n=15) were treated with a single dose of 250 mg/kg cyclophosphamide intraperitoneally to induce cytopenia, subsequently mice received 7.8 µg/kg G-CSF twice a day for 4 days. Retro-orbital blood samples were drawn to determine leukocyte counts by Siemens Advia 120 analyser, while leukocyte differentials were evaluated by microscopy.
Results. The neutrophil granulocyte count upon completion of G-CSF treatment increased significantly in WT and KO mice respectively to 28.3 G/L and 47.7 G/L, while the monocyte counts were 2.0 G/L and 4.1 G/L and no changes were observed in eosinophil counts. Four days after the last G-CSF treatment, both strains displayed considerably reduced neutrophil (1.8 G/L in WT and 9.8 G/L) and monocyte (0.4 G/L and 1.6 G/L) counts, the values always being higher in KO animals. Contrary, eosinophil granulocyte values became elevated only at this sampling time, being 0.5 G/L and 1.2 G/L in WT and KO mice respectively.
Conclusions. The lack of PSGL-1 results in higher myeloid cell counts after G-CSF treatment with prolonged effects on eosinophils. The differences are most probably caused by faster mobilization from the bone marrow and delayed extravasation in the peripheral vessels.

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PERFORMANCE OF A NEPHELOMETRIC ASSAY WITH AUTOMATED ANTIGEN EXCESS DETECTION FOR SERUM FREE LIGHT CHAINS

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Background. Specific serum assays for immunoglobulin free light chains (FLC) are available for different analysers. For monoclonal immunoglobulins and FLCs cases of antigen excess have been reported. Although a rare occurrence, these FLC assays have been criticised for their susceptibility to antigen excess. New reagents with automated antigen excess detection (AAED) for use on the Siemens BN™II are now available. The aim of this study was to compare performance of the new assay with AAED to the standard assay for free kappa and free lambda in the daily routine.
Methods. Performance of assay with AAED was compared to the standard assay regarding correlation and expenditure of time to gain the final FLC result on the Siemens BNII. Antigen excess assessment was carried out with samples previously shown antigen excess with the standard BNII kit by using additionally to the standard dilution (1/100) also the antigen excess check dilution (1/2000).
Results. Coefficient of determination(r^2) was for free kappa r^2= 0,928 and for free lambda r^2 = 0,988 .The time needed to gain the final FLC result was for the assay with AAED shorter than for the standard assay. All samples showing antigen excess were determined correctly by the AAED assay.
Conclusions. The study demonstrated a good performance of the assay with AAED and will help to increase the reliability to cover antigen excess when measuring monoclonal FLC on the BNII. Further investigations with a higher number of samples showing antigen excess are necessary.

Haematology

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GLUTAMATE DEHYDROGENASE ACTIVITY IN LYMPHOCYTES OF B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS

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Background. We investigated the pattern of glutamate dehydrogenase (GLDH) activity, *GLUD1* and *GLUD2* expression in peripheral blood mononuclear cells (PBMC) of untreated B-chronic lymphocytic leukemia (CLL) patients, in healthy individuals (HI), and patients with infectious mononucleosis (IM). Gene expression results in CLL patients were further analysed regarding the presence of *TP53* deletion; del(17)(p13).
Methods. GLDH activity was determined in a supernatant obtained from pelleted PBMC. *GLUD1*, *GLUD2* and reference *ABL1* mRNA expression was analysed by quantitative real-time polymerase chain reaction. Fluorescence *in situ* hibridization (FISH) was used to detect del(17)(p13).
Results. The highest GLDH activity was found in PBMC of CLL group followed by HI group and IM group. PBMC GLDH activity was higher in 60 % of the CLL patients according to the established reference interval for HI (2.17-5.70 mkat/g protein). The median value of *GLUD1/ABL1* expression was the highest in IM group (n=11), followed by HI (n=14) and CLL group (n=59) (median 4,69/3,78, p<.005 and4,69/2,91, p<.0005, respectively). *GLUD2/ABL1* expression didn't differ significantly between groups. The lower median *GLUD1/ABL1* gene expression level was found in CLL patient with deletion del(17)(p13) (del(17)(p13) positive (N=5),*GLUD1/ABL1* 2,02 vs. *GLUD1/ABL1*2,85, del(17)(p13) negative (N=23); p= 0.024).
Conclusions. The increased GLDH activity is specific for the PBMC of CLL patients. The *GLUD1* but not the *GLUD2* gene expression pattern is different comparing PBMC of IM and CLL patients. *GLUD1* gene expression level was lowered in CLL patients with deletion del(17)(p13).

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EVALUATION OF NEW PROGNOSIS MARKERS IN B-CLL

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Background. The clinical course of B-cell chronic lymphocytic leukemia is highly variable. To predict the individual risk of disease progression a newmarkers need to be identified.
AIM: The aim of the present study is to investigate the expression and possible prognostic value of the CD305 (LAIR-1) and CLLU-1 (CLL UPREGULATED GENE 1) in 31 patients affects by B-CLL.
Methods. Flow cytometry was used to analyse CD305, and the results are expressedas a percentageofleukemic cellspositive. Moreover, was determined the expression levels of CLLU1 by quantitative RT-PCR with kit Profile*Quant* (IPSOGEN).
Results. The obtained results show a statistically significant difference between a controls and patients both for CD305 (range 0- 91% vs 63% -100%) and CLLU-1 (range 134-1553788 RNA bp vs 5-296). We didn'tfound a statistically significant difference between CD305 expression and sex, clinical stage and the others prognostic markers (cytogenetics, IgVH mutational status, ZAP-70, CD38). Furthermore, significant difference was found in CLLU1 expression levels and clinical stage while no difference were with age, sex and the others marker.
The use of the tree algorithm, a good discriminator performance of CLLU1 in prognostic classification in patients with favorable cytogenetic and younger than 70 years was obtained.
Conclusions. CLLU1demonstratesthefeasibility ofit canbe usedas a prognostic marker.
Our data demonstrate that CD305 can't be used like a prognostic marker in BCLL, but CLLU1seems to be particularly promising like a new biomarker in BCLL.

Haematology

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SCREENING OF ANAEMIA CASES IN STUDENTS

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Background. The world's population at all ages suffering from anaemia in roughly 30%. Around half of these cases are caused by iron deficiency, while approximately 20% are caused by vitamin B_{12} and folate deficiencies.

Methods. The aim of the study was screening for anaemia cases in students participating in the sistematic examination in period from 1991-2009and complete blood count (CBC) was determined in 4299 students.

Results. Statistically significant difference was found (p<0,05) in minimal haemoglobin values, which are higher in last period of examination.In both sexes minimal hemoglobin values and maximal erythrocytes values showed statistically significant difference (p<0,05) in a view of higher values in last year. Results was shown that student's population have a constant mean values of CBC and we established own reference values: men erytrocytes 4,30-5,94 x 10^{12}/L ; women 3,80-5,28 x10^{12}/L,   haemoglobin men 138-179 g/L; women 119-158 g/L. Statistically significant difference was found (p<0,05) in distribution of anaemia with lower percent of appearance of anaemia in both sexes in last year.

Conclusions. These data suggest that screening of anaemia is necessary in student's population, primary prevention is very important and can achieved through lifestyle changes, promotions of health way of life, supplementation of food with iron and vitamins, active diagnosis of preanaemic states and anaemia and therapeutic intervention of established clinical cases.

0565

ERYTHROBLAST COUNT IN HEALTHY NEWBORNS

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Background. Our aim was to determine erythroblast count in healthy newborns with two Methods.

Methods. Erythroblast count was determined on haematology counter Sysmex XE2100 and by microscopic slide examination. We analysed 122 blood samples from healthy newborns aged 1-3 day.

Results. Erythroblast count on haematology analyser ranged from 0 - 0,90x10^9/L (0-4,3%) and by microscopic slide examination ranged from 0-5/100 leucocytes. This is in agreement with recommended reference range.

Conclusions. Comparing the results, we noticed that the haematology analyser is more analytically sensitive because there are numerous samples in which erythroblasts were not found by microscopic slide examination while haematology analyzer counted >0%.

Haematology

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INFLUENCE OF THE NEW ORAL ANTICOAGULANTS ON COAGULATION ASSAYS

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Background. Dabigatran and rivaroxaban are new oral anticoagulants with major influence on almost all coagulation assays. The forthcoming wide-spread clinical use in therapeutic dosage will face every lab with these interferences. We present a systematic evaluation of the influence on a wide spectrum of coagulation assays.
Methods. Plasma pools were prepared from normal volunteers, ICU and cumarin patients. Dabigatran or rivaroxaban were added to these pools giving a final concentration of 1000 μg/l, followed by a geometric dilution: 500-250-125-67.5-31.25-0 μg/l. On each pool and dilution the following assays were measured with Roche and Siemens assays on a STA-R and a BCS analyzer, respectively: PT, APTT, Fibrinogen derived/Clauss, TT, Batroxobin Time, AT, D-Dimer, PS clotting/free, PC clotting/chromogenic, DRVVT, Factor II/V/VII/VIII/IX/X/XI/XII/XIII, vWF antigen/activity. Results are expressed as deviation (%) from basic value (0 μg/l). Drug concentrations of 31.25, 250 and 1000 μg/l were selected as representative for through level, Cmax and overdosage/cumulation.
Results. For rivaroxaban through level deviation ranged from 80-134% and 87-146% for Roche and Siemens assays, respectively. Cmax levels from 37-271% (R) and 54-290% (S) and overdosage levels from 7-846% (R) and 17-303% (S). For dabigatran through level deviation ranged from 69-167% (R) and 75-168% (S), Cmax levels from 8-482% (R) and 17-275% (S) and overdosage levels from 1-765% (R) and 11-759% (S), respectively.
Conclusions. The influences on coagulation assays depend on plasma concentration, assay and patient characteristics. At Cmax levels and in overdosage/cumulation a reliable measurement is almost impossible. Most reasonable seems to be measurement at through levels taking assay characteristics and a deviation of about -20 to +50% for rivaroxaban and of -30 to +70% for dabigatran into account.

0567

DONOR CELL LEUKEMIA AFTER 17 YEARS OF ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background. Relapse of acute leukemia after allogeneic hematopoietic stem cell transplantation represents rarely a severe complication. The acute leukemia can develop de novo in engrafted cells of donor origin.
Methods and Results. We report a case of a female patient with APL (FAB type M3) who developed AML M5 of donor origin 17 years after allogeneic BMT from his HLA-matched sister. We investigated the origin of haematopoiesis by analysis of 21 different STR markers. Several factors like chemotherapy and radiotherapy, the preparation before the transplantation, as well as the deficitary immune response of the recipient and the stress originated by the allogenic response of the donor in the recipient's bone marrow microenvironment, leading to the production of genetic abnormalities in the recipients cells have been involved in the pathogenesis of this complication. Leukemia cells were studied by FISH not being found the t (15; 17) translocation, however it was detected a 11q23 deletion. Other studies have proven that abnormalities in the 11 chromosome, specially the 11q23, are related to the administration of topoisomerase II inhibitors.
Conclusions. We believe that a more exhaustive research of the post-transplantation leukemic process must be done by the combination of several methods (flow cytometry, FISH and molecular biology). A deep analysis of this process will provide a more accurate diagnosis and help to reveal the mechanisms that take part in the origin of the leukemias.

Haematology

0568

MEASUREMENT OF ERYTHROCYTE SEDIMENTATION RATE (ESR) IN EDTA AND CITRATE BLOOD – COMPARISON OF VESMATIC 30, VESMATIC CUBE 30 AND VESMATIC CUBE 80 ANALYZERS

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Background. The erythrocyte sedimentation rate (ESR) is the most widely used laboratory test for monitoring the course of infections, inflammatory diseases and some types of cancer. Commonly, the ESR is performed in diluted citrate blood but more recent the EDTA blood is used for this test.
Methods. VesMatic 30 analyzer measures ESR from diluted Sodium Citrate anticoagulated blood sample. VesMatic Cube 30 and VesMatic Cube 80 measures ESR from K_2 or K_3 EDTA anticoagulated whole blood.
Results. Comparison study of VesMatic 30with VesMatic Cube 30 (n=196) showed Spearman's correlation coefficient ρ=0.92; Passing-Bablok linear regression: slope 0.875 (95% CI: 0.809 to 0.941) and intercept -3.125 (95% CI: -4.470 to -2.071); Bland-Altman analysis: bias (6.1) and limits of agreement (-13.5 to 25.7).
Results of comparison of VesMatic 30 with VesMatic Cube 80 (n=120) were: Spearman´s correlation coefficient ρ=0.95; Passing-Bablok linear regression: slope 1.200 (95% CI: 1.130 to 1.272) and intercept -2.200 (95% CI: -3.409 to -1.174); Bland-Altman analysis: bias (-1.8) and limits of agreement (-15.4 to 11.8).
VesMatic 30 showed slightly better correlation with VesMatic Cube 80 than with VesMatic Cube 30. Even more, VesMatic Cube 30 showed greater bias and wider limits of agreement.
Conclusions. VesMatic Cube 30 and VesMatic Cube 80 are reliable systems for automatic measurements of ESR in EDTA blood samples when compared to VesMatic 30. The advantages of the EDTA blood samples used for ESR measurement are avoidance of a dilution step and the need for an extra blood sample for hematology analyses.

0569

DIRECT CALCULATION OF REFERENCE LIMITS FOR COMPLETE BLOOD COUNT INCLUDING WBC DIFFERENTIAL ON SYSMEX XE 2100

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Background. In line with different regulations, clinical laboratories have to check their reference limits.
The goal of our study was to estimate reference limits for Complete Blood Count (CBC) including WBC differential on the Sysmex XE 2100 analyser in accordance to CLSI/IFCC procedures.
Methods. CBC results from healthy volunteers participating in clinical trials of the SGS Life Science Services Clinical Pharmacology Unit Stuivenberg (Antwerp, Belgium) (n= 1000, 18-60 years old, 80% males and 20% females) were collected retrospectively over a time period of 12 months. We calculated reference intervals according to CLSI C28-A3 guidelines, using MedCalc and RefVal software.
Results. For RBC, Hb and HCT gender specific reference intervals were established.
Small differences were observed between manufacturer's and calculated reference limits for Hb (M:133 -166; F: 113-150 g/L), HCT (M: 0.40-0.49; F: 0.37-0.45 L/L), RBC (M: 4.4-5.6; F: 3.9-5.0 10E12/L), MCV (82.2-95.9 fl), MCH (27.7-32.2 pg), MCHC (31.8-35.3 g/dl), platelets (157-341 10E9/L) and monocytes (5.3-13.8%). Differences of more than 10% were found for WBC (3.7-9.2 10E9/L), neutrophils (43.3-74.0%), lymphocytes (16.1-44.3%), eosinophils (0.4-6.3%), and basophils (0.1-1%).
Conclusions. We established reference limits for CBC including WBC differential on the Sysmex XE 2100 platform, using a large number of reference individuals. For most parameters, calculated values corresponded well with those provided by the manufacturer; however for some parameters differences of more than 10% were observed.

Haematology

0570

MOLECULAR MONITORING IN PAEDIATRIC PATIENTS WITH CHRONIC MYELOID LEUKAEMIA; DETECTION OF BCR-ABL TRANSCRIPT USING THE GENEXPERT SYSTEM

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Background. The tyrosine kinase inhibitors (TKI) have revolutionized the management of chronic myeloid leukaemia (CML), improving the outcome of patients significantly. Molecular monitoring, based on detection of BCR-ABL mRNA levels, has become an important criteria for evaluate the molecular response to treatment. The real-time quantitative polymerase chain reaction (RQ-PCR) techniques require optimization for measurements of BCR-ABL transcript. Values can vary widely among molecular laboratories, and many European organizations have proposed recommendations for harmonizing the differing methodologies.
Patients and Methods. Peripheral blood and/or bone marrow aspirate specimens were obtained from 4 paediatric CML patients on TKI therapy for an overall of 10 evaluations of molecular monitoring. Quantitative BCR-ABL transcript was performed by the reference method and using the Cepheid GeneXpert System. The GeneXpert performs the RQ-PCR using an automated procedure for all of the RNA purification, amplification and detection steps, in a closed system, using a single cartridge and with a quick turnaround time.
Results. The BCR-ABL results obtained with the GeneXpert assays showed a good correlation with those converted to IS and obtained using the reference method. Statistical analysis performed by the Passing Bablock method showed an intercept= -0,011 (95% CI -0,03 to 0,01), slope= 1,85 (95% CI 1,45 to 2,37). The coefficient of determination, r^2 was 0,954.
Conclusions. The Xpert BCR-ABL Monitor assay is a reliable method for detecting the BCR-ABL transcript in molecular monitoring of CML patients. It should provide a standardized method for international harmonization of BCR-ABL assessment to monitor the treatment response in patients with CML.

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EVALUATION OF CELL-DYN RUBY HAEMATOLOGY ANALYSER COMPERED TO ADVIA120 AND TO BLOOD SMEAR MICROSOPIC EXAMINATION

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Background. The purpose is to validate the Abbott CELL-DYN Ruby haematology analyser analytical performance and compare it to ADVIA120 haematology analyser with the same parameters. 5-part differential from both analysers was separately compared to blood smear microscopic examination.
Methods. The CBC test (n=165) parameters WBC, RBC, HGB and PLT were compared between the analysers and the 5-part WBC differential (n=77) from both analysers was compared to blood smear microscopic examination.
Results. Intra-assay correlation between Ruby and ADVIA 120 for WBC was r=0,991; for RBC r=0.986; for HGB r=0.994 and for PLT r=0.967.
Interassay CV`s for WBC were 2.2(3.2); 1.8(4.3) and 0.9(2.7)% at 3.56; 6.18 and 10.24*10^9/L; for RBC were 1.0(1.0); 1.1(1.5) and 0.7(0.9)% at 1.94; 4.49 and 5.25 *10^12/L; for HGB were 0.6(0.71); 0.9(1.2) and 0.5(0.8)% at 67; 137 and 165g/L; for PLT were 9.5(18.8); 2.4(2.8) and 2.0(3.8)% at 78; 254 and 328 *10^9/L respectively. In brackets are presented the results by ADVIA120.
Correlation of 5-part WBC differential from Ruby compared to blood smear microscopic examination was as follows: NEUT r=0.967 (r=0.955), LYMPH r=0.972 (r=0.960), MONO r=0.634 (r=0.557), EOS r=0.758 (r=0.674). Results presented in brackets are for ADVIA 120.
Conclusions. Correlation between the analysers was excellent. Correlation of 5-part WBC differential from Ruby compared to blood smear microscopic examination for NEUT and LYMPH was excellent, for EOS good and for MONO satisfying.

Haematology

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ALTERATION OF PLATELET PARAMETERS IN MALARIAL INFECTION

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Background. Malaria is a global health problem with an annual incidence of 300 million people with one million deaths.Symptoms of malaria include fever, shivering, arthralgia, vomiting, anemia, hemoglobinuria, retinal damage, and convulsions. Microscopic examination of blood film shows infected erythrocyte for diagnostic criteria. Thrombocytopenia has been reported in many studies; therefore platelet parameters obtained from automated blood cell analyser were interested in this study.
Methods. The EDTA blood samples were taken from 34 malaria infected patients and 34 healthy volunteers (age and sex matched). Hematological parameter results were obtained from Beckman Coulter AcT 5 Diff blood cell analyzer. Parasitemia was determined from 1,000 erythrocytes of thin film stained with Wright-Giemsa.
Results. The results showed that 19 patients (55.88%) were infected with *P. falciparum*, 12 patients (35.29%) with *P. vivax*, 1 patient (2.94%) with *P.malariae* and 2 patients (5.88%) with *P. knowlesi*. Red blood cell count, hemoglobin, and hematocrit in the patients were lower than in the control group, whereas white blood cell count was significantly higher. Platelet count (98.41 ± 53.64 vs. 248.64 ± 53.40 x10^9/L) and MPV (9.33 ± 1.63 vs.10.64 ± 0.97 fL) in the patients were significantly decreased as compared to the control group. The relationship between parasitemia and platelet parameters was not found in this study.
Conclusions. Decreased in platelet count and MPV indicated the alteration of platelet parameters in malarial infected patients which may provide the information for healthcare service.

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STUDY OF EXPANSION OF CD34+ UMBILICAL CORD BLOOD IN TWO AND THREE-DIMENSIONAL CULTURE MEDIA ON MESENCHYMAL STEM CELL

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Background. Umbilical cord blood transplantation (UCBT) in adults is limited by the small number of primitive hematopoietic stem cells (HSC). Expansion of cord blood HSCs in-vitro is a solution. Mesenchymal stem cells (MSCs) as stromal cell would support proliferation of cord blood HSCs in culture media.
Methods. Mononuclear cells from bone marrow were isolated by density gradient centrifugation. Cells were cultured and the adherent cells were allowed to attach to the flask. Non adherent cells were removed and adherent cells were kept for 10-14 days. Umbilical cord bloods (UCB) CD34+ HSCs were isolated with immunomagnetic separation system. these cells were expanded for 14 days in three circumstances: 1) Stroma free culture containing TPO, SCF, FL2) Two dimensional condition containing MSCs and cytokines 3) Three dimensional condition containing MSCs co-culture with cytokines on calcium phosphate scaffold. On days 3, 7 and 14 aliquots of cultured cells were harvested and subjected to cell count, colony forming unit assay and flowcytometric analysis.
Results. In the stroma free culture media the mean of fold increase of CD34+, number of colony forming unit and the percentage of CD34+ cells after 14 days were 31±2.4, 31±4 and 10.56±4.3% respectively. This data was 389±18, 305±4 and 67.28±7.4% for 2D culture, and 247±13, 186±5 and 41.46±7.1% for 3D culture
Conclusions. The results showed that 2D and 3D co-cultures led to significantly higher expansion of UCB HSCs than cultures without MSCs and supplemented only with cytokines.

Haematology

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MEAN RETICULOCYTE VOLUME AND SOLUBLE TRANSFERRIN RECEPTOR-FERRITIN INDEX EVALUATION IN PATIENTS WITH B-CELL NON HODGKIN LYMPHOMA

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Background. New laboratory parameters are continuously reported for establishing exact etiology of anemia in oncological patients. In this work we evaluated the mean reticulocyte volume (MRV) and soluble transferrin receptor-ferritin index (sTfR/F) in patients with B-cell non-Hodgkin lymphoma (B-NHL).
Methods. The study included a control group (CG) of 12 healthy donors, 14 patients with diagnosed iron deficiency anemia (IDA) and 35 patients with diagnosed B-NHL presenting anemia without ferropenia. Blood cell counts, iron, ferritin, C-reactive protein, sTfR and MRV were determined. Means and standard deviations were evaluated; t-test was used for group comparison and ROC curve analysis to evaluate performance of MRV and sTfR/F.
Results. B- NHL patients presented an MRV of 105.9±9.2 fL (mean±sd) and an sTfR/F of 1.37±1.02, IDA group an MRV of 89.7±7.9 fL and an sTfR/F of 3.05±2.01, the CG an MRV of 106.9±4.9 fL and an sTfR/F of 0.61±0.18. MRV was significantly higher in the B-NHL patients than in the IDA group ($p<0.001$), unlike between B-NHL patients and CG ($p=0.73$). MRV was also significantly higher ($p<0.0005$) in CG when compared to IDA group. sTfR/F in B-NHL patients presented lower values than IDA group ($p<0.004$) and no significant difference from CG ($p=0.09$). IDA patients presented higher sTfR/F than the CG ($p<0.0009$). sTfR/F had better performance in differentiating both IDA (AUC=0.9881) and B-NHL (AUC=0.6620) from CG that MRV (AUC=0.9467) and (AUC=0.5590) respectively.
Conclusions. MRV and sTfR/F may be an additional tool in establishing the anemia etiology in B-NHL patients and differentiate patients with IDA.

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CELL SURFACE POSITIONING OF L-SELECTIN AND CD44 DEPENDS ON THEIR TRANSMEMBRANE DOMAINS

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Background. During inflammation and immune surveillance, initial contacts (tethering) between free-flowing leukocytes and the endothelium are vitally dependent on the presentation of the adhesion receptor L-selectin on leukocyte microvilli. Determinants that regulate receptor targeting to microvilli are, however, largely elusive.
Results. Therefore, we systematically swapped the extracellular (EC), transmembrane (TM) and intracellular (IC) domains of L-selectin and CD44, a hyaluronan receptor expressed on the cell body and excluded from microvilli. Electron microscopy of transfected human myeloid K562 cells showed that the highly conserved TM domains are responsible for surface positioning. The TM segment of L-selectin forced chimeric molecules to microvilli, and the CD44 TM domain evoked expression on the cell body, whereas the IC and EC domains hardly influenced surface localization. Transfectants with microvillus-based chimeras showed a significantly higher adhesion rate under flow but not under static conditions compared with cells with cell body-expressed receptors. Substitution of the IC domain of L-selectin caused diminished tethering but no change in surface distribution, indicating that both microvillus positioning and cytoskeletal anchoring contribute to leukocyte tethering.
Conclusions. These findings demonstrate that TM domains of L-selectin and CD44 play a crucial role in cell adhesion under flow by targeting receptors to microvilli or the cell body, respectively.

Haematology

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MEAN MONOCYTE VOLUME AND MEAN NEUTROPHIL VOLUME ARE ASSOCIATED WITH MARKERS OF FOLIC ACID DEFICIENCY

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Background. Diagnosis of folic acid (FA) and vitamin B12deficiencies is difficult and, because there is no single best parameter, may require the concomitant use of several markers. A novel haematological flow cytometric device allows for the determination of mean neutrophil volume (@MNV) and mean monocyte volume (@MMV).
Aim: To determine, whether @MNV and @MMV are associated with markers of FA and vitamin B12 deficiency.
Methods. Subjectively healthy individuals $\geq$ 60 years were consecutively included. Using the UniCel DxH 800 Coulter Cellular Analysis System (Beckman Coulter, Miami, FL, USA), MNV and MMV were measured and distribution width of these parameters (NEV-DW&MMV-DW) was calculated. Further, erythrocyte MCV&MCH, FA, homocysteine, vitamin B12, active B12, methyl malonic acid (MMA), ferritin, cystatin C and CRP were determined.
Results. Among the 100 included individuals (60 female/40 male; age 71$\pm$7 years), a correlation was seen between serum FA and both,@MNV (r=-0.285;p=0.004) and @MMV (r=-0.389;p<0.0001). NEV-DW (r=-0.237;p=0.02) and MMV-DW (r=-0.281; p=0.005) were associated with homocysteine. From all markers of B12 status, a significant correlation between MMV-DW and active B12 (r=0.215;p=0.03) was observed. ROC-analysis revealed @MMV to possess a higher accuracy than @MNV (AUC=0.832 vs.0.664;p=0.05) in recognizing a FA concentration $\leq$10nmol/L. MCV and MCH did not show any association with the investigated markers of vitamin deficiency.
Conclusions:@MMV and @MNV are associated with markers of folic acid status. In suited instruments, these parameters can be easily determined at reasonable costs. A possible role for @MMV in screening for folic acid deficiency is suggested.

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DETERMINATION OF THE ERYTHROCYTE SEDIMENTATION RATE (ESR) ON TWO AUTOMATED SYSTEMS USING EDTA SAMPLES IN PATIENTS WITH RHEUMATOLOGIC DISEASES

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Background. Monitoring disease activity in patients with rheumatologic diseases includes determination of ESR. The use of Westergren method with diluted citrate blood is recommended, which takes 60 minutes. In order to reduce time for determination but still get reliable results for ESR, we test two automated systems using non diluted EDTA samples.
Methods. Citrate and EDTA blood samples were obtained from 80 patients suffering from rheumatologic diseases. We measured ESR on automated system Ves-Matic cube 30 (Diesse, Italy) and on Roller Test-1, (Alifax, Italy), which both use the infrared reading system. We used modified Westergren method as a reference.
Results. Bland Altman analysis for Westergren vs Ves-Matic: limits of agreement between -7,7 and 10 mm/h, bias 1,2 mm/h, for Westergren vs Roller Test-1: limits of agreement between -31,5 and 22,8 mm/h, bias -4,4 mm/h. Passing Bablok regression for Westergren vs Ves-Matic: slope 1,0000 (95% CI 0,9000 to 1,0435), intercept -1,0000 (95% CI -1,7391 to 0,3500), for Westergren vs Roller Test-1: slope 1,2000 (95% CI 1,0381 to 1,3898), intercept -0,2000 (95% CI -2,1186 to 2,1333). Spearman's coefficient of rank correlation for Westergren vs Ves-Matic: r=0,928 (95% CI 0,8896 to 0,9533, p<0,0001), for Westergren vs Roller Test-1:r=0,905 (95% CI 0,8534 to 0,9384, p<0,0001).
Conclusions. Both automated systems use for determination EDTA blood, so no extra citrate tube is required. On both we achieve the results faster and they both show high correlation with the reference method, but results on Roller Test-1 sometimes deviate too much from the real value.

Haematology

0578

THE NATURE OF HIGH OXYGEN AFFINITY IN THE HUMAN HEMOGLOBIN COIMBRA

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Background. The aim of this work was to investigate the molecular mechanism responsible for the high oxygen affinity of Hb-Coimbra, a human Hb variant characterized by the Asp99βàGlu replacement which *in vivo* results in polycythemia to its carrier. This mutation is located at the $\alpha_1\beta_2$ interface of the molecule which undergoes major modifications during the oxygenation process. The structural characterization of this variant complements studies of functional activity and allows identification of the mechanisms involved in the cooperativity and affinity of Hb with O_2, contributing to a better understanding of the pathophysiology and diagnosis of hemoglobinopathies.

Methods. The structures of deoxy and oxy-HbCoimbra were determined by homology modeling, taking the coordinates of human Hb at 1.25 Å as templates. The generated models were submitted to 2ps molecular dynamics and then validated.

Results. Analysis of the structure of deoxy-T HbCoimbra showed that the Glu99β is greater in a CH_2 group, which lies compressed into a polar environment interface and thus acts as a spring that pushes the Hb to the R conformation with greater space on this interface. In oxy-R HbCoimbra, the Glu99β is better accommodated and also makes a hydrogen bond with the Asn97α, which is not present on native Hb, further stabilizing this conformation.

Conclusions. The mutation in HbCoimbra destabilizes the T form and stabilizes the R form of the molecule, shifting the allosteric equilibrium to the conformation of high affinity, resulting in increased affinity for O_2 as observed in *in vitro*studies.

0579

SILENCING OF THE PIPKIIA ENZYME GENE IN K562 CELLS

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Background. Phosphatidylinositol phosphate kinases (PIPK) belong to a family of enzymes that generate various lipid messengers. The PIPK subfamilies are divided into type I (a, β and γ), type II (a, β and γ) and type III. In a recent study in our laboratory, the PIPKIIa gene was differentially expressed in reticulocytes from 2 siblings with hemoglobin (Hb) H disease. Expression of both the PIPKIIa and b-globin genes were higher in the patient with the higher Hb H level, suggesting a possible relationship between PIPKIIa and the production of globins.

Methods. We performed gene knockdown of the PIPKIIa gene in K562 cells by RNA interference-based gene silencing using plasmids that encode short-hairpin RNA molecules specific to this gene in an attempt to elucidate its role, particularly insofar as the expression of a and g globin genes is concerned. We used quantitative real-time PCR to assess gene expression. PIPKIIa protein was quantified by Western blot.

Results. Analysis of the results of three independent experiments revealed a 78.59% reduction in PIPKIIa gene expression, as well as a 2.65-fold and 2.6-fold increase in a and g globin gene expression, respectively.We also observed a 75% reduction in PIPKIIa protein level.

Conclusions. These data suggest that the reduction in PIPKIIa gene expression somehow influenced the a and g globin gene expression. Our findings show that in K562 cells there may be, at least in vitro, a regulatory mechanism that acts on a and g genes in response to the reduction in PIPKIIa gene expression.

Haematology

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IN VITRO ASSESSMENT OF DEXAMETHASONE EFFECT ON DRUG RESISTANCE IN LEUKEMIC BLASTS FROM CHILDREN WITH CALL AND PERIPHERAL BLOOD LYMPHOCYTES (PBL) FROM HEALTHY VOLUNTEERS

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Background. The aim of the study was to test if dexamethasone, used in leukaemia and other diseases in combination therapy, increases the expression and activity of P-gp (P-glicoprotein) presented on PBL and leukemic lymphoblasts and reduces intracellular drugs concentration.
Methods. 9 children at the day of diagnosis of cALL and 19 healthy volunteers were included in the study. Cells were isolated by gradient centrifugation. P-gp expression was measured after staining with MoAb anti-P-gp (Immunotech), anti-CD2 and anti-CD19 (Beckman Coulter) on flow cytometer Coulter EPICS XL. Rhodamine test was performed after incubation of cells with Rhodamine-123 (Sigma-Aldrich) and then with/without dexamethasone and verapamil. Cells where stained with MoAb anti-CD2 and anti-CD19, measured on flow cytometer Coulter EPICS XL.
Results. The percentages of normal PBL B and T expressing P-gp+ were similar (about 25%), the frequency of P-gp on leukemic blast was lower (14.5%). Dexamethasone slightly increased P-gp+ PBL and decreased the number of P-gp+ lymphoblasts, but the differences were not significant. Dexamethasone stimulated activity of the P-gp pump as number of cells excreting rhodamine significantly increased after incubation with dexamethasone compared to control: 2.6% vs. 4.3% for blasts, p=0.015; 12.8% vs. 14.7% for PBL CD2+, p=0,031; 5.0% vs. 7.6% for PBL CD19+, p=0.017.
Conclusions. Dexamethasone may increase cytostatic drug resistance in leukemia. Combining membrane P-gp expression test together with the study of P-gp activity allows for more complete evaluation of drug resistance in leukemia.

0581

CUSTOMIZING SYSMEX FLAGS FOR A SPECIFIC PATIENT POPULATION USING STATISTICAL METHODS

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Background. The absolute neutrophil count (ANC) is utilized to guide chemotherapy treatment in oncology patients. Although automated methods to determine ANC may be adequate, samples from oncology patients often trigger flags leading to manual review. These flags may not affect the automated ANC and could therefore potentially be ignored. This study uses statistical methods to identify WBC-differential flags associated with a discrepancy between manual ANC (manANC) and automated ANC (autoANC) and determines the rate of manual review if only these flags are used.
Methods. A manual and automated WBC differential was performed on 341 samples flagged by WBC-differential related flags by Sysmex XE/XT hematology analyzers (Sysmex, Kobe, Japan). ManANC and autoANC were calculated. A multivariable logistic regression model was created to identify flags predictive of discrepancy between manANC and autoANC. Discrepancy was defined as > 20% difference. WBC count was included in the model to control for confounding. 9 WBC differential related flags were analyzed.
Results. 71 cases had a discrepancy between the manANC and autoANC. The final regression model revealed the Immature Granulocyte and Abnormal Lymphocyte flags to be significantly associated with a discrepancy, p-value 0.001 and 0.0143, respectively. The sensitivity and specificity of this method are 68% and 53%. Using only these flags, 174 cases would be flagged.
Conclusions. Sysmex flagging criteria can be tailored to meet the needs of specific patient populations. Statistical models can identify parameter specific flags predictive of automated and manual result discrepancy. Using this method, cases requiring manual review could be decreased.

Haematology

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PLATELET ACTIVATION PARAMETERS IN SEPSIS

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Background. Platelets (Plt) play a pivotal role in the complex interaction between inflammation and coagulation. The understanding of essential aspects of platelet activation and aggregation can help to better define the pathogenesis of sepsis.The aim of our study was to analyze the main platelet functions: aggregation, adhesion and secretion in septic patients.
Methods. Routine Plt parameters; platelet count, mean platelet volume (MPV,fL) and parameters of activity, mean component concentration (MPC,g/L), platelet component distribution width (PCDW, g/L) were measured by SIEMENS ADVIA 120 System in 30 critically ill patients with sepsis and 30 healthy controls. Agonist-induced platelet aggregation, ADP (%), Epinephrine (Epi%) and Collagen (Col%) were measured in platelet-rich plasma.
Results. A decrease in platelet density, measured by reduction in MPC (248.2 ± 21.95 in sepsis vs. control group 270.0 ± 15.0) indicates platelet activation and reflects a granules release. The PCDW, as an indicator of morphological changes, was decreased (51.1 ± 4.6 in sepsis vs. control group 54.5 ± 5.0). Values for MPV increased significantly (10.7 ± 1.50 in sepsis vs. control group 9.0 ± 1.1) reflecting shape change. Results for ADP, Epi and Col were significantly decreased (84 ± 9.2, 73 ± 20.4, 74 ± 16.8 in sepsis vs. control group 90 ± 10.2, 86 ± 12.2, 88 ± 9.3) indicating platelet degranulation.
Conclusions. Our results suggest that sepsis alters the hemostatic function of the platelets and increases the risk of thromboembolic complications, which seriously worsens the outcome of illness.

0583

DETECTION OF DYSPLASTIC NEUTROPHILS WITH CELL POPULATION DATA ON THE UNICEL DXH800 COULTER CELLULAR ANALYSIS SYSTEM

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Background. The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic disorders characterized by peripheral blood cytopenias and cellular dysplasia. One of diagnostic criteria for MDS is the type and degree of dysplasia involving one or more myeloid cell lines, which is subjective. The detection of dysplastic leucocytes in laboratory practice with automated hematology analyser may improve the reliability of these features.
Methods. The UniCel DxH 800 Coulter Cellular Analysis System performs Flow Cytometric Digital Morphology analysis of leukocytes with measurement of cell volume (impedance), internal complexity and nucleo/cytoplasm ratio (cell conductivity using radio-frequency current) and granularity (measurement of 5 angles of light scatter). All these measurements (Mean and Standard Deviations (SD)) are reported as numerical values (Cell Population Data, @CPD) for every sample. We evaluated the DxH800 for routine haematological analysis on 190 normal patients and 12 MDS patients with neutrophil dysplasia.
Results. ROC curve analysis with MedCalc Software (Mariakerke, Belgium) showed that dysplastic neutrophils can be detected with: @NE Vol SD (neutrophil anisocytosis) (AUC=0.995, cut-off>17.81, sensitivity=100% , specificity=94.2%), @MO Vol SD (monocyte anisocytosis) (AUC=0,969, Cut-off>25,41, sensitivity=83.3%, specificity=100%) and several parameters of neutrophil light scatter – mean values of Low Angle Light Scatter (@LALS, AUC=0.910, cut-off<=114, sensitivity=91.7% , specificity=96.3%), Low Medium Angle Light Scatter (@LMALS, AUC=0,914, cut-off<=114, sensitivity=91.7% , specificity=97.9%) and Medium Angle Light Scatter (@MALS, AUC=0.911, cut-off<=125, sensitivity=91.7%, specificity=96.3%).
Conclusions. These preliminary results demonstrate that automated leukocyte morphology may be useful for the screening for neutrophil dysplasia.
@-For Research Use Only. Not for use in diagnostic procedures.

Haematology

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DETECTION OF ABNORMAL LYMPHOCYTES WITH CELL POPULATION DATA ON THE UNICEL DXH800 COULTER CELLULAR ANALYSIS SYSTEM

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Background. Chronic Lymphocytic leukaemia (CLL) is a neoplasm composed of monomorphic small, round to slightly irregular B lymphocytes in the peripheral blood, bone marrow, spleen and lymph nodes. As CLL is the most common leukaemia of adults in Western countries, and abnormal lymphocytes are present in peripheral blood, any new method to detect abnormal lymphocytes will improve the flagging of pathological samples.
Methods. The UniCel DxH 800 Coulter Cellular Analysis System performs Flow Cytometric Digital Morphology analysis of leukocytes with measurement of cell volume (impedance), internal complexity and nucleo/cytoplasm ratio (cell conductivity in the radio-frequency current) and granularity (measurement of 5 angles of light scatter). All these measurements, called Cell Population Data (@CPD) are reported as numerical values (Mean and Standard Deviations (SD) for every sample. We evaluated the UniCell DxH800 for routine haematological analysis of the group of normal patients and the group of CLL patients with abnormal lymphocytes.
Results. ROC curve analysis with MedCalc Software (Mariakerke, Belgium) showed that the best CPD for the detection of changes in lymphocyte population are: @LY Mean Volume (AUC=0.736, cut-off<=83, sensitivity=75% , specificity=80.5%), @ LY Volume SD (Lymphocyte anisocytosis) (AUC=0,989, Cut-off>16,04, sensitivity=100%, specificity=96.3%) and mean values for Lymphocytes Axial Light Loss (@AL2, AUC=0.979, cut-off<=74, sensitivity=100% , specificity=83.2%) and Lymphocytes Low Angle Light Scatter (@LALS, AUC=1.00, cut-off<=19, sensitivity=100% , specificity=100%)
Conclusions. These preliminary results show that cytometric digital leukocyte morphology may be useful for the screening of changes in the lymphocyte population.
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0585

MEASURING HOMOCYSTEINE, FOLIC ACID AND B$_{12}$ VITAMIN LEVELS IN THALASSAEMIA AND SICKLE CELL ANEMIA: AHEPA UNIVERSITY HOSPITAL BIOCHEMICAL LAB EXPERIENCE

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Background. Homocysteineisasulfur-containing aminoacid.It is a metabolic derivative of methionine and is not nutritionally received.For itsremethylationtomethionine,theenzyme methylenetetrahydrofolatereductase, folicacidandB$_{12}$, arenecessary.
Theincreasedlevelsof homocysteine are related to high risk of atherosclerosis .
Aim. Theaimofthis studywas to evaluate serum levels of homocysteine, folicacidandB$_{12}$ in patients with thalassaemia and sickle-cell anaemia.
Methods. We studied 59 patients ,20 with omozygous β-thalassaemia,10 with intermedia and 29 with sickle cell anemia.We also used an age-matched control group of 30 healthy subjects.Homocysteine levels were determined by FluorescencePolarizationImmunoassay whereas folicacidandB12 levels by Electrochemiluminescence Immunoassay .
Results. Homocysteine levels are significantly lower in patients groups and present statistically significant difference compared to the healthy group (p<0,05). Levels of B$_{12}$ vitamin are similar in all patients groups.
Serum levels of folicacid were increased both in patients with omozygous β-thalassaemiaand in patients with sickle cell anemia presenting statistically significant difference (p<0,05) compared to the healthy group.Any correlation wasn't found between the levels of homocystein and folicacid in all patients.
Conclusions. Patients with omozygous β-thalassaemia,intermedia thalassaemia and sickle cell anemia present low serum levels of homocysteine that protect vessels and decreases the risk of thrombotic events and cardiovascular diseases. Our conclusions agree with international guidelines that suggest in these patiens daily therapy with folicacid and vitamins of complex B for keeping low the levels of homocysteine and preventing thrombosis.

Haematology

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OSTEOCALCIN, A SPECIFIC MARKER OF BONE TURNOVER IN SICKLE CELL ANEMIA

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Background. Osteocalcin is a noncollagenous protein found in bone.It is secreted by osteoblasts and it is often used as an indicator for the bone formation.
Aim: To evaluated bone biochemical marker,osteocalcin in adults with sickle cell anemia (SCA). Sickle cell anemia is one of the most common hereditary haematological disorder. The bone in SCA is affected by microinfarction, osteopenia, osteoporosis, osteomyelitis, and osteonecrosis.
These complications are the major source of morbidity and highly impact on patients' quality of life.
Patients and Methods. We studied *sixteen* adults(9 men-7 women) with severe manifestations of SCA (frequent hospitalizations or need for chronic red cell transfusions) living in the area of Northern Greece. We also used an age-matched control group of 16 healthy subjects. We evaluated their bone health by measuring serum osteocalcin (BGP) with electrochemiluminescence immunoassay.
Results. The median age of the study population was 45,18 years (20-68 years). SerumlevelofBGPwas significantly lower (17,26±8,71 ng/ml vs 21,38±7,52 ng/ml,p<0,05)) compared to the healthy group.It is noticeable that lower serum BGP levels(<13 ng/ml) were observed in 37,5% of the patients.
Conclusions. 1.The patients with sickle cell anemiahavelowerboneanabolism compared to thehealthy group. 2. Osteocalcin (BGP) consists a very useful marker in the biochemical assessment of bone metabolism in patients with sickle cell anemia. These preliminary results indicate that measurements ofserum BGP might be helpful in predicting bone changes in sickle cell hemoglobinopathies and also useful in the early detection of skeletal complications of the disease.

0587

BCR-ABL/ABL RATIO INCREASE THAT CORRESPONDS TOPRESENCEOF*BCR-ABL* MUTATION IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED BY IMATINIB

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Background. Currently there is no consensus in definition what level of *BCR-ABL/ABL* ratio increase predicts presence of point mutations in *BCR-ABL* gene.
Methods. Among 531 chronic myeloid leukemia(CML) patients on imatinib, in 47 ones *BCR-ABL* mutation detection was performed. Point mutations in the *BCR-ABL* gene were detected by RT-PCR and direct sequencing. Elevation of *BCR-ABL/ABL* was calculated by dividing of *BCR-ABL/ABL* value at the time point (TP) where mutation detection was performed to the *BCR-ABL/ABL* value at TP prior to mutation screening. Threshold level was defined by ROC curve analysis. Positive and negative predictive values (PPV, NPV), sensitivity, specificity and overall correct prediction (OCP) were calculated.
Results. 10 different point mutations of *BCR-ABL* gene were detected, including 3 ones in P-loop, 2 in imatinib-binding site, 3 in A-loop, and 2 mutations outside the KD. Patients were divided into two groups: with (n=18) and without (n=29) *BCR-ABL* mutations. Groups did not differ in age, sex distribution, type of *BCR-ABL* transcript, frequency of cumulative achievement of complete hematological, complete cytogenetic, major molecular responses and level of *BCR-ABL/ABL* increase. ROC curve analysis determined that increasing of *BCR-ABL/ABL* level in 5.5-fold corresponds to 92.9% of NPV. Area under curve was 68% (95% CI 50-95%) (p=0.022). Sensitivity, PPV and OCP were relatively low (40.6%, 40.6%, 56.5%, respectively) while specificity was high (92.9%).
Conclusions. In our series increasing of *BCR-ABL/ABL* level lower than 5.5-fold clearly indicates group of CML patients that lacks the point mutations in *BCR-ABL* gene. It helps to avoid unnecessary mutation tests.

Haematology

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THE ROLE OF ERYTHROCYTE SUBSETSIN THE DIFFERENTIAL DIAGNOSIS OF MICROCYTIC ANEMIA AND THALASSEMIA SCREENING

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Background. Cell counter–based formulae have been used in the differential diagnosis of microcytic anemia.
The measurement of red cells subsets is now available on the Sysmex XE 5000 analyzer (Sysmex Corporation, Kobe, Japan). Derived from the percentages of microcytic and hypochromic red cells the authors describe the new formula
%microcytic -%hypochomic-RDW
The aim of this study was to assess the discriminant value of this index in the differential diagnosis of microcytic anemia and thalassemia screening, compared to the published England & Fraser, Eshani, Green & King, Mentzer, Ricerca, Shine & Lal, Sirdah and Srivastava indices.
Methods. the indices were calculated for a set of 370 iron deficiency anemia (IDA) patients and 350 β-thalassemia carriers. Receiver operating characteristic (ROC) curves analysis was applied.The diagnostic performance of the new index was confirmed in a set of 200 consecutive patients with microcytosis including β-thalassemia, α-thalassemia and IDA.
Results. % microcytic - % hypochromic –RDW index showed the best area under the curve (AUC, 0.997), 98.1 % sensitivity, 97.1 % specificity, cut off value >-5.1
A cut off >-7.6 provided 100% sensitivity and 92.6% specificity.Green & King index ranked second (AUC 0.990).
96 % of IDA, 100 % β-thalassemia and 95 % α-thalassemia patients were correctly classified
Conclusions. %microcytic - %hypochomic - RDW is a reliable index for differential diagnosis of microcytic anemia and β-thalassemia screening with high sensitivity and specificity. It could be a useful tool to select samples for further analysis to confirm the diagnosis of the disease.

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%HYPO-HE POTENTIAL MARKER OF IRON AVAILABILITY

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Background. This study evaluates the potential utility of %Hypo-He reported by the Sysmex XE5000 analyser in the diagnosis of iron deficiency in presence of inflammation .
Methods. Samples from 90 healthy subjects, 65 patients with CKD and 85 ACD patients receiving therapy and 91 IDA patients previous to therapy were analysed.
t test was performed to detect statistical deviations between the groups and Receiver operating characteristic (ROC) curve analysis was utilized to illustrate diagnostic performances; iron deficiency was defined by soluble transferrin receptor (sTfR)> 21 nmol/L.
The ACD patients were subdivided based on (sTfR) levels. ACD patients with sTfR higher than 21 nmol/L were considered to have iron deficiency associated (n=28) and patients with normal sTfR were considered to have functional iron deficiency (n=57).
Results. In ferropenic patients (IDA and ACD accompanied with IDA) the values obtained for %Hypo-He showed no statistical difference (p=0.5037). The differences were significant between when those groups where compared to CKD and iron sufficient ACD patients.
ROC analysis results for %Hypo-He in the detection of iron deficiency: area under curve (AUC) 0.929; at a cut off threshold 3.6 % sensitivity 91.5% and specificity 80.9 %. AUCs obtained for ferritin 0.698, iron 0.722 and MCH 0.838.
Conclusions. Comparing the results obtained by this parameter with those obtained with sTfR the reliability of %Hypo-He in distinguishing functional iron deficiency from IDA in patients with anemia of inflammation has been stated.
In conclusion, our study shows that %Hypo-He is useful for diagnosing iron deficiency.

0590

MYELOID-ANTIGEN POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA-INCIDENCE AND CYTOGENETIC CHARACTERISTICS

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Background. Leukemic cells from a significant number of adults with acute lymphoblastic leukemia (ALL) express protein antigens characteristicof both lymphoid and myeloid cells. We studied incidence and the relations of myeloid-antigen (My) expression to cytogenetic features of ALL and to outcome.
Methods. Leukemic blasts from 33 newly diagnosed adults with untreated ALL were examined for myeloid surface antigen expression.The simultaneous expression of lymphoid-associated antigens and at least one of three myeloid-associated antigens (CD33, CD13, and CD14) on cells were detected by a standard two-color direct immunofluorescent assay.
Results. My(+) ALL was established in 39% of cases, all of them B-lineage ALL. Immunologic subtyping of B-ALLs revealed an association between common B phenotype and coexpression of myeloid antigens-53% of My(+)ALL (P<0.05). Cytogenetic abnormalities that have been associated with My(+) ALLs were common, including t(9;22), 11q23 abnormalities, del 4p, and del 12p. The common ALL phenotype was associated with a high incidence of bcr-abl rearrangements - 24% of all common- B cases. Leukemic blast of one patient presented untypical for t(1;19) more mature immunophenotype with expression of CALLA and myeloid antigens. No differences in complete remission rate(P=0 .3) or 5 years survival (P=0.5) were observed between My(+) and My(-) patients expressing B-cell antigens.
Conclusions. We concluded that the higher frequency of poor prognostic cytogenetic markers in My(+) ALL may influence outcome of disease.Further studies will be required to determine the significance of karyotype abnormalities in My(+) ALL.

0591

SIGNIFICANT EFFECT OF DIFFERENCES BETWEEN ELECTROPHORETIC EXTRA- AND M-GRADIENTS ON IGA-κ- AND IGA-λ-SERUM CONCENTRATIONS IN IGA-MONOCLONAL GAMMOPATHIES

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Background. A recently promising and novel method of diagnostic relevance for monoclonal gammopathies is measurement of Immunoglobulin heavy chain-κ- and -λ-serum concentrations.
Methods. IgA-κ- and IgA-λ-serum concentrations in 19 controls and 44 patients with IgA-gammopathies and electrophoretic extra- or M-gradients divided into the subgroups (a) IgA-κ- and (b) IgA-λ-gammopathies were analyzed. To evaluate diagnostic significance compared to electrophoretic findings, κ/λ-free light chainand total IgA serum concentrations were tested.
Results. IgA-κconcentrations were elevated (11.1 g/l, controls 1.55 g/l, p<0.0001) in IgA-κ- and decreased in IgA-λ-gammopathies (0.33 g/l, p<0.0001). There is elevation of IgA-λ levels in IgA-λ- (8.37 g/l vs. 1.15 g/l, p<0.0001) and decrease in IgA-κ-gammopathies (0.31 g/l vs. 1.15 g/l, p<0.0001). Total IgA concentrations are strongly positive correlated and reveal no systematic biases with the sum of IgA-κ- and IgA-λ-levels (controls 3.02 g/l, all patients 10.8 g/l, p<0.0001; r=0.96, p<0.0001), as well as κ/λ-free light chain ratio compared with IgA-κ/IgA-λ ratio (r=0.81, p<0.0001). The electrophoretic differences of extra- and M-gradients have a stronger significant effect on the IgA-κ- and IgA-λ-concentrations compared to κ- and λ-free light chain levels in IgA-κ-gammopathies (all p<0.05). In particular, in IgA-λ-gammopathies electrophoretic findings do strongly influence the IgA-κ- (p=0.0222) and IgA-λ-levels (p=0.0055), whereas for free light chains, these differences do not reach significance (p>0.5).
Conclusions. Our data of an ongoing study reflect IgA-κ and IgA-λ in serum as reliable method able to identify specific monoclonal immunoglobulin pattern amenable for the monitoring and diagnosis of monoclonal gammopathies. For validation, larger sample sets must be analyzed in subsequent studies.

Haematology

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THE PREVALENCE OF HEMOGLOBINOPATHY IN THE WESTERN LOWLANDS OF ALBANIA

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Background. Hemoglobinopathies are inherited disorders in which mutations in or near multiple genes alter either the structure or the rate of synthesis of a particular globin chain. These are one of the most common pathologies in the Western Lowlands of Albania. The rate mortality of this disease was very high in this area. Identification of Hemoglobin disorders in the Western Lowlands of Albania.
Methods. In this study were involved 1100 outpatients. Cases were collected from subjects coming in center of hemoglobinopathie in Lushnja for B-screening ,in the period March 2008-March 2010.
The samples were analyzed for full blood count. We use Mythic 18. For determination of iron we use autoanalyser A-15. Reference value 60-175 ng / ml. Serum ferritin was determined on Elexys 2010(Roche diagnostic) .Reference value 10-106 ng / ml Hemoglobin electrophoresis was carried out in Hydrasis automatic system. The collected blood was maintained at 2-8oC, and determined within 5 days of receipt. The blood collected in heparin and K3EDTA tubes.
Results. Anemia was noted in 30% of cases examined where:
· 16% beta thalassaemia minor,
· 7% HbS trait
· 0.9% homozygote HbS
· 0.35% beta thalassaemia major
· 0.5% sickle cell beta -thalassaemia
· 0.45% thalassaemia intermedia
· 1 case is HBC.
Conclusions. Awareness of the population in these areas has significantly reduced morbidity from hemoglobinopathies. Electrophoresis plays a very important role to discover this disease.

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POST TRANSFUSION ENDOCRINE DYSFUNCTION IN MULTITRANSFUSED PATIENTS IN THALASSAMIA CENTER IN LUSHNJE, ALBANIA

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Background. Thalassemia is characterized by the reduction of synthesis of β chain of globin. This is one of the most common pathologies in the Western Lowlands of Albania. The combination of therapy and transfusion has significantly prolonged the longevity of thalassemic patients. On the other hand, frequent blood transfusions can lead to increased levels of ferritin which is associated with endocrine complications. The aim of this study was to identify the prevalence of endocrine disorders in thalasemic patient's correlation between levels of ferritin and hemoglobin, ferritin and thyroid hormone.
Methods. 101 patients that had received frequent transfusion in Hemoglobinopathies Center in Lushnje participated in this study. All patients were examined; Mythic 18 was used for complete blood count and Elexys 2010(Roche Diagnostic) was used to measure ferritinemia, TSH and FT4. The levels of ferritin and hemoglobin were followed for 1 year period October 2009 to October 2010. The blood was collected in K3EDTA and heparin tubes. Patients were divided in four groups based on their age respectively 1-13 years old, 14-24 years old, 25-44 years old and >44 years old.
Results. Group I has presented with lower Hb levels, 6.5+/-1.62 gr/dl.
There is a stronger association FE-FT4 on group III, (r=0.6), there is no associatin FE-FT4 (r=0) on group IV, FE-Hb(r=-0.67) on group III.Fe-TSH (r=0.6), on group IV.
The prevalence of hypothyreosis, is more strongly expressed on group IV(16%) whereas that of hyperthyreosis is expressed more on the patients of group I.(16%)
Conclusions. In the Western Lowlands of Albania , endocrine disorders don't correlate with the frequence of transfusions.

Haematology

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HEMOGLOBINOPATHIES AND HEMOGLOBIN ANOMALIES IN THE WESTERN REGION OF GERMANY

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Background. In Germany, no definite data exist to date on the prevalence of hemoglobinopathies and hemoglobin anomalies despite the fact that a considerable proportion of the population consists of people with immigrant background and foreigners from geographical regions with a high incidence of hemoglobinopathies and hemoglobin anomalies.
Methods. We retrospectively analyzed hemoglobin chromatographies performed at the University Hospital Bonn between 2004 and 2010. We analyzed patient data, diagnoses, laboratory test results and any additional findings as retrieved from our laboratory data base, the University Hospital's data base and laboratory request forms.
Results. Between 2004 and 2010, 439 hemoglobin chromatographies were carried out. This corresponds to 72 cases per year. In 29.28% of these hemoglobin chromatographies, findings were pathological. In total, 353 patients of foreign origin were examined and in 34.28% of these patients, hemoglobin chromatography produced pathological findings. In the 86 examined patients of German origin, 9.3% of the findings were pathological. Iron deficiency was diagnosed in 40 patients.
Conclusions. In our University Hospital, incidence of pathological hemoglobin is relatively high. The proportion of patients of non-German origin roughly corresponds with that found in the population of Germany.

Haemostasis

0595

EVALUATION OF CLOT LYSIS TIME WITH PLATELETS: CORRELATION WITH CARDIOVASCULAR RISK MARKERS

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Background. Elevated plasma clot lysis time (CLT) increases risk of venous and arterial thrombosis and reflects levels of all fibrinolytic factors except t-PA. Platelets are important for development of cardiovascular and ischemic events. Platelets contain fibrinolytic factors as plasminogen, TAFI, PAI-1, and t-PA, however, the platelets role in fibrinolysis have not been studied extensively.
Material and Methods. Our laboratory has developed a modification of the CLT which incorporates platelets as a source of Tissue Factor and phospholipid surface for assembly of coagulation factors. Our aim was to study the relationship between the CLT with platelets and markers of cardiovascular risk. We studied 100 individuals between 18 to 66 years with no history of cardiovascular events. Fibrinolytic activity was measure with resting and activated platelet. PAI-1 and usPCR were measured by ELISA. Plasma Fibrinogen and cholesterol were measured using standard laboratory methods.
Results. The CLT with activated or resting Platelets Rich Plasma (PRP) was increased compared with Platelets Free Plasma. The CLT in PRP activated with Ristocetin shows significant correlation with cholesterol (r=0.503 p< 0.0001), PAI-1(r=0.543 p< 0.0001), fibrinogen(r=0.526 p< 0.0001), usPCR (r=0.231 p=0.034) and with age (r=0.494 p< 0.0001).
Conclusions. Our CLT modification gives an accurate overview of fibrinolytic activity including the role of platelets in fibrinolysis and it might be a useful tool for the evaluation of thrombosis risk.

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EXAMINATION OF REFERENCE VALUES OF COAGULATION PARAMETERS IN PREGNANCY

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Background. Pregnancy, delivery, and puerperium are associated with many hemostatic complications as well as significant morbidity or mortality to both mother and fetus. Physiological changes that occur during pregnancy may affect biochemical parameters. However, there is no differentiation which value might be a hint for pathological changes. The aim of this study was to establish reference values for standard coagulation parameters at time going to delivery room.
Methods. We examined the coagulation parameters listed below of women with normal pregnancy (n= 1768) at time going to delivery room. All tests were performed according to the manufacturer's protocols on CA 7000, CA 1500 (Dade Behring) and AU 640 (Olympus) with reagents from Dade Behring and Trinity Biotech.
Results. After statistical analysis, we took the 2.5% and 97.5% percentile or the 95% percentile as a reference range. This resulted in the following reference ranges: thromboplastin time: 82-100%, PTT 23.0 – 30.8 s, fibrinogen 3.65 – 9.02 g/l, antithrombin 77 – 120% , D-dimer 0.4 – 2.7 µg/l, protein S 12 – 59%, protein C 74 – 161% , DRVVT ratio 0.97 – 1.37. Distribution of factor VIII activity showed two peaks at 130% and 200% and a reference range of 93 – 341%.
Conclusions. Compared with the established reference range of non-pregnant women, our results showed lower protein S activity and higher concentration or activities of D-dimer, fibrinogen and factor VIII. We think this study can help in distinguishing normal and pathological activation of the coagulation system to prevent thromboembolic complications both at the timepoint and after delivery.

Haemostasis

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DO PHYSICIANS FOLLOW SUGGESTED ALGORITHM FOR THROMBOPHILIA TESTING: OUR EXPERIENCE FOR APCR AND FVL

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Background. Two discoveries made a major advance in the laboratory assessment of thrombotic risk, an inherited form of resistance to the activated protein C (APCR) and a missense mutation in the factor V gene (FVL). Many factors, such as age, gender, pregnancy and estrogen use influence sensitivity to APC. Familiar APC resistance is predominatly caused by factor V Leiden. New, modified coagulation assays for APC resistance are highly sensitive and specific for FVL, and thus laboratory diagnostic algorithm for thrombophilia screening prefers APCR funtional test to be done before FVL DNA-based test. Functional APCR test is quicker, easier and more cost-effective than FVL genotyping.
Countinuos increase of FVL genotype requests in our laboratory last few years suggest that physicians do not follow suggested algorithm for thrombophilia screening.
Methods. Data were collected from Laboratory Information System for the period from January 2007 till December 2010.
Results. 534 FVL genotype and 22 APCR requests were recieved. Among APCR requests 7 patients had increased APCR; 2 was confirmed as heterozygots for FVL and 1 was not genotyped. One patient had normal APCR and genotyping was not performed. 512 FVL genotypes were done without prior APCR test.
Conclusions. Although there are a few reasonable conditions (eg. oral anticoagulant therapy) in which FVL genotyping is suggested rather than APCR functional assay, our observation has shown that further efforts in education of physicians should be done.

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PLASTIC VERSUS GLASS TEST TUBES FOR COAGULATION ASSAYS

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Background. Samples for coagulation testing have usually been taken into glass siliconised test tube. For better safety of laboratory personnel, plastic test tubes take place in everyday practice. This leads us to the question, whether this change could have an impact on coagulation testing due to exposure of coagulation proteins to the surface which might activate coagulation cascade.
Methods. Samples were taken in duplicate. One in glass Becton Dickinson, and another in plastic Greiner Bio-one vacuette double layer test tube. Both containing 3.2% sodium citrate. Measurements of APTT, PT, TT and fibrinogen were performed on Siemens BCS coagulation analyzer from both samples, one after another.
Results. Samples were taken from 95 patients. Passing and Bablock regression and Bland-Altman plot showed no statistically significant difference between measurements for APTT, PT and TT. Passing and Bablock regression equation for fibrinogen: y=-0.200 (CI 95%-0.200 to -0.011) +1.000 (CI 95%1.000 to 1.056)X, indicates that values are lower for 0.2g/L in plastic test tubes. With reference interval for fibrinogen of 1.8–3.5g/L, and within subject biological variation for fibrinogen of 10.7%, this is not clinically relevant for values above the upper limit of interval. For pathologically low values this difference is clinically significant. The same conclusion was made based on Bland-Altman analysis.
Conclusions. The results of this study suggest that plastic test tubes can be used instead of glass tubes. Because of lower values of fibrinogen in plastic test tubes, the clinician should be informed about such change.

Haemostasis

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MEASUREMENT OF DIRECT THROMBIN INHIBITORS: WHICH ASSAY TO USE?

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Background. Direct thrombin inhibitors (DTI) should require less monitoring, since their half life is very short. Still, when bleeding problems occur assays to determine the level of direct thrombin inhibitor remain necessary. Although being considered superior to aPTT, dedicated tests as the ecarin clotting time (ECT) are still poorly standardized.
Methods. In this study we testedthe effect of DTI's argatroban, lepirudin andbivalirudin in different concentrations with a maximumof 5mg/mL on the APTT (kaolin, Roche Stago), prothrombin time (PT, Neoplastin Plus, Roche Stago), ECT (Stago) and Hemoclot (Hyphen Biomed) on a Roche STA-R evolution.
Results. Influence of DTI's on APTT was dose dependent (linear >1mg/L) and the gradual increase of the APTT was not significantly different between DTI's. The PTwas dose dependent and showed a linear increase with increasing concentrations of DTI's. The largest increase was shown for argatroban, an intermediate increase was seen for Bivalirudin and a mild increase was observed for lepirudin. The ECT showed similar results as the PT, however with an increased sensitivity for all DTI's. Even lepirudin showed an increase upto a 5-fold of the baseline value. The Hemoclot assay showedthe highest dose dependent increase for all DTI's, without apparent differences in increase between DTI's.
Conclusions. The two dedicated DTI-monitoring assays ECT and Hemoclot show a dose-dependent linear increase with increasing DTI-concentration. Only the Hemoclot assay shows the same kinetic response to the different DTI's. Therefore, full standardisation canonly be achieved for the Hemoclot assay, irrespective of the DTI used.

0600

FIBRINOLYSIS INHIBITORS IN END STAGE RENAL DISEASE

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Background. Abnormalities of coagulation and fibrinolysis have been reported in patients with end stage renal disease (ESRD) as a risk factor for both, thrombosis complications and bleeding abnormalities. The aim of this study was to determine the level of impaired fibrinolysis inhibitors and affected platelets aggregation.
Methods. In this study 65 ESRD patients and 60 control ones were examined (age and sex matched). Blood samples were taken before the hemodialysis session. Fibrinolysis inhibitors were performed by immunochemistry methods for: antitrombin III, a_1 antitripsin and a_2 macroglobulin and platelet aggregation (in presence of ristocetin). None of the patients was given anticoagulant therapy, except the heparin during the hemodialysis.
Results. Fibrinolysis inhibitors were found decreased in ESRD patients: for antitrombin III - 0.19±0.11 g/L v.s. control group - 0.24±0.08 g/L ($p<0.05$) for a_1 antitripsin 1.92±0.5 g/L v.s. control group - 2.30±0.21 g/L ($p<0.01$) and for a_2 macroglobulin 2.26±0.9 g/L v.s. control group - 3.25±0.61 g/L ($p<0.001$). Platelet aggregation (platelet aggregates) in ESRD patients showed increased value of 17±12 % v.s. control group, 4±0.3 % ($p<0.01$).
Conclusions. The ESRD patients are exposed to prolonged hypercoagulability due to the decreased fibrinolysis and increased platelet aggregation which may lead to vascular complications followed by a higher risk of morbidity and mortality.

Haemostasis

0601

CLINICAL SIGNIFICANCE OF PLATELET INDICES (PDW, MPV, P-LCR%) IN PATIENTS WITH SEPSIS AND SYSTEMATIC INFLAMMATORY RESPONSE SYNDROME AFTER CARDIAC SURGERY

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Background. One ofthe mechanismsin the pathogenesis ofmultiorgandysfunctioninsepsisand Systemic Inflamatory Response Syndrome (SIRS)is athrombotic microangiopathy, inwhichthere isinitialactivationand consequentlyconsumptionofcoagulation factorsand platelets. As acompensatorymechanismmegakaryocytesinbone marrowproducemoreimmatureplatelets, whicharemorphologicallylarger and moreactive. There are multiple methodsfor evaluation of theplateletfunctionand its activation(ADP aggregation, flow cytometry, Pselectin), someofthemsignificantly expensive.

Most of the hematology analyzers provide information regarding platelets morphology such as platelets volume (MPV), distribution (PDW), and percentage of platelets with a volume greater than 12 fl (P-LCR%), which can be used as more cost effective alternative method for indirect assessment ofthe plateletfunction in conditions associated with thromboticmicroangiopathy. .

Methods. This prospective study included assessment of 20 patients (Group I) with SIRSand sepsiscompared to 30 patients (Group II) without evidence of SIRS and sepsis.The platelets and their indeces was analyzed with Sysmex SF-3000. As a marker of thrombotic microangiopathy was analyzed D-dimers with COBAS INTEGRA.All data are presented as mean and standart deviation (SD). Statistical comparisons of samples were performed by Student's t-test.

Results. Mean MPV, PDW and P-LCR% were higher or lower in Group I comparing to Group II. (p<0.05).

Conclusions. The analysis of theplateletsandtheirindices,especiallyP-LCR%is areliable indicator of immature platelets release and can be used as more cost effective surrogate marker for platelets activationindiseasesassociated withthromboticmicroangiopathy.

0602

ROTATION THROMBOELASTOGRAPHY FOR ASSESSMENT OF HYPERCOAGULATION IN PATIENTS WITH CARDIOVASCULAR DISEASES

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Background. Hypercoagulation is not detected in clinical practice with routinely performed blood coagulation tests. More advanced laboratory analyses to detect or monitor hypercoagulation have not yet been introduced into routine clinical management. Thromboelastography assesses the influence of plasmatic factors and platelets during all phases of haemostasis, thus permits evaluation of hypo- and hypercoagulation state.

Methods. This prospective study included assessment of 26 patients with thrombotic complications and cardiovascular diseases (II -nd group), compared with 21 healthy controls(I -st group). Haemostasis was analyzed with routine clotting tests (PT, APTT, Fibronogen, Platelets) and rotation thromboelastography (ROTEM) with measuring time to 20 min. All data are presented as mean and standart deviation (SD). Statistical comparisons of samples were performed by Student's t-test. The sensitivity and specificity ofThrombodynamic potential index(TPI) was calculated. The "cut off" point of TPI was calculated by using the Receiver operator characteristic (ROC) curves for two groops.

Results. There was significant difference (p <0.05) observed in the parameters of ROTEM: CFT, α-angle, MCF and TPI in the patient population compared to the healthy controls. No significantdifference was observed in CT (ROTEM) and routine coagulation tests when the two groups were compared.

Conclusions. Rotation thromboelastography analysis demonstrated to be a reliable method for diagnosis of hypercoagulable state. The value of TPI above 3.2 define as hypercoagulation with measuring time to 20 min.

Haemostasis

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EXPERIENCIES WITH THE ROUTINELY USE OF THE ENDOGENOUS THROMBIN POTENTIAL

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Background. Endogenous thrombin potential (ETP, area under the curve) seems to be an independent predictor of recurrent venous thrombembolism. However, the use of this parameter routinely in patients with and without anticoagulation has to be established.
Methods. So far, 662 platelet poor plasmas (314 from males, 348 from females) were analysed. Peak thrombin and ETP were determined with the Technothrombin TGA kit (Technoclone GmbH), the reagent TGA RC low and the software from the supplier. The prothrombin time (PT) was analysed with Neoplastin on STA-R Evolution analysers (Roche Diagnostics).
Results. ETP and peak thrombin were not measurable in 22 of 426 (5.2 %) samples with normal PT ($\geq$ 70 %, INR $\leq$ 1.20), in 24/80 (30 %) specimens were the PT was between 41 % and 69 % (INR > 1.20 and < 2.00) and 97/156 (62 %) samples with adequate anti-vitamin K anticoagulation (PT $\leq$ 40 %, INR $\geq$ 2.00). When considering the 2.5th and 97.5th percentile in the 404 samples with normal PT (73.1 % – 120.8 %) then both ETP (mean 3916, 1757 – 5612) and peak thrombin (mean 406 nM, 109 – 701 nM) showed much higher variations (45 – 143 % and 27 – 189 %). About two thirds of the peak thrombin variations have no analytical cause (controls). The correlation between ETP and PT overall is high (r 0.804, n 519) but much lower in samples with normal PT values (r 0.253, n 404).
Conclusions. ETP determination with the reagent TGA RC low should be used only for specimens without any anticoagulation therapy. ETP is prefered against peak thrombin as a biomarker of thrombotic risk.

0604

COMPARISON OF TWO REAGENTS MEASURING HEPARIN (UFH, LMWH)

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Background. Heparins (UFH, LMWH) are the most frequently used antithrombotic drugs. LMWH treatment does not require a laboratory control, except in some patient groups (e.g. pregnancy, chronic kidney disease). The choice of method to measure heparin level in patient plasma is a chromogenic assay based on FXa inactivation.
Methods. Our aim was to compare the IL Liquid Heparin and the Roche/Stago STA Rotachrom Heparin one stage kit (use the patient endogenous AT) and compare the stability of the samples stored for a longer time at -20°C, the effect of endogenous AT on heparin measurement.
Results. We used 100 routine samples anticoagulated with trisodium citrat (0,129M) collected before LMWH administration, and for 4 hours after the treatment. Centrifugation was made within 1 hour after sample collection, and at that point the first measurement was carried out. The plasma was stored in Eppendorf tubes at -20°C until reevaluation on the 7th and 30th days..
In low measurement range <0,2 IU/ml the results with two tests showed a remarkable deviation (r=0,883). The Roche/Stago STA Rotachrom reagent had a good analytical achievement, inter-assay CV 12,3 % at 0,245 IU/ml, error 3,4 %. In contrast the IL kit had no control in this measurement range. In a medium and high measurement range the two methods showed good correlation (r=0,977). Freezing the samples at -20°C for up 1 month had no effect on the measurements.
Conclusions. Both tests are suitable for follow-up the heparin treatment.

Haemostasis

0605

HEMOSTASIS AND CBC SHIFTS CAUSED BY ENTOTOXICOSIS AT VAGINAL DYSBIOSIS

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Background. At vaginal dysbiosis (VD) the normal vaginal microflora substitutes with gram-negative bacteria containing lipopolysaccharide (LPS). Excessive release of LPS into blood flow may lead to various clinical disorders. Blood cells and hemostasis factors are among primary targets of LPS action.
Methods. CBC and basic coagulation tests were performed together with specific methods showing level and immune response to LPS.
Results. At healthy women (n=24) serum levels of LBP and LPS anti-core IgG antibodies were 8.1 ± 1.8 µg/ml (reference level - up to 10 µg/ml) and 76.1 ± 17.8 IU/ml consequently. At confirmed VD (n=37) same parameters elevated to 19.7 ± 3.1 µg/ml and 149 ± 11 IU/ml reflecting immunityactivation. Some aPTT elongation (37.8 ± 0,7 s comparative to 32.7±2,1 s at healthy women), slight PT (14.3 ± 0.7 s and 12.8 ± 2.4 s) and fibrinogen (3.8 ± 0.2 g/l and 3.6 ± 0.2 g/l) changes were also observed. The blood picture parameters at VD remained within reference levels (RBC – 4.15 ± 0.05 ×10^{12}/l , PLT – 235.0 ± 3.8 ×10^9/l) although WBC slightly elevated (in average to 7.01 ± 0.34 ×10^9/l) . The RBC indexes (MCH, MCHC, RDW) were also within normal ranges.
Conclusions. VD not accompaniedby severe system endotoxinemia and symptpomatic intoxication does not lead to considerable changes of CBC and basic coagulation tests. Further investigations are to be performed for estimation of "cut-off" levels of endotoxinemia and specific immune response that may lead to WBC and PLT activationand reflect hemostasis.

0606

NATIVE MULTIMER ANALYSIS OF PLASMA AND PLATELET VON WILLEBRAND FACTOR (VWF) COMPARED TO DENATURING SEPARATION: IMPLICATION FOR THE INTERPRETATION OF SATELLITE BANDS

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Background. The analysis of vWF multimers by electrophoretic separation is an important laboratory tool for distinguishing the subtypes of vWD. We present Blue Native Electrophoresis as a new method to perform vWF multimer analysis and compare it to the commonly used SDS Agarose-Gel Electrophoresis.
Methods. The method described here is a 2-D Blue Native/SDS gel electrophoresis combining a first-dimensional separation of the multimeric vWF protein in its native state followed by a denaturing separation in the second dimension.
Results. The major difference between this method and the commonly used SDS-Agarose Gel Electrophoresis is the lack of satellite bands in the high-resolution native gel. In the second dimension a pattern was obtained, where each protein sub-unit from the first dimension dissociates into three distinct sub-bands. These bands confirm the triplet structure which consists of an intermediate band and two satellite bands. Our method separates the triplet structure into a higher resolution than the commonly used SDS-Agarose Gel Electrophoresis does. This helps considerably in the classification of ambiguous vWD-subtypes. The 2D- Blue Native/SDS gel electrophoresis has the additional advantage of being able to resolve the triplet structure of platelet vWF multimers, which previously has not been identified through conventional SDS-Agarose-Electrophoresis multimer analysis. This potential enables us to compare the triplet structure, from platelet and plasmatic vWF and may help to find out whether structural abnormalities concern the vWF molecule in the platelet itself, or are due to the physiological processing. of vWF shedded into circulation.
Conclusions. Due to its resolution and sensitivity, this native separation technique offers a promising tool for the classification of von Willebrand's Disease (vWD)-subtypes.

Haemostasis

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NOVEL PHOTOMETRIC ASSAY FOR QUANTIFICATION OF FACTOR XIII ACTIVITY ON AUTOMATED COAGULATION ANALYZERS*

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Background. Coagulation Factor XIII (FXIII) is essential for blood clot formation. Reduced FXIII plasma levels are associated with bleeding and impaired wound healing. Monitoring of FXIII substitution therapy requires a widespread availability of adequate diagnostic tests. Today, FXIII activity is typically quantified using a photometric method (1) that measures FXIII-dependent NADH consumption at a wavelength of 340 nm. However, the application of this method is limited to selected coagulation analyzer models, which prevents broad application of the method.

Methods. Here, we describe a prototype of a novel photometric assay for quantification of FXIII activity on virtually all automated coagulation analyzers. The assay is based on the method developed by Fickenscher et al., but NADH is replaced by the analogue Thio-NADH. Consumption of this substance can be monitored at 405 nm.

Results. The assay can be performed at 405nm, a wavelength present on virtually all coagulation analyzers. Using FXIII-deficient plasma as diluent for the calibrator, the prototype assay exhibits an improved recovery in the lower measuring range, an issue previously reported for NAD(P)H-based FXIII assays. Moreover, preliminary performance data indicate a reduced susceptibility of the assay prototype to HIL interference when compared to an NADH assay format, while maintaining an excellent correlation and standardization.

Conclusions. This preliminary assay prototype therefore has the potential to measure FXIII activity accurately on a broad range of automated coagulation analyzers.

* Under feasibility evaluation. Not available for sale.

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0608

ULTRASENSITIVE ASSAY PROTOTYPE FOR THE DETECTION OF RESIDUAL ACTIVITIES OF COAGULATION FACTORS IN DEFICIENT PLASMAS SUITABLE FOR DIAGNOSTIC AND PHARMACEUTICAL APPLICATIONS*

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Background. Functional blood coagulation assays often have a limited sensitivity and require high sample volumes. We recently described a new assay design based on the Luminescent Oxygen Channeling Immunoassay (LOCI®) technology that overcomes these limitations (EP2177624A1).

Methods. Such LOCI blood coagulation assays are based on two latex bead reagents and a biotinylated thrombin-sensitive peptide containing an epitope tag. One bead reagent (sensibead) is coated with streptavidin and contains a photosensitive dye. A second bead reagent (chemibead) is coated with an anti-epitope tag monoclonal antibody and contains a chemiluminescent dye. During an assay the three reactants combine to form a bead-aggregated immuno-complex. Illumination of the complex by light at 680 nm generates singlet oxygen from sensibeads, which channels into adjacent chemibeads, triggering a chemiluminescent reaction that is measured at 612 nm. Coagulation activation of a plasma or whole blood sample that is combined with the LOCI reactants leads to degradation of the thrombin-sensitive peptide, and a reduction in signal that is proportional to the amount of active thrombin generated.

Results. In the present study we used this setup for detection of residual FVIII or FIX activity in deficient plasmas, and found that plasmas containing 0.5 % FVIII or FXI activity can be robustly discriminated from immunodepleted plasmas. In addition, this setup also allowed the detection of deficiency in factors II, V, VII, X, and XI.

Conclusions. This ultrasensitive assay prototype may be used e.g. for detection of factor deficiency or for discrimination of moderate from severe hemophilia A or B.

* Under feasibility evaluation. Not available for sale. LOCI is a trademark of Siemens Healthcare Diagnostics

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THE INFLUENCE OF IMPAIRED PRIMARY HEMOSTASIS ON THE RESULTS OF THE INNOVANCE® PFA P2Y CARTRIDGE

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Background. The new INNOVANCE® PFA P2Y cartridge (Siemens, Marburg, Germany) was developed to monitor the inhibitory effect of ADP P2Y receptor antagonists (e.g. clopidogrel or prasugrel). However, pre-existing disorders of primary hemostasis could affect results independently from specific antiplatelet medication.
Methods. Prospectively, we measured the closure time (CT, normal < 106 s) of the new cartridge in 103 consecutive patients with assumed hemostatic disorders. We compared these results with the routine work-up for the evaluation of primary hemostasis (collagen/ADP and collagen/epinephrine PFA-100® cartridges, light transmission aggregometry, von Willebrand parameter).
Results. In 39 patients, primary hemostasis showed abnormalities. 10 of 17 patients with confirmed von Willebrand disease showed prolonged CTs in the new cartridge (detection rate 59%; collagen/ADP 82%, collagen/epinephrine 71%). 4 patients without any medication had impaired platelet aggregation: one patient with decreased epinephrine stimulation suffered from essential thrombocytosis and had prolonged CTs in the INNOVANCE® PFA P2Y and the collagen/epinephrine cartridge; one patient with decreased ADP-induced aggregation and two patients with decreased epinephrine-induced aggregation had normal CTs in all cartridges. One patient with confirmed M. Glanzmann type I had a CT >300 s in the new cartridge. 11 patients under various medications (e.g. losartan, ramipril or allopurinol) showed abnormalities in at least one of the primary hemostasis tests, four of them in the INNOVANCE® PFA P2Y cartridge.
Conclusions. Pre-existing impaired primary hemostasis can have influence on the INNOVANCE® PFA P2Y results which must be considered in the use of the cartridge.

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RAPID CHANGE OF THE FIBRIN MONOMER COMPLEX LEVEL DURING THE PERIOPERATIVE PERIOD FOR EARLY DIAGNOSIS OF VENOUS THROMBOLISM

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Background. This study was to clarify the rapid changes of the fibrin monomer complex (FMC) and D-dimer levels during the perioperative period for early diagnosis of venous thromboembolism (VTE).
Methods. The 72 patients underwent spine surgery. The FMC and D-dimer levels were measured at induction of general anesthesia, just after implantation or during surgery, immediately following surgery;,1 day, 3 days and 7 days after surgery. All were examined with duplex ultrasonography assessments of both lower extremities and with lung perfusion scintigraphy 7 days after surgery.
Results. There were no patients with clinical signs of DVT and PE, but 6 showed VTE, among whom 5 had DVT and 3 had PE. Patients with VTE had significantly higher FMC levels 1 day after surgery, compared with those without VTE (55.9 ± 17.2 µg/ml vs 11.1 ± 2.89 µg/ml; $p < 0.01$). Patients with VTE had significantly higher D-dimer levels 7 days postsurgery, compared with those without VTE (12.5 ± 2.95 µg/ml vs 4.3 ± 0.39 µg/ml; $p < 0.01$). Receiver operating characteristic analysis showed that the FMC result was more useful than the D-dimer assay for diagnosis of VTE. When the cutoff value was set to 20.8 µg/ml for FMC, sensitivity was 100% and specificity was 86.3%.In this study the prevalence of VTE after spine surgery was 8.3%.
Conclusions. The FMC measured 1 day after spine surgery is considered to be useful as an indicator of VTE.

0611

THE ALTERATION OF ANTITHROMBIN III FUNCTION DETERMINED BY CALIBRATED AUTOMATED THROMBOGRAM

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Background. The way of an estimation of functional activity of an antithrombin III by means of Calibrated Automated Thrombogram is offered. We propose to determine it as an inhibition degree of thrombin generation in conditions of activated ATIII.
Methods. We investigated citrate plasma of 20 cancer patients with the confirmed thrombosis/thromboembolism; 37 cancer patients, without thrombosis/thromboembolism.16 healthy volunteers formed the control group.A buffer solution and an ATIII activator to the two parts of investigated plasma sample were added. For each part of the sample thrombin generation was measured.Inhibition degree of thrombin generation was calculated by the next formula: [100x(ETP1-ETP2)] / ETP1, where:ETP_1 – Endogenous Thrombin Potential, measured without addition of the activator,ETP_2 – Endogenous Thrombin Potential, measured in the presence of the activator.Definition of thrombin generation test was performed on Fluoroscan Ascent (Thermo Electron Corporation). As activating ATIII agent the specific activator bound exclusively with ATIII was used.
Results. There were no differences between group's ETPs, measured without addition of activator. At calculation of inhibition degree of thrombin generation significant differences as between control group and group with the confirmed thrombosis/thromboembolism [18,5 % (15,0 have been taped; 34,8) vs. 6,2 % (2,8; 13,5); p = 0,001], and also between group of patients without thrombosis/thromboembolism and group with the confirmed thrombosis/thromboembolism [21,0 % (8,6; 37,9) vs. 6,2 % (2,8; 13,5); p = 0,014] were found.
Conclusions. We conclude that the offered method reflects the alterations of ATIII and requires further investigations.

0612

DEVELOPMENT OF REFERENCE METHODS FOR THE EVALUATION OF PLATELET RESPONSE TO ASPIRIN. THE LACK OF ASPIRIN RESISTANCE AMONG HEALTHY VOLUNTEERS

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Background. Aspirin is the most frequently used antiplatelet drug in the secondary prevention of myocardial infarction and stroke. Studies using various laboratory methods and cut-off values demonstrated Aspirin resistance in a wide range (1-45%) of patients. The aim of this study was to develop reference methods (RMs) for the detection of platelet response to Aspirin, to determine the frequency of Aspirin resistance among healthy volunteers by RMs and to evaluate other laboratory methods routinely used for the detection of Aspirin resistance.
Methods. Healthy volunteers (n=105) took 100 mg/day enteric coated Aspirin for one week. Blood samples were collected before, 24 hours and 1 week after the first dose. The response to Aspirin was detected by two RMs and by a number of routinely used assays.
Results. Two RMs were developed for detecting the effect of Aspirin. RM1 determines arachidonic acid (AA) induced thromboxane B_2 production by platelets. RM2 used monoclonal antibodies reacting with acetylated or non-acetylated cyclooxygenase1 (COX1) for establishing the state of COX1 Ser529 acetylation. None of the volunteers demonstrated Aspirin resistance by either RM. The same results were obtained by AA-induced platelet aggregation/secretion and VerifyNow Aspirin Assay. ADP, epinephrine and collagen induced aggregation/secretion and PFA-100 closure times demonstrated false positive Aspirin resistance in 3-58% of the cases.
Conclusions. Only reliable methods should be used for the detection of response to Aspirin. Aspirin resistance, if it exists, must be a rarity. The main aim of investigating the response toAspirin is to detect non-compliance and drug interference.

Haemostasis

0613

PROGRESSIVE ANTITHROMBIN ASSAY: ITS ROLE IN THE CLASSIFICATION OF ANTITHROMBIN DEFICIENCIES

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Background. Antithrombin (AT) deficiency, a severe thrombophilia, is classified as type I (decreased AT concentration) or type II (functional defect) deficiency. The latter could involve the reactive site, the heparin binding-site (HBS) or could exert a pleiotropic effect. With the exception of IIHBS deficiency, homozygous mutations are lethal and heterozygous genotype represents severe thrombosis risk. Homozygous IIHBS deficiency causes severe thrombophilia, heterozygousity represents only minor thrombosis risk. Despite its clinical importance, the diagnosis of IIHBS AT deficiency is not part of the routine laboratory work-up.
Methods. 26 IIHBS AT deficient patients (with p.Leu99Phe or p.Pro41Leu mutation), 5 patients with type I deficiency and 23 non-deficient relatives were involved in the study. A chromogenic anti-FXa assay was designed for the parallel measurement of progressive and heparin cofactor AT activity. Anti-FIIa AT activity and AT antigen were also measured.
Results. In all type IIHBS heterozygotes (n=16) the anti-FXa heparin cofactor activity was below (58.2-70.0%), while the progressive activity was above (83.9-152.3%) the lower limit of reference interval (80%). In some type IIHBS homozygotes (n=10) the progressive activity was moderately decreased, however it was very much higher (56-94%) than the heparin cofactor activity (9.4-22.7%). In non-deficient healthy individuals all AT values were above 80%. Anti-FIIa heparin cofactor assay could not detect AT deficiency in 85% of IIHBS heterozygotes and in 20% of homozygotes.
Conclusions. The parallel use of progressive and heparin cofactor anti-FXa AT assays is required for the diagnosis of IIHBS AT deficiency. Anti-FIIa assay cannot detect the majority of these deficiencies.

0614

A SPECIFIC ACQUIRED INHIBITOR DISTURBING TESTS FOR UNRELATED SINGLE CLOTTING FACTORS

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Background. An 11 years old girl was admitted to our clinic with seizures of unknown origin. Haemostaseological testing revealed a prolongation of activated partial thromboplastin time (aPTT). There was no evidence for previous bleeding episodes.
Methods. To identify the cause of aPTT prolongation, lupus anticoagulant testing, plasma mixing tests as well as measurement of single clotting factors (VIII, IX, XI, XII) have been performed.
Results. Clotting factors VIII, IX, XI, and XII were significantly diminished. In the plasma mixing test we observed an immediate aPTT inhibition. After incubation for 1 h at 37 °C this finding was essentially unchanged. Lupus anticoagulant assay was negative. In the plasma mixing test, we observed an increase of factor VIII, IX and XII activity to subnormal levels in a mixture of 1/4 (patient/normal plasma). Factor XI activity exhibited no increase in plasma mixing assays with all patient/normal plasma ratios tested. Based on the assumption of an inhibitor of factor XI, we performed a Bethesda assay. The inhibitor was quantified with 20 BE.
Conclusions. Based on the described results, we identified a strong factor XI inhibitor that resulted in false-pathological testing for clotting factors VIII, FIX and FXII.

Haemostasis

0615

DIRECT THROMBIN-INHIBITORS LEAD TO UNDERESTIMATION OF THROMBOTIC RISK BY INFLUENCING FUNCTIONAL APC-RESISTANCE TESTING

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Background. Screening for resistance against activated ProteinC (APC) is usually performed by functional coagulation tests. Recently, it was shown that emerging new treatment strategies (e.g. direct thrombin-inhibitors) for preventing thromboembolic events exert dose-dependent interferences with routine coagulation assays. We aimed at investigating the influence of clinically attainable concentrations of two direct thrombin-inhibitors, dabigatran etexilate and argatroban, on two APC-resistance tests which are in routine clinical use.
Methods. Sixteen plasma samples (wildtype: n=7, heterozygous: n=7, homozygous: n=2) were assayed in the absence or presence of either 0.12µg/ml dabigatran or 0.5µg/ml argatroban. The clinically relevant concentrations were attained by spiking the patients' samples with CE-labelled plasma calibrators containing dabigatran or argatroban. APC-resistance testing using the Hyphen Hemoclot®Quanti-V-L and Coatest® APC™ ResistanceV kits was performed on a BCS-XP analyzer (Siemens, Germany).
Results. Wild-type samples supplemented with 0.12µg/ml dabigatran or 0.5µg/ml argatroban were correctly identified in both test systems. In the Coatest® APC™ five out of seven heterozygous specimens were misclassified as indeterminate (ratio between wild-type and heterozygous), two out of seven heterozygous samples erroneously produced a wild-type result. The two homozygous samples were misclassified as heterozygous. Similar results were observed for the Hyphen Hemoclot®Quanti-V-L assay: All heterozygous samples were misclassified as indeterminate between heterozygous and wild-type, whereas the two homozygous samples produced a heterozygous result.
Conclusions. We have shown for the first time that the use of direct thrombin-inhibitors leads to underestimation of the thrombotic risk when functional assays for APC-resistance testing are used. Molecular genotyping is strongly recommended in patients under therapy with this novel class of anticoagulants.

0616

PURIFYING OF BLOOD CLOTTING FACTORS WITH THE USE OF BIOSPECIFIC CHROMATOGRAPHY ON SILICA SORBENTS FOR DIAGNOSTIC AND THERAPEUTIC USING

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Background. For the receipt of the purified preparations of clotting factors are using: blood plasma, products of its processing, and also extracts or extracellular liquids, gene engineer construct of producents. In the process of purifying of factors more frequent is using alcoholic, salt or polyethylenglycol-fractionation, chromatographic methods, anti-viral treatment.
Task. Receipt of silica chromatographic sorbents which can be suitable for an effective selection and purifying of clotting factors from blood plasma.
Methods. Synthesis of chromatographic sorbents: macroporous silica matrix Diasorb aminopropyl was modified by active triazine and by vinilsulphones dyes. A feedstock for researches were fractions II+III and III by Cohn from blood plasma. Efficiency of sorbtion and desorbtion from sorbents and also the purifyness of the received preparations was estimated after such parameters: specific activity on the hydrolysis of chromogenic peptide substrates, electrophoretic description in the PAAG-SDS-system.
Results. From 22 synthesized sorbents 10 appeared suitable for obtain and purifying of that or other factor. Due to application of various terms of sorbtion and desorbtion, and also by combination of different (after properties) sorbents it was succeed to receive the purifyed preparations of factors II, VII, IX, X in analytical researches.
Conclusions. Macroporous silica matrixes with ligands - active dyes are comfortable in the using of sorbents for the effective selection of blood clotting factors for diagnostic and therapeutic using. Technology of receipt of preparations is easily combined with the methods of anti-virus treatment (for example, solvent-detergent).

0617

THE EFFECT OF FACTOR XIII POLYMORPHISMS ON THE RISK OF MYOCARDIAL INFARCTION IN YOUNG ADULTS

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Background. Coronary artery disease (CAD) is the leading cause of death in developed countries. Previous studies have shown that polymorphisms of haemostatic proteins could influence the risk of CAD. Among factor XIII (FXIII) polymorphisms Val34Leu polymorphism of FXIII A subunit (FXIII-A) has been intensively investigated and metaanalysis of the reported data demonstrated a protective effect against myocardial infarction(MI). Most studieswere carried out on elderly patient group. Our goal was to analyze the effect of FXIII-A and FXIII B subunit (FXIII-B) polymorphismson the riskof MI in young population.
Methods. The patient population consisted of individuals who had coronary sclerosisproven by angiography and suffered MI below the age of 40 (n=109). Patients' results were compared to results obtained in an age matched control group (n=109) without coronary sclerosis. FXIII-A Val34Leu and Tyr204Phe polymorphisms and FXIII-B His95Arg and intron K nt29576C>G (IVS11+144) polymorphisms were determined by real time PCR using melting point analysis and FRET detection. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Parameters for adjustment were calculated by a logistic regression model.
Results. Among the investigated polymorphisms only intron K nt29576C>G in the FXIII-B gene influenced significantly the risk of MI in the study population. Non-adjusted OR was 0.514 (CI: 0.278-0.952). OR adjusted for smoking, HDL-cholesterol and BMI was 0.298 (CI: 0.127-0.702).
Conclusions. The intron K polymorphism, leading to a novel splice acceptor siteand a protein 15 amino acids longer at the C-terminus, provides significant protection against MI in young adults.

0618

NATURAL IGM ANTIBODIES REDUCE THE PRO-COAGULATORY EFFECT OF MICROPARTICLES IN HUMAN PLASMA

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Background. Microparticles (MP) are small membrane-derived vesicles shed by activated cells or cells undergoing apoptosis. They are present in plasma and play an important role in cardiovascular disease by promoting blood coagulation. Although tissue factor (TF) expression is important, TF-negative microparticles can also have pro-coagulatory activities.
Methods. Circulating MP (CMP) were isolated from a human plasma pool, red blood cell derived microparticles (RBMP) from a pure erythrocyte concentrate. The plasma pool was produced from 20 healthy volunteers. The natural IgM-antibodies (E06, LR01, LR04) used are directed against epitopes of oxidized LDL (oxidized phosphatidylcholine, oxidized cardiolipin, and malondialdehyde). Antibody binding to microparticles was measured with flow cytometry. The coagulation inducing properties of microparticles were evaluated by measuring the peak thrombin with a commercial thrombin generation assay (TGA) in human microparticle free plasma.
Results. We could show that E06 and LR04 bind to RBMP and E06, LR01 and LR04 bind to CMP. Furthermore, incubating these antibodies with MP at a concentration of $100\mu g.mL^{-1}$ the MP-induced peak thrombin could be reduced by 20-40% in comparison to a set up with no antibody. The addition of a control antibody which did not show any binding to MPs did not have any reducing effect on the peak thrombin.
Conclusions. We conclude that natural IgM antibodies binding MPs play a role in haemostasis and might reduce coagulability of human blood by blocking the procoagulatory effect of microparticles present in circulation.

Haemostasis

0619

MEASUREMENT OF SOLUBLE FIBRIN IN PERIPARTUM PHASE

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Background. During pregnancy levels of fibrinogen and d-dimer are elevated and are therefore not suitable markers in diagnosing thromboembolic diseases. The newly introduced soluble fibrin test is discussed to overcome this limitation.
Methods. A total of 23 women were included in this pilot study. Sample collection was done once during pregnancy (week 34-36) and twice postpartum (week 1 and week 8-12). Routine blood coagulation measurements were performed on STA-R Evolution instrument (Roche Diagnostics), soluble fibrin was tested on the Cobas 8000 system (Roche).
Results. There was no significant difference in routine coagulation testing for prothrombin time and activated partial thromboplastin time. During pregnancy fibrinogen and d-dimer showed mean levels of 5,10g/l and 1.6μg/ml respectively, and decreased significantly postpartum from 4,90g/l to 3,04g/l* and 1.7μg/ml to 0.7μg/ml*. Corresponding mean levels of soluble fibrin were found to be 42μg/ml before delivery, 42μg/ml and 24μg/ml* after delivery.
(Asterisk* indicates level of significance p< 0.05)
Conclusions. These preliminary data of an obstetric cohort with an increased thromboembolic risk show a similar dynamic range for soluble fibrin compared to the established coagulation markers. The diagnostic value in this population has to be established in further studies.

0620

DETECTION OF AN ADDITIONAL, NEWLY DETECTED POLYMORPHISM IN THE FACTOR V GENE WITH A HETEROZYGOUS FACTOR V LEIDEN GENOTYPE SHOWING A NORMAL APC-RESISTENCE TEST RESULT

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Background. A female patient showed discrepant results regarding Factor V Leiden (FVL) testing: A normal activated Protein C (APC) resistance ratio in conjunction with a genetically determined heterozygous FVL allele.
Methods. Factor V Activity (Coagulation factor V Deficient Plasma and Thromborel S, Siemens Healthcare Diagnostics) and APC Resistance (Pefakit APC-R Factor V Leiden, DSM - Pentapharm) were measured on the BCS XP coagulation system (Siemens). Sequencing was performed in two independent laboratories using 310A and 3130 sequencers employing the big Dye RR Terminator Cycle Sequencing Kit (Perkin-Elmer, ABI).
Results. In the index patient, who carried the FVL mutation, a new heterozygous missense mutation 5326G>A (Gly1718Ser) in exon 16 (FV *Graz*) was detected. Coagulation measurements gave a normal APC resistance ratio of 3.2 [cut off ≥ 3.0] and a factor V activity of 76.9% [normal range >70%]. Also a normal APC resistance ratio (3.4) and reduced factor V activity (58%) in conjunction with the heterozygous mutations for FVL and FV *Graz* were found, when testing the mother. When the father was tested, normal APC resistance ratio (3.9) and normal factor V activity (123%) were found. Sequencing revealed wild types for FVL and FV *Graz*.
Conclusions. The new missense mutation FV *Graz* seems to protect the patient with the FVL genotype from the expected APC-resistance phenotype. As routine molecular testing can miss additional functional polymorphisms, functional coagulation testing might be advantageous to get a more holistic view of the individual thromboembolic risk.

Haemostasis

0621

DETERMINATION OF THE PROCOAGULANT ACTIVITY IN PATIENTS WITH PULMONARY HYPERTENSION USING THE NEW STA®-PROCOAG-PPL KIT AND THE NEW INNOVANCE® TEST

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Background. Circulating microparticles (MP) and endogenous thrombin potential (ETP) are important mediators in thrombogenesis bypromoting procoagulant activity. In patients with pulmonaryhypertension (PH) their roles as functional participants indisease processes is poorly investigated.

Methods. A total of twenty platelet-poor plasma samples derived from patients undergoing right heart catheterization were tested, patients were grouped in PH (10) and non-PH (10). Concentration of microparticles was determined functionally using the new automated STA®-Procoag-PPL kit (Diagnostica Stago) on the STAR-Evolution instrument (RocheDiagnostics) based on the principle of measuring clotting-time.ETP was determined using thenewly introduced CE-IVD labelled Innovance®ETP test (Siemens Healthcare Diagnostics) on the BCS-XP instrument (Siemens). Results were normalized against standard human plasma concentrations and the two groups were then compared. In addition, prothrombin time (PTZ) and activated partial thromboplastin time (aPTT) were tested in all samples.

Results. When clinical samples from patients with PH were tested, a mean MP concentration of 78 seconds was found compared to 76 seconds mean concentration of MP in the non-PH group; *p=0.748*. ETP testing revealed a mean value of 19% in the PH group compared to 64% in the non-PH group; *p=0.006*. No significant differences in PTZ and aPTT were found.

Conclusions. In patients with PH a significant lower thrombin generation was found compared to non-PH patients whereas MP concentrations did not differ significantly. Larger studies to further investigate these findings are needed.

0622

THE ROLE OF ENDOGENOURS THROMBIN POTENTIAL AND PLASMA MICROPARTICLE CONCENTRATION IN OBESITY: RESULTS FROM THE STYJOBS / EDECTA STUDY

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Background. Obesity is often associated with early development of atherosclerosis and thrombembolic events. Increased potential of endogenous thrombin generation (ETP) and circulating microparticles (MP) may trigger the pathogenesis of secondary diseases in such risk patients.

Methods. Plasma samples taken from the STyrian Juvenile OBesity (STYJOBS) / Early Detection of Atherosclerosis (EDECTA) Study cohort (n=420) were tested. This study collective represents a well defined cohort of obese subjects and matched normal healthy controls (5-50 years of age). The study participants are grouped related to their atherosclerosis risk-level.

For determination of ETP the newly introduced CE-IVD labelled Innovance®ETP test (Siemens Healthcare Diagnostics) on the BCS-XP instrument (Siemens) was employed. Concentration of microparticles was determined functionally using the new automated STA®-Procoag-PPL kit (Diagnostica Stago) on the STAR-Evolution instrument (Roche Diagnostics) based on the principle of measuring clotting-time.Results obtained by the two test methods were correlated to other major risk factors for atherosclerosis.

Results. In the obese study group, ETP levels were found to be significantly higher compared to healthy controls (*p*= 0.003) and there was significant correlation with the following risk factors for atherosclerosis: Oxidized LDL, BMI, WHR and systolic blood pressure.

The PPL-based clotting time, representing MP plasma concentration, was significantly shorter compared to healthy controls (*p*=0.038) and there was significant correlation with the following risk factors: Carotis intima-media thickness, WHR, BMI and systolic blood pressure.

Conclusions. There is positive correlation of increased ETP and MP plasma levels with risk factors of atherosclerosis in obese study subjects.

Haemostasis

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COMPARISON BETWEEN HEMOSIL D-DIMER HS AND HEMOSIL D-DIMER HS 500 REAGENTS ON AN ACL TOP 500

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Background. Elevated D-dimer levels are associated with DIC and thromboembolism, but are also found in solid tumor patients including cervical, lung, prostate and colorectal carcinoma.

Methods. 63 adequate plasma samples were randomly processed for D-Dimer evaluation using DDHS and DDHS500 reagents from IL. Two groups were established based on DDHS cutoff value (278 ng/ml): ≤ 278ng/ml; >278ng/ml. Ratios were obtained considering cutoff values for each reagent DDHS (278 ng/ml) and DDHS500 (500 ng/ml).

Means and standard deviations of the ratio differences (DDHS500-DDHS) were evaluated. Student's t-test and Pearson's correlation were performed for result analysis.

Results. Mean ± SD of the differences on the different groups:
- DDHS ≤ 278ng/ml: 0,3701±0,1425
- DDHS > 278ng/ml: 3,9462±5,2969
- All samples:1,9028±3,9209

All groups showed a good Pearson´s correlation:
- DDHS ≤ 278ng/ml: 0,9038
- DDHS > 278ng/ml: 0,9894
- All Samples: 0,9910

T-test analysis:
- DDHS ≤ 278ng/ml: p<0.0001
- DDHS > 278ng/ml: p=0.0008
- All Samples: p=0.0003

12 samples evidenced values below the cutoff using DDHS but were above the cutoff for DDHS500.

Conclusions.These results suggested a consistent higher D-dimer ratio when using DDHS500 for all samples tested. T-test analysis revealed statistical difference between both reagents in all groups studied.

The existenceof 12 discrepantsamples might point to a higher specificity of the DDHS500, thus diminishing false negative Results.

0624

APPLICABILITY OF THE VASP ASSAY AND MULTIPLATE ELECTRODE AGGREGOMETRY FOR MEASUREMENT OF PLATELET AGGREGATION IN HEMODIALYSIS PATIENTS

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Background. Thrombosis is a frequent finding in patients under hemodialysis treatment and is due to increased platelet aggregation and hypercoagulability. Hemodialysis patients frequently receive platelet aggregation inhibitors for treatment and/or prevention of thromboembolic complications. Multiple electrode aggregometry (MEA) and the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay can be used to monitor antiplatelet therapy. The suitability of both platelet function tests in predialysis samples is largely unknown.

Methods. Fifteen blood samples obtained from healthy volunteers and were diluted 1:2 and 1:3 with pooled plasma samples of 25 hemodialysis patients without antiplatelet therapy. Samples were analyzed with the VASP and the MEA method after two exposure times. Blood samples obtained from healthy subjects served as control.

Results. The mean platelet reactivity index (PRI, assessed by the VASP assay) was 77.6 % in 1:2 and 78.8 % in 1:3 mixed (uremia) samples and 79.4% in 1:2 and 78.2% in 1:3 mixed (healthy) samples (P =0.117, P =0.540). Also no significant differences between 1:2 and 1:3 mixed and unmanipulated whole blood samples (P =0.130, P =0.945) were detected.

However, the results of MEA ADPtests showed significant differences in the means of simulated (healthy) samples group (56 U) and of unmanipulated whole blood group (82U) (P=0,000). This may suggest that our approach of simulating samples is not compatible for MEA.

Conclusions. Our preliminary data suggest that the VASP assay is not influenced by the presence of uremia and can be used in predialysis samples, whereas MEA needs to be investigated in further studies.

Haemostasis

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STATISTICAL ANALYSIS OF HAEMOSTATIC PARAMETERS IN MULTIPLE MYELOMA PATIENTS

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Background. Patients with multiple myeloma (MM) have an increased risk of both bleeding and thrombosis. The dominant factor of thrombosis risk is the immunomodulatory therapy. Our aim was to highlight the laboratory parametersrelevant inthrombosis risk by statistical methods in MM patients.
Methods. We investigated the parameters of thrombosis facility as well as the activation markers of coagulation in 19 untreated and 30 treated patients(median age 67 years). Tests were performed by methods of coagulation, biochip technology and molecular genetics.Besides univariate descriptive statistics we applied multivariate analysis.
Results. Comparing treated and untreated MM patients we found that von Willebrand Factor antigen and fibrin monomer levels increased significantly in both groups, while plasminogen activator inhibitor-1, D-dimer and thrombin-antithrombin complex were higher only in treated patients. We observed elevation of cytokines IL-6 in the treated group.Activation marker of platelets (PF4) was elevated in patients without treatment.
Conclusions. Our study suggests that the risk of thrombosis is increased in our treated myeloma patients. By elevation of activation markers, individual setting of antithrombotic protection may be recommended. However, larger group of patients should be analyzed to confirm this observation.

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DECIPHERING PHOSPHOTYROSINE-MEDIATED SIGNALING IN ADP-ACTIVATED HUMAN PLATELETS

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Background. Phosphorylation-dependent signal transduction regulating platelet activation and aggregation plays a crucial role in haemostasis, thrombus formation and atherothrombosis. Among several platelet activators, adenosine diphosphate (ADP) induces shape change and aggregation of platelets via P2Y1 and P2Y12 receptor signaling. ADP-dependent signal transduction in platelets is significantly governed by tyrosine phosphorylation prompting us to investigate phosphotyrosine-dependent signaling networks in activated platelets on a global phosphoproteomic scale.
Methods. Applying 38 SH2-domains as phosphotyrosine-specific probes, changes in the global state of tyrosine phosphorylation were analyzed in ADP-stimulated, human platelets by Far Western blot analysis and mass spectrometry.
Results. To gain detailed insights in phosphotyrosine-driven signaling networks, we performed global SH2-profiling after treatment of platelets with ADP in combination with the P2Y receptor inhibitors MRS and ARC (Cangrelor), respectively, and the prostacyclinanalogue Iloprost. Depending on the drug and time of treatment, we observed highly specific and reproducible SH2-domain binding patterns reflecting differential activation of tyrosine phosphorylation of signaling proteins with different dynamics. Guided by SH2-profiling, we performed selective SH2-domain pull-down assays in combination with mass spectrometry. In a first screen, we identified numerousproteins potentially involved in P2Y1- and P2Y12-regulated signaling.
Conclusions. Here we demonstrate that important biological information on the state and dynamics of phosphotyrosine-dependent signaling of platelets can be obtained by global SH2-profiling. In combination with mass spectrometry, this profiling approach may serve as a powerful strategy for the deciphering of signaling cascades and reconstruction of signaling networks in activated platelets and other phosphotyrosine-dependent, cellular signaling systems.

Haemostasis

0627

DIFFERENTIAL EXPRESSION OF PAI-1 IN NORMAL AND MALIGNANT PROSTATE CELL LINES

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Background. Up-regulation of plasminogen activator inhibitor-1 (PAI-1) impairs the pro-oncogenic action of plasminogen activation cascade. However, current data regarding the expression of PAI-1 in prostate cancer cells which differ in their invasive potentials are quite inconsistent.

Methods. In the present study we examined the expression of *PAI1* gene in normal and three prostate cancer cell lines using flow cytometry, ELISA and RT-qPCR techniques.

Results. In comparison to non-cancerous prostate epithelial cells (PrEC), PAI-1 expression is reduced in human prostate cancer cell lines. In invasive DU-145 and PC-3 cell lines, the PAI-1/β-actin mRNA ratio were 0.2% and 17% of that found in PrEC. In less invasive LNCaP cells, PAI-1 expression was reduced to 30% in comparison to PrEC. After treatment of cells with 5-aza-2´-deoxycytidine the PAI-1 mRNA level was enhanced by ~2-fold in DU-145 cells suggesting that DNA methylation is implicated in the regulation of PAI-1 expression in this cell line. Additionally, activated protein C (aPC) from human plasma dose-dependently decreased the PAI-1 activity in conditioned medium. Furthermore, both normal and malignant prostate cells were able to activate protein C (PROC) in a thrombin-dependent manner and itself to express PROC.

Conclusions. The study shows that (i) PAI-1 is differentially expressed in prostate cancer cell lines, (ii) epigenetic mechanisms such DNA hypermethylation are involved in the regulation of PAI-1 at least in DU-145 cells, and (iii) endogenous PROC expression and generation of aPC in prostate cancer cell lines may provide an additional mechanism controlling PAI-1 activities.

0628

ROTATION TROMBELASTOMETRY DETECTION OF FIBRINOLYTIC ACTIVITY

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Background. The performance of perioperative fibrinolytic monitoring during liver transplantation becomes possible with the development of the rotation thrombelastometry method ROTEM®.

Methods. Perioperative haemostatic monitoring during transplantation periods R1-R6, was performed to 30 patients undergoing orthotopic liver transplantation (13 male (42 %) and 17 female (58 %) age: 21±17 years. STA Compact (Diagnostica Stago-Roche) was used to determine PT/INR, APTT and fibrinogen. ROTEM® analyzer (Pentapharm) performed rotation thrombelastometry analyses in citrated blood and activation with thromboplastin tissue factor for EXTEM and aprotinin for APTEM.

Results. The highest percentage of patients with increased fibrinolytic activity (33,33%) and with hyperfibrinolytic activity (8,7 %) were determined during the anhepatic period (R4).16 % from the group with heavy preoperative coagulopathy displayed hyperfibrinolytic activity in the anhepatic period R4, while no patient from the moderate preoperative coagulopathy group developed hyperfibrinolysis during all perioperative periods R1-R6. During the anhepatic period (R4)the percentage of adults with increased fibrinolytic activity was significantly higher than the percentage of children (adults 41,6% and children 25%).The correlation between MCF and A15 parameters was *high* for EXTEM, INTEM and FIBTEM tests (r=0,98 p<0,001).

Conclusions. EXTEM and APTEM parameters could be used for determing the increased fibrinolytic and hyperfibrinolytic activity during the preanhepatic, anhepatic and reperfusion periods (R3, R4, R5) of liver transplantation.

Patients with considered preoperative heavy coagulopathy are more risky to develop hyperfibrinolysis during the preanhepatic and anhepatic periods (R3-R4).

The application of A15 is satisfactory for the monitoring of fibrinolytic activity.

Haemostasis

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QUANTIFICATION OF FACTOR XIII ANTIGEN ON AUTOMATED COAGULATION ANALYZERS*

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Background. Coagulation Factor XIII (FXIII) is essential for fibrin clot stabilization. Substitution therapy of patients with low plasma levels of F XIII requires diagnostic quantification of the factor before and during therapy.
Methods. Here we describe a prototype of a preliminary research immunoassay for quantification of F XIII antigen on automated coagulation analyzers*. The prototype assay is based on a monoclonal antibody to FXIII A chain, which is coupled to latex beads. FXIII in a patient sample causes agglutination of the latex particles, which can be quantified turbidimetrically.
Results. The assay prototype demonstrates a good correlation to the Berichrom® Factor XIII activity assay (r = 0.94) with 169 samples, with excellent instrument consistency (r=0.98). Coefficients of variation ranged from 2.2% CV to 3.4%. Linearity was excellent over the entire range tested (12 - 121% FXIII), and analytical sensitivity was 0.51% FXIII on BCS XP and 0.44% FXIII on CA-1500. No interference (> 10% bias) was observed with hemoglobin (up to 400 mg/dL), cholesterol (up to 300 mg/dL), bilirubin (up to 60 mg/dL) or triglycerides (up to 3,000 mg/dL) on both platforms. HAMA and RF interferences are minimized by the use of an active blocking antibody that is part of the reagent.
Conclusions. We conclude that the assay prototype has the potential for accurate determination of FXIII antigen levels in human plasma.
* Under feasibility evaluation. Not available for sale

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HEMOSTASIS ABNORMALITIES IN PATIENTS WITH GAUCHER DISEASE

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Background. Gaucher disease (GD) is a recessively inherited lysosomal storage disorder that is caused by the deficiency of the β-glucosidase. Beside the bleeding due to thrombocytopenia and clotting factors deficiencies, changes in the haemostatic system occur as one of the clinical symptoms. The aim of our study was evaluating parameters of coagulation and fibrinolysis in 26 GD patients (non-splenectomized and splenectomized) at baseline and during enzyme replacement therapy (ERT) in a period of two years.
Methods. Plasminogen (PLG) was measured with spectrophotometric method, D-dimer levels were determined with a imunoturbidimetric method, both on Siemens BCS XP System. Thrombin-antithrombin complex (TAT) and Prothrombin fragment (F_{1+2}) were measured by ELISA Siemens Healthcare Diagnostics kit.
Results. Markers for activation of coagulation (TAT and F_{1+2}) and fibrinolysis (D-dimer) were significantly elevated at baseline while PLG was within reference range. We obtained following median (range): PLG 108 (79–141%); D-dimer 187 (100–1984 µg/L); TAT 4.00 (1.900–8.60 µg/L); F_{1+2} 0.81 (0.34–6.70 nmol/L). There was no significant deference between non-splenectomized and splenectomized GD patients at baseline period. During the two years of ERT there were significant decreases of: PLG (p=0.00002, χ^2=24.82258), TAT (p=0.0000, χ^2=28.47594) and F_{1+2} (p=0.00003, χ^2=23.65263).
Conclusions. Our results indicate ongoing activation of coagulation in patients with GD at baseline, which is balanced by simultaneous activation of the fibrinolytic system. ERT partially corrected these abnormalities. Further studies are necessary to elucidate the mechanism behind this response.

Haemostasis

0631

A CLINICAL APPLICATION OF THE SCREENING SYSTEM FOR PROTEIN S TYPE II DEFICIENCY

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Background. Unlike Caucasians, a polymorphism of factor V, factor V Leiden, is not the major risk factor for venous thromboembolism (VTE) in Asian population and instead, protein S type II deficiency mainly accounts for the disease. Thus a simple, rapid and quantitative assay system for protein S has long been desired for thrombophila, especially in Asian countries.
Methods. Using a newly developed automated protein S assay system, we have measured the total protein S activity and antigen levels in plasma samples.
Results. Reference intervals (average ± 2SD) of healthy male (n = 98) were 19.3 – 32.8 µg/mL for total protein S antigen and 18.8– 32.3 µg/mL for total protein S activity, respectively, and those of female (n = 89) being 16.0 – 29.0 µg/mLand 14.8 – 28.8 µg/mL, respectively. The mean specific activity, the ratio of protein S activity to total protein S antigen level, was 0.99 for healthy individuals but was ≤ 0.69 (mean - 3SD) in those with a protein S type II deficiency and administered warfarin. The total protein S antigen level in the subject taking estrogen was significantly decreased, to 4.2 µg/ml, but the specific activity was 1.0. The protein S gene analyses revealed that three healthy subjects, with the specific activity ≤ 0.69, are heterozygous for protein S Tokushima (K155E).
Conclusions. Our automatedprotein S assay system is an effective screening tool for protein S type II deficiency. Applications of this novel system would enable early detection of protein S type II deficiency, and thereby prevention of thrombosis.

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NEW QUANTITATIVE TOTAL PROTEIN S ASSAY SYSTEM FOR DIAGNOSING OF PROTEIN S TYPE II DEFICIENCY

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Background. The incidence of venous thromboembolism (VTE) has rapidly increased in Asia due to lifestyle change and population aging. Protein S deficiency is the major risk factor for VTE in Asia. Currently available methods of protein S measurement are not precise enough for screening the deficiency.
Methods. (1)Total Protein S Activity Assay: C4bBP is removed from the protein S-C4bBP complexes in plasma samples before colorimetric measurement of total activity. The assay is comprised of three solutions, Reagent 1 (APC), Reagent 2 (FVa) and Reagent 3 (FXa, prothrombin and S-2238). (2) Total Protein S Antigen Assay: The second assay system consists of two reagents, the free protein S in samples being complexed with C4bBP, and then the amount of protein S molecules is assayed by latex agglutination using an anti-protein S monoclonal antibody. Both assays are readily applicable to automated analyzers.
Results. Total activity and content of protein S was determined on specimens from 50 healthy subjects. Correlation of our activity assay with Asserachrom® Total Protein S was excellent (r=0.93) and intra- and inter-assay C.V. were 1.6 - 4.5 % and 2.8 – 5.6 %, respectively. Similarly, correlation of our antigen assay with Asserachrom® was excellent (r = 0.97), and intra and inter-assay C.V. were 0.4 - 1.2% and 0.8 - 1.4%, respectively.
Conclusions. There are conflicting reports on how protein S is pathologically involved and part of this disagreement may result from methodology. Our new protein S assay system, especially previously unavailable activity assay, will facilitate resolving these issues and give us new insights into the pathology of thrombosis with this assay system.

Haemostasis

0633

ROLE OF THE LABORATORY IN MONITORING PATIENTS RECEIVING DOUBLE ANTIPLATELET THERAPY

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Background. Recent studies have shown that the high occurrence of resistance to aspirin and clopidogrel is rare if response to antiplatelet drugs is evaluated by specific tests. We devised a laboratory-based approach helpful to support the clinician in detecting patients at risk to develop thrombotic events.
Methods. 180 patients, in double antiplatelet therapy (aspirin and clopidogrel) after angioplasty and stent implantation, were studied by PFA100® with collagen/epinephrine (CoEPI, cut-off 165s) cartridge and by Multiplate® impedance analyzer using arachidonic acid (ASPItest, pos <862 AUC), ADP (ADPtest, pos <417 AUC) and collagen (COLtest, pos <607 AUC).
Results. Only 67 out of 173 patients with ASPI<862 displayed a prolonged CoEPI (>165s) and up to 65 patients had normal CoEPI despite ASPI<300 (75th percentile of ASPI distribution). Patients with ASPI<300 had significantly lower COL (p<0.001) than patients with ASPI>300. 138 patients displaying ADP<417 had significantly lower COL (p<0.001), but not CoEPI, than those with ADP>417. Association between COL and ADP remained after ASPI stratification. Accordingly, clopidogrel administration in patients with suboptimal (ASPI 300-892) or maximal (ASPI<300) response to aspirin increased COL positivity respectively from 9.5% to 58.8% and from 47.6% to 82.7%.
Conclusions. A combination of specific tests may be useful in identifying patients where prescribed drug interacts with its molecular target (ASPI<300 and ADP<417) or those with poor compliance or drug-resistance who potentially may benefit from therapy change. COLtest allows double antiplatelet therapy response evaluation and may be helpful in detecting patients with high residual reactivity platelet at risk to develop thrombotic events.

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PREANALYTICAL AUTOMATED PLASMA VOLUME DETERMINATION IN COAGULATION TESTING BY AUTOMATE 2500

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Background. Recognition of underfilled coagulation samples is usually done by visual inspection. This step could be automated by a plasma volume determination with a sample-processing-system. Using the AutoMate 2500 not the total sample, but the supernatant volume is measured, reflecting the citrate-plasma-ratio. In contrast to total sample volume with the citrate-plasma-ration a more reliable parameter for coagulation testing is available.
Methods. Supernatant volume detection is a specific feature of the Tube Inspection Unit of the AutoMate 2500 Family. A light beam, (halogen lamp) passes the sample tube and is detected by a Infrared sensor. In the cruor region of the tube infrared light is completely absorbed. In contrast the plasma area shows wavelength-dependent absorption. By scanning the whole tube the supernatant height is measured and its volume calculated. The accuracy of plasma volume detection was tested for the Sarstedt Coagulation Tube (3ml) containing 0.3 ml citrate.
Results. Reading through three labels is possible. The accuracy for bottom of tube and upper level between liquid and air is ±38μl. The accuracy for the lower level between serum or plasma and cruor or gel is -151μl/+76μl. For Sarstedt Coagulation tube means this a maximum error of the Serum volume of -189μl and +114μl.
Conclusions. The tested sample-processing-system allows a precise detection of supernatant volume. This is a reliable preanalytical tool for a total volume independent coagulation testing in samples with correct citrate-plasma-ratio.

Haemostasis

0635

CHANGES IN FIBRINOLYTIC PARAMETERS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION OLDER THAN 45 YEARS

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Background. Disturbances in various components of fibrinolytic system may account for development of acute myocardial infarction (AMI), and are considered as valuable prognostic factors.
Methods. Study compared fibrinogen, plasminogen, plasminogen activator inhibitor 1 (PAI-1) and factor XII levels between the group of 114 patients with AMI, older than 45 years (76 men, 38 women), and control group of 59 age-matched healthy persons (28 men, 31 women). Plasma samples were analyzed by standard methods and results were compared using Student's t-test.
Results. Patients had significantly higher fibrinogen (p=0.000) and plasminogen (p=0.000), and significantly lower PAI-1 (p=0.039) and factor XII (p=0.01) compared to healthy controls. Men with AMI had significantly higher levels of fibrinogen (p=0.000) and plasminogen (p=0.001), and significantly lower levels of PAI-1 (p=0.01), than healthy men. No significant difference in factor XII levels was observed between male patients and healthy men. Women with AIM had significantly higher fibrinogen (p=0.000) and significantly lower factor XII (p=0.036) levels, than healthy women, while there were no significant differences in plasminogen and PAI-1 levels.
Conclusions. Fibrinogen, plasminogen and factor XII levels were higher, and PAI-1 levels were lower in older patients with AMI. Significant difference was observed in plasminogen and PAI-1 levels between healthy men and men with AMI, while there is no such difference for women. Significant difference was observed in factor XII levels between healthy women and women with AMI, while there is no such difference for men. Both men and women had significant difference in fibrinogen levels between two groups.

Infectious diseases (acute and chronic)

0636

SIC11A1 AND INOS EXPRESSION IN SKIN BIOPSY OF LEPROMATOUS LEPROSY MEXICAN PATIENTS

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Background. Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae, an intracellular parasite in the monocyte-macrophage system, which cannot eliminate the bacilli effectively. Solute carrier family 11a member 1 (Slc11a1), and the inducible nitric oxide synthase (iNOS) molecules play major roles in host defense against mycobacterium. In mice, Slc11a1 transports Fe^{2+} from the cytosol to phagolysosomes and generate hydroxyl radicals with bactericidal activity and deprive bacteria of Fe^{2+}, limiting growth. In absent of Slc11a1, iNOS production is drastically diminished. iNOS catalyzes production of nitric oxide (NO) able to kill mycobacterium during early infection. However, these molecules have been scarcely studied during natural infection with M. leprae.
Methods. Slc11a1 and iNOS were studied by immunohistochemistry in skin paraffin-embedded tissues from lepromatous leprosy patients and controls.
Results. We found strong immunolabeling against both Slc11a1 and iNOS in leprosy lesions compared with healthy controls. The intensity of labeling (subjectively estimated) and the number of Slc11a1 and iNOS cells were very high in approximately 60% of the leprosy samples. Abundant intracitocytoplasmic and around vacuoles NRAMP1 and iNOS granules were observed in the macrophage. In samples with abundant bacilli, the Slc11a1 and iNOS expression were moderate and high.
Conclusions. Our observations indicate an overexpression of Slc11a1 and iNOS in cells infected by M. leprae. However, this overexpression does not appear to be sufficient to eliminate the pathogen causing of lepromatous leprosy in humans.

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PREVALENCE OF CHAGAS INFECTION IN PREGNANT WOMEN NATIVE OF SOUTH-AMERICA IN AN AREA OF NORTH-EAST SPAIN

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Background. The aim of the study was to determine antibodies against Trypanosoma cruzy in pregnant women native of South-America and to detect congenital infection in their children in a non-endemic area in north-east of Spain.
Methods. The study included screening all the pregnant women native of South-America living in our area, in order to detect antibodies against Trypanosoma cruzy.
We performed two diferent serological techniques: Indirect inmunofluorescence detecting IgG antibodies and a chromatographic immunoassay detecting IgG antibodies by using specific recombinant antigen.
We considered Trypanosoma Cruzy infection when both serological techniques were positive.
In infected women, we performed DNA amplification by the polymerasechain reaction (PCR) for specific detection of Trypanosoma cruziin the newborn.
Results. We studied 108 pregnant women coming from South-America between july 2008 and december 2010. We found 8,18% of women with Chagas disease, most of whom were born in Bolivia. We found no case of vertical transmission.
Conclusions. Since the vector transmision doesn' t exist in our community and all blood donations from donnors coming from endemic areas are screened, the mother-to-child transmision remains the most important way of Chagas disease transmision in our area.
Althoug no primary prevention is possible during pregnancy it is indeed necessary to detect the infection in order to prevent late complications of the disease and to make an attempt at breaking the chain in future pregnancies.

Infectious diseases (acute and chronic)

0638

SUPPLEMENTAL RECOMBINANT IMMUNOBLOT ASSAY (RIBA) TESTING IS NOT REQUIRED FOR ELECSYS ANTIBODY TO HEPATITIS C VIRUS (ANTI-HCV) ASSAY RESULTS WITH A CUTOFF INDEX ≥150

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Background. This study aimed to identify thecut-off index (COI) for the Elecsys anti-HCV assay that accurately predicts true positivity, thus obviating the need for supplemental RIBA testing.
Methods. Samples reactive (COI ≥1.00) using the Elecsys anti-HCV assay were retested using the Chiron RIBA HCV 3.0 Strip Immunoblot Assay. Positive percentage agreement (PPA), based on a 95% reactive agreement level between the two tests, was determined.
Results. Samples from 506 subjects initially tested on the *cobas e* 411 were retested. The PPA for samples with a COI ≥150 was 95.46 with a 95% confidence interval of 93.15–97.17 for all subjects, 94.17 (90.24–96.86) for 239 symptomatic subjects and 96.67 (93.54–98.55) for 267 asymptomatic subjects. 509 samples tested on the *cobas e* 601 were retested. The PPA for samples with a COI ≥150 was 95.28 (92.94–97.02) for all subjects, 94.64 (90.83–97.20) for 240 symptomatic and 96.87 (92.53–98.00) for 269 asymptomatic subjects. 514 samples tested on the MODULAR ANALYTICS E170 were retested. The PPA for samples with a COI ≥150 was 95.07 (92.70–96.85) for all subjects, 94.25 (90.36–96.90) for 242 symptomatic and 95.85 (92.50–97.99) for 272 asymptomatic subjects. With a COI ≥150 approximately 90% of supplementary RIBA testing can be avoided.
Conclusions. A COI ≥150 using the Elecsys anti-HCV assay is highly predictive (≥95%) of infection status as determined by supplemental RIBA testing, avoiding 90% of supplementary RIBA testing. The result was confirmed in symptomatic and asymptomatic patients and on three different Elecsys analyzers.

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GENOTYPIC PREDICTION OF HIV-1 CORECEPTOR TROPISM FROM PLASMA AND PERIPHERAL BLOOD MONONUCLEAR CELLS IN THE CLINICAL ROUTINE LABORATORY

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Background. HIV-1 infection is initiated by binding of viral particles to CD4 and coreceptor molecules, mainly CCR5 and CXCR4, on host cells. Maraviroc, which is a CCR5 antagonist to block HIV-1 from entering host cells, has been introduced into clinical practice. As Maraviroc treatment is restricted to patients harboring viruses using only CCR5 as coreceptor, tropism testing is required prior to therapy.
Methods. HIV-RNA from plasma or DNA from PBMCs wereextractedon the MagNA Pure LC instrument. RT-PCR or PCR were performed followed by sequencing with BigDye termination technology on the ABI instrument 3130. Coreceptor tropism was determined with the geno2pheno coreceptor bioinformatic on-line tool.
Results. Detection limit for sequencing from plasma was obtained at 500 RNA copies/ml. Sequencing results were obtained from all HIV-1 group M subtypes of the AREVIR reference panel and of CRF01_AE and CRF02_AG from clinical samples. Examination for accuracy and the testing of 145 plasma and 260 PBMC samples revealed reliability and robustness of the assay. Examination of 100 pairs of plasma and PBMC samples from identical collection tubes showed 91%and 95% concordance of the tropism results depending on the cut-off used within the geno2pheno bioinformatic tool for tropism prediction.
Conclusions. The new assaycovers the most frequent HIV subtypes across the world and allows testing of samples with undetectable viral loads, too. With the limitations of population based sequencing and of using a bioinformatic tool for tropism prediction, the assay was found robust, reliable and suitable for the routine clinical laboratory.

Infectious diseases (acute and chronic)

0640

PRELIMINARY QUANTITATIVE ASSESSMENT OF SERUM HBSAG LEVELS USING THE ACCESS® HBSAG ASSAY ON UNICEL® DXI 800

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Background. Quantitative measurement of serum hepatitis B surface antigen (HBsAg) has been proposed as a predictor of response to antiviral treatment.
Methods. The Access HBsAg was tested with a documented quantitative panel (10 samples from 2.04 to 0.03 ng/mL) from Etablissement Français du Sang (EFS) and the WHO 00/588 diluted (from 100 to 0.09 IU/mL). A linear regression was calculated. Linearity was studied with a diluted high-titer sample. All samples were also tested with the quantitative Abbott ARCHITECT* HBsAg assay and correlation calculation was performed.
Results. The regression calculation with the EFS quantitative panel indicated a R²= 0.9945 for Access HBsAg (the R² observed with ARCHITECT was 0.9804). Regression calculation with the diluted WHO standard showed a R² = 0.9999 up to 25 HBsAg IU/mL and 0.9813 up to 100 HBsAg IU/mL. A dilution study with HBsAg high-positive sample demonstrated excellent linearity of the Access HBsAg assay with R² = 0.9921 up to 50 ng/mL. The correlation coefficient between DxI HBsAg and Architect HBsAg ranged from 0.9898 to 0.9997 for the EFS quantitative panel and diluted (WHO, EFS high-titer) HBsAg samples.
Conclusions. These preliminary data demonstrate that the Access HBsAg assay, currently available as a qualitative assay for HBsAg, could be adapted for quantitative HBsAg determination**. Additional investigation with clinical samples should support algorithm definition and performance with this new application.
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COMPARISON OF THE ARCHITECT VCA-IGM, VCA-IGG, AND EBNA IGG PROTOTYPE ASSAYS TO THE DIASORIN LIAISON EBV PANEL

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Background. Serological EBV assay panels detecting specific IgG and IgM antibodies to viral capsid antigens (VCA) and IgG antibodies to the EBV nuclear antigens (EBNA) are routinely used as an aid in the diagnosis of primary EBV infections. In this study we compared two automated random access analyzer EBV panels regarding their ability to detect or rule out primary EBV infections.
Methods. A diagnostic population (n=519) that included 15 known acute infections was evaluated for EBV serology. All samples were tested for anti-VCA IgG, anti-VCA IgM and anti-EBNA IgG antibodies using three prototype Abbott ARCHITECT assays. Results were compared to results obtained with the respective panel of Diasorin Liaison assays. Specimens were staged as EBV negative, suspected acute, acute, transient, past infection or rated as unresolved (VCA IgG only or for EBNA IgG only reactive).
Results. Overall agreement in infection staging between the two panels was 90%. Diasorin rated 19 and ARCHITECT 16 specimens as acute infection, both detecting all 15 known acute infections. Largest disagreement between the methods resulted in the 'unresolved' group: 11% of Liaison results were rated as unresolved (2% EBNA only, 9% VCA-G only) whereas Architect rated 4% of results as VCA-G only (0% EBNA only).
Conclusions. The ARCHITECT EBV prototype assays showed a high agreement in infection staging to the corresponding Diasorin EBV assay panel. All acute infections were identified by both assay panels while the ratio of unresolved EBV serology results was lower for the ARCHITECT EBV panel.

Infectious diseases (acute and chronic)

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PROCALCITONIN IN INFANTS UNDER 3 MONTHS WITH FEVER OF UNKNOWN ORIGIN

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Background. Fever of unknown origin (FUO) is one of the main reasons for emergency consultations. In some cases there is no clear source of infection and FUO manifests with fever and non specific symptoms that may end up endangering the life of patients with bacteremia, meningitis or pyelonephritis. We evaluate the usefulness of procalcitonin (PCT), with other markers, in the interpretation of FUO.

Methods. In those infants who attended the emergency with fever we determined the following parameters: PCT (ng/mL), C reactive protein (CRP, mg/dL), total leukocyte (Cells/L) and percentage of neutrophils (%) and presence of blood, urine and spinal fluid culture positive. Statistical analysis was performed using the SPSS 15.0 for Windows using chi-square test for qualitative variables, the non-parametric Wilcoxon test for quantitative variables and COR curves.

Results. PCT median and interquartil range for infants with urine infection was 0.25 [0.11 - 0.91] and for those without infection was 0.14 [0.10 - 0.23], p = 0.0033. We also found significant differences for urine culture in leukocytes (p = 0.0002) and CRP (p = 0.0045); and for blood culture in percentage of neutrophils (p = 0.0123). We improved the PCT diagnosis ability in urinary infections by establishing a cutoff point, based on the ROC curve, in 0.190ng/mL (AUC= 0.677, Sensitivity = 69.7%, Specificity = 71.1%).

Conclusions. PCT, with leukocytes and CRP were found to be good markers in the diagnosis of urinary tract infections in infants under 3 months with FUO.

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SHIGELLA DYSENTERIAE TYPE-1, A LEADING CAUSE OF BACTERIAL HEMORRHAGIC DYSENTERY AMONG CHILDREN

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Background. Bacterial dysentery in children with bloody diarrhea and mucus is mainly caused by *shigella species*. Among them *shigella dysenteriae* type 1 is mostly endemic in Southeast Asia region. This study was aimed to find out the role of *Shigella dysentery* type-1 in bacterial hemorrhagic dysentery among children in Kathmandu, Nepal.

Methods. A prospective study was carried out from July, 2006 – October, 2008 at Om Hospital & Research Center (pvt) ltd, Nepal. 645 children (125 < 1 year and 520 < 12 years) visiting out patient department with complain of bloody diarrhoea were included in this study. Rectal swab and stool specimens were collected in sterile container. Specimens were collected before the therapy was instituted. These specimens were cultured in deferentially selective media (SS agar) and MacConkey agar plate. Culture identification, antibiotic sensitivity and serotyping were performed by following the protocol of American Society of Microbiology (ASM).

Results. Out of 645 specimens, 85 (13.17%) were bacterial isolates. Among them 71 (83.52%) were *shigella species*, followed by 9 *salmonella species*, 3 *E. coli* and 2 *campylobacter species.* Prevalence of *shigella dysentery* type 1 was 69.41% (59/85) among total bacterial isolates. 11 *shigella dysentery* type 1 were isolated from infants and 48 from children above 1 year age. Rate of infection was found to be high in summer season (May- September). 78 % shigella *dysentery* type 1 were resistant to Ampicillin, followed by 67% to chloramphenicol and 62% to tetracycline. Ciprofloxacin and nalidixic acid were most effective drug among all isolates.

Conclusions. The result shows, around 70% of bacterial hemorrhagic dysentery in children is caused by *shigella dysentery* type 1 with increasing resistant pattern to commonly used antimicrobials. Routine culture of stool specimen and antibiotic sensitivity testing are strongly recommended for empirical treatment of bacterial hemorrhagic dysentery among children.

Infectious diseases (acute and chronic)

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MICROALBUMINURIA AND URINARY RETINOL BINDING PROTEIN AS MARKERS OR SUBTLE RENAL INJURY IN VISCERAL LEISHMANIASIS: SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF THE IMMUNOTURBIDOMETRIC TECHNIQUE

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Background. Sudanese visceral leishmaniasis (VL) is a disease of children that is characterized by fever, hepatosplenomegaly, lymphadenopathy, pancytopenia, and renal injury. Microalbuminuria (MA) and urinary retinol binding protein (urRBP) are useful markers for glomerular and tubular dysfunctions, respectively.Paromomycin® (PM), an amino glycoside antibiotic that is under assessment as an alternative treatment for VL is known to be nephrotoxic. The nephrotoxicity is dose related.
Methods. We report the frequency of subtle renal affection of VL and PM treatment in 46 parasitologicaly confirmed VL patients enrolled for random treatment with different PM doses (15mg/kg /day for 28 days or 20mg/kg /day for 21 days)) in a prospective, hospital-based and comparative study. And introduce the turbidometric measurement for MA as a simple and field-based technique. Blood and urine were collected before and after treatment for hematological, biochemical profiles in addition to MA and urRBP measurement using competitive solid phase, sandwich enzyme-linked immune sorbent assay (ELISA), and immunoturbidometry.
Results. All patients (46/46; 100%) had normal serum urea and creatinine levels. more than fifty per cent of patients had pre treatment MA detected by ELISA, whereas 54% were reactive with turbidometry. 4.3% of the patients had pre urRBP detected by ELISA those patients were randomized to the 20 mg/kg/day for 21 days PM. Post treatment MA was seen in more than 80% of patients who were treated with 20 mg/kg/day for 21 days PM. While 100% of the patients who were treated with 15mg/kg/day were lost their pre-treatment reactivity. The sensitivity, specificity, positive and negative predictive values for MA turbidometric technique was calculated as 100%; 86%; 85% and 100% respectively.
Conclusions. subtle renal injury in VL is mainly glomerular. use of the 20 mg/Kg/day PM should be critically investigated before implementation in routine use. Turbidometry for MA measurement is a simple inexpensive, sensitive, and specific technique with high predictive values.

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HEPATOTOXIC EFFECTS OF VISCERAL LESHMANIASIS: CYTOCHROME C AS A MARKER OF HEPATOCYTES APOPTOSIS

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Background. Visceral leishmaniasis (VL) is an important cause of morbidity and mortality that affects multiple organs. Fever and hepato-splenomegaly are important manifestations of the disease. Hepatocytes apoptosis greatly contribute to VL morbidity. This study aimed to evaluate the extent of liver damage in patients with visceral leishmaniasis as evidenced by apoptosis and lipid metabolism derangement.
Methods. This was a prospective, hospital-based and case-control study. Following informed consent eighty patients with parasitologically confirmed VL patients and eighty apparently healthy volunteers were enrolled in the study. Serum cytochrome C was measured by ELISA technique, while the lipid profile was assessed using the A15 chemistry auto-analyzer.
Results. Cytochrome c concentrations in VL patients were significantly increased compared to apparently healthy volunteers. There was no significant change in the serum cytochrome c concentrations in patients's pre and post treatment samples. There was marked hypocholestereamia, very low serum levels of LDL and HDL. Most VL patients showed markedly increased triglycerides levels.
Conclusions. The increased serum cytochrome c in VL patients is most probably released following hepatocytes apoptosis. On the other hand, derangement in lipid metabolism in VL patients could be due to sequestration and/or degradation of lipoproteins in the enlarged liver/spleen.

Infectious diseases (acute and chronic)

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COMPARATIVE STUDIES OF THE ACCESS® HIV COMBO ASSAY

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Background. The objective of the study was to assess the performance of the Access HIV combo assay (Bio-Rad) on the Beckman Coulter Immunoassay Systems in terms of specificity and sensitivity using serum and plasma samples. The results were compared to Architect® HIV Ag/Ab Combo (Abbott) assay.
Methods. Comparison studies were performed on either Access 2 or UniCel DxI systems with 1674 selected, 518 non-selected hospital samples, 200 pregnant women, 555 positive HIV-1 / HIV-2 samples, 12 seroconversion panels and 17 early seroconversion samples. Comparative analysis was performed with the Architect Ag/Ab Combo assay.
Results. The specificity was 99.82% on selected serum samples, 99.80% on non-selected serum samples and 100% on serum samples from pregnant women. The concordance with Architect was 100%. The clinical sensitivity for all positive samples HIV-1 & HIV-2 antibodies, subtypes and variants of HIV-1 was 100% for Access 2, UniCel DxI and Architect. For seroconversion samples, the sensitivity was the same for both assays for patients at the phase of early seroconversion and better than Architect for the 12 commercial seroconversion panels.
Conclusions. The performance of the Access HIV combo assay on the Access immunoassay system family was excellent in terms of specificity and sensitivity. The agreement with the Architect HIV Ag/Ab Combo assay was 100% for the specificity and clinical sensitivity. The seroconversion sensitivity was better on UniCel DxI than on Architect. The new Access HIV combo assay is fully suited for the screening of HIV infection in diagnostic laboratories.

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PERFORMANCE OF INTERFERON GAMMA RELEASE ASSAYIN PATIENTS WITH HIV

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Background. Study evaluated the performance of Quantiferon-TB Gold Assay in HIV patients, analyzed the risk factors, CD4 count effects on QFT Results.
Methods. 124 participants were enrolled. The median age was 34±9 years, and 81 participants (65 %) were men. The median CD4 count was 262±212 /ml. 25 patients (20.2 %) had undetectable HIV-1 RNA (<50 copies/ml). Patients were classified in 4 groups according CD4 count: A) 300 /ml and more, B) 200 - 299, C) 100 - 199, D) less than 100.
Results. 8 (6.5 %) patients were positive, 103 (83 %) negative, 13 (10.5 %) had an indeterminate result. A positive and negative QFT result was not statistically associated with any of the risk factors (age › 35, male sex and CD count less than 100/ml) studied. No significant association was detectable between CD4 nadir cell counts and QFT positive or negative Results. Indeterminate results were significantly associated with low CD4count.Frequency of indeterminate tests was 21.4% (6/28) in patients with CD4 count less than 100/ml, compared with 7.7% (6/26),5.9% (1/17), and 7.1% (3/42) in those with CD4 count between 100-199, 200 - 299 and greater than 300/ml (P< .005). Overall sensitivity in patients with active tuberculosis was 57% (95%CI:0.25-0.84), specificity – 96% (95%CI:0.9-0.99), positive likelihood ratio:15.3(95%CI:4.8-48.6), negative likelihood ratio:0.45(95%CI:0.19-1.1), diagnostic OR:34.3(95%CI:5.7-207.7).
Conclusions. In HIV patients indeterminate results were caused by low positive control IFN-g. Test performance was impaired by low CD count and this may limit the potential of QFT in diagnosis of active TB.

Infectious diseases (acute and chronic)

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EVALUATION OF A NEW METHOD TO DETERMINE CORE ANTIGEN OF HEPATITIS C VIRUS AND ITS COMPARISON WITH THE DETERMINATION OF RNA-HCV

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Background. Detection of antibodies against Hepatitis C virus (HCV) and detection of RNA-HCV by PCR are the most common used methods for detecting exposure to HCV in both blood screening and diagnostic laboratories. Quantitative determination of HCV Core Antigen could be useful to substitute or complement this valoration

The aim of this study is to evaluate the utility of the quantitative determination of HCV Core Antígen by means a new automated assay and its correlation with the RNA-HCV quantification

Methods. Quantification of HCV Core Antigen was measured in 145 serum samples by a new chemiluminescent microparticle immunoassay in an Architect Autoanalyzer (Abbott Diagnostics). RNA-HCV quantification was determined in a Cobas Taqman System (Roche Diagnostics). Sensitivity and lineality was evaluated with 112 chronic hepatitis anti-HCV positive serum samples. Specificity of the method was evaluated with the study of 33 negative anti-HCV serum samples. We considered a positive HCV-Core Ag. sample those with values over 10 fmol/L

Results. We have detected HCV Core Ag. in 100 (89.5%) of the 112 RNA-HCV positive samples. All the 33 negative anti-HCV serum samples showed undetectable RNA-HCV and HCV Core Antigen values.

HCV Core Antigen levels showed a very good correlation with RNA-HCV levels: lLogRNA-HCV = 1.0449 x log HCV-Core Ag + 2.7579, R = 0.9633

Sensitivity of the method was about 89%, specificity and the positive predictive value of 100%, and the negative predicitive value of 70.4%. The price of the HCV-Core Antigen determination was 4 times less than the one for RNA-HCV.

Conclusions. HCV Core Antigen quantification by the Abbott immunoassay is a simple quick method to evaluate the HCV replicative activity. The excel.lent correlation with the RNA-HCV quantification allows it to be a perfect alternative to this determination, with a lower economic cost.

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CHRONIC EPSTEIN-BARR VIRUS INFECTION IN ASSOCIATION WITH HERPES SIMPLEX VIRUS TYPES 1 AND 2, HUMAN CYTOMEGALOVIRUS

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Background. A person may be infected with several viruses of the family *Herpesvirida,*because they have *c*ommon epidemiological patterns.

We studied patients with chronic Epstein-Barr virus (EBV) infection ($n = 345$), age (40 ± 11) years on the presence of serological markers of herpes simplex virus types 1 and 2 (HSV½) and human cytomegalovirus (CMV). Patients with various combinations of herpesviruses were compared by parameters of immunity.

Methods. We used the «EUROIMMUN» test (Germany) to detect the human IgG against HSV½, CMV in serum. The lymphocyte subpopulations were determined using «Beckman Coulter» flow cytometry (USA).

Results. Specific IgG against antigens of HSV½ and CMV were detected in 93% and 89% accordingly. EBV Monoinfection was detected in 2%. Correlation of coinfection by age ($p = 0,05$; $r = 0,47$) was found. Different structure of coinfection did not influence lymphocytes subpopulation. Spontaneous production of IFN-α was within the reference range. Spontaneous production of IFN-g in coinfected patients due to EBV+HSV½+CMV was (76 [37; 159]) pg/ml and it was higher than for patients with coinfection caused by EBV+CMV (34 [10; 59]) pg/ml and EBV+HSV½ (26 [7; 45]) pg / ml (p<0,05). Patients infected by EBV+CMV had the lowest level of induced production of IFN-g (374 [247; 385]) pg/ml (p <0,05).

Conclusions. We conclude that the patients with chronic EBV infection need an examination of serological markers of HSV½, CMV. The age is predisposing factor for coinfection. The production of IFN is various for patients with different coinfection caused by herpes viruses.

Infectious diseases (acute and chronic)

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LEISHMANIA: PROBABLE GENETIC HYBRIDDS BETWEEN SPECIES IN SUDANESE ISOLATES

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Background. The existence and the role of genetic exchange in Leishmania has been debatable for decades. The consensus is that on a population level Leishmania is essentially clonal, this does not rule out the possibility of genetic exchange. Recently,hybridization was demonstrated in the vector experimentally. Several studies reported the isolation of New World strains that have been characterized as putative hybrids between different, though closely related, Leishmania subgenus Viannia species. In the Old World, a hybrid between the closely related L. infantum and L. donovani was found. Only recently, genetic exchange between different Leishmania strains of one species was definitively demonstrated in vitro..
Methods. One hundred and sevenLeishmania donovani isolates were collected from lymph nodes/bone marrow aspirates, from patients from Sudan and Ethiopia. DNA was isolated from leishmania parasites using the High Pure PCR Template Preparation Kit. PCR products were sequenced using BigDye Terminator chemistry and analyzed using the ABI 3100 or 3730 Genetic Analyzers. Sequences were analyzed using CodonCode program (CodonCode Corporation) and MEGA. Sequences were submitted to Genbank and are accessible under accession numbers HM117696-HM117699.
Results. Evidence for hybridization between two divergent Leishmania species, L. donovani and L. major, was detected in isolates from Sudan and Ethiopia. Such hybridization may have clinical implications with respect to parasite fitness, vector adaptation and response to treatment.
Conclusions. Genetic exchange probably exists between divergent leishmania species in East Africa and may have implication in treatment.

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REDUCED NEED FOR CONFIRMATORY TESTING OF REACTIVE ROCHE ELECSYS HEPATITIS B SURFACE ANTIGEN II RESULTS

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Background. To assess if the COI values from the Roche Elecsys HBsAg II assay can reliably predict the results of confirmatory testing and thus reduce the number of samples requiring confirmatory testing.
Methods. The Roche assay uses a cutoff index or COI <0.9 to define non reactivity and the manufacturer suggests confirmation of all repeatedly reactive (COI >=0.9) samples. We presently confirm all samples with COI 0.9-20 using the Elecsys HBsAg confirmatory test based on antibody neutralisation. COI values and confirmatory results from 2 years of HBsAg testing using the Roche e601 immunoassay analyser were extracted from the laboratory database for statistical analysis using Analyse-It add-on for Microsoft Excel.
Results. From Dec 2008 to Dec 2010, 37062 HBsAg tests were performed, of which 35218 were non reactive. 187 of the remaining 1844 samples had COI 0.9-20 and underwent confirmation testing. 12 were indeterminate, 23 were non-reactive, 131 were positive and 21 were non-valid. All 47 samples with COI 6-20 were positive. ROC analysis to separate positive from other results showed a COI of 5.21 to give sensitivity of 41.7%, specificity 100%. Performing confirmatory testing only on samples with COI 0.9-6 would reduce confirmatory testing by 92.3% of all samples with COI >= 0.9 and by 24.6% in the COI 0.9-20 group.
Conclusions. Limiting confirmatory testing to samples with COI 0.9-6 can markedly reduce the number of samples requiring additional testing while avoiding false negative Results. This approach can reduce cost and improve result turnaround time.

Infectious diseases (acute and chronic)

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HOW WELL CAN THE ROCHE ELECYS HIV COMBI TEST PREDICT WESTERN BLOT FINDINGS?

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Background. Present government policy requires reactive HIV screen results to be confirmed by Western Blot analysis prior to reporting. This can cause delays and difficulties for immediate patient management (e.g. needlestick injuries). This study assesses the ability of the cutoff index (COI) of the Roche Elecsys HIV Combi assay to predict the confirmation report.
Methods. TTSH uses the Roche Elecsys HIV Combi assay on the e601 analyser. All repeatedly reactive samples (COI >=0.9) are sent to the HIV reference laboratory for confirmatory testing. Anonymised details of the HIV Combi COI and Western Blot analysis results for the last 2.5 years were extracted from the laboratory database for statistical analysis.
Results. 501 samples were sent, of which 3 were reported as inconclusive, 27 indeterminate, 113 negative and 358 positive. The mean COI (and SD) for inconclusive, indeterminate, negative and positive samples was 21.3 (30.3), 118.8 (121.2), 4.0 (6.1), 352.6 (179.6) respectively. Assuming a pretest probability of 50%, the following post-test probabilities were calculated: 100 or 95% chance of positive result if COI >= 338.1 or 180.8, 100 or 95% chance of negative result if COI <= 0.94 or 82.01, 0 or 5% chance of positive result if COI <= 58.42 or 116.30 respectively.
Conclusions. COI values from the Roche Elecsys HIV Combi assay can be used to advise clinicians on the likely Western blot result. This is useful in the acute care setting when decisions on initiation of anti-retroviral therapy cannot await the outcome of Western blot testing.

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PROCALCITONIN UTILITY IN THE DIAGNOSIS AND PROGNOSIS OF INFECTION AFTER CARDIAC SURGERY IN CHILD POPULATION

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Background. Procalcitonin (PCT) is a key marker in the diagnosis and prognosis of infections in Intensive Care Units (ICU). In patients undergoing surgery it may be useful to differenciate post-surgical systemic response from infectious complications that may occur.
Methods. 225 samples were collected from patients undergoing cardiac surgery. The following parameters were analyzed: PCT (ng/mL), C- Reactive Protein (CRP, mg/dL) and leukocytes (x1000/µL) at baseline (on admission to the unit) and 24 hours later; infection (bacteremia, endotracheal tube colonization), mortality and type of cardiac surgery performed. Depending on the kind of surgery, they have been divided into 4 groups according to the incidence of infection: (1) cyanotic heart disease;(2) with left-right circuit non-cyanotic;(3) with univentricular physiology and (4) others. Statistical analysis was performed using SPSS 15.0 for Windows using the chi-square test, non-parametric Wilcoxon test and Kruskal-Wallis, and COR curves.
Results. The median and interquartile range of the baseline PCT in patients who had bacteremia was 0.44 [0.19-2.44], in patients without sepsis was 0.16 [0.09-0.72], p = 0.002. In patients with endotracheal tube colonization was 0.48 [0.12-4.63], in patients without infection the value was 0.20 [0.09-0.81], p = 0.04. In terms of mortality, the results were 0.18 [0.09-0.81] in the alive group and 0.94 [0.28-5.91] in the deceased group, p = 0.005. We also found significant differences regarding the type of surgery, p<0.0001.
Conclusions. PCT is useful to diagnose infections and to predict the evolution of post-surgical process.

Infectious diseases (acute and chronic)

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SERUM SOLUBLE TRANSFERRIN RECEPTORS IN RELATION TO IRON STATUS IN EGYPTIAN CHRONIC VIRAL HEPATITIS C PATIENTS WITH AND WITHOUT SCHISTOSOMAL HEPATIC FIBROSIS

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Background. Egypt contains one of the highest rates of hepatitis C virus (HCV) and schistosomiasis infection in the world. Co-infected patients experience earlierdeterioration of liver functions. Iron overload can damage the liver when combined with chronic viral hepatitis, whereas iron deficiency could occur due to bleeding, haemodilution, hypersplenism… Serum soluble transferrin receptors (sTfR) are a promising tool for detecting iron deficiency. This study aimed at evaluation of sTfR in relation to other iron status parameters in chronic HCV patients with and without schistosomal hepatic fibrosis (SHF).
Methods. 61 subjects were included in this study: 16 HCV, 12 SHF, 18 coinfected patients with HCV and SHF and 15 controls. Serum iron (SI), total iron binding capacity (TIBC) were assessed colorimetrically, transferrin immuno-nephelometrically , ferritin and sTfR by ELISA and erythropoietin by CLIA.
Results. SI and ferritin didnot differ among the studied groups while TIBC was lower in patients and was lowest in the coinfected group (mean +/- S D 215.61+/- 51.03 vs 299.67+/- 23.37 ug /dl for controls). The same was found for transferrin (1.7+/- 0.47 vs 2.68 +/- 0.75 g/l for coinfected and control groups respectively). sTfR, sTfR/ferritin index and erythropoietin were lowest in HCV patients compared to all other subjects(sTfR 0.59+/-0.41vs 1.22+/-0.3mg/l, sTfR/ferritin 0.24+/-0.16 vs 0.58+/-0.12, erythropoietin 7.94+/-3.2 vs 11.28 vs 0.95 mIU/ml in HCV patients and controls respectively).
Conclusions. Lower sTfR in HCV patients may be attributed to the decrease in bone marrow activity, this is supported by the lower erythropoietin levels in these patients.

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SIMILAR LEVELS OF INFLAMMATORY MARKERS IN NON-NEUTROPENIC PATIENTSWITH CANDIDEMIA AND BACTEREMIA

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Background. Fever of bacterial and fungal origine cannot be distinguished clinically.
Methods. From 2006 to 2010, at a single secondary care hospital, 9299 blood cultures were analyzed. Subgroups of 25 episodes of candidemia and 20 cases of bacteremia in febrile non-neutropenic patients were compared retrospectively. A total of 188 serum samples, drawn before, at, and after the onset of fever, was examined for C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), and lipopolysaccharide binding protein (LBP).
Results. The prevalence of*Candida* spp. among blood isolates was 2.2% (45/2053) with *Candida albicans* comprising 53.3%, *Candida glabrata* 20.0%, *Candida parapsilosis* 15.6%, and others 11.0%. Candidemia patients needed more intensive care (16.6 vs. 4.4 days, p=0.02) and had a higher mortality (40 vs. 5%, p=0.007) than bacteremia patients. Age, sex, underlying disease, and hospital time matched well (p>0.05). At the onset of fever, patients with candidemia and bacteremia had similar, mean elevated levels of body temperatures (38.5 and 38.8°C, p=0,28), leukocytes (13.8 and 15.1Gpt/L, p=0,64), CRP(179 and 157mg/ml, p=0.43), PCT (1.6 and 11.0ng/ml, p=0.12), IL-6 (394 and 550pg/ml, p=0.56), and LBP (28.4 and 28.8 ng/ml, p=0.93). Furthermore, evaluations of maximum values including three cut-offs for PCT (0.5, 2.2, and 5.5 ng/ml) and of all samples failed to disclose any significant differences between the two subgroups (p>0.05 for all the markers).
Conclusions. There was a trend towards higher PCT levels in bacteremia. The levels of fever, leukocytosis, CRP, IL-6, and LBP were similar in candidemic and bacteremic febrile non-neutropenic patients.

Infectious diseases (acute and chronic)

0656

HIGHLY EXPRESSION OF S1 SUBUNIT OF PERTUSSIS TOXIN IN *E.COLI*

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Background. *Bordetella pertussis* is a Gram-negative bacterium causing the respiratory disease whooping cough. Pertussis toxin is the main component of current acellular pertussis vaccine and the S1 subunit is the most important immunogenic part of it. Thus, S1 has been the target of many studies as a useful candidate component of acellular vaccine against *Bordetella pertussis*, lacking the side effects of whole cell based vaccine.
Methods. S1 gene was amplified and inserted in a few expression vectors. The inserted gene was controlled with different Methods. The possibility and level of expression of these vectors were examined in BL21 (DE3) strain of *E.coli* as expression cell.
Results. The best result was with pET-22b (+)-rS1 as vector. The highest level of expression achieved 6 hours post induction with 0.2 mM IPTG in LB broth with shacking in 250 rpm. Recombinant S1 was observed in two distinct separated proteins with 28 and 31 KD estimated molecular weight. These proteins after of purification by nickel column were confirmed by western blot analyze with monoclonal antibodies against pertussis toxin.
Conclusions. S1 is the major subunit of pertussis toxin. In spite of the high level PT and S1 toxicity in the *E.coli*, a high amount of S1 was expressed in *E.coli*. Production of rS1 as two different SDS-PAGE bands could be the result of leader peptidase or nonspecific peptidase enzymes of *E.coli* activity on recombinant S1. The recombinant protein could
be used as a suitable candidate component of acellular vaccine or as an antigen for detection of *Bordetella pertussis*.

0657

EVALUATION OF THE NEW VITROS® HIV COMBO ASSAY (IN DEVELOPMENT) IN A CLINICAL LABORATORY

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Background. Fourth generation immunoassays designed to detect simultaneously HIV p24 antigen and antibodies have become the new standards for detecting acute and chronic HIV infection. This study was performed to assess the performance of the VITROS HIV Combo assay in a clinical setting.
Methods. Antibody detection in the VITROS assay is achieved using recombinant transmembrane envelope proteins for HIV-1, HIV-2 and HIV-1 Group O. p24 antigen is detected using monoclonal antibodies (MAbs) against HIV-1 p24. Biotinylated envelope proteins and MAb, bound to the microwells coated with streptavidin, are used in the first step to capture antibodies to HIV or p24 antigen in the sample. HRP conjugates of the envelope proteins and anti-p24 MAbs are employed in the second step. After a final wash bound HRP conjugate is measured by a luminescent reaction.
Results. Specificity assessed by using 997 blood donor samples and 100 clinical samples, was 99.60% for the blood donors and 100.0% for the clinical samples. 80 HIV-1 and 5 HIV-2 positive samples were all positive. Inter-assay precision ranged from 2.5 to 4.3% for the various reactivities. When tested on 7commercially available seroconversion panels the VITROS assay was positive an average of 7.6 days before a 3rd generation assay. The lower limit of detection of p24 antigen was 0.83 IU /ml against the WHO international standard. The assay was reactive with samples containing p24 antigen from various HIV-1 subtypes.
Conclusions. The VITROS HIV Combo assay demonstrated excellent performances for identifying infections with various HIV-1 and HIV-2 strains.

Infectious diseases (acute and chronic)

0658

PERFORMANCE OF THE VITROS® HIV COMBO ASSAY (IN DEVELOPMENT) ON THE VITROS ECI, VITROS 5600 AND VITROS 3600 SYSTEMS

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Background. To assess the performance of the VITROS HIV Combo assay across the VITROS ECi Immunodiagnostics, the VITROS 5600 Integrated and VITROS 3600 Immunodiagnostics Systems. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection and is designed for use on any of the VITROS ECi, 5600 or 3600 systems.
Methods. The following areas of performance of the HIV Combo assay were tested across the 3 VITROS systems: specificity using 2098 random blood donor samples; serocoversion sensitivity using 11 commercially available panels; Ab and Ag sensitivity using commercially available and internal panels; sensitivity using 100 anti-HIV-1, 50 anti-HIV-2, 5 anti-HIV-1 Group O and 5 p24 known positive samples and precision performance.
Results. Specificity performance across the 3 systems was equivalent and was 99.90%. Seroconversion sensitivity was also equivalent across systems and positive an average of 6 days before a 3rd generation assay (the VITROS Anti-HIV 1+2 assay). HIV-1 p24 Ag and anti-HIV Ab dilutional sensitivity was equivalent across the 3 systems and matched or, for some reactivities, exceeded another 4th generation HIV assay. All of the known positive samples were positive in the VITROS HIV Combo assay and had equivalent results across the 3 systems. Precision of the assay across the 3 systems ranged from 1.1 to 4.4% for the various reactivities.
Conclusions. The VITROS HIV Combo assay has equivalent performance across the VITROS ECi, VITROS 5600 and VITROS 3600 systems.

0659

MUTUAL CONNECTION BETWEEN PROTEINS ACUTE PHASE IN BACTERIAL INFECTIONS

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Background. Infection is the presence and propagation of infectious agents in the human body if we speak about presence of the agent bacteria then it is bacterial infection. If infectious agents are enough pathogenic, toxic and invasive and act as triggering factors and lead to the synthesis of acute phase proteins, whose concentration changes during the disease.
Methods. In the group of infected patients of both sexes with overt form of bacterial infection (n=55), with age group of $\overline{X}$age = 55.6±17.4 years were measured concentration of: C-reactive protein (CRP, turbydimetry), fibrinogen (Fb, Clauss metodh), erythrocyte sedimentation rate (SE, Westergreen), leukocytes (Le), neutrophiles (%) at parts diff. analyzer.
Results. In the examined group of acute phase all reactants are statistically significantly differ from those in the control group (n=28). Bacterial infections are manifested by a marked increase of CRP ($\overline{X}$=66.03±59.7mg/L), accelerated SE ($\overline{X}$=38.22±25.3 mm/1h), wich arise due to changes in dielectric properties of plasma and leads to their aggregation, leukocytosis ($>11 \times 10^9$/L) as a consequence of expressed neutrophils ($\overline{X}$=80.41±5.4%). Nonparametric treatment of values in the group of examined patients, showed a significant correlation of CRP and Fb (t=0.15;p=0.26), CRP and neutrophils (t=0.31;p=0.022). Fb was significantly correlated with SE (t=0.5;p=0.002).
Conclusions. CRP is a good diagnostic and prognostic indicator of infection and it has a high sensitivity. It is considered the more sensitive parameter than the number of leukocytes and agility of erythrocyte sedimentation rate, due to the faster growing concentration and faster decline in recovery. It is mandatory to check the value of CRP in suspected infection.

Infectious diseases (acute and chronic)

0660

EVALUATION OF TREPONEMA PALLIDUM IMMUNOBLOT AND ITS REACTIVITY PATTERN IN ROUTINE CONFIRMATION TESTING FOR SYPHILIS

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Background. Immunoblots have been introduced for confirmation of syphilis screening tests. Reactivity pattern in routine sera is of interest, to reevaluate blot criteria, and for antigen composition of new screening tests.
Methods. *Treponema pallidum* LINE (Virotech) blots for IgG and IgM with recombinant antigens 47kDa, 44.5kDa (TmpA), 17kDa, and 15kDa, were used to confirm positive SERODIA TPPA (Fujirebio) screened sera, followed by VDRL (Siemens) and IgM ELISA (Euroimmun).
Results. 456 of 89054 sera were TPPA reactive (0.51%), 444 underwent full diagnostic algorithm. 387 sera revealed positive IgG blot (87.2%) of which 41 also had positive IgM blots. 5 positive IgM blots were seen for 18 equivocal and 39 negative IgG blots. Therefore syphilis for a total of 492 sera (88.3%) was confirmed. IgM ELISA gave positive values for 36/46 positive, 2/16 equivocal, and 6/340 negative IgM blot sera. 177 of 444 sera were VDRL positive (39.9%). All 18 IgG equivocal sera showed 17kDa IgG together with one other IgG specificity: 13 15kDa, 3 TmpA, 2 47kDa. 15 IgG negative/IgM blot equivocal sera showed IgM single reactivity: 8 15kDa, 5 47kDa, 1 TmpA, 1 17kDa. From 21 IgG/IgM negative blots 9 showed no reactivity, 1 15 kDa IgM, 5 15kDa IgG, 4 17kDa IgG, and 2 47kDa IgG. Isolated IgM for 15kDa and 47kDa without subsequent seroconversion was observed.
Conclusions. Results point onto high sensitivity for 17kDa antigen. Revision of blot criteria to "positive" for 17kDa IgG in conjunction with other reactivity, and "equivocal" for isolated 17kDa reactivity, is proposed.

0661

DIAGNOSTIC USEFULNESS OF C-REACTIVE PROTEIN IN DIFFERENTIATION OF PLEURAL EFFUSIONS

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Background. Classification of pleural effusions(PE) into exudative(E) and transudative(T) is currently based upon Light's criteria. In order to achieve a specific diagnosis, especially in the E effusion group, several biochemical test have recently been applied. C-reactive protein (CRP) is an acute phase protein widely used as a marker of inflammation and tissue injury.
Aim: To investigate the diagnostic usefulness of pleural fluid CRP levels (CRPp) and pleural fluid to serum CRP ratio (CRPp/s) for differentiating E from T pleural effusions and to distinguish inflammatory PE from other types of effusion.
Methods. We analysed 58 patients with PE who where classified as E(45) and T (13) by Light's criteria. Pleural fluid and serum CRP levels were measured using an immunoturbidimetric method on Olympus AU-400 autoanalyser.
Results. Our results showed significant increase of CRPp level and CRP p/s ratio in E(35.18±4.57 and 0.58±0.18) in comparison with T(14.37±4.77and 0.31±0.05), p< 0.001. In E group: 16 patients had malignant effusions, 9 tuberculous pleurisy, 18 parapneumonic effusion, and 2 undetermined. The CRPp/s ratio was significanthly higher in the parapneumonic effusion subgroup(0.69±0.09) then in the malignant subgroup (0.37±0.16), p< 0.01. Using cut-off value > 10 mg/L for CRPp and 0.26 for CRPp/s ratio (sensitivity 82% and specificity 87.5% and positive predictive value 95.5%) effectively separated E from T.
Conclusions. Based on these results we can conclude that determination of CRP may be useful in differentiation of pleural effusion into E and T. In the differential diagnosis of PE, higher CRP levels may prove to be a rapid, practical and accurate method of differentiating parapneumonic effusions from other E types.

Infectious diseases (acute and chronic)

0662

CORRELATION BETWEEN LOW PCT SERUM CONCENTRATIONS AND POSITIVE GROWTH CULTURES

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Background. Procalcitonin (PCT) is a polypeptide which increases in serum in response to proinflammatory stimuli produced by bacterium infections. PCT levels under 0.5 ng/mL suggest a very low risk of progressing to an acute systemic infection (sepsis or septic shock). However, these values are possible in localized infections and during the beginning of the sepsis.
Methods. We studied 463 patients having at least one PCT (Brahms) determination, which was measured in Cobas e411 (Roche Diagnostics). C-reactive protein (CRP) values were also considered. Microbiological culture results for each patient were checked. We focused on patients with PCT<0.5 ng/mL and positive growth in blood culture.
Results. First, we found 40 cases (8.6%) with PCT<0.5 ng/mL and one positive non-blood culture, corresponding to patients with localized infections, and 26 cases (5.6%) with PCT<0.5 ng/mL and one positive blood culture. These were classified into three groups according to data in medical record, culture contamination (18 cases), infection without septicaemia (6 cases) and clinical criteria of sepsis (2 cases). Most contaminations were caused by coagulase negative *Staphylococcus*, especially *S. epidermidis* (13 cases). Strangely, a new PCT determination was not requested for the two patients who developed sepsis. Finally, we observed high CRP concentrations (>8 mg/L) in the 8 patients with infection or sepsis, while normal PCR values were present in 5 patients of blood culture contamination group.
Conclusions. Our data suggest that PCT is not useful for managing local infections and that a positive blood culture with PCT<0.5 ng/mL is normally due to a contamination.

0663

STUDY ON ANTIBICROBIOL ACTIVITY OF HUMAN HEMOGLOBIN AND ITS FRAGMENTS BOTH IN VITRO AND IN VIVO

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Background. Here we isolated human hemoglobin and its fragments, compared their antimicrobial activity in vitro and researched their antimicrobial activity in vivo.
Methods. The α and β chains of hemoglobin were separated by cation exchange chromatography and gel chromatography; and then cleaved by cyanogens bromide respectively. The cleaved fragments were purified by reverse phase high performance liquid chromatography, and antimicrobial activity of hemoglobin and its fragments was determined by agrose radial diffusion assay. After establishment of E. coli vaginal infection model, the rats were randomized into the experimental group (hemoglobin group) and the control group. The histologically pathological section was observed.
Results. Hemoglobin, α/β chain and their fragments had similar antibacterial activities in vitro, which were mainly against Gram-negative bacteria E. coli.,butα1-32 had a comparatively lower antimicrobial activity in vivo.
A comparison between the hemoglobin group and the matrix control group (no treatment after infection) was executed, and the surface layer of vaginal stratified squamous epithelium was smoother, inflammatory cells were significantly reduced in the lamina propria and congestion was obviously decreased.
Conclusions. Human hemoglobin and its fragments had antibacterial activity in vitro, and hemoglobin might relieve the inflammation of E. coli vaginal infection in rats moreover.

Infectious diseases (acute and chronic)

0664

COMPLEX MUTATIONS IN S GENE AND REVERSE TRANSCRIPTASE REGION RELATED TO COEXISTENCE OF HEPATITIS B SURFACE ANTIGEN AND ANTI-HBS ANTIBODIES IN CHINESE CHRONIS HEPATITIS B VIRUS PATIENTS

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Background. We aimed to determine the prevalence of the coexistenceof HBsAg and anti-HBsAb, analyze the clinical and virological features, includingaa pattern of S gene and reverse transcriptase (RT) region in Chinese CHB patients.
Methods. 54CHB patients concomitantly carried both HBsAg and anti-HBs were test and sequences have beenobtained from 52 of them and48 from the control group. S gene and RT region sequences were amplified and sequencedusing in-houseprotocols.
Results. There was no significant difference between patientswith and without anti-HBsAb regard to age, gender, alanineaminotransferase level, HBeAg positive proportion and HBcAbpositive proportion. The proportion of anti-HBeAb positive (p=0.027) and Genotype C (p=0.001) is significantly morefrequent inHBsAg+/anti-HBsAb+ individuals. In S gene, the number of mutated residues in the HBsAg+/anti-HBsAb+groupwas markedly increased than in control patients(1.88 versus 1.02 substitutions per 100 amino acids,p=0.022). The amino acid exchange occurredmostly within the N-terminal region(2.15 versus 0.87 substitutionsper 100 amino acids, p=0.023) and the"a" determinant(3.61 versus 1.56 substitutions per 100 aminoacids,p =0.049) in two groups.In the RT region, the meannumber of substitution per 100 aa showed a tendency to besignificantly higher in HBsAg+/anti-HBsAb+ patients than incontrols (2.34 versus 1.46, p=0.04).
Conclusions. This study has shown a prevalence of anti-HBsAb coexistence in HBsAg-positivepatients in Chinese CHB population. We observed anincreased aa variability of HBV strains within both HBsAg andRT involving functionally important regions of those proteins.

0665

***LEGIONELLA SPP.* IN 8 HOTELS OF ALBUFEIRA, ALGARVE, PORTUGAL**

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Background. Legionella spp. is a frequent event in touristic accommodations, been responsible for many cases of legionnaire disease. Albufeira is a high tourist activity district of Algarve, were has been reported Legionella outbreaks.
Methods. Eight hotels were studied from 2003 to 2007. Samples from all water systems were collected. The possible relation between the presence of Legionella spp. and local of sample collection, season of the year, water temperature and free residual chlorine, was analyzed in 996 samples. Descriptive analysis and Chi-square tests were made.
Results. Legionella spp. was detected in 9% of the samples, being present in the water systems of 7 hotels; Legionella pneumophila was the predominant specie (95%), the type 1 strains were identified in 42% of the contaminated samples. The presence of Legionella spp ranged from 11.1% (2003) to 7.9% (2005) in the samples analyzed during all over the year and was related with water temperature (P = 0.026), water free residual chlorine (P = 0.003) and with the local of collection (P = 0,000); The showers and the hot water reservoir were the most frequently contaminated locals (11% and 21%).
Conclusions. The risk of legionnaire disease can be avoided with a control programme for water systems.

Infectious diseases (acute and chronic)

0666

PROCALCITONIN AND C-REACTIVE PROTEIN IN PATIENTS WITH LOCAL AND SYSTEMIC INFECTIOUS DISEASES

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Background. Procalcitonin (PCT) and C-reactive protein (CRP) are acute phase proteins, useful for differentiation of bacterial and viral, local and systemic infectious diseases.
Aim. Compare and correlate PCT and CRP in local and systemic infectious diseases in our clinical setting.
Methods. PCT and CRP were determined in sera of 113 patients first 24 hours after admission to our Hospital. Patients were divided into groups on the basis of clinical and microbiological findings. PCT was measured with electrochemiluminescence on a Elecsys 2010, Hitachi, Roche Diagnostics, and CRP turbidimetrically on a Beckman Coulter AU640 analyzer. Statistical analysis was done with SigmaStat.
Results. PCT and CRP values showed a significant difference between groups (ANOVA on ranks, post Dunn's test, p<0,001). PCT and CRP values (median, 25th-75th percentile) were as follow: sepsis group (n=11) 11,09 (1,82-27,12) µg/L; 135,6 (98,6-177,6) mg/L; bacterial pneumonia (n=20) 1,94 (0,21-9,12) µg/L; 104 (65,9-137,4) mg/L; adenovirosis (n=17) 0,31 (0,23-0,47) µg/L; 49,7 (33,5-87,3) mg/L; gastroenterocolitis (ROTA +, n=19) 0,16 (0,09-0,31) µg/L; 10,0 (3,7-15,7) mg/L; bacteriemia (n=10) 2,00 (0,44-2,86) µg/L; 110,0 (45,5-145,0) mg/L; acute bronchiolitis and respiratory catarrh (n=28) 0,22 (0,07-0,42) µg/L; 15,9 (4,6-52,2) mg/L and acute pyelonephritis (n=8) 1,10 (0,15-2,98) µg/L; 94,3 (41,4-118,1) mg/L. Spearman's coefficient of rank correlation for PCT and CRP was 0,741 (95%CI 0,642 – 0,816, p<0,0001).
Conclusions. PCT is a very useful marker as an aid in early diagnosis of sepsis, bacteriemia, pneumonia and acute pyelonephritis in our clinical setting. In some infections like upper respiratory tract infections PCT remains low, while CRP is increased.

0667

DIAGNOSTIC VALUE OF SERUM AND BRONCHOALVEOLAR LAVAGE FLUID LEVELS OF PROCALCITONIN, INTRLEUKIN-6 AND TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 IN PATIENTS WITH VENTILATOR-ASSOCIATED PNEUMONIA

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Background. The aim was to examine the diagnostic value of serum and bronchoalveolar lavage (BAL) fluid levels of procalcitonin (PCT), interleukin-6 (IL-6) and triggering receptor expressed on myeloid cells-1 (TREM-1) in patients with ventilator-associated pneumonia (VAP) caused by bacteria.
Methods. The study included 31 surgical intensive care unit (ICU) patients with VAP and 52 ICU patients without VAP. The concentrations of PCT, IL-6 and TREM-1 were determined in serum and BAL fluid from patients with VAP and in serum of no VAP patients. PCT was determined with an immunofluorescence assay (KRYPTOR, BRAHMS, Germany). IL-6 was measured with a chemiluminescent enzyme immunoassay (IMMULITE1000, Siemens, Germany). TREM-1 was determined with an enzyme-linked immunosorbent assay (R&D systems, Minneapolis, USA).
Results. In serum, the medians of PCT, IL-6 and TREM-1 concentrations were significantly elevated in patients with VAP compared with no VAP patients: 0.680 *vs* 0.055 ng/mL, P<0.0001 (PCT); 343 *vs* 85 pg/mL, P=0.0006 (IL-6); 75.6 *vs* 39.5 pg/mL, P=0.0010 (TREM-1). In BAL fluid samples from patients with VAP, PCT concentrations were extremely low (0.020 ng/mL, 95%CI 0.02-0.04), IL-6 concentrations were significantly lower than in serum (92.8 pg/mL, 95%CI 56-355, P=0.008), while TREM-1 concentrations were significantly higher than in serum (408 pg/mL, 95%CI 290-544, P<0.0001).
Conclusions. Serum concentrations of PCT, IL-6 and TREM-1 have been significantly increased in patients with VAP caused by bacteria. However, in BAL fluid only TREM-1 receptor has been shown as useful in the diagnosis of VAP, suggesting bronchoalveolar TREM-1 level to be a potential marker of bacterial pulmonary infection.

Infectious diseases (acute and chronic)

0668

CONCENTRATION OF B-TYPE NATRIURETIC PEPTIDE (BNP) DISCRIMINATES DIFFERENT STAGES OF SEPTIC SYNDROME

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Background. The aim of the study was to examine the concentrations of B-type natriuretic peptide (BNP) in different stages of septic syndrome in surgical intensive care unit (ICU) patients.

Methods. 133 ICUpatients were classified into five groups according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) criteria: without infection (52), systemic inflammatory response syndrome (SIRS, 20), sepsis (36), severe sepsis (9) and septic shock (16). BNP concentration was determined in serum with an enzyme-linked immunosorbent assay (Biomedica, Austria).

Results. The median of BNP concentrations in patients without infection (685.5 fmol/mL; 95%CI 568-778) was significantly lower (p<0.05) compared with medians in all other groups of patients: SIRS(969 fmol/mL; 95%CI 801-1677), sepsis(1048 fmol/mL; 95%CI 813-1626), severe sepsis (1362 fmol/mL; 95%CI 673-4326) and septic shock (1504 fmol/mL; 95%CI 1338-3453). BNP concentrations were significantly lower (p<0.05) in SIRS and sepsis compared with septic shock, suggesting a correlation with the severity of sepsis.

Receiver operating characteristic (ROC) analysis showed excellent diagnostic accuracy in differentiating patients without infection and those with septic shock with area under the curve(AUC) 0.932, sensitivity (Se) 93.7%, specificity (Sp) 98.5% and optimal BNP cut-off value above 1135 fmol/mL. ROC analysis between other groups of patients showed good discrimination: without infection-severe sepsis: AUC=0.793, Se=77.8%, Sp=88.5%; without infection-sepsis: AUC=0.742, Se=68.6%, Sp=73.1%; SIRS-septic shock: AUC=0.772, Se=93.7, Sp=65.0%.

Conclusions. BNP concentrations showed a strong relationship with the severity of sepsis, with most increasein patients with the most severe stages of sepsis: severe sepsis and septic shock.

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EPIDEMIOLOGY AND TREATMENT OF COMMUNITY ACQUIRED PNEUMONIAS IN A HEALTH AREA

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Backgroun. To evaluate the epidemiology of community acquired pneumonias (CAP) and their treatment.

Methods. Descriptive study. Population in a Health Area (120,000 inhabitants). Period: 2008-2009. Data Base: data from medical records.

Results. Incidence was 0.28% (284/280 cases, respectively). Distribution: <14 years: 55/30 cases (19.3/10.8%); >65 years: 193/204cases (68/78%); 14-65 years: 36/46 cases (13/16%). The diagnosis was clinical in the children (without requesting routine microbiology diagnosis). They were successfully treated with empirical cefuroxime. In 2008, 21 bloodstream infections (7% of the CAP): 12 gram positive cocci (G+), among them, 8 *S. pneumoniae* multisensitive, 3 *H. Influenzae* and the remainder other gram negative bacilli (G-). There were 4 multiresistant isolations (19%). In 2009, 24 bloodstream infections (8.6%): 6 (25%) were due to 100% penicillin sensitive *S. pneumoniae*, 14 due to other cocci and 4 G- bacilli. There were 7 (27%) resistant to quinolones/cephalosporins, with the empirical treatment being modified.Atypical pneumonias predominated in 2008-2009: *Mycoplasma* (13/14 cases, respectively), in patients <40 years, *C. pneumoniae* (4/16 cases), *C. psitacci* (0/5 cases), Influenza A (0/8 cases), Influenza B (0/1 cases). No seroconversions to *Legionella, Adenovirus*, *Respiratory syncytial virus*, or *Coxiella,* were observed. There were 35 patient deaths due to pneumonia / year (12.3/12.5%), elderly, except 3 adults with an underlying disease.

Conclusions. Incidence of CAP is stable despite the appearance of Influenza H1N1 in 2009. It appears in a higher proportion in elderly males, with an increased mortality rate. Classic aetiology: *S pneumoniae, Mycoplasma, C. pneumoniae* and outbreaks of *C. psitacci* and *Influenza A.*

Infectious diseases (acute and chronic)

0670

DIFFERENCIAL DIAGNOSIS OF PLEURAL EFFUSION

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Background. Biochemical studies of pleural effusion are objects of many investigations. The diagnosis problem of tuberculous pleuritis is emmhasized as tuberculosis is a frequent pathology and due to the difficulties of its diagnosis confirmation.The aim of this study was to evaluate the usefulness of biochemical examinations of different pleural exudates for acute diagnosis.
Methods. The routine study of pleurae fluid included the following biochemical testing of pleure/serum:total proteins,albumine, gl ucose,cholesterol,triglyceride and lactate dehidrogenase on automated analyzer Vitros 350, adenosine deaminase (ADA)-spectrophotometric method and lysozime-turbidimetric method.The first step in biochemical examinations of pleural effusions is separation ezudates from transudates according to Light criteria.
Results. The parameters were assayed in the serum and pleural fluid of 60 patients out of which 40 were with tuberculous pleurisy and 20 with primary and metastatic pleuropulmonary malignancies.Lysosime as a macrophage product showed the highest concentration in serum915,6+9,6mg/l and effusion (39,7+13,5mg/l)of patients with tuberculous etiologt,statistically different from other group(p<0,001).Adenosin deaminese(ADA) is also very effective immunologic parameter in the diagnosis of the tuberculous effisions. The mean(+SD)ADA concentration was 35,4+25,5 U/L.This was much higher(p<0,001 by students test) than in pleuropulmonary malignancies (16,5+6,5U/L)
Conclusions. Biochemical assay should not be considered as an alternative to biopsy and culture but rather as a screening test to guide fyrther diagnostic procedures and management of an exudative pleural effusion.

0671

DESIGNING AND STUDYING OF DEFENSIN PEPTIDES ANALOGUES AS A MUCOSAL ADJUVANT/MICROBICIDE: A STUDY TOWARDS THE DEVELOPMENT OF PEPTIDE BASED VACCINE AGAINST AIDS

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Background. The mechanisms of resistance to HIV infection in the human oral cavity are incompletely understood while salivary components have been implicated in protection. There are growing evidences that human defensin peptides originating in the oral epithelial cells may be playing an important role in the prevention of HIV infection.
Methods. We have synthesized HIV and Defensin peptides and their corresponding analogues by making some modifications in the natural sequence. We have done Anti HIV, Anti-microbial and other characteristic study of defensins to prove them active. Then, immunized theses formulations in Outbred and Two different Inbred mice (H^{2b}, H^{2d}) through IN route using Microsphere as delivery vehicle. We have studied Humoral Response of HIV peptides with and without Defensins by estimating antibody levels (IgG/IgA) in the serum as well as in lung, intestinal, vaginal and rectal washes till day 120. For cell mediated immune response, peptide specific T cell proliferation and cytokine/chemikine levels were studied in the cells isolated from the three different mucosal sites i.e. spleen, lamina propria and peyer's patches of the primed mice. Simultaneously, we have done Cytolytic activity analysis, by estimating IFN-γ/Perfofin secretion by CD4+ also through FACS, which was checked by IFN-g/Perforin secretion.
Results. The HIV peptides alone in microsphere showed low peptide specific response of peak titre in sera and different washes while the presence of defensins increased significantly this titre both in sera (1,02,400-4,09,600) as well as in washes (800-12,800) (p<0.05) Very interestingly, we have found that the cellular immunogenicity of all the HIV peptides with defensin peptides in different formulations showed a significantly higher (upto 2 fold ranging from 10-50 stimulation index) (p<0.001) proliferation response as compared to HIV peptide alone. The cytokine measurement profile showed mixed Th1 and Th2 type of immune response. The FACS analysis data revealed that CD8+/ CD4+ T cells showed significantly higher Cytolytic activity in the HIV with Defensin peptide formulations. Surprisingly, CD4+ T-cells were also showing Cytolytic property.
Conclusions. We have shown from the present study that these defensin peptides and their analogues are markedly enhanced the antigen specific immune response even at very low concentration. Thus, the results reported here demonstrate the effectiveness of synthetic defensin peptide analogues to induce strong and long lasting humoral and cellular immune response through intranasal route using PLG- microsphere as a delivery vehicle. Our findings may have implications in the development of new antiviral agent for AIDS therapy.

Infectious diseases (acute and chronic)

0672

STUDY OF DEFENSIN PEPTIDES ANALOGUES AS A MUCOSAL ADJUVANT/MICROBICIDE WITH THE PEPTIDE ANTIGENS OF HIV: AN APPROACH TOWARDS THE DEVELOPMENT OF VACCINE AGAINST AIDS

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Background. There are growing evidences that human defensin peptides originating in the oral epithelial cells may be playing an important role in the prevention of HIV infection. Defensin molecules have shown to be active against HIV even at very low (100μM) concentration through direct virolysis, suppression of transcription and due to its lectin like activity.
Methods. All the HIV peptides were selected from the core, envelope and regulatory proteins of HIV-1 subtype 'C while out of few isoforms of defensin two active variants of α and β-defensin were selected for the present study. Both the HIV and defensin peptides were chemically synthesized using F-moc chemistry while all the defensin peptides were folded correctly using regio-selective method. To increase their anti-HIV activity, two arginine residues were added at the amino terminal of the defensin peptides. All the HIV peptides were immunized alone and with defensins in the form of conjugate and by physical mixing in microsphere. The antibody levels (IgG/sIgA) were detected in the serum as well as in lung, intestinal, vaginal and rectal washes till day 120.
Results. The HIV peptides alone in microsphere showed low peptide specific response while the presence of defensins in different formulations showed significantly higher peptide specific response both in sera as well as in washes (Highest in physical mixing). The increase in the peptide specific immune response was observed at a very low dose (1μg) of defensins while the higher dose produced poor humoral response.
Conclusions. Our findings may have implications in the development of new antiviral agent for AIDS therapy.

0673

DEVELOPMENT OF A SYPHILIS TPA ASSAY* FOR THE VITROS® ECI/ECIQ IMMUNODIAGNOSTIC SYSTEMS, THE VITROS 3600 IMMUNODIAGNOSTIC SYSTEM AND THE VITROS 5600 INTEGRATED SYSTEM

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Background. Syphilis is a sexually transmitted disease caused by the spirochete *Treponema pallidum*. The presence of antibodies to *Treponema pallidum* (TP) antigens, in conjunction with non-treponemal laboratory tests and clinical findings may aid in the diagnosis of recent, past or treated syphilis infection.
Methods. The method is a qualitative immunoassay technique, which involves the reaction of IgG, IgM or IgA antibodies present in samples with biotinylated TP antigen and horseradish peroxidase (HRP)-labeled TP antigen conjugate The bound HRP conjugate is directly proportional to the concentration of anti-TP antibody present. The performance of the VITROS Syphilis TPA assay was compared to that of other commercially available tests.
Results. Assay results are expressed as a ratio of the signal at the clinical cut off. Precision at the cut off to strong positives averaged 1.6% (range 0.9 – 5.0%) within run, 3.8% (range 1.9 – 5.8%) within calibration and 4.3% (range 3.0 – 6.5%) within lab. Clinical performance was assessed on a population of 1831 random donor and 233 clinical samples against a currently marketed assay. Sensitivity was 100% (193/193) and specificity 99.77% (1748/1752) after resolution of 2 discordant samples by testing in two other Methods. 100% agreement was obtained with commercially available performance panels and no interference was seen with a wide range of potential interferents or patient samples with other infectious agents present.
Conclusions. We conclude the VITROS Syphilis TPA assay meets current clinical practice needs for a fully automated assay.
Under Development

Infectious diseases (acute and chronic)

0674

SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUES OF ADENOSINE DEAMINASE IN TUBERCULOUS DISEASES

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Background. Adenosine Deaminase (ADA) is an enzyme in the purine metabolism that catalyzes the conversion of adenosine to deoxyadenosine with the release of ammonia. The present study evaluates the cut off values, sensitivity, specificity, positive and negative predictive values of ADA in cerebrospinal fluid (CSF) and pleural fluid (PF) as a diagnostic marker in tuberculous diseases as compared with tuberculous non-disease.
Methods. Total 98 patients visiting Department of Biochemistry in our hospital for ADA test from July 2009 to August 2010 were enrolled in the study. CSF and PF samples were drawn after proper informed consent from the patients by trained Medical Personnel. ADA was measured by the spectrophotometric method described by Guisti and Galanti, 1975. Tuberculosis was confirmed by culture or acid fast bacilli (AFB) positivity.
Results. Our study showed CSF ADA values with sensitivity 100% at 95% CI (0.69 - 1.00) and specificity 93.8% at 95% CI (0.698 - 0.998), cut off values were 8.83 IU/L and Area under curve (AUC) was 1.00, positive predictive value (PPV) 90.9% and negative predictive (NPV) value 100%. PF ADA values showed AUC 0.96 with a cut off value of 44.00 IU/L with 94.7% specificity at 95% CI (0.823-0.994) and sensitivity 97.2% at (0.855-0.995), PPV 94.7% and NPV 94.4% respectively.
Conclusions. Body fluid ADA can be used as an early diagnostic marker for tuberculous disease, tubercular meningitis and tubercular effusions. This study highlights the role of ADA as a sensitive, diagnostic and screening marker for tuberculous disease.

0675

LC-MS/MS METHOD FOR QUANTIFICATION OF 14 DIFFERENT DRUGS USED IN HIV TREATMENT

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Background. Highly active antiretroviral treatment (HAART) led to a dramatic decrease in HIV-associated mortality. HAART consists in most cases of at least three different antiretroviral drugs. Those drugs are often metabolized by Cytochrom P450 or UGT1A1 pathways, leading to a high rate of interaction either between the antiretroviral drugs themselves or other drugs. While too low level of the drugs can lead to insufficient activity, too high drug levels increase the rate of adverse reactions.
Methods. We developed a HPLC-MS/MS method for quantification of the following 14 drugs: Ritonavir, Lopinavir, Nevirapin, Saquinavir, Nelfinavir, Indinavir, Amprenavir, Atazanavir, Maraviroc, Raltegravir, Darunavir, Etravirine, Tipranavir and Efavirenz. Single step extraction of Plasma samples, controls and calibrators is performed after addition of internal standard (A-86093.0) using Methanol/Acetonitril v/v 80/20%. An aliquot of the supernatant is analyzed using a Shimadzu prominence HPLC with an 5 cm RP18 (Eurospher) Phase with a acetonitril gradient (0,7 ml/min) and an AB SCIEX API 4000Q TRAP mass spectrometer using electro spray ionisation.
Results. Linear range of the method is from 25 ng/ml up to 20000 ng/ml. The extraction recovery was tested with spiked plasma samples and always showed values above 96%. Intraassay CVs were between 1.3% and 6.0%, interassay CVs were between 4.4% and 10.4%.
Conclusions. Our method is suitable for therapeutic drug monitoring (TDM) of HIV treatment regimens. The method can be used to asses treatment adherence, complex drug interactions and exposure to specific drugs, thus helping the physician to improve treatment outcome and reducing adverse effects.

Infectious diseases (acute and chronic)

0676

COMPARISON OF TWO FULLY AUTOMATED SEROLOGIC TESTS FOR LUES ANTIBODIES

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Background. While TPPA (Treponema pallidum particle agglutination) or TPHA (Treponema pallidum hemagglutination) are still seen as the gold standard assays when it comes to syphilis screening they both are not easily and reliable automatable. This leads to a high interest in alternative assays in specialized laboratories with a high number of requests for syphilis screening.
Methods. We compared the Abbott ARCHITECT Syphilis TP (Micro particel enzyme immuno assay) versus the Roche/Sekisui Cobas Mediace TPLA (Treponema pallidum latex agglutination test), which is a latex enhanced immunoturbidimetric assay. We analyzed 617 fresh serum samples sent to the Medizinisches Labor Dr. Berg for routine diagnostics and 300 samples from healthy blood donors.
Results. We observed an accordance between the results of both assays for all the samples of 94%. 21% of the samples were detected positive for antibodies against Treponema pallidum in both assays and could be confirmed by TPPA . Only 1.1% of the samples showed a positive result in the Architect assay while having a negative result in the Cobas assay. Of those 10 samples, 6 could not be confirmed as positive for Lues antibodies using TPPA, VDRL and Immunoblot. 5.3% of the cobas c 6000 positive results could not be confirmed in the Abbott assay.
Conclusions. Both assays show excellent performance in terms of sensitivity. We conclude that both assays are valuable tools for syphilis screening. Nevertheless, positive results should be confirmed by an additional assay.

0677

EXTENDED-SPECTRUM B-LACTAMASE (ESBL) AMONG *ENTEROBACTERIACEAE* ISOLATED IN A PEDIATRIC HOSPITAL IN PARAGUAY

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Background. Extended-spectrum β- lactamase (ESBL) are enzymes produced by *Enterobacteriaceae* that confere resistance to an important group of antibiotics. In Latin America there is a high rate of ESBLs, althoughin our country there have been few reports. Pediatric infections caused by ESBL producing bacteria are associated with high mortality rates, so the phenotypic and genotypic identification of these pathogens is essential to avoid clinical treatment failures, achieving infection control and interpret epidemiological spread.
Methods. We present the first results concerning the detection of ESBL-encoding genes: Bla_{CTX-M2}, bla_{PER-2}, bla_{TEM} and bla_{SHV} in 67 isolates of *Enterobacteriaceae* analyzed for 15 monthsin the laboratory of a teaching Hospital (CMI-FCM) and presented with ESBL phenotype determined with the double disc method. The molecular characterization of these isolates was performed by chain reaction technique (PCR) using specific primers.
Results. 90.3% of these isolates came from inpatients biological materials. *Klebsiella pneumoniae* was the ESBL-producing germ most frequently (55.2%). Bla_{CTX-M2}, bla_{PER-2}, bla_{TEM} and bla_{SHV} gene were detected respectively in 38.8, 7.5, 20.9 and 16.4% of the isolates. In *K. pneumoniae* were detected in addition to β-lactam resistance, resistance to amikacin and gentamicin, respectively, 32 and 68% of the isolates.
Conclusions. Not detected in this period carbapenem-resistant *Enterobacteriaceae*. This is the first report of genotypic characteristics of ESBL-producing *Enterobacteriaceae* in pediatric population of Paraguay.

Infectious diseases (acute and chronic)

0678

PERFORMANCE EVALUATION AND SYSTEM CONCORDANCE OF THE NEW SIEMENS HBSAGII ASSAY FOR QUALITATIVE DETECTION OF HBS ANTIGEN ON THE ADVIA CENTAUR AND ADVIA CENTAUR CP SYSTEMS

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Background. The ADVIA Centaur® HBsAgII assay is an in vitro sandwich chemiluminometric immunoassay for qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma using the ADVIA Centaur and ADVIA Centaur CP Systems.
Methods. Analytical sensitivity was determined using serial dilutions (WHO Second International HBsAg Standard) run in singlicate using two assay kit lots on each system. Precision was determined using a CLSI EP5-A2–type protocol using two of each system, 2 runs/day for 20 days. Diagnostic sensitivity and specificity were measured using 1105 patient samples (100 confirmed positive). Each sample was tested with 2 reagent lots on both systems in singlicate. Centaur results were compared to patient status, and CP results to the Centaur. Mutation sensitivity was tested using 25 cell lysates from recombinant HBsAg mutants. Systems concordance was tested in singlicate using 15 commercial seroconversion panels.
Results. Calculated analytical sensitivities for each kit lot were 0.033IU/ml and 0.040IU/ml on the Centaur, 0.035IU/ml and 0.037IU/ml on the CP. Pooled CV[within-run] was 2.1–7.3, Centaur and 2.3–6.7, CP. CV[Total] was 2.5–11.5 Centaur, 6.1–13.3 CP. Sensitivity and specificity were 100% on both systems. Concordance and mutant sensitivity were 100%. Fourteen of 15 seroconversion panels were reactive on the same bleed. The Centaur CP reported reactivity 1 bleed earlier than the Centaur for 1 panel.
Conclusions. The Siemens HBsAgII assays for the ADVIA Centaur Systems are sensitive, specific, and concordant. They show good precision and analytical sensitivity, and successfully detect HBsAg mutants.

0679

THE VALUE OF INFLAMMATORY TEST IN SEPSIS

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Background. Procalcitonin (PCT) is a marker of the inflammatory response. The study aimed was to assess the value of white blood cell count (WBC), C-reactive protein (CRP), PCT for the diagnosis of systemic inflammatory response syndrome (SIRS) and sepsis. In Albania, PCT has been used as a serum marker for bacterial sepsis since May, 2010.
Methods. We evaluated: WBC, CRP, and PCT in 67 patients presented in our hospital. We used Elycsys BRAHMS PCT test Electro Chemiluminescent Immunoassay (ECLIA) for the determination of Procalcitonin. We evaluate those parameters in three subgroups: 20 patients with sepsis, 32 patients with SIRS, and 14 patients as control group. The cut off for positivity was ≥2 ng/ml.
Results. In sepsis subgroup mean age was 46.5 years old ±20.7 SD. Average value of PCT, PCR and WBC were respectively 23.65ng/ml, 97.5ng/l and 12.79×10^3cells/mm³. In SIRS subgroups mean age was 41.9 years old with ±15.9 SD and the average value of PCR, WBC were 74.3ng/l and 10.137×10^3cellc/mm³, but PCT was ≤2ng/ml. In control group mean age was 43.6 years old with ±26,4 SD, and WBC, PCR and PCT were in normal value respectively ($3.5\text{-}10 \times 10^3$cells/mm³), (0-5ng/l) and (0.5-2ng/ml).
Conclusions. The serum concentration of PCT is specifically elevated in sepsis patients. However, the evidence of it in infectious diseases is limited. The diagnostic model based on the laboratory parameters, using the combined predictors of PCT, CRP and WBC, can be a useful means for predicting early-onset of sepsis.

Infectious diseases (acute and chronic)

0680

THE HIV AVIDITY INDEX FOR IDENTIFYING RECENT HIV INFECTIONS USING THE VITROS ECI HIV 1-2 IMMUNOASSAY

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Background. The Avidity Index (AI) for HIV antibodies identifies recent HIV infections (RHI, ≤6 months from seroconversion) and established HIV infections (EI, >6 months from seroconversion). Using AxSYM HIV1/2 gO and Architect HIV Ag/Ab Combo immunoassays (Abbott) the AI cutoff was 0.80. This study analyses the performance of different cutoffs for identifying RHI (pre-established 'windows period' of 180 days), using Vitros Eci HIV1-2 immunoassay (Ortho Clinical Diagnostics).
Methods. We collected 115 serum samples from 34 HIV-positive individuals. The seroconversion date was estimated as the midpoint between the last negative and the first positive HIV test. Specimens were classified as recent when the AI value was below orequal the cutoff, and established when the AI value was above the cutoff. We analyzed the accuracy by ROC analysis.
Results. Of the 115 specimens, 72 (62.6%) were RHI. The value AI mean was 0.37±0.25 and among RHI samples and 0.69±0.21 among EI. Considering a 'windows period' of 180 days, the following results were obtained with three selected cutoffs:
-0.40: sensitivity 56.9%, specificity 90.7%, ROC area73.8%
-0.50: sensitivity 80.6%, specificity 79.1%, ROC area79.8%
-0.60: sensitivity 87.5%, specificity 62.8%, ROC area 75.1%
The overall accuracy using the 0.50 cutoff was 80.0%. Of the 72 RHI samples, 7.8% were misclassified as EI, and of 43 EI samples 12.2% were misclassified as RHI.
Conclusions. The AI for HIV antibodies can be used with various immunoassays. However, the AI cutoff may differ between immunoassays, stressing to need to established the best performing cutoff on panels of HIV seroconverters.

0681

PARVOVIRUS ANTIBODIES DETECTION BY THE NEW LIAISON ASSAY

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Background. Parvovirus infection in pregnancy can cause foetal anaemia and hydrops fetalis. Accurate and prompt detection of the virus antibodies is highly important. The aim of the present study was to evaluate new assays for parvovirus antibody detection.
Methods. We used 120 sera samples for determination of the performance of three kits for parvovirus IgG and IgM antibodies: two ELISA assays one CLIA assay tested on the random access Liaison analyzer. Discrepant results for IgG antibodies were confirmed by IFA (Biotrin) and discrepant results for IgM antibodies were confirmed by PCR.
Results. Ninety five samples were positive and 21 samples were consensus negative for IgG with all three assays. Two discrepant samples were positive by IFA, rendering a false negative result with each of the examined assays. Two more discrepant samples had negative IFA result, rendering two false positive results for each of the ELISA assays. As for IgM antibodies, 35 and 56 samples had consensus positive and negative results, respectively. Twenty nine samples had discrepant Results. 23 out of them were positive and six of them were negative by PCR. One of the ELISA assays revealed one false negative result and 4 false positive results while the other ELISA assay had 23 false negative results and one false positive result. The CLIA test had only one false positive result.
Conclusions. The DiaSorin CLIA (Liaison) parvovirus antibody assay exhibited the best performance (among the evaluated kits) and was found to be suitable for random access detection of antibodies to parvovirus.

Infectious diseases (acute and chronic)

0682

STUDY OF MARKERS PROCALCITONIN AND C-REACTIVE PROTEIN IN THE DIFFERENTIATION OF SYSTEMIC INFLAMMATORY RESPONSE SYNDROME AND SEPSIS IN POSTANESTHESIA CARE UNIT

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Brackground. In the Postanesthesia Care Unit (PACU) patients occasionally arrive in critical condition and we must know if they suffer from a systemic inflammatory response syndrome (SIRS) for the surgery performed or if the patient is suffering from sepsis or systemic shock.
Methods. The study was conducted for 19 months in a total of 88 patients by eliminating patients immunosuppressed transplant or cancer processes. The samples were collected on admission to the PACU and 24 hours and analyzed the following parameters: procalcitonin (PCT) (ng/mL) and C-reactive protein (CRP) (mg/dL) and the ratio of PCT / CRP. It was analized in Cobas e601 (Roche). Patients have been classified into two groups: SIRS and Sepsis. Statistical analysis was performed using the SPSS 15.0 for Windows using theno-parametric testWilcoxon and COR curves.
Results. We have obtained in the study an average PCT in the SIRS group is 3.02 ± 4.65, while the average of PCT in sepsis group is 36.21 ± 37.70 (p <0.001).
The area obtained in the COR curve group SIRS / Sepsis for PCT was 0.910 ± 0.37 (p <0.001) and the ratio of PCT / CRP is 0.862 ± 0.50 (p <0.001).
PCT cutoff more than 5.73 with a Sensitivity = 89.2%, Specificity = 76.9%, Positive Predictive Value = 84.62% and Negative Predictive Value = 83.33%.
Conclusions. Using the PCT cutoff in our study over 5.73 we can differentiate whether patients are in a situation of sepsis or SIRS, so treatment will be different.

0683

LABORATORY-BASED SURVEILLANCE OF SEXUALLY TRANSMITTED INFECTIONS IN ITALY

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Background. To assess the prevalence of *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoeae* (Ng) and *Trichomonas vaginalis* (Tv) infections based on data provided by a laboratory-based sentinel surveillance system.
Methods. The sentinel network is composed of 13 microbiology laboratories which report data on individuals who undergo testing for Ct and/or Ng, and/or Tv, on ano-genital specimens. Socio-demographic, clinical and behavioral information is also collected. Every individual can undergo microbiological testing for more than one pathogen.
Results. From April 2009 to August 2010, 8,490 individuals were tested for Tv infection, 7,333 for Ct, and 5,178 for Ng. The median age of all tested individuals was 34.0 years; 50.9% had genito-urinary symptoms at the time of sample collection. The prevalence of Ct, Tv, and Ng was 3.2%, 0.7%, and 0.4%, respectively. Positivity for Ct was significantly (p-value<0.001) associated with young age (7.9% among persons aged 15-24 years vs. 2.4% among persons aged ≥25 years), having had two or more partners in the previous six months (≥ 2 partners 17.4% vs. 0-1 partner 2.3%), and being symptomatic (presence of symptoms 4.3% vs. no symptoms 2.1%). No significant association was observed among patients with Tv or Ng.
Conclusions. These preliminary results show a low circulation of Tv and Ng. A sustained prevalence of Ct is observed, especially among young persons, which needs to be monitored for a longer period.

Infectious diseases (acute and chronic)

0684

HEPATITIS C ANTIBODY ASSAY BASED ON MULTIEPITOPE PROTEINS AND FLUORESCENT LANTHANIDE CHELATES

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Background. Conventional immunoassays for hepatitis C require several capture polypeptides with different epitopes for reliable detection of geographically distributed genotypes. However, rare genotypes can still remain undetected. A previously developed recombinant multiepitope protein (MEP) incorporates epitopes from four HCV antigens recognizing different genotypes. Using this MEP as the capture antigen eliminates the need for several separate antigens.
Methods. We have developed two immunoassays for anti-HCV antibodies based on the MEP and time-resolved fluorometry. An *in vivo* biotinylated version of the MEP immobilized on streptavidin microtiter wells was used as a capture and MEP or a secondary antihuman antibody conjugated to a europium chelate as tracer.
Results. The MEP recognized the samples from all six HCV genotypes in a worldwide performance panel. Using the MEP as tracer, the sensitivity with a patient sample panel was 88.5% (77.9-94.6%) and specificity 100% (92.2-100%). With a secondary antibody tracer, sensitivity was 93.8% (86.0-97.7%) and specificity 100% (92.2-100%).
Conclusions. When the MEP-conjugate was used as tracer, both the solid-phase and tracer binding reactions were HCV antibody specific. Thus, background originating from high excess of non-virus specific antibodies was eliminated. However, the assay using MEP both as capture and tracer was strongly affected by the low affinity seen in unmatured antibody responses, explaining the lower diagnostic sensitivity especially with samples from acute infections. With the antihuman antibody conjugate tracer, the low affinity was largely compensated by the avidity effect caused by the bivalent binding of the antibodies to the capture MEP.

0685

REFERENCE LEVELS OF PNEUMOCOCCAL ANTIBODY GLOBAL SERUM ASSAYS (IGG & IGG$_2$) AND SPECIFIC ANTIBODIES IN HEALTHY CHILDREN AND ADULTS

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Background. In children, specific antibody levels against pneumococcal capsular polysaccharide represent the acquisition of natural immunity. The determination of pneumococcal antibodies is an important tool in search of immunodeficiencies.
Methods. Antibody levels specific for capsular polysaccharides of Streptococcus pneumoniae were measured in serum samples of age-stratified subjects. We determined a representative panel of specific pneumococcal antibodies (serotype 4, 5, 6, 7, 14, 18, 19, and 23) by ELISA and global pneumococcal IgG and IgG2 antibodies by EIA. The study group consists of 502 healthy adult volunteers and hospitalized children undergoing elective surgery. Of these, 87 had received at least one dose of pneumococcal vaccine in advance.
Results. In vaccine-naïve subjects, the initial pneumococcal IgG geometric mean concentration of 13.1-14.4 *µ*g/ml was low in the first year of life and increased over the time, reaching adult levels (70.5 *µ*g/ml) at age eight to 12 years. In parallel, PnC antibodies of the IgG2 subclass increased from 20.7% (0.5-1 year old) to adult proportion (33.8%) in four to eight year old subjects. We found a good correlation between the pneumococcal IgG screening assay and our specific pneumococcal antibody levels (Pearson's coefficient r= 0.4455; p<0.001). Our data provide reference ranges for the interpretation of specific antibody determinations in the clinical setting.
Conclusions. The global pneumococcal IgG/IgG2 assay is a suitable screening tool and correlates well with the determination of serotype-specific pneumococcal antibodies as determined by ELISA.

Infectious diseases (acute and chronic)

0686

CHLORINATIONAL LEVEL OF WATER AND PREVALENCE OF VIRAL HEPATITIS IN FLOOD AFFECTED AREAS OF VADODARA CITY, INDIA

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Background. A Chlorination level of water was in various field areas of flood affected areas of one of the city of Gujarat state in India to check the correlation of viral hepatitis with the contamination of water using various laboratory tests.**Objectives :** To know the chlorination level in Municipal Water Supply System-Administrative ward wise distribution post flood. To Study pattern of Viral Hepatitis cases post flood
Methods. Sample selection : Purposively selected households from each of the 10 wards of Vadodara city.
Study period : Chlorination of water - july 4th to july 20th 2005 and disease prevalence – july '05 to Oct '05.
Results. Measuring the chlorine level in each of the 10 administrative wards of Vadodara city showed initial low level of chlorine (<0.5 ppm) in north and south zone followed by east and west zone upto 8th july 2005 which then significantly improved after regular update and followup action daily till 12th july , but east zone (ward no. 1,2,9) continued to a problem of low chlorination level throught study period. Higher prevalence of viral hepatitis cases reported in the month of august-nov. 2005, which is in relation to low chlorination level of water in all zones, significantly higher in east zone throught the study period.
Conclusions. Low level of chlorine in water was associated with higher number of viral hepatitis all the wards of Vadodara city.

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LATENT TUBERCULOSIS INFECTIONS (LTBI): TUBERCULIN SKIN TEST AND WHOLE BLOOD IFN-Γ AS SURROGATE MARKERS IN DEVELOPING COUNTRIES

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Background. Identification of surrogate markers for progression of latent TB to overt disease among household contacts (HHC) of patients with active pulmonary TB can markedly improve case detection rates in developing countries. Tuberculin skin test (TST) with its drawbacks is still a cheap, simple and reasonably sensitive test of latent TB infections compared to whole blood IFN-γ levels.
Methods. In thisprospective, cross-sectional and community-based study, consented 98 house hold contacts (HHC) of patients with pulmonary TB and 138 community controls (CC) were enrolled. Tuberculin skin test and whole blood culture IFN-γ responses to the *M.tuberculosis* antigens: early secretary antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10), TB 7.7 and purified protein derivative (PPD) were performed.Data was entered, checked and analyzed using Epidemiological information (Epi Info) software version 3.4.3.0.
Results. The male female ratio was 1:3 with an overall mean age of 35±15. The mean ages of HHC (30± 11) and CC (29± 10) were not significantly different (*p>0.05*). Forty one per cent of HHC were reactive in TST HHC compared to 6% for CC. Fifteen per cent (37/236) of the volunteers had IFN-γ levels above the cut-off compared to 22.8% volunteers (54/236) who were reactive in TST. In the HHC the IFN IFN-γ reactivity was seen in 28.6% compared to 6.5% in CC. There was significant concordance between IFN-γ secretion and TST in all volunteers (p=0.000). Increased IFN-γ levels but not TST reactivity, was associated with the duration of exposure to TB index cases (p=0.0001). Four of the HHC developed smear positive pulmonary TB, but only one of these had an increased IFN –γ at the enrollment stage.
Conclusions. TST reactivity is a more sensitive marker of exposure to *M.tuberculosis* compared to IFN-γ levels among house hold contacts, while IFN-γ level is a good marker for the duration of exposure.

Infectious diseases (acute and chronic)

0688

EVALUATION OF A FULLY AUTOMATED METHOD TO DETERMINE CHAGAS DISEASE ANTIBODIES

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Background. Due to growing numbers of South American immigration in our area a new guideline for detection of Chagas antibodies in pregnant women has been set by our local government .
Methods. We retested 60 samples from pregnant women with the new Abbott Chagas test for the i2000. Thist test detects total antibodies against T.cruzi, we followed the instructions for use included in the kit for interpretation of Results. We also tested 38 samples of healthy individuals who had never been in South America to evaluate the possibility of false positive Results.
Results. We found NO discordances between the methods used as routin tests until that moment (Immunochromatography and IFI), and none of the control patients appeared to be positive for Chagas antibodies. A 20% of samples of native people from endemic areas resulted positive for T.cruzi antibodies. No false positive results were found in our study.
Conclusions. We conclude that the performance of the new Chagas kit manufactured by Abbott diagnostics is at least as good as the kits we had been using with the great advantage of being fully automated and the objectivness in interpretetaion of results because all negative samples by this method were clearly negative while other methods were prone to need interpretatation.

0689

LATENT MYCOBACTERIAL INFECTIONS AMONG HEALTHCARE WORKERS IN CENTRAL SUDAN: TUBERCULIN SKIN TEST AND WHOLE BLOOD IFN-Γ LEVELS AS INFECTION MARKERS

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Background. Overt Mycobacterium tuberculosis infections are an important health problem, while latent infections can be an important source of new cases. This study aimed to determine the prevalence of latent mycobacterial infections among healthcare workers in Greater Medani, Gaziera State. The study also aimed to determine the role of Tuberculin skin test (TST) and whole blood cytokines as markers of latent mycobacterial disease.
Methods. The tuberculin skin test (TST) and whole blood IFN-γ assays using Quantiferon test (QFT-G tests) were performed for all consenting volunteers.
Results. One hundred and fifty healthcare workers (HCW) were approached to participate in the study. Only fifty consented and agreed to participate, the mean age of the participants was 41.6 ± 16.6 years. A quarter (13/50; 26%) of participants had received BCG vaccination. Forty eight per cent (24/50) were reactive in TST with an induration ≥15 mm. Thirty two per cent (16/50) were positive for IFN-γ. Only 20% (10/50) were positive for both IFN-γ and TST. Thirty two per cent (16/50) had high IL-4 and 12% were positive in both IFN-γ and high in IL-4.
Conclusions. Latent mycobacterial infections ranges between 30% and 48% among healthcare workers. TST and QFT-G tests are good complementary surrogate markers of latent mycobacterial infections. TST had a high reactivity compared to QFT-G test.

Infectious diseases (acute and chronic)

0690

CRP, PROCALCITONIN, SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR LEVELS IN HOSPITALIZED PATIENTS AND THEIR RELATION WITH MORTALITY

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Background. Infection and sepsis are the most frequent causes of mortality in hospitalized patients. Although CRP is a sensitive marker in early diagnosis, it has low specifity and therefore, was replaced by more sensitive and specific procalcitonin (PCT) in follow-up of infection and sepsis since it increases early in the course and has prognostic value. Soluble urokinase plasminogen activator receptor (suPAR) secreted by inflammatory cells during infection-sepsis is also used for the same purpose and has importance particularly in assessment of mortality and complications. In the present study we investigated; 1) levels of CRP, PCT and suPAR, 2) their relation with mortality, and 3) oxidative stress in patients hospitalized for infection-sepsis.
Methods. Serum CRP, PCT, suPAR, TAS and TOS levels were measured in 62 patients (m/f: 38/24, age: 37.8 ± 29.3 years) hospitalized for infection and/or sepsis.
Results. CRP, PCT, suPAR, TAS and TOS levels were 10.41± 10.49 mg/dl, 5.57±19.48 ng/ml, 7.38±5.78 ng/ml, 2.17±0.57 mmol/l and 24.04±10.94 umol/l, respectively. CRP correlated significantly with mortality (r=0.319, p<0.05), suPAR (r=0.279, p<0.05), and PCT (r=0.382, p<0.01). Neither PCT nor suPAR correlated significantly with mortality. They did not correlate significantly with each other, either. TAS and TOS levels didn't correlate significantly with mortality and other parameters.
Conclusions. Present findings demonstrated a significant relation of CRP with mortality in patients hospitalized for infection and/or sepsis. It was concluded that further studies with larger numbers of patients are needed to clarify the relations of suPAR and PCT with mortality.

0691

EVALUATION OF INFLAMATION/SEPSIS MARKERS IN EMERGENCY DEPARTMENTS PATIENTS WITH SUSPECTED BACTEREMIA

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Background and Methods. We retrospectively evaluated C-reactive protein (CRP), procalcitonin (PCT) levels, white blood cell (WBC), neutrophil and lymphocyte count and the neutrophil-lymphocyte count ratio (NLCR) in 60 patients of Emergency Departments. CRP was measured by immunoturbidimetry method on Architect ci8200 (Abbott Diagnostics), PCT by electrochemilumi-nescence immunoassay on Cobas e411 (Roche) and blood cells on Sysmex XE-5000 (Roche).
Results. Significant differences between patients with positive and negative blood cultures were detected with respect to the CRP level (198±127 mg/L vs 137±103; p=0.046), PCT (11.2±22.4 ng/mL vs 1.9±6.2; p=0.046), WBC (15.0±8.6 vs 10.5±5.5; p=0.019), neutrophil count (13.6±8.1 vs 8.5±5.3; p=0.0055) and NLCR (21.6±1.8 vs 9.0±9.7; p< 0.0001) and NLCR (20.9±3.3 vs 13.2±14.1; p=0.026) but not in lymphocyte count.
In bacteremic patients PCT (cutoff 2.0 ng/mL) sensitivity was 65% (95%CI 0.4-0.8) and specificity 87% (95% CI 0.75-0.97) with a negative likelihood ratio (LR-) 0.39, CRP (cutoff 50 mg/l) sensitivity was 93% (95%CI 0.4-0.8) and specificity 20% (95% CI 0.76-0.99) with (LR-) 033, WBC count sensitivity was 69% (95% CI 0.5-0.8) and specificity 80% (95% CI 0.6-0.9) with (LR-) 0.43, neutrophilcountsensitivity was 63% (95% CI 0.4-0.8) and specificity 80% (95% CI 0.6-0.9) with (LR-) 0.46, NLCR (cutoff greater than 4) sensitivity was 97% (95% CI 0.8-1.0) and specificity 37% (95% CI 0.2-0.6) with (LR-) 0.09.
Conclusions. The studied markers could be used for identification patients with risk of sepsis and help in the diagnosis of sepsis. Further work and prospective validation is needed in other settings.

Infectious diseases (acute and chronic)

0692

RAP-ID – A NEW DEVICE FOR RAPID MOLECULAR DIAGNOSTICS OF VENTILATOR-ASSOCIATED PNEUMONIA

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Background. Ventilator-associated pneumonia (VAP) is a feared nosocomial infection associated with high mortality and morbidity rates. Rapid and accurate determination of the causing pathogen and its antibiotic resistance is crucial for an adequate and successful treatment. RAP-ID (*R*eal-Time *A*rray *P*CR), a new device for molecular diagnostics of VAP, was evaluated compared to conventional culture and PCR Methods.

Methods. RAP-ID uses a novel hybrid technology for multiparametric pathogen and antibiotic resistance testing, combining targeted multiplex amplification of nucleic acid sequences (up to 50-plex) with real-time detection on microarray to reach a sensitivity and dynamic range comparable to quantitative single-plex PCR (qPCR). Proprietary buffer chemistry and Forbidden angle detection technology allows the integration of all steps into a single-use cartridge and automated workflow.

Results. The content of the RAP-ID assay consisted of 15 relevant bacterial species and 16 resistance determinants for e.g. Extended-Spectrum-Beta-Lactamases, Methicillin-resistant *Staphylococcus aureus*, carbapenemases, Vancomycin-resistant Enterococci and aminoglycosides, respectively. A total of 109 clinical specimen from the lower respiratory tract were tested (bronchoalveolar lavage n = 22; tracheal secretion n = 87) with RAP-ID in comparison to the results obtained by standard microbiology methods, qPCR and sequencing. Taking relevant bacterial load and clinical significance into account, the overall concordance of RAP-ID with standard methods was 96%, the detection limit was 100 cfu/ml, and time-to-result including specimen processing was 4 hours, respectively.

Conclusions. We conclude that RAP-ID is a promising tool for rapid pathogen and resistance detection in critical ill patients. Currently, a two-site study with ICU patients is performed.

0693

THE IMPACT OF LRT INFLAMMATION ON SERUM GLUCOSE LEVELS AMONG ID PATIENTS WITH DIABETES

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Background. Correlation between serum glucosa levels and its mutual
impact to infections is well known, especially with dermal, renal and low respiratory tract inflammations (LRTI).

Aim. This investigation was established to estimate the impact of LRTI (Pneumonia, Pleuropneumonia, exacerbated COPD, Bronchiectasiones inflamata) to insulin daily doses among ID (insulin dependent) diabetic patients through the acute phase of the inflammation.

Methods. In 5 year long period a total number of 871 patients with LRT inflammation were healed at the department of respiratory diseases in our hospital. 116(13.3%) of these patients had controlled and regulated ID diabetes before the time of hospitalization. The routine biochemical testing at admission showed quite increased levels of serum glucose among 89(76%) patients, despite the correctly proceeded insulin therapy. Parallel with the antimicrobial therapy, correction of insulin doses was essential (in a consultation with endocrinologist, based on glucose profile) in a manner of increasing doses of prompt and lente insulin combination or the daily dose scedual in a goal of serum glucose level normalization.

Results. After resolving of inflammatory lung process within approximately 15 days of hospitalization (9-25 days) only 7(7.8%) patients needed a correction of the insulin doses up to levels that were established before the onset of the LRT inflammation.

Conclusions. The impact of LRT inflammation on serum glucose levels among ID diabetic patients origins frequent and continuously careful glucose profile control and additional correction of the insulin therapy as needed during the hospitalization and release, with further precise establishment of the insulin dosage control.

Kidney disease

0694

METHANOLIC EXTRACT OF CNIDOSCOLUS ACONITIFOLIUS ATTENUATES RENAL DYSFUNCTION INDUCED BY CHRONIC ETHANOL ADMINISTRATION IN RATS

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Background. The present work studied the modulatory role of methanolic extract of Cnidoscolus aconitifolius leaf (MECA) in rat model of renal dysfunction induced by chronic ethanol administration.

Methods. Forty-two male Wistar rats were distributed into 7 groups of 6 animals each. Some groups were pre-treated with MECA (100 and 200 mg/kg) or kolaviron (KV) (200 mg/kg) for 2 weeks before simultaneous administration of MECA or KV and 20% ethanol (7.9 g/kg) for 8 consecutive weeks. Others were given ethanol or MECA (200 mg/kg) or KV alone.

Results. In ethanol-treated rats, serum urea, creatinine, urinary glucose, gamma-glutamyl transferase and protein increased by 59, 81, 70, 148 and 63%, respectively, while creatinine clearance significantly ($P < 0.05$) decreased by 79%. MECA significantly attenuated the above biochemical indices to near normal. Also, the levels of serum and kidney lipid peroxidation (LPO) increased by 102 and 143%, respectively. Ethanol intoxication caused a significant decrease in the levels of catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) in kidney of the rats. MECA attenuated the ethanol-induced increases in serum and kidney LPO, and also enhanced the antioxidant status of the rats by increasing the levels of CAT, SOD and GSH. The protective effect of MECA was comparable with KV at 200 mg/kg. The biochemical findings were corroborated by histopathological examination of the kidney slides.

Conclusions. The results suggest that the renal protective effect of Cnidoscolus aconitifolius leaf extract is by attenuating oxidative stress induced by chronic ethanol administration.

0695

SERUM SELENIUM LEVELS OF CHRONIC RENAL FAILURE PATIENTS ON PERITONEAL DIALYSIS

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Background. The kidneys play an important role in selenium homeostasis. It has been found that plasma isoform of glutathione peroxidase is mainly synthesized in renal proximal tubular cells. Several authors have found low levels of glutathione peroxidase and selenium in blood components of patients with chronic renal failure (CRF). Most published data agree that serum selenium levels are significantly decreased in patients with end-stage CRF compared with control groups whereasother studies have found no significant differences.

The aim of this work is to determinewhetherthere issignificant difference betweenselenium levelsof CRF patientson peritoneal dialysisisandhealthypopulationofour area.

Methods. Selenium was determined in serum samplesfrom 23 healthy subjects and 21 CRF patients on peritoneal dialysis, using a Varian-300 SpectrAA spectrometer equipped with a Zeeman-effect background correction system.

Control group: 23 subjects with ages between 19 and 74y (median: 37), 11 women, 12 men.

Patient group: 21 subjects between 31 and 74 (median: 50), 9 women, 12 men.

Results. Selenium levels in control group: 70.2 - 148.9 µg/L. Mean: 85.1µg/L. Selenium levels in patient group: 47.7 - 102.2 µg/L. Mean: 74.5 µg/L. Mann-Whitney U= 118.0, p = 0.004

Conclusions. There is a statistically significant difference between selenium concentrations of the two groups, with a certainty of 99.6%. Whether selenium deficit should be correctedmust be investigated.There is growing evidence of a relationship between decreased levels of selenium and various types of cancer.

Kidney disease

0696

INFLAMMATION, ENDOTHELIAL DISFUNCTION AND COAGULATIONSTATE IN END STAGE RENAL DISEASE HEMODIALYSIS PATIENTS

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Background. Accelerated atherosclerotic process in end-stage renal disease (ESRD) may involve several interrelated processes, such as oxidative stress, a constant low-grade inflammation, endothelial dysfunction , vascular calcification and alterations in coagulation. However, relationships between these processes in end stage renal disease hemodialysis patients are unknown. Objective of the study was to investigate the levels of inflammation markers [C-reactive protein (CRP)], of endothelial disfunction [as assessed by von Willebrand factor (vWf)] and coagulation state [as assessed by fibrinogen and D-dimer], in end stage renal disease hemodialysis.
Methods. A total of 21 patients, (meanage 47,5±16.6 years) in hemodialysis were enrolled to the study. Patients were compared with 20 healthy subjects matched for age and ethnicity. Coagulation and endothelial function parameters were measured in an automated analyser (ACLTop) and CRP with electroluminescence.
Results. D-dimer, vWf and CRP (all P< 0.01), but not fibrinogen were raised in hemodialysis patients compared with the healthy subjects. There was positive correlation between CRP and D-dimer (r=0,480, p=0,044) and between CRP and vWf (r=0,544, p=0,019) in hemodialysis patients .
Conclusions. 1) Raised plasma levels of D-dimer, vWf and CRP in patients compared with healthy subjects implies that inflammation, endothelial dysfunction and alterations in coagulation are present in end stage renal disease hemodialysis patients . 2) Positive correlations between CRP and D-dimer leads us to speculate that inflammation may be important in coagulation activation in these patients, or vice versa. 3) Correlation of vWf with CRP implies that inflammation may be responsible for endothelial disfuction.

0697

STUDY OF THE NEW PLATELET INDICES AND LEVELS OF HOMOCYSTEINE IN END STAGE RENAL DISEASE IN HEMODIALYSIS PATIENTS

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Background. Large platelets present greater functionality because produce more thromboxane A2 , are more easily aggregated and present increased expression of receptors on their membrane. Increased mean platelet volume (MPV) and platelet distribution width (PDW) have been connected to morbidity and cardiovascular mortality, especially MPV has been recognized as a risk factor for myocardial infarction. Hyperhomocysteinaimia has been identified as an independent risk factor of cardiovascular disease and of thromboembolic incidents. Aim of our study was the evaluation of the levels of new platelet indices (MPV, PDW) and homocysteine (Hcy) in end stage renal disease hemodialysis patients.
Methods. A total of 21 patients on dialysisfor end-stage renal disease (meanage 47,5±16.6 years) were enrolled to the study. Patients were compared with 20 healthy subjects matched for age and ethnicity. In all subjects MPV and PDW were calculated with SYSMEX XE2100 analyzer and Hcy using immunofluropolarimetry with AxSYM plus analyzer.
Results. Mean values of MPV, PDW and Hcy, in patients presented statistically significant difference (p<0,05) with that of the healthy group. A positive correlation was found between the values of MPV and of PDW (r=0,648, p<0,01) while no positive correlation was found between the value of Hcy and the value of MPV or that of PDW.
Conclusions. The combination of measurement of levels of Hcy in blood serum and of MPV and PDW may be useful for their evaluation as possible risk factors of cardiovascular incidents in end stage renal disease hemodialysis patients.

Kidney disease

0698

THE APPLICATION OF LABORATORY RESULTS IN CLINICAL MEDICAL PRACTICE: HOW WELL ARE INTERNATIONAL EXPERT GUIDELINES AND DECISIONS OF NATIONAL ASSOCIATIONS COORDINATED? AN EXAMPLE WITH ESTIMATED GLOMERULAR FILTRATION RATE (EGFR)

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Background. Confusion arises between statements like "…does automatic reporting of the eGFR…influence clinical practice?" (2010) and "…population–based screening for chronic kidney disease (CKD)…are not cost effective" (2011). Did the application of eGFR in clinical practice fail? Since 2009 the National Kidney Disease Education Program (NKDEP) strives for global standardisation.
Methods. Creatinine, freshly extracted from morning serum, was evaluated following Roche enzymatic method (crea-plus2) ID-MS traceable INTEGRA model 411(CI95%). 1912 patients, age 17-96 (827 male, 43,3%; 1085 female, 56,7%), were tested. Modification of Diet in Renal Disease (MDRD) study (with 5 equations including quadratic and Cockroft-Gualt) were compared with the new (2009) Levey CKD-EPI (CKD-Epidemiology Collaboration). Predictor variables such as serum creatinine, age, gender, were adjusted for BMI, body-weight, -size, -height, -surface, log(creatinine), log(age), log(eGFR).
Results. CKD-EPI uses two equations for each gender. eGFR for male patients correlated to MDRD study, whereas female eGFR were significantly different. Male: MDRD 86(22) CV25; CKD-EPI 86(24) CV28. Female: MDRD 116(27) CV27; CKD-EPI 89(22) CV24.
Conclusions. The prevalence of creatinine results within normorange shows its irrelevance to eGFR values. The so-called "creatinine blind range" is evidently false, causing a misuse of interpretation within normal range. Results exclusively within a defined cut-off are insufficient. A test allowing diagnostic as well as monitoring the progression of the disease and the response to therapy is essential in clinical diagnostics. eGFR costs little. The clinical evaluation of kidney function is part of the medical routine and eGFR is considered the best index for general health status.

0699

ESTIMATION OF GLOMERULAR FILTRATION RATE (GFR) IN CHRONIC RENAL FAILURE (CRF) PATIENTS WITH DIFFERENT CATEGORY OF BODY MASS INDEX (BMI)

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Background. There are several equations available for estimating GFR. The most of them are derived from serum creatinine level, such as Cockcroft-Gault (C&G), MDRD (Modification of Diet in Renal Disease), and CKD-EPI formula, and some of them derived from serum cystatin C. Obesitas may influence estimation of GFR by these equations. Aim of this study was the evaluation of BMI impact on GFR estimation using different equations.
Methods. Serum cystatin C (immunoturbidimetric method), serum creatinine and creatinuria (standard biochemical metods), were measured in 46 CRF patients with BMI 25-29.9 kg/m2, and 27 CRF patients with BMI<25 kg/m2,and in 27 CRF patients with BMI>30 kg/m2. In all subjects we calculated C&G, MDRD, CKD-EPI, cystatin C-based GFR equation and creatinine clearance (CrCl).
Results. In patients with BMI>30 kg/m2 the GFR estimated by C&G was significantly higher than GFR estimated by CrCl (90.2 ± 33.1 vs. 70.2 ± 29.9, p=0.027). The mean level of CKD-EPI, MDRD and cystatin C based equation were 64.7± 23.3, 63.4 ± 21.2, and 74 ± 32.5 mLx min–1 x (1.73 m2)–1 respectively. In patients with BMI <25 kg/m2 and BMI 25-29.9 kg/m2 there were not significantly differences beetwen levels of GFR estimated by different equations and by CrCl.
Conclusions. In CRF patients with BMI>30 kg/m2 C&G formula was overestimate GFR (compared to CrCl), while MDRD, CKD-EPI, and cystatin C-based equation were not.

Kidney disease

0700

IMPROVEMENT OF IOHEXOL DETERMINATION BY USING POLYMERICALLY BONDED AND DOUBLE END-CAPPED COLUMN IN RENAL AND LIVER DISEASES PATIENTS

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Background. Several investigators have developed HPLC methods to determine serum iohexol, but the chromatograms of iohexol using prior techniques show two peaks overlapping of iohexol isoforms. Improvement of the method may give better data integration and minimize interference. We evaluated and compared a new RP column, Alltech altima C18, with a previously described standard column for serum iohexol determination.

Methods. Serum samples spiked with iohexol ex-vivo were extracted and separated by HPLC using 2 different columns. The performance of the standard separation method using a single end-capped column, Nucleosil C18 column, was compared to polymerically bonded and double end-cap column, Alltech altima C18 column. Effects of interference from high creatinine and bilirubin concentrations obtained from renal and liver diseases were assessed.

Results. Retention times of Alltech altima, and Nucleosil were 9.41 and 9.32, respectively. Alltech altima produced the best characteristic chromatogram and symmetry peak. Resolution of A and B isoforms of iohexol for both columns were 1.5 and 0.9, respectively. The recovery of Alltech altima and Nucleosil ranged from 99.85 ± 0.20% to 100.33 ± 2.32% and 93.29 ± 2.46% to 100.19 ± 1.09%, respectively. Intra-assay and inter-assay variations ranged from 0.94% to 1.54% and 2.58% to 5.42%, respectively. For Alltech altima, samples with creatinine >7.0 mg/dL and bilirubin >27.8 mg/dL were not influence on chromatograms and percent recoveries (p> 0.05).

Conclusions. Using double end-capped column is more suitable for iohexol determination than single end-capped column, especially in renal and liver disease patient samples. This column produces simple, reliable, precise and accurate method and got clear resolution chromatogram.

0701

QUANTITATIVE DETERMINATION OF HUMAN PLASMA AND URINE NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) VALIDATION OF AN AUTOMATED TURBIDIMETRIC IMMUNOASSAY

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Background. Acute Kidney Injury (AKI) is a major clinical problem in hospitalized patients. Currently, the most used method of detecting early signs of AKI is serum creatinine which is an unreliable indicator during acute changes in kidney function. Recently NGAL and other tubular markers have been suggested as a valuable alternative to serum creatinine in the early management of AKI showing increased expression and excretion in urine.

In the present study a sensitive automated turbidimetric immunoassay for fast determination of human plasma and urine NGAL was validated.

Methods. Immune complexes were formed, by mixing antigen and Mouse anti human NGAL covalently conjugated to polystyrene particles. The resulting changes in transmitted light, was quantitied by a Hitachi 917 analyser. The measuring range was 25 – 5.000 ng/mL. Calibration was performed using a 6 point calibration curve.

Results. The estimated within and total imprecision were below 5.5% and 6.0%, respectively. Analysis of serial dilutions of a urine pool in the range 72 – 4834 ng/mL showed good linearity (less than 10% deviation from the expected value) in the assay. Detection limit was estimated to 12 ng/mL. The security range was tested up to a concentration 40000 ng/mL without erroneous Results.

Conclusions. This assay represents an automated, fast and precise alternative to other immunoassays for determination of human NGAL in plasma and urine and may prove a valuable tool in the early diagnosis of AKI.

Kidney disease

0702

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN(NGAL) AND ACUTE KIDNEY INJURY AFTER ABDOMINAL AORTIC SURGERY

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Background. Acute Kidney Injury (AKI)occurs frequently after abdominal aortic surgery. Although serum creatinine (Screatinine) is routinely used as a marker of renal function, it isn't accurate in detecting acute changes in glomerular filtration rate. Many studies showed that NGAL is an effective biomarker of AKI.
Methods. Serialsamples of blood and urine were obtained from 25 patients undergoing both open and endovascular surgical repair of abdominal aortic aneurysm. Screatinine and urinary NGAL (uNGAL) levels were measured at baseline, at 4 hours, and at 18 hours after time 0 (aortic declamp or intra-operative administration of iodinate contrast). uNGAL was determined by a chemiluminescent microparticle immunoassay (ARCHITECT i1000SR®, Abbott Diagnostics), Screatinine was determined by a colorimetric assay, Jaffé reaction (Cobas c501, Roche/Hitachi). AKI was defined according to the RIFLE criteria (increase by 50% in Screatinine or reduction of at least 25% of GFR from baseline). uNGAL levels were compared between patients with and without postoperative AKI.
Results. 7 patientsdeveloped AKI. Screatinine values were: at baseline, 0.87 ± 0.17 mg/dl (non-AKI) and 0.90 ± 0.11 mg/dl (AKI), p=0.628; at 4 hours, 0.82 ± 0.20 mg/dl (non-AKI) and 0.91 ± 0.24 mg/dl (AKI), p=0.337; at 18 hours, 0.88 ± 0.21 mg/dl (non-AKI) and 1.29 ± 0.18 mg/dl (AKI), p=0.0004. uNGAL values were: at baseline, 24.8 ± 26.1 ng/ml (non-AKI) and 11.3 ± 7.6 ng/ml (AKI), p=0.059; at 4 hours, 12.3 ± 9.3 ng/ml (non-AKI)and 7.7 ± 5.8 ng/ml (AKI), p=0.667;at 18 hours, 18.1 ± 16.0 ng/ml (non-AKI) and 41.6 ± 41.8 ng/ml (AKI), p=0.193.
Conclusions. Our results doesn't lead to affirm that uNGAL, after abdominal aortic surgery, provides additional information with respect to Screatinine.

Kidney disease

0703

URINARY PROTEINS AS BIOMARKERS OF CHRONIC KIDNEY INJURY

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Background. Proteinuria is one of the most commonly used markers of kidney damage. High excretion of albumin suggests increased glomerular permeability and/or tubular damage. The excretions of alpha-1-microglobulin (A1M), b-2-microglobulin (B2M), cystatin C (CYSC) and beta-trace protein (BTP) have been used in detection of proximal tubular injury.
Methods. The aim of this study was to investigate and to compare the clinical usefulness of the urinary proteins (albumin, A1M, B2M, CYSC and BTP) for detection of renal injury in 179 patients with different stages of chronic kidney disease (CKD). These proteins were measured by immunonephelometry in 24-hour urine samples.
Results. Comparison analysis (Mann-Whitney U-test) showed that concentrations of urinary proteins were significantly higher in diabetic nephropathy than in other disease groups. BTP and CYSC significantly increased from CKD stage 2 to stage 5, A1M and B2M from CKD stage 3 to stage 5 and albumin only showed significant increase in end stage of CKD. Correlation analysis (Spearman) showed the highest value among A1M, CYSC and BTP (coefficients were: 0.793-0.899) and very low values between albumin and tubular proteins. ROC analysis showed high diagnostic accuracy of all proteins except for albumin for detection of reduced renal function in CKD stages (for GFR <60 mL/min/1.73 m2). AUCs were: 0.869, 0.861, 0.825, 0.806 and 0.650 for A1M, BTP, CYSC, B2M and albumin, respectively.
Conclusions. The results of this study showed that A1M, CYSC and BTP may be useful and reliable urinary biomarkers for identifying magnitude of renal dysfunction in chronic kidney injury.

0704

LIPID PEROXIDATION EVALUATION IN HEMODIALYSIS PATIENTS UNDER SUPPLEMENTATION THERAPIES

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Background. Oxidative stress is common in hemodialysis (HD) patients. The aim of the clinical trial was to consider the oxidative stress by lipid peroxidation level in HD patients during the supplementation therapies.
Methods. A number of 82 HD patients were divided in 4 groups: I - control group; II - on L-carnitine therapy; III - on erytropoietin therapy and IV - both therapies, L-carnitine and erytropoietin. In a period of 6 months, supplementation therapies were: Erytropoietin (Epreg-Cillas-Janssen) was given s.c. after HD, 20-50 U/kg weekly and L-carnitine (Sigma-Tau) was given i.v. after HD, 1gr. Lipid peroxidation assay was performed by modified fluorimetric method by using the end product malonildialdehyde. For L-carnitine enzymatic UV method was used (Roche Diagnostic GmbH). The patients were evaluated at 0, 3, and 6 months.
Results. Lipid peroxidation showed decreased levels in both examined groups: in II group from 4.46±1.1mmol/L - 0 month to 4.10±0.6mmol/L - 6th month and in III group from 4.63±0.8mmol/L - 0 month to 4.26±0.9mmol/L - 6th month. However statistically decreased value was found only in the IV group from 4.70±0.8 mmol/L - 0 month, 4.40±0.6mmol/L - 3th month, to 4.10±0.4mmol/L - 6th month (p<0.05). L-carnitine increased in II group from 5.3±1.7mg/L - 0 month to 16±5.7mg/L - 6th month (p<0.01) and in IV group from 4.62±2mg/L - 0 month to 15.9±6.1mg/L - 6th month (p<0.01).
Conclusions. We may conclude that erythropoietin and L-carnitine supplementation therapies have synergistic effect on decreasing oxidative stress level in HD patients.

Kidney disease

0705

THE HAPTOGLOBIN PHENOTYPE DETERMINES THE RISK OF CUTANEOUS SQUAMOUS CELL CARCINOMA IN KIDNEY TRANSPLANT PATIENTS

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Background. Skin cancer is a major dermatological health problem in renal transplant patients causing substantial morbidity. Cutaneous squamous cell carcinoma (SCC) is the most frequent skin cancer after organ transplantation. This study investigated the influence of the haptoglobin phenotype on the development of non-melanoma skin cancer in 291 kidney transplantation patients with a median follow-up time of 94 months [interquartile range: 39.5- 159].
Methods. Haptoglobin phenotyping was performed on hemoglobin (Hb)-supplemented starch gel electrophoresis. High performance gel permeation chromatography was used in case of low Hp concentrations.
Results. Kaplan-Meier analysis and Cox regression analysis (adjusted for age, gender and mediterranean origin) showed a significant association of the Hp 1-1 fenotype with a higher risk of multiple primary SCC's ($p<0.001$ and $p=0.006$, respectively). Kaplan-Meier analysis for the development of SCC showed no significant difference in the Hp phenotype in the first 10 years following renal transplantation. However, after 10 years Kaplan-Meier and Cox regression analysis showed a significant association between the Hp 1-1 phenotype and the occurrence of SCC ($p=0.008$ and $p=0.003$, respectively).
Conclusions. Renal transplant patients with the Hp 1-1 phenotype are at increased risk for the development of (multiple) SSC. This finding is in agreement with the decreased inflammatory capacity of the Hp 1-1, favouring persistent infection with oncogenic viruses and may be an important predictor in identifying a subset of patients at increased need for preventive measures.

0706

INVESTIGATION OF URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) AND CYSTATIN-C (CYS-C) AS MARKERS FOR HEMOFILTRATION THERAPY IN SEPTIC PATIENTS OF AN INTENSIVE CARE UNIT

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Background. Post-surgical patients in intensive care may develop sepsis and acute renal failure requiring intermittent hemofiltration. We investigated whether urinary NGAL, a new marker of acute kidney injury, or Cys-C could serve as indicators of imminent hemofiltation therapy.
Methods. 31 consecutive patients of a surgical intensive care unit were examined. 23 patients developed sepsis as defined by clinical criteria and/or procalcitonin (PCT) > 0,5 ng/ml, 7 patients had acute renal failure stage 3 according to AKIN criteria resulting in hemofiltration therapy, 6 of these were septic. Urinary NGAL (Abbott) and Cys-C (Siemens) were examined in relation to subsequent hemofiltration treatment. Duration of intensive care was up to 100 days.
Results. In the study group as a whole urinary NGAL was in the range of 15-19,761 μg/g creatinine (median: 356 μg/g), Cys-C was 0.47-2.76 mg/l (median: 1.18 mg/l) and PCT was 0.00-106.0 ng/ml (median: 3.73 ng/ml), none of these were correlated with each other. Subjects with sepsis and hemofiltration therapy had NGAL values of 121-19,761 μg/g (median: 214 μg/g) prior to intervention as compared to 19-9,758 μg/g (median: 1,323 μg/g) in septic patients without hemofiltration ($p=0.98$, n.s., Mann-Whitney). Cys-C concentrations were 0.77-2.70 mg/l (median: 1.04 mg/l) and 0.47-2.76 mg/l (median:1.13 mg/l), respectively. In non-septic patients NGAL was 35-451 μg/g (median: 79 μg/g) ($p= 0.006$ compared with all septic patients).
Conclusions. Data from this small study indicate that urinary NGAL is increased in sepsis but, in the presence of sepsis, does not correlate with acute renal failure.

Kidney disease

0707

A COMPERATIVE ANALYSIS OF THE IRIS IQ200 WITH MANUAL LIGHT MICROSCOPY AS A DIAGNOSTIC TOOL FOR DYSMORPHIC ERYTHROCYTES IN URINE

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Background. Microscopic analysis of erythrocytes in urine is a valuable diagnostic tool for identifying potential glomerular hematuria. Indicative of glomerular hematuria is prevalent amounts of cellular casts and poly- and dysmorphic erythrocytes (DE) whereas in nonglomerular hematuria, erythrocytes are mono- and isomorphic, without cellular casts. Manual microscopy has been the prevailing technique for examining DE. This labor intensive method requires significant expertise to ensure consistent and accurate analysis. The aim of the study was to investigate the use of the iQ200 as an automated platform for screening of DE.
Methods. 250 urine specimens with suspected DE were fixed and initially examined using manual microscopy. The same specimens were then processed with the iQ200 using the cell-recognition function for classifying dysmorphic eyrthrocytes.
Results. Our findings show that the iQ200 was able to accurately identify specific pathological DE notably acanthocytes (G1-or D1- and D2-cells) . However, the iQ200 was not able to recognize D3 cells and other dysmorphic forms. The iQ200 images did not provide sufficient resolution to distinguish these cell types.
Conclusions. The iQ200 has proven to be an efficient and reliable asset for our routine urinalysis. It was able to detect notable specific pathology dysmorphic cells but excluded other forms of DE. Further investigations warrant inclusion of the iQ200 using more standardized criteria when comparing with manual microscopy. Despite its current draw-backs, with software improvements to enhance the image quality, in time the iQ200 could prove to be of value for high throughput analysis of all types of DE.

0708

CONCENTRATION OF SERUM PARATHORMON AT HEMODIALYSIS PATIENTS

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Background. Hyperparathyroidism occurs at a large number of patients in advanced phase of chronic renal insufficiency because of the lack of or insufficient activity of vitamin D active form. Parathyroid glands are stimulated to free PTH which is a consequence of the low level of circulating calcium. The aim of this paper is examining the influence of the hemodialysis duration to the frequency and degree of hyperparathyroidism.
Methods. The examination included 80 patientsof the average age of 56, who were approximately 66 months (1-27 years) at a chronic dialysis programme. Among the biochemical parametres determined from the serum taken before the dialysis, PTH was done (with the referent value of 10±69 pg/ml), calcium and phosphate with the use of commercial tests produced by "Siemens".
Results. Hyperparathyroidism is present at 38% (x =180±69pg/ml) of the patients with less than 5 years on dialysis (N=35), while in the group of the patients with more than 5 years of dialysis, 60% had the value of PTH above the referent (x =320±81 pg/ml), that is, 15% of the examinees had the value higher than 400 pg/ml and the necessity of parotidectomy had to be taken in consideration.
Conclusions. PTH enlarges osteoblast and osteoclast activity; the creation and reasorption of bones is accelerated and makes a picture of osteitis fibrosa, which can cause skeleton deformities or pathologic bone breaks that occur at 2-45% of the patients with more than 5 years on hemodialysis. This is influenced by the appropriate therapy, discipline of the patients during the use of the medicines as well as the quality of the hemodialysis.

Kidney disease

0709

UNDERCARBOXYLATED AND CARBOXYLATED OSTEOCALCIN IN RELATION TO BONE MINERAL DENSITY AND CALCIFICATION SCORE IN END STAGE RENAL DISEASE PATIENTS

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Background. Osteocalcin, a vitamin K dependent protein is expressed by osteoblasts. Its undercarboxylated (ucOC) form is considered a marker of vitamin K status. Osteocalcin concentrations are highly elevated in hemodialysed patients. It is reported that increased circulating ucOC is associated with osteoporosis and vascular calcification. We evaluated the relationship between serum carboxylated osteocalcin (cOC), ucOC and bone mineral density (BMD) and calcification score (CaSc) in hemodialysed patients.
Methods. We included 68 hemodialysed patients, 29 women, 39 men. Serum cOC, ucOC were measured by ELISA (TAKARA, Japan) and iPTH using Nichols method. CaSc was assessed using multi-slice spiral computed tomography and BMD by dual energy X-ray absorptiometry.
Results. In the whole studied group we observed negative correlation between ucOC and BMD of femoral neck (r=-0,37, p=0,0035) and positive correlation with CaSc (r=0,34; p=0,0051) without any correlations neither between cOC and BMD and CaSc nor between cOC, ucOC and BMD of lumbar spine. Additional analysis showed the correlation between ucOC and BMD (r=-0,38; p=0,025 and between ucOC/cOC ratio and BMD of femoral neck (r=-0,52; p=0,0017) and BMD of lumbar spine (r=-0,37; p=0,0297) in men without correlation between any osteocalcin form and CaSc. In women we found the correlation between ucOC (r=0,50; p=0,0068), cOC (r=0,38; p=0,0486), ucOC/cOC (r=0,47; p=0,0118) and CaSc and the only correlation between ucOC/cOC and BMD of femoral neck (r=-0,43; p=0,0240).
Conclusions. Serum undercarboxylated or carboxylated osteocalcin is strongly elevated in hemodialysed patients and associated either with bone mineral density or with vascular calcifications in those patients depending on gender.

0710

THE RELATIONSHIP BETWEEN CARBOXYLATED OR UNDERCARBOXYLATED OSTEOCALCIN AND LEPTIN LEVELS IN HEMODIALYSIS PATIENTS DEPENDS ON GENDER AND PTH CONCENTRATIONS

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Background. Osteocalcin, present either as carboxylated (cOC)or as undercarboxylated (ucOC)form is a non-invasive marker of osteoblast activity and bone formation. Fat cell-derived leptin via sympathetic tone stimulates carboxylation of osteocalcin and in that way inhibits its hormonal activity. In animal studies leptin and osteocalcin regulate energy metabolism. The aim was to assess the relationship between osteocalcin and leptin serum concentrations in hemodialysis patients.
Methods. We included 57 hemodialysed patients, 24 women, 33 men, 23 subjects with intact PTH ≤300 pg/ml and 34 subjects with intact PTH >300 pg/ml, i.e. with secondary hyperparathyroidism. cOC, ucOC were measured by ELISA (TAKARA, Japan), leptin by ELISA (R&D Systems) and iPTH using Nichols method.
Results. There was increase in cOC and ucOC serum concentrations in end stage renal disease patients as compared to reference values in humans. We observed statistically significant correlation between cOC and leptin in hemodialysed men (r=-0,31; p=0,0443) and between cOC, ucOC and leptin in hemodialysed patients with iPTH>300 pg/ml (r=-0,36; p=0,0187 and r=-0,39; p=0,0097 respectively). No such correlations in the whole studied group were found.
Conclusions. Our results may confirm the existence the axis between bone and adipose tissue in hemodialysis patients. Additionally it should be stressed that those relationships strongly depend on sex and development of secondary hyperparathyroidism.

Kidney disease

0711

IMMUNOLOGICAL MONITORING AFTER KIDNEY TRANSPLANTATION: RESEARCH TO IMPROVE DIAGNOSIS OF REJECTION AND TO ESTIMATE RISK OF INFECTION

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Background. Not early enough treated immunological complications like infections and rejections can important compromise the success of kidney transplantation. For this reason a sensitive diagnosis is very important. The eligibility of selected cytokines and marker of inflammation to detect this complications was evaluated.

Methods. 32 patients was involved in our study. The determination of the serum parameter soluble interleukin 2-receptor (IL-2R), interleukine 10 (IL10) and CD30 as well as the urinary interleukine 6 (IL6), interleukine 8 (IL8), myeloperoxidase (MPO) and monokine induced by interferon gamma (MIG) carried out before transplantation and consecutively biweekly first month, than once monthly till 3 months after. A total of 314 serum and 309 urinary patient probes were obtained.

Results. The studied laboratory parameter contributed to diagnosis of complications in different way. Rapid decline of IL-2R, CD30 and MIG as well as no rise of IL10, IL6, IL8 and MPO characterized a uncomplicated course after transplantation. Rising concentrations of IL-2R and CD30 accompanied systemic bacterial and viral infections. Viral infections maybe cause a specific IL10-increase. UTI`s cause pathological values of urinary IL6, IL8 and MPO. There are no UTI without rising IL8 and MPO. To detect rejection crisis urinary MIG is superior to the other parameters, but it is not specific. Also rising MIG-level were detected during periods of systemic bacterial and viral infections.

Conclusions. Preoperative determined values of the tested laboratory parameters didn`t give a reference to an individual risk of transplant rejection or to the future transplant function. Unlike reported, MIG isn`t a specific marker of transplant rejection.

0712

VALUE OF URINARY NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) AS BIOMARKER OF ACUTE KIDNEY INJURY AFTER KIDNEY TRANSPLANTATION: A PILOT STUDY

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Background. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is produced in the distal nephron and its synthesis is up-regulated in response to kidney injury. The purpose of this pilot study was to evaluate the use of urinary NGAL the biomarker of acute kidney injury (AKI) in kidney transplant recepients.

Methods. Urinary NGAL was measured by chemiluminescent microparticle immunoassay –CMIA (Architect analyzer; Abbott Diagnostics). Verification of performance for precision was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) Document EP15-A2. In the low concentration level the obtained within-laboratory precision was 12.2% and 1.8% in the high concentration level, respectively. Diagnosis of AKI was based on renal biopsy as a gold standard for establishing the diagnosis. In this pilot study 61 protocol (at 1, 3, 6 and 12 months) or indication kidney biopsies were performed in 37 adult patients after kidney transplantation.

Results. Acute kidney injury was diagnosed in 33 biopsies while in 28 biopsies did not. Highly increased urine NGAL concentrations (79.7-486.8 μg/L) were found in 21% of cases with AKI, moderately increased concentrations (above 10 μg/L) in 58% and below 10 μg/L in 21% of cases, respectively.

There were no association between urinary NGAL concentrations (3.2- 154.0 μg/L) and tubular atrophy and interstitial fibrosis in kidney transplant recipients.

Conclusions. NGAL is a biomarker of AKI in kidney transplant recipients. However more data are needed to determine whetherthe concentration measurement of urinary NGAL could lead to accurate noninvasive diagnosing of acute kidney injury after kidney transplantation.

Kidney disease

0713

COMPARATIVE ASSESSMENT OF CALCIUM TO CREATININE, OXALATE TO CREATININE, AND CITRATE TO CREATININE RATIOS IN RANDOM MORNING URINE SAMPLES OF RECURRENT URINARY STONE FORMERS AND THEIR CONTROLS; A CASE-CONTROL STUDY

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Background. Urolithiasis is a common disease in Iran and other parts of the world. Urinalysis is at cornerstone of its diagnosis and treatment. Determining and calculating different ratios and combined indices of urine electrolytes from random morning urine samples, can be a useful method besides 24-hour urine specimen analysis, if we can show there is a difference between patients and normal controls.
Methods. In a case-control study with 106 recurrent calcium stone-former males from Urolithiasis Clinic of Labbafinezhad Hospital (Shahid Beheshti Medical University, Tehran, Iran) and 109 controls, random morning urine calcium, creatinine, oxalate, and citrate were assessed in the laboratory and the difference between these ratios (Uca/Ucr; Uox/Ucr; Uci/Ucr) in 2 groups was tested statistically.
Results. There was a statistically significant difference in serum total calcium between 2 groups (P=0.02); but no statistically significant difference was seen between serum ionized calcium (P=0.37). Mean calcium, oxalate, and citrate levels in random morning urine sample showed no differences (P=0.3, 0.6, 0.2 respectively); but after calculating ratios, there was statistically significant difference between these ratios (Uca/Ucr; Uox/Ucr; Uci/Ucr) in 2 groups (P=0.006, 0.017, 0.001 respectively) and the upper limits for the ratios (using 97% percentiles) were 0.18, 0.004, and 6.03 respectively.
Conclusions. Determining these ratios helps us to diagnose possible cases of (recurrent) urolithiasis. Because of difficulties that patients encounter in collecting 24-hour urine samples, analysis of random morning urine sample can be a helpful replacement. Physicians can use upper limits calculated as 97% percentiles to guide assessments for diagnosis and treatment.

0714

COST-EFFECTIVENESS OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN FOLLOWING CARDIAC SURGERY

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Background. Acute Kidney Injury (AKI) is a serious postoperative complication of Coronary Artery Bypass Grafting (CABG) and an early diagnosis of AKI may improve outcome. uNGAL is a novel biomarker for the early detection of AKI. Objectives were to compare cost-effectiveness of uNGAL+Standard Monitoring (uNGAL-SM) vs. SM alone in CABG patients in Italy. Diagnostic schemes were:1) uNGAL: 5 consecutive determinations within the first 36h; 2) SM consisting of serum creatinine and clearance, sodium and urea tests: 2 assays in the first 24h, then 1set per day of hospitalisation.
Methods. A decision model was adapted based on probabilities of developing AKI (RIFLE criteria), progression to chronic kidney disease, post-discharge recovery of renal function and death, decreased life expectancy, direct medical costs and patient health utility values. Treatment included medical support and renal replacement therapies. The basecase considered a 71 year old male and assumed that 25% of risk cases are prevented from progressing AKI. The model included data from literature and Italian experts' interviews. Univariate and probabilistic sensitivity analyses were performed.
Results. Expected costs were: €1,399 for uNGAL-SM vs €1,501 for SM alone; expected QALYs were 10.26 vs 10.22, thus resulting in €136/QALY for uNGAL-SM and €147/QALY for SM. uNGAL-SM was dominant in all risk reduction scenarios. Sensitivity analyses showed that the model is very robust to variations of all variables tested with the exception of patients' life expectancy.
Conclusions. uNGAL is an effective and cost-effective biomarker to be possibly recommended in the early diagnosis of AKI after CABG.

Kidney disease

0715

PLASMA LEVEL OF ATRIAL NATRIURETIC PEPTIDE (1-28) IN PATIENTS WITH SEVERE HEMORRHAGIC FEVER WITH RENAL SYNDROME

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Background. Atrial natriuretic peptide (ANP 1-28) significantly influences the function of kidneys and cardiovascular system. Both systems are considering most damaged at hemorrhagic fever with renal syndrome (HFRS) – pathology associated with Hantaviral infection.
Methods. The plasma level of ANP 1-28 was measured using ELISA (Peninsula Inc.) in 33 mail patients suffering from severe HFRS and 12 healthy men.
Results. The observed levels of ANP (Me; P25-P75) in HFRS patients were: oliguric phase (n=13) - 55.1 ng/ml (36.4–75.7); polyuric phase (n=11) – 33.0 ng/ml (25.9–49.4); early reconvalescence phase (n=9) – 52.8 ng/ml (49.4–54.5). In healthy men (n=12) the ANP level comprised of 85.4 ng/ml (79.0–94.3). The ANP decrease was statistically significant in all HFRS phases – oliguric (U=30.0; Z=2.6; p=0.009), polyuric (U=0.0; Z=4.1; p=0.000049) and early reconvalescence (U=0.0; Z=3.8; p=0.00012). Perhaps, atrial tissue hemorrhages usually found at severe HFRS may cause rapid release of ANP into blood flow in early phases of HFRS and at the same time lead to subsequent decline in peptide synthesis and secretion.The general decrease of plasma ANPconcentration may also be related to raise of the plasma protease activity in cytolysis and DIC.
Conclusions. The decrease of ANP 1-28 plasma level observed may be of some pathogenetic role in development of water-salt metabolism disordersatsevere HFRS. Anyway it should be further investigated, as well as ANP possible role as a marker of HFRS phase and severity.

0716

CAN BE THE RISK OF RENAL DAMAGE PROGRESSION ASSESSED USING SOME EXCRETED URINE PROTEINS?

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Background. Thedegreeoftubulointerstitialfibrosisisoneofthemainprognosticfactorsofkidneydamageprogression. Itcanbeassessedbyhistologicalfindings in renalbiopsy. Someexcreted urine proteinswereevaluatedas a potentialmarkerofinterstitialfibrosis.
Methods. 43 patients,age 22 to 75yearswithchronicalglomerulardiseasesundergone to renalbiopsydividedinto 4 groupsto a 4-pointsemiquantitativescaleon base ofhistologicalfindings in renalbiopsy (1-none tubularatrophyorfinterstitialfibrosis, 2-minimal, 3-mild, 4-atrophy > 50% orgreatfibrosis). Urine total protein (protein-creatinine-ratio, PCR), albumin (albumin-creatinine-ratio, ACR), alpha-1-microglobulin (A1m), serum and urine creatinineweremeasured, eGFR (CKD-EPI) and A1m/Albumin ratio as a laboratorymarkerofinterstitialfibrosiscalculated.Thelaboratory and histologicalfindingswerecompared in groups and evaluated (ANOVA, Mann-Whitney and T-tests and Pearson and Spermancoefficientsofcorrelation).
Results. significantdifferences in medianvalues A1m/Albamonggroupswereobserved not onlyifcomparedgroupswithlesssignificant (Group 1+2) to more significanthistologicalfindings (Group 3+4): 0,017 in comparison to 0,050, (P < 0,005), as among single groups: 0,053 (Group 4), 0,026 (Group 3), 0,018 (Group 2), 0,013 (Group 1), P variedfrom< 0,05 to < 0,001. Strongcorrelationsbetweenhistologicalfindings and A1m/Alb (P < 0,001) andbetweenhistologicalfinding and eGFR (P < 0,001)werefound, ingeneral. Correlationbetween A1m/Alb.andagewas not significant (P=0,905).
Conclusions. A1m/Alb in urineappeared as a laboratorypredictorofinterstitialfibrosisdegreeand couldbeused in clinicalpractice.

Kidney disease

0717

IS THERE A ROLE FOR NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) IN THE DIAGNOSIS OF THE BIPHOSPHONATES NEPHROTOXICITY?

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Background. biphosphonates are nephrotoxic drugs and renal function should be monitored for acute renal injury (AKI) recognition during intravenous treatment. NGAL is a promising biomarker for AKI. Aim of this work is to evaluate the usefulness of NGAL measurements during treatment for osteoporosis.
Methods. plasma and urine NGAL were measured in 31 patients treated with intravenous zolendronate, at the following time points: T0 (before treatment), T1 and T2 (5 hours and 7 days after the treatment). P-NGAL was measured by POCT NGAL test (Biosite); U-NGAL was measured on Architect (Abbott). P-Creatinine and P-Cistatin C and U-albumin, U-alpha-1-microglobulin and U-creatinine were also measured.
Results. one patient showed an increase in P-Creatinine >60% and so was diagnosed as having AKI. P-NGAL concentrations were all within the cut off (149 ng/mL), the AKI patient included. U-NGAL concentrations were above the cut off limit (132 ng/mL) in 9 patients, in one or another of the time points; three of them showed an elevated U-NGAL at T0. The patient with AKI showed a P- NGAL value close to the cut off limit (155 ng/mL at T2). The other plasma and urine biomarkers were abnormal only in the AKI patient; in particular, U-albumin and U-alpha-1-microglobulin were normal in patients with high U-NGAL.
Conclusions. in this clinical setting, NGAL did not prove to be an early and sensitive marker of AKI. The study has two main limitations: the low number of the examined patients and the possible not appropriate choice of the time points.

0718

THE FIRST HARMONIZATION STEP ON CYSTATIN C, WITH THE AIM TO INTRODUCE THE FIRST ERM-DA471/ IFCC CYSTATIN C CALIBRATOR AND ONE WORLDWIDE COMPANY-INDEPENDENT EGFR EQUATION

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Background. The first Certified Reference Material for cystatin C (ERM-DA471/IFCC) was releasedJune 2010. However, this is just the first step in the standardization of all commercially available assays and the cystatin C based GFR estimation.
Three assays were selected as the most comparable based on linearity studies: Gentian, Sentinel and Siemens:
• Siemens vs Sentinel $Y = 0.9814X + 0.1257$, $R^2 = 0.9989$
• Gentian vs Sentinel $Y = 0.787X + 0.1035$, $R^2 = 0.9994$
• Gentian vs Siemens $Y = 0.8725X + 0.1133$, $R^2 = 0.9985$
Based on our knowledge of the different assays, we know that there was a calibration-shift for Siemens between 2006 and today.
Results. After optimization of the set-up of the Gentian and Sentinel assays we found alinear equation: Gentianvs Sentinel $Y = 1.066X-0.08$. After additional fine-tuning,the equation was: Gentian vs Sentinel $Y = 1.025X - 0.049$, $R^2 = 0.9996$, with CV below 2%.
Conclusions. Thus, the Sentinel results at a cystatin C concentration of 1 mg/L will only be 0.024 mg/L higher than the Gentian and at 2 mg/L almost identical cystatin C results will be found.
We have clearly shown that it is achievable to reach an excellent comparability between different commercial cystatin C assays.
Our aim is to help producers of cystatin C assays to reach the same type of comparability as shown above.
Our next step will be to produce a very well documentedcompany-independent equation for cystatin C based estimation GFR in relation to 3000 Iohexol GFR determinations.

Kidney disease

0719

CROSSLINK OF LIPOPROTEIN (A) WITH INFLAMMATION, D-DIMER AND VON WILLEBRAND FACTOR IN PATIENTS ON LONG TERM HEMODIALYSIS

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Background. Haemodialysis patients are at an increased risk of cardiovascular mortality. Inflammatory and procoagulant markers are potentialmediators for the cardiovascular risk of kidney disease. We investigated the relationship between Lp(a), as an important risk factor for cardiovascular disease , inflammation and homeostatic variables in hemodialysis patients.
Methods. In 40 hemodialysis patients (22 male and 18 female), we determined Lp(a), CRP as a sensitive marker of inflammation,D-dimer, a marker of intravascular coagulation, von Willebrand factor, a marker of intravascular injury, fibrinogen and serum albumin. The same parameters, were determined in 40 healthy people (20 male and 20 female), as a control group.
Results. The mean Lp(a) level was increased in hemodialysis patients compared with controls (25.42 mg/dl versus 18.18 mg/dl). Among hemodialysis patients, 32% had an Lp(a) level higher than 30 mg/dl, which was significantly higher than the percentage in the control group (10%). Serum concentration of CRP, fibrinogen, D-dimer and von Willebrand factor were significantly higher (37,44 mg/L versus 10,9 mg/L; 3.88g/L versus 2.25g/L; 2.28 µg/ml versus 0.50µg/ml; and 173.9% versus 85,55%, p<0.001) whereas the concentration of serum albumin was significantly lower in hemodialysis patients than in controls (27,81 g/L versus 40,50 g/L; p<0.001). Lp(a) correlated positively with CRP (r=0.63), fibrinogen (r=0.61) and D-dimer (r= 0.64) and negatively with serum albumin (r= - 0.58), in group of patients but not in healthy controls.
Conclusions. These results indicate that the Lp(a) is closely related to the acute phase reaction and hypercoagulability in patients on long term hemodialysis.

0720

FRACTION EXCRETION OF SODIUM AS A MARKER OF TUBULAR INJURY – IS IT SENSITIVE ENOUGH?

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Background. The only promptly available test for early diagnosis of acute kidney failure, so far, is fraction excretion of sodium (FE_{Na}). Recent studies identified neutrophil gelatinase-associated lipocalin (NGAL) as a promising biomarker for such state. The aim of our study was to compare diagnostic usefulness of those analytes in acute tubular impairment caused by bacterial urinary tract infection in children.
Methods. We analyzed 25 children with static scintigraphy confirmed diagnosis of acute pyelonephritis. Serum and urine sodium were measured by ISE, serum creatinine by enzymatic method and urine creatinine by Jaffe's method (OLYMPUS AU640, BECKMAN COULTER) in order to calculate FE_{Na}. Urine NGAL measurement was performed by CMIA method (ARHITECT i1000, ABBOTT).
Results. FE_{Na} values were in range from 0,040% to 0,795% (median 0,290), which, according to the literature, are all normal values. NGAL values were in range from 17,9 ng/ml to 1768,4 ng/ml (median 205,3), which, according to expected normal range of used assay (131,7), points to acute tubular injury for in 60% of patients.
Conclusions. Although FE_{Na} is in use as a test for diagnosis of acute renal failure, it is not sensitive enough for detection of minor damage presented in early stage of acute kidney injury. On the other hand, NGAL revealed better sensitivity, and seems to be a better biomarker of minor tubular injury.

Kidney disease

0721

ASSAY-DEPENDENCY OF CREATININE CLEARANCE AND COMPARISON WITH CKD-EPI ESTIMATED GLOMERULAR FILTRATION RATE FORMULA

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Background. Besides the use of estimated (e)GFR-formulas, a creatinine clearance (CrCl) is still often requested to estimate the GFR in clinical practice. The diversity of serum and urine creatinine (Scr, Ucr) assays might however lead to various CrCl-
Results. CrCl obtained with different creatinine assays were compared with each other and with the eGFR calculated by means of the CKD-EPI-formula.
Methods. Urine volume, Ucr and Scr were obtained in 589 patients, aged between 18 and 75 years, assayed by Roche enzymatic assay (E), Roche compensated kinetic Jaffe assay (CJ) and Roche kinetic Jaffe assay (J). 24h-CrCl was calculated and corrected for body surface area. Deming regression was used for comparing CrCls with each other, ordinary linear regression for comparison with CKD-EPI-eGFR.
Results. Comparison of CrCls: (a) CrCl-J/CrCl-E: slope=0.791($p<0.05$), intercept=4.28($p<0.05$), (b) CrCl-CJ/CrCl-E: slope=1.031($p<0.05$), intercept=0.01($p>0.05$). Comparison of CrCl and CKD-EPI-eGFR: (a) CrCl-E/CKD-EPI: slope=1.079($p<0.05$), intercept=6.35($p<0.05$), mean bias=23%. (b) CrCl-CJ/CKD-EPI: slope=1.143($p<0.05$), intercept=4.97($p<0.05$), mean bias=26%. (c) CrCl-J/CKD-EPI: slope=0.842($p<0.05$), intercept=9.85($p<0.05$), mean bias=9%. The explained variance R^2 is in all three cases lower than 0.66, demonstrating the very large scatter of the data around the regression line.
Conclusions. CrCl isextremely assay-dependent. Individual and systematic differences between CrCl-CJ/CrCl-E are rather small as opposed to the very large differences between CrCl-J/CrCl-E. Although the trend of many labs to switch from Jaffe assays to IDMS-traceable assays (E and CJ) for Scr and Ucr determinations, the deviation from the CKD-EPI-equation illustrates that CrCl for clinical practice remains questionableand this in addition to the known lack of accuracy of the urine collection.

0722

MONITORING OF OXIDATIVE STRESS MARKERS AFTER KIDNEY TRANSPLANTATION

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Background. Oxidative stress (OS) belongs to risk factors for high cardiovascular morbidity and mortality in renal transplant recipients.The level of OS can be monitored by evaluation of total antioxidant status (TAS) and parameters which result from damaged molecules, such as advanced oxidation protein products (AOPP) or oxidized low density lipoproteins (OxLDL).
The aim of this study was to monitor OS in patients within one year after kidney transplantation.
Methods. Patients after cadaverous kidney transplantation were involved in the study (N = 56; age 54,0 ± 10,4 years). Blood samples were collected before kidney transplantation (KT) and at days 1, 7, 30, 90, 180 and 360 after KT, respectively.
Determination of AOPP was based on spectrophotometric assay according to Witko-Sarsat. TAS was evaluated using Randox commercial kit and the OxLDL concentrations were measured using Mercodia Oxidized LDL ELISA test.
Results. Patients before KT have high level of AOPP (208,4±129,5 μmol/l) At the seventh day after transplantation, study participants showed a significant decrease in AOPP (108,9±80,1 μmol/l , P< 0.0001) compared to levels before KT. The decrease in AOPP lasted until one year after KT. There was not found a significant decrease in OxLDL levels at any time period after transplantation.
Conclusions. Successful KT reduced however did not normalize AOPP levels, perhaps due to effects of immunosupression. The OS monitoring seems to be a very important tool for improvement of KT patiens health.

0723

PREGNANCY ASSOCIATED PLASMA PROTEIN-A (PAPP-A) AS A MORTALITY PREDICTOR OF LONG-TERM HEMODIALYSIS PATIENTS

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Background. Patients with chronic kidney disease have a significantly higher mortality rate compared with the general population, and cardiovascular complications are the major cause of death. Pregnancy-associated plasma protein A (PAPP-A) is a biomarker related to vascular damage. We focused on PAPP- A and its relationship to the prognosis of long-term hemodialysis patients (HD).
Methods. 261 long-term hemodialysis patients were included in this prospective observational cohort study and followed up for five years (2003-2008). The control group consisted of 66 healthy subjects.During the follow up, 146 patients (i.e. 56%) died, in 71 of these death was due to cardiovascular causes and in 42 patients due to infection. Pregnancy-associated plasma protein A (PAPP-A), cardiac markers and nutritional and inflammatory parameters were measured at the beginning of the study and tested as predictors. PAPP-A was measured by time resolved amplified cryptate emission (TRACE).
Results. PAPP-A was significantly increased in HD patients compared to controls (27.6±15.5 mIU/L vs. 9.4±2.5 mIU/L, p<0.001). Increased PAPP-A was found to be a significant independent predictor of overall mortality and mortality due to infection in multivariate Cox analysis (HR (95% CI): 1.237 (1.060-1.444), p=0.007, and 1.416 (1.115-1.798), p=0.004, per standard deviation, respectively). PAPP-A was not related to cardiovascular mortality.
Conclusions. This study demonstrates PAPP-A as an independent predictor of overall mortality and mortality due to infection in hemodialysis patients. Our results suggest superior relationship of PAPP-A to infection-inflammation than to cardiovascular risk in HD patients.

0724

COMPARATIVE ANALYSIS OF TRADITIONAL AND NEW BIOMARKERSOF RENAL FUNCTION MEASURED IN SERUM AND URINE SAMPLES OF PATIENTS AT RISK OF ACUTE KIDNEY INJURY

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Background. Early diagnosis of acute renal injury is essential for rapid therapeutic intervention. Due to low sensitivity of traditional renal parameters, nem biomarkers including neutrophil-gelatinase-associated lipocalin (NGAL)) and (N-acetyl-beta-D-glucosaminidase (NAG)) are suggested as possible tools for early recognition of acut kidney injury, or detection of acute tubular damage.
Methods. In patients with chronic kidney disease (CKD) or subjected to chemotherapy (n=41 and 65, respectively) serum creatinine, blood urea nitrogen (BUN), beta-2-microglobulin, cystatin C, microalbumine, and CRP levels were related to those of NAG and NGAL.In addition, these parameters with CRP and procalcitonin were also monitored in 7 septic patients. Traditional parameters and NAG were measured with commercially available kits on an OLYMPUS 2700 chemical analyser; NGAL assays were performed on an ARCHITECT analyser by electro-chemiluminescency.
Results. In CKD patients correlation was observed between creatinine and beta-2 microglobulin or cystatin C (r = 0.85, p<0.001 and r = 0.93, p<0.001, respectively) and between urinary NAG and beta-2-microglobuline (r=0.76, p<0.01). In patients with lympho- and myeloproliferative diseases NGAL associated with creatinine and with NAG (r = 0,712, p<0.001, and r = 0,729, p<0.001, respectively). In sepsis NGAL, CRP and procalcitonin values fluctuated parallel during disease progression, but no association was demonstrated.
Conclusions. NAG and NGAL kinetics differ from those of traditional renal parameters in patients with risk for acute renal injury. This finding raises the notion that they may provide additional information about renal function, but further studies should clarify the diagnostic value of these new biomarkers.

0725

VALIDATION OF THE SEDIMAX SEDIMENT ANALYSIS SYSTEM WITHIN A LABORATORY OF GENERAL PRACTITIONERS

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Background. Inside the National transmural appointments Chronic kidney damageproduced by Dutch Association of general practitioners (NHG), Dutch Association of Internal Medicine (NIV) and the Dutch Federation of Kidney Disease(nfN)is described that diagnostic of cell cylinders and/or dysmorphic erythrocytes is the responsibility of the family doctor.Saltro, a laboratory for general practitioners, has chosen for an analyzer system (SediMax, Menarini) that runs sediment automatically. The named images can be corrected manually after analysis and including archived footage. The validation of the SediMax is a collaboration between the Saint Antonius Hospital and Saltro.
Methods. 141 (general patients) and 11 urine samples (nephrologic patients) have been collected within the hospital. Urine samples have been split in two and the urine sample intended for the SediMax (Menarini) was stabilized with Stabilur. The results obtained with the SediMax (Saltro) was compared with the manual microscopic assessment (St. Antonius). This was performed during 3 consecutive days. Also a within-day-variation was measured.
Results. 1 patient result of 141 general patient was different between manual method and the Sedimax. There were 3 discrepancies of 11 nephrologic patient samples. A second opinion was performed and only 1 discrepancy remained.
Within-day-variation: erythrocytes high: average 300 (sd 22, vc 7.4) and low: average 2.35 (sd 1.1; vc 46,62); leucocytes high: 422 (sd 74; vc 17.6) and low: average 14.8 (sd 2.4; vc 16.5).
Conclusions.The SediMax and the manual method give similar Results. The Sedimax standardize the entire process (pre-processing and interpretation) and has the possibility of a second opinion.

0726

PRESENCE, CONCENTRATION AND COMBINATION BETWEEN DIFFEERENT PLASMA PROTEINS IN THE URINE OF NORMOALBUMUINURIC AND MICROALBUMINURIC PATIENTS WITH HYPERTENSION

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Background. Search for new candidate serum proteins detected in the urine to reliably evaluate the development of decreased renal function in the early hypertensive nephropathy (urinary albumin to creatinine ratio - uACR<30 mg/mmol). The investigated urinary proteins differ in the molecular size : proteins of molecular weight >50 kD (albumin, alfa-1-antitrypsin, transferrin) and proteins with low molecular weight ((retinol-binding protein, alfa-1-microglobulin).
Methods. Proteins were assayed by single radial immunodiffision in the urine of 102 hypertensive patients. The lowest detectable study protein concentration in the urine (mg/dl) were: albumin (0.14), alfa-1-antitrypsin (0.10), orosomucoid (0.13), transferrin (0.23), retinol-binding protein (0.04), alfa-1-microglobulin (0.25).
Results. Urinary presence (percentage of whole tested group) of proteins above the detection limit were as follows: albumin (76.4%), alfa-1-antitrypsin (9.8%), orosomucoid (12.7%), transferrin (6.6%), alfa-1-microglobulin (27.5%), retinol-binding protein (2.3%). Significant (p<0.05) correlations were demonstrated between concentrations of proteins of molecular weight >50kD (albumin,alfa-1-antitrypsin, transferrin). Alfa-1-microglobulin concentration in the urine significantly correlated with orosomucoid and albumin. No significant association was established between urinary protein concentrations and e-GFR (p>0.05). Orosomucoid correlates with the e-GFR at p=0.078.
Conclusions. Conventional immunologic test for investigation of urinary concentrations of individual serum proteins are not sensitive enough to detect early deterioration of renal function in hypertension.

Kidney disease

0727

DETERMINATION OF VITAMIN B$_{12}$ AND FOLIC ACID IN PATIENTS ON HEMODIALYSIS AND ERITROPOETIN THERAPY

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Background. Vitamin B12 and / or Folic acid deficiency can cause a clear eritropoietin resistance in the treatment of renal anemia in hemodialysis patients.Vitamin deficiency is being developed by poor food intake, reduced intestinal absorption and constant loss during dialysis, so monitoring of these parameters could take place in manageing therapy for anemia in hemodialised patients.
Methods. The stady included 15 heathy subjects (5 females and 10 males, mean age 41 ± 13 years) and 20 patients on hemodialysis (8 males and 12 females, aged 53 ± 12 years, on hemodialysis treatment three times a week, during 4.9 ± 4.4 years). Vitamin B12 and folate levels were determinated by Microparticled enzyme immunoassay (MEIA) method.
Results. The average serum vitamin B12 concetracion in hemodialysis patients was 296.05 ± 137.21 pmol / L, whereas in control group was 273 ± 165.51 pmol / L. The average serum folate level in hemodialysis patients was 19.41 ± 12.8 nmol / L, whereas in the control group was 20.04 ± 6.62 nmol / L. Mean concentration of folic acid in erythrocytes in hemodialysis patients was 1729.27 ± 1282.0 nmol / L, whereas in the control group was 841.38 ± 274.78 nmol / L.
Conclusions. Vitamin B12 and folate serum levels in hemodialysis patients did not differ significantly compared to values in the control group. Red blood cells folate in hemodialysis patients was significantly higher to values obtained in healthy people. These parameters should be monitored for deficiency prevention and prompt correction in vitamin supplementation.

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SERUMFREELIGHT κ AND λ CHAINSANDMULTIPLEMYELOMA

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Background. For 150 years, the presence of Bence Jones protein (immunoglobulin free light chains – FLCs) in the urine has been an important diagnostic marker for multiple myeloma (MM). Indeed, it was the first cancer test. Over the last few years, nowever, interest in FLCs has undergone a renaissance. Development of serum tests for free kappa (κ) and free lambda (λ) has opened the door to new applications and increased their clinical importance.
From a physiological viewpoint, blood tests for small molecular weight proteins have clear advantages over urine tests.
Methods. Evaluating the concentration of a soluble antigen by turbidimetry involves the addition of the test sample to a solution containing the appropriate antibody in a reaction cuvette. A beam of light is passed through it. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration.
Results. In the past 8 months we measure 30 patients with MM. The age was between 25 and 70 years old. Approximately 20% of all patients with myeloma have light chain or nonsecretory myeloma. Among the remaining patients, those who produce intact monoclonal immunoglobulins, FLCs are abnormal in 95% at disease presentation.
Conclusions. General statements have been formulated on the clinical applications of cancer markers: differential diagnosis in symptomatic patients; clinical staging of disease; estimating tumor burden; prognostic indicator for disease progression; evaluating the success of treatment; detecting the recurrence of cancer; screening symptomatic patients; screening the general population.

Kidney disease

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CYSTATIN S – BIOMARKER TO DETERMINE THE RENAL FUNCTION

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Background. Cystatin C is a biomarker to determine the renal function.
Cystatin C is an extracellular cysteine protease inhibitor that belongs to the cystatin superfamily. Encoded by the CST3 gene on the short arm of chromosome 20, it is a non-glycosylated protein of 120 amino acids with a predicted molecular mass of 13 kDa. Cystatin C is produced in all tissues and present in all biological fluids. Because of its small size, Cystatin C is freely filtered by the glomerulus.
Methods. The assay employs the quantitave sandwich enzyme immunoassay technique. A monoclonal antibody specific for Cystatin C has been pre-coated onto a microplate. After the required incubation an enzyme-linked monoclonal antibody are added; followed by substrate solution, which develops color reaction in proportion to the amount of Cystatin C in the serum.
Results. In period of one year we measure 80 patients suspected for renal diseases. The age was between 20 and 70.
In 68 of them levels of Cystatin C were high. They were with renal dysfunction diagnosed with creatinine, urea, total protein, albumin levels, followed by renal echography. In the rest 12 cases we found no deviation from the normal levels.
Conclusions. Cystatin C is a biomarker with important value and is used for diagnostics of renal function. It serum concentration correlates closely to the glomerular clearance rate. It may be superior to creatinine due to the fact that its serum concentration is not affected by other factors, such as gender, age and muscle mass.

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ROUTINE SERUM CREATININE MEASUREMENTS ANNO 2010: HOW WELL DO WE PERFORM?

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Background. The aim of the study was to investigate the accuracy and variation of serum creatinine measurementsbetween local clinical laboratories using serum pools. This is a major advantage over the non-commutable control materials typically used in (inter)national proficiency studies.
Methods. 26 routine instruments were included, representing 13 different types of analyzers from 6manufacturers and covering all current methodologies. Target values of five serum pools (creatinine concentrations: 35 - 933 μmol/L) were assigned using isotope dilution gas chromatography/mass spectrometry (IDMS).
Results. Intra-laboratory CV (n = 5) was >5% for 8/26 analyzers. Inter-laboratory CV was 12.8% for pool 1 (35.0 ± 0.6 μmol/L) and <5% for pool 2 to 5 (higher concentrations). Linear regression analysis did not show major slope or intercept deviations from 1 and 0 respectively. Bias calculations showed analytical inaccuracy in the normal creatinine concentration range. For pool 1 only 8/26 analyzers met the <3.8% desirable bias criterion and at least 16/26 analyzers failed the <5.7% minimal bias specification (Ricos-Fraser); for pool 2 (69.9 ± 0.4 μmol/L) this was 13/26 and 6/26 respectively. Pool 3 (111.8 ± 0.8 μmol/L) and the two pools with pathological creatinine concentrations (296.5 ± 1.9 μmol/L and 933.5 ± 1.7 μmol/L) showed less deviation from the target value.
Conclusions. Although most assays claim to be traceable to IDMS, large inter-laboratory differences still exist. The inaccuracy in the lower concentration range is of particular concern and may lead to clinical misinterpretation (e.g. children). Further effort to improve harmonization between methods is required.

Kidney disease

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RENAL INVOLVEMENT IN LEPROSY PATIENTS AFTER MULTI DRUG THERAPY: A RETROSPECTIVE ANALYSIS OF RENAL PROFILE

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A retrospective study was conducted using historical data obtained from sequential biochemical investigations of renal functional profile from 920 leprosy cases to test the involvement of renal function impairment. Of the 920 Leprosy cases 459 were multi bacillary type, 380 cases were Pauci bacillary leprosy and the remaining 81 cases had leprosy with reaction.

The data obtained from this study revealed possible involvement of renal functional impairment. Further analysis of the data suggest a statistically significant ($P<0.01$) renal involvement in 25% cases in the age group of 50-70 years. Hence it is observed that significant of renal involvement is noticed across the spectrum of the disease, irrespective of the disease severity and anti leprosy chemotherapy (MDT).

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TRIGLYCERIDES/HDL-CHOLESTEROL RATIO IN PATIENTS WITH DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE

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Background. According to NCEP ATPIII guidelines secondary therapeutic goal in hypertriglyceridemic states, tipically associated with decrease of HDL-C level, is non-HDL-C. However, in patients with chronic kidney disease non-HDL-C is not enough valuable, because of simultaneous decrease of total cholesterol (C). Moreover, the levels of LDL-C and apoB are lowered. The aim of this study was the evaluation of TG/HDL-C ratio and logTG/HDL-C ratio (Atherogenic Index of Plasma) in hemodialysed (HD) and non-HD chronic renal failure patients.

Methods. In 46 HD, 50 non-HD patients (creatinine clearance 59.9 ± 26.7 ml/min/1.73m^2), and 48 control subjects, triglycerides, total-C, HDL-C, urea, creatinine, (standard biochemical methods), apolipoprotein (apo) A-I, apo B, (immunoturbidimetric method) were evaluated, and LDL-C, non-HDL-C, LDL-C/HDL-C, non-HDL-/HDL-C, TG/HDL-C, and, AIP were calculated.

Results. TG/HDL-C ratio and AIP were significantly higher in HD compared to non-HD patients (p˂0,05), and in HD compared to control group (p˂0,001), as well as in non-HD patients compared to control group (p˂0,05). The values of AIP˃0.11 (intermediate and high risk) we found in 71.7% of HD patients, 46% of non-HD patients, and in 31.2% subjects of control group. Also, there were significant differences in the levels of TG, HDL-C, LDL-C, non-HDL-C, total C and apo A-I between HD and non-HD patients, and between HD patients and controls.

Conclusions. AIP and TG/HDL-C ratio are the most suitable for evaluation of lipid disturbances in different stages of CRF. In addition to, non-HDL-/HDL-C ratio, and levels of apo A-I, HDL-C and TG are important markers in HD patients.

Kidney disease

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PHARMACOKINETICS OF TACROLIMUS IN RENAL TRANSPLANT RECIPIENTS

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Background. Area Under Curve (AUC)-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. Tacrolimus has a narrow therapeutic drug and bioavailability is known to vary considerably between renal transplant recipients. Most of the transplant centers still rely on measurement of trough levels, but there are conflicting reports on the correlation between tacrolimus trough levels and systemic exposure, as measured by the area-under-the-concentration-over-time curve ($AUC_{(0-12h)}$). The main objective was to study intrapatient variability in the drug concentrations in blood versus different doses.
Methods. We studied 15 renal transplant recipients. ELISA method is used (of TMB as a substrate) to estimate tacrolimus concentration**.**
Results. Bayesian forecasting with a two-point sampling strategy, a trough level, and a second sample obtained four hours post-dose (C4) significantly high concentration in 12 patients[80 %] the squared correlation with the $AUC_{(0-12h)}$ (r^2= 0.94).the concentration of four hours post-dose blood is proportional to the increase of the drug dosage . Compared with trough level monitoring only, this approach reduced the 95%-prediction interval by 50%. The Bayesian approach proved to be feasible in clinical practice, and provided accurate information about systemic tacrolimus exposure in individual patients. In the AUC-guided dosing cohort the apparent clearance of tacrolimus decreased gradually over time, which was not reflected in corresponding trough levels.
Conclusions. Patients (80%) showed higher concentration of tacrolimus at C4 Present study shows that C4 level may be used to monitor tacrolimus levels in renal transplant recipient when compare with trough levels.

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KIDNEY STONE ANALYSIS IN A MEDITERRANEAN POPULATION

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Background. Kidney stones are increasingly more commonly in the western civilization due to changes in socio-economic status and diet habits. The identification of the calculi composition allows a better treatment, decreasing costs and increasing the quality of life of the patient. The number and composition of the kidney stones analyzed in a reference laboratory at the south of Spain (Murcia) was evaluated.
Methods. We analyzed 2291 calculi received at the Biochemical Clinical Analysis Laboratory, between 2005 and December 31, 2010, using interferometry with Fourier transformation. Prevalence and relative composition were studied. For an aleatory portion in the years 2009 and 2010 (n=475), we have also studied the relationship between age, gender and composition of the stones.
Results. The prevalence for the period 2005-2010 was 0.38. The stone composition obtained was Calcium Oxalate Monohydrate 41.44%, Calcium Oxalate Dihydrate 7.61%, Anhydrous Uric Acid 12.41%, Uric Acid Dihydrate 6.70%, Urates 1.36%, Carbonate-apatite 2.58%, Mixtures 24.64% and Others 2.67%.The disease is more common in men (1,93 times) and for all compositions, except for Carbonate-apatite (3,75% of all calculi in women and 1,27% in men; M/W=0,67).
Conclusions. We conclude there is a tendency for an increase of Calcium Oxalate Monohydrate and Mixtures and a decrease of Anhydrous Uric stones. We observed prevalence below the expected but also that men have a higher prevalence than women. A relevant difference regarding gender was found for Carbonate-apatite. Stones follow a Gaussian distribution throughout life-time, with particular incidence between 40 and 49 years of age.

Kidney disease

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A COMPARISON OF ESTIMATED CREATININE CLEARENCE AND MEASURED GLOMERULAR FILTRATION RATE (TC[99M]DTPA CLEARANCE) IN INDIANS

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Background. The aim of this study was to compare measured glomerular filtration rate (GFR) with estimates of GFR derived from various estimated creatinine clearance methods of Jelliffe, Cockcroft and Gault, and 4-MDRD equations in Indian population.
Methods. We enrolled 80 patients in the study. GFR was determined by technetium-99m diethyl triamine penta-acetic acid (Tc[99m]DTPA) clearance . Height, body weight and serum creatinine were measured, and GFR and creatinine clearance (CrCl) estimates calculated by various equations. spearman correlation was used to assess relationships between measured GFR (Tc[99m]DTPA clearance) and estimated clearances using the three formulae. Difference between the measured GFR and estimated clearances compared with measured GFR were examined to determine whether prediction error was independent from measurement magnitude. Analyses of differences were used to determine bias and precision. Bias was assessed by mean percentage error (MPE), calculated as the percentage difference between the estimated clearances for each formula and measured GFR. A positive bias indicates overestimation of GFR, and a negative bias indicates underestimation. Relationships were also assessed by gender and varying levels of renal function: GFR <60 ml/min, and GFR >60 ml/min.
Results. The mean measured GFR was 77.2 ml/min (range 17–152 ml/min). The mean bias (mean percentage error) was-4.9,-10.3 and -1.57% respectively for the, Jelliffe , Cockcroft and Gault, and 4 -MDRD formulas, respectively.   The 4 MDRD formula overestimates the GFR in patients having GFR<60ml/min, where as underestimates for GFR>60ml/min.
Conclusions. 4-M DRD equation seems to be best for estimating GFR in Indian population.

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DYNAMIC CHANGES OF INTACT PTH, DURING SIX-MONTH SERUM STORAGE IN HEMODIALYSIS PATIENTS

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Background. Determination of intact PTH in hemodialysis patients (HP) is a key test for the diagnosis and management of renal osteodystrophy. The aim of this study is to estimate the stability of intact PTH in serums drawn from dialysis patients.
Methods. Serums of 27 HP were divided into 4 aliquots. The first aliquotwasused to determine baseline values of PTH, while remaining were frozen and stored at -20˚C. After 1.5, 3 and 6 months, aliquots were thawed in order to measure PTH. In control group (CG), PTH was assayed at once, after 1.5 and 3 months. All samples were analysed on automated system Elecsys 2010 and presented as a ratio of measured to baseline PTH values.
Results. After 1.5 and 3 months storage, PTH levels in HPdiffer significantly bothamong themselves and baseline (p<0.001). Changes inPTHvaluesbetweenthe first (-15.4±6.3%) and the second (+0.3±3.9%)three-month intervalshowed statistically significantdifference. PTH decline in CG during the first 1.5 months was significantly higher compared to the second 1.5-month interval (p<0.001), while not in HP (p=0,684). PTH showed significantlygreater decreasein the CG for the first 1.5 month interval (p=0.00092) opposite to HP.
Conclusions. The extensive pace of lowering PTH during the first 3 months of storage, indicates relatively rapiddegradation of remaining molecules of PTH (1-84) in HP. Higher percentage of unsteady PTH (1-84) in the total measured concentration provokes significantly faster reduction of assayed PTH during the first 1.5 months in CG compared to HP.

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A COMPARATIVE STUDY OF FORMULAE FOR DERIVING ESTIMATED GLOMERULAR FILTRATION RATES BASED ON THE MEASUREMENTS OF ENZYMATIC SERUM CREATININE, URINE CREATININE AND SERUM CYSTATIN C, IN AN ASIAN PATIENT POPULATION WITH SUSPECTED RENAL DISEASE

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Background. Chronic kidney disease is a worldwide health problem. Creatinine has traditionally been used for assessment of kidney disease but its utility has been limited by pre-analytical factors and analytical limitations of the Jaffe method. We implemented the Isotope Dilution Mass Spectroscopy-traceable enzymatic creatinine method in 2010.
Methods. Serum creatinine, serum cystatin C and 24-hour urine creatinine were measured on the Cobas501 analyser(Roche Diagnostics, Switzerland). Creatinine clearance and estimated Glomerular Filtration Rate using both Modified Diet in Renal Disease (eGFR-MDRD) and cystatin (eGFR-Cystatin) were calculated.
Results. The study population comprised 178 individuals (99 males, 79 females; aged 17-103 years). Using eGFR-MDRD as reference, CCT yielded a sensitivity of 92% and specificity of 32%. For males, sensitivity and specificity were 87% and 39% respectively; for females, sensitivity and specificity were 97% and 25% respectively. Cystatin C was measured in 128 individuals in our study. Using eGFR-MDRD as reference, we obtained a sensitivity of 66% and specificity of 82%. For eGFR-(Cystatin), a cut-off of >60 mL/min yielded sensitivity and specificity of 55% and 93% respectively. Using >90 ml/min, sensitivity and sensitivity changed to 79% and 71% respectively.
Conclusions. Our results show that CCT is more sensitive than Cystatin C and eGFR-Cystatin, especially in females, while cystatin has superior specificity. We recommend that both markers be used jointly when screening patients for suspected renal disease. Additionally, increasing the cut-off for eGFR (Cystatin) from 60 ml/min to 90 ml/min does not yield any advantage.

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FIBROBLAST GROWTH FACTOR-23 LEVELS IN CHRONIC RENAL FAILURE PATIENTS ON DIFFERENT DIALYSIS TREATMENTS AND IN PATIENTS WITH RENAL TRANSPLANTATION

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Fibroblast Growth Factor-23 (FGF-23)-Klotho axis plays an important role in mineral homeostasis. FGF-23 inhibits renal 1α-hydroxylase expression and regulates phosphate reabsorption in kidney. In the present study serum FGF-23 levels were determined in chronic renal failure (CRF) patients on different dialysis treatments and in patients with renal transplantation (RT).
Serum FGF-23, intact parathormon (PTH), 1,25-dihydroxy vitamin D3 (1,25(OH)$_2$Vit-D3), phosphorus calcium and calcium X phosphorus ratio were measured in CRF patients on hemodialysis (HD-CRF; n=27, 50.9±16.9 years) or on peritoneal dialysis (PD-CRF; n=51, 50.9±16.5 years), in RT patients (n=25, 40.5±11.9 years) and control subjects (n=41, 46.3±11.5 years).
Phosphorus levels of all patient groups were significantly higher than control group, whereas calcium levels decreased significantly only in dialysis groups compared to control. FGF-23 levels were 1252.65±310.73, 872.96±526.99, 86.89±200.85 and 153.38±222.66 RU/ml in HD-CRF, PD-CRF, RT and control groups, respectively. Both FGF-23 and PTH levels were significantly higher in HD-CRF group compared to other groups. PTH levels in patient groups were significantly higher than control group. 1,25(OH)$_2$Vit-D3 levels decreased significantly in dialysis groups compared to both RT and control groups. FGF-23 and 1,25(OH)$_2$Vit-D3 levels did not correlate significantly in study groups. FGF-23 correlated significantly with both PTH and P in PD-CRF, but not in HD-CRF or RT groups.
In the present study finding of increased PTH levels together with very high FGF-23 levels might reflect the resistance of parathyroid cells to FGF-23 due to decreased Klotho suppression in hyperparathyroidism. Present results also suggested that FGF-23 resistance is augmented in CRF patients on hemodialysis.

Kidney disease

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CKD-EPI, MDRD AND COCKROFT-GAULT EQUATIONS' ACCURACY IN PREDICTING PERITONEAL DIALYSIS-DELIVERED CREATININE CLEARANCE

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Background. Various formulae (CKD-EPI, MDRD six variables- MDRD-6, MDRD four variables-MDRD-4 and Cockroft-Gault) are widely used for renal function prediction. Peritoneal creatinine clearance (Ccr) with 24-hour dialysate collection is included in chronic Peritoneal Dialysis (PD) patients' evaluation. The aim of this study was to determine whether these formulae would accurately predict measured Ccr (residual renal plus peritoneal Ccr) in PD patients.
Methods. A group of 23 consecutive PD patients were enrolled in the study. All were treated by Automated PD. Sixteen out of twenty-three had residual kidney function. Primary renal disease was Diabetic Nephropathy in 6, Chronic Glomerulonephritis in 5, Nephrosclerosis in 3, Cystic Kidney Disease in 1, IgA Nephropathy in 1, Amyloidosis in 1, Reflux Nephropathy in 1, Interstitial Nephropathy in 1 and unknown Nephropathy in 4. Ccr was determined from 24-hour dialysate and urine collections and also estimated by Cockroft-Gault, MDRD (4 and 6) and CKD-EPI formulae.
Results. CKD-EPI and MDRD-6 estimation results were similar to those measured by 24-hour dialysate and urine collection Ccr (9,01±3,9 and 9,54± 2,98 vs 8,63±3,73 ml/min/1.73m², P=0,49 and 0,09, respectively). Additionally, neither the presence nor the volume of residual urine affected the accuracy of the prediction. In contrast, Cockroft-Gault (12,50±5,81 vs 8,65±3,72 ml/min/1.73m², P<0,001) and MDRD-4 (10,00±4,20 vs 8,64±3,76 ml/min/1.73m², P=0,03) formulae were not accurately predictive of the measured Ccr and differed significantly from the latter.
Conclusions. CKD-EPI and MDRD-6 equations could be used with accuracy and precision .

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AN ERROR PROPAGATION FORMULA MUST BE USED TO CORRECTLY CALCULATE UNCERTAINTY IN EGFR

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Background. Uncertainty in eGFR (U_{eGFR}) is commonly calculated using the 95% confidence limits in measured creatinine. Our objective was to evaluate the impact of using an error propagation formula for calculating eGFR uncertainty.
Methods. Creatinine variability (mean and SD for the all methods group) was obtained from CAP C-A 2010 survey and from RIQAS (16 consecutive challenges in 2010). Regression equations (SD versus mean) were used to determine creatinine variability at specific levels of interest. Uncertainty in eGFR was calculated by two approaches: 1) using 95% confidence limits for creatinine to calculate uncertainty limits for eGFR and 2) using expanded fractional uncertainty of creatinine in the following published error propagation formula ($Ue_{GFR} = 1.154 \times U_{Cr} \times eGFR$). Uncertainties in eGFR were then expressed as percent uncertainties for comparison.
Results. At 60 ml/min/1.73m², eGFRuncertainties were 25.4% and 23.4% (from CAP and RIQAS data, respectively) using the confidence limits approach compared with 18.3%% and 18.1% using the error propagation formula. An even greater difference was obtained with these two approaches at 90 ml/min/1.73m², yielding uncertainties of 30.1% and 35.2% using confidence limits versus 14.8% and 16.7% using the error propagation formula.
Conclusions. Uncertainty in eGFR is overestimated by 1.3 to 2.1 fold when calculated using confidence limits in measured creatinine. Calculation of uncertainty by error propagation analysis indicates that eGFR uncertainty is less at 90 ml/min/1.73m² than at 60 ml/min/1.73m². Hence, the current practice of not reporting discrete numeric values for eGFR greater than 60 ml/min/1.73m² should be revisited.

Kidney disease

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INTEREST OF THE COMBINED DOSAGE OF SELECTED URINARY PROTEINS IN THE DIAGNOSIS APPROACH IN NEPHROLOGY

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Background. Determination of the protein composition of urine is a non-invasive method helping to diagnose renal lesions and evaluate therapeutic interventions. We selected five observations (C1-C5) that highlight the performance of a proteinuria analysis combining selected glomerular and tubular protein dosage.
Methods. Total urine protein level and selective dosage of urinary IgG, albumin, a-1 microglobulin and retinol binding protein were performed on a spot urine by immunonephelometry (Beckman-Coulter). The results were normalized to urine creatinine level and integrated in the MDI interpretation software that provides a "urine protein profil" (UPP).
Results. Sequential UPP were performed in two patients with drug-induced tubular toxicity. C1 resolved after drug withdrawal. C2 concomitantly presented glomerular lesions and repeated UPP was warranted to follow evolution of the distinct renal lesions. C3 and C4 illustrate two clinical situations in patients with multiple myeloma, respectively myeloma cast nephropathy and toxic acute tubular necrosis. Those differential diagnoses were early anticipated by UPP that suspected the presence of large amount of urinary monoclonal light chain excretion in C3 before urine electrophoresis and renal biopsy availability. In a patient with membranous nephropathy and renal function deterioration (C5), we compared sequential UPP and kidney biopsies and observed a good correlation between glomerular and tubular protein excretion and progression of the renal lesions.
Conclusions. The use of routine UPP that includes selected glomerular and tubular markers is warranted in the clinical practice, especially to detect and follow remission of tubular defects, as well as to anticipate mixed glomerular and tubular injury.

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SERUM URIC ACID CONCENTRATION AND ENDOTHELIAL DYSFUNCTION IN NEWLY DIAGNOSED HYPERTENSION

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Background. Intima media thickness (IMT), uric acid (UA) and sICAM concentrations are well recognized risk factors for cardiovascular diseases and have been positively associated with hypertension. The aim of study was to assess the relationship between UA, sICAM and IMT in patients with newly diagnosed nontreated hypertension.
Methods. Study included a group of 49 patients aged 45-65 years with nontreated hypertension (NH) and age-matched healthy controls (n=49) (C). The diagnosis of hypertension was based on the definitions of the European Society of Hypertension (2007). Blood pressure was measured by ABPM; IMT of the carotid artery was examined by ultrasound. Blood analyses were performed for UA, sICAM and hsCRP and urinary albumin was measured.
Results. Albuminuria (>20 mg/L) was found in three out of all NH patients. Among subjects with NH two subgroups were selected: with albuminuria <10 mg/L (n=31)(NH1) and ≥10 mg/L (n=18)(NH2). Significantly higher values of hsCRP, sICAM and IMT have been found in NH2 compared with controls (n=46) with urinary albumin concentration <10 mg/L. However, the most characteristic feature in NH2 subgroup compared with controls was significantly elevated UA concentration that was not observed in patients of NH1 subgroup. A significant negative correlation between UA and sICAM (r= -0,55; p<0,005) and UA and hsCRP (r= -0,57; p<0,005) was found in NH2 subgroup.
Conclusions. Circulating uric acid, even at low albuminuria (≥10 mg/L) may be regarded as a marker of early endothelial dysfunction in newly diagnosed nontreated hypertension.

Kidney disease

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MICROALBUMINURIA IN INDIVIDUALS WITH NEWLY DIAGNOSED HYPERTENSION AND NORMOTENSION

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Background. Microalbuminuria (MAU) has been recognized as an early sign of renal damage and a predictor of end-stage renal disease in hypertensive patients. MAU can be estimated using random spot urine sampling. MAU is generally adjusted to creatininuria to account the variation in urinary flow rate and concentration and the test is considered positive for the albumin creatinine ratio (ACR) of 30–300mg/g, corresponding to 3.4–33.9mg/mmol. The aim of this study was to examine and compare the extent of microalbuminuria in hypertensive and normotensive individuals.

Methods. Spot urine sample was collected from newly diagnosed hypertensive patient as a case and normotensive person as a control. In spot urine sample albumin was measured quantitatively and adjusted to creatininuria then ACR of 3.4–33.9 mg albumin/mmol creatinine interpreted as MAU.

Result. The mean systolic blood pressure/diastolic blood pressure of case and control were 144.04±14.17/96.60±7.18 and 113.58±6.75/74.52±5.14 respectively. The mean of quantitative spot urinary albumin (mg/dl) of case and control were 60.64±12.97 and 21.62±6.67 respectively which were statistically significant (P-value 0.001). The mean of spot urinary ACR (mg/mmol) between case and control were 11.02±14.45 and 3.14±3.89 respectively which were statistically significant (P-value 0.001). The prevalence of microalbuminuria with ACR cut off 3.4 was 51.88% out of 212 cases and 22.64% out of 212 controls which were statistically associated (P-value 0.001).

Conclusions. By showing strong associations between MAU and newly diagnosed hypertension, MAU could be a useful marker of hypertensive patients to help identify patients in need of intensified kidney disease risk management.

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PERITONEAL DIALYSIS AND LABORATORY

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Background. Since our Department for Dialysis performs the Peritoneal Equilibration Test (PET) to monitor peritoneal dialysis adequacy, we have been asked to collaborate on calculating clearances and transport characteristics of patients.
This collaboration is ongoing since 2003., despite the Baxter-PD Adequest software having appeared.

Methods. For each of the 76 patients we have dialysates samples (0, 2 and 4 hours of dwell time = PET I, II, III), serum, nightly and 24-hourly dialysates and 24-hourly urine,if possible. We determine glucose, creatinine and urea and calculate peritoneal and renal clearances for creatinine and urea. We also calculate dialysates creatinine / serum creatinine ratio and dialysates glucose / dialysate glucose 0 ratio to determine membrane transport type.
Since creatinine determination is encumbered by recommendations for correcting elevated creatinine levels because of the influence of glucose in PETs,we compared resultes of dialysates determined as serum (Jaffa kinetic) and as urin (what are dialysates actually ?).

Results. We got the results in these ranges : 42 Kt/V urea/week 1,33-4,75; 34 peritoneal Kt/V urea/week 1,070-2,60 ; 42 CrCl L/week 38,3-241,7. Transporters :10,8 % low, 41,9% average low, 40,5 % average high and 6,7 % high.
Dialysates creatinine determined as serum and as urin belong ,statistically, to the same set . An Indipendent T-test reports that P = 0,8350.

Conclusions. Having the knowledge of the method of determining creatinine and clearance formulas we hold that laboratories should collaborate with the Department for Dialysis and thus make monitoring peritoneal dialysis adequacyeasier.

Kidney disease

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VIABILITY ASSESMENT OF DONOR KIDNEYS: IMPEDED BY COAGULATION IN RENAL VASCULATURE

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Background. Non-heart beating (NHB) donors are a valuable source for kidney transplantation. The organs, however, sustain substantial warm ischemic damage that may jeopardize the transplant outcome. Organ viability can be assessed by perfusion characteristics of kidneys preserved by machine pulsatile perfusion and measurement of tissue injury markers in the preservation solution, such as lactate dehydrogenase (LDH) and glutathione S-transferase (GST)
Methods. After one hour perfusion, a sample of preservation solution was taken and centrifuged at 900g at 4°C for 10min. Total GST activity and LDH concentrations were measured in perfusate from six uncontrolled and six controlled NHB kidneys. Tandem mass spectrometry was used to identify perfusate proteins present on a 2D-gel.
Results. LDH concentration (339 ± 20 U/L/100g vs 193 ± 45 U/L/100g; p=0.021) and total GST activity (48 ± 9 U/L/100g vs 19 ± 4 U/L/100g; p=0.015) are significantly higher in preservation solution from uncontrolled compared to controlled NHB kidneys. Furthermore, identification of perfusate proteins on a 2D-gel revealed that almost 70% were blood-borne (e.g. albumin, transferrin and haptoglobin).
Conclusions. Uncontrolled NHB donors experience a longer period of warm ischemia, leading to prolonged coagulation and thus trapping of blood in the renal microcvasculature as reflected by the presence of blood-borne proteins in the perfusate as compared to controlled NHB donors. In contrast to GST, LDH seems not specific for renal tissue injury, since damaged erythrocytes will also release LDH. However, LDH remains useful as an indicator of organ viability reflecting tissue injury as well as coagulation in the microvasculature.

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EVALUATION OF AUTION MAX AX-4030 AND 9UB URIFLET, 10PA AUTION STICKS URINE DIPSTICKS IN THE AUTOMATED URINE TEST-STRIP-ANALYSIS

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Background. Aution Max AX-4030, a test strip analyzer recently introduced to the market, represents an upgrade of the Aution Max AX-4280 widely employed for urinalysis. This new instrument model can allocate two different test strips at the same time. In the present study the two instruments have been compared together with the usage of Uriflet 9UB and the recently produced Aution Sticks 10PA urine strips, the latter presenting an additional test area for the measurement of urinary creatinine.
Results. Measurements of chemical-physical parameters on urine controls, by Aution Max AX-4030, employing Uriflet 9UB strips showed always good repeatability. Similar results were obtained when evaluating within-run imprecision on urine pools employinf both strips. Creatinine measurements obtained by Aution Max AX-4030, employing Aution Sticks 10PA strips showed very good repeatability. For all analytes data were within one set point or distributed between 2 contiguous set-points.
Correlation between instruments, evaluated for urine protein and glucose was always strong and accuracy evaluated for protein, glucose and creatinine, comparing the semi-quantitative results to those obtained by quantitative methods was also very strong.
Conclusions. Aution Max AX-4030 presents comparable performances with the earlier model with the main advantage related to the possible allocation of two strips at the same time. In particular it can employ, the Aution Sticks 10PA, that demonstrated reliability of creatinine measurements, making these strips eligible for urine creatinine determination whenever necessary. Further studies should be carried out to evaluate effectiveness and appropriateness of the usage of creatinine semiquantitative analysis employing this technology.

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N-TERMINAL PROBNP AND HIGH SENSITIVITY CARDIAC TROPONIN I CONCENTRATIONS IN HAEMODIALYSIS PATIENTS

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Background. Assessment ofserum biomarkers that are useful in stratification of early mortality and cardiovascular risk is very important in the treatment of patients on chronic haemodialysis. This study examinedthe relationship between high sensitivity cTnI and NT-proBNP according to glomerular filtration rates and vascular access (native AV-fistula or permanent catheter) in patients on haemodialysis.
Methods. NT-proBNP and cTnI were measured by Vitros 3600 Immunodiagnostic System (Orthoclinical Diagnostic, Johnson & Johnson Company, USA) in 100 patients on haemodialysis.
Results. Concentrations of cTnI and NT-pro BNP were increased in 35% and 100% of patients. According to their residual renal function, patients were divided in three groups: oligoanuric, 02-05 L/24H and 05 –1 L/24h. The highest concentrations of NT-proB-NP were in the first group and the third group showed the lowest NT-proBNP levels ($p<0.05$). The cTnI concentrations showed no statistically significant differences between these groups. Patients with fistula accesses had lower levels of cTnI and NT-proBNP than patients with permanent catheter, but these differenceswere not statistically significant.
Conclusions. In patients with end-stage renal disease, interpretation of NT-proBNP levels should take into account residual diuresis. On the basis of our results, we suppose that high levels of cTnI should be interpreted only as a consequence of myocardial necrosis.

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LIPID ABNORMALITIES AND LDL OXIDATION IN END-STAGE RENAL FAILURE PATIENTS ON DIALYSIS

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Background. Dyslipoproteinaemia and oxidative modification of LDL (oxLDL) are common symptoms in patients with chronic renal failure and contribute to the development of oxidative stress. In this study we compared the serum levels of oxLDL, lipids and lipoproteins in hemodialysis (HD) patients, peritoneal dialysis (PD) patients and in normal controls (NC). We also compared the concentrations of HDL-C subclasses, which are affected by oxidative stress.
Methods. The study was carried out with end-stage renal failure patients which were on stable HD (N=31, 16 males and 15 females, age 55±3 years, mean ± SEM) and on PD (N=24, 11 males, 13 females, age 50±4 years). A control group of 21 healthy individuals was also included (age 40±5 years, 11 males, 10 females). HDL2 and HDL3-C subclasses were isolated from serum according to a single-step precipitation method following a homogenous HDL-C assay The oxLDL concentration was measured by ELISA.
Results. In dialysis patients, HDL-C was significantly lower than in NC (PD: 42±3 md/dl, HD: 40±2, NC:57±3, p<0.001 vs.NC). The concentration of HDL3 subclass in patients was significantly lower than in NC (p<0.001) but did not differ between dialysis groups (PD: 10±1 md/dl,HD:11±0.5,NC:23±1). The levels of oxLDL in the groups of patients were significantly higher than in NC (PD: 2.43±0.17 mg/l, HD: 1.41±0.45, NC: 0.22±0.05, p<0.05 vs. NC).
Conclusions. The decrease of HDL-C and especially of HDL3 subclass concentration together with the increase of oxLDL levels may possibly indicate an acceleration of atherosclerosis process in dialysis patients.

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PROTEIN DETERMINATION FROM SPONTANEOUS URINE: STUDY OF SIGNIFICANCE OF PROTEIN DETERMINATION WITH STIX AS SCREENING TEST

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Background. Protein determination from spontaneous urine is regularly applied in the diagnosis of renal impairment. Available procedures include protein test stix or nephelometric albumin determination. Since the stix test primarily detects albumin in the protein range, this is mainly suitable for the detection of glomerular kidney damage. Advantages of this method include easy handling, rapid results and low costs. Focus of our study was to assess significance of the stix test compared to the nephelometric method, currently the best procedure for albumin detection in urine.

Methods. Study subjects were recruited from newly appointed staff at the University Hospital Bonn presenting at the occupational health office without previously known kidney disease (n=361). In each participant, stix protein determination was carried out with Urisys 2400 (Roche Diagnostics®) and nephelometric comparison measurement with BN ProSpec (Siemens Healthcare Diagnostics®).

Results. For evaluation, participants were divided into three groups. In 319 participants, no pathological albumin excretion was found (nephelometrically determined albumin concentration <20 mg/l). Here, 315 participants were correctly found to be negative with stix (specificity 98.7%). In 39 participants, microalbuminuria was nephelometrically detected (albumin 20-200 mg/l). Here, the stix method failed to determine pathological protein excretions in 22 participants (sensitivity 43.6%).

Macroalbuminuria was found in three participants (albumin >200 mg/l), whereby one had a single positive and two a double positive test strip result.

Conclusions. The stix test was found to have a relatively low sensitivity, especially in the microalbumin range. Therefore, nephelometric microalbumin determination should be the preferred screening method in risk patients.

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CHEMICAL COMPOSITION OF ENCRUSTATES ON DOUBLE "J" URETERAL STENT IN RECURRENT UROLITHIASIS

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Background. Ureteral stent occlusion by encrustation is a major problem in long-term artificial urinary drainage. In this paper are demonstrated results of the investigation the physical chemical analysis of extracted encrustates to the stents for some patients with recurrent urolithiasis.

Methods. We analyzedthecomposition of encrustates to 20 removed doubleJstentsinpatientswith recurrenturolithiasis. Analysis ofthe encrustates compositionweremadebythermogravimetric analysis (Mettler TG 50) andFT-IRspectroscopy (Shimadzu 8400).

Results. The results obtainedwere compared with encrustatescompositionof the primarycompositionof kidneystonesbypatientspreviouslyexcreted. Encrustatessampleswere takenfromthree differentplaces on thestent todeterminesample homogeneity,considering thediversity ofenvironmentthrough which thestent is situated. The results indicate composition diversity ofthe ofthe sampleencrustatedwiththe samestent. Analysis composition encrustates revealed struvite and carbonate hydroxyapatite as most frequent minerals.

Conclusions. Based on results ofanalysis ofthe compositionencrustatesdepositedat thestentin patients with recurrent urolithiasis we can concludedthat it is not homogeneus and is not identical with the composition of primary kidney stones who is patients previously extracted.

0751

PROTEIN: CREATININE RATIO IN RANDOM URINES ACCURATELY PREDICT 24 HOUR PROTEIN LOSS IN NEPALESE PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background. Assessment of proteinuria is important in chronic kidney disease (CKD). Spot urine protein:creatinine ratio (PCR) have been widely used as alternative to 24h urine protein (UTP). The aim of this study was to examine the ability of PCR to predict urinary 24h protein loss in Nepalese patients with CKD.
Methods. This Study comprises 584 CKD patients (111 had stage 1 CKD, 144, 139, 99, and 91 patients with stage 2, 3, 4, and 5 respectively). 24h urine and spot urine was collected and were subjected for determination of UTP and spot PCR. Urine protein and creatinine was estimated by pyrogallol and Jaffe's method (Human, Germany) respectively.
Results. 24.3%, 25%, 46.8%, 75.7% and 90.1% of stage 1, 2, 3, 4 and 5 CKD respectively had proteinuria as determined by UTP (>150 mg/day). There was good correlation between UTP and PCR, with correlation coefficients(r) of 0.923 (r= 0.858, 0.873, 0.916, 0.926 and 0.943 in stage 1, 2, 3, 4 and 5 respectively) (p<0.001 for all). Receiver operator characteristic (ROC) curve analysis showed PCR to be a good predictor of proteinuria, with area of 0.973 (95% CI;0.961-0.985 p<0.001). Areas under ROC curve were 0.947, 0.967, 0.964, 0.937 and 0.995 in stage 1, 2, 3, 4, and 5 groups respectively. At PCR cutoff of 20 mg/mmol sensitivity of 93% and specificity of 92% can be achieved. The optimal cutoff varies among stage of CKD.
Conclusions. By careful choice of cutoffs, PCR can be used in patients with CKD to identify significant proteinuria.

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DYSLIPIDEMIA AFTER RENAL TRANSPLANTATION

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Background. Kidney transplantation is the treatment of choice for most patients with end-stage renal disease. Immunosuppression has improved graft and recipient survival in transplantation but is accompanied by several adverse effects like dyslipidemia and cardiovascular disease. Herein, lipid abnormalities including increased total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL) have been frequently reported in renal transplantation and could be involved in the high frequency of cardiovascular diseases in this population.
Methods. Forthy patients were included in this study. Twenty five patients received tacrolimus (Tac), and fifteen patients cyclosporine (CsA). Lipid parameters were assessed the day of transplantation and 1 year posttransplantation.
Results. Comparing serum lipid levels between two groups TC and LDL were significantly higher in the CsA group (6.14 ± 1.37 vs 5.28 ± 1.32 mmol/L; $P < .05$ and 3.98 ± 1.05 vs 3.26 ± 1.03 mmol/L; $P < .05$ CsA vs Tac, respectively). TG were comparable in both groups (1.86 ± 1.07 vs 1.62 ± 0.92 mmol/L; $P = .55$; CsA vs Tac). Incidence of de novo hypercholesterolemia was significantly higher in the CsA group (28 vs 8%) whereas incidence of hyperTG was similar in both groups. Prevalence of LDL was significantly higher in the CsA group (65% vs 31%; $P < .001$), whereas there was no difference in high density lipoprotein (HDL) levels.
Conclusions. Lipid disorders are frequently observed in renal transplant recipients. CsA, but not Tac, significantly increases incidence and prevalence of high TC and LDL. Conversion from cyclosporine to tacrolimus is recommended for kidney transplant patients.It leads to stabilization or even improvement of transplant function and a reduction in cardiovascular risk factors.

Kidney disease

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SERUM APOLIPOPROTEIN H AS A MARKER OF RENAL INJURY, A PILOT STUDY

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Background. Apolipoprotein H (beta2 glykoprotein I, 48 kDa) is a plasma plasma glycoprpotein synthetized in the liver. ApoH interferes with blood coagulation and has some effetc in autoimmune disporders. Precise fuctions are unknown but it is speculated about urine ApoH as a marker of kindey diseases. No valid data on ApoH serum method for laboratory diagnosis of nefropathy s exist.
Aim. development and validation of the new ELISA test for serum ApoH measurements and its testing in individuals with diabetic nefropathy.
Methods. serum samples were used from 43 patients of nephrologic out patient centre (27 with diabetic nefropathy, 16 with normal kidney functions). ELISA was developed, validated and performed for ApoH (specific goat polyclonal anti-human ApoH)from urine, serum and urea, creatinine, were measured in sera and albumin/creationin index, alpha 1 microglobulin and GGT in urines.
Results. ELISA test for serum ApoH DKK-1 measurement had optimal analytic characters (limit of detection 1 ng/l, ,dilution linearity recovery 95%, spiking recovery 97%, interassay and interassay CV < 10%). Serum ApoH serum values were significant higher in individuals with diabetic nefropathy (195 vs.326.6 ug/l, P<0.01) and test had high diagnostic efficacy (ROC 0.95, sensitivity 90, specificity 100%, LR+ 15). Differences were significant after adjustment for age, sex and urine or serum kidney markers. Serum ApoH correlated with serum kreatinine and cystatin C and albumin/kreatinin index, GGT, alpha 1 microglobulin in urine. Urine ApoH did not correlated with kidney markers and did not differ in individuals with nefropathy. Results were verified with freguency chart and with serum ApoH were 93% of individulas correctly clasified..
Conclusions. ELISA test for serum ApoH was developed. Serum ApoH is promising marker for laboratory diagnosis of diabetic nefropathy. Largerstudieswillbecarried outinthe future.

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LACK OF COMPARABILITY OF PTH MEASUREMENTS – THE URGENT CLINICAL NEED FOR IMPROVEMENT

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Background. Renal physicians strive to maintain PTH concentrations for patients with chronic kidney disease (CKD) within guideline limits, but poor method comparability, producing wide discrepancy in PTH results, can result in serious risk of clinical misinterpretation. The potential for under-or over-treatment is significant, representing a major challenge to patient safety.
Method. At a meeting in September 2010, attended by representatives of relevant clinical and scientific professional organisations and manufacturers of most PTH assays, the current status of PTH measurement was reviewed and priorities for improvement identified.
Results. In the short-term, raising awareness of clinical implications of method-related differences in PTH is essential. Agreeing and adopting assay-specific PTH action limits for CKD patients, as an interim measure, is highly desirable and has been achieved in Scotland. Establishing pre-analytical requirements for each PTH assay is a priority. Mid to longer term, re-standardization of PTH methods in terms of an appropriate International Standard (IS) is required. Provided commutability can be demonstrated, the recently established IS 95/646 for PTH1-84 is a suitable candidate. Establishment of a well-characterized panel of samples of defined clinical provenance to enable manufacturers to determine appropriate reference intervals and clinical decision points is also recommended and will provide an invaluable resource. Recent developments in mass spectrometry mean that a candidate reference measurement procedure for PTH is now achievable and represents major progress. Concurrently, evidence-based recommendations on clinical requirements and performance goals for PTH measurement are required.
Conclusions. Improving comparability of PTH results requires support from many stakeholders but is achievable.

Kidney disease

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BETA-2 MICROGLOBULIN INTERMEDIATE IN SERUM BY CAPILLARY ELECTROPHORESIS

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Background. The beta 2 microglobulin (B2 m) conformer has been proven to be an amyloidogenic intermediate using capillary electrophoresis(CE) in several studies . However, this technique has never been used to determine the presence of the B2 m conformer in clinical specimens, such as hemodialysis(HD) patients serum. We established measurement of amyloidogenic intermediate B2m(I-B2m) and native B2m(N-B2m) in serum by CE.
Methods. All experiments performed on a P/ACE 2100 CE system (Beckman Coulter). Before the CE analysis, the serum samples were centrifuged for 30 min at 2,500 g using a Sartorius Ultrafilter Centrisart I (Sartorius) to exclude proteins over 20,000 Da in the sample.
Results. Standard B2m and all serum samples showed two peaks, major and minor, with migration time of 9.8 and 10.2 min, respectively. We judged the major peak to be the N-B2m and the minor peak to be the I-B2m using affinity column of B2m polyclonal antibody. Linearity up to 100mg/L for N-B2m and 50 mg/L for I-B2m. Detection limits was 0.38 mg/L. The within-assay and between assay precision were less than 6.5% (N=10). Recovery studies were performed 96–104% was achieved. The concentrations of N-B2m and I-B2m were: 29.4± 6.8 and 2. 8±1.4 mg/L in HD patients(N=31) and 1.2 8 ± 0.1 and 0.2 ± 0.1 mg/L in healthy subjects(N=5). In addition, dialysis related amyloidosis(DRA) patients(N=13) of I-B2m concentration were significantly lower than non-DRA patients (N=13, p<0.004).
Conclusions. The proposed method suggested that can be new tool for diagnosis of DRA.

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COMPARISON OF CYCLOSPORINE A LEVELS BETWEEN THE NEW ABBOTT ARCHITECT AND DIAGNOSTICS TDX CSA ASSAYS

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Background. A new Abbott ARCHITECT Cyclosporine assay has been made commercially accessible recently. The objective of this study was to examine the analytical performance of this assay and the correlation with the Abbott Diagnostics TDx CsA assay.
Methods. We compared cyclosporine levels on the ARCHITECT ci82000 analyzer based on a chemiluminescent microparticle immunoassay (CMIA) method and the TDx analyzer based on a fluorescence polarization immunoassay (FPIA) using 46 whole blood samples obtained from the kidney recipients. The imprecisions were analysed using the low (74.9 ng/mL) and high (310 ng/mL) levels of Abbott Immunosuppressant-MCC.
Results. The within-run and between-run imprecision (CV) were 14.50%, 8.87 % and 11.8%, 10.84 %, respectively. A new Abbott ARCHITECT CsA whole blood results from the kidney recipients (n = 46) were significantly lower than the TDx CsA results (*p*<0.001). The mean difference was -103.25 ng/mL. The Deming regression analysis revealed the equation as following: ARCHITECT = -8.6077 + 0.6569 TDx with correlation coefficience (*r*) = 0.9966. The 95% confidence interval [CI] of intercept, slope and *r* were -17.0143 to -0.1987, 0.6176 to 0.6962 and 0.9938 to 0.9981, respectively.
Conclusions. A new Abbott ARCHITECT CsA assay demonstrates adequate performance characteristics for routine clinical use.

Kidney disease

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LIPID PEROXIDATION AND FERRIC REDUCING ABILITY OF PLASM BEFORE AND AFTER HEMODIALYSIS IN CHRONIC RENAL FAILURE PATIENTS

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Background. Renal failure is associated with several metabolic disturbances and increasing evidences support a role of oxidative stressin these patients.Oxidative stress is a reaction during which the balance between antioxidant and antioxidant agents in biological systems are disturbed. The susceptibility to oxidative stress in patients is mediated by lower antioxidant defences. lipid peroxidation is one of the most well-known biological effects of oxidative stress. In hemodialysis patients due to increased probability of producing free radicals from various sources, the conditions of above reaction is increased. The purpose of this study was to determine the effect of hemodialysis on changes of lipid peroxidation with status antioxidant capacity of plasma.
Methods. Thirty patients undergoing hemodialysis were included in this study. Erythrocye MDA (marker of lipid peroxidation) concentrations were determined according to the method of thiobarbituric acid-reactive substances. The antioxidant capacity of plasma were measured by method of Benzie & Strain (FRAP assay).
Results. According to our findings, Erythrocye MDA levels were significantly higher after compare to before hemodialysis in patients ($p<0.001$).FRAP levels in plasma of hemodialysis patients significantly decreased after than before hemodialysis ($p<0.001$) .
Conclusions. These findings indicate that oxidative stress in hemodialysis patients followed by increased lipid peroxidation and decreased total antioxidant capacity of plasma. Hence, considering the important role of oxidative stress and progression of complications of hemodialysis in creating strategies for inhibiting This reaction can be an effective step taken to further improvement of these patients.

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ALBUMINURIA IN THE ASSESSMENT OF THE DETERIORATION OF RENAL FUNCTION IN EARLY HYPERTENSIVE NEPHROPATHY

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Background. Investigation of laboratory parameters in hypertensive patients with normoalbuminuria and microalbuminuria (urinary albumin to creatinine ratio - uACR <30 mg/mmol) for early detection of kidney damage. Assessment of albuminuria and estimated glomerular filtration rate (e-GFR) were used in combination as two factors linked to kidney function.
Methods. Albumin in the urine were evaluated in 102 hypertensive subjects with urinary albumin to creatinine ratio (uACR) <30 mg/mmol. e-GFR was calculated using Modification of Diet in Renal Disease.
Results. No statistically significant association was etablished between urinary albumin concentration (mg/dl) and e-GFR (ml/min/1.73 m^2) values ($p>0.05$). Decreased e-GFR values significantly ($p=0.017$) correlated with gradual increases in uACR (mg/mmol) values. For e-GFR >90 - median (range) uACR was 0.68 (0.10-24.65), for e-GFR 76-89 - uACR 1.43 (0.19-14.34), for e-GFR 60-75 – uACR 1.22 (0.17 – 6.08), for e-GFR<60 – uACR 3.86 (0.66 -12.93). Disperssion of uACR values within e-GFR groups were considerable.
Conclusions. uACR in spot urine is better test to detect the decreased e-GFR in early hypertensive nephropathy than albumin concentration in the urine.

Kidney disease

0759

NITRIC OXIDE FORMATION AND ACID-BASE BALANCE ALTERATIONS EARLY FOLLOWING KIDNEY TRANSPLANTATION: RECIPIENTS VS. LIVING DONORS

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Background. Ischemia /reperfusion injury is commonly seen in renal surgery or transplantation. The purpose of the present study was to examine relationship between acid-base balance and nitric oxide (NO) formation within the first week following kidney transplantation, comparing recipients vs. donors, both having single kidney after surgical procedures of similar severity.
Methods. Arterial blood samples were taken before, after anesthesia and surgery (0-time) as well as 1, 2, 3, and 7 days after transplantation in recipients (R group, n=8) and living donors (D group, n=8). Measurements of pH, pCO_2, actual bicarbonate (AB) and excess base (EB) were performed by ABL System (Radiometer, Copenhagen), while nitrites+nitrates (NOx) were analyzed by capillary electrophoresis.
Results. Control limits were as follows: pH 7.366-7.440units, pCO_2 35.6-42.0mmHg, AB 23.3-27.1mmol/L, EB -1.1-2.1μmol/L and NOx 21.0-37.0μmol/L. In D group all parameters were within control limits throughout the examined period, except transient pH and EB decrease at 0-tim. In R group before transplantation NOx (55.4±14.7*μ*mol/L) was above, pH (7.323±0.042units), and EB (-7.0±3.0mmol/L) below control limits. Early after transplantation NOx, pH and other parameters were brought to the control limits, but EB stay below (p<0.05) control limits and corresponding time interval in D group until the end of examined period. Moreover, EB negatively correlated with NOx (r= -0.738, p<0.001).
Conclusions. Respiratory compensated metabolic acidosis, indicated by decreased pCO_2, normalized pH and prolonged negative EB suggest prolonged compensated metabolic acidosis early after transplantation. Interestingly, EB was negatively related to NO production and that has to be considered in therapy.

0760

EARLY TIME-COURSE ALTERATIONS OF ARGININE METABOLISM IN PATIENTS FOLLOWING KIDNEY TRANSPLANTATION

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Background. This study aimed to quantify early changes of plasma arginine (Arg), a semi-essential amino acid, mainly synthesized in the kidney, phenylalanine (Phe), reflecting net protein catabolism, citrulline (Cit) and ornithine (Orn), involved in Arg metabolism, asymmetric dimethylarginine (ADMA), an endogenously produced competitive inhibitor of endothelial NO-synthase, and nitric oxide (NO) production, within two weeks following kidney transplantation in human.
Methods. We investigated patients (n=8) before and after kidney transplantation (living donors). Controls were healthy subjects (n=8). Arterial blood samples were taken before, after anesthesia and transplantation (0-time) and 1, 2, 3, 7 and 14 days after transplantation. Amino acids (Arg, Phe, Cit, and Orn) were analyzed by an amino acid analyzer (AAA-400, Ingos, Czech), ADMA by Elisa-test, nitrites+nitrates (NOx) by capillary electrophoresis.
Results. Before transplantation plasma Arg (89.6±14.6*μ*mol/L vs. control 102.6±5.5*μ*mol/L) was significantly (p<0.05) below, Cit (94.4±16.9*μ*mol/L vs. control 30.2±5.3*μ*mol/L), ADMA (0.693±0.158*μ*mol/L vs. control 0.432±0.035*μ*mol/L) and NOx (55.4±8.0*μ*mol/L vs. control 29.0±8.0*μ*mol/L) above, while Phe and Orn were within control limits. Immediately after transplantation (0-time) decrease of all parameters occurs. After that Arg and Cit levels were below, ADMA above control limits, while NOx normalized and Phe increased comparing to 0-time. Molar Arg/Phe ratio was not altered.
Conclusions. Decreased plasma Arg level associated with increased Phe and unaltered molar Arg/Phe ratio suggest increased Arg utilization with increased net protein catabolism. Elevated ADMA level indicate increased risk of atherogenesis throughout examined period in these patients that has to be considered in their therapy.

Liver and gastrointestinal diseases

0761

EFFECTS OF LONG-TERM LOW DOSE ASPIRIN INTAKE ON GASTRODUODENAL MUCOSA AND PROTECTION BY GSH-VITAMIN C AND E

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Background. Inflammation and oxidative stress may be the important events that lead to gastroduodenal mucosal damage induced by aspirin (ASA). It is hypothesized that GSH-Vitamin C-E may protect to the upper gastrointestinal mucosa against aspirin's adverse effects.

Methods. The adult male rats were randomly divided into three groups of 8 each. Rats were fed regular diets and maintained for 40 days in the control group, the ASA group, which was given aspirin (1.44 mg/kg/day) administered orally by feeding tube to rats, or the ASA with antioxidant supplement group, to whom 1.44 mg of aspirin/kg/day + a solution that contained 50 mg vitamin C, 25 mg vitamin E and 25 mg GSH was administered orally by feeding tube to rats every day. After the treatments, blood, stomach and duodenum were taken for pathological and biochemical analysis.

Results. The stomach and duodenum of the ASA group rats had higher scores of pathological findings compared with the control group, whereas the scores of the antioxidant-suplemented group were lower than the ASA group. In addition, the gastric mucosal myeloperoxidase (MPO) activity and heat shock protein (Hsp) 70 levels in the ASA group were significantly higher than the control, but antioxidant suplementation lowered the values of MPO and Hsp 70 in the antioxidant supplemented group (p<0.01).

Conclusions. A simultaneous intake of GSH-vitamin C- E along with aspirin attenuated the gastric mucosal injury. GSH-vitamin C and E could play a protective role in the stomach against gastric damage resulting from long-term low dose aspirin intake.

0762

BIOPSY OR BIOMARKERS: WHICH IS THE SAMPLE OF CHOICE IN ASSESSMENT OF LIVER FIBROSIS?

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Background. The aim of the study is to assess the diagnostic value of fibrotest and hyaluronic acid in discriminate between insignificant and significant fibrosis. Also, to find out if these parameters could replace liver biopsy which is currently used for selection of chronic hepatitis C patients eligible for antiviral therapy.

Methods. This study was conducted on 52 patients with HCV RNA detected by polymerase chain reaction (PCR) who had undergone liver biopsy and attending the internal medicine clinic at Ain Shams University Hospital. Liver fibrosis was evaluated according to the METAVIR scoring system on a scale of F0 to F4. Biochemical markers assessed were: alpha-2 macroglobulin (α2-MG), apolipoprotein A1 (Apo-A1), haptoglobin, gamma-glutamyl transferase (GGT), total bilirubin (TB) and hyaluronic acid (HA). The fibrotest score was computed after adjusting for age and gender. Predictive values and ROC curves were used to assess the accuracy of fibrotest and HA Results.

Results. For fibrotest, the observed area under curve for the discrimination between minimal or no fibrosis (F0-F1) and significant fibrosis (F2-F4) was 0.6736 for cutoff value 0.19 with sensitivity of 84.2% and specificity of 85.7%. For HA, the sensitivity was 89.5% and specificity was 85.7% and area under curve was 0.540 at the best cutoff value 71 mg/dL. Multi-use of both parameters, HA at 71 mg/dL with fibrotest score at 0.22 give a sensitivity 89.5%, specificity 100 and efficacy 92.3% (AUC 0.895).

Conclusions. The use of both fibrotest score and HA could be as alternative to biopsy in most patients with chronic hepaitis C putting in consideration some limitations of the proposed markers in evaluating liver fibrosis.

Liver and gastrointestinal diseases

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7-ALPHA-HYDROXY-4-CHOLESTEN-3-ONE CAN BE A CLINICALLY USEFUL CIRCULATING MARKER FOR LIVER AND INTESTINAL DISEASE

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Background. Several biochemical tests for liver and intestinal dysfunction exist, yet there is a need for additional tests. We have previously demonstrated that the levels of 7-alpha-hydroxy-4-cholesten-3-one (C4) in plasma reflect rates of bile acid synthesis in man. Analysis of C4 has since then been widely used to study short- and long-term effects of various factors, such as drugs, individual bile acids, diets, alcohol etc. on bile acid production in humans. However, analysis of C4 has clinically mainly been limited to diagnose patients with chronic diarrhoea caused by bile acid malabsorption. Since the rate of bile acid synthesis depend on the condition of both the liver and the intestine, we have now studied the possibility to clinically use C4 as a more general marker for liver and intestinal diseases in patients.
Methods. Plasma levels of C4 from patients with various liver and intestinal diseases were analysed by HPLC or LC-MS/MS.
Results. The results show that most patient groups with liver disease had subnormal levels of C4 and patient groups with ileal dysfunction or ileal resection had pathologically elevated levels. Many of the patients with fat malabsorption had, however, normal levels of C4. A rapid and sensitive LC-MS/MS method for analysis of C4 in plasma was developed, which permits a large number of samples to be processed.
Conclusions. The results show that analysis of C4 in plasma can provide valuable information about the condition of the liver and intestines when investigating patients with suspected liver or intestinal dysfunction.

0764

IMPORTANCE OF CALCULATION OF FIBROSIS INDEX IN DIFFERENT LIVER DISEASES

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Background. Different methods can be used to evaluate liver fibrosis. Liver biopsy is the most frequently used, but it has some drawbacks. Nowadays, researches are directed into development of noninvasive approaches in assessing stage of liver fibrosis. We used the index-NAFLD fibrosis score, which was evaluated in patients with nonalcoholic fatty liver disease to distinguish between patients with and without advanced fibrosis. The index is based on six parameters: age, body mass index, hyperglycemia, aspartate aminotransferase/alanine aminotransferase ratio, platelet count and albumin.
Methods. We enrolled 136 patients with undefined cirrhosis, alcoholic cirrhosis, alcoholic hepatitis and cholangitis. We used weight and height to calculate BMI and took them blood for the determination of glucose, AST, ALT, albumin and platelet count. We calculated an average index of certain group and statistically compared indices of four groups.
Results. The average fibrosis index for undefined cirrhosis was 0,81; 1,94 for alcoholic cirrhosis; 2,73 for alcoholic hepatitis and -1,46 for cholangitis. The aim of the statistical comparison of the average indices of these four groups was to determine whether there is a statistical difference among the groups. We used an additional test to determine where the statistical difference occurs.
Conclusions. The results show that there was a statistical difference in the average fibrosis indices among the groups. The most withdrawal of all four groups was the group with cholangitis. This group was statistically different compared to the undefined cirrhosis, alcoholic cirrhosis and alcoholic hepatitis. The test showed no differences among other three groups.

Liver and gastrointestinal diseases

0765

ANTI-CHLAMYDIA PNEUMONIAE ANTIBODIES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Background. Primary biliary cirrhosis (PBC) is a chronic cholestatic, autoimmune liver disease. We studied the role of environmental agents in the pathogenesis of PBC. The aim of the work was to determine anti-Chlamydia pneumoniae antibodies (anti-Cpn) level in sera of patients with primary biliary cirrhosis.
Methods. Cpn IgG, IgM, IgA were determined by ELISA kit in 92 well established PBC patients and 92 control patients (PSC - primary sclerosing cholangitis, AIH - autoimmune hepatitis, alcoholic liver disease - ALD, healthy controls).
Results. Antibodies to Cpn (IgG) were detected in 74% patients with PBC. The mean level of of Cpn IgG in PBC group and group with other liver diseases were significantly higher than those in healthy control (82-78 Ru/ml,61-57 Ru/ml vs 46-41 Ru/ml). Seroprevalence of Cpn IgG in PBC group was significantly higher than those in PSC, AIH, ALD and healthy control and were: 74%, 50%, 29%, 33% and 30%, respectively. There was remarkably elevated seroprevalence of Cpn IgA and IgM patients with PBC (30%,27% respectively) compared to the other groups. For the PBC patients versus the healthy control, the odds ratios (ORs) of the presence of Cpn IgG , IgM and IgA were 3.8 9 (95% CI 1.4-10.4), 7.09 (95% CI 0.9-55.8) and 8.31 (95% CI 1.1-65.2) .
Conclusions. We didn't support the high association between C. pneumonia infection and PBC, but we suggest molecular mimicry as one possible mechanism underlying the breakdown immune tolerance subsequent autoimmunity disease.

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HELICOBACTER PYLORI ASSOCIATED WITH CHRONIC URTICARIA

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Background. Helicobacter pylori are the most important cause of gastritis and peptic ulcer but also have been associated with several extra digestive diseases. Since the prevalence of infected persons with Helicobacter Pylori and chronic urticaria disease in Kosovo is high, the aim of this study was to evaluate correlation between Helicobacter Pylori antibodies IgG and IgA in appearance of chronic urticaria.
Materials and Methods. In this study were examined 105 persons (18 – 65 Years old) from which 62 were diagnosed with chronic urticaria (23 Male, 39 Female).The patients with chronic urticaria were clinically diagnosed by dermatologist. Blood samples were collected and analyzed for levels of serum IgG and IgA antibodies against H. pylori with ELISA method.
Results. In 62 patients with chronic urticaria 71 % (n=44) had elevated titer of both H.Pylori IgA and IgG antibodies. H.Pylori IgG antibodies were found elevated in 47.13 % of patients, while only IgA antibodies were elevated in 43.55 % of patients. Helicobacter Pylori antibodies together in the chronic Urticaria patients were statistically associated (*P*<0.002). Among group without chronic urticaria we found elevated values of H.Pylori antibodies IgA and IgG in 39.6 % of patients. H.Pylori IgG antibody was higher than normal value in 41.9 % and Anti-H.Pylori IgA was increased in 37.4 %.
Conclusions. Overall results indicate a significant relationship with chronic hives and both IgG and IgA together in patients, indicating the possible role of *H*. pyloriin symptoms of chronic urticaria.

Liver and gastrointestinal diseases

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SEROLOGICAL MARKERS OF GASTRIC PATHOLOGY

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Background. To assess how serological tests, Pepsinogen I [PGI], Pepsinogen II [PGII], Gastrin 17 [G17] and anti Helicobacter pylori IgG antibodies [HP-IgG] (Gastropanel), provide useful information on the gastric mucosa.
Methods. Results were formed by ELISA techniques (Biohit, Helsinki, Finland and Euroclone Milan, Italy), 01 March 2009-12 January 2010, (Cut-offs:PGI:40-100µg/L;PGII:2,5-10;ratio PI/PII > 3; G17:2,0-7,0;HP-IgG: <32 IU). Data were retrieved for 1049 tests performed on 685 women (mean age± SD: 48,1± 38,5) and 364 men (mean age ± SD:49,1±18,2).
Results. the population was clustered: Class1: 616 patients: Normal Gastric Mucosa (59%:mean age± SD: 45,5±17,3; females/males 392/224); PgI (median,5-95° percentiles):67,2; 44,6-129 µg/L; PgII:6,1; 3,1-9,5 µg/L;G17:1,4; 0,2-18,2 pmol/L; IgG-Hp:2,7; 0-88,7U/L including 361 patients with probably gastroesophageal reflux (mean age ± SD: 45,7±16,4; females/males 218/143);PgI:64,8; 44,8-105,9 µg/L;PgII:5,5;2,9-9,2 µg/L;G17:0,6;0,1-1,8;IgG-Hp:2,0;0-77,4. Class2:310 patients: Non Atrophic Gastritis (PgII>10 µg/L)(30%: mean age±SD:53,6±16,5; females/males 198/112),118 of which were Hp positive (mean age±SD:52,7±14,74; females/males 76/42): PgI:111,5;41,5-261,3 µg/L;PgII:16,3;10,8-50,2µg/L;G17:7,8;0,8-59,8pmol/L;IgG-Hp:87,2;43,4-229,7U/L, and 191 Hp negative (mean age±SD:54,1±17,5; females/males 121/70) PgI:145,0;37,4-271,2 µg/L;PgII:14,9: 10,2-53,1µg/L;G17:8,0;0,2-40,0pmol/L;IgG-Hp:4,3;0,03-34,6U/L-0). Class3: 123 patients: possible Atrophic Gastritis (PgI <40 µg/L) (11%: mean age±SD: 44,4±21,2; females/males 98/25) including 37 patients with probable atrophic gastritis of the gastric body (mean age±SD: 56,8±17,9; females/males 26/11) PgI:17,7;3,7-37,1 µg/L; PgII: 8,3:2,7-33,5 µg/L; G17: 40,0;7,3-84,0 pmol/L; IgG-Hp:8,82;0,1-100U/L, and 86 patients with widespread atrophic gastritis (mean age±SD: 39,1±20,3; females/males 72/14) PgI:33,0; 7,5 -39,3 µg/L; PgII:3,6:1,5-7,8 µg/L; G17: 0,7; 0, 0 -5,4 pmol/L; IgG-Hp: 2,6;0,0-87,1U/L.
Conclusions. The classes need a specific diagnosis and treatment The clinical usefulness of Gastropanel might be extended to a more reliable planning of additional invasive testing.

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ROLE OF VITAMIN C IN THE MECHANISM OF CHOLESTEROL GALLSTONES FORMATION IN MAN

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Background. Chile has the highest frequency of gallstones published in the world. A deficiency of vitamin C, in experimental animals, drives to a supersaturation of biliary cholesterol and to the gallstones formation. Biliary cholesterol is transported by vesicles and micelles, but the cholesterol microcrystals derived from physicochemically unstable vesicles. The aim of this study is to establish a possible relation between serum levels of vitamin C and the formation of cholesterol gallstones formation in patients with cholelithiasis.
Methods. Our patients (n=13) were treated with vitamin C (1 g, two times a day) for 2 weeks before surgery. The control group (n=49) was composed of cholecystectomy patients without administration of vitamin C. We determined plasma concentrations of vitamin C and lipid profile. In the biliary samples we analyzed the cholesterol saturation index, crystalization time, and vesicular and micellar cholesterol content, separated by gel filtration chromatography.
Results. The vitamin C supplementation did not change significantly the plasma and biliary lipid composition. However, a significant reduction in vesicular cholesterol content (6.5 ± 4.8% vs. 17.9 ± 14.0%, in the control group, p< 0.05) and particularly a lower vesicular cholesterol/phospholipid ratio (0.71 ± 0.53 vs. 1.36 ± 1.15, p< 0.05) was obtained in the treated group.
Conclusions. These results suggest that oral vitamin C administration change the biliary cholesterol crystalization process, the first step in the cholesterol gallstones formation. We hypothesized that vitamin C status may be a risk factor for human gallbladder disease.

Liver and gastrointestinal diseases

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EVALUATION OF EXOCRINE PANCREATIC FUNCTION USING PANCREOLAURYL AND ELASTASE-1 TESTS

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Background. The chronic pancreatitis (CP) is a gradual destruction of the pancreatic parenchyma leads to the impairment of exocrine and endocrine functions. The diagnosis is usually based on clinical features and imaging techniques. The biochemical pancreatic function tests are: pancreolauryl test in serum (PL, measurement a pancreas-specific enzyme cholesterol ester hydrolase), and elastase-1in stool (Ela-1, determination of pancreatic isoenzyme). It has been used to evaluate exocrine pancreatic capacity and assessment of pancreatic insufficiency.
The objective was to evaluated clinical usefulness PL and Ela1, in CP patients.
Materials-Methods. Serum and stool of 41 patients: CP (n=13 49±10years; 5men/8women) and no pancreatic digestive disease-NPD (n=28; 54±15 years, 8men/20women). PL was determined photometrically and Ela1 by ELISA (BioservDiag).
Results.

- significant decrease of PL($P<0.001$) and Ela1($P<0,05$); PL below normal value (4,5 g/l); 7 patients presented Ela1 below reference value(200ug/gr); and significant positive association ($P<0.05$) PL with Ela1 (r=0.59-IC(0.037-0.865); in CP respect to NPD patients
- significant positive association ($P<0.05$) PL with Ela1(r=0.38-IC(0.083-0.616), and Ela1 showed ($p<0,01$):sensitivity=54%(IC:0.25- 0.81) and specificity=89%(IC:0,72-0,98), in all patients studied.

Conclusions.

- The decrease of PL and Ela1, and the positive association between them, which specially when the PL is less than the cut-off value, suggest of pancreatic hypofunction in CP patients.
- Ela1 shows low sensitivity, because polyclonal antibody was used; for this reason we suggest using a monoclonal test.
- We recommend the use of the serum pancreolauryl test as a helpful tool in the diagnosis of CP.

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DIAGNOSTIC ACCURACY OF COELIAC SEROLOGICAL TESTS

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Background. The current guidelines, as a first diagnostics step of coeliac disease (CD), still recommend use of either IgA anti-transglutaminase (TTG) or IgA antiendomisial (EMA) antibodies as well as total serum IgA antibodies. The aim of this study was the evaluation of diagnostic accuracy of IgA anti-TTG antibodies in CD.
Methods. 50 patients of both sexes (21 male and 29 female), average age 39 years for male and 47.4 years for female, with clinical features of CD were tested for IgA anti-TTG (BioSystems, Spain) and endoscopic biopsy was done. Basic biochemical and hematological parameters were done using standard Methods.
Results. CD was confirmed by endoscopic biopsy in 4 patients (2 male & 2 female) while IgA anti-TTG were positive in 3 patients (1 female was falsely negative due to hypoproteinemia and IgA deficiency). In our risk group sensitivity was 75%, specificity 100%, positive likelihood ratio was 75% while negative likelihood ratio was 25%. Prevalence of CD in our group was 8,2%. In male subgroup significant differences between patients with and without CD were present in mean values of MCV (96.5±7.7 vs. 78.6 ±11.3; $p<0.05$), MCH (36.9±4.6 vs. 25.9±4.9; $p<0.01$), MCHC (382.5 ±16.3 vs. 326.9±19.1; $p<0.005$) total proteins (47.5 ±16.3 vs. 68.3 ± 7.6; $p<0.01$), albumins (24.6±9.5 vs. 42.1 ± 6.9; $p<0.01$), and HDL-cholesterol (0.42.±0.12 vs. 0.90±0.30; $p<0.05$). In female subgroup there were no significant differences between patients with and without CD.
Conclusions. Our results have shown high correlation IgA anti-TTG with gold standard (endoscopic biopsy).

0771

NON-INVASIVE TEST TO ASSESS HEPATIC FIBROSIS. COMPARISION OF APRI, FORNS AND FIB-4 INDEX WITH TRANSIENT ELASTOGRAPHY IN CHRONIC HEPATITIS C

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Background. Non-invasive biochemical markers have been proposed for the evaluation of the severity of hepatic fibrosis, such the AST-to-platelet ratio index (APRI), the Forns test and FIB-4 index. We compared these indices with a promising and reproducible method that measure liver stiffness to assess liver fibrosis: the transient elastography.
Methods. Indices were calculated using the formulas taken from the original publications and values of GGT, AST, ALT, platelet count, cholesterol and age of 133 patients with chronic hepatitis C. No laboratory values were used if beyond 3 months from the date of FibroScan measurement. The liver stiffness was measured using FibroScan and the results were expressed in kilopascals. We used the application of ROC analysis to evaluate these different Methods. Statistical analysis was performed with SPSS 17.0.
Results. FibroScan values ranged from 2,1 to 75,0 kPa (median 6,9). To stage we used the cut-off values currently accepted (F0-1:≤ 7,0 kPa; F2: 7,1-9,4 kPa; F3: 9,5-14,5 kPa and F4: >14,5 kPa). The areas under the ROC curve of APRI, FORNS and FIB-4 were 0,657, 0,702 and 0,666 respectively, for F≥2; 0,775, 0,806 and 0,789 respectively for F≥3; and 0,956, 0,926 and 0,932 for F=4.
Conclusions. Our results suggest that FORNS is the index that presents the best concordance with the results of FibroScan to differentiate significant fibrosis (F0-F1 versus F2-F3-F4) and advanced fibrosis (F0-F1-F2 versus F3-F4). For assessing cirrhosis (F0-F1-F2-F3 versus F4) the three indices were proven to be in concordance with FibroScan results, being the most significant the APRI index.

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PORTAL HYPERTENSION IN LIVER CIRRHOSIS: DINUCLEOTIDE (GT)N POLYMORPHISM OF HMOX1 AND THE INFLAMMATORY PROFILE

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Background. Portal hypertension as complication of Liver cirrhosis has recently shown a mayor inflammatory and oxidative stress influence, HMOX1 is the catalytic enzyme of HEME into CO, Bilirrubin and Fe^{++}, these products and the enzyme have been studied due to their antioxidant and anti-inflammatory properties, therefore it is of great interest to determine the relation between dinucleotide (GT)n polymorphism of HMOX1 in Mexican population and the development of Portal hypertension and liver cirrhosis.
Methods. Polymorphism was determined by PCR and poly acrylamide electrophoresis, small (S) repetitions consider under 125 bp and large (L) repetitions above 125 bp, resulting in tree genotypes possible: L/L, S/L, S/S. The serum values of HMOX1 and the inflammatory cytokines: IL-1β, TNFα, IL-10 as anti-inflammatory was determined by ELISA. The genotype frequency was determined and for ELISA results, correlations were established.
Results. Genotype L/L, has higher frequency 57.6%, p=0.007 compared to control group where genotype S/L has a higher frequency 60%. L/L, is predominant in male population 63.2 %, p=0.006, and alcoholism as main etiology, 70.8%, p=0.005, IL-10 values were higher than the Cirrhosis group, p=0.002, HMOX1, TNFα and Bilirrubin values were higher in Cirrhosis group than control p=0.031, p=0.008 and p=0.009 respectively.
Conclusions. L/L genotype showed correlation to the development of Liver Cirrhosis and Portal hypertension specially on alcoholism as etiology in male population, HMOX1, Bilirrubin and TNFα showed a positive correlation, IL-10 and IL-1β showed a negative correlation, HMOX1 increases in the presence of inflammation despite genotype in cirrhosis with or without hypertension.

Liver and gastrointestinal diseases

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EFFECT OF THE IBD-ASSOCIATED NONSYNONYMOUS CODING VARIANT RS3197999 IN MACROPHAGE STIMULATING PROTEIN ON MACROPHAGE FUNCTION

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Background. The association of the common MSP-SNP rs3197999 with IBD (inflammatory bowel disease) was shown by several authors. MSP (macrophage stimulating protein) modulates macrophage motility, shape changes and phagocytotic activity. It is known to be involved in inflammation and carcinogenesis; however, its potential role in IBD has not been investigated.

Methods and Results. C2078T results in the substitution of an arginine by a cysteine at position 689 of the MSP protein. SIFT Genome predicts functional consequences of Arg689Cys based on sequence homology and the physical properties of the amino acids.

Human wild type and mutant type MSP cDNAs were cloned and expressed in CHO-K1 cells. The effect of MSP on migration properties of THP-1 cells (AML cell line, macrophage-like phenotype) was investigated by boyden chamber experiments, the effect on proliferation was investigated by BrdU-incorporation assay. Both wild type and mutant MSP stimulated migration and proliferation but the mutant type was significantly more effective: MSP^{mut} stimulated THP-1 migration and proliferation in lower concentrations and reached a higher maximal stimulatory effect. MSP dependent apoptosis was excluded by TUNEL staining and caspase activity assay. Western Blot and inhibitor experiments suggested that this effect is mediated by the MAPK-pathway.

Summary and Conclusions. Arg689Cys increases the stimulatory function of MSP on macrophage migration and proliferation. While the association of rs3197999 with IBD was confirmed several times previously, we here show for the first time physiological effects of MSP on macrophages which are known to play an essential role in the pathogenesis of IBD.

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PANCREATIC CANCER – SURVIVAL DEPENDING OF SUCCESSFULNESS OF THERAPY

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Background. Pancreatic cancer is the fourth most common cause of death due to cancer as it is usually detected in an advanced form when a radical operation is no longer possible. The role of CA 19-9 marker has been investigated on patients with pancreatic cancer for many years. There are some recommendations regarding the role of CA 19-9, however, its clinical relevance needs to be yet investigated and confirmed.

Methods. We enrolled 34 patients with pancreatic cancer. The diagnosis was confirmed by ultra sound and/or CT scan. The values of CA 19-9 marker were determined by chemiluminiscence immunological sandwich method. Correlations were investigated using statistical methods between the values of CA 19-9 and survival rate.

Results. CA 19-9 concentrations before therapy and at lowest point can be used as predictive factor for the disease. The correlations found were ($p = 0,015$, $s = -0,472$) for the whole group and ($p = 0,034$, $s = -0,669$) in a group without metastases. The results show that there is a strong negative correlation between the lowest concentration of CA 19-9 and survival ($p < 0.0005$, $s = -0,813$)

Conclusions. CA 19-9 values are mainly much higher in the group with metastases than in the group without metastases. In the group without metastases CA 19-9 values decrease close to the reference limit during therapy, whilst in the group with metastases this was not the case. The patients where the first order chemotherapy was inefficient received second order chemotherapy which prolonged the survival rate.

Liver and gastrointestinal diseases

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THE ACTIVITY OF ALCOHOL DEHYDROGENASE ISOENZYMES AND ALDEHYDE DEHYDROGENASE IN THE SERA OF PATIENTS WITH ACUTE AND CHRONIC PANCREATITIS

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Background. Acute and chronic pancreatitis is a major complication of alcohol abuse The pancreas can metabolize ethanol via oxidative pathway involving the enzymes – alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as well as the nonoxidative pathway. Pancreatic tissue contains various ADH isoenzymes and possess also ALDH activity.In thisstudy, we investigated the effect of pancreas cells inflammation and damage in acute and chronic pancreatitis on the serum activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase.
Methods. Serum samples were taken from 46 patients suffering from acute and 32 patients from chronic pancreatitis. Class I and II ADH isoenzymes and ALDH were measured by fluorometric method with specific substrates. For measurement of class III, IV and total ADH activity we employed photometric method.
Results. A significant increase of class III ADH isoenzymes was found in the sera of patients with acute (2.876±1.534 mU/l)and chronic pancreatitis (2.812±1.752 mU/l). The median activity of this class isoenzyme in the total patients group increased about 35 % in the comparison to the control . The total ADH activity was also significantly higher (23.5 %) among patients with pancreatitis (695±466 mU/l)than healthy ones (522±230 mU/l). The activities of other tested ADH isoenzymes and total ALDH were unchanged. The activity of ADH I was significantly higher in the sera of heavy drinkers with pancreatitis.
Conclusions. These results demonstrate that serum activity of class III ADH is an indicator of pancreatic cells destruction and may be useful in the diagnosis of acute and chronic pancreatitis.

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DIAGNOSTIC UTILITY OF SIMPLE NONINVASIVE MARKERS AND INDEXES FOR NONALCOHOLIC STEATOHEPATITIS

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Background. Diagnosis of the nonalcoholic steatohepatitis (NASH) with noninvasive markers and indexes is an important issue in routine clinical practice. Therefore, we aimed to determine the diagnostic utilities of prolidase, ALT, AST, AST/ALT ratio, APRI, FIB-4, FORNS and API indexes for the prediction of NASH.
Methods. Grading of steatohepatitis for liver biopsy performed 57 patients with nonalcoholic fatty liver disease was carried out according to the Pathology Committee of the NASH Clinical Research Network Scoring System.
Results. Prolidase, AST, ALT and APRI index were statistically significant among the NASH subgroups (p=0.007, p=0.010, p=0.019 and p=0.021, respectively). Prolidase values were [1051(829-1325) U/L, 1289(1137-1644) U/L and 1375(1225-1512) U/L)]; AST values were [25(21-36) U/L, 25(23-29) U/L and 38(32-46) U/L)]; ALT values were [30(19-48) U/L, 31(23-38) U/L and 71(37-88) U/L]; APRI index values were [0.25(0.18-0.37), 0.26(0.21-0.32) and 0.34(0.30-0.49)], median(25th-75th interquartile range) for grade 0, 1 and 2 steatohepatitis, respectively. Prolidase, AST and ALT were correlated significantly with the grade of steatohepatitis (r=0.392, p=0.003; r=0.284, p=0.032; r=0.265, p=0.049, respectively). The areas under the ROC curve for prolidase, AST and ALT were 0.806, 0.560 and 0.555, respectively. Multivariate linear regression analysis revealed that prolidase enzyme activity was the best indicator for NASH (p=0.009).
Conclusions. This study demonstrated that prolidase enzyme activity could be a useful non-invasive biomarker for the prediction of NASH, but AST, ALT, AST/ALT ratio, APRI, FIB-4, FORNS and API indexes could not.

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VITAMIN B12 AND FOLATE STATUS IN *HELICOBACTER PYLORI* SEROPOSITIVE SUBJECTS

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Background. Recent studies have showed thatvarious micronutrients absorption may be diminished in case of gastrointestinal dysfunction during *Helicobacter pylori* (HP) infection. The aim of this study was to investigate the relationship between HP infection, estimated by serological test, and serum levels of vitamin B12 and folates.
Methods. Groups of103 HP seropositive subjects (70 female and 33 male) (IgG>50 U/ml) and 103 gender matched controlHP seronegative subjects (IgG<35 U/ml) were investigated. HP IgG antibodies were determined by ELISA method (Virion/serion, Germany). Levels of vitamin B12 were determined using MEIA method (Abbot, USA). Folate levels were determined by ion capture assay (Abbot, USA).
Results. In whole HP seropositive subject group, serum levels of vitamin B12 (269.85±139.11 vs. 306.42±178.79) and folates (18.44±7.71 vs. 19.87±8.64) were lower than HP seronegative ones, but without significance. Lower levels of vitamin B12 were found in HP seropositive females (283.97±156.08 vs. 304.21±193.26) and, significantly, in males (239.90±88.29 vs. 311.10±146.05, p<0.025) than coresponding control HP seronegative subjects. Non-significantly lower levels of folates were found in HP seropositive females (19.66±8.29 vs. 20.27±9.10) and males (15.85±5.56 vs. 19.04±7.65), too. Pathological low levels of vitamin B12 were found in 13.59% (12.86% females and 15.15% males) and folates in 48.54% (40.00% females and 66.67% males) HP seropositive subjects. Significant univariate correlation analysesbetween vitamin B12 and folate levels and HP IgG titers were not found.
Conclusions. Decreasing of vitamin B12 levels, which were found in HPseropositive subjects, were significant only in male subgroup. These findings need further investigations.

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ASSESSMENT OF THE DIAGNOSTIC APPLICABILITY OF QUANTITATIVE IMMUNOCHEMICAL FAECAL OCCULT BLOOD TESTS

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Background. Today, faecal occult blood (FOB) tests are the most commonly used screening tests for identifying gastrointestinal diseases. The use of these tests is of exceptional importance, as they allow us to discover potential malignant transformations at an early stage.
Methods. The study included 83 symptomatic patients undergoing endoscopic and/or histological examinations. The presence of FOB was detected using three different laboratory Methods. a qualitative colour test (Hema-Screen Occult Blood Test, Stanbio Laboratory), a qualitative immunochemical test (Diaquick FOB Cassette, Dialab) and the latest quantitative immunochemical test (FOB Gold, Sentinel Diagnostics) using the Modular Roche biochemical analyzer. Patients suffering from polyps, malignant diseases or observed bleeding during endoscopy were determined to be affected patients.
Results. The ROC curve analysis found the cut-off value of quantitative immunochemical test to be 55 ng/mL. The results of all three laboratory methods match at 62.7% (52/83).The quantitative immunochemical test matches the two tests in 75.9% of cases. The Kruskal-Wallis test was used to illustrate statistically significant differences in the tests (P = 0.004). Diagnostic sensitivity was calculated for the qualitative colour test, the qualitative immunochemical test and the quantitative immunochemical test (51.6%; 74.2% and 45.2% respectively), as well as diagnostic specificity (80.8%, 71.2% and 92.3%), the positive predictive value (61.5%, 60.5% and 77.8%), the negative predictive value (73.7%, 82.2% and 73.9%) and the diagnostic efficiency (70%, 72% and 75%).
Conclusions. The quantitative immunochemical test is the most appropriate test for establishing FOB, as it has the highest diagnostic specificity and a positive predictive value.

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DETERMINATION OF HEPATOCYTE GROWTH FACTOR IN PATIENTS WITH ACUTE PANCREATITIS

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Background. Acute pancreatitis (AP) is self-limiting disease in most patients, but its necrotizing form develops in up to 20-30% cases. Early diagnosis of necrotizing AP has been considered a key requirement for successful therapy. The aim of this study was to evaluate the diagnostic value of Hepatocyte Growth Factor (HGF) as a new predictor of AP complications.
Methods. Thestudy included 40 patients with AP (28 edematous, 12 necrotizing); 24 (60%) males and 16 (40%) females, mean age 47 years, admitted to Department of Surgery. HGF concentrations were measured by ELISA (R&D Systems). IL-6, CRP, SAA, fetuin A and albumin were also measured. Non-parametric statistical tests (Mann-Whitney and Spearman, as appropriate) were used; $p < 0,05$ was considered statistically significant.
Results. Serum HGF concentrations was significantly higher in severe than in mild AP on 1st, 3rd and 5th day after admission (median7,61 vs 3,30; 7,19 vs 3,43; 5,76 vs 2,42 ng/mL, respectively; $p < 0,05$). On 3rd day, HGF correlated significantly with Glasgow and Imrie scores (R=0,57; R=0,51). The HGF correlations with IL-6 were increasingly stronger on each day of the study (R=0,61; 0,77 and 0,85 on 1st, 3rd and 5th day). Starting on the 3rd day, HGF strongly positively correlated with CRP (R=0,93; 0,80 on 3rd and 5th day) and SAA (R=0,78; 0,82) but negatively with fetuin A (R=-0,60; -0,45) and with albumin (R=0,72 on 3rd day).
Conclusions. Serum HGF correlates with inflammatory markers and clinical scores in patients with AP and can be considered a tool in assessing disease severity.

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HELICOBACTER PYLORI DNA IN LIVER TISSUES FROM CHRONIC HEPATITIS C EGYPTIAN PATIENTS

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Background. Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease in Egypt, which may progress to cirrhosis and hepatocellular carcinoma (HCC). Previous studies have documented an association between *Helicobacter pylori* (*H.pylori*) infection and liver cirrhosis with or without HCC.However, the frequency of *H.pylori* in chronic hepatitis C (CHC) was not thoroughly investigated in Egypt. The aim of this study was to determine whether *H.pylori* DNA was associated with CHC in Egyptian patients.
Methods. Fifty-two Egyptian CHC patients were enrolled in this study. Plasma anti-H.pylori IgG was assessed with ELISA. Liver biopsies were tested for presence of *Helicobacter* DNA by genus specific nested polymerase chain reaction (PCR) and species was identified by sequencing.
Results. Anti-*H.pylori* IgG was detected in 31 out of 52 CHC (59.6%) patients while *Helicobacter* DNA was detected in 6 (11.5%) patients; all were H. pylori by sequencing. *Helicobacter* DNA was more frequent in patients with high stage liver fibrosis (33.3%) than in those with low stage fibrosis (2.7%) (P=0.006). All *H.pylori* DNA positive cases were positive for *H.pylori* antibodies. There was no association between the presence of *H.pylori* DNA in the liver and age, gender of patients, liver function tests, and viral load or AFP levels.
Conclusions. These data confirm the presence of *H.pylori* DNA in liver of CHC Egyptian patients and suggest an association of this bacterium with progression of liver fibrosis. Further studies are needed to ascertain whether *H.pylori* plays a role in the development of HCC.

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INTERLEUKIN-6 AS A NECROSIS INFECTION MARKER IN SEVERE ACUTE PANCREATITIS

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Background. Severe acute pancreatitis (TAP) is an acute inflammation of the exocrine pancreas with local complications or organ failure. Mortality of severe acute pancreatitis ranges from 10-20% (for sterile necrosis, SN) after 20-85% (for infected necrosis, IN). Resolution SN and IN is clinically very important, but often difficult and therefore they are still searching for a new laboratory markers for their differentiation. Our retrospective study aimed to evaluate the contribution of interleukin-6.
Methods. Included were 59 patients hospitalized with severe acute pancreatitis (classification Atlantic 1992) in the surgery department of Hospital Sand in 2000-2006. Every day has been investigated by C-reactive protein (CRP) and interleukin-6 (IL-6), the data was sufficient in 42 patients: 14 with IN (microbiological or CT), 28 with SN. Statistical analysis using Fisher's test and chi-square test.
Results. It was found statistically significant discriminatory ability of daily averages of IL-6 5th and 6th + 7th and 8th day of hospitalization between the IN and SN (p = 0.0014 for day 5 and 6, respectively. p = 0.0009 for day 7 and 8). Developing IN realistically set in the most between 5 and 9 day. CRP did the ability of discrimination, the levels were between 2 8.dne to significantly increased steadily. Due to the characteristic concentrations of IL-6, where the input was present in significant elevation irrespective of subsequent development of IN and from the second day of hospitalization decreased significantly. Further increase in IL-6 was only present in patients with the development of IN. For the cut-off of IL-6 (valid after the 3rd day of hospitalization) 100 ng / L was P <0.005 for 150 ng / lp <0.01.
Conclusions. Interleukin-6 significantly discriminates infected and sterile necrosis in TAP.

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PSEUDOMYXOMA PERITONEI SECONDARY TO APPENDICEAL MUCINOUS TUMOR: A CASE REPORT

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Background. Pseudomyxoma peritonei (PMP) is an uncommon clinical entity with a incidence of one to two cases per million per year. Classically it is characterized by diffuse intra-abdominal gelatinous collections with mucinous implants on peritoneal surfaces. Many cases are unexpectedly discovered at laparoscopy/laparotomy during the investigation or staging of another pathological entity. The tumour slowly spread throughout the peritoneal cavity, as the disease progresses, eventually resulting in symptoms of bowel obstruction, dyspnoea and malnutrition. Consequently, death results unless the patient undergoes corrective surgery.
Methods and Results. A 81-year old male patient presented with hypogastric and lower quadrant abdominal pain, asthenia, acute weight loss (not quantified) and a progressive increase of the abdominal diameter, without other pathological findings in the clinical examination. Laboratory tests on admission showed: WBC: 6900/mm3 (with normal type), Hb: 9.8 g/dl, Platelets:282000/mm3 and normal coagulation values. CEA: 256.2 ng/mL and CA-125:68.5 U/mL. Abdominal CT scan showed high quantity of free liquid located in the Douglas area and normal internal organs. Colonoscopy reported normal results and endoscopy was hindered by a high amount of food in the stomach. Despite of the anatomopathological analysis of the liquid was not possible and malnutrition and weakness of the patient prevent a laparoscopy/laparotomy analysis, pseudomyxoma peritonei secondary to appendiceal mucinous tumor was proposed as the most probable diagnosis.
Conclusions. PMP is an uncommon entity generally originates from a perforated appendiceal tumour. The optimal treatment consisting of aggressive cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has a curative intent.

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IMPORTANCE OF SEROTONIN DETERMINATION IN PATIENTS WITH ESOPHAGEAL AND GASTRIC FUNDUS VARICES

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Background. The aim of the work was to determine potential effects of free serotonin concentrations in plasma on development of esophagial and gastric fundal varices.
Methods. The study was included 33 patients with liver cirrhosis and 24 healthy controls. Measurment of free serotonin concentration in plasma by HPLC techniques, and ultrasonography were carried out in bought groups of subjects.
Results. The mean plasma free serotonin level was much higher in liver cirrhosis patients than in healthy controls(219.0 ± 24.2nmol/L,vs 65.4 ± 18.7nmol/L,$p<0.0001$). There was no significant correlation between serotonin concentration in plasma and the platelet count according to Spearman coefficient of correlation ($r_s=0.158$,$p>0.05$. The mean plasma free serotonin level was higher in patients with esophageal varices than in patients without varices ($t=-2.301$, $p<0.05$). The correlation of free plasma serotonin concentration and fundal varices was highly significant ($r_s=-0.601$, $p<0.01$). The mean plasma free serotonin levels was much higher in patients with esophageal and gastric fundal varices, than in patients who had only esophageal varices ($t=-5.862$, $p<0.01$.
Conclusions. Free serotonin is significant in pathogenesis of portal hypertension, especially in development of fundal varices, indicating the clinical value of serotonergic receptor blockers in these patients.

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BIOCHEMICAL MARKERS IN ACUTE BILIARY PANCREATITIS

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Background. Acute Pancreatitis(AP),is an inflammatory pancreas disease, the pathophysiology is intensitivity investigated. The etiology is mainly biliary. Biochemical markers of: -global evaluation are Amylase(Amy), lipase(Lip), glucose(Glu) and insuline(Ins); -inflammation are C-ReactiveProtein(CRPhs) and pancreatitis-associated protein(PAP), and -exocrine functionality is: Elastasa-1(Ela1).
Acute Biliary Pancreatitis(ABP), is produced by pancreatic duct obstruction by gallstone impacting, and consequentlypancreatic hyperstimulation. The ABP can be also evaluated by hepatobiliary enzymatic marker of cholestasis: transaminases(ALT/AST), alkaline phosphatase(ALP), gammaglutamyltranspeptidase(GGT) and 5Nucleotidase(5NT); during the bile obstruction the biliary macroenzyme of ALP (bALP) is produced.
The objective wasto evaluated: Amy,lip,Glu,Ins,PAP,Ela1 and CRPhs and to analyze its association with the cholestasis markers,in ABP and controls patients
Materials and Methods. Serum and stool of 26 patients: Controls(C-n=11, 58 ± 12years, 3Males/8Females) and ABP(n=15,24-48hs.evolution;69 ± 17years;5Males/10Females).
Biochemical Parameters: serum: Amy,Lip,Glu,ALT,AST,ALP,GGT,5NT,CRPhs (recommended automated methods); bALP(Electrophoresis); PAP and Ins(EIA); and stools: Ela1(Elisa)
Results. We observed:
- significantincreases($P<0.05$): Amy,Lip,PAP and CRPhs and not significant increase of Glu and Ins; significant decrease($P<0,05$) of Ela1, in ABP with respect to C.
- positive associations($P<0.05$): Amy,Lip,PAP with bALP and GGT; CRPhs with ALP,bALP,GGT and 5NT; Ela1 and Ins did not correlate with any parameters; presented bALP; andincreased: Amy,Lip,Ins, PAP and not different parameters of cholestasis in women relation to men, in ABP patients.
Conclusions. These results are related with the acute process, the biliary obstruction and probable endocrine compromise. We suggested Ela1 serumdetermination, and bALP showed more specificity than totalALP, in ABP patients. Although the sample size should be increased, its suggested that women would suffer more severe ABP, related to hormonal influence.

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NOVEL ENTEROVIRULENT *ESHERICHIA COLI* CONFFERING R-PLASMID MEDIATED RESISTANCE TO FLUOROQIONOLONE ANTIBACTERIAL AGENTS IN BENIN CITY

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Background. *Escherichia coli,* as a normal Biotype inhibiting the gastrointestinal tract (G.I.T) exhibit symbiotic relationship with the host. The seemingly pathogenic role, with its concomitant resistance to the fluoroquinolone antibacterial agents, is worrisome to chemical microbiologist world over. The purpose of this study therefore, was to characterize the enterovirulent *Escherichia coli* strains isolated from diarrheic patients attending various hospitals in Benin City.
Methods. Stools specimens from three hundred diarrheic patients comprising infants, below three years old and adults above three years of age., attending these hospital were cultured using routine methods in University of Benin Teaching Hospital. Enterovirulent *Escherichia coli* strains isolated from the specimens were identified to species levels by biotyping using the standard Methods. They were also subjected to serotyping using *Escherichia coli* anti-sera and were used according to the manufacturers instructions. Antibiotic susceptibility spectrum was carried out on the isolates using agar diffusion methods of stokes. R-Plasmid DNA analysis was also carried out on the strains using alkaline lysis protocol of Takahashi and Nagano.
Results. Nine enterovirulent strains were isolated from the specimens and exhibited very high minimum inhibitory concentration (MIC) to the fluoroquinolones antibacterial agent and some other antibiotics and were thus screened for the presence of Transferable conjugate R-Plamid mediated resistance. The R-plasmid DNA analysis showed two bands greater than 23.1KB compared with the reference molecular weight plasmid, as revealed by agarrose gel electrophoresis on a horiszontal apparatus.
Conclusions. We present in this study the isolation of enterovirulent *Escherichia coli* strains that exhibited transferable conjugative R-Plasmid mediated resistance to the fluoroqiunilone antibacterial agents and some other antibiotics substance in Benin City, Nigeria.

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ANALYSIS OF SPINDLE CELL TUMORS OF GASTRO-INTESTINAL TRACT – A HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Background. Gastro-intestinal stromal tumors (GIST) are mesenchymal tumors. It is postulated that GISTs originate from the CD- 34 positive stem cells and differentiate towards the pace maker cell phenotype. Majority of GIST show somatic mutation of CD117 which is produced by GISTs in contrast to other spindle cell tumors of the Gastro-Intestinal tract (GI T).
Methods. In this 14 years retrospective study, primary mesenchymal tumors of GIT were reviewed with routine Haematoxylin & Eosin sections and immunohistochemistry with Vimentin, Smooth muscle actin, Desmin, s-100, CD34 and CD-117.Antibody against CD117 (1:50 dilution; Dako), S-100 protein (1:1 500; Dako), desmin (1:50;Dako), and Smooth Muscle Actin(SMA) (1:100,Dako)were used.
Results. There were 40 primary mesenchymal tumors of the GIT with equal male to female ratio. Majority of patients were in the fourth decade. Small intestine was the most common site followed by stomach. Other sites like mesentery, retroperitoneum, pelvis, and appendix were also encountered. Fifteen cases (37.5%) were CD117 positive whereas 25 cases (62.5%) were negative. CD34 in 36 (90%), SMA in 34 (85%), s-100 in 22 (55%) and desmin in 16(40%) cases showed positivity respectively. CD117 negative cases showed leiomyomatous (9), neural (1), dual (12) and null (3) differentiation.
Conclusions. In our study CD34 was positive in 90% cases, which re-establishes the fact that GISTs originate from the CD- 34 positive stem cells. Majority were CD117 negative and in small intestine in contrast to reported cases in literature.

Liver and gastrointestinal diseases

0787

SERUM HYALURONIC ACID IN PATIENTS WITH LIVER DISEASE

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Background. Hyaluronic acid (HA) is a glycosaminoglycan, produced mainly by mesenchymal cells. HA plays a prominent role in
the pathogenesis of liver fibrosis; thus serum HA concentrations itself or in combination with other parameters (Hepascore) have
been proposed as a non-invasive biomarker of liver fibrosis. The aim of this study was to evaluate the relationship of serum HA
levels to the severity of histologicaly proved liver fibrosis.
Methods. 86 patients with chronic liver disease confirmed by liver biopsy (59 men; mean age 49,6±14,7 years) were enrolled.
The etiology of liver disease was NASH (15 pat.), chronic hepatitis C (32 pat.), alcoholic liver disease (30 pat.) and other (9 pat.),
Liver fibrosis was determined by METAVIR score. Hepascore was computed as published in literature. Serum HA was assayed
using the latex agglutination method (Hyaluronic acid LT, Latex Agglutination Method, Wako, Germany).
Results. Serum concentration HA (ng/ml; median; IQ range) in patients with different stages of fibrosis were: F0 (9 pat.): 28,1
(13,4-58,3); F1 (10 pat.): 20,1 (12,6 – 27,2); F2 (11 pat.): 32,7 (26,1 – 46,4); F3 (9pat.): 59,9 (38,7 – 71,3); F4 (47 pat):188,70
(167,3 – 307,92).There was statistically significantly difference in HA levels in patients with F1 to patients with F4 ($p < 0,001$). The
Hepascore correlated significantly ($p < 0,001$) with fibrosis stage.
Conclusions. Serum HA is a useful non-invasive marker of liver fibrosis. The combination with other parameters adds signifi-
cance and could reduce the need for liver biopsy.

0788

EFFECTS OF UNFRACTIONATED HEPARIN ON PLASMA LIPASE ACTIVITY

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Background. Increase of amylase and lipase acitivities in human plasma is considered to be the best indicator of pancreatic dam-
age. In our hospital, amylase and lipase are usually requested together.
Methods. Lipase was determined using 1,2-diglyceride as substrate and H_2O_2 as indicator, amylase acitivity with EPS-Substrate
after inhibition of salivary amylase by specific antibodies, both with an Abbott ci8200. Selection criterium for samples was an
activated partial thromboplastin time (aPTT) > 180 sec (reference range 26-40 sec). Spiking of control samples was carried out
with final heparin concentrations between 1 and 5 IE/ml (N=24). Relative enzyme acitivities were obtained for lipase by division of
actual activity by 43, for amylase by 30,5. Reference values of lipase are 8-78 U/l, for amylase 8-53 U/l (37°C).
Results. It was observed that lipase was often increased in samples with an aPTT > 180 sec or was pathological, while amylase
remained normal. On the average, first measured relative lipase activity was with 4,3±2,3 significantly (p=<0.0001) elevated if
compared to amylase (1±0,6). Time course of enzyme activities substantiated these observations. In vitro effects of heparin on
lipase activity were excluded by spiking of control samples with heparin and subsequent measurement of lipase acitivity.
Conclusions. On the basis of organ specificity, lipase is considered as the better parameter for pancreas diagnostics. However,
unfractionated heparin binds to lipase and releases lipase from liver, muscle, adrenals, ovaries and testes. Considering these
data, a specific pancreatic amylase test seems to take over the favourite position in pancreatic diagnosis of heparinized patients.

Liver and gastrointestinal diseases

0789

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF GHRELIN ON CCL4-INDUCED EXPERIMENTAL ACUTE LIVER INJURY IN RATS

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Background. The aim of this study was to evaluate the mechanisms of the protective effects of ghrelin in rats with CCl4(carbon tetrachloride) induced acute liver injury.

Methods. In experimental studies,24 Spraque-Dawley albino rats from genus were divided into three groups equally as follows:Control,CCl4 and CCl4+Ghrelin.4 ml/kg olive oil was administered intraperitoneally (i.p.) to the control group,4 ml/kg CCl4 (1.1) dissolved in olive oil)was administered i.p. to the animals in other two group.After three and six hour,80 mcg/kg ghrelin was administered i.p. to the CCl4+Ghrelin group.Twenty four hours after administrating CCl4,all of the rats were sacrificed.Biochemical assessments were performed using serum AST,ALT,MDA(Malondiadehyde),tissue MDA,MPO(Myeloperoxidase) and NO(Nitric Oxide)levels.Histopathological assessments were performed using Haematoxilen &Eosine staining in light microscope.

Results. Serum AST,ALT, MDA and tissue MDA,MPO levels all increased in CCl4 group.But they were decreased in group treated with ghrelin.Tissue NO levels decreased in CCl4 group, but they were a more limited decrease in group treated with ghrelin. Histopathological comparison of the groups showed that a decrease in vacuolar degeneration and necrosis of hepatocytes,hemo rrage,sinusoidal congestion,PMNL(polymorphonuclear leukocytes) and MNL(mononuclear leukocytes) infiltration in group treated with ghrelin .

Conclusions. Our study supports that ghrelin prevents experimental acute hepatic injury by preventing oxidative stress.

0790

PLASMA LEVELS OF SOLUBLE CD30 AND CD40L IN PEDIATRIC PATIENTS AFTER LIVER TRANSPLANTATION

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Background. It has been found that plasma levels of such immunological markers as soluble CD30 (sCD30) and soluble CD40L (sCD40L) are associated with graft dysfunction of kidney and heart. There are few reports about relationship between these markers and some liver diseases. The aim of the study was to find association between plasma levels of sCD30 and sCD40L in children with end-stage liver disease (ESLD) after living-donor liver transplantation(LDLT) and graft dysfunction.

Methods. The study included 65 children with ESLDaged 14±6 (4-36)months before and after LDLTand 38 adult living donors aged 37±19 (18-56)years. Plasma concentrations of sCD30 and sCD40L were measured by ELISA.

Results. In children with ESLD pre-transplant plasma levels of sCD30 (78.3±36.3 ng/ml) and sCD40L (3.2±1.8 ng/ml) were significantly higher than in healthy donors (31.1±11.7 ng/ml, $p<0.01$ and 0.9±0.6 ng/ml, $p<0.01$ resp). After LDLT concentrations of sCD30 and sCD40L were significantly decreased (56.4±19.0 ng/ml, $p<0.01$ and 1.7±0.6 ng/ml, $p<0.01$ resp).

Pre-transplant plasma level of sCD30 was 83.3±34.1 ng/ml in children, who had graft dysfunction on days 26-32 after LDLT (n=12). It was increased to 106.5±15.9 ng/ml, $p<0.05$ in 18-21 days after transplantation. Elevation of the concentration of sCD30 was observed 2-5 days before increasing of liver enzyme activity. In these children pre-transplant plasma level of sCD40L was significantly higher (5.7±2.1 ng/ml, $p<0.05$) than in common group.

Conclusions. Elevated plasma levels of sCD40L before LDLT and sCD30 after LDLT are associated with graft dysfunctiondevelopment in children after LDLT.

Liver and gastrointestinal diseases

0791

THE PROTECTIVE EFFECTS OF GLYCINE ON BILE DUCT LIGATION-INDUCED CHOLESTASIS

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Background. Glycine, a nonessential amino acid, has been reported to improve survival in rat liver transplantation, and protect hepatocytes against hypoxia. The mechanism of glycine protective effects remains unclear. This study aimed to determine whether intraperitoneal injection of glycine would blunt bile duct ligation-related injury and to elucidate the protective mechanism of glycine.

Methods. Bile duct ligation was performed on male guinea pigs, sham-operated as the control group. The animals were sacrificed and examined at day-14 after bile duct ligation. Serum concentrations of total antioxidant capacity (TAC) were measured by luminal-enhanced luminescence. Ischemia-modified albumin (IMA) was determined by cobalt-binding assay. Serum bilirubin concentrations and activities of AST, ALT, ALP, and GGT were measured using a Roche modular system. Liver caspase 3 activitywas determined by using Ac-DEVD-AMC as the fluorogenic indicator and oxidative damage indicator malondialdehyde (MDA) by high performance liquid chromatography.

Results. Glycine supplement improved the survival of bile duct-ligated animals, and reduced liver damage. Serum IMA increased after bile duct ligation [mean(SD) 0.582(0.020) vs. 0.532(0.020), $p<0.01$] and glycine treatment restored IMA to the control level. Serum TAC decreased after bile duct ligation [23.9(18.8) vs. 56.5(25.0) micromol/L, $p<0.05$] and back to control level with glycine treatment. Serum bilirubin and liver enzymes also increased significantly after bile duct ligation.Treatment of glycine would prevent against these changes. Similar changes were found in liver MDA and caspase 3 activities.

Conclusions. Our study showed that glycine provides protective effects against cholestasis-induced apoptosis and oxidative stress.

0792

PLASMA PHOSPHOLIPID FATTY ACID PROFILE IN CHILDREN WITH CELIAC DISEASE BEFORE AND AFTER TREATMENT

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Background. Fatty acids (FA) play a role not only as energetic material but also participate in the course of inflammation. It is known that omega-3 FA and their derivates have potential anti-inflammatory effect in contrast to omega-6 which are mainly pro-inflammatory compounds. Therefore omega-3 and omega-6 FA as well as eicosapentaenoic acid (EPA), docosahexaenoic (DHA), arachidonic acid (AA) in children with celiac (CD) has been estimated.

Methods. Plasma phospholipid FA were analyzed by capillary gas chromatography.

Results. The mean concentration values of saturated FA (SFA), monounsaturated FA (MUFA), omega-3 FAs, omega-6 FAs, the ratios of omega3/omega6 and AA/EPA in celiac patients before treatment was significantly lower ($p<0,05$) and AA/EPA was significantly higher ($p<0,05$) than in control. Six months after gluten free diet (GFD) the mean concentration values of SFA, MUFA reached the control values. Mean concentration of omega-3 FAs, omega-6 FAs, omega-3/omega-6 ratio, AA/EPA ratio in celiac patients who have been using GFD for several years was comparable with control.Total phospholipid fatty acid concentration in each celiac group was significantly lower ($p<0,05$) than in control.

Conclusions. Gluten free diet used for several years restores normal concentration of SFA, MUFA, omega-3 FAs, omega-6 FAs and the ratio of omega-3/omega-6, AA/EPA, AA/DHA but doesn't restore the total concentration of phospholipids.

Liver and gastrointestinal diseases

0793

FUNCTIONAL CAPACITY OF ALBUMIN IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

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Background. Ischemia modified albumin (IMA), a marker of myocardial ischemia, was reported recently as a simple albumin function test.This study aimed to compare functional capacity of albumin in albumin solutions and to investigate the effect of albumin infusion on IMA in patients with cirrhosis.
Methods. IMA was measured by albumin cobalt binding test on a Beckman Spectrophotometer.IMA stability was examined in pooled serum stored at 4°C, -20°C and -70°C for 28 days. IMA in commercial human albumin solutions,AlbuRx and TBSF (CSL, Behring) and frozen plasma were determined.The total antioxidant capacity was determined on a Luminometer. Blood samples from normal subjects (N=50), patients with liver dysfunction (N=17) anddecompensated cirrhosis (N=9) were analyzed. Albumin and uric acid levels were determined on a Roche DP Modular analyzer.
Results. The inter- and intra-assay imprecision was less than 7% for the IMA.IMA was stable in pooled serum at all storage conditions.Citrate level at 0.038 % or more lowered the IMA Results. However, uric acid up to 10.8 mg/dLdid not interfere the assay. IMA level was higher in frozen plasma, compared to albumin solutions. IMAR was markedly elevated in patients with cirrhosis (0.22±0.072), and liver dysfunction (0.20±0.107), compared to normal subjects (0.10±0.014).The supplement of albumin solutions in cirrhotic patients had significant effects on serum albumin (p<0.01) and IMAR (p<0.05).
Conclusions. In this study, an IMA assay to measure albumin function was established.These data indicate that treatment with albumin solutions would improve the functional capacity of albumin in cirrhosis patients.

0794

VARIABILITY OF BILIRUBIN VALUES IN SAMPLES SERUM WITH HIGH TRIGLYCERIDES; INTERFERENCE OR CONGENITAL LIVER SYNDROMES

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Background. A recurring theme in work of laboratory medicine, the interference in laboratorytesting as falsely or normal results, can occurs leading to delays disease recognition. The aim of this work was to identify and to interpret the variability of bilirubin values in serum samples together or isolated with high values of Triglycerides and variable values of Cholesterol, excluding congenital liver syndromes from interference, to patients presented in private laboratory for a routine pre-clinical control.
Methods. In our prospective study, in time of 30 days, we have analyzed 160 patients, performing 10 major tests; 5 substrates/chemistries: cholesterol, total bilirubin, direct bilirubin, indirect bilirubin, triglycerides and 5 enzymatic tests, AST, ALT, ALP, LDH and GGT, measured on automatic analyzer Hitachi 907, hepatic viral markers (Ag HBS, Anti HCV), Elisa method, and electrophoresis proteins working on automatic method. Additional samples of hemogram with differential count and reticulocytes, were accomplished on Coulter analyzer with 22 hematological parameters. From the total patients 70 were females (20-30 years, mean age= 26, SD=2.6) and 90 males (25-36 year, mean age 30, SD=2.8).
Results. Laboratory studiesrevealed the following:
- the health young patients with all normal analysis were registered in 90 % percent (n=.144).
- an isolated increased Total bilirubin(1.1-7.7 mg/dl, mean=1.99 mg/dl) and an increase of Indirect bilirubin (1-4.9 mg/dl, mean =1.45 mg/dl, biologic reference=0.0-1.1 mg/dl, SD=0.10;CV=0.18, p=0.012, results correlated with normal liver enzymes, but with high Triglycerides(cut off dilution = 243 mg/dl, in samples without macroscopic aspect of turbidity), to patients from cohort studywere registered in 6.8%. After performing tests of Indirect bilirubin in dilution 1/5, only 2.8% of tests were with normal values (negative predictive value=66%) and 4% of tests have had the same high results, meaning the congenital liver syndromes (positive predictive value=72%).
Conclusions. Elevated values of triglycerides, correlated with increased levels of total bilirubin in these cases, can present a differential diagnosis with liver congenital syndromes with isolated high indirect bilirubin values (Gilbert syndrome or Crigler-Najjar syndrome).

Liver and gastrointestinal diseases

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A NEW LIQUID REAGENT FOR DIRECT BILIRUBIN ON ROCHE CLINICAL CHEMISTRY ANALYSERS

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Differential diagnosis of an icterus requires analytical discrimination between the water-insoluble prehepatic unconjugated α-bilirubin and the water-soluble post-hepatic fractions comprising glucuronidated β- and γ-bilirubin as well as covalently protein-bound δ-bilirubin. A new direct bilirubin reagent was designed to have improved specificity, stability, and more convenient reagent handling.

Test principle is the reaction of water-soluble bilirubin fractions with 3,5-dichlorophenyl diazonium ion. The reagent is liquid ready-to-use with an on-board stability of 6 weeks without recalibration and more than 12 months shelf life.

The method was highly specific: In samples containing only water-soluble bilirubin, the direct bilirubin equalled the total bilirubin Results. In neonatal samples containing only unconjugated bilirubin from 250-350 μmol/L, direct bilirubin was <10 μmol/L.

Method comparisons between the liquid ready-to-use formulation and already existing direct bilirubin reagents showed very good correlations (r ≥ 0.997) and negligible intercepts (<2 μmol/L). Assessment of intra-assay precision gavecoefficients of variation well below 1%. The linear measuring range was up to 300 μmol/L. There was no interference by coagulants, heparin or EDTA plasma as well as serum can be used as samples.

The standardisation is traceable to the Doumas method for Total Bilirubin and is established using low bilirubin human sera spiked with direct reacting ditaurobilirubin.

In summary, the new BILD2 reagent offers a ready-to-use liquid stable formulation with high specificity, good precision and good correlation to existing methods (no change of reference ranges!) on all Roche analysers.

0796

AESKULISA AND H-TTG/DGP SCREEN FOR CELIAC DISEASE DIAGNOSIS

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Background. This study was aimed to verify the clinical performance of two screen tests in serum samples for celiac disease (CD) diagnosis: one provided by Inova (IL, Italy) which identifies IgA and IgG anti DGP (deamidated gliadin peptides) and tTG (h-tTG/DGP), and another provided by Aesku.Diagnostics (Grifols, Italy), which identifies IgA and IgG anti neo-epitopes of tTG, (Aeskulisa).

Methods. We retrospectively analysed a series of 500 children (173 M, 327 F) consecutively subjected to EGDS. CD was histologically diagnosed (n=232) or ruled out (n=268).

Results. The areas under the ROC curves (AUC) ± SE were 0.96±0.09 for h-tTG/DGP and 0.94±0.011 for Aeskulisa. 32.66 U/mL and 45.1 U/mL were identified as best cut-off for h-tTG/DGP and Aeskulisa respectively. The following results were found for h-tTG/DGP and Aeskulisa respectively: 94.8% and 94.3% sensitivity; 89.1% and 85.8% specificity; 90.7% and 88.3% PPV; 93.8% and 93.0% NPV. Among CD children, both h-tTG/DGP and Aeskulisa results correlated with the degree of duodenal atrophy (Marsh Oberhuber criteria), the highest levels being recorded among those with type IIIa-c than those with type I-II (F=18.8, p<0.0001 and F=14.7, p<0.0001 respectively). Considering children's age quartiles the AUCs of h-tTG/DGP and Aeskulisa were: 0.97±0.018 and 0.95±0.023 in the 0-4 yrs group; 0.967±0.013 and 0.96±0.017 in 5-8 yrs group; 0.94±0.029 and 0.92±0.038 in 8.5-11 yrs group; 0.947±0.020 and 0.93±0.023 in > 11 yrs group.

Conclusions. The new screen tests appear sensitive and specific enough to be proposed as first step in the flow-chart of CD diagnosis in children of any age.

Molecular diagnostics

0797

MOLECULAR CHARACTERIZATION OF THALASSAEMIA IN SUDANESE PATIENTS

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Background. Thalassaemias are a group of genetic disorders of haemoglobin synthesis, resulting from a reduced rate of production of one or more of the globin chains of haemoglobin. They are divided into different types according to the polypeptide chains affected, the most common types are α thalassaemia and β thalassaemia. Most thalassaemias are inherited in mendelian recessive fashion.

The β thalassaemias are the most important type, they occur in a belt extending from the Mediterranean and parts of north and west Africa through the Middle East and the India subcontinent to south east Asia.Thalassaemia is now commonly seen in Sudan than before and involved ethnic group out side the thalassaemia belt. . The type of mutations was not identified because thalassaemia was not studied before at molecular level in Sudan.

β-thalassaemia associated with total absence or reduced β-globin chains, most of these result from point mutations. A mutation may be defined as permanent change in the DNA. Mutations can be genome mutations, chromosome mutations and point mutations. α-thalassaemia result from deletions of one or both α-globin chains, and there are many different sized mutations.

The aim of this study is to determine the pattern of mutations and the genotype in β- thalassaemia in Sudan.

Methods. Screening for thalassaemia was done, by CBC, measurement of HbF, and HbA_2.Moleculardiagnosis by doing DNA isolation, in vitro amplification,(PCR), and Hybridization using β-Globin Stripassay to identify the mutation and determine the genotype of different mutation.

Results. 40 samples was screened and diagnosed as thalassaemia, different types of mutations identified, the most common mutations are; (110), (1.1), (1.6), (-87), and (1.5), which represent (40%), (20%), (20%), (15%) and (5%) respectively. These mutations are the most common mutations in the Mediterranean and middle east.

Conclusions. Thalassaemia in Sudan has a similar molecular characterization as other Mediterranean and Middle East regions.

0798

LANTHANIDE CHELATE COMPLEMENTATION AND HYDROLYSIS ENHANCED LUMINESCENT CHELATE IN REAL-TIME PCR ASSAYS FOR PROSTATE SPECIFIC ANTIGEN TRANSCRIPTS

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Background. The aim of this study was to optimize a recently developed time-resolved fluorometric method (lanthanide chelate complementation) for real-time PCR based on two probes moieties carrying lanthanide ion and light absorbing antenna and compare the results with a previously described reference Methods. time-resolved fluorometric real-time PCR method based on hydrolysis enhancement and quenching of intact probes.

Methods. Reverse transcription real-time PCR assays were done for standard series of mutant prostate specific antigen (mm-PSA) and prostate specific antigen (PSA) mRNA with both Methods. PSA mRNA was also quantified in a series of blood samples, spiked with different numbers of LNCaP cells (10, 10^2, 10^3, or 10^4 LNCaP cells were added to 2.5 ml of healthy female blood). mmPSA was used as internal standard.

Results. Same limit of quantification (1 copy per μl of sample for standards and 10 cells per 2.5 ml blood for LNCaP spiked blood samples) was obtained for PSA with both systems. Comparison of C_t values between methods demonstrated two cycles earlier detection with lanthanide chelate complementation method. A higher amount of probe was required in the lanthanide chelate complementation method but the background was 20 times lower and signal-to-background ratio was three times higher when compared with the reference method.

Conclusions. The new reporter method provided similar sensitivity as the reference method in this reverse transcription real-time PCR assay for PSA. Substantially the lower background and the improved signal-to-background ratio in lanthanide chelate complementation method could possibly be of additional value for samples with lower purity.

Molecular diagnostics

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ABOUT A CASE: DIAGNOSIS OF POORLY DIFFERENTIATED CARCINOMA BY FLOW CYTOMETRY

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A 61 years old patient with a history of herniated disc, smokes 2 packs of cigarettes a day, chronic furunculosis, hyperuricemia and hairy cell leukemia diagnosed in 2005 in therapeutic abstention. Go to the emergency room with a fever (39ºC), epitaxis, more intense aches in the loins, chronic skin lesions of unknown etiology. No cough, no abdominal pain or other symptoms. On the examination we highlight the presence of bilateral inguinal lymphadenopathies and hepatomegaly. The blood count showed leucopenia of 1010 leukocytes/mL with 50% of LUC and the biochemical analysis include elevated liver enzymes and LDH. Morphology in the bone marrow aspirate revealed the presence of blasts that might fit with those described in Acute Megakaryioblastic Leukemia. However, the result of immunophenotyping revealed the presence of 94% of cells CD56+, CD117+, EpCAM +, CD90+, CD81+, CD99+, CD15weak, CD13-, CD33-, CD19-, CD20-, MPO-, CD41-, CD45-, CD61-. The CD45 antigen is known as Leukocyte Common Antigen, which is negative in these cells. Furthermore, the EpCAM antigen positivity indicates the presence of tumor cells derived from epithelial cells. According to these results we can orient the diagnosis toward a poorly differentiated carcinoma, which is confirmed by the results of the biopsy (EMA +).

0800

PRINCIPLE OF RECOVERYELISA – A NOVEL IMMUNOASSAY CONCEPT FOR MONITORING OF TREATMENT WITH THERAPEUTIC ANTIBODIES

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Therapeutic use of proteine-based pharmaceuticals-therapeutic antibodies- cause the problem of difficulties in laboratory monitoring of drug and target. Aim of the study was to find a cost-effective and rapid control for therapeutic proteins.

The *recovery*ELISA combines a Sandwich-ELISA (solid phase immuno enzymetric assay = IEMA) for the antigen with a competitive ELISA (Enzyme immuno assay = EIA) for the therapeutic antibody. Under certain assay conditions the recovery of the antigen is systematically decreased in dependence to the concentration of the therapeutic antibody in relation to a calibration curve. That can be shown by a recovery curve. In samples of unknown composition the recovery can be estimated from the addition of a certain amount of the antigen.

By use of a standard curve for the antigen and a recovery curve for the therapeutic antibody the *recovery*ELISA can estimate the concentration of the therapeutic antibody as well as of the free antigen in serum of patients.

The application of the *recovery*ELISA was demonstrated on clinical serum samples of patients treated with Omalizumab (Xolair)/ IgE and for Adalimumab (HUMIRA)/TNF-a. The assays run in diluted sera (1:20 resp. 1:10). The detection range for IgE was 1.6 – 4760 ng/ml, for Omalizumab 0.4 – 80 μg/ml, respectively. For Humira-treatment the detection limits are 20 – 600 ng/ml for TNF-α, and 2 – 180 μg/ml for Adalimumab, resp., which cover the expected therapeutic ranges.

Thus *recovery*ELISA is expected to provide fundamental information necessary for the therapeutic application of therapeutic antibodies.

Molecular diagnostics

0801

EXPERIENCES IN SIMULTANEOUS DETECTION OF FACTOR V LEIDEN, FACTOR II G20210A, MTHFR C677T AND MTHFR A1298C MUTATIONS IN PATIENTS WITH THROMBOPHILIA

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Background. Mutations factor V Leiden (FV G1691A), factor II G20210A (FII G20210A), as well as C677T and A1298C in the methylenetetrahydrofolate reductase (MTHFR) gene are hereditary risk factors associated with thrombophilia.
Methods. Study enrolled 60 patients (29 men and 31 women). Genomic DNA was extracted from blood collected with citrate, using Illustra blood genomicPrep Mini Spin Kit®. Combined molecular analyses were performed using ThromboType®plus test. Chi-square test was employed for statistical evaluation.
Results. Testing revealed 12 heterozygous carriers of FV Leiden, 9 heterozygous carriers of FII G20210A, 6 homozygous and 27 heterozygous carriersof MTHFRC677T and 6 homozygous and 29 heterozygous carriersof MTHFRA1298C mutations. Gender related differences were significant only in distribution of FV Leiden mutation, with higher incidence in males (p=0.009). Among observed genotypic combinations, following are the most important: FV 1691GA/FII 20210GA/MTHFR 677CT/MTHFR 1298AC (1 patient), FV 1691GA/FII 20210GG/MTHFR 677CT/MTHFR 1298AA (1 patient), FV 1691GA/FII 20210GG/MTHFR 677CT/ MTHFR 1298AC (3 patients), FV 1691GA/FII 20210GG/MTHFR 677CC/MTHFR 1298AC (3 patients), FV 1691GA/FII 20210GG/ MTHFR 677TT/MTHFR 1298AA (2 patients), FV 1691GA/FII 20210GG/MTHFR 677CC/MTHFR 1298CC (1 patient), FV 1691GA/ FII 20210GG/MTHFR 677CC/MTHFR 1298AA (1 patient), FV 1691GG/FII 20210GA/MTHFR 677CT/MTHFR 1298AC (3 patients), FV 1691GG/FII 20210GA/MTHFR 677CT/MTHFR 1298AC (2 patients), FV 1691GG/FII 20210GA/MTHFR 677CC/MTHFR 1298CC (2 patients) and FV 1691GG/FII 20210GA/MTHFR 677CC/MTHFR 1298AA (1 patient).
Conclusions. Our experiences imply that simultaneous detection of above mentioned mutations improve evaluation of hereditary risk factors in patients with thrombophilia.

0802

IDENTIFICATION OF POLYMORPHIC SITES IN THE MCM6 GENE IN PATIENTS INVESTIGATED FOR ADULT-TYPE HYPOLACTASIA IN STOCKHOLM, SWEDEN

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Background. Lactose intolerance is due to a genetic or acquired deficiency of the enzyme lactase in the mucosa of the small intestine, causing diarrhoea, flatulence and abdominal distension. A polymorphism in the nucleotide position 13910, in intron 13 within the MCM6 gene on chromosome 2q, affects lactase synthesis. Adult-type hypolactasia in Caucasians is associated with the 13910 (C/C) genotype. During the last years it has been discovered that populations in Africa and the Middle East seem to have acquired lactose tolerance by allelic variants in adjoining areas. Our laboratory offers analysis of the polymorphism in position 13910 of the MCM6 gene. Samples that do not show expected results are further investigated. Herein we present the additional mutations found.
Methods. Analysis of 13910C>T is based on TaqMan technology using 7500 Fast RealTime PCR System (Applied Biosystems). Samples with a reduction in the signal intensity were further investigated by DNA sequence analysis (Sanger technology, Macrogen).
Results. Of the 14,390 samples analysed by real-time PCR, 155 (1.1%) were further investigated and showed additional mutations outside the 13910 nucleotide position, primarily in positions 13915 (T/G) and 13907 (C/G) but also 13913 (T/C) and 13914 (G/A). Most of these cases are 13910 (C/C) and were not of Scandinavian descent.
Conclusions. The occurrence of C/C in position 13910 in the MCM6 gene might not always predict adulttype hypolactasia, especially if the patients originate from populations in regions where convergent evolution introduced additional genetic aberrations. This could confer the ability to consume milk at adult age.

Molecular diagnostics

0803

A SIMPLIFIED REAL-TIME PCR-BASED METHOD FOR INTERLEUKIN (IL)28B GENOTYPING FROM DIFFERENT BIOLOGICAL SPECIMENS

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Background. Several recent studies reported a close correlation between the presence of polymorphisms (SNPs) in the IL28B gene and the rates of spontaneous and treatment-induced HCV clearance. Therefore, simple, sensitive and rapid methods to determine the IL28B genotype on different biological samples are urgently needed.
Methods. We developed a real-time PCR method suitable for very small DNA quantities derived from different biological specimens. The PCR target was rs12979860, the best characterized IL28B SNP in Western populations. PCR was carried out by a Taqman assay (Applied Biosystems) using a Rotor Gene 3000 thermal cycler (Corbett). The reaction was performed in a volume of 10 microliter containing 5 ng of DNA.
Results. Reliable IL28B genotyping was obtained from different sources of starting material: whole blood, buccal swab, serum samples, paraffin-embedded liver tissue. At least two different specimens from 50 patients were analyzed with consistent Results. A preliminary analysis of IL28B genotype prevalence in 122 patients with chronic HCV infection from our area is consistent with data reported in Caucasian populations (TT: 16.4%, TC: 46.7%; CC: 36.9%).
Conclusions. Real-time PCR was used to develop a simple, rapid and reliable method for IL28B genotyping suitable for non invasive samples as buccal swab or archival materials as serum or paraffin-embedded tissue samples. This method may be useful for large-scale epidemiological studies or retrospective cohort studies of the natural history of HCV infection in relation to IL28B genotype.

0804

COMPARISON OF TWO MULTIPLEX PCR ASSAYS FOR THE DETECTION OF RESPIRATORY TRACT INFECTIONS

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Background. A broad spectrum of pathogens is causative for respiratory tract infections, but symptoms are mostly similar. Therefore, the identification of the causative viruses is only feasible using multiplex PCR or several monoplex PCR tests in parallel.
Methods. The sensitivity of two multiplex PCR assays, RespiFinder-19 and xTAG-Respiratory-Virus-Panel-Fast-Assay (RVP), were compared to monoplex real-time PCR. Both assays include the most common viruses (adenovirus, coronavirus, human metapneumovirus (hMPV), influenzavirus, parainfluenzavirus (PIV),respiratory-syncytial-virus (RSV), rhinovirus).
Results. To compare the sensitivity of both multiplex assays, samples were inoculated with 14 different viruses at different concentrations. Concordant results were received for rhinovirus, whereas the RVP detectedinfluenzavirus, RSV and hMPV more frequently in low concentrations. In contrast, the RespiFinder-19 showed a higher sensitivity for adenoviruses and coronaviruses. However, the detection of all 14 viruses was only achieved using monoplex PCR.
Additionally, the incidence of respiratory viruses was compared in tracheal secretion (TS) samples (n=100) ofmechanically ventilatedpatients in winter (50 TS) and summer (50 TS). In winter, respiratory viruses were detected in 32 TS samples (64%) by RespiFinder-19, whereas the detection rate with RVP was only 22%. The most frequent viruses were adenovirus (32%) and PIV-2 (20%). Multiple infections were detected in 16 TS samples (32%) by RespiFinder-19. Fewer infections were found in summer (20% with RespiFinder-19; 6% with RVP). All positive results were verified using monoplex PCR.
Conclusions. Multiplex PCR tests have a broad spectrum of pathogens to test at a time, but a lack of sensitivity in comparison to monoplex tests.

Molecular diagnostics

0805

PROBABILITY MODEL FOR PREDICTING *BRCA1* AND *BRCA2* MUTATIONS IN SPANISH FAMILIES WITH BREAST CANCER

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Background. *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185I) genes are major responsible of genetic inherited susceptibility to breast/ovarian cancer (BC/OC).

Objectives. 1) To know the prevalence of *BRCA1* and BRCA2 mutations in Spanish BC/OC families. 2) To assess the association between mutation status and individual and family characteristics. 3) To estimate the probability of *BRCA1/BRCA2* mutation detection.

Patients and Methods. We screened index cases from 2.908 BC/OC families for *BRCA1/BRCA2* germ-line mutations. Molecular results were correlated with individual and familiar history. A logistic regression model was fitted for both genes.

Results. We identified mutations in 702 families (24.5%), 355 *BRCA1* (119 different) and 347 in *BRCA2* (138 different). Proband characteristics related to mutation were: a) *BRCA1*: OC+BC (OR=7.7 and 9.6 for uni or bilatBC), OC (OR=2.8) and bilatBC (OR=2.2). Family characteristics were: OC (OR=3.5 for 1 member, OR=7.3 for ≥ 2 members) and bilatBC (OR=1.54 for ≥ 1 members). b) *BRCA2*: bilatBC and OC (OR=8.5) and male BC (OR=3.1). Family characteristics were male BC (OR=6.9 for ≥ 1 members), BC (OR=1.9 for ≥ 2), OC (OR=1.6 for ≥ 1 members). Ages at diagnosis were inversely associated with risk of *BRCA1/BRCA2* mutations. Areas under the ROC curve were 0.77 for *BRCA1* and 0.71 for *BRCA2*.

Conclusions. Knowledge of individual and familiar variables associated to the presence of a mutation will allow a more effective detection strategy.

Molecular diagnostics

0806

IDENTIFICATION OF *ETFDH* GENE MUTATIONS USING HIGH-RESOULTION MELTING ANALYSIS

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Background. Multiple acyl-CoA dehydrogenase deficiency (MADD) or gluaric aciduria type II is an autosomal recessive disease caused by defects in mitochondrial electron transfer system and metabolism of fatty acid. Recently, *ETFDH* mutations were reported to be major causes of riboflavin-responsive MADD. The present study is aimed at screening *ETFDH* mutations.
Methods. High resolution melting (HRM) analysis was performed to screen *ETFDH* mutations. Genomic DNA was extracted from peripheral blood samples of the 9 patients with MADD and normal controls. Total 13 exons of *ETFDH* were screened by HRM analysis. The results were subsequently confirmed by direct DNA sequencing.
Results. This diagnostic strategy proved to be feasible in detecting 3 known (c.250G>A, c.380T>A, c.524G>T) and 1 novel (c.1831G>A) *ETFDH* mutations. Each mutation could be readily and accurately identified in the difference plot curves. We estimated the carrier frequency of the hotspot mutation, c.250G>A, in the Taiwanese population to be 1:125 (0.8%).
Conclusions. HRM analysis can be successfully applied to screen *ETFDH* mutations. Since riboflavin-responsive MADD is often treatable, especially with mutations in *ETFDH*, identifying *ETFDH* mutations is crucial for these patients.

0807

IDENTIFICATION A NOVEL MISSENSE MUTATION P.F128S IN A TAIWANESE PATIENT WITH RIBOFLAVIN-RESPONSIVE MULTIPLE ACYL-COA DEHYDROGENASE DEFIENCY

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Background. To date, the mutations in *ETFA*, *ETFB* and *ETFDH*, encoding α- and β-subunits of ETF and ETFDH, respectively, have been identified in most MADD patients. Among these causative genes, *ETFDH* mutations have been reported as the major causes of riboflavin-responsive MADD. Until now, there are approximately 60 mutations in *ETFDH* reported in the literatures.
Methods. Genomic DNA was isolated from peripheral blood samples and all exons of *ETFDH* were screened for mutations by High Resolution Melting (HRM) analysis.The results were subsequently confirmed by direct DNA sequencing.Eighty peripheral blood samples from the general population of Taiwanese were used in control experiment for *ETFDH* mutations identified by HRM analysis.Besides, we predict the protein 3D structure of wild-type and mutant form using MODELLER.
Results. We describe a patient with riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency with a novel T>C point mutation at position 383 in the *ETFDH* gene, leading to a substitution of phenylalanine for serine in amino acid position 128 (p.F128S) using HRM analysis.*In silico* analysis showed that position 128 was conserved in all orthologues. The SIFT tool analysis revealed a score of <0.05 and predicted that the replace amino acid is potentially damaging and would not be tolerated.
Conclusions. In this study, we expand the spectrum of *ETFDH* gene mutations in Taiwanese population.

Molecular diagnostics

0808

DIVERSITY OF THE HUMAN *GLA* GENE: IMPROVEMENT OF ANDERSON-FABRY DIAGNOSIS

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Background. The human *GLA* gene encodes the enzyme alpha-galactosidase A. Mutations within the *GLA* gene are responsible for Anderson-Fabry disease (AFD), an X-linked lysosomal storage disorder. To date more than 600 different mutations have been identified. Contrariwise there is less information available about genetic polymorphisms in this gene.
Methods. Using sequence analysis 100 healthy individuals (48 males/52 females = 152 X-chromosomes) were investigated for the presence of mutations within the entire *GLA* gene (promoter, exons and introns). Conventional cloning with pGEM®-T Easy Vector Systems were applied for identification of a certain heterozygous insertion within intron 1.
Results. Among 100 healthy subjects, only 54 showed the *GLA* wild-type sequences (i.e. no mutation). The other 46 individuals displayed at least one genetic variation (either hetero-, homo- or hemizygous). In total 39 (22 novel/17 known) different genetic variations were found with frequencies ranging between 1-25 among the 152 tested X-chromosomes. The most polymorphic genotype identified showed 17 different mutations. Detailed analysis of the sequence variations revealed some remarkable linkages, suggesting these variations to represent statistically associated sets of polymorphisms or different haplotypes.
Conclusions. The *GLA* gene is highly polymorphic. Knowledge of polymorphisms within the *GLA* gene is important for appropriate molecular diagnosis in patients with suspected AFD.

0809

DETECTION OF LOW LEVEL *ADENOMATOUS POLYPOSIS COLI(APC)* GENE MUTATIONS BY WILD-TYPE BLOCKING-PCR AND HIGH RESOLUTION MELTING ANALYSIS

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Background. There is a growing interest of low-level mutation and sequence variant screening in clinical samples. Nevertheless a reliable method for screening of hotspot mutations in the *APC* gene within wild-type background, being relevant for colon cancer diagnosis, is not yet found. Here we present a new combination of two methods to enrich the mutated sequence within a high background of non-mutated DNA and to detect the mutation in clinical samples.
Methods. Colon cancer cell line DNA containing mutations in exons 1309 (LS1034) and 1554 (COLO678), respectively, of the *APC* gene and a plasmid containing the exon 1465 mutation hotspot were serially diluted with wild-type DNA (HCT116). To enrich the mutations three DNA fragments encompassing the aforementioned exons were amplified by Wild-type Blocking (WTB)-PCR. Subsequently the PCR products were used for Real time PCR and screened by High Resolution Melting Analysis (HRMA). Furthermore DNA from colon biopsies was analyzed as well.
Results. Whereas the achieved detection limit for the mutated *APC* exon 1309 hotspot in wild-type background was only 1 %, we were able to detect 0.01 % of mutated DNA in case of exons 1465 and 1554. Mutations were also detected in 7/14 colon biopsies analyzed for exon 1309, in 1/11 cases tested for exon 1465 and 4/11 samples screened for exon 1554, respectively. These results were confirmed by sequencing.
Conclusions. The combination of WTB-PCR and HRMA is basically a highly sensitive and accurate tool to detect mutations and sequence variants of interest at low abundance within a high background of non-mutated DNA.

Molecular diagnostics

0810

MALARIA INFECTION DIAGNOSIS: COMPARISON BETWEEN TRADITIONAL METHODS AND A NEW FREEZE-DRIED PCR MULTIPLEX TEST

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Background. Malaria is the most diffused parasitic disease that affects humans. A rapid and accurate diagnosis is a prerequisite for effective treatment. To solve the temperature storage requests of common molecular tests and PCR reagents, a freeze-dried self-containing test for the screening and the typing of malaria was considered.
Methods. The performances of the STAT-NAT Malaria Screening and Typing for Malaria infections in immigrants (I) and international travellers (IT) suspected to be infected were compared to the internationally accepted gold standard (microscopy).
Results. In this study 35 patients with suspected malaria were diagnosed in the San Matteo Hospital in Pavia. 25 subjects were IT, and the remaining 10 were I. The microscopy identified 18 of them as positives. 11 of them were *P. falciparum* positives (5 IT, 6 I), 3 were *P. vivax* (3 IT), 2 were *P. ovale* (1I, 1 IT), and 2 were *P.malariae* (2 I). No co-infections were diagnosed. The STAT-NAT Malaria screening confirmed all the 18 positives, as well as the 17 negatives (100% sensitivity). In one case the STAT-NAT identified a *P. ovale* instead of a previously diagnosed *P. vivax* . The epidemiological analysis (the subject came from Cameroun, were *P. vivax* is not present), and a new microscopic diagnosis, confirmed the infection as caused by *P. ovale* type (100% specificity).
Conclusions. We conclude that the association of the freeze-dried STAT-NAT tests could be determinant for the correct detection and typing of malaria, mainly where the temperature controlled storage could be problematic.

0811

ANALYSIS OF DNA LESIONS INDUCED BY ULTRAVIOLET RADIATION WITH TWO-DIMENSIONAL ELECTROPHORESIS

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Background. The main DNA damage caused by ultraviolet radiation (UV) are base changes that cause bending of DNA molecules. They include cyclobutane pyrimidine dimers, pyrimidine (6-4) pyrimidone photoproducts and its Dewar isomers. Two-dimensional electrophoresis, a method that can separate bent DNA from normal DNA, was used to analyse DNA lesions in complex DNA samples and cell cultures after UV radiation.
Methods. Human genomic DNA was digested with *Mbo*I and radiated with UVB (312 nm, 5-30 J/cm^2) in a droplet on a Petri dish. HeLa cell culture was radiated with UVB (15-45 J/cm^2), the DNA isolated and digested with *Mbo*I. The samples were analysed using Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) and Two-Dimensional Conformation-Dependent Electrophoresis (2D-CDE). *Ava*I digested lambda DNA was UVA radiated (44 J/cm^2) and analyzed in the same manner.
Results. DNA induced by UVB migrated in front of normal DNA on 2D-CDE gel, due to bending caused by the DNA lesions. UVA radiation of lambda DNA also caused migration of DNA in front of normal DNA with 2D-SDE. Surprisingly, 2D-SDE analysis of DNA radiated with UVB revealed formation of a DNA arc behind normal DNA. The ratio of this arc increased with increased UVB dosage up to 43%. The same effect was detected in DNA from UVB induced cell culture.
Conclusions. 2D-SDE and 2D-CDE can be used to assess DNA lesions associated with UV-radiation. The DNA arc caused by UVB radiation may be explained by cross-links or formation of A-helix DNA due to either base lesions or deoxyribose oxidation to ribose.

Molecular diagnostics

0812

STUDY OF DNA FRAGMENTATION AND CELL VIABILITY BY FLOW CYTOMETRY IN HUMAN SPERM

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Background. The major problems of public health are the reproductive difficulties which affecting 15% of couples in the world with two thirds of cases, an associated or exclusive male cause. These varied and often multifactorial anomalies resulting in 61% of cases by abnormal quantitative and / or quality of sperm. The objectives of this study were to measure viability and DNA fragmentation in human sperm by flow cytometry.
Methods. First, we used flow cytometry to assess the viability of 47 samples of fresh semen from patients consulting for a review of the evaluation of infertile couples whose average age was 37 ± 6.37 years and 8 samples of semen (4 fresh and 4 frozen). Secondly we measure sperm DNA fragmentation index (DFI) of 51 samples of fresh human semen from patients consulting for the same reasons and with a mean age of 36.5 ± 5.23.
Results. Our results suggest that a relationship exists between sperm viability and spermatic parameters such as concentration (p = 0.01), morphology (p <0.01) and mobility (p <0.01).The comparative study of the results about cell viability and DNA fragmentation obtained by flow cytometry and epifluorescence microscopy showed a significant correlation (p <0.01).We also note that the process of freezing and thawing significantly alters the semen characteristics.
Conclusions. The study of DNA fragmentation could be applied in clinical (therapeutic choice to avoid unnecessary treatment) and reproductive toxicology (study of the effects of chemical, physical or biological germ cells).The use of flow cytometry technique must go through standardized protocol inter-laboratories for a better choice of therapeutic approaches.

0813

EVALUATION OF A NEW COMMERCIAL PCR ASSAY, GENOMERA™ MRSA CONFIRM, FOR RAPID DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM OVERNIGHT CULTURE

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Background. Methicillin-resistant Staphylococcus aureus (MRSA) is a significant cause of morbidity, mortality and increased healthcare costs. Therefore rapid and reliable MRSA screening and confirmatory tests are needed. We evaluated the GenomEra™ MRSA Confirm (Abacus Diagnostica Oy, Turku, Finland), a new automated molecular assay, which amplificates simultaneously the well-known marker of methicillin resistance (mecA) and a S. aureus-specific (SA) DNA segment.
Methods. The test was applied on 230 well-characterized MRSA isolates consisting of multiple different genotypes and on 90 non-MRSA staphylococci and other bacterial isolates from overnight cultures on different agar plates. The sample preparation consisted of transferring cells from single bacterial colonies to sample buffer tubes and adding the sample suspensions to dry-reagent test chips. The thermal cycling and homogeneous detection were performed in the GenomEra CDX™ instrument with results reported within an hour. Results were compared to another well-established in-house assay, based on amplification of mecA gene and SA specific nuclease gene (nuc).
Results. No false positive or false negative results occurred during the evaluation implicating that both the specificity and sensitivity were 100 % when the test was performed from overnight cultures. The PCR-inhibition rate was 0.3 % (1/320).
Conclusions. The GenomEra MRSA Confirm is a promising new tool for rapid and reliable MRSA detection supporting clinical decision making as well as urgent MRSA prevention. A user-friendly application, fast turnaround time, and scarce space requirement makes this small-scale system highly suitable to small or middle sized laboratories, too.

Molecular diagnostics

0814

MLPA-BASED EVIDENCE FOR GENOMIC DUPLICATIONS/AMPLIFICATIONS NEEDS METHODOLOGICALLY INDEPENDENT VALIDATION

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Background. Multiplex ligation-dependent probe amplification (MLPA) is increasingly used in routine genetic diagnostics to identify genomic copy number aberrations. An apparent deletion is an unambiguous finding (after sequencing-based exclusion of small mutations in the MLPA probe binding site), whereas an apparent duplication/amplification is harder to interpret. Still, MLPA-based identification of these latter mutations is frequently claimed without further validation.
Methods. With the aim to confirm MLPA-based evidence for duplicated/amplified sequence as obtained in research screening of monogenic diseases, we re-analysed 11 pertinent samples by a set of independent approaches. These included (i) application of additional MLPA probes for the critical exons, (ii) long range PCR to reveal existence of the presumed breakpoints, (iii) cDNA amplification, (iv) analysis of similarly affected family members, (v) analysis of independently collected secondary samples, and (vi) Southern hybridisation.
Results. Method (i) was compatible with the primary finding in all samples. Methods (ii) and (iii) revealed ultimate evidence for existence of a duplicated segment for four samples; method (iv) confirmed five additional cases. In the remaining two samples, both associated with apparent high level amplifications, method (v) excluded presence of gained sequence. Finally, method (vi) suggested a contamination with PCR product for the corresponding exons as an explanation for the discrepancies between MLPA data for primary and secondary samples in these two cases.
Conclusions. Intra-analytically controlling for contamination is not possible in MLPA. Evidence for sequence gain, therefore, needs independent validation, especially in cases with apparent high level amplification.

0815

ANALYSIS OF URINARY BIOMARKERS IN PATIENTS WITH DIABETIC NEPHROPATHY USING AU CHIP TECHNOLOGY

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Background. Diabetic nephropathy (DN) is the leading cause of chronic kidney disease in patients starting renal replacement therapy. Early intervention and consequent therapy may prevent or at least delay the development of end-stage renal disease. The aim of this study was to establish a proteomic method for identification of DN-related biomarkers in urine that may serve as diagnostic tool for DN.
Methods. We employed urinary proteomics of Au Chip (ProteinChip Gold Array) combined with surface-enhanced laser desorption-ionization time of flight mass spectrometry (SELDI-TOF-MS) to detect protein profilings, which were from 178 patients with DN and 213 non-DN subjects. From signatures of protein/polypeptide mass, an artificial neural networks (ANNs) model was established for diagnosing the presence of DN. Some differential proteins were identified by molecular mass compared with the standard protein and electrospray ionization quadrupole time of flight mass spectrometry (ESI-Q-TOF-MS).
Results. Total of 35 distinguished protein peaks were obtained between DN and the control groups ($P<0.01$). The intensities of 12 detected peaks appeared up-regulated, whereas 23 peaks were down-regulated more than twofold in the DN group compared with the non-DN groups. The algorithm identified a diagnostic DN pattern of six protein/polypeptide masses. On masked assessment, the predictive model based on these proteins/polypeptides achieved a sensitivity of 98.7% and specificity of 97.3%. Six specific peaks (m/z at 11 735, 15 150, 2 2871, 23 770, 67 650 and 80 045) were identified and considered as β_2-microglobulin, hemoglobin, α_1-antitrypsin, α_1-microglobulin, albumin and transferrin, respectively.
Conclusions. The results show that the capability of the Au chip and SELDI technology for rapid biomarker detection from urine that indicate DN, especially early protein alterations that signal the initiation of renal damage.

Molecular diagnostics

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LANTHANIDE CHELATE COMPLEMENTATION IN HOMOGENEOUS WELL-BASED FOUR-PLEX NUCLEIC ACID ARRAY

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Background. Conventional fluorescent labels have been used in microarray detection of nucleic acids. Herein we describe a new technology, lanthanide chelate complementation, for separation-free four-plex nucleic acid array.
Methods. Four oligonucleotide probes, with biotin at 5' and non-fluorescent Eu^{3+}-carrier chelate at 3', were spotted by contact printing onto streptavidin-coated microtitration wells, four spots per well. Four probes with light absorbing antenna ligand at 5' and up to four complementary target sequences were added into the wells in a total volume of 60 μL. After hybridization of the spotted and liquid-phase probe adjacently onto the target sequence, a luminescent complex was formed into the respective spot. The spots were visualized by measuring luminescence from the bottom of the wells at 615 nm in time-resolved mode by scanning a 10x10 raster with 0.5 mm distance between measuring points with Victor 2030 (PerkinElmer, USA). Out of the 100 points, 9 were selected to present one spot area and an average signal was counted for all four spots.
Results. When all four target sequences were present in the well, four luminescent spots were formed. The spots were well distinguished from each other and had a signal-to-background (S/B) ratio of 30–90. When only one target sequence was present in the well, only one luminescent spot with similar S/B ratio was formed.
Conclusions. We have demonstrated that lanthanide chelate complementation can be used for qualitative detection of four specific oligonucleotide sequences in wash-free well-based nucleic acid array. Higher multiplexing of the array could be possible.

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MICROFLUIDIC FLOW-THROUGH DNA PURIFICATION FOR CONTINUOUS MONITORING APPLICATIONS

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Background. The ability to continuously monitor nucleic acid content in a dynamic sample would allow significant progress in numerous fields, such as monitoring of bioprocesses, air, water, extracorporeal blood,and many other samples. Until now, a device for continuous extraction of DNA from a crude sample has not been available and was hence developed by our lab.
Methods. A mixture of cell lysate and superparamagnetic DNA-binding beads is continuously injected into a microfluidic chip. Circularly arranged microchannels guide different buffer flows around a central rotating permanent magnet. The magnet attracts the DNA carrying beads towards the inner part of the channels and ensures their transfer across the laminar interface, thus continuously performing the three essential steps in DNA purification: separation, washing, elution.
Results. Genomic DNA from *E. coli* lysate was continuously purified on-chip. Syringe pumps controlled the flow of sample and extraction reagents. An inlet flow of 11.9 mm·s^{-1} (0.75 μl·s^{-1}) led to an average bead velocity of 0.7 mm·s^{-1} and a sample transition time of approximately 2 minutes. Subsequently, the extracted DNA was amplified off-chip via qPCR. In dilution series, the continuous on-chip purification showed linearity over 7 orders of magnitude and recovered 150 ± 50 % of total DNA compared to batch-wise reference purifications.
Conclusions. We have successfully established a microfluidic platform for flow-though DNA extraction from lysate. With appropriate surface modification of the magnetic beads the chip also allows for continuous purification of other biomolecules such as RNA, proteins or even cells, including their subsequent real-time analysis.

Molecular diagnostics

0818

LABORATORY PERSONNEL IS A HIGH-RISK GROUP FOR LATENT TUBERCULOSIS INFECTION: EVALUATION BY INTERFERRON-GAMMA RELEASE ASSAY AND TUBERCULIN SKIN TEST IN AN INTERMEDIATE INCIDENCE SETTING

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Background. To evaluate the prevalence of latent tuberculosis infection (LTBI) in the laboratory personnel in an intermediate incidence setting.
Methods. We recruited 173 laboratory personnel, and performed QuantiFERON-TB Gold In-Tube test (QFT-G) and tuberculin skin test (TST).
Results. QFT-G was positive in 21.4% of the enrolled laboratory personnel, and TST was positive in 33.3%. The agreement between the two tests was fair ($\kappa = 0.234$). With multivariate analyses, household contact with TB patients ($P = 0.013$), the laboratory sections of microbiology ($P = 0.045$) and chemistry/immunology ($P = 0.014$) were significantly associated with positive QFT-G Results.
Conclusions. Our data shows the high prevalence of LTBI in the laboratory personnel and emphasizes the importance of LTBI screening for the laboratory personnel. QFT-G seems to be superior to TST for the LTBI screening.

0819

CENTRIFUGAL MICROFLUIDIC PLATFORMS FOR MOLECULAR DIAGNOSTICS

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Background. We present a microfluidic Lab-on-a-Chip platform for integration and automation of diagnostic and biochemical protocols, including nucleic acid analysis and immunoassays.
Methods. Laboratory processes are automated in disposable microstructured polymer test carriers. These labs-on-a-chip are processed and readout in centrifugal devices. Centrifugal processing of nucleic acid applications is realized on the commercially available Rotor-Gene platform.
Results. Fourteen real time PCR reactions are performed in parallel in microstructured foil substrates. The detection of < 10 copies of the antibiotics resistance marker Exf A is demonstrated in a foil featuring pre- and main amplification. Isothermal amplification of DNA by recombinase polymerase amplification (RPA) has also been demonstrated in our lab-on-a-chip system. Automated parallel testing of 30 samples with sensitivities < 20 copies and time-to-result of 20 minis achieved. Furthermore, a polymer cartridge for immunoassays is shown for the quantification of estradiol and IL8 with sensitivities down to 125 pg/mL. 24 assays can be processed in parallel. The immunoassay cartridge can be complemented with a blood plasma separation module for sample preparation extracting 4 μL blood plasma out of 10 μL whole blood with a CV of 6 %. Furthermore, an automated DNA extraction is developed, allowing to extract DNA out of a bacterial sample with a yield comparable to the laboratory reference method.
Conclusions. The development of a microfluidic platform for manifold applications in the field of diagnostics and biochemistry is shown and a successful implementation of assays for DNA and protein analysis as well as sample preparation is demonstrated.

Molecular diagnostics

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TYROSINEMIA TYPE I: A CASE REPORT

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Background. Tyrosinemia type 1 is an autosomal recessive disease that results from absence or deficiency of the enzyme activity fumarylacetoacetate hydrolase (FAH) encoded by FAH gene located in 15q 23-25.
The intracellular accumulation of fumarilacetoacetate, succinilacetoacetate and succinilacetoacetone is toxic to the cell, producing cell damage and apoptosis.
It usually presents in young infants (frecuently before the first year of life) with liver dysfunction and renal tubular acidosis. Death in untreated child occurs before ten years.
Molecular genetic testing by analysis of the more common FAH mutations is clinically available.
Methods. We present the case of a three months infant with hepatic vascular malformation who was admitted to the pediatric intensive care unit of our hospital with anasarca and important ascites, respiratory and liver failure and hyponatremia. During her PICU stay, she remained hemodynamically stable and showed an improvement of the pulmonary function but persisted an electrolyte imbalance, a coagulation disorder and renal tubular dysfunction. High levels of tyrosine, methionine and succinylacetone were found in serum and urine. The patient died after an episode of severe pulmonary and gastrointestinal bleeding. Sequence analysis of the FAH gen was performed, finding a compound heterozygosity c554-1G> T and c1062 +5 G> A, inherited from his mother and father respectively.
Conclusions. Genetic counseling was provided to the parents, as carriers, including discussion of potential risks to offspring and the reproductive options, which includes prenatal and preimplantation genetic diagnosis.

0821
HIGH THROUGHPUT TETRAPLEX REAL-TIME PCR ANALYSIS FOR SIMULTANEOUS DETECTION OF INFLUENZA A, B AND H1N1 2009 STRAINS

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Background. In 2009 the Influenza H1N1 pandemia was a challenging period for medical laboratories to deliver fast and accurate Results. To cope with this we developed a tetraplex real time PCR analysis for simultaneously determination of Influenza A and B and H1N1 2009 pandemic strain.
Methods. Based on CDC public protocol for detection of Influenza A and the porcine H1N1 strain we developed a new protocol complemented with primers and probe for Influenza B. Our primer mix included an internal control of extraction and RT-PCR. The extraction of viral RNA was performed with either EasyMag (bioMérieux) or M2000sp (Abbott) while the RT-PCR reactions were validated on both RotorGene 6000 (Qiagen) and LightCycler 480 (Roche).
Results. Up to 5 batches of 96 samples a day could be analysed at peak time with only one lab technician. The specificity was >99% and sensitivity >95% for InfA H1N1. Viral RNA could be detected easily until 6 days after beginning of symptoms. However, typing of H1N1 usually failed for samples taken 5 days after the initial symptoms because of insufficient viral load.
Conclusions. Our development allows rapid determination of Influenza A and B and H1N1 with a high throughput of samples per day.

Molecular diagnostics

0822

MICROARRAY BASED ANALYSIS OF THE GENETIC RISK FACTORS HLA-DQ2/DQ8IN THE DIAGNOSIS OF CELIAC DISEASE

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Background. Celiac disease is a chronic indisposition predominantly of the small intestine. It is caused by the ingestion of gliadin containing wheat, rye, and barley. In genetically predisposed persons deamidated fragments of gliadin lead to an inappropriate immune response in the intestinal mucosa. The disease is strongly associated with the presence of the heterodimeric human leukocyte antigens (HLA) DQ2 and DQ8.

Methods. In order to identify DQ2 and DQ8 sequence specific amplification of selected HLA-DQ gene sequences is achieved by two parallel multiplex PCRs with simultaneous fluorescence labeling of the reaction products. PCR mixtures arehybridized to a microarray with allele-specific probes on aslide using TITERPLANE incubation technique. Spot intensities areanalyzed bythe microarray scannerand genotypes automatically deduced by the EUROArrayScan software.

Results. The result output shows whether a patient is negative or positive for HLA-DQ2/DQ8 and discriminates in positive cases between DQ2 only, DQ8 only, and the presence of both genotypes.

In a study with 101 DNA samples from blood donors (precharacterized by DNA sequencing) and 44 reference DNA samplesfrom the "International Histocompatibility Working Group" (containing all positive genotypes as well as DQ2/DQ8 negative probes) themicroarray test showed 100% concordance with all reference genotypes.

Conclusions. The newly developed test system is easy and fast to perform, accurate and reliable. HLA-DQ2 and -DQ8 markers can be unambiguously determined based on the sophisticated array design which includes also non disease associated alleles. The output of the results is fully automated.

0823

WHAT IS THE ADDED VALUE OF MOLECULAR MICROBIOLOGY IN SEPSIS?

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Background. Sepsis is severe acute condition. Immediate initiation of therapy is essential as well as fast detection of a pathogen. Traditional cultivation methods take 24-48 hours, new molecular methods 6 hours or less.

Methods. We compared detection of pathogenic microorganisms in blood by cultivation methods – blood culture (BC) with examination by LightCycler SeptiFast Test MG using the principle of real-time PCR and high resolution melting curve analysis. SeptiFast Test detects DNA of most frequent etiological agens of sepsis including fungi.

Results. Our dataset included 81 children and 31 adult cancer patients. BC was positive in 41 samples of 268 (15.3%). SeptiFast Test was positive in 68 samples of 224 (30.4%). Positivity of both methods was in 22 samples (25.3%) of all positive samples (n=87), in 19 samples (21.8%) was positive only BC and in 46 samples (52.9%) only SeptiFast Test, 94 samples were consistently negative. Most frequent findings were *coagulase-negative* Staphylococcus *spp.*, *Escherichia coli,Klebsiella pneumoniae* and *Candida albicans*. Using of SeptiFast Test was the greatest benefit for detection of mycotic infection. SeptiFast Test detected fungi in 7 samples, while BC were negative. Reason for negativity can be slow growth and necessity of specific cultivation conditions.

Conclusions. SeptiFast Test is more sensitive than BC and may lead to earlier introduction of specific therapy. However it is not a substitute for cultivation Methods. SeptiFast Test detects only limited number of pathogens and cannot provide bacterial resistance profile.

Molecular diagnostics

0824

MUTATION PATTERN OF *KRAS* AND *BRAF* ONCOGENES IN COLORECTAL CANCER PATIENTS OF CROATIA

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Background. Colorectal cancer (CRC) is one of the leading malignancies worldwide and has been reported to show geographical variation in its incidence. Activation of *KRAS* and *BRAF* oncogenes has been implicated in colorectal carcinogenesis. Theirmutations can be detected in approximately 30-40% and 5-15% of all patients with CRC, respectively. The aim of this study is to identify *KRAS* and *BRAF* gene mutations in CRC patients among the Croatian population, and to assess whether they are linked with the clinicopathological features of patients and tumors.
Methods. All specimens were obtained during routine surgery of patients with CRC. The diagnoses were established by standard procedures and confirmed histopathologically. After DNA extraction,mutations were analyzed using Real-Time PCR. The presence of mutations is compared with age, gender, tumor size, differentiation and Dukes' stage.
Results. Our preliminary results shows that *KRAS*gene mutations are detected in 18 samples (33,3%). The most frequent *KRAS* mutation isp.G12Vdetected in 9 samples (50%). *BRAF*gene mutation p.V600E is detected in 4 samples (7,4%).Statistical analysis revealed a significant association (p=0,04) between the *KRAS* mutation and Dukes' stage with least frequency in Dukes' A. We found no correlation between mutations and other clinicopathological features.
Conclusions. The first data about *KRAS*and *BRAF* mutational status in the sample of Croatian population with CRC shows that the incidence of *KRAS*and *BRAF*mutations is within generally valid limits. Prospective studies are to be continued in order to determine whether these mutations play a role in the progression of CRC.

0825

IMPACT OF GERMLINE MUTATIONS IN TUMOUR SYNDROME SUSCEPTIBILITY GENES ON THE DEVELOPMENT AND DIGNITY OF PARAGANGLIOMAS (PGL) AND PHAEOCHROMOCYTOMAS (PHAEO)

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Background. PGL and Phaeo are rare, mostly benign tumours originating from the neural crest, frequently caused by germline mutations.
Methods. 110 index patients were analysed for mutations in the *SDHB, C* and *D* gene by conventional sequencing and MLPA. 74 patients presented with PGL, 7 with a malign PGL, 26 with Phaeo, and 3 with multiple Phaeo and PGL. Wherever no *SDHB, C or D* mutation was found sequencing was extended to *RET*, *VHL* and *TMEM127* (Phaeo patients) or to *SDHA* and *SDHAF2* (PGL patients).All PGL patients with erythrocytosis were tested for the H374R mutation in the*PHD2* gene.
Results. In 40 of the 110 patients disease causing mutations were detected: In the PGL collective (81) 38% were affected (*SDHD>>SDHB = SDHC>>SDHA*). Malignant PGL was associated with truncating mutations in *SDHB* and *SDHD* and a gross deletion of *SDHB*. In the Phaeo collective (26) mutations were detected in 23% of the cases (4 RET, 2 VHL). 1 of the 3 patients with multiple Phaeo and PGL showed a truncating *SDHD* mutation, 2 showed a gross deletion in *SDHB*.
Conclusions. Familial cases of PGL and Phaeo are more common than assumed in the past. Genetic characterization of PGL patients is essential to facilitate genetic counselling. Even if the index patients shows few symptoms, one of his relatives may be affected seriously and profit from early diagnosis. Complementing conventional sequencing of *SDHB, C* and *D* by the analysis of these genes for gross deletions is strongly recommended.

Molecular diagnostics

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ANALYSIS OF MUTATIONS IN THE HFE GENE IN PATIENTS WITH HFE TYPE 1 IRON OVERLOAD WITHOUT COMMON HFE MUTATIONS

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Background. Hereditary hemochromatosis is a common autosomal recessive disorder of iron metabolism. Mutations in the *HFE* gene can cause increased iron absorption leading to iron overload in various of organs. If untreated this disorder results in diabetes, arthritis, cardiomyopathy, and liver cirrhosis, ultimately leading to death. Detected at an early stage hereditary hemochromatosis can be successfully treated.

Methods. We used nucleotide sequence analysis for examination of the *HFE* gene in 23 patients with suspected hereditary hemochromatosis negative for the *HFE* C282Y, *HFE* H63D and *HFE* S65C mutation. Source material was genomic DNA isolated from patients´ whole blood and amplified by PCR before sequencing.

Results. We detected two heterozygous exonic mutations *HFE* 5228G>A (exon 1, R23H) and *HFE* 9047G>A (exon 3, L118L) and three intronic mutations IVS2+4T>C, IVS4+48G>A and IVS4+115T>C. These were present in heterozygous and/or homozygous form in 20 patients. Among eight patients these five mutations were found in different combinations.The mutations IVS2+4T>C, IVS4+48G>A and IVS4+115T>C represented published polymorphisms, R23H is a novel, potentially causative mutation. Three patients did not show any mutations.

Conclusions. Our study demonstrates that Austrian patients with suspected hereditary hemochromatosis seldom exhibit rare, potentially causative mutations in the *HFE* gene. In contrast, genetic polymorphisms can be frequently found.

0827

OPTIMIZATION OF PCR FOR DETECTION OF BRUCELLA IN MILK

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Background. Brucellosis causes considerable economic losses in food animal industry. Milk excretion of Brucella is an important factor in the spread of infection. In this study, several combinations of DNA extraction and PCR protocols were tested for the accurate detection of Brucella in milk.

Methods and Results. Fresh bovine milk spiked ten-fold log-phase culture dilutions of B. melitensis was used as the study material. Brucella DNA was extracted from several compartments of milk, by DNeasy tissue kit (Qiagen) and two modifications of phenol-chloroform-isoamyl-alcohol protocols (PCI-1, PCI-2). PCRs were performed by using five different primer sets in their original protocols.. The higher concentration of DNA was obtained from non-fatty compartment of milk when PCI-1 protocol was used. Non-fatty compartment of milk and PCI-1 protocol gave higher DNA yield. In spiked-milk samples, detection limits of PCR protocols designated with primer names for B.melitensis were as follows; 6.4×10^1 CFU for F4-R2, 1.2×10^2 CFU for Ba148/928 , 10^3 CFU for IS711 and 10^4 CFU for 31ter/sd and JPF/JPR primers. In optimized conditions, PCR was able to detect lower numbers of Brucella in milk when compared with bacteriological culture.

Conclusions. Non-fatty compartment of milk, DNA extraction with PCI-1 protocol and F4-R2 primers were found to be optimal conditions for the detection of Brucella in raw milk. When optimal conditions were used PCR was found to be more sensitive than conventional culture technique.

Molecular diagnostics

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PRESERVATION OF CELL-FREE DNA IN STORED BLOOD SAMPLES FOR THE ANALYSIS OF THE ^{m}SEPT9 COLORECTAL CANCER SCREENING MARKER ENABLES SAMPLE SHIPMENT BY MAIL

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Background. The detection of the biomarker ^{m}SEPT9 in plasma is strongly associated with the presence of colorectal cancer (CRC). ^{m}SEPT9 analysis for the early detection of colorectal cancer requires plasma generated from whole blood samples. Sample logistics and shipment by standard mail would facilitate broad adoption of this parameter in the routine.
Here we evaluated long term storage of full blood samples at room temperature in S-Monovette CPDA (Sarstedt) and Cell-Free DNA BCT (Streck) blood collection tubes for subsequent analysis of ^{m}SEPT9 with the Epi *pro*Colon Early Detection Assay in blood plasma generated from the stored samples.
Methods. Blood draws from 20 healthy donors were spiked with methylated DNA and stored at conditions simulating mail transport at room temperature (18 to 25 °C for 48h). The samples were analyzed according to the instructions for use of the Epi *pro*Colon assay.
Results. All results derived from S-Monovette CPDA tubes spiked with methylated DNA were consistently ^{m}SEPT9 positive. A comparison of the real-time PCR threshold cycle values (CP values) for beta-actin (ACTB) showed a trend of decreasing CP values (i.e. larger amount of ACTB) with increasing storage times. Blood draws collected in Cell-Free DNA BCT tubes failed the quality criteria in 14 out of 40 samples.
Conclusions. The study clearly demonstrates that ^{m}SEPT9 can be consistently detected in plasma samples derived from whole blood samples stored at 18 to 25 ºC for 48 hours. Study results did not support the usage of Cell-Free DNA BCT tubes for this application.

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AN AUTOMATED WORKFLOW FOR THE ANALYSIS OF THE ^{m}SEPT9 COLORECTAL CANCER EARLY DETECTION MARKER IN BLOOD PLASMA: EPI *PRO*COLON EARLY DETECTION ASSAY WITH THE INVIGENIUS®

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Background. Colorectal cancer (CRC) screening has a low compliance rate, although prognosis for CRC patients is more likely to be favorable when disease is detected early. Epi *pro*Colon, a blood-based IVD assay available in many countries accepting CE-marking, is a new alternative for CRC early detectionand has potential to increase screening compliance.
Epi *pro*Colon detects bisulfite-converted methylated DNA within the Septin 9 gene (^{m}SEPT9) in plasma. Assay performance was analyzed in multiple case control studies and prospectively enrolled CRC screening guideline-eligible individuals. These studies demonstrated that detection of ^{m}SEPT9 in plasma is strongly associated with presence of CRC.
For adoption of ^{m}SEPT9 testingin clinical routinethere is need for automation of the assay procedure. Here we present preliminary data from an automated version of the Epi *pro*Colon test using the InviGenius® platform.
Methods. Concordance between manual and automated workflows was assessed. Five technical samples (plasma samples spiked with methylated DNA), and ten clinical samples (plasma pools from CRC patients and healthy donors) were processed manually and on the robot.
Results and Conclusions. mSEPT9 sample measurementsdetermined with the automated version were concordant with manual version Results. The automated workflow enables standardized processing of 12 samples per batch and up to 24 samples per day. The InviGenius system allows complete traceability of samples, reagents, tips and waste requiring a minimum footprint (~1m2) and minimal manual interaction.
These results confirm that the^{m}SEPT9 workflow can be easily automated to meet increasing demands for blood-based CRC early detection.

Molecular diagnostics

0830

FAST MICROARRAY TEST FOR DETERMINATION OF HLA-B*27 ALLELES DIRECTLY FROM WHOLE BLOOD

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Background. HLA-B27 is associated with several rheumatic diseases including Bechterew's disease (spondylitis ankylosans). Around 90%of patients with spondylitis ankylosans carry alleles encoding the B27 subtype. More than 1000 different HLA-B alleles are known. Seventy-seven of them correspond to HLA-B*27 (B*27:01 - B*27:65N). To facilitate HLA-B27 determination, a microarray system was developed that can be performed directly from whole blood without prior DNA isolation.
Methods. After blood samples are briefly incubated (1 minute) with extraction buffer,exon 2 and 3 of HLA-B*27 alleles are amplified by multiplex PCR. PCR products are analyzed by microarray hybridization using TITERPLANE incubation technique. The microarrays are evaluated automatically with a microarray scanner andEUROArrayScan software. Besides the HLA-B*27 status the report documents validity of each test result based on controlsassessing sample integrity,PCRand hybridization conditions.
Results. The two-exon-approach makes it possible to detect all HLA-B*27 alleles known worldwide and to indicate whether the not disease associated subtypes (B*27:06 and B*27:09)are involved.
Validation of the test system was based on 100 DNA samples from blood donors and 55 reference DNA samples from the IHWG including HLA-B*27 positive and negative cases. These studies revealed 100% specificity and sensitivity of the microarray test. All determinations were successful.
The handsontime per blood sample is 1.5 minutes.
Conclusions. The direct use of blood as sample material, the fully automated analysis procedure and ready-to-use reagents make performance of this microarray assay fast,easy and robust. No additional reagents for DNA isolation are required.

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THE EFFICIENCY OF DOUBLE-STRANDED DNA FORMATION IN CDNA SYNTHESIS CAN BE CRUCIAL FOR PRECISION OF MICROARRAY EXPRESSION ANALYSIS

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Background. The quality and reproducibility of microarray expression experiments within and between laboratories are compromised by the complexity of the samples and procedures. We used Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) to investigate the quality and reproducibility of the cDNA synthesis. 2D-SDE can measure double-stranded DNA the essential template for T7 RNA polymerase.
Methods. SuperScript III reverse-transcriptase was used to synthesize cDNA from Universal Human Reference RNA (Stratagene) using T7(dt) primer. The samples were synthesized both according to kit protocol and according to our improved methodology. The amount of dsDNA was mesured with 2D-SDE. The cDNA was transcribed with T7 RNA Polymerase and the labeled aRNA hybridized to Agilent microarray containing probes from 230 genes. Each gene was represented by 10 probes and each probe was replicated six times to estimate the variability of the experiments. Results were analyzed using the R software.
Results. The amount of double-stranded cDNA after synthesizing the same RNA samples six times was very variable ranging between 0 and 73%. Microarray experiments showed that the higher the dsDNA percentage the fewer probes had significant variability between samples. Approximately 15% of probes showed variability (p = 0,05) between samples when the dsDNA percentage was between 12% and 35%. In contrast, only 3% of probes showed variability between samples when the dsDNA percentage was 69% and 73%.
Conclusions. Our results indicate that an important component in imprecision in T7 RNA polymerase-based microarray expression analysis can be explained by the amount of double-stranded cDNA synthesized.

Molecular diagnostics

0832

QUANTITATIVE DETERMINATION OF PROTEIN BIOMARKER ISOFORMS USING MASS SPECTROMETRY IMMUNOASSAY

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Background. Proteins play important role in biological processes. Protein isoforms that serve as potential biomarkers may provide enhanced clinical utility. Therefore, developing new and rapid methods for analysis of these isoforms is of great importance for determining their role in the onset of disease, progression and response to therapy.
Methods. Presented in this work is a two step mass spectrometric immunoassay for quantitative analysis of several proteins and protein variants in serum and plasma samples. In the first step, immunoaffinity pipettes were used to isolate the protein of interest from the biological sample. Then, MALDI-TOF-MS was utilized to analyze and quantify the protein.
Results. Fully quantitative assays for cystatin C, beta-2-microglobulin, transthyretin and retinol binding protein were developed and characterized in terms of their precision, linearity and recovery characteristics. Furthermore, the novel methods were compared to conventional ELISAs. Then, the assays were utilized to determine the individual concentration of protein variants across larger cohorts of samples, demonstrating the ability to fully quantify all individual forms of post-translationally modified proteins. Additional proteolytic digestions using trypsin and endoproteinase Arg-C were performed for samples containing genetic heterogeneity in order to identify the point mutations..
Conclusions. The novel mass spectrometric immunoassay method provided a fast, accurate and high-throughput quantitative analysis for all proteins when compared to conventional ELISAs. Moreover, this method allowed detection and quantitation of protein isoforms, which can be used as part of specific protein biomarker discovery/rediscovery undertaking and can be a step forward in protein analysis in clinical practice.

Molecular diagnostics

0833

DEVELOPMENT AND CLINICAL IMPLEMENTATION OF NEXT GENERATION SEQUENCING FOR MULTI-GENE DIAGNOSTIC PANELS

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Background. Next generation, high throughput sequencing technologies (NGS) have emerged during the past six years. They share a fundamental process in which clonally amplified DNA templates, or single DNA molecules, are sequenced in a massively parallel fashion in a flow cell. Sequencing occurs in either a stepwise iterative process, or in a continuous real time manner. By virtue of the highly parallel process, each clonal template or single molecule is individually sequenced and can be counted among the total sequences generated. The high throughput combination of qualitative and quantitative sequence information generated has allowed analyses that were previously not technically possible or cost prohibitive. While NGS has experienced wide dissemination throughout biomedical research, its translation into molecular diagnostics is in the early phases. One growing area of clinical application for NGS is the targeted resequencing of multiple genes whose mutational spectra result in overlapping clinical phenotypes. A second, clinical research application is the use of exome and whole genome sequencing for identifying causative variants and genes in affected probands in family studies.

Methods. We are developing multi-gene panels for diagnostic applications and utilizing exome and genome sequencing for clinical research. Sample preparation for multi-gene panels includes enrichment of target genes from human genomic DNA by long range PCR or highly parallel microdroplet PCR using RainDance Technology. Fragmentation of enriched target sequences, prior to library preparation, is achieved by Covaris focused acoustic technology. Libraries are produced either manually or with the Beckman SPRI-TE automated library preparative instrument and sequenced on the Illumina HiSeq 2000 platform. For exome sequencing, coding regions are enriched using either the Agilent SureSelect or Nimblegen SeqCap EZ in solution oligonucleotide capture reagents. Bioinformatic analyses are conducted with a combination of freeware and commercial softwares including CLC-Bio. Identified variants are cross referenced against several databases including dbSNP, the Human Genome Mutation Database, OMIM and relevant locus specific databases. Softwares employed for functional significance of variants include SIFT, PolyPhen-2 and pMUT.

Results. Several multi-gene panels are in different stages of development and implementation with first panels focused on hypertrophic cardiomyopathy (HCM), aortopathies, and mitochondrial disorders. Initial work on hypertophic cardiomyopathy, employing target gene enrichment with long range PCR coupled with Illumina sequencing, demonstrated feasibility of this combined approach as confirmed by Sanger sequencing of identified variants. Enrichment of HCM associated genes by RainDance technology is under evaluation. Feasibility of exome capture coupled with Illumina sequencing has also been demonstrated and is being utilized for clinical research.

Conclusions. Development and implementation of NGS into the clinical laboratory poses unique technical workflow challenges, notably due to the multi-step processes required for target gene enrichment, whether for multi-gene panels or exomes. Bioinformatic analyses of generated data require expertise and flexibility of approach to arrive at consensus variant calls. This process complexity needs to be accompanied by multiple quality control steps to achieve reproducible Results. NGS offers a powerful approach for complex genetic analyses and is increasingly being adopted into the clinical laboratory. Updates on progress will be presented.

Molecular diagnostics

0834

ARRAY-IN-WELL TYPING ASSAY FOR ADENOVIRUSES USING ENHANCED PHOTON UPCONVERSION

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Background. A multiplexed oligonucleotide hybridization assay was developed for detection of the most common adenovirus genotypes causing human infections. The study demonstrates surface enhanced anti-Stokes photoluminescence from white microtiter well plates compared to transparent well plates. The study also provides a demonstration of the advantages and potential of the upconverting phosphor (UCP) reporter technology in multianalyte assays.

Methods. Eleven adenovirus genotype-specific 5'-aminomodified probe spots were printed covalently in array format on transparent and white microtiter wells. The oligonucleotide probes were hybridized with the biotinylated asymmetric qPCR-products and the bound biotinylated products were detected with streptavidin-coated UCPs. The assay was measured with a CCD-based anti-Stokes photoluminescence imager using 30 s exposure per well for transparent wells and 1 s exposure per well for white wells. The image analysis was performed with ImageJ software. Samples were sequenced to assure the assay result.

Results. The instrument background signal was same with both plate types. With white plate the specific mean intensity values were between 7000–45500 and with the transparent plate the specific mean intensity values were between 5500–18000. However, the signal-to-background ratios were not improved with the white plate as the signal outside the spots originating from non-specific binding of the label was also increased in the white wells.

Conclusions. High-throughput and specific detection of adenovirus genotypes can be performed with white plates. The advantage of the white plate is that the exposure time is 30 times shorter compared to the transparent plate making the assay implementation more user-friendly.

Neurological diseases (acute and chronic)

0835

OXIDATIVE STRESS MARKERS IN BIPOLAR DISORDER

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Background. Recent data suggest that oxidative stress may be involved in the pathophysiology of bipolar disorder (BD). In the present study, we evaluated serum markers of oxidative stress and the relation of these markers with serum excitatory amino acids in patients with BD.

Methods. The study included 60 bipolar patients diagnosed according to DSM IV and 20 healthy controls. Serum advanced oxidation protein products (AOPP), total sulphydryl (SH), total antioxidant capacity (TAC) and total oxidative status (TOS) levels were determined by using colorimetric Methods. The OSI value was calculated according to the following formula: TOS/TAC*10. Serum aspartate and glutamate levels were measured by LC-MS/MS.

Results. Serum AOPP levels were significantly higher in BD patients than controls (16.67 ± 7.36 mmol/L vs 11.94 ± 4.38 mmol/L $p<0.05$). Serum SH, TOS,TAC and aspartate levels did not significantly differ among patients and controls. Serum glutamate levels significantly higher in BD patients compared with controls (136.58 ± 58.8 µmol/L vs 83.02 ± 42.4 µmol/L, $p<0.05$). In patients with BD, AOPP levels were significantly correlated with TOS, SH and OSI levels ($p< 0.01$, $r= 0.614$; $p< 0.01$, $r= 0.412$; $p< 0.01$, $r= 0.742$, respectively). Aspartate levels were correlated with TOS levels ($p< 0.05$, $r= 0.299$) while no correlation was observed between glutamate and all parameters (all $p > 0.05$).

Conclusions. Our study suggest that oxidative stress still do not clarify completely the BD pathophysiology. Since serum levels were measured, our results may not reflect the brain levels.

0836

CHANGES IN LEUKOCYTE SUBPOPULAIONS IN MULTIPLE SCLEROSIS PATIENTS UNDER VARIOUS MEDICATIONS

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Background. Modern medicines for the treatment of the multiple sclerosis (MS) influence the immune system and significantly diminish certain leukocyte subpopulations. In this study galatrimeracetate, natalizumab and laquinimod, three modern MS-medicines, are compared to each other.

Methods. Flow cytometric analysis of immune cell populations (immunstate) was performed using a Beckman Epics flow cytometer in patients with MS during therapy. The measurements were compared with regard to their therapeutic regimen.

Results. Above mentioned novel medicines influence the subpopulations of leukocytes. Significant shifts in lymphocyte subpopulations are seen. The medicines mentioned above suppress the development of lymphocyte subpopulations. Two populations of CD3+ T lymphocytes were observed in patients treated with natalizumab. Further, in addition to the normal CD3+CD8+ population, a second CD3 negative CD8+ population emerged. Also, the mean of the HLA DR positive monocyte population decreased significantly under natalizumab. The number of NK cells increased, as measured in relative percentage, but absolute NK cell numbers remained stable during therapy. The amount of B lymphocytes declined significantly.

Conclusions. Regular flow cytometric measurements are recommended for patients with MS and therapy with one of the substances investigated to monitoring the therapy sufficiently.

Neurological diseases (acute and chronic)

0837

QUANTIFICATION OF LACTIC ACID IN CEREBROSPINAL FLUID TO DIAGNOSE NOSOCOMIAL BACTERIAL MENINGITIS AFTER NEUROSURGERY

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Background. Nosocomial bacterial meningitis (NBM) after neurosurgery are common complications. They are life treating events and the diagnosis is sometimes difficult. The aim of this study was to analyze the value of CSF lactic acid (LA) as an adjuvant biomarker in the diagnosis of NBM after neurosurgery, and to calculate the operational characteristics of CSF LA to discriminate bacterial.
Methods. CSF LA was quantified in 96 CSF samples by the Dimension AR (Dade Behring, Deerfield, IL, USA), distributed into five groups:
1. Proved bacterial meningitis after neurosurgery, all cases with bacteria isolated by CSF culture (n 13); 2. Possible bacterial meningitis after neurosurgery (n 6); 3. Non bacterial meningitis after neurosurgery (n 10); 4. Normal CSF after neurosurgery (n 10); 5. Normal CSF, from patients that not underwent neurosurgery(n 57).
Results. CSF LA (median; IQR) in group 1 (5.4; 2.9-9.4 mmol/L); group 2 (4.4; 3.1-9.3 mmol/L); group 3 (2.9; 1.8-3.5 mmol/L); group 4 (1.7; 1.5-2.2 mmol/L); group 5 (1.7; 1.4-1.9 mmol/L) ($P \leq 0.0001$). CSF LA (cutoff of 3.5 mmol/L) for the diagnosis of NBM showed sensitivity of 69%, specificity 90%, positive (PPV), negative predictive value (NPV) and efficiency of 82%, Youden index 0.6, positive likelihood ratio of 6.9.
Conclusions. Determination of CSF LA is rapid, inexpensive and easy to perform. It is specific and have high PPV and NPV to identify bacterial meningitis after neurosurgery.

0838

EVALUATION OF ANTIPSYCHOTIC DRUGS IN RELATION TO THEIR EFFECT ON METABOLIC RISK FACTORS

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Background. The high incidence of metabolic abnormalities such as diabetes and hyperlipidemia, seen in schizophrenic patients are result of complex interaction of number of factors including lifestyle and antipsychotic medication. This study indicates the effect of various drugs which includes both conventional and atypical drugs in their effects in causation of metabolic abnormalities leading to metabolic syndrome.
Methods. Efforts were made to select 210 (divided in 30-30 each group)subjects who were given the antipsychotics i. e. Olanzapine, Clozapine ,Quietapine, Risperidone, Amisulphride , Aripriazole (as atypical antipsychotics)and Haloperidol (as conventional drug) in therapeutic flexible dose as per clinical regimen. Patients were observed for the changes in the anthropometric and biochemical parameters after 16 weeks of Anti psychotic treatment and were subjected to ATPIII defined criteria for metabolic syndrome.
Results. Highly significant(p<0.001) weight gain was observed in patients receiving Olanzapine(3.2 Kg) followed by Clozapine (2.8Kg). An average weight gain of1.9Kg,1.8Kg, 1.06 Kg ,was observed in patients with Quietapine,Risperidone and Haloperidol medication respectively. No significant weight gain(p>0.05) was observed in patients receiving Aripriazole or Amisulphride. Percentage of patients with metabolic syndrome ($\geq$ 3 risk factors) increased form 3.3% at baseline to 36.6% after Olanzapine medication. However,no increase in metabolic risk factors was observed in patients after receiving Haloperidol, Aripriazole or Amisulphride. A highly significant (p<0.001) increase in blood glucose levels from baseline(82.73$\pm$8.27 mg/dl) to after 16 weeks(103.17+ 12.12 mg/dl) of olanzapine medication was observed.
Conclusions. Regular monitoring of weight and metabolic risk factors is important in patients with antipsychotic medication.

Neurological diseases (acute and chronic)

0839

PARANEOPLASTIC MOTOR-NEURON-DISEASE IN A PATIENT WITH LYMPHOPLASMOCYTIC LYMPHOMA ASSOCIATED GAMMA HEAVY-CHAIN DISEASE, A CASE REPORT

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Background. Paraneoplastic motor-neuron-disease (MND) is a rare neurological disorder. heavy-chain disease (HCD) is a rare haematological presentation. We report a patient with a lymphoplasmocytic lymphoma associated gamma HCD presumably causing paraneoplastic MND.
Methods. We describe a 74 years old women presenting 09/2009 with a very slowly progressive generalised muscle-weakness of all limbs over the last 3 years. At the time of presentation the patient was hardly able to climb one floor and reported restriction of walking distance to about 1000 meters. Investigations including clinical neurological assessment, electrophysiology, spinal tap, MRI and clinical chemical parameters were analysed.
Results. Neurological examination showed a mild, slightly asymmetric tetraparesis and generalised muscle atrophy with brisk reflexes. EMG revealed acute denervation activity and fasciculation in all examined muscles (proximal and distal of all extremities and paravertebral) with normal ENG. MRI and spinal tap showed normal Results. With these findings we diagnosed a MND. A monoclonal gammopathy was detected with isolated massive elevation of gamma-heavy chain and we discussed a paraneoplastic character of the MND. After intensive haematological diagnostic the underlying disease – a lymphoplasmocytic lymphoma – could be identified not before almost one year later and specific therapy with Rituximab and Bendamustin was initiated 07/2010, also hoping to have a positive effect for the otherwise not treatable MND.
Conclusions. We report – to our knowledge – the first case of paraneoplastic MND associated with a gamma HCD caused by a lymphoplasmocytic lymphoma and underline the benefit of cooperation between different clinics and institutes in diagnostic and therapeutic means.

0840

DEVELOPMENT OF A BIOCHIP ASSAY FOR THE SPECIFIC DETECTION OF APOLIPOPROTEIN E4 IN BIOLOGICAL SAMPLES

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Background. Human apolipoprotein E4 (ApoE4) is one of 3 major isoforms of apolipoprotein E (ApoE). ApoE is encoded by the three alleles ApoE-ε2, ApoE-ε3, and ApoE-ε4, determining the three major isoforms, namely ApoE2, ApoE3 and ApoE4, in six phenotypes. Genetically, the ApoE-ε4 allele is associated with both familial late-onset and sporadic Alzheimer's disease (AD), which accounts for more than 95% of AD cases. Therefore it is renowned as the best known genetic risk factor for AD. In addition, several studies have linked the ε4 allele with greater risk of cardiovascular disease and with significantly greater progression of disability in multiple sclerosis, though not susceptibility to it. We report the development of a biochip assay for the specific detection of ApoE4 in biological samples, which will facilitate the diagnosis of Alzheimer's disease and the monitoring of its progression.
Methods. A sandwich chemiluminescent immunoassay is employed for the detection. The biochip represents the solid-phase where the capture molecules are immobilized and stabilized and the vessel for the immunoreactions. The assay was applied to the semi-automated Evidence Investigator analyser. The system incorporates the software to process, report and archive the data generated.
Results. The assay has a measuring range of 0-400ng/ml and exhibits <1% crossreactivity with ApoE2 and <1% crossreactivity with ApoE3. Within-run precision was <6% for standard level concentrations.
Conclusions. Results indicate that this biochip assay is highly specific for the detection of ApoE4 genotypes, which can be applied to the diagnosis and monitoring of AD and other disease states.

Neurological diseases (acute and chronic)

0841

THE REFERENCE LEVEL OF S-100β PROTEIN SERUM CONCENTRATION FOR POOR PROGNOSIS IN PATIENTS WITH INTRACRANIAL EXTRACEREBRAL HEMATOMA

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Background. S-100β protein, blood-brain barrier permeability marker, is one of a few biochemical indicators useful in the evaluation of traumatic brain injury. Our aim was to correlate S-100β serum concentration with clinical condition and CT head scan findings as well as to estimate the level of the protein significant for clinical outcome prediction.
Methods. The cohort of 41 subjects underwent clinical examination by the neurosurgeon, consciousness was evaluated with Glasgow Coma Scale (GCS). Diagnosis was established on the basis of CT head scans. Venous blood samples were collected before surgery. Serum concentration of S-100β protein was estimated using ECLIA method (Cobas 6000, Roche). Clinical outcome was measured applying Glasgow Outcome Scale (GOS). Finally, data were analyzed with Statistica, v. 8.0.
Results. The average S-100β level was 0,95±1,75 μg/L. Statistical analysis revealed significant correlation between the level of S-100β and GCS, GOS and D-dimers level (p<0,001, Spearman correlation test). There were statistically significant differences in the level of S-100β depending on the presence of brain edema (1,29±2,02 vs. 0,06±0,03; p<0,01, Mann-Whitney test) or contusion foci (1,37±1,77 vs. 0,72±1,92; p<0,01) in CT scans. The level of S-100β = 0,288 μg/L was determined as a cut off point for clinical outcome prediction (ROC, p<0,001).
Conclusions. Dependences between S-100β serum level and clinical, radiological or laboratory findings prove its usefulness as a diagnostic marker for assessment of brain trauma severity. The level of the protein >0,3 μg/l is connected with poor prognosis.

0842

EVALUATION OF THE NEW MARBURG CEREBROSPINAL FLUID (CSF) MODEL WITH HUMAN SPONDYLOPATHIES

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Background. Marburg CSF Model is based on the theorem: To few proteins (<200 mg/L), filtered from blood through molecular sieve of blood-CSF barrier (BCB) into brain ventricles, blood proteins are added in lymph moving backward from paravertebral lymph vessels into spinal CSF, elevating proteins up to <450 mg/L. Normally, CSF flows off here, but upright human position reverses equilibrium between CSF and lymph pressures allowing minimal lymph leakage into lumbar CSF: 0.3 ml lymph elevates CSF proteins from 200 to 400 mg/L. Spondylopathies altering CSF backbone clearance, are used to evaluate Marburg CSF model.
Methods. Lumbar Solutrast® myelography supplied with radiological diagnosis and CSF to be analyzed for total proteins (biuret reaction of TCA precipitated proteins, CV <8%, inaccuracy <11%), albumin; IgG (immunological nephelometry CV <9%, inaccuracy <11%); significant mean differences* with t-test p<0.05.
Results. In lumbar CSF total proteins >450 mg/L were found with lumbar prolapses* (n=319), mass prolapses* (n=18), presacral prolapses (n=41), osteochondrosis (n=45) (*significance to protrusions). Molar IgG/Alb ratio above BCB one was significantly higher with lumbar and mass prolapses.
Conclusions. Molar IgG/albumin ratios above BCB IgG/albumin ratio indicate serum protein leakage into lumbar CSF confirmed with lumbar and mass prolapses: Addition of 1μL of serum to 1000μL CSF, filtered through BCB, is sufficient to elevate IgG/Albumin ratio significantly. The prolapses increase lymph leakage into lumbar CSF, thus giving evidence of Marburg CSF model which explains origin of 3 CSF types: molecular-sieved CSF of ventricles, spinal CSF with blood proteins, albumin-enriched CSF diffused through inflammatory-destroyed blood-brain-barriers.

Neurological diseases (acute and chronic)

0843

THE P-SELECTIN, GP IBA AND GP IIB/IIIA FLOW CYTOMETRY MAESURING UPON PLATELET'S SURFACE IN PATIENTS WITH DIFFERENT VARIANTS OF ISCHEMIC STROKE

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Background. The mechanisms of ischemic stroke are not studied completely. The interactions between platelet's receptors are pivotal in patients with different pathogenetic variants of stroke. Flow cytometry was performed with CYTOMICS FC 500 for detection of P-selectin expression, GP IIb/IIIa and GP 1ba receptors upon the platelet's surface, using fluorescent labeled antibodies CD62P-PE, CD61-FITC and VM16d-FITC in 18 healthy donors, patients with atherotrombotic stroke due to macroangiopathy and lacunar stroke due to microangiopathy (27 and 23 individuals, respectively) and 22 subjects with pathological tortuosity of brachiocephalic arteries, but without ischemic stroke.
Results. Density of GP Iba doesn't significantly differ in investigated groups – 3.84±0.38; 3.59±0.25; 3.55±0.24; 3.65±0.29 whereas level of GP IIb/IIIa was significantly higher in donors compared with macroangiopathy (8.98±0.53 and 7.49±0.37, p=0.02) and in donors compared with pathological tortuosity of brachiocephalic arteries (8.98±0.53 and 7.37±0.32, p=0.006). The inverse correlations were revealed between GP Iba and GP IIb/IIIa numbers upon the platelet's membrane in group with microangiopathy (R=-0.41; p=0.05) and between quantity of GP Iba and age of all examined individuals (R=-0.24; p=0.03). The change of P-selectin expression after 10mkM ADP induction was significantly varied in studied groups - 57±9%; 71±5%; 61±7%; 76±6% (p=0.02). Accordingly the least increasing in level of P-selectin (and respectively in platelet's activation) was found in donors and the highest – in subjects with pathological tortuosity of brachiocephalic arteries.
Conclusions. there was platelet's activation in individuals with pathological tortuosity of brachiocephalic arteries and P-selectin expression measuring by flow cytometry might reveal this activation to prevent acute cerebral ischemia.

0844

COPPER LEVELS AND OXIDATIVE STRESS IN ALZHEIMER DISEASE

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Background. Some researchs involved oxidative stress (OE) as a decisive factor in Alzheimer disease (AD) and even suggest that is present in initial phase of Mild Cognitive Impairment (MCI). Copper (Cu) due to its redox properties has an important role as cofacor in antioxidative enzymatic activity. At the same time, OE can be evaluated measuring the production of oxidated molecules such as malondialdehyde (MDA).
Methods. In this assay it was studied 36 patients with possible AD, 18 patients with MIC and 33 people as control group (CG). It was obtained EDTA plasma and free methal serum samples from each one and It was measured the concentration of malondialdehyde (HPLC, Chromsystems) and copper (ICP-MS). The results of each group were treated with statistic program SPSS 15.0.
Results. It was found significantly higher copper levels in patients with AD (1006.6+/-182.8 ug/L) or MCI (931.4+/-185.4 ug/L) than the control group (878.8+/-234.5 ug/L), (p=0.038). Similar behaviour had got levels of MDA. They were higher in groups with cognitive disease, AD group (19.0+/-8.1 ug/L), and MCI group (19.4+/-6.5 ug/L), than the control group control (12.5+/-4.9 ug/L), (p<0.001). Moreover, it was found that both studied parameters had positive correlation in whole studied population (r=0.340; p=0.001).
Conclusions. To the best of our knowledge, copper showed a direct relation with neurological damage progresion. Secondly, it was associated worsening cognitive function with oxidative stress through MDA concentration and also, its direct and significant relation with copper concentration.

Neurological diseases (acute and chronic)

0845

SERUM RESISTIN LEVELS ARE REDUCED BY IMMUNOMODULATORY TREATMENT OF RELAPTING-REMITTING MULTIPLE SCLEROSIS

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Background. Adipose tissue is recognized as an important component connecting immune and central nervous system. Among pleiotropic actions of adipokines the modulation of immune system is associated with autoimmune diseases. In the course multiple sclerosis (MS) the abnormalities in proinflammatory adipokines have been shown as a link between immune system and metabolic status. Thus, the effects of immunomodulatory treatment on adipokines levels may play important role in the clinical course of MS. The aim of the study was to evaluate the effect interferon beta and glatiramer acetate treatment on circulating adipokines in patients with relapting-remitting multiple sclerosis RR-MS).

Methods. The study included 55 RR-MS patients treated with interferon beta and 16 with glatiramer. The blood samples were withdrawn before initiation of immunomodulatory treatment, at 1st and 6th month of interferon beta and glatiramer treatment. Serum concentrations of leptin, resistin and adiponectin were estimated by means of ELISA.

Results. Immunomodulatory treatment of RR-MS with interferon beta led to lowering of resistin concentrations on 1st (19325, 14971-28793pg/mL, median, interquartile range, P=0.0104) and 6th month (13902, 11049-18980pg/mL,P<0.0001) comparing to baseline (29403, 17284- 37336pg/mL). Glatiramer acetate decreased resistin concentration on 6th month of treatment (25141± 5965pg/mL) comparing both to baseline (41638± 14942mL) and 1st month (39428± 15342mL). Immunomodulatory treatment not affected serum leptin and adiponectin concetrations.

Conclusions. Interferon beta leads to reduction of serum resistin concentrations already during first month of treatment, while glatiramer acetate causes prolonged effect. Immunomodulatory treatment of RR-MS have no effect on serum leptin and adiponectin concentrations.

0846

ONCONEURONAL AND ANTI-NEURONAL ANTIBODIES IN BENIGN OVARIAN TUMORS

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Background. Neurological paraneoplastic syndromes (NPS) in females are frequently associated with malignant gynecologic and breast tumors. The aim of this study was to evaluate underlying benign ovarian tumors in female patients with suspicion of NPS and neurological deficits, onconeuronal or anti-neuronal antibodies.

Methods. The study included 201 women selected from 395 patients with suspicion of NPS hospitalized in Department of Neurology in Poznan (Poland). NPS were diagnosed basing on Graus criteria in 113 females. Indirect fluorescence (EUROIMMUN, Germany) was performed as a screening test and Western blotting (EUROIMMUN) as a confirmation test for the presence of onconeuronal antibodies in patients' sera. Patients with ovarian tumors (n=85) hospitalized in Department of Gynecological Surgery in Poznan were included as well in the study. Nonteratomous benign ovarian tumors and ovarian teratoma patients were analyzed separately.

Results. Neurological paraneoplastic syndromes were found in 10% of nonteratomous benign ovarian tumors and in malignant tumors in 37% (P=0.0063). The percentage (5%) of nonteratomous benign ovarian tumor patients with classical NPS was lower (P=0.0392) comparing to malignant ones (37%). Patients with teratomas manifested only non-classical NPS. Benign, nonteratomatous tumors patients with NPS symptoms had anti-neuroendothelium antibodies, and asymptomatic subjects – anti-amphiphysin, anti-Ma, anti-NMDA, anti-myelin and anti-neuroendothelium antibodies. Teratoma patients with clinical signs of NPS had anti-neuroendothelium antibodies, while asymptomatic patients - anti-NMDA antibodies.

Conclusions. Onconeuronal and anti-neuronal antibodies can be found in patients with benign ovarian tumors and clinical symptoms of NPS as well as in asymptomatic subjects.

Neurological diseases (acute and chronic)

0847

FACTOR V LEIDEN, FII G20210A, PA1-1 (-4G/5G), FIBRINOGEN G(-455)A, FXIII VAL34LEU AND ACE I/D POLYMORPHISM IN STROKE: A CASE-CONTROL STUDY

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Background. Stroke is a multifactorial disease with predisposing genetic and traditional risk factors, such as hypertension, smoking, diabetes and male gender. The aim of this study was to determine whether there is a difference in frequency of these traditional risk factors between stroke patients and control group.

Methods. This retrospective study included250 stroke patients and 100 healthy individuals that served as a control group. Five polymorphisms of the genes included in coagulation were determined: Factor V Leiden and FII G20210A using melting curve analysis; PA1-1 (-4G/5G), fibrinogen G(-455)A and FXIII Val34Leu using PCR-RFLP method. Also, angiotensin-converting enzyme I/D polymorphism was determined using electrophoretic separation of PCR amplified products. Data on traditional risk factors (gender, hypertension, smoking and diabetes mellitus) were obtained from patient's medical history.

Results. There were no differences in genotype frequencies of any of the tested polymorphism between study groups (all P > 0.05). However, some traditional risk factors were more frequent in the stroke than in control group: diabetes 26.8% *vs.* 8.1% (P<0.001; OR (95% CI) = 3.6 (1.6-8.0)), hypertension 80.8% *vs.* 59.0% (P<0.001; OR (95% CI) = 2.8 (1.6-4.8)) and male gender 52.8% *vs.* 36.0% (P=0.006; OR (95% CI) = 2.3 (1.4-3.9)). Interestingly, there was no statistically significant difference in smoking status (P=0.938).

Conclusions. Our results didn't reveal any association between tested genetic polymorphisms and stroke, while well established traditional factors were identified as significant risk factors. Probably, a larger cohort would detect smaller gene effects of tested polymorphisms.

0848

VITAMIN B12 IN CLINICALLY STABLE MULTIPLE SCLEROSIS PATIENTS FROM NORTHERN GREECE

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Background. Many studies suggest that vitamin B12 plays role as a co-factor in myelin formation and has important immunomodulatory and neurotrophic effects. The aim of this study was to evaluate serum vitamin B12 levels in patients with multiple sclerosis (MS) from northern Greece.

Methods. The study included 100 MS patients (with relapsing-remitting or secondary-progressive courses) in a phase of clinical inactivity and 70 healthy controls. The patients (68 women / 32 men) median age was 42,5 years and the mean MS duration was 10±1,5 years. The control group had almost the same distribution of gender and age. Serum samples were obtained from patients and control group. Commercially available immunoassay was used for Vitamin B12 calculation (Roche, Modular E170).

Results. Vitamin B12 concentration was lower in MS patients (12/100, 12%) compared to control group (3/70, 4,2%), but the difference between the two groups did not reach statistical significance. The concentration of vitamin B12 was significantly lower (p<0,05) in women with MS (10/68, 14,7%) compared to men (2/32, 6,25%).

Conclusions. We observed no statistical significant difference as regard vitamin B12 between clinical inactive MS patients and healthy controls. These results are conflicted with data from other studies and could be explained by differences in the population studied or by different detected Methods. Further investigation on a larger sample size, for a better understanding of the possible role of vitamin B12 in MS pathology should be performed.

0849

CAPILLARY ELECTROPHORETIC ANALYSIS OF β-TRACE PROTEIN AND β2-TRANSFERRIN τ FRACTION IN CEREBROSPINAL FLUID

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Background. Laboratory analysis of cerebrospinal leakage is based on composition differences between cerebrospinal fluid (CSF) and other body secretions.β-Trace Protein (βTP) and β2-Transferrin τ fraction (β2Tr) features CSF.
βTP is a brain-specific protein and it is the second most abundant protein in CSF.The β2Tr,or asilaotransferrin, is a brain specific variant of transferrin that lacks neuramidic acid.
Capillary electrophoresis was applied to the analysis of this proteins in CSF.
Metods. 10 CSF samples were taken by lumbar puncture from patients with various neurological disordes according to Helsinki declaration.The samples were analyzed in capillary electrophoresis on V8 system manufactured by Helena Labs.βTP and β2Tr were detected by immuno-electrophoresis subtraction (IFE/s) with rabbit anti-human Prostaglandin D Synthase polyclonal antibody,unconjugated (βTP) from Abcam Company and with polyclonal rabbit anti-human Transferrin (β2Tr)from DAKO Company.
Results. The peaks of βTP and β2Tr disappear in IFE/s electropherograms on all CSF samples .
Comclusions. Detection of CSF leakage is important to prevent possible infection of central nervous system.Capillary electophoretic analysis of βTP and β2Tr identify CSF from serum or other body secretions.The reference CSF
pattern provided by the instruments makes the interpretation of the immunosubtraction very easy.

0850

DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE SPECIFIC RECOGNITION OF THE WILD TYPE FORM OF GLUTATHIONE S-TRANSFERASE OMEGA 1

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Background. Glutathione S-transferase omega 1 (GSTO-1) exists as both a wild-type (*wt*) and a mutant (*mut*) enzyme due to a polymorphism in a single amino acid Ala140Asp. The role that this gene product plays in connection with a range of disease conditions has been investigated. An antibody capable of specifically detecting wtGST would be of clinical significance to further elucidate the role that wtGST01 plays in disease states. The aim of this work was to develop a highly specific monoclonal antibody to wtGSTO 1, which will be used as a tool in the development of immunoassays.
Methods. Sheep were immunized with a motif, which housed the single amino acid difference between wtGST and mutGST conjugated to bovine thyroglobulin (BTG) as a carrier. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of generic antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by direct binding ELISA to determine their specificity for wtGST and mutGST.
Results. Cross reactivity studies show <1% cross reactivity with the mutant variant form of GST01.
Conclusions. Data indicate that thismonoclonal antibody is specific for the wtGSTO 1 and creates a new analytical tool for the determination of the wtGST01 form and it is suitable for the development of immunoassays for applications to studies of its role in disease states.

Neurological diseases (acute and chronic)

0851

SOD LEVELSON ERYTHROCYTES FROM PAF PATIENTS SHOW INCREASED OXIDATIVE STRESS

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Background. Familial amyloid polyneuropathy, caused by a mutant transthyretin is particularly frequent in Portugal, where almost all cases are related to the same mutation TTRv30M. Recent studies have revealed that free radical injury are involved either in the amyloid formation process or in post-fibrillar modification in several types of amyloidosis. Superoxide dismutase (SOD) is an important antioxidant enzyme that protects cells exposed to oxygen from superoxide radicals aggression. This study evaluate the oxidative state in patients with familial amyloidotic polyneuropathy.
Patients and Methods
Patients: Forty symptomatic PAF and 45 asymptomatic carriers from outpatient neurological PAF-Unit clinics of the Centro Hospitalar do Porto and 26 healthy controls were recruited.
Methods. Erythrocyte SOD levels were evaluated by the Randox Kit (RANSOD). Statistical analysis was done using SPSS®software.
Results. SOD activity was calculated as the medium of three replicates. The values were as follows: healthy controls (1208±254 U/g Hb), asymptomatic carriers (1436±62 U/g Hb), PAF patients (1455±350 U/g Hb).
SOD activity was significantly increased in the group of PAF, as well as asymptomatic carriers compared with control group (P= 0.003), (P= 0.013), respectively. No significant difference between patients and asymptomatic carriers was observed. A significant correlation (P = 0.013) between SOD and C- reactive protein levels was observed only for symptomatic PAF.
Conclusions. The disturbances in SOD activity apparently indicates that PAF patients are exposed to oxidative stress to a greater degree than controls. This phenomenon can influence the pathology of the disease and it should be studied more accurately.

0852

THE LIPID LEVELS IN PATIENTS WITH ISCHEMIC STROKE AND VASCULAR DEMENTIA

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Background. The study was to determined concentrations of cholesterol, triglycerides HDL and LDL cholesterol in patients' serum after stroke.
Methods. It is 150 subjects, 50 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase (initial 24-48 hospitalization hours), 50 patients diagnosed with vascular dementia and 50 healthy subjects. Lipids were determined using DIMENSION LxR automatic analyser of DADE BEHRING.
Results. Our results show that the concentration of HDL cholesterol was significantly lower in the group with ischemic stroke and vascular dementia than in the control group. Averageconcentrationsof cholesterolandLDLcholesterolweresignificantlyhigherin thegroupwithischemicstrokeandvasculardementiathanin the controlgroup.In the group with ischemic stroke, the concentration of LDL cholesterol was higher than 4.3 mmol / L in about 56% of patients, whereas in the group with vascular dementia, the concentration of LDL cholesterol was higher than the upper reference value in about 48% of patients.The meanconcentration oftriglyceridewassignificantlyhigher inthe stroke group.
Conclusions. Increasing concentrations of LDL and atherogenic index values in the serum of patients with ischemic stroke affects the further development of atherosclerosis and the development of new stroke.

Neurological diseases (acute and chronic)

0853

THE SERUM HOMOCYSTEINE CONCENTRATION AT PATIENTS WITH CEREBROVASCULAR DISEASES

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Background. Homocysteine is an independent risk factor for developing peripheral vascular, cerebrovascular and coronary heart disease.
Methods. The study covered 150 subjects, 50 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase and post-acute phase. The other study group was 50 patients diagnosed with vascular dementia developed as a consequence of ischemic brain stroke and healthy group with 50 subjects. Homocysteine concentration in serum was measured by AXSYM apparatus of ABBOTT Company, on the basis of fluorescent polarisation measuring.
Results. The study demonstrated that hiperhomocysteinemia was present in the patient group with ischemic brain stroke 30% (44 % men and 13 % women), and in the group of patients with vascular dementia 62% (81.4% men and 39.1% women). Homocysteine increases during the postacute phase of ischemic stroke after 7 days for 1.54 mmol / L and 14 days for 3.66 mmol / L compared to the concentration of homocysteine after the first hours of hospitalization. Using the Wilcoxon signed ranks test we got significant difference between homocysteine concentration at acute phase and post-acute phase of ischemic stroke. According to our results using the Mann-Whitney test the concentrations of homocysteine in the acute phase of ischemic stroke showed no significant difference with control group, a significant difference between concentrations of homocysteine in the acute and post-acute phase of ischemic stroke and vascular dementia.
Conclusions. The homocysteine concentration rises significantly during the acute phase of ischemic brain stroke, and it is significantly increased during post-acute phase, which is a precondition for further development of vascular dementia, or a new ischemic brain stroke.

0854

THE CORRELATION BETWEEN HOMOCYSTEINE AND URIC ACID IN THE ACUTE AND CONVALESCENT PHASES AFTER STROKE

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Background. The aim of study was to investigate correlation of homocisteine and uric acid at patients with stroke.
Methods. The study covered 150 subjects, 50 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase (initial 24-48 hospitalization hours), and post-acute phase (after 7±1 days, and after 11-14 hospitalization days). The homocysteine concentration in serum was measured by AXSYM apparatus of ABOTT Company, on the basis of fluorescent polarisation measuring and uricacid, was determined by means of DIMENSION LxR automatic analyser of DADE BEHRING Company. Determinationof uricacidbasedon theenzymaticmethod.
Results. After 24-48 hours we got results of homocysteine and uric acid correlation: correlation coefficient was r = 0,376 and regresion line had a slope 7,2801 and a y axis intercept of 226,78. According to our results, after 7 days coefficient was r = 0,396 and regresion line had a slope 7,0652 and a y axis intercept of 235,99 and after 14 days coefficient was r = 0,313 and regresion line had a slope 5,54 and a y axis intercept of 260,49. The correlation coefficient show good correlation.
Conclusions. The reason for increased concentration of uric acid can be explained by the metabolic connection purine bases adenosine and homocysteine. Therefore is increased concentrations of homocysteine is associated with the increasing concentration of uric acid at patients with ishemic stroke.

Neurological diseases (acute and chronic)

0855

SERUM BIOMARKERS OF ISCHEMIC NEUROLOGICAL COMPLICATIONS BEFORE AND AFTER CARDIAC SURGERY

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Background. Besides neurological examination and a magnetic resonance imaging (MRI) the biomarkers attained attention in the last few years for monitoring neurological complications after cardiac operations.
Methods. We included 20 patients who required surgical revascularization of the heart with the use of the heart-lung machine (cardiopulmonary bypass-CPB). The patients were divided in two groups: group of 10 patients received a potential neuroprotective human recombinant erythropoietin and control group. Neurological complications were monitoring by measuring serum concentrations of N-methil-D-aspartate (NMDA) receptors antibodies (NR2Ab), S100B and NSE before and in the first 5 days after surgery, comparing predictive ability for neurological outcome/deficits with MRI examinations.
Results. The correlation between comparing pairs of pre and postoperative serum concentration of S100B, NSE and NR2Ab of the erythropoietin-treated and untreated control group clearly proved that significant correlation occurred only in the case of NR2Ab and S100B in erythropoietin-treated group. We observed postoperative fresh ischemic lesions in 4 of 10 patients without rHuEpo therapy. Two of them had lesions larger than 5 mm and experienced delirium (cerebrovascular insult). In both patients the preNR2Ab levels were above 6.0 μg/L (3 times reference value of 2.0 μg/L). MRI scans revealed no fresh ischemic brain lesions in patients treated with human recombinant erythropoietin. with in average of about 20% lower pre and postoperative serum concentrations of NR2Ab in comparison with patients of untreated control group.
Conclusions. The analysis of pre&poststroke serum concentrations of glial-neuronal tissue derived proteins might be promising strategy to monitor and evaluate neuroprotective stroke treatment.

0856

β-TRACE PROTEIN AS A MARKER FOR DETECTION CEREBROSPINAL FLUID LEAKAGE

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Background. Head trauma, scull-base surgeries may cause liquorrhea but can also occur spontaneous as a consequence of inflammation or tumors. β-trace protein (βTP) is a brain-specific protein. The logic of using βTP for diagnostic CSF in body fluids is based on its high concentration in CSF.
Methods. Ventricular CSF, lumbal CSF, samples from nasal secretion (NS) together with corresponding serum samples were obtained from patients of the Institute of Neurosurgery. The samples were analyzed according to the manufactures instructions with the Dade-Behring βTP assay (Dade-Behring, Marburg, Germany).
Results. The mean of the βTP concentrations in ventricular CSF was 2.45 mg/L (n=22); in normal lumbal CSF was 17.58 mg/L (n=35); in normal serum was 0.52 mg/L (n=57). In 4 patients with acute bacterial meningitis βTP in CSF were very low, and after administration of the antibiotics they raze to their normal values on 7th day. The clinically confirmed cases of CSF rhinorhea (n=6) showed βTP concentrations in the material collected from nose to be between 0.92–11.9 mg/L.
Conclusions. The study demonstrates that βTP is an accurate marker of CSF leakage. Determination of βTP in both serum and secretion is recommended. The βTP test is a noninvasive, method that can be used as a marker for the detection of CSF in NS.

Neurological diseases (acute and chronic)

0857

PROSTATIC ACID PHOSPHATASE (PAP) IN NEUROTRANSMISSION

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Background. In addition to the secretory PAP we recently described a novel spliced variant of PAP mRNA encoding type 1 transmembrane PAP (TM-PAP). In contrast to general belief, we showed that PAP is not a prostate-specific protein but is widely expressed in non-prostatic tissues. It is neither cancer-associated as stated.
Methods. We have generated PAP$^{-/-}$ mice to study physiological function of PAP.
Results. TM-PAP functions as a 5'-ectonucleotidase that produces adenosine and activates A_1-adenosine receptor. Our recent finding shows that A_1-receptor activation leads to PIP2-depletion. In confocal microscopy and yeast-two-hybrid analyses we have detected interaction between PAP and SNARE-complex. Disruption of the function leads to neuropathic pain. Recently prepulse inhibition test showed these mice to have a deficit in sensomotor gating, which is typical symptom seen in schizophrenia patients. This finding is supported by our results of increased striatal synthesis and extracellular amount of dopamine. TM-PAP is expressed in striatal neurons.
Conclusions. TM-PAP functions in purine nucleotide metabolism and pathophysiological findings of PAP$^{-/-}$ mice are due to disrupted production of adenosine suggesting disrupted neurotransmission.

0858

D-DIMER AS A POTENTIAL BIOMARKER FOR CNS MALIGNANT INFILTRATION

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Background. Several markers have been studied for their ability to make the central nervous system (CNS) infiltration diagnosis earlier and more precise. We investigated the measurement of D-dimer in cerebrospinal fluid (CSF) and evaluated them as a potential biomarker of neoplastic infiltration within the CNS.
Methods. The study was performed on 87 CSF samples divided into three groups: patients with carcinoma (n=10) and different lymphoid malignancies (n=43) with clinical or biological evidence of CNS involvement, and controls (unsolved headache) (n=34). D-dimers were measured by VIDAS automated immunoassay system. Cell counts were performed by optical microscopy (Fuchs-Rosenthal chamber), and glucose, lactate and total proteins were determined by standard tests on Beckmann automated analyzer.
Results. Highly increased levels of D-dimers (mean=3.80 mg/L) were demonstrated in all CSF samples from patients with carcinoma, but in group of patients with lymphoid malignancies increased levels of D-dimer were found only in 32% CSF samples, and the values were lower (mean=0.52 mg/L). In all control CSF samples, D-dimer values were <0.05 mg/L.
Conclusions. Although this test is not specific for neoplastic affections, our preliminary data suggest that the measurement of D-dimers in CSF may be useful in the diagnosis of CNS involvement of neoplastic cells, especially in patient with carcinoma, and also in monitoring intrathecal therapy.

Neurological diseases (acute and chronic)

0859

6-BIOMARKER ALGORITHMS IDENTIFY ALZHEIMER'S DISEASE AT HIGH ACCURACY

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Background. Late-onset Alzheimer's disease (LOAD) is a multifactorial disease. It is characterised by neurofibrillary tangles and amyloid plaques and can only be accurately diagnosed post-mortem. The ε4-allele of apolipoprotein E is a confirmed but insufficient risk factor.

Methods. To find new Alzheimer's disease biomarkers, platelets, which are peripheral model cells of neurons, were investigated. 42 Alzheimer's disease patients and 43 age- and sex-matched individuals were analysed by 2-dimensional differential gel electrophoresis and statistically evaluated. Algorithms of the most significant LOAD-biomarkers were generated using logistic regression and were assessed by Receiver operating characteristic (ROC) curves and Area under the curve (AUC).

Results. Several algorithms that contain five or six of the LOAD biomarkers apolipoprotein E4 (ApoE4), monoamine oxidase B, coagulation factor XIIIa, glutathione S-transferase omega wild type (wtGSTO-1) and mutant (mutGSTO-1), and tropomyosin showed AUC above 0.9 but differed in the weighting of these biomarkers. Best AUC were obtained when our finding that wtGSTO-1 is prominent in nonApoE4 LOAD patients was taken into consideration: Algorithm 1: AUC=0.938 (95% confidence interval 0.8839-0.9923) and Algorithm 2: AUC = 0.952 (95% confidence interval 0.8799-1.0000)

Conclusions. A combination of only six LOAD biomarkers is sufficient to differentiate between LOAD patients and controls at a considerably high accuracy.

Nutrition

0860

VITAMIN B12 AND HOLOTRANSCOBALAMIN LEVELS IN PATIENTS WITH HELICOBACTER PYLORI INFECTION

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Background. *Helicobacter pylori* is one of the most common causes of peptic ulcer disease worldwide and a major cause of chronic superficialgastritis leading to atrophy of gastric glands. It is suggested that there may be a casual relationship between *H pylori* and food-cobalamin malabsorption. Recently, new markers for vitamin B12 deficiency, holotranscobalamin, have been introduced. Because only B12 attached to transcobalamin (holotranscobalamin) is able to enter all the cells of the body, holo transcobalamin may be a more useful marker than total B12 in serum.
Patients and Methods. All patients undergoing upper GI Endoscopy were selected, while patients of active peptic ulcers, malignancy, varices, malabsorption and recent blood donation were excluded. Blood samples for vitamin B12 and holotranscobalamin were collected and H.pylori status was ascertained by histopathological determination on gastric biopsy.
Results. *Helicobacter pylori* and associated gastric histological changes were detected in 77 (66.9 %) of 115 patients. Significantly low levels of vitamin B12 and holotranscobalamin (p <0.001) were found in patients with H. pylori infection. In receiver operating characteristic curves for detection of definite vitamin B12 deficiency, holoTC had a slightly greater area under the curve (AUC) compared with vitamin B12 in all participants (AUC=0.886 vs 0.864; *p* =0.638). There is significant correlation between vitamin B12 and holotranscobolamin values is (r= 0.536, p<0.001).
Conclusions. The findings provide strong evidence that *H. pylori* infection is associated with cobalamin deficiency,HoloTC and total vitamin B12 have equal diagnostic accuracy in screening for metabolic vitamin B12 deficiency in patients with H. pylori infection.

0861

ADIPOSITY EFFECTS OF CENTRALLY ADMINISTERED GHRELIN IN AGED RATS

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Background. Ghrelin is known to have a significant influence on adipose tissue content, energy balance and is an important factor in pathophysiology of obesity. The aim of our study was to determine the effects of intracerebroventricularly (ICV) administered ghrelin on white adipose tissue content (WAT) and lipid metabolism in WAT.
Methods. Five injections of rat acylated ghrelin or PBS (n=10 per group) were administered once per day (0.15 nmol of ghrelin in 5 µL of PBS), into lateral cerebral ventricle (ICV) of free feeding aged Wistar male rats. Body weight (BW) and food intake (FI) were measured daily, while ending WAT content as well as blood samples for glucose (Glu), triglycerides (TG), cholesterol (CH), HDL, LDL and free fatty acids (FFA) were obtained.
Results. Centrally applied ghrelin significantly increased FI (p<0.05), BW (p< 0.05), WAT (p<0.05) content by 10.6%, 66.8% and 95.2% respectively, compared to controls.The blood concentration of TG, CH, HDL, LDL and FFA significantly increased (p<0.05) by 107.4%, 37.5%, 27.6%, 11.8% and 43.3% while Glu level significantly decreased (p<0.05) by 12.8% compared to untreated rats.
Conclusions. The results clearly demonstrate that daily subnanomolar doses of ICV ghrelin during five consecutive days significantly increased: FI, BW and WAT content. Increased TG, CH, HDL, LDL, FFA concentrations and decreased glucose concentration in blood indicate intensive anabolic processes in white adipose tissue.Central ghrelin strongly stimulates lipid metabolism in WAT.

Nutrition

0862

URINARY CD71, A POTENTIAL MARKER FOR IGA NEPHROPATHY

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Background. IgA nephropathy (IgA-N) and Henoch-Schönlein Purpura nephritis are among the most common causes of primary glomerulonephritis in Asians and Caucasians. Recently, a IgA1 receptor, expressed on mesangial cells was characterized. This receptor, CD71 or transferrin receptor (TfR) is upregulated in mesangial cells of patients with IgA-N. To assess the diagnostic value of CD 71, this study investigated the possibility to measure CD71 in urine.
Methods. 78 samples were analyzed (30 controls, 26 patients with IgA-N, 22 patients with other glomerulopathies, including idiopathic glomerulonephritis, diabetic nephropathy, membranoproliferative glomerulonephritis, ANCA-associated vasculitis, systemic lupus erythematosus and focal segmental glomerulosclerosis). Samples were concentrated 10-fold using a Vivapore® 5 (Sartorius) concentrator (cut-off: 7.5 kDa) and consequently nephelometrically assayed for IgG and CD71 by a modified CD71 method developed on BNII (Siemens). An analytical sensitivity of 0.1 μg/L was calculated.
Results. For the control group, reference limit was 0.4 μg/L. Low to moderate levels of CD71 were detected in IgA-N and other glomerulopathies (range 0.0-20.0 μg/L). A significant difference was found between the values of the controls and the IgA-N group ($p<0.001$). No significant correlation was found between urinary and iron status (serum TfR concentrations, serum ferritin concentration). Severe proteinuria is a confounder, as soluble TfR is a 85 kDa plasma glycoprotein. To correct for proteinuria, the ratio CD71/IgG was calculated.
Conclusions. This study shows that CD71 can be assayed in urine. CD71 may be regarded as a promising urinary marker for IgA nephropathy.

0863

EFFECT OF NARINGENIN ON LIPOPROTEIN LIPASE EXPRESSION

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Background. Lipoprotein lipase (LPL) plays an important role in the metabolism of plasma lipid. It catalyzes the hydrolysis of triglycerides from very low-density lipoprotein and chylomicrons into glycerol, diacylglycerol and free fatty acid and the free fatty acids are supplied to tissues as sources of metabolic energy or stored as triglycerides after re-esterification. Defects of LPL can cause hypertriglyceridemia, and are also related to many diseases including obesity, coronary heart disease, pancreatitis and atherosclerosis. Naringenin is a flavonoid aglycone and abundant in grapefruits and could reduce triglyceride production in primary rat hepatocytes. The aim of this study is to explore if hypolipidemic effect of naringenin is through the enhancing LPL expression.
Methods. Human embryonal kidney 293 cells were cultured in DMEM/HAM'S F12 with 10% fetal calf serum. Then these cells were treated with 0, 100 and 200μM of naringenin, respectively. Medium and cell lysates were harvested after 48 hours incubation. Their LPL activities were determined with hydrolysis of VLDL, and the liberated free fatty acids were measured by NEFA kit.
Results. A significant endogenous LPL expression was observed with 200μM of naringenin. The increments were 1.6-fold ($p=0.017$) and 1.7-fold ($p=0.025$) in cell lysates and media, respectively.
Conclusions. These results indicated that hypolipidemic effect of this natural compound could be by enhancing LPL expression.

Nutrition

0864

COMPARISON OF THE FORMATION OF DEFINITE METABOLIC SYNDROME BY HIGH FRUCTOSE FEEDING BETWEEN 6-WEEK- AND 14-WEEK-OLD SPONTANEOUS HYPERTENSION RATS

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Background. Spontaneous hypertension rats(SHR) showing not only hypertension and insulin resistance but also hypertrophy of fat cells are used as a model animal of the metabolic syndrome (MS). It is known that when young SHR receive high fructose diet (HFD)feeding for two weeks, in addition to progressed hypertension and insulin resistance, disorder of lipid metabolism occurs, whichleads to definite MS formation.The blood pressure of SHR begins to increase around 5 weeks of age and the increase almost reachesa plateau around 15 weeks of age. Therefore, we compared definite MS formation by HFD feeding between6-week- and 14-week-old SHR.
Methods. Male 6-week-old SHR (6WSHR) and 14-week-old SHR (14WSHR) were fed HFD containing 60% fructose for four weeks. Systolic blood pressure was measuredone day before sacrifice. Serum separated from blood collected under fasting was used for the measurementsof glucose, insulin, total cholesterol, triglyceride, free fatty acids, adiponectin, TNF-alpha, lipid peroxide, and uric acid.
Results. Four weeks of HFD feeding increased serum insulin, total cholesterol and free fatty acids levels and HOMA-R valuein 6WSHR and 14WSHR. The HFD feeding increased serum triglyceride, lipid peroxide, uric acid,and systolic blood pressure levelsin 6WSHR but did not affect those levels in 14WSHR. The HFD feeding reduced serum adiponectin level in 6WSHR and 14WSHR but did not affect serum TNF-alpha and glucose levelsin 6WSHR and 14WSHR.
Conclusions. These results suggest that definite MS is formedby HFD feeding in 6WSHR more easily than in 14WSHR.

0865

THE EFFECTS OF INFLAMMATION ON THE SERUM CONCENTRATION OF ESSENTIAL TRACE METALS

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Nutrition

0866

EFFECT OF JERUSALEM ARTICHOKE CONSUMPTION ON BLOOD BIOCHEMICAL PARAMETERS

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Background. The Jerusalem artichoke (*Helianthus tuberosus*) is a species of sunflower native to the eastern United States. It is also cultivated widely across the temperate zone for its tuber, which is used as a root vegetable. Artichoke extract has been shown to improve digestion, liver function and help lower high LDL cholesterol levels and prevent atherosclerosis and heart disease. We investigate the effect of consumption of fresh Jerusalem artichoke that cultivated in the area of the northeast of Thailand on the blood biochemical parameters.
Methods. The three groups of subject including healthy, overweight and those with dyslipidemia were recruited. The written consent forms were given. Blood glucose, parameters for renal and liver function as well as lipid profile were assessed and compared between two time points; before and after 4 weeks of consumption.
Results. Significant decrease in blood level of LDL cholesterol and increase in HDL cholesterol were observed in dyslipidemic group. There were slightly changes in blood level of both LDL cholesterol and HDL cholesterol in the overweight group. Other blood parameters were not different.
Conclusions. Consumption of fresh Jerusalem artichoke provides a beneficial effect for health by improving blood lipid level, especially in dyslipidemic subjects.

0867

EFFECT OF FRUCTOSE ON SERUM TRIGLYCERIDESIN NORMAL, HYPERTRIGLYCERIDEMIC, AND HYPERINSULINEMICS SUBJECTS: A META-ANALYSIS OF CONTROLLED FEEDING TRIALS

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Background. Health agencies have expressed concern that fructose may contribute to hypertriglyceridemia. In a previous meta-analysis of controlled feeding trials, we showed that isocaloric fructose raised triglycerides only under specific conditions (starch-comparator, dose>60g/d, follow-up ≤4wk) in type 2 diabetes. Whether these conditional effects hold true in other cohorts is unclear. We report a meta-analysis of the effect of fructose on triglycerides in non-diabetic subjects.
Methods. We searched MEDLINE, EMBASE, CINAHL, and the Cochrane Library for relevant trials ≥7d. Data were pooled by the generic inverse variance method and expressed as standardized mean differences (SMD) with 95% CI. Heterogeneity was assessed (c^2) and quantified (I^2).
Results. Twenty-six isocaloric (n=302) and 5 hypercaloric (n=43) trials met the eligibility criteria. Primary analyses in the isocaloric trials showed that fructose had a small triglyceride-raising effect (SMD=0.16 [0.06, 0.27], P=0.002) without significant heterogeneity in normal subjects, while it had no effect with significant heterogeneity (I^2>65%, P<0.05) in hypertriglyceridemic and hyperinsulinemic subjects. In the hypercaloric (+18-35%E) feeding trials, fructose had a large triglyceride-raising effect (SMD=0.90 [95% CI: 0.22,1.58], P=0.01) with significant heterogeneity (I^2=86%, P<0.001). A priori subgroup analyses explained the heterogeneity in the isocaloric trials showing a triglyceride-raising effect (P<0.05) of fructose under specific conditions in the hypertriglyceridemic (starch-comparator, high study quality) and hyperinsulinemic (starch-comparator, dose>60g/d) subjects. Subgroup analyses failed to explain the heterogeneity in the hypercaloric trials.
Conclusions. Isocaloric and hypercaloric fructose show conditional triglyceride-raising effects in normal, hypertriglyceridemic, and hyperinsulinemics subjects. Confounding from excess energy cannot be ruled out in the hypercaloric trials.

Nutrition

0868

XENIN IN OBESE CHILDREN AND CHILDREN WITH IBD

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Background. Xenin is a peptide hormone produced by Kcells in the duodenum. The same cells secrete GIP, and xenin potentates GIP`s incretin action. In humans, xenin is released into circulation after a meal. In animals xenin`s infusion increases satiety.Little is known about the xenin secretion and action in humans. Therefore the aim of the study was to determined xenin concentration in several groups of patients.
Methods. Xenin was studied in obese children, in children with exacerbation of inflammatory bowel disease (IBD) and in age matched healthy children (control group). In all children xenin was measured in fasting blood plasma (RIA, Phoenix Pharm. Inc), in obese group also 60 and 120 minutes after the meal stimulation.
Results. The mean plasma concentration of xenin in healthy children was 35,2 ± 11,7 mcg/ml. There was no statistical difference between values observed in IBD children in acute stage of the disease (44,3 ± 12,3 mcg/ml). The plasma concentrations of xenin in those both group were significantly higher than that observed in obese children (fasting: 1,8 ± 2,7 mcg/ml; p<0,001). There were no differences in fasting and postprandial plasma xenin levels.
Conclusions. The disturbances in distal gut do not affect plasma xenin levels, and probably do not influence the secretion of this peptide from proximal gut. The significantly lower fasting plasma xenin levels in obese children than in lean controls and no influence of meal on postprandial xenin concentrations suggests the role of this satiety peptide in the obesity development.

0869

OBTAINING OF AN ANTIOXIDANT CONCENTRATE FROM *CYNARA SCOLYMUS* FOR THE ATTENUATION OF LIVER DISEASES

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Background. Since several hundred years ago, Cynara scolymus has been used in traditional medicine in order to treat gallbladder and liver diseases.
Methods. The aim of this research was to obtain a vegetal product from Cynara scolymususing an extractor in fluidized bed, with ethanol as solvent. Different concentrations of solvent were used during the extraction: 25%, 50%, 70% and 97% ethanol. The extracts obtained were concentrated so as to eliminate the solvent, in a Buchi rotavapor. The concentrated solutions were lyophilized in a freeze-dryer Alpha 1-2D. For both phases of the product (liquid and solid) the total antioxidant activity was determined using the indirect spectrophotometric method that used 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a generating system for the stable free radical DPPH•. The total phenolic content was also determined using the reagent Folin – Ciocalteau, as well as the reduction capacity and linking capacity of the free radicals.
Results. The fluidized bed extraction in the extractor, using 70% ethyl alcohol as solvent, represented the optimal variant. It determined the obtaining of an effective extract from the point of view of the antioxidant effect. In this case, the largest quantity of phenols and flavonoids and the highest reduction power were obtained. The antioxidant activity was high, ranging between 80 - 85%. The maximum antioxidant effect in case of using freeze-dried powder of 0,5 mg/ml was of 90%, which is a high value. Very good results were obtained for the other chemical species which were determined, resulting that this is the optimal concentration which can be used to obtain functional antioxidant products.
Conclusions. The quality of the final product significantly improved by using the extractor, as well as by means of lyophilization, that did not determine the loss of antioxidant properties.

Paediatric clinical chemistry

0870

HIGH SENSITIVITY CRPH SYNCHRON® SYSTEMS ASSAY (BECKMAN COULTER) AND PNEUMATIC TRANSPORT: A RELIABLE AND ECONOMICAL ALTERNATIVE TO CRP POINT-OF-CARE METHOD IN PEDIATRIC POPULATION

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Background. C-reactive protein (CRP) is found valuable in the differentiation between bacterial and viral infections, but venous blood sampling causes many problems in children. Point-of-care testing (POCT) methods were developed for CRP measurement in capillary blood, but require new equipments, clinical staff training, are time consuming and costly. Utilising our hospital pneumatic transport system, we have developed a reliable alternative procedure.
Materials. Glass capillaries (20μL) with plug and plunger; microtubes: Minicollect® Closure Cap with Cross Cuts (GreinerBio-One) containing 100μL of haemolysing solution: Triton-X100 (0.1%) and anti-foam B (0.1%) in PBS (Sigma-Aldrich®).
Procedure. After pricking and filling the glass capillary, the blood is applied in microtube by pushing through the cap (useless wiping). After shaking and being carried by the pneumatic system in laboratory, it is directly analysed with ultrasensitive CRPH Synchron® method (Beckman Coulter).
Methods. Whole Blood from hospitalised patients were analyzed by our procedure according to the VALTEC protocol validation.
Results. Intra-assay CV's were 5.9% and 5.2% and Inter-assay CV's 6.6% and 6.0% at 9.2g/L and 87.9g/L respectively, with detection limit <2 mg/L and linearity >400mg/L; no hook-effect, no interference with bilirubin nor turbidity and excellent correlation (Passing-Bablock: y=0.99x-0.38; r=0.99; n= 50) with QuicKRead® CRP method (FumouzeDiagnostics).
Conclusions. Its good performances thus allow us to use this simple, reliable and economical procedure, useful for all the clinical services. The results can be consulted within 30mn, time-limit a little longer than near-patient tests, but compatible with clinical investigations and the main goal is to reduce the venous blood samplings.

0871

HEALTHY PEDIATRIC REFERENCE VALUES OF SERUM OSTEOCALCIN AND B-CROSSLAPS LEVELS

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Background. Reference values forβ-crosslaps (β -CTx) and osteocalcin (OC) levels are highly dependent methods used for their determination. In addition, they are largely different in children compared to those in the adult. The aim of our study was to establish age-depentent healthy pediatric reference ranges for commercially available Roche kits.
Methods. We measured serum OC and β-CTx levels in 453 healthy subjects (207 males and 266 females, aged of 4-29 years) using Roche immunochemistry on a Hitachi Modular E170 ECLIA automatic system. Specific reference values were established according for age- and gender-specific age cohorts.
Results. OC and β-CTx levels peaked in early puberty and adolescence. For girls between 8 and 13 years of age cohorts median OC and βCTx values ranged between 105 and 120 ng/ml and 1150 and 1400 pg/ml, respectively. For boys between 10 and 15 years of age cohorts median OC and βCTx values ranged between 113 and 132 ng/ml and 1350 and 1500 pg/ml, respectively. In late adolescence and young adulthood, a delayed decline of OC and β-CTx levels was observed in girls compared to boys; however, median adult OC and β-CTx levels reference values were reached earlier in girls than in boys (by 18 vs 22 years, respectively).
Conclusions. These results reinforce earlier findings that specific reference values for OC and β-CTx are required for children and adolescents. Pediatric OC and β-CTx age-specific reference ranges specific for test used should be considered when bone-turnover parameters are evaluated in children.

Paediatric clinical chemistry

0872

USEFULNESS OF C-REACTIVE PROTEIN IN ADMISSION TO LOCALIZE SITE OF URINARY INFECTION IN CHILDREN

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Background. Urinary tract infections (UTI) are among the most prevalent infections in the pediatric population and its location has serious implications both in therapy and prognosis, because acute pyelonephritis (APN) may cause irreversible renal scarring. Technecium-99m dimercaptosuccinic acid (DMSA) scintigraphy is the gold standard method for diagnosis of APN. The purpose of this study was to investigate the value of C-reactive protein (CRP) to predict the presence of abnormal DMSA scintigraphic findings in children with first episode of UTI.
Methods. 40 patients (23 females and 17 males, aged from 1 to 55,4 months (median age: 5,04 (7,2) months) with first UTI and suspicion of PNA were evaluated. In all patients, CRP was measured in admission, a urine sample was collected for culture and renal ultrasonography and scintigraphy were performed within the first seven days after admisión.
Results. 17 children (40.2%) had scintigraphic alterations compatible with APN. These children, in admission, had higher CRP that children with lower UTI (160 mg/L vs 39,3 mg/L (p=0,006).However, no single cutoff was appropriate to rule-out and predict simultaneously renal parenchymal involvement. For a level of CRP < 25 mg/L, negative predictive value to exclude the renal involvement was 92% and for a level of CRP ³ 175 mg/L, positive predictive value to predict the renal involvement was 90%.
Conclusions. If new infection markers as procalcitonin is not available, CRP may be a useful tool to exclude and predict renal parenchymal involvement, using a strategy with two cutoffs.

0873

EVALUATION OF BILIRUBIN ADSORBENT FOR THE IN-VITROHEMOPERFUSION IN SEVERE NEONATAL JAUNDICE

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Background. To determind the best adsorbent for in-vitro hemoperfusion therapy of neonatal pathological jaundice, fourcandidates including NKA-9 resin, NK110 resin, immuno-affinity adsorbant and chitosan were evaluated according totwo criteria: bilirubin adsorptive efficiency, influence on other plasma components
Methods. Plasma samples that obtained from 2 to 4days new born with bilirubin range (TB 226.49±73.60 umol/L and DB6.84±3.41umol/L) were chosen to apply hemoperfusion. Adsorptive efficiency (AE) and adsorptive capability (AC) were compared among the four adsorbent materials. Other plasma components and plasma index were detected in a time lapse manner (0, 10, 20, 30, 40, and 60 min) during hemoperfusion therapy.
Results. NKA-9 resin that owns the highest AE (TB 82.2%±6.1% and DB 38.8%±23.8%) and largest AC (TB 0.32±0.05umol/g and DB 0.024±0.01umol/g) is chosen as the adsorbent in hemoperfusion therapy. The decrease percentage of TB and DB are 43% and 24.7% during the first 10min hemoperfusion. However, the declined velocity of plasma TB (20min 16% and 60min 5%) and DB (20min 16% and 60min13%) slows downgradually. For other plasma components, TP, ALB, ALP, CK, nCa, HCO3-, FIB, IgM, and IgG decrease statistically(P<0.05),. Hemoperfusion treated ALT, AST, GGT, LDH, BUN, UA, K+, IgE, PLT, C3, Glucose and TC have no significant changes(P>0.05). whereas PT, APTT and TT have been on the rise
Conclusions. NKA-9 resin absorbs bilirubin efficiently without significant changes to most other components in plasma.
Therefore, NKA-9 is an ideal adsorbent in hemoperfusion therapy for neonatal pathological jaundice.

Paediatric clinical chemistry

0874

DIAGNOSTIC VALUE OF URINE NGAL IN ACUTE PYELONEFRITIS OF CHILDREN

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Background. Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein expressed in renal tubules where it is considerably induced in ischemic or nephrotoxic injury. The aim of this study was to assess diagnostic value of urine NGAL as a biomarker of acute ascending urinary tract infection in children.
Methods. We analyzed 52 children which were admitted to the Pediatric Clinic. 33 of them had acute pyelonephritis, while 19 who represented controls had cystitis or fever of other etiology. Besides routine laboratory analysis (CBC, CRP and urine analysis), urine culture, kidney ultrasound and static scintigraphy were also done. Urine NGAL was measured using CMIA method (ARHITECT i1000, ABBOTT). We compared NGAL concentrations in children with acute pyelonphritis towards those from control group. Statistical calculation was performed using Mann-Whitney Rank Sum Test.
Results. NGAL concentrations were significantly elevated in children with acute pyelonephritis in comparison with controls (median=119,3 ng/ml vs 10,2 ng/ml, p<0,001).
Conclusions. Since urine NGAL is fast test, easy to perform, and require noninvasive sample, it could be useful biomarker of acute pyelonephritis in children.

0875

TRACE ELEMENTS IN BRAEST MILK FAT

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Background. Pediatrics is an area where the importance of trace elements is difficult to overestimate. Their dosage is needed for controlling the supplementation of bioelements as part of clinical nutrition programs and for adequate correction of mineral metabolism. However, breast milk, especially colostrum, contains fats (triglycerides and *trans*-lipids), which may eventually interfere with trace elements determinations. Depending on the concentration they can block the capillar during spectrofotometric determinations making the results unstable. On the other hand, mechanical separation of the fats can lead to unexpected removal of trace elements from the sample. The aim of this study is to analyze fat bioactive cations in breast milk.
Method. Five postpartum healthy mothers volunteered to provide human milk samples for this study. The specimens (2 g) were digested with 65% nitric acid for 20-50 min, until a totally transparent yellowish solution was obtained. Upon cooling, a small globule of undigested fat was formed on its surface. This fat was carefully removed using a platinum loop, then absorbed on a paper filter and tested by X-ray dispersive analysis (EDAX) using a Princeton Gamma Tech PGT instrument provided with SiLi monochromator. The experiments were carried out in triplicate.
Results. The EDAX spectrum was dominated by *Ka* lines of Na, K and Ca. Meanwhile zinc, copper, manganese and other bioelements were not detected even in minimal quantities.
Conclusions. There is no danger of underestimating trace elements concentrations when solidified milk fat is mechanically separated from the mixture to be analyzed.

Paediatric clinical chemistry

0876

CERULOPLASMIN DOSAGE DURING CARDIAC SURGERY IN CHILDREN

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Background. Kinetics of copper-containing enzymes may be considered as an additional tool for the monitoring of free radicals dynamics during cardiac surgery, especially in children. However, data interpretation is not easy, since blood volume changes significantly when cardiopulmonary bypass is a part of the circulation system. In this case, the ceruloplasmin levels should be obligatory referred to the hemodilution arising from the additional volume included in the circuit.
Methods. The serum ceruloplasmin levels were measured by a nephelometric technique. In order to take the hemodilution into account, the calculations were carried out by using a relationship linking the patient initial and end volemia with the initial and end hematocrit. This study enrolled 21 children, with congenital heart diseases, which underwent cardiac surgery with cardiopulmonary bypass, at Santa Casa Hospital, Campo Grande, Brazil.
Results. It was shown that there is a strong correlation observed between mean values of serum concentrations of free copper and ceruloplasmin before and during key moments of cardiac surgery, which biochemically mark the process of the procedure ($p<0.001$, $r \geq 0.87$). Average serum ceruloplasmin concentrations expected for hemodilution in relation to the cardiopulmonary bypass were shown to be good markers. At the end of cardiopulmonary bypass usage, the influence of hemodilution tends to be less pronounced as long as total plasmatic volume returns to normal.
Conclusions. Corrections for hemodilution applied during the calculation of serum ceruloplasmin levels allow to describe its dynamics adequately, and can be recommended in all procedures when cardiopulmonary bypass is involved.

0877

FATTY ACID COMPOSITION OF HUMAN MILK AT 3,7, 28. DAYS AFTER TERM AND PRETERM DELIVERY IN TURKISH WOMEN

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Background. Lipids are the main source of calories in the human milk. Fatty acids are considered important in infant development.
Methods. Human milk samples were obtained after term (n=15) and preterm (n=15) delivery at days 3, 7, 28. In our study, the fatty acid composition of human breast milk was determined longitudinally by high resolution gas liquid chromatography.
Results. Some fatty acids[sature fatty acid C14:0 and C16:0], [monosature fatty acid C18:1] and [poly-unsature fatty acid C20:5] significantly increased in preterm group than term group (respectively, p=0.041, p=0.046, p=0.027, p=0.033) whereas myristoleic acid (C14:1) and eicosanoic acid (C20:0) significantly decreased in preterm group (respectively, p=0.015, p=0.048).
Conclusions. Term and preterm milk have got different concentration of fatty acids. As a general conclusion, breast milk provides the lipid requirements of infants.

Paediatric clinical chemistry

0878

ACTIVITY OF G-6-PD IN INFANTS WITH HYPERBILIRUBINEMIA OF UNDETERMINED ETIOLOGY IN THE REPUBLIC OF MACEDONIA

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Background. Approximately 200 million people worldwide are estimated to suffer from G-6-PD deficiency. In Macedonia the incidence of G-6-PD deficiency was estimated 6% among Romains male children, and 1% among Macedonians. Infants with G6PD deficiency suffer from hyperbilirubinemia significantly more frequently than controls. The screening method for G-6-PD deficiency used at our Clinic showed low level of detection. Aim of study was to introduce a new, more sensitive quantitative method for G-6-PD detection and evaluate the level of G-6-PD in a group of infants with indirect hyperbilirubinemia of undetermined etiology.
Methods. A group of 30 infants that were hospitalized at the University Pediatric Clinicneonatal department was prospectively evaluated. The study involved a questionnaire, clinical examination of subjects and laboratory analyses. Inclusion criteria were: term newborns, excluded other determined causes of jaundice, serum bilirubin level ≥257 μmol/l (15 mg/dL)and/or prolonged jaundice after one month. A quantitative spectrophotometric method was used for G-6-PD detection.
Results. The study group included 17 (56.7%) boys and 13 (43.3%) girls with median (interquartile range) age of 30 (19-42.5) days. The mean +-SD of G-6-PD was 217.69 (+-53.29) mU/10^9 Er and the median (interquartile range) serum levels of G-6-PD were 0.125 (0.05-0.35) mU/ml in the studied group.
Conclusions. These are preliminary results of G-6-PD testing in Macedonian infants with jaundice. A larger group has to be evaluated in order to produce reference values for G-6-PD in neonates and infants from the Republic of Macedonia and to estimate the incidence rate of this enzyme deficiency.

0879

BIOCHEMICAL BIOMARKERS OF BONE CELL ACTIVITY IN CHILDREN AND ADOLESCENTS WITH UNTREATED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In children with diagnosed untreated acute lymphoblastic leukemia (ALL) disturbances in mineral homeostasis and bone mass were reported. The purpose of this study was to evaluate bone metabolism in children and adolescents with newly diagnosed acute lymphoblastic leukemia by assessing biomarkers of bone cell activity.
Methods. 24 Caucasian children and adolescents (14 boys 4-21 years, 10 girls 4-16 years) with newly diagnosed, untreated ALL and twenty four age, gender- and pubertal stage-matched controls were included. Propeptide of type 1 procollagen (P1NP), osteocalcin (OC), C-terminal telopeptide of type 1 collagen (CTX) and tartrate resistant acid phosphatase 5b (TRAP 5b) were assayed.
Results. Bone formation, in particular, (Me P1NP 51,9 vs 433,4 µg/L and OC 16,1 vs 80,5 µg/L; p<0.0001) and bone resorption (Me CTX 0,454 vs 1,225 µg/L and TRAP 5b 2,8 vs 5,6 U/L; p<0,001) were significantly reduced in ALL children and adolescents compared to controls. P1NP positively correlated with OC (r=0,56 p=0,01) and CTX correlated with TRAP 5b (r=0,54 p=0,02) in children and adolescents with ALL. Median P1NP and OC concentrations in ALL children (4-9 years) were dramatically reduced compared with healthy ones (10-fold and 9-fold, respectively) whereas in adolescents with ALL (10-21 years) both bone formation markers were reduced in a lesser degree in comparison to healthy adolescents.
Conclusions. Acute lymphoblastic leukemia influences bone metabolism which is related to age of onset. There are more significant disturbances in bone turnover, particularly in bone formation, in children with untreated ALL in comparison to ALL adolescents.

Paediatric clinical chemistry

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GLUTATION PEROXYDASE (GPX) IN PREMATURE NEWBORN INFANTS

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Background. Premature newborn infants are at risk of „oxygen radical disease". This could be due to their exposure to supplements and/or to decrease in their antioxidant defence systems. A number of complication in preterm newborn infants may be caused by increased oxidative stress.
An increasing of data are accumulating, demonstarting the role of oxidative in these conditions, especially in chronic lung disease. Important intracellular defence system are the antioxidant enzymes, superoxide dismutase, glutation peroxydase and catalase.
To test any statistical significant changes, we measured glutation peroxidase (Gpx) serum concentrations in 21 premature newborn infants (11 / 52,4 % male and 10 / 47,6 % female) during first week of life (1th and 4th day).
Methods. Twenty one low birth weight premature newborn infants receiving standard medical treatment at the Gynecologycal and obsternical vlinic "Narodni front", Belgrade, Serbia, were prospectively studied from March to June 2006. At 1th and 4th day of age, blood was taken by venipuncture. Erythrocites were separated and washed three times in isotonic saline to remove the uffy coat. Weight (between 1680 and 2500g), gestational age, average daily fractional oxygen exposure (FiO_2), total bilirubin and dietary intakes were obtained from subjects medical records.
Results. N=21; 1th day mean = 49,89 +/- 9,68; male N=11 mean = 50,52 +/- 11,79; female N=10 mean 49,2 +/- 7,26; 5th day N=21 mean = 49,9 +/- 7,97; male N=11 mean = 47,35 +/- 5,61; female N=10 mean = 52,7 +/- 9,46.
Conclusions. Examination of glutation peroxydase can be useful parameter for oxidative stress in premature newborn infants status.

0881

HYPERTENSION RISC IN PREAGNENT WOMEN

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Background. Hypertethnsive disorders complicating pregnancy are common and form one of the deadly triad, along with hemorage and infection that contribute greatly to maternal morbidity and mortality.
The term gestational hypertension is used now to describe any form of new – onset pregnancy – related hypertension.
It is clear that wenn blood pressure begins to rise, both mother and fetus are at increased risk. Proteinuria is a signe of worsening hypertensive disease, specifically preeclampsia.
Overt and persistent preeclampsia, overt and persistent proteinuria further increases maternal and fethal risk.
The minimum criteria for the diagnosis of preeclampsia are hypertension plus minimal proteinuria. The combination of proteinuria and hypertension during pregnancy markedly increases the risk of perinatal morbidity and mortality.
Material and Methods. Thity-five pregnant women with gestational hypertension(GH) in 3 groups. I group: second (24+/-3 gestational weeks), II group: third trimester (36+/- gestational weeks), and III group; one month (30+/-10 days) after delivery. To test any statistical significant changes, we measured creatinine in serum and urin, and creatinine clearance in 33 pregnant women.
Unpaired t-test was used to test the difference between the groups. A probability value of p<0,05 was considered significant.
Results. N = 33;
Creatinine (s): I group mean 65,27 +/- 6,96 mmol/L; II group mean = 65,24 +/- 6,71 mmol/L; III group mean 77,61 +/- 10,61 mmol/L.
Creatinine (u): I group mean 5,59 +/- 2,58 mmol/L: II group mean 5,22 +/- 2,68 mmol/L; III group mean 7,23 +/- 2,83 mmol/L.
Creatinine clearance: I group mean 1,49 +/- 0,79 ml/s; II group mean = 1,47 +/- 0,59ml/s; III group mean 1,33 +/- 0,43 ml/s. Uric acid: I group mean 185,8 +/- 59,1 nmol/L; III group mean 226,8 +/- 60,3 nmol/L; III group mean 261,5 +/- 56,9 nmol/L.
Conclusions. Our results showed that exist significant statistical changes between II and III group in all paramerters. Our work sugest that determination of creatinine and uric acid in serum, creatinine in urine and creatinine clearance could be important in diagnostic and therapeutic approach.

Paediatric clinical chemistry

0882

ACTIVE IMMUNITY DEVELOPEMENT IN NEWBORNS- CHANGES OF CBC AND ANTIBODY SYNTETIC SYSTEM

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Background. The baby has some immunoglobulins at birth, but the sheltered intra-uterine existence limits the need for learned immune responses to specific antigens. Of immunoglobulins only IgG is small enough to cross the placental barrier. At birth the baby's levels of IgG are equal to or slightly higher than those of the mother. That is why we have studied immunological responce of full-term newborns to antigenic stimulation of their new eviroment through changes of CBC and IgM synthesis.

Methods. Comparison of CBC and IgM concentration of two groups normal, full-term immunologicaly uncompromited (CRP 4.3 ± 4.2 mg/L), newborn infants (1st group- 30 newborns 1.3 ± 0.6 days after birth (d.a.b.); 2nd second group- 21 newborns 4.3 ± 1.2 d. a. b.).

IgM conc. measuremet- imuno-turbidimetric method, Olympus AU 400 analyser.

CBC was performed on ADVIA™ 120 haematology system, Siemens healthcare division.

Results. Statistically significant changes of CBC ($p > 0.05$)

1st group: WBC $17.06\pm 5.8 \times 10^9$/L; Mo $8.7\pm1.6\%$; Neut $64.8\pm6.7\%$; Neut/Mo 7.5

2nd group: WBC $11.1\pm 2.9 \times 10^9$/L; Mo $12.4\pm2.2\%$; Neut $48.4\pm10.41\%$ Neut/Mo 3.9

There is no changes of IgM production (p= 0,00018)

1st group: 0.18 ± 0.05 g/L; 2nd group: 0.20 ± 0.08 g/L

Conclusions. Despite drastical changes in CBC (neutrophile/monocite switch) full-term, healthy newborn babies does not begin to synthesize IgM antibodies at an increased rate soon after birth. Levels of IgM at term are 20% those of the adult, taking 2 years to attain adult levels (elevation of IgM levels at birth are suggestive of intra-uterine infection).

0883

THE USE OF PROCALCITONIN IN EARLY DIAGNOSIS

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Background. The Procalcitonin (PCT) importance in early diagnosis of neonatal sepsis is still very unclear. The importance and performance of serical biomarkers PCT and C-Reactive Protein (CRP) has been determined in newborns (RN) during their first 48 hours.

Methods. 219 RN, in a 6 month period with sepsis risk factors. We identified the cutoff points and compared the results of the tests PCT and CRP, in three moments of collection (<12h, 12-24h, 24-48h).

Results. 219 RN studied, 38.4% were classified with documented sepsis and 61.6% without sepsis (p=0,000). PCT and CRP values were related in the three moments (r_1=0,685**, r_2=0,759** and r_3=0,339) (**p<0,01). Cutoff points were identified according to sensitivity for 25%, 50% and 75%, depending on being sepsis or non sepsis, calculating to their efficiency (Ef=(specificity + sensitivity)/2), using the ROC curves method. In the first moment the results were $PCT_{75\%}$: CP=0.1650, Ef=79.80% e $CRP_{75\%}$: CP=0,045, Ef=74.54% in the second moment CRP is more effective and in the third moment effectiveness values are similar. With RN divided into intervals according to cutoff points and two biomarkers related, the results were K=0,276 for both markers, K=0,369 in the second and K=0,268 in the third.

Conclusions. In the first and second moments PCT and CRP present a strong relation among themselves, although in the first moment (<12h) the efficiency is greater in PCT. No common results exist between the three collection moments, but if we related with Sepsis or not Sepsis and risk factors there are some interesting Results.

Paediatric clinical chemistry

0884

PLASMA B-TYPE NATRIURETIC PEPTIDE (BNP), SERUM N-TERMINAL-PRO-BNP (NTPBNP) AND LVEF LEVELS WITH HEART FAILURE CHILDREN

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Background. Clinical studies have shown excellent correlation between BNP and NT pBNP levels in adults. However, there are not many reports with neonates and children. Therefore, this study, we evaluated serum NTpBNP levels in serum and BNP levels in plasma for heart failure (HF) neonates and children.

Materials and Methods. Samples for HF patients (N=142, Age 0~ 20 , 88 males and 54 females) were obtained from the Toyama University Hospital. NTpBNP levels were analyzed an Elecsys E170 (Roche Diagnostics K.K., Tokyo) and BNP levels were analyzed MIO2 automated analyzer (Sionogi Co. Ltd,, Osaka). Left ventricular ejection fraction (LVEF were analyzed an iE-33 ultrasonograph (Philips Electronics Japan, Tokyo).

Results. Both NTpBNP and BNP levels for less than 5 years old HF patients were higher(mean : NTpBNP 970 pg/mL ,BNP 78 pg/mL),(N=61)than up to 5 years old HF patients (mean :NTpBNP 126 pg/mL and BNP 28 pg/mL),(N=81,p<0.0001). Especially, neonate(less than one years old) HF patients of NTpBNP and BNP levels were very high (2076 pg/mL and 88 pg/mL,N=8).Furthermore, no significant difference were observed between LVEF, NTpBNP and BNP levels in less than5 years old HF patients, respectively. Other hand, excellent correlations were observed between NTpBNP, BNP levels and LVEF with up to 5 years old HF patients (> r=0.77, P< 0.0001), respectively **.**

Conclusions. In this study, proposed data suggested that does not apply in judgment basis of adults BNP and NTpBNP levels for HF patients with neonates and less than5 years old children.

0885

SIMULTANEOUS ANALYSIS OF 7 STEROID HORMONES BY ISOTOPE-DILUTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS) AND ESTABLISHMENT OF NEW PEDIATRIC REFERENCE INTERVALS

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Background. We have developed an assay for the accurate and simultaneous measurement of serum cortisol, corticosterone, 11-deoxycortisol, 17-hydroxyprogesterone, 21-hydroxyprogesterone, androstenedione and testosterone. These steroids are clinically important markers for the rapid and sensitive diagnosis and monitoring of congenital adrenal hyperplasia. Accurate reference intervals based on a healthy pediatric population are essential for the interpretation of the Results. Current reference intervals are based on older Methods. This can lead to multiple repeat testing and unnecessary delay in diagnosis.

Methods. The samples analyzed in this study were collected from non-hospitalized ethnically diverse healthy children in the Greater Toronto Area (GTA). 235 serum samples between the ages of 0 and 18 years were analyzed. The concentrations were measured using an LC-MS/MS method, developed at the Hospital for Sick Children on an API4000 QTRAP mass spectrometer using atmospheric pressure chemical ionization (APCI). The data was analyzed for outliers and age- and gender-specific partitions using the CLSI/IFCC C28-A3 guidelines. Parametric and non-parametric methods were used to establish the 2.5th and 97.5th percentiles for the reference intervals (90% confidence interval).

Results. Age- and gender-specific reference intervals were established for 17-hydroxyprogesterone, androstenedione and testosterone. The reference intervals for the remaining analytes did not require partitioning and were combined. Detailed reference intervals will be presented for all analytes.

Conclusions. We used a sensitive, accurate and rapid method for the simultaneous measurement of 7 serum steroids to establish new pediatric reference intervals in healthy children that will contribute to improved diagnostic specificity and sensitivity in pediatric diseases.

Paediatric clinical chemistry

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PEDIATRIC NORMATIVE DATA ON ABBOTT, ROCHE, AND ORTHO CLINICAL CHEMISTRY SYSTEMS: INSTRUMENT AGREEMENT AND EQUIVALENCE DETERMINATION UTILIZING PEDIATRIC SAMPLES

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Background. The study aimed to determine if the pediatric CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) normative data is comparable for analytes measured on two or three instruments.
Methods. Serum and plasma samples from metabolically healthy and ethnically diverse children (N=3825; 0-18 years) attending outpatient clinics at The Hospital for Sick Children were collected. Samples were analyzed to determine reference intervals (CLSI C28-A3) for chemistries and immunoassays on: Vitros® 5600, ARCHITECT ci8200 and Cobas ® 6000. Fifty five analytes were measured; only those measured on 2 or 3 instruments were statistically compared. Following outlier removal and age- and sex-matching participants across instruments, the samples (n=2931) were split into 20 age categories (0-<6 months, 6 months-<1 year, plus one group for each subsequent year) and statistically compared across instruments using Bland and Altman and percent difference plots, intraclass correlation (ICC) or kappa (k) coefficients, and equivalence trial plots.
Results. Nineteen analytes were measured on 2 instruments. Analytes with strong to perfect agreement (ICC/k>0.6) were DHEA-S (0.65), total T4 (0.73), pre-albumin (0.83), phosphate (0.96) and LH (0.97), while equivalence was found for DHEA-S, pre-albumin, LH, triglycerides and ferritin. Nine analytes were measured on 3 instruments, and six had an ICC>0.6: calcium (0.84), FSH (0.81), creatinine (0.94), total T3 (0.79), free T4 (0.64) and testosterone (0.69). Of these nine analytes, only FSH and total bilirubin showed full equivalence.
Conclusions. Most of the normative or reference interval data collected in healthy children was not equivalent across instruments.

0887

HEMOLYSIS IS A MAJOR CAUSE OF VARIABILITY IN INSULIN MEASUREMENT DURING ORAL GLUCOSE TOLERANCE TEST IN CHILDREN

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Background. The insulin measurement during the Oral Glucose-Tolerance Test (OGTT) has a central role in the clinical practice for a diagnosis of insulin resistance. Therefore an extensive characterization of known interferences it is mandatory. In this study we evaluated the effects of hemolysis on the measurement of insulin in serum of children at risk for insulin resistance.
Methods. Plasma glucose concentration (ADVIA 2400; Siemens) and insulin responses (ADVIA Centaur; Siemens) during the OGTT were measured in 35 unselected children.
Results. The negative interference of hemolysis on insulin assay increased not only in proportion to the degree of hemolysis, but even if the hemolyzed sample was not immediately processed. Increasing levels of hemolysis (from 0.1 up to 10 g Hb/L) caused important negative interference with insulin immunometric assay, already after 3 minutes of incubation at room temperature, respectively from 5% to 80%. Moreover, hemolysis had a great impact on the variability of insulin measurement, with a mean variability of insulin levels measured increasing from 40% to 90%, respectively in non-hemolyzed and hemolyzed samples.
Conclusions. Based on these results we consider as mandatory: 1) to report insulin values with an automatic-generated note in the presence of hemolysis; 2) to analyze blood samples of OGTT immediately after sampling. These recommendations may be particularly relevant in pediatric patients, where the prevalence of hemolyzed samples is particularly high due to the difficulty of blood sampling.

Paediatric clinical chemistry

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LABORATORY STUDY OF LIPID PATTERNS IN CHILDREN WITH FAMILY HISTORY OF CARDIOVASCULAR DISEASE OR HEMOGLOBIN DISEASES

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Background. Evaluation of children's lipid profile when family history of cardiovascular disease (cvd) is positive or hemoglobin diseases are present.

Materials. In a 4 year period (2006-09) 176 children (84 girls-92boys, aged 3-14 years) were studied. Group A: 84 children with positive family history of cvd, Group B: healthy individuals (30) with free family history (11 boys,19 girls), Group C: patients of hemoglobin diseases (62)

Methods. Serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), were measured spectrophotometrically while apolipoprotein-B (apo-B), apolipoprotein-A(apo-A) and lipoprotein (a) (Lpa) were detected nephelo-metrically andlow density lipoprotein cholesterol (LDL-C) was computed by Friedwald equation.

Results. In group A: 46,6% had abnormal values of TC, 50% of LDL-C, 7,2% of apo-B and 25% of Lpa. In 95,2% normal values of HDL-C were accompanied by elevated values of TC and LDL-C. In group B pathological values were observed in 16,6% for TC, 23,3% for LDL-C. Increased values for Lpa were detected in 25% and 6,6% for group A and C respectively. TG measurements were normal for groups A and B though in group C were pathological for 14,6%. In group C HDL-C<35mg/dl was found in 27,4%.

Conclusions. LDL-C values are better prognostic markers than TC values for cvd. Lipid profile screening should be performed during school years in order to intervene regulating meals, body exercise and even lifestyle to reduce the chance of cvd early in adult life. In group C an association termed as hypertriglyceridemia-thalassaimia syndrome was found.

Patient and laboratory management

0889

SPERM HSP 70 LEVELS IN VARICOCELE

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Background. The elevated expression of heat shock protein 70 (Hsp 70) has been described in patients with astheno-and oligoasthenospermia. However, there is not any investigation on Hsp 70 in patients with varicocele. Thus we aimed to investigate sperm Hsp70 levels and its association with sperm paramaters in varicocele patients.

Methods. The study group consisted of 26 patients with varicocele and 12 patients with oligoasthenospermia without varicocele. The control group comprised 16 age-matched fertile men. Sperm Hsp 70 levels were measured by using commercially available ELISA kit, and sperm parameters were analyzed.

Results. Sperm Hsp 70 levels were not different in varicocele group from those of controls. But in oligo and/or asthenospermic patients without varicocele, sperm Hsp 70 levels were higher than those of the controls (p=0.0001) and varicocele patients (p=0.005, ANOVA post hoc Tukey test).

We further divided the patient groups as oligoastheno- and asthenospermics. In only oligoasthenospermia group (n= 22), sperm Hsp 70 levels were significantly higher than those of normospermic (n= 20, p=0.001) and asthenospermic patients (n= 12, p=0.009).

There were significant negative correlations between Hsp 70 and motility (r=-0.50, p=0.0001) and between Hsp 70 and sperm concentration (r=-0.55, p=0.0001) in total study group.

Conclusions. The present study indicated that sperm Hsp 70 levels were negatively associated with sperm motility and concentration but not with varicocele. This may indicate that when sperm motility and concentration decrease, Hsp70 increases (probably as a compensatory mechanism) because of its antiapoptotic property.

0890

ASSESSMENT OF OUTCOMES OF DIABETIC PATIENTS BY USING DATA FROM LABORATORY/HOSPITAL INFORMATION SYSTEM

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Background. Outcome analysis by using data from laboratory/hospital information system (LIS/HIS) provides useful information for quality health care. Outcomes for diabetics related to testing such as HbA1c, lipid profile and renal markers have been measured in combination with risk factors such as demographic characteristics, duration of diabetes, smoking status. Our purpose was to evaluate laboratory tests and characteristics of diabetics by using the data obtained from LIS/HIS, and to improve the data mining capacity of the LIS/HIS for recovering knowledge about health outcomes of diabetics.

Materials and Methods. The data was obtained from the LIS. Study outcomes were HbA1c measurements, lipid profile and renal function markers. The glycemic control status was evaluated according to the ADA2010 criteria.

Results. The number of patients who had at least 2 measurements during last six months was 827. Forty-nine had three measurements. The significant differences were determined for HbA1c (p values) (0.008,0.011,0.001); for glucose (Not significant-NS,0.003,NS); for BUN (0.0001,NS,NS); for HDL-C (0.0001,0.008,NS); for creatinine (0.000,NS,NS); for TG (0.0001,0.016,NS) between the 1st and 2nd; 1st and 3rd; and 2nd and 3rd admissions, respectively. The percentages outside the treatment goals were as follows: HbA1c (24,23,29); T-Chol (37,28,27); TG (37,38,27); LDL-C (22,20,15); HDL-C(male) (38,35,35); HDL-C(female) (47,46,47) for the first three admissions, respectively.

Conclusions. The analysis of health care databasescould greatly help to extract useful information for the assessment of health care delivery. However, their mining capacity should be improved.

Patient and laboratory management

0891

REFLEX TESTING TO DEFINE TRULY CRITICAL RESULTS FOR A COMMUNITY LABORATORY

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Background. Tests may be added to requests using algorithms. Critical results may be divided into truly critical requiring to be phoned 24/7 and those required to be phoned during the day. Community based requestors prefer not to be contacted unless a result is truly critical and contacting out of usual office hours requires substantial staff resource. We derived algorithms to define those results that are truly critical.
Methods. A critical phoning list was agreed. Algorithms with reflex tests added where required, were devised to help define a truly critical result. To justify the reflex tests, each algorithm was costed, by audit of results in the laboratory database and test costs.
Results. These algorithms identified 18 out of 51 tests per month as being truly critical. The total cost of the reflex tests added is €29 per month. Addition of s-bicarbonate identified 10% of s-glucose > 24.9 mmol/L as truly critical. Addition of s-creatinine identified 65% of s-lithium > 1.49 mmol/L as truly critical. Addition of s-potassium, calcium & magnesium identified all s-digoxin > 2.49 nmol/L as truly critical. Addition of s-creatinine & potassium identified all s-creatine kinase > 4999 u/L as truly critical. Addition of s-potassium & calcium identified all s-magnesium < 0.31 as truly critical.
Conclusions. The use of reflex tests with critical results helped inform a two tier critical result phoning system. This system both avoids disturbing requestors unnecessarily & conserves staff time at night.

0892

CORRELATION BETWEEN THE EQUATIONS MDRD-4 AND CYSTATINE C TO EVALUATE RENAL FUNCTION IN CHRONIC RENAL DISEASE

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Background. Assess in chronic renal disease (CRD) the relation between glomerular filtration rate (GFR) based on creatinine (cr) and in cystatine c (cy).
Evaluation of renal function is important to determine the beginning, development and severity of renal disease, to evaluate the therapy efficiency and to adjust the portion of drugs excreted by the kidney. MDRD4 equation estimates GFR considering the concentration of cr and demographical variables but it has limitations. Recent researches have showed cy as a reliable marker. National Kidney Foundation recommends the use of equations using cy, as a FG marker, in replacement of cr.
Methods. Prospective study during 2010 in 70 CRD outpatiens Nephrology Service (ULSM). Majority of patients were male (40). Average age was 70 years, ranging from 52 to 94 years. Each patient provided a serum sample where cr was determinated by spectrophotometric assay (alkaline picrate) and cy by turbidimetric immunoassay (PETIA).TFG was determined by cr (MDRD4 equation): $175 \times (cr)^{-1.154} \times (age)^{-0.203} \times (0.742$ if female$) \times (1.210$ if African American$)$ (1) and by cy ($71/cy^{1.28}$) (2). (1) Ranging from 12.42 to 59 mL/min/1.73m^2 and (2) from 16.48 to 100.88mL/min/1.73m^2. Statistical analyses were performed with SPSS (vs 18).
Results. Following Pearson correlation, we achieved a moderate correlation (r. 600; p<.001) between (1) and (2).
Conclusions. GFR based on cy has the advantage of no interference of some factors like the age.Considering our results, GFR based on cy needs to be improved, although helps to evaluate CRD.

Patient and laboratory management

0893

HOW TO MEASURE PROTEINURIA IN CHRONIC RENAL DISEASE: 24-HOUR URINE COLLECTION OR PROTEIN CREATININE RATIO IN A RANDOM URINE SAMPLE?

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Background. Determine Protein Creatinine ratio (PC) using random urine or 24 hours urine protein (UP) in Chronic Renal Disease (CRD) in order to eliminate 24 hours collection.
Proteinuria requires timed urine collection for its quantification. 24 hours urine collection is often incomplete and unreliable due to wide variation in creatinine excretion, changes in physical activity and dietary protein intake that make this assumption unreasonable and inaccurate.Difficulties of 24-hour urine collection are well recognized, especially for children and elderly people for whom a means of estimating protein excretion from a single casual unit sample would be preferable.
Methods. Prospective study, during 2010, in 30 CRD outpatients of ULSM Nephrology Service (ULSM),majority male (19). Average age was 74 years, ranging from 59 to 94 years. Patients provided 24-hour urine collections with concomitant random urine specimens. UP was quantified by turbidimetric assay (Benzethonium chloride) and urine creatinine by spectrophotometric assay (alkaline picrate). The PC ratio (0.06 - 16.51) of an aliquot obtained from a patient random urine was compared to total protein excretion over the 24 hour period (39.1 - 12409.8 mg/24h). Statistical analyses were performed with SPSS (vs 18).
Results. Following Pearson correlation regression analysis yielded a higher correlation coefficient (r .979, p<.001).
Conclusions. We find a strong correlation. Admittedly our population sample is small and may not be generalizable to entire population it validates published findings supporting the use of random urine PC for estimation proteinuria in DRC.

0894

PERFORMANCE OF BD VACUTAINER® GLUCOSE TUBES CENTRIFUGED WITH HIGHER G FORCE

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Background. Manufacturers recommend different centrifugation conditions for different blood collection tube types. These differing centrifugation requirements can have an adverse impact on laboratory efficiency, particularly where laboratory work flow is automated. This study was conducted to evaluate the performance of
BD Vacutainer® Glucose tubes when centrifuged at 2000g as per BD Vacutainer® SST™ II *Advance* rather than the recommended 1300g.
Methods. Blood from 30 apparently healthy adult donors was collected into two of each of the following BD Glucose tubes with different glycolytic inhibitors (fluoride/oxalate (F/Ox); fluoride/EDTA (F/EDTA); heparin/iodoacetate (Hep/Iod)). One was centrifuged at 1300g for 10 minutes, the other at 2000g for 10 minutes. Glucose was measured for each of the tubes using a Roche Integra 400 at initial time (t_0) and after 24 hours (t_{24}) storage at room temperature.
Results. Mean (SD) for the glucose measurements in mg/dl were F/Ox 1300g t_0: 5.833 (2.733), F/Ox 2000g t_0: 5.785 (2.736); F/Ox 1300g t_{24}: 5.816 (2.739), F/Ox 2000g t_{24}: 5.758 (2.739); F/EDTA 1300g t_0: 5.832 (2.731), F/EDTA 2000g t_0: 5.816 (2.718); F/EDTA 1300g t_{24}: 5.803 (2.741), F/EDTA 2000g t_{24}: 5.801 (2.701); Hep/Iod 1300g t_0: 5.695 (2.756), Hep/Iod 2000g t_0: 5.592 (2.673); Hep/Iod 1300g t_{24}: 5.699 (2.716), Hep/Iod 2000g t_{24}: 5.605 (2.699).
Conclusions. BD Vacutainer® Glucose tubes with fluoride/oxalate, fluoride/EDTA and heparin/iodoacetate glycolytic inhibitors that been centrifuged at 2000g rather than the recommended 1300g gave clinically equivalent results for the measurement of glucose using a Roche Integra 400 instrument at initial time and after 24 hours storage at room temperature.

Patient and laboratory management

0895

A MONITORING PROCESS TO ASSESS THE IMPACT OF PREANALYTICAL VARIABLES ON SAMPLE QUALITY FOLLOWING A CHANGE IN BLOOD COLLECTION SYSTEM

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Background. The Charité University Hospital, Berlin wanted to assess whether there was an impact on sample quality when switching from the Sarstedt Monovette® to the BD Vacutainer® blood collection system across its multiple sites and wards.
Methods. Preanalytical Reviews were conducted for both blood collection systems. Observations were recorded on a form with standardised responses ensuring consistency between observers. Free haemoglobin (fHb) measurements were used as an indicator of sample quality.
Results. The reviews showed a significant difference ($p<0.001$) between the two systems for underfilling (<75% filled), with 109 of the 241 (45%) observed tubes collected with the Sarstedt system being underfilled compared with 41 out of 482 (9%) with the BD system. Analysis of the fHb data showed higher haemolysis levels in Emergency than in other wards: (median fHb values in mg/dl (N)): Sarstedt Site 1 Emergency 31.0 (1307), Non Emergency 6.0 (11263), Site 2 Emergency 15.0 (1948), Non Emergency 6.0 (15196); BD Site 1 Emergency 42.0 (1125), Non Emergency 5.0 (13208), Site 2 Emergency 11.0 (1924), Non Emergency 5.0 (17064). Further analysis showed that 61% of the BD samples exhibited a lower fHb, 6% were approximately equal, and 33% had higher fHb, when compared to the Sarstedt system Results.
Conclusions. The sample quality was not impacted as a result of the switch in suppliers. The Preanalytical Review and use of fHb identified factors that could impact sample quality and allowed a quantitative comparison of the two systems. The study identified previously unknown differences between wards at the hospital.

0896

AUTOVERIFICATION OF CLINICAL LABORATORY RESULTS

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Background. Laboratory results need post analysis processing. This data processing includes analytical quality control, plausibility checks, checking for clinically relevant test results to be re-checked or communicated to the attending physician without delay, authorisation and reporting. This process used to be time consuming and sensitive to errors. In order to improve both laboratory quality and efficiency we developed, within the framework of a laboratory information system, a concept to automate parts of the post analysis data processing.
Methods. The concept is based on result-based flaggings and decision rules. Each laboratory result is accompanied by a flagging which refers to norms (measuring rang, reference range, pathological range, critical values), delta checks, analyzer flags, or quality control rules. Using decision rules, these severities may trigger e.g. reflex testing, authorisation, and addition of comments on reports.
Results. Many parts of our laboratory data processing could be automated. The authorisation process was translated to a set of decision rules and is now performed semi-automatically. Exceeding of critical values and stat results are notified by the system. All data are logged and listings of results to be paged are produced automatically. The system is in compliance with the requirements of the recently published CLSI AUTO10-a document on autoverification of clinical laboratory test Results.
Conclusions. The execution of decision rules is performed consequently. Approximately 90% of the test results are now confirmed and validated automatically. The overall turnaround time was reduced considerably, while the workload of the technicians has been slightly reduced.

Patient and laboratory management

0897

MONITORING OF THE C-REACTIVE PROTEIN PRESCRIPTION AT THE EMERGENCY DEPARTMENT OF BICHAT-CLAUDE BERNARD HOSPITAL

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Background. C - reactive protein (CRP) was inappropriately and systematically prescribed in the emergency department (ED) (33 CRP per day; 1CRP for 1.33 Creatinine in 2008). Since inadequate allocation and waste of financial resources could affect patient care, a consensus between physicians and clinical chemists emerged to improve quality of CRP prescription.
Methods. Both physicians from ED and clinical chemists were involved to implement a specific pre-testquestionnaire with CRP prescription restricted to indications modifying patient's care and to participate in training the staff. Staff training and controlled CRP prescription started in July 2008.To assess the impact on CRP prescription, CRP and Creatinine -reflecting the global biologic prescription of the department- numbers were recorded from July 2008 to March 2009 and compared to those from July 2007 to March 2008.
Results. A global reduction in CRP prescription (- 72.77%) was observed, although ED global activity slightly increased in (+6.8%). In the meantime, CRP prescription decreased only by 7.6% and Creatinine by 0.4% in the whole hospital. Until May 2010, CRP mainly prescribed for suspected intra-abdominal sepsis (51%), remained low and stable (10 CRP per day; 1CRP for 2.25 Creatinine). Physicians strongly adhered to the protocol. No adverse events directly related to the non-prescription of CRP were described. Cost saving allowed us to introduce Procalcitonin.
Conclusions. Systematic/unjustified CRP prescription was sharply reduced without affecting patient's care. Our experience shows the importance to formalize and repeat regularly adequate prescription recommendations. This approach will be extended to improve cost effectiveness of other biomarkers.

0898

REDESIGN OF LABORATORY INVENTORY SYSTEM RESULTS IN SIGNIFICANT SAVINGS AND IMPROVED STAFF MORALE

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Background. Difficult access to and storage of inadequate or excessive quantities of reagents is common problem. This leads to wastage of reagents, technologist time, space and frustration. This was the situation in our laboratory before we designed and implemented a simple solution using abandoned filing cabinets from a refurbished clinic and a cheap inventory control system.
Methods. "Spacesaver®" filing cabinets were used to organise all of our inventorystored at room temperature. The laboratory is now stocked weekly, by a clerk, with a one week supply of reagents. Access to the inventory room is restricted to senior technologists or above to ensure all reagents entering and leaving are documented. "Red Beam®" software is used to track inventory and to reorder supplies. We compared hours worked, reagent wastage, space occupied and out-of-stock occurrences, for one year pre and post implementation.
Results. The following annual savings were observed: Technologist time searching for reagents, 20 days; Senior technologist counting and ordering reagents, 62 days; Laboratory inventory stocking, 45 days. Time spent unloading reagents was unchanged. In-house reagent storage decreased from an average of 3 months to 6 weeks.Rush orders decreased from 18 to 2 per year. Spacesaved 161 m^2. Reagent wastage decreased from an estimated 20-30kits to 1 kit per year.Staff working conditions have improved due to less laboratory clutter, less futile activity and increase in staff pride.
Conclusions. This re-organisation has saved us space, eliminated wastage and saved significant salary reagent costs and should be seen as a priority by laboratories.

Patient and laboratory management

0899

PREANALYTICAL ERRORS ASSOCIATED WITH SAMPLE COLLECTION

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Backgrounds. In an era where mechanization of laboratory automation has drastically reduced the errors due to the analytical phase of testing, errors due to the preanalytical phase are largely responsible for the decrease in quality of clinical laboratory results

Methods. The present study was conducted at one of the tertiary care hospital at State Capital to find out the incidence of pre-analytical errors between inpatients and outpatients samples collection either into vacuated (Plain and EDTA) tubes or without evacuated (Plain and EDTA) tubes.

Results. Sample ID/Tracking related errors in inpatients, outpatients, BD Vacutainer® tubes and without evacuated tubes were 17.25%, 12.40%, 11.06% and 15.74%. Sample insufficient/leakage/spillage/ rejected errors in inpatients, outpatients, BD Vacutainer® tubes and without evacuated tubes were 6.90%, 4.98%, 3.96% and 6.51%. Sample clotted/volume related errors in inpatients, outpatients, BD Vacutainer® tubes and without evacuated tubes were 11.41%, 9.73%, 8.85% and 11.05. The incidence of hemolysis/fibrin clot in samples collected in inpatients, outpatients, BD Vacutainer® open collection and without evacuated tubes open collection was 10.50%, 7.64%, 4.65% and 10.62%. The frequency of total errors observed was 25.80% and 27.96% for inpatients samples collected either into BD Vacutainer® tubes or without evacuated tubes respectively while for outpatients it was 20.75% and 29.27%. The total errors (30.24%) irrespective of the blood container used were 34.92% and 25.55% for inpatients and outpatients respectively.

Conclusions. Preanalytical variables can produce unpredictable and unfavorable impacts on the wellbeing of patients because of preanalytical variables which could affect more than 30% of laboratory Results.

0900

ASSESSING SAMPLE TYPE SUSCEPTIBILITY TO HEMOLYSIS IN THE ROUTINE CLINICAL LABORATORY

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Background. Hemolysis is a common problem in clinical laboratories and is promoted by pneumatic tube sample transportation. However, it is not clear if certain samples types are more susceptible to hemolysis than others.

Methods. Paired routine blood samples in lithium heparinate and serum gel separator tubes from the same venipuncture were sent through the pneumatic tube system to the laboratory. The hemolysis index (HI) was measured in 157 lithium heparinate plasma samples with a HI above 20 and in the paired serum samples because HI values above the threshold value of 20 can interfere with the selected assays. Creatine kinase, creatine kinase-MB and alanine aminotransferase activities were determined in plasma and serum.

Results. The median HI in lithium heparinate plasma was 85.0 (5th and 95th percentiles: 28.8; 326.3) and 33 (5th and 95th percentiles: 11.0; 128.2) in the paired serum samples (p<0.001). The median HI difference between paired plasma and serum was 46 (5th and 95th percentiles: -35.2; 253.4; p<0.001). The creatine kinase and creatine kinase-MB activities were determined significantly higher in plasma than in serum (p<0.001) whereas the alanine aminotransferase activities were determined slightly lower in plasma than in serum (p=0.017).

Conclusions. Blood samples in lithium heparinate tubes are substantially more susceptible to hemolysis than blood samples in serum tubes. The results have organisational implications for the selection of sample types in clinical laboratories.

Patient and laboratory management

0901

A COLLABORATIVE APPROACH TO ADDRESS THE DIAGNOSTIC PATHOLOGY WORKFORCE CRISIS

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Background. Workforce shortages in pathology are not new, yet the area still struggles to attract suitably qualified staff, particularly new graduates. An Australian Government report stated that training medical scientists in a university environment is not always attractive due to the high cost of course delivery. This, coupled with the shortage of medical doctors, has seen a shift away from medical laboratory training to pre-medical degrees, with less emphasis on expensive laboratory training. Thus, graduates from medical science degrees often do not have suitable skills for employment in a diagnostic pathology laboratory. In Canberra, Australia, ACT Pathology, the University of Canberra (UC) and Siemens Healthcare Diagnostics are collectively attempting to address this issue.
Methods. Collaborative, tripartite discussions between senior staff of ACT Pathology, UC and Siemens identified the needs of new graduates and modified the UC curriculum to better deliver the skills and knowledge needed by industry. The revised curriculum incorporates on-line learning components developed by Siemens and a work placement program using ACT Pathology and Siemens facilities. Specialist units are taught by staff employed by ACT Pathology at the Canberra hospital and university campus.
Results. The updated course commenced in 2009 with successful work placement programs. Revamped units in clinical chemistry commenced in 2010, with haematology, transfusion and histology to be introduced in 2011.
Conclusions. There has been an increase in students undertaking this undergraduate program who intent to seek employment within the diagnostic pathology industry.

0902

REDUCING IDLE TIME FOR PATIENTS IN THE EMERGENCY WARD – ARE FURTHER IMPROVEMENTS ALWAYS POSSIBLE?

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Background. The clinical chemistry laboratories of the two university hospitals in Stockholm (located in Solna and Huddinge) both had a tradition of highly automated work processes and focussing intently on turn-around-times. When the two hospitals merged, a programme was started to increase throughput in the emergency wards.
Methods. Separate project groups, each containing staff from both the laboratory and the emergency ward, were started under the same leadership. The groups had access to data compiled from various systems, thus making it possible to time all phases of a patient´s visit to the emergency ward. The work was inspired by "lean" principles.
Results. Initial data analysis suggested two major areas for improvement: reducing the number of haemolytic samples (described separately) and reducing the time from test ordering to result delivery. Detailed flow-charts were constructed covering all steps from ordering to result delivery. Various changes in the working processes were tried and evaluated. Over a two-year period, the mean time from ordering to result delivery was reduced by approx 13 % (from 122 to 104 min in Solna and from 110 to 98 min in Huddinge). The mean turn-around-time (90 percentile in brackets) for CRP stat samples in the laboratory was reduced from 38 (52) to 30 (40) min in Solna and from 50 (71) to 38 (46) min in Huddinge.
Conclusions. Optimization of preanalytical activities is of great importance in reducing idle time. Using a structured approach, further improvements are always possible, the reduction of outliers being of particular importance.

0903

RECENTRIFUGATION OF PRIMARY GEL TUBES WITHIN 16 HOURS OF INITIAL CENTRIFUGATION DOES NOT CAUSE SIGNIFICANT PSEUDOHYPERKALEMIA

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Background. There is some confusion as to whether recentrifugation of primary tubes causes pseudohyperkalaemia. Clinical laboratories receive both centrifuged and uncentrifuged primary tubes and are often asked to add tests to previously centrifuged specimens. When using a pre-analytical module, centrifugation of all tubes, whether previously centrifuged or not, simplifies the workflow. Studies have shown that recentrifugation after 24 hours causes pseudohyperkalaemia. However, it is unclear whether this effect is seen if recentrifugation occurs within 24 hours. Our policy has been to centrifuge all tubes on reception regardless of if they had been previously centrifuged. We therefore designed a study to verify if our practice was causing pseudohyperkalaemia and if we needed to separate all incoming tubes into centrifugation and non centrifugation racks.
Methods. Primary tubes, that had been centrifuged at time 0, were analyzed for potassium immediately before and after recentrifugation at 4, 8, 12, 16, 20 and 24 hours (n = 40-50 at each time interval). Differences were analyzed using Method Validator ® software.
Results. Significant pseudohyperkalaemia was found following 20 and 24 hours delay, with a mean increase of 0.62 and 2.13 mmol/L respectively. At 4, 8, 12 and 16 hours delay the mean increase observed was 0.08, 0.04, 0.06 and 0.16 mmol/L respectively. The effect of hematocrit on potassium differences was not significant.
Conclusions. If it can be established that initial centrifugation was perform within 16 hours, the workflow can be simplified by centrifuging all samples received without concern of significant pseudohyperkalaemia.

0904

MANAGEMENT OF DIABETES MELLITUS – CLINICAL PATHWAY FOR THE PREVENTION OF SECONDARY COMPLICATIONS IN THE PRIMARY HEALTH CENTRE "SAVSKI VENAC" BELGRADE

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DiabetologycareinSerbiais defined througha NationalStrategyfor prevention and Diabetes control. According tothe National Programmefor prevention and diabetes controlinSerbia, prevention, diagnosis, initial therapyandmonitoring are taking placeat the primarylevel.
Clinical Pathway represents a detailed, precise planof activities which has to be undertakenduringthe treatment so diagnostic and therapeutic options could be used efficiently andeconomically. It allowsrecording ofallrelevant and applied clinicalprocedures, as well asthe reasonswhy theapplicationfailed. It relieson the Nationalguide.
Clinical Pathway forthe preventionof secondarycomplications ofD Mellitusdefinesthe type anddynamicsof examination and the dynamicsof laboratorycontrols which includesthe controlof similar parameters: fasting glucose, postprandialblood glucose, HbA1C, total cholesterol, triglycerides, HDL, urea, creatinine, proteinuria, microalbuminuria andGFR.
An importantactivitythat Clinical Path provides is education of a patient and making recommendations about nutrition andphysicalactivity.
Developed by all the experts which participating in the treatment of diabetic patient removes all doubts in terms of organization, activities, responsibilities, control and provides management of diabetes at the primary health care level.

Patient and laboratory management

0905

COMPARISON OF FASTING AND 2-HOUR GLUCOSE TO HBA$_{1C}$ FOR DIAGNOSING OF DIABETES MELLITUS IN THE ELDERLY

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Background. The aim of this study was to compare fasting plasma glucose (FPG) and plasma glucose 2-hour after 75gr glucose load (2hPG) to HBA$_{1C}$, as screening tools for early detection of diabetes mellitus (DM) in the elderly.
Methods. FPG, 2hPG and HBA$_{1C}$ were estimated in 90 elderly subjects of 60-75 years. Diabetic type was defined when FPG was 125mg/dl or higher and/or 2hPG was 200mg/dl or higher. Normal type was defined when FPG was below 110mg/dl and 2hPG below 140mg/dl. Borderline type included those who were neither diabetic nor borderline type. The HBA$_{1C}$ cutoff level of > or =6,5% was used.
Results. Normal, borderline and diabetic type was identified in 70, 3 and 17 subjects, respectively. HBA$_{1C}$ above cutoff levels was determined in 17/70 (28,3%) normal type, 3/3 (100%) borderline type and 17/17 (100%) diabetic type subjects. HBA$_{1C}$ below cutoff levels was determined in the remaining population of 53 normal type.
Conclusions. The evaluation of HBA$_{1C}$ levels represents a lower diagnostic validity for DM, than FPG and 2hPG.

0906

LIPOPROTEIN (A) IN HEALTH AND TYPE 2 DIABETES MELLITUS

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Background. Lipoprotein (a) is an independent risk factor for Ischaemic Heart Disease. The aim of this study was to evaluate the concentration of Lp(a) in subjects with type 2 diabetes (T2DM) and non-diabetic subjects living in urban areas from northern Greece.
Methods. An over-night fasting blood sample from 68 T2DM subjects (25 men/43 women) and 219 non-diabetic subjects (64 men/155 women), was taken for Lp(a) determination Serum Lp(a) concetration >30mg/dl was considered abnormal. Both diabetic and non-diabetic subjects were grouped into five age groups: 20-40 (n=35), 40-50 (n=27), 50-60 (n=45), 60-70 (n=73) and>70 (n=107) years. All patients with type 2 diabetes fulfilled World Health Organization criteria for the diagnosis of T2DM.
Results. Of the 287 subjects examined, 40 (13,9%) had high Lp(a) concentration. The prevalence of high Lp(a) concentration did not differ significantly between men 10,1% (9/89)and women 15,6% (31/198) or between age groups [(5,7% (2/35), 7,4% (2/27), 13,3% (6/45), 16,4% (12/73), 16,8% (18/107), respectively]. Non-diabetics patients had significantly higher Lp(a) concentration($p<0,05$) [16,8% (37/219)] compared to T2DM subjects [4,4% (3/68)]. Among non-diabetic subjects women presented with significantly higher Lp(a) concentration compared to men ($p<0,05$) [men 10,9% (7/64), women 19,3% (30/155)]
Conclusions. Our results support that Lp(a) is elevated in healthy people compared to T2DM patients, and they are conflicted with data from other studies. This difference is based probably on the insufficient sample sized of our study. Therefore, determination of Lp(a) should be cautiously exercised among diabetic and non-diabetic people.

Patient and laboratory management

0907

MULTIDRUG RESISTANT, EXTENDED SPECTRUM BETA-LACTAMASES AND AMPC BETA LACTAMASES PRODUCING UROPATHOGENES AMONG CHILDREN: HOSPITAL BASED STUDY

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Background. Gram negative bacilli are the common isolates from UTIs in children. Extended spectrum beta-lactamases (ESBL) and AmpC beta-lactamases (AmpC) are the most significant enzymes involved in conferring resistance to Beta-lactam antibiotics in Gram negative bacteria. This study was aimed to finding the prevalence of multidrug resistant (MDR), ESBL and AmpC producing isolates among children with UTI in Nepal.
Methods. A prospective study was carried out from July 2006 to March 2008 at the Department of Microbiology, TUTH, Nepal. 820 urine specimens were obtained from clinically suspected UTI children (age<12 years, female to male ratio 2.1:1). Isolates resistant to two or more antibiotics were defined as MDR, among them resistant to third generation cephalosporins were further tested for ESBL and AmpC phenotypes.
Results. Among 820 urine samples, 24.51% (201/820) had significant bacterial growth with 184 (91.54%) non-repeatgram-negative isolates in which most were *Escherichia* coli (58.15%) followed by Klebsiella species (15.2%).The prevalence of MDR, ESBL and AmpC were 115 (62.5%), 43 (23.36%) and 15 (8.15%) respectively. Maximum incidence of ESBL producer was *Escherichia* coli(39.5%) followed by Klebsiella (16.2%). High AmpC producing species were Klebsiella(40%) and Pseudomonas(26.6%). Imipenum and piperacillin were most effective drug among ESBL producers and non producers. Infection was more common in age group 6±2.3 years with female to male ratio 2.04:1.
Conclusions. Result shows high percentage (62.5%) of MDR pathogens in childhood UTI. Preponderance of ESBL and AmpC enzymes seems leading to medical cumbersome and treatment failures in childhood UTI cases.

0908

CAN A FRIEDWALD TYPE EQUATION (FTE) BE USED WHEN TRIGLYCERIDE CONCENTRATIONS [TG] =/> 400 mg/dL?

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Background. FTEs can estimate LDL-cholesterol (eLDL-C) when [TG] <400 mg/dL. Otherwise LDL-C is directly measured [mLDL-C]. We investigated whether a simple FTE could be used for [TG] ≥400 mg/dL.
Methods. All assays used the Xpand Plus analyzer (Siemens; Deerfield, IL). Excel software (Microsoft; Redmond, WA) was used for multiple linear regression (MLR).
Results. Data from 93 consecutive specimens with [TG] between 400 and 599 mg/dL, MLR and median values of the TC:HDL-C and TC:TG ratios generated equation (1).
$eLDL\text{-}C \approx TC - HDL\text{-}C - .138 \cdot [TG]$ (1)
This suggested that the [TG] should be divided by 7 or 8. An FTE using the latter value was applied to an independent sample of 84 patients. The median absolute difference (MAD) between eLDL-C and mLDL-C was 13.875 mg/dL. Sixty-nine patients (.821) were in the same NCEP risk category using m or eLDL-C, 11 (.131) differed by one category and four differed by two categories. The latter four had eLDL-C >150 mg/dL. suggesting an algorithmic approach where eLDL-C was accepted if ≤150 mg/dL and a mLDL-C performed otherwise. Using this program 60 of 84 values of eLDL-C would have been accepted while mLDL assays would have been reduced from 84 to 24 (71%).
Conclusions. Whether the derived FTE varies with analytical system and whether a non-linear equation would improve risk stratification are open questions but the current study, although small, raises the possibility of using a FTE to significantly reduce the number of mLDL-C assays in the [TG] range of 400-599 mg/dL.

Patient and laboratory management

0909

BENCHMARKING: A LABORATORY MANAGEMENT TOOL

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Background. In times of economic uncertainty and budget constraints laboratory managers need sound costing information to make decisions about which methods of testing provide the most cost effective options. The Benchmarking in Pathology Program provides managers with a tool to address such issues.
Methods. Initially a total cost methodology program was designed but a lack of high level costs available to laboratory managers and inefficient information systems made determining costs using this methodology impossible. As a consequence an 'avoidable cost' methodology was introduced.
Results. The 'avoidable costs' methodology has assisted laboratory managers in knowing the individual test costs for the range of tests they perform and has enabled them to benchmark these costs against their peers. Having this information readily available has resulted in management applying 'what-if' analysis and making decisions to change technology with the confidence of knowing what costs will be incurred. This may permit improvements in quality of testing while minimising cost. Information provided by the Program has assisted laboratory managers to accurately calculate costs when tendering for additional external testing. Supplementary staffing information, also provided by the Program, provides management with comprehensive costing details relating to all activities performed by members of staff including research, teaching and training, professional obligations and clinical duties in addition to duties associated with laboratory testing.
Conclusions. The Program is continually being expanded and refined in response to changing laboratory practice and participant feedback to address the issues faced by laboratory managers and is now generally accepted and utilised throughout Australia.

0910

DIRECT CALCULATION OF REFERENCE LIMITS FOR 17 SERUM ANALYTES USING THE VITROS 5.1 FS CHEMISTRY SYSTEM

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Background.
We established reference intervals for 17 routine serum biochemistry analytes, measured with the Vitros® 5,1 FS MicroSlide assays (Ortho Clinical Diagnostics). According to ISO 15189 laboratories should check the appropriateness of their reference intervals, and prove that these are suitable for the intended population. In general there is little data on reference limits for parameters analysed with the multilayer film technique.
Methods.
We calculated reference intervals according to CLSI C28-A3 guidelines, using MedCalc and RefVal software. Retrospectively routine biochemistry results from healthy volunteers participating in clinical trials of the SGS Life Science Services Clinical Pharmacology Unit Stuivenberg (Antwerp, Belgium) (n= 1000, 18-60 years old, 80% males and 20% females) were collected during a time period of 12 months.
Results.
For ALT, AST,GGT, creatinine and urea we established gender-based reference intervals.
For most analytes, differences were observed between calculated lower and or upper reference limits and those currently proposed by Ortho Clinical Diagnostics. Differences up to 10% were found for calcium, chloride, creatinine, LDH, phosphate, potassium, sodium and total protein. For bicarbonate, magnesium, ALP and urea, deviations between 10 and 20% were observed. Differences over 20% were calculated for albumin, ALT, AST, GGT, and total bilirubin.
Conclusions.
We established reference intervals for biochemistry analytes measured with the multilayer film technique on a large number of reference individuals. For some analytes, calculated reference limits corresponded well to manufacturer's limits, however for a number of parameters important differences were observed.

Patient and laboratory management

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THE LABORATORY AS INFORMATION PROVIDER. THE CASE OF GLYCATED HEMOGLOBIN (HBA1C)

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Background. Laboratory tests are useful when they provide information that positively helps the clinician with diagnostic decisions. Post-test probability of patient having a disease should be increased respect pre-test. Results of target positive patient should fall outside those of normal people or differentiate target positive from target negative patients with same symptoms and signs attributed to the disorder. Analytically valid results are an essential requirement. Second, but not last, is evaluation of the "limit" between normality and illness. The objective was to evaluate usefulness of HbA_{1c} test in diabetes diagnosis as it is reported by participants of an EQAS, taking into account methodologies used and a suggested cut-off.
Methods. 6 whole blood pools (repeated twice each) were sent to 145 laboratories. Laboratory Coefficient of Variation (LCV%) and Bias % (B%) were calculated and compared to quality requirements. Laboratories sent their cut-off values and answered a questionnaire about interpretation of a clinical case, with several diagnostic options to choose.
Results. Methods reported were mostly immunoturbidimetry (81%), ion exchange chromatography (7%) and HPLC (9%). 25% participants presented a LCV% <3.8 and Bias% <3.1. Reported cut-off values ranged between 4.8% and 8.0% (median 6.0 %). Submitted answers for the same clinical case ranged from patients with diabetes (6.5%) to patients low risk of having the disease (41.3%).
Conclusions. Post-analytical interpretation of results should be capable of classifying patients on a defined category of disease. HbA_{1c} results show that, even with reliable analytical quality, patient classification outcome depends on accurate information provided by laboratories.

0912

GRAPHICAL PRESENTATION AND INTERPRETATION OF CAPILLARY ZONE ELECTROPHORESIS PROFILE AND ASSOCIATED PROTEINS

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Inexpensive capillary zone electrophoresis (CZE) allows rapid and automated protein separation. It is potentially a cheap screening tool to optimize DRG diagnosis, because the obtained patterns help to diagnose and follow up many frequent disorders, for instance acute and chronic inflammation, antibody deficiency, protein synthesis or loss, nutritional status or detect a monoclonal gammopathy. In a recent continuing education session, however, 2/3 of 107 laboratory professionals were not able to identify an apparent monoclonal gradient.
CZE produces digital absorbance data that is appropriate for mathematical analysis. This allows to identify typical protein profiles as well as curve irregularities, i.e. monoclonal components and also automate immunotyping.
We therefore developed the knowledge base "MDI LabLinkCZE⁺" that combines mathematical analysis with a color-coded electropherogram. An overlay of the previous consultation identifies changes from the previous visit. Electrophoresis, however, becomes only a component in patient work-up if, for instance, once a monoclonal component is detected. Therefore, other relevant information, including free light chain values, immunofixation results, M-gradients, immunoglobulin values, as well as urine data are additionally graphically represented to rapidly differentiate pathological from reference range values. A large number of often incomplete follow-up data accumulate not only for patients presenting with an established diagnosis of a monoclonal malignancy, but especially in cases where results are inconclusive.An additional dedicated follow-up page summarizes up to a 5-year time span of relevant data.
This combination of mathematical analysis, visual presentation and disease related information provides rapid, objective criteria for better and faster clinical diagnosis and disease follow up.

Patient and laboratory management

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INFIRMARY SATISFACTION SURVEY

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Background. Survey of laboratory customer satisfaction is one of the Norma ISO 9001:2008 requisits. The opinion of infirmary users is very valuable to improve our preanalytical processes, because patient preparation, samples taken and delivery absolutely depend of this professional group.
In a 24 hour laboratory (LAC) the response time and easy access to information are essential aspects about which is important to know our infirmaryopinion.
Methods. Satisfaction survey performed for infirmary in our hospital CHUAC during the first half of 2010.
Statistic analysis using MS Excel.
Results. Different stages for the performance of the satisfaction survey:
A) Quote: Group selection between the Services mostly demanding 24 hour laboratory petitions. Emergency Room, Anesthesia, Intensive Care Units, Cardiology, General Surgery, Digestive, Internal Medicine, Paediatrics, Neumology and Nefrology.
B) Questionnaire preparation, related to previous surveys and the new implementation we would like to evaluate. The previous year we had started the use of special security bags for samples transport.
Nine questions are prepared to de valued from *0* to *10*.
The tenth point encourages the nurse to make suggestions or complaints to help us to improve our service.
C) Delivery of the questionnaire and determination of the time of reply (15 days) questionnaires are given in envelopes identified by clinical Services to each infirmary supervisor. (386 questionnaires in total)
D) Statistical analysis and satisfaction report
Replied: 264. Our sample size allows the estimation of satisfaction percentage, with a 95% of reliability and a precision of 3.5 %.
Population Standard deviation: 1.399
Confidence level: 95%
We consider satisfaction level: 5 or higher.
Global score of survey: 6.74 improving the last result obtained (6.20).
Participation also has rised from 39,60% to 65.7%.
91.29% of the infirmary users are satisfied 241 with confidence interval 87.7-94.9%.
E) Results about determined questions:
· security bags for simples transport: 7.28 (±2.56) [median 8 range 0 to10]
· Results time: 6.02 (±2.31) [median 6 range 0 to 10] has increase comparing to previous survey.(5.90)
· Computer access to laboratory Results. 7.31 (±2.43) [median 8 range 0 to 10].This is the best valued question of the whole questionnaires, and also improved since the previous survey (6.67)
Conclusions. Satisfaction survey to infirmary group allows the analysis to improve our processes, especially those related to preanalitical stages. Identification of the questionnaires by departments allows the identification of particular problems needing some continuous improving activity (need of enlarging the neumatic tube, specific problems of neonatal samples, and so on)

Patient and laboratory management

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CLINICAL LABORATORY REDESIGN AIMED AT IMPROVED TURNAROUND TIMES

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Background. Disproportionate turnaround times (TATs) constitute a serious problem in clinical chemistry. In the recent past, total and laboratory TATs in our University Hospital were unacceptably long. In order to improve our Department's performance, we proposed a number of changes aimed at Lean processing and a laboratory TAT <60 min and <120 min for stat and routine samples, respectively.
Methods. We measured initial laboratory TATs, divided into specific activities including time on the Roche Modular Pre-Analytics (MPA) and either of two Modular P800 chemistry systems. Then several changes were implemented: (i) partition of samples for clinical trials versus routine diagnostics, by obtaining laboratory equipment dedicated to trials; (ii) elimination of unnecessary waiting time and movements, with handling according to the one-piece flow principle (i.e., no batching); (iii) optimisation of MPA settings; (iv) optimisation of analyser use by reallocation of assays between devices, depending on production volumes. Finally, TATs were measured again.
Results. Initially, average TAT (from arrival of the chemistry tube at the laboratory until availability of results to the clinician) measured in a random set of samples (n=63) was 84 min (range 46-165 min). After implementation of all measures, mean TAT (n=63) was 55 min (range 35-95 min). With tolerance set at 60 min, the fraction of samples within the tolerance limits over several days improved from 79% (n=2042) to 87% (n=890; 1.07 to 1.25 sigma).
Conclusions. There was a large improvement in performance. Yet, we recommend additional measures, including quality and rapidity awareness training for our employees.

0915

HOW CAN SIX SIGMA BE APPLIED IN IMPROVING THE SERVICE OF MEDICAL LABORATORIES TO PATIENTS?

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Background. Six Sigma is one of the different quality tools within the Health Care industry. It is a methodology to reduce errors and defects by applying different statistical and managerial tools. Although Six Sigma can be applied to various areas of medical laboratories, this study focused on improving customer turnover times at the phlebotomy sites of our laboratory. Customer turnover time at phlebotomy consists of times from arrival of patient to registration, from registration to phlebotomy and the actual time of phlebotomy. Recent internal audit revealed that the latter two time components were sufficiently short, but prolonged customer waiting times were mostly due to the length of registration.
Methods. We investigated with Six Sigma the variation of registration times on a day to day basis. Results were analysed with boxplots, homogenity of variances ANOM and ANOVA methods and we tested normality with Minitab. Registration times were examined by Weibull analysis too.
Results. Average patient registration time was significantly longer (13 min) on Mondays and Tuesdays, than on any other days (4.5-6 min). Registration time was more prolonged in the early hours of the days due to higher traffic of patients, in spite of scheduled appointments, for which several causes were identified.
Conclusions. This pilot project led to an action plan to improve patient turnover time and customer satisfaction and served as a pilot to demonstrate how Six Sigma methods can be used in quality improvement of medical laboratory services.

Patient and laboratory management

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THE DEVELOPMENT OF THE URINE LABORATORY IN BULGARIA SINCE THE YEAR OF 2000

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Background. During the second half of the 20-th century the health system in Bulgaria is owned by the state. Since 2000. The system is reformed. Laboratories with different forms of ownership are created. It is interesting to clear the tendencies in the development of the urinelabs and estimate the technical and analytical practice there.
Methods. We use a questionnaire method is. 72 laboratory workers answered questionnaires about their analytical methods, reading apparatus, microscopes and the sediment analyses
Results. At the beginning of the period 95% of the participants analyze urines with wet tests. Sediment is made without coverglass on a low quality microscope. Less than 5% use a good microscope for urines. Phase contrast is not used. At the end 1/3 answered that they use strip readers (two say they possess a reader, but not in use because of high consumable prices). In 2010 in Bulgaria 5 fully automated stripreaders are in use, automated sediment analyzers are not yet put into practice. We estimate an improvement of the quality of microscopes – excellent in 8%, good – 43%, 5% possess phase contrast microscope, one – with polarized light. 100% of the technicians and 57% of laboratory physicians would participate in a urinalysis teaching course.
Conclusions. We estimate a positive development in the conditions in the urine sector. The low level of the situation at the beginning is because of the low reimbursement of the urinalyses and the priority of development of hematology and clinical chemistry during the first years.

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QUALITY INDICATORS TO IMPROVE TURN-AROUND-TIME AND EFFICIENCY OF INTEGRATED PREANALYTICAL AND ANALYTICAL AUTOMATION SYSTEMS

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Background. Modern diagnostic laboratories are expected to deliver high quality and cost-effective services. To achieve these aims, we developed 30 pre-analytical, analytical and post-analytical quality indicators for monitoring the performance of key laboratory processes. The most frequently used indicator is turn-around-time (TAT). There are various ways of calculating TAT and the different components incorporated should reflect the total diagnostic process and be clearly defined to allow deeper analysis and benchmarking.
Methods. Various components of TAT were studied on an integrated pre-analytical and analytical automation system (Roche) using process analysis. Time data for each step in the process were retrieved from the laboratory information system. After obtaining baseline TAT data, changes were made to workflow and TAT was calculated again. Other parameters, such as the efficiency of analyzer utilization and quality control were also investigated.
Results. Analysis of individual TAT components linked to process analysis helped optimizingthe workflow of integrated automation systems and led to an apparent, 8-18% improvement in routine TAT. Analysis of internal quality control process indicators decreased the ratio of control/patient measurements from 6.2% to 2.7%, without compromising the quality of patient Results.
Conclusions. Detailed analysis of individual components of TAT on automated systems helps the optimization of workflow, resource utilization and cost-effectiveness of laboratory services, and results in significant shortening of TAT itself.

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LABORATORY PROCESS IMPROVEMENT BY WORKFLOW ANALYSIS

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Background. Process improvement is an important management goal for laboratory managers. Workflow analysis helps to making the correct decision and to meet the desirable outcome. This study has applied this approach to enhance laboratory process performance.

Methods. The experience involved chemistry/immunochemistry section of Laboratory Medicine department, processing 1800 samples and 17200 tests daily, by total laboratory automation with Modular Preanalytics and no. 2 Modular Analytics SWA (Roche Diagnostics, Basel, Switzerland). The workflow analysis, using the software Leonardo (Roche Diagnostics), was applied to the workload before and after the flow changes, choosing a day as representative sample.

Results. The baseline evaluation showed the sample delivery and check in within 11 a.m. for 53% of total workload. This produced at 12 a.m. an overload of maximum capacity for preanalytical and analytical modules by 50% and 8%, respectively. The turn-around time value, from check in to result about 90th sample percentile, resulted 115 min. The workflow analysis suggested intervention on sample transport timing. So the collection routes from external phlebotomy centers were modified, a new run was added and top hospital users were prioritized along laboratory processing. The results were the sample check in within 11 a.m. for 65%, a turn-around time reduction of 24% to 88 min and the absence of instrumentation overload at any time.

Conclusions. The study shows that workflow analysis makes the laboratory process improvement more effective. Moreover, in total laboratory automation layout the preanalytical changes have significant effect on the analytical phase, as well on the overall process.

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THE REFERENCE RANGES OF BIOCHEMICAL ANALYTES IN LITHIUM HEPARINIZED PLASMA SAMPLES

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Background. The reference range is one of most important value to help clinicians interpreted laboratory Results. Recently, Thai laboratories trend to increasing use lithium heparinized (LH) plasma. The biochemical results provided from serum and plasma, might be different in reference ranges, which affected to clinical interpretation. The study established the reference ranges of blood biochemical analytes from LH plasma and evaluated stability of biochemical analytes in LH plasma, which were affected by time and temperature during preparation and storage. The concentration of biochemical analytes were compared between healthy volunteers and patients.

Methods. The blood samples from 120 healthy volunteers and 30 patients were collected to prepare serum and LH plasma (except sodium fluoride, NaF plasma for glucose determination). All samples were measured for 18 biochemical tests by using the automated analyzer including glucose, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, uric acid, total bilirubin, direct bilirubin, total protein, albumin, aspatate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), sodium, potassium and chloride. The Non-parametric reference intervals were used following the recommendation from CLSI C28-A3/IFCC, and pair *t*-test used to comparing data between different groups. All statistic tests were calculated by SPSS program.

Results. The finding reference ranges were established and presented along with manufacturer reference ranges. Stability of blood glucose concentration from LH plasma was decreased when standing after 2 hours at room temperature. Potassium concentration in LH plasma was increased when stored after 3 hours in a refrigerator (2-8 ⁰C). The comparison of 18 analyte concentrations between serum (NaF plasma for glucose) and LH plasma samples from 30 healthy volunteers and 30 patients (with diabetes mellitus, liver disease and renal disease for 10 patients each) were shown that LH plasma samples could be used for glucose, HDL-cholesterol, direct bilirubin, AST, ALT and creatinine instead of using serum samples.

Conclusions. The finding may provide the information to suggest that Medical Technologists and Clinicians should be careful to use the appropriate reference ranges, when performing at individual laboratory, along the interpretation of laboratory Results.

Analyte (Units)	Metabolism (mg/dL)								Proteins (g/dL)	
	GLU	CHOL	TG	HDL	LDL	UA	TB	DB	TP	ALB
	71-97	144-209	40-198	M= 29-76 F= 38-102	64-137	M= 4.2-8.5 F= 2.6-6.1	0.3-1.0	0.06-0.26	6.8-8.5	4.0-5.0

Analyte (Units)	Enzymes (mg/dL)			Kidney Function (mg/dL)		Electrolytes (mmol/L)		
	AST	ALT	ALP	CRE	BUN	Na	K	Cl
	15-39	9-35	32-77	M=0.71-1.02 F=0.48-0.87	6.2-17.0	132.6-139.5	3.46-4.47	100.4-106.3

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WEBSITE REFLECTIVE TESTING IN PRIMARY CARE

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Background. Laboratory examination in primary care is often used to monitor patients with known diseases. Furthermore, laboratory testing is applied for screening patients, since the differential diagnosis in primary care is often not very specific. In such cases, abnormal results can be found that may indicate some unexpected pathology. Recognition and interpretation of abnormal results by the laboratory specialist may be helpful for general practitioners. The laboratory specialist can interpret the abnormal test results and determines whether additional tests are needed. In most cases comments are added to the report. This way of consultation is called reflective testing. The newly launched website www.reflectivetesting.com provides detailed information on reflective testing in primary care.
Methods and Results. The procedure of reflective testing was introduced by our laboratory to 155 local general practitioners in June 2006. In 2010 the website was launched in Dutch. The website was designed to be used in several ways: 1) search for a specific laboratory test, or 2) search for a specific disorder/disease. Information is given about possible follow up research (e.g. additional tests), rationale (using evidence based guidelines as much as possible), comments to be used to complete a laboratory report and an accompanying example. The website is now available in English; in the future we plan to translate the website also into Spanish.
Conclusions. The website is a sophisticated, useful and workable tool for laboratory specialists to support the procedure of reflective testing in primary care.

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REFLECTIVE TESTING HAS A FAVORABLE EFFECT ON ASSESSING CASE REPORTS BY GENERAL PRACTITIONERS

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Background. One way of providing consultation by the laboratory concerns addition of tests and/or comments to laboratory reports (also called reflective testing). The Atrium Medical Centre in Heerlen introduced this procedure to local general practitioners (GPs) in 2006. GPs linked to the JeroenBoschHospital in Den Bosch are not familiar with this procedure. We compared assessment of case reports by GPs in both regions, and examined whether this was influenced by reflective testing.
Methods. A list of 13 cases was prepared and sent to the GPs. They were asked about their working hypothesis and subsequent action(s) they would take. The lists were judged by their agreement with the suspected diagnosis as determined after adding additional tests.
Results. 56 GPs from Heerlen and 31 from Den Bosch returned the list. Assessment of case reports was very diverse: 81.6% of GPs (Heerlen and Den Bosch) had an appropriate working hypothesis in the case of sub-clinical hypothyroidism, while only 5.9% correctly assessed the case of diagnosing M-protein. Furthermore, an adequate working hypothesis could be made by a significantly higher percentage of GPs in Heerlen (50.8%) compared to GPs in Den Bosch (38.2%; p<0.001). The difference in assessment between the regions was most pronounced in the case of hemochromatosis.
Conclusions. GPs that were familiar with reflective testing assessed case reports more accurately compared to GPs that are not provided with this way of consultation. Adding commentary to laboratory reports possibly leads to a learning effect by GPs, especially in less common diseases.

Patient and laboratory management

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IMPROVING WORK FLOW BY THE IMPLEMENTATION OF LEAN 6 SIGMA PROCESSES

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Background. Lean and 6 Sigma are process improvement methodologies which have been applied to a number of sectors including health care. In pathology, the improvements resulting from implementation of Lean 6 Sigma include work flow and turnaround time (TAT), quality, error rates and cost reduction.
Methods. This project used a process of team building, workflow analysis, client and staff surveys and use of Define, Measure, Analyse, Improve, Control (DMAIC) methodology with the aim of improving workflow through the Core Laboratory and by extension the turnaround time for specimens and the quality of the work performed as measured by errors rates.
Results. A 'Lean Team' was formed via an expression of interest and this team has identified problems within the Core Laboratory and suggested solutions. This process identified a number of areas for improvement which have undergone the DMAIC process. A number of Lean activities have been undertaken including a pre-analytical (phlebotomy and specimen processing) workflow analysis, client and staff surveys, telephone audit, 5S activities (Sort, Set, Shine, Standardise and Sustain), implementation of 6 Sigma based Quality Control processing and a restructuring and automation of the Central Processing Unit (CPU) all of which have led to an improvement in specimen processing, TAT and error rates within the Core Laboratory. However, as predicted, some enthusiasm has been lost as the 'low hanging fruit' activities have been completed and more difficult projects identified.
Conclusions. Undertaking a Lean 6 Sigma journey can be useful, even for small laboratories, with many benefits to be gained.

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ACCREDITATION ACCORDING TO ISO 15189 – OUR EXPERIENCE

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Background. Quality medical laboratory are an integral part of health care, medical research and the key partners in patient safety and public health system. Key component these action is the enforcement of quality assurance services through accreditation by ISO standards.
Methods. ISO 15189, based upon ISO 9001 and ISO 17025, requires that medical laboratories comply with requirements for quality management and technical requirements, including pre- and post-analitical phases, as well as the analytical process itself. Laboratory ethics and safety are also included.
In Serbia, an ISO 15189 accreditation system was started in 2009. Laboratories of Center for medical biochemistry Clinical Center of Serbia are the leading in our country and they had previous of long standing experience with ISO 9001 certification and 17025 accreditation.
Results. Our laboratories was accredited for 500 tests and accreditation scopes contains methods for testing parameters of clinical chemistry, hematology, hemostasis, and immunology. Quality management system is documented and processed electronically. Since 2008. our laboratory has LIS system. Also, we participate in 5 different PT programs and like as reference institution in our country, we organizes and perform national external quality control. Over 100 laboratories participate in this program, 2 cycles per year.
Conclusions. Accreditation, data management, personnel education, external quality control programs and demonstrate of competence to a third party assessor improve laboratory services and business processes, increases the quality of the results, motivates personnel and is beneficial for all interested.

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EVALUATION OF LABORATORY PERFORMANCE IN PROFICIENCY TESTING PROGRAMS FOR IDENTIFICATION OF PRE-ANALYTICAL ERRORS IN CHEMISTRY

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Background. Advances in laboratory automation have led to significant reduction in analytical errors. Reports indicate that most laboratory errors are now in the pre- and post- examination phases. Proficiency testing (PT) has traditionally been used to assess analytical quality. PT performance may reveal lapses in pre- and post-examination phases creating opportunities for continual improvement.
To identify pre-analytical errors by comparing the laboratory performance in 2 PT programs for Chemistry.
Methods. Analysis of performance for chemistry parameters in the two PT programs between January and August 2010 was done. HUQAS uses Liquid stable PT material while RIQAS uses lyophilized samples. RIQAS samples are analyzed every fortnight while in HUQAS 5 PT samples are run at a go every 4 months. Results were analyzed as Standard Deviation Index (SDI) based on consensus means. SDI more than +3 or less than – 3 is considered to show significant bias. Non concordance between RIQAS and HUQAS biases was noted.
Results. More random biases were reported from RIQAS than HUQAS. In 80% of analytes showing significant bias on RIQAS , the HUQAS report for the same analyte was within acceptable performance limits . Furthermore, the subsequent RIQAS report showed the analyte within acceptable limits of performance in 95% of the cases. Pippetting errors were identified as cause of difference in PT performance.
Conclusions. Comparison of PT performance enables the laboratory to identify pre-analytical errors if the PT materials undergo different processing steps. Results can be used as a tool for continual quality improvement.

Pharmacogenetics/pharmacogenomics

0925

DIFFUSE ALVEOLAR HEMORRHAGE ASSOCIATED WITH *VKORC1* AND *CYP2C* POLYMORPHISMS

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Background. Diffuse alveolar hemorrhage (DAH) can be a life-threatening complication. Recently, we found that DAH is associated with vitamin K epoxide reductase (*VKORC1*) and cytochrome P450 *CYP2C9* and *CYP2C19* variant alleles in patients using oral anticoagulation therapy (Wijnen, P.A. *et al.* Mol Diagn Ther 2010;14:23-30).
We hypothesized that the DAH occurring in eight patients after inhalation of paint thinner, turpentine, textile protector and/or cleaning product fumes, that can work as so-called superwarfarin, also might be associated with these polymorphisms.
Methods. *CYP2C9**2/*3(C430T/A1075C), *CYP2C19**2/*3(G681A/G636A), and *VKORC1* (G-1639A/C1173T) single nucleotide polymorphisms were genotyped, using real-time PCR on a LightCycler.
Results. In all eight patients (mean age 32.6±13.2; 7 males), at least one relevant variant allele was found. Five out of eight patients (62%) displayed a combination of variant alleles, compared with 43% (74/173) in healthy volunteers. In three patients *CYP2C9* heterozygote allelic variants (one *1/*2 and two *1/*3) were observed and one patient had a *CYP2C19**1/*2, together with *VKORC1* variant alleles. In one patient *CYP2C9**1/*2 and *CYP2C19**1/*2 variant alleles and no *VKORC1* variant alleles were found. Three patients displayed either *CYP2C9**1/*2, *CYP2C19**1/*3 or *VKORC1* AA/TT variant alleles.
Conclusions. DAH due to toxic fume inhalation might be associated with *VKORC1*, *CYP2C9*, and *CYP2C19* polymorphisms. In cases of unexplained DAH, genotyping for these polymorphisms is recommended.

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TACROLIMUS-IMMUNOSUPPRESSIVE DRUGS IN AN ORGAN TRANSPLANTATION

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Background. Genetic factors play an important role in individual variability of pharmacokinetic or pharmacodynamic characteristics of immunosuppressant drugs. All existing immunosuppressant have a very narrow range of ordinariness. To low doses of the drug can lead to rejection of the graft, while to high doses lead to infection, nephrotoxicity, cardio toxicity, hypertension, hyperlipidemia, diabetes mellitus and even cancer. Tacrolimus is indicated for the prevention of organ rejection in patients receiving liver, kidneys or heart.
Methods. Two methods are available for analysis of tacrolimus-MEIA and ELISA. Both use the same monoclonal antibody for tacrolimus primary compound. The whole blood matrix and samples should be collected in tubes containing EDTA as anticoagulant. Samples not analyzed immediately should be stored in a refrigerator and analyzed within 3 days, and if should be kept longer be frozen a -20ºC for no longer than 12 months.
Results. Concentrations of tacrolimus vary the most during the first week of dosing. During the first 3 months the concentration is maintained between 7-20 ng/ml and 5-15 ng/ml in 1 year. In pediatric patients with liver transplants are required and tolerated higher doses (0.3 mg / kg/ day orally) than in adult patients to achieve similar concentrations in the blood.
Conclusions. Monitoring of tacrolimus blood with other laboratory and clinical parameters are considered an essential aid to the patient. Afterthe operatingperiod, measuringtacrolimusshould beonevery1-3days. After the patient leaves the hospital the frequency of monitoring will decreased with time after transplantation.

Pharmacogenetics/pharmacogenomics

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REDUCED OVERALL SURVIVAL OF CYP2C19 *2/*2 HOMOZYGOTES AFTER MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Patients with allelic variations of cytochrome P450 enzymes have been shown to have worse outcomes after hematopoietic stem cell transplantation (HSCT). CYP2C19 is responsible for the metabolism of many drugs and null variants such as *2 and *3 are common. Patients are classified as extensive (EM) (*1/*1), intermediate (IM) (*1/*2, *1/*3) and poor metabolizers (PM) (*2/*2, *2/*3, *3/*3). We hypothesized that allelic variations in CYP2C19 may lead to worse outcomes after HSCT due to altered metabolism of CYP2C19 substrates.
Methods. We analyzed DNA samples from 344 patients who received cyclophosphamide conditioning and total body irradiation prior to allogeneic HSCT. Genotyping was performed to detect the *1,*2 and *3 CYP2C19 alleles. Progression-free survival (PFS) and overall survival (OS) were determined for EM, IM and PM.
Results. CYP2C19 PM had significantly worse OS and PFS than EM and IM – nine of the twelve *2/*2 homozygotes died within a year of HSCT (adjusted HR=2.78, p=0.004, and 2.38, p=0.008, respectively). Of the drugs metabolized by CYP2C19, seven of the nine patients who died received voriconazole and four received omeprazole. None of the three surviving patients received either drug. PM have significantly higher concentrations of voriconazole than EM and IM, which may lead to direct voriconazole toxicity and exacerbate drug-drug interactions and altered concentrations of immunosuppressants and antibiotics.
Conclusions. Further investigation into the causes of poor outcomes in this patient subset is warranted. In the interim, we propose testing for the CYP2C19 *2 allele in patients undergoing HSCT and careful monitoring of drug levels.

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CYP2C19 MRNA EXPRESSION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS STIMULATED WITH RIFAMPIN IN VITRO

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Background. Cytochrome P450 (CYP)2C19 enzyme metabolizes many pharmaceuticals, while it is well known to have an inherited single nuclear polymorphism. The frequency of the CYP2C19 poor metabolizer (PM) phenotype is about 20% in Japanese. CYP genetic variants affect the pharmacokinetics. Some studies have suggested that the use of CYP gene mRNA expression in peripheral blood mononuclear cells (PBMC) as surrogates for measuring hepatic enzyme activity. We have measured CYP2C19 mRNA in PBMC of extensive metabolizer (EM) and PM with real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR).
Methods. Peripheral blood samples from healthyadults (phenotype of EM and PM of CYP2C19) were used in this study. Human PBMC were isolated by Ficoll-Conray density gradient centrifugation and washed with in phosphate-buffered saline. Human PBMC (2x10^{6}cells) in RPMI 1640 medium were treated with or without CYP2C19 enzyme-induciblerifampin, and then incubated at 37°C with 5% CO_2.After incubation, the cells were collected and applied to RT-PCR. Product of cDNA were amplified real-time PCR to determine mRNA of CYP2C19 and *h*GAPDH as an internal standard.
Results. When PBMC were stimulated by rifampin, the expression of CYP2C19 mRNA was observed. The expression of CYP2C19 mRNA in EM was higher than that in PM under the experiment.
Conclusions. The measurement of CYP2C19 mRNA levels in PBMC with real time RT-PCR seems useful for distinguishing EM and PM in human population.

Pharmacogenetics/pharmacogenomics

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ASSOCIATION BETWEEN ULTRARAPID CYP2D6 POLYMORPHISMS AND ADVERSE DRUG REACTIONS AMONG WOMEN WITH BREAST CANCER TREATED WITH TAMOXIFEN

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Background. Association between genetic variants of CYP2D6 (Poor Metabolizer PM) and ineffective therapy with tamoxifen (TAM) in women treated for estrogen receptor positive breast cancer was recently reported. In this study we evaluated whether CYP2D6 variation (Ultrarapid Metabolizer UM) is associated with adverse reactions in women treated with TAM.
Methods. 25 patients on therapy with TAM, with breast cancer (stages I-III), no chemotherapy or endocrine treatment other than tamoxifen, no metastases at diagnosis, estrogen receptor positive, were analyzed for the presence of 16 genotype variants of CYP2D6 by INFINITI™ CYP2D6 assay, which utilizes AutoGenomics proprietary film-based microarray technology.
Results. The prevalence of Extensive, Intermediate, Ultrarapid and Poor Metabolizers (EM, IM, UM, PM) were respectively 44% (11/25), 36% (9/25), 12% (3/25) and 8% (2/25), comparable with what has been previously reported in the Caucasian population. Tamoxifen adverse reactions evaluated (hot flashes, endometrial thickening, headache, spotting, cramps and varices) were present in all UM patients (3/3) but only in 2 out of 11 (18%) EM patients (difference statistically significant, p=0.027).
Conclusions. Based on these results a pharmacogenetic approach (right dose for the right patient) in the treatment with TAM might be economically justified in the near future not only by improving the effectiveness of TAM therapy in PM patients, but also by potentially reducing adverse reactions in UM patients and hence cost management.

0930

DETECTION OF CYP2C19 GENE POLYMORPHISM FROM NONINVASIVE SAMPLES BY CYCLING PROBE TECHNOLOGY

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Background. The frequency of cytochrome P450 (CYP)2C19 poor metabolizers (PM) is much higher in the Japanese population than in European populations. The CYP2C19 exhibits significant gene polymorphism, and consequently a higher incidence of side effects among patients with CYP2C19 substrates might be increased. Cycling probe technology (CPT) is a simple signal amplification technique for specific target DNA sequence. CPT utilizes a chimeric DNA-RNA-DNA probe that is cleaved by the enzyme RNase H. In this study, we detected the CYP2C19 gene polymorphism from noninvasive samples by CPT to determine whether Extensive Metabolizer (EM) or PM of CYP2C19.
Methods. DNA samples were extracted from hair, buccal mucosa and blood cells according to the manufactured instruction. Primer and cycling probe were designed for specific region of G636A for exon 4 and G681A for exon 5 reported for gene polymorphism of CYP2C19. DNA samples were genotyped by CPT andcompared with a commercial kit of TaqMan probe method.
Results. DNA gene extracted from hair follicle cells andbuccal epithelial cells was the same from invasive blood collection. All of the sample were successfully identified the genotype of CYP2C19 for EM or PM, and also the results were identical to Taq Man method.
Conclusions. We havesuccessfully detected the two gene polymorphisms of CYP2C19 from noninvasive samples using CPT. DNA results from hair follicle cells, buccal epithelial cells and whole blood cells are comparable.

Point of Care Testing

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VKORC1, CYP2C9 AND CYP4F2 GENETIC BASED ALGORITHM FOR WARFARIN DOSING. PRELIMINARY RESULTS OF A PROSPECTIVE ITALIAN STUDY

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Background. We have previously developed a pharmacogenetic algorithm (PG) for warfarin dosing based on *VKORC1*, *CYP2C9* and *CYP4F2* gene polymorphisms, Body Surface Area and age. We verified in a prospective randomized study whether our algorithm (PG arm) has any clinical advantage over standard warfarin dosing (STD arm).

Methods. 38 patients (age >18y; atrial fibrillation; target INR2-3) were enrolled from October 2009. Randomization: 17 STD arm, 16 PG arm, 5 drop out. *VKORC1* (-1639G>A SNP), *CYP2C9* (*1,*2,*3 alleles) (INFINITI™ Analyzer, Medical System) and *CYP4F2* (*1,*3 alleles)(Taqman chemistry) polymorphisms were analysed within 24 hrs from enrolment. INR was monitored on days 0,5,7,9,12,15 and 19 of treatment.

Results. INR findings over 3.5 were higher in the STD (7/102) than in the PG arm (1/96)(p=0.039). This difference was confirmed in patients under low maintenance dose <=20 mg/week (p=0.05), not in the other cases. Their relative risk of an INR finding >3.5 was lower in PG than STD arm (OR=0.828; 95% CI 0.68-1.00). To estimate in which arm INR results were closer to the target values, we applied the 6-sigma formula. After 12 treatment days higher 6-sigma values were always recorded among PG than STD arm (2.8 and 1.1 at day 15; 2.3 and 1.9 at day 19).

Conclusions. These preliminary findings suggest that the application of the PG algorithm might significantly reduce the risk of bleeding especially in patients requiring very low warfarin dosing and it could also allow INR to better fit within the defined range.

Point of Care Testing

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COMPARISON OF SIEMENS RAPIDLAB® POCT DEVICE VS SIEMENS DIMENSION VISTA® FOR ELECTROLYTE MEASUREMENT DURING CLINICAL ROUTINE

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Background. Point-of-care (POCT) electrolyte measurement is standard procedure in intensive care and emergency patients. While electrolyte measurements often influence acute therapeutic decisions, few reports have been published on deviations between measurements from POCT devices and laboratory analyzers.

Methods. We examined data from intensive care and emergency patients where within one hour electrolyte measurement was performed with POCT device (arterial full blood analysis, Siemens Rapidlab®) andlaboratory analyzer (serum, Siemens Dimension Vista®). No pre-analytical or therapeutic data were collected. In total, we examined 351 records of sodium, 353 of potassium and 231 of chloride. Furthermore we compared 280 POCT measurements of ionized calcium in full blood with laboratory measurements of total calcium.

Results.POCT measurements of sodium and potassium were significantly (f-test, $p<0.0001$) lower than laboratory measurements (median bias sodium: -2.2% [IQR -3.4% - -0.9%]; median bias potassium: -6.6% [IQR -10.1% - -3.9%]). Chloride POCT measurements were slightly, but significantly ($p<0.0001$) higher than laboratory measurements (median bias chloride +0.9% [IQR -3.2% – +5.2%]. Ionized and total calcium measurements cannot simply be compared. However, only in 95 (65%) of 184 patients with normal ionized calcium levels (POCT) were normal total calcium levels confirmed in the laboratory. 75/88 (85%) hypocalcemia cases diagnosed by POCT and 5/8 hypercalcemia cases were confirmed by laboratory measurements.

Conclusions. Despite some limitations (pre-analysis, nonspecific therapy), our examination highlightsseveral considerable deviations between POCT full blood measurements and laboratory serum analysis. Since some of these deviations may affect therapy, laboratory control measurements remain indispensable despite the advantages of POCT diagnosis.

0933

FIRST EXPERIENCES WITH THE SENSITIVE TRIAGE(R) CARDIAC TROPONIN I ASSAY IN THE EMERGENCY SETTING

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Background. Cardiac troponins are the preferred biochemical markers in the diagnosis of cardiac injury. Rapid determination is crucial in the emergency setting and a sensitive point-of-care (POC) devise might be of great interest. But the available POC devises often do not fulfil the criteria of standard laboratory Methods. In this study we compared the new sensitive Triage cTnI with the VITROS® 5600 cTnI.

Methods. In 206 patients of the emergency department with suspect myocardial injury, cTnI was measured on-side with the sensitive Triage cTnI assay and simultaneously in the central lab on a VITROS 5600. Both assays were compared to their diagnostic specificity using the lower detection limit (<0.01 ng/ml for the Triage and <0.012 ng/ml for the Vitros) and the 99th upper reference limit (<0.02 ng/ml and <0.034 ng/ml, respectively) as the cut-off.

Results. Using the lower detection limit, 120 patients were negative and 60 positive in both assays. In 22 patients the Vitros and in 3 patients the Triage were only positive. Using the upper reference limit, 144 were negative and 56 positive in both assays, 2 only with the Vitros and 4 only with the Triage. Correlation was cTnI (Vitros) = 1.101 x cTnI (Triage) + 0.045 (r = 0.966). Within the groups with discrepant results one patient out of each had a NSTEMI.

Conclusions. The sensitive Triage cTnI assay is a promising new assay for rapid and accurate determination of cTnI with a similar diagnostic efficiency and accuracy compared to a standard laboratory assay.

0934

INFLUENCE OF PO$_2$ AND HEMATOCRIT VALUES ON GLYCAEMIA MEASURED BY POINT OF CARE TESTING (POCT) GLUCOMETERS.

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Background. POCT glucometers are very widely used in clinical department, allowing fast diagnosis in emergency and better follow up without recurrent blood punction in intensive care unit. Results are considered correct if between ±20% of results obtained with standard laboratory techniques. In any case, results may be influenced by abnormal pO$_2$ or hematocrit, both frequent in hospitalized patients.
Methods. We compared the results of glycaemia obtained about 95 blood samples by Abbott-Optium-Xceed®, Nova-Biomedical-StatStrip-Xpress® and Roche-Accu-Check-Performa®, with those obtained in the laboratory using the hexokinase method on a Roche Cobas 6000 Analyser (reference method). PO$_2$ and hematocrit were measured on a GEM 4000 Analyzer.
Results. Correlations between glycaemia obtained by reference method and by POCT were good, r=0.933 for Xceed, r=0.982 for Xpress and r=0.958 for Accu-Check, respectively.
The influence of pO$_2$ differed among POCT analyzers. For Xceed, glycaemia was overestimated in 42% cases, when pO$_2$ was <8 kPa, and underestimated in 11% cases when pO$_2$ was >12 Kpa. For Accu-Check, glycaemia was underestimated in 24% cases whatever the pO$_2$ value, and for Xpress, glycaemia was overestimated in 4% of cases when pO$_2$ was >12 kPa.
The hematocrit also influenced Results. for Xceed, glycaemia was overestimated in 29% cases when hematocrit was <0.5 and for Xpress, glycaemia was underestimated in 5% cases when hematocrit was >0.37. For Accu-Check, glycaemia was underestimated in 21% cases whatever the value of hematocrit
Conclusions. Correlations with reference methods were good but glycaemia measurements obtained with POCT glucometers were influenced by pO$_2$ and hematocrit

0935

PERFORMANCE OF THE STATSTRIP GLUCOSE HOSPITAL METER IN PATIENTS WITH DIABETIC NEPHROPATHY

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Background. Interference of hemoconcentration/hemodilution in point-of care glucose testing is well described and correction algorithms used to overcome this problem have limited effects. StatStrip Hospital Glucose Meter provides unique correction for hematocrit, which is simultaneously measured with glucose on the same strip.
Methods. The performance of the point-of-care StatStrip Glucose Hospital Meter was assessed in patients with various stages of diabetic nephropathy, associated with anemia (decreased hematocrit levels). Results were compared to the reference hexokinase procedure on Olympus AU400 analyzer.
Results. Total of 74 diabetic patients with confirmed diabetic nephropathy were included in this evaluation [age-range=23–83, median= 62,5 years; male/female= 52/22, HbA$_{1c}$ range=4,5 to 10,4, median=6,8%]. Hematocrit levels ranged from 0,236 – 0,489 L/L, with 54,1% results below lower limit of reference intervals, and plasma glucose range was 2,0-17,6 mmol/L. Median glucose values were 9,2 and 9,0 mmol/L for hexokinase and StatStrip, respectively, with 0,2 mmol/L absolute bias, which is in accordance with desirable specifications for bias derived from intra- and inter-individual biologic variation (2,2%). Agreement with the hexokinase method was excellent: y = 0.9318x + 0.06705 (Passing Bablok regression) with no significant deviation from linearity (P>0,10). In a subgroup of patients with low hematocrit Passing-Bablok regression also showed excellent agreement with the hexokinase method (y = 0.9355x − 0,01613) with no significant deviation from linearity (P>0,10).
Conclusions. StatStrip Glucose Meter can be used as an accurate and reliable tool for monitoring glucose levels in patients with diabetic nephropathy associated with anemia.

Point of Care Testing

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COMPARATIVE STUDY OF FECAL CALPROTECTIN (FC) RAPID TEST WITH THE ESTABLISHED ELISA METHOD

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Background. FC is a useful marker for diagnosing inflammatory bowel disease (IBD). However, no automated assay is available yet and widely adopted ELISA methods show turnaround time of≥4h. We compared the point of care (POC) Quantum Blue FCimmunochromatographic test (Buhlmann) to the Calprest ELISA (Eurospital) routinely used in our laboratory.
Methods. Stool specimens of 67 consecutive patients were analyzed after extraction using recommended device. FC was determined on fresh samples by POC, while for the ELISA stool samples were frozen at -20 °C, and thawed, extracted, and assayed within 2 weeks from collection. We first compared results by Passing Bablok regression and then estimated diagnostic agreement by adopting 90 µg/g feces as cut-off for ELISAand an experimentally recalculated cut-off for POC.
Results. Method comparison, performed on 28 paired results higher than the POCdetection limit(30 µg/g), showed a slope of 2.24 (ELISA in x-axis), with not significant intercept. On the basis of this finding, the POC cut-off was established at 200µg/g. FC was positive on 20 samples by ELISA and on 17 samples by POC, with a 92.5% agreement. Among fivepatients showing discrepant results, 4 were positive on ELISA (two borderline – 94 and 98 µg/g – results) and one positive on POC. This POC "false-positive" related to a 16-years old girl, with chronic diarrhea but no IBD diagnosis.
Conclusions. Our preliminary results suggest that POC may replace the standard ELISA and make FC testing more rapid (<20 min), effective, and suitable for any laboratory setting.

0937

THE EFFECT OF INTERFERING SUBSTANCES IN POINT OF CARE GLUCOSE MEASUREMENTS

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Background. During treatment of patients, possible interfering substances might be present for Point of Care (POC) glucose measurements. Four POC glucose meters of different companies were tested for interfering substances and the correlation with the lab method.
Methods. The interference of Hematocrit, Vitamine C, Galactose, Maltose en HAES (plasma expander) was investigated with the Accucheck I (Roche), Accucheck II (Roche), Statstrip (Nova biomedical) en de 201 DM (Hemocue) POC glucose meters. All substances were measured at different concentrations and at 3 blood glucose concentrations. The Beckman DXC860i was used as reference method. The correlation between the 4 POC meters and the Beckman method was studied with 20 samples.
Results. Raising Hematocrit percentage underestimated the glucose concentration with the Accucheck I. Vitamin C overestimated the glucose with both Accucheck I and II. Galactose also interfered on both the Accucheck I and II, while Maltose only interfered with the glucose measurement at the Accucheck I. Both Statstrip and 201 DM glucose meters showed no or little effect on the glucose measurement in presence of the interfering substances. HAES caused a small underestimation of the glucose concentration at all POC meters. The method correlation was $R^2 > 0.996$ for all 4 meters, with a bias within the ISO 15197 limit.
Conclusions. All meters performed well in a correlation study with the Beckman DXC860i glucose assay. Both 201 DM and Statstrip meters were the least affected by the possible interfering substances, which might be a critical point in the selection for a POC meter.

Point of Care Testing

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CLINICAL AND ANALYTICAL EVALUATION OF THE EPOC BLOOD-GAS ANALYZER

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Background. The analyzer "Epoc Blood Analysis" (Alere Srl, Venice, I) is a bedside single cartridge system for the determination of blood gases, electrolytes and glucose. It consist of a base unit for the analytical process and a handheld palm wireless connected PC that can receive the data near the patient. Our study assessed the analytical performance and potential clinical efficacy of this tool.
Methods. 436 consecutive patients who accessed the accident and emergency department needed arterial blood gases. 38% of them were identified as red code priority on triage, 54% as yellow code and 8% as green or white code. The most common clinical presentations were: dyspnea 45%, disorders of consciousness 16%, chest pain 10%and others. The variables examined were pH, pCO2, pO2, hematocrit, Na, K, ionized calcium, glucose. Readings from the Epoc instrument were compared to those of a reference laboratory analyzer in 103 of these cases. We investigated imprecision, accuracy, and bias; timeliness and therapeutic benefit from the possibility to obtain the results earlier on the Epoc instrument served as outcomes.
Results. The correlation coefficients vs. reference instrument for pH, pCO2, pO2, K, glucose results were higher than 0.96. The imprecision level was consistent with the state of the art for these tests. Sigma performance metrics provided a value above 4 for the main variables.There was definite clinical benefit deriving from earlier treatment in 35% of patients, probable benefit in 30%.
Conclusions. the Epoc Blood Analysis can be safely applied within the context of critical care.

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NOVEL LABORATORY SYSTEM FOR RECORDING COLOUR REATIONS GENERATED BY POINT-OF-CARE TESTING DEVICES

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Background. In the field of clinical diagnostics, point-of-care testing (POCT) devices such as β-hCG, urine toxicology are frequently used in routine laboratories as their analytical method. ISO 15189 and other accreditation bodies require laboratories to keep the reaction results of their assays for extended periods eg. 2 years. Unfortunately, the colour reaction of many of POCT methods deteriorate with time and it is therefore not useful to keep the original cassette. As different devices use the presence or absence of lines as either a positive or negative result, the possibility of the misreading the cassette is significant. In the case of either a patient or a doctor challenging a result, there is no original record, only the worksheet or the result in the LIS (laboratory information system). Therefore, we developed a system to record the POCT colour reaction interpreted by the technologist.
Methods. Patients' laboratory barcodes are used to identify the individual POC cassettes. Once the technologist has entered the results in the LIS, an image is taken using a Webcam and transferred to our laboratory scanner. The laboratory barcode is the basis for recording and retrieving the cassette image. The saved images archived by the scanner are backed up nightly, as part of the hospital-wide network maintenance.
Results. On evaluation, the system proved to be 100% reliable for transfer, backup and retrieval of the images.
Conclusions. We developed a cheap, fast, easy-to-use system from commonly used laboratory software and hardware to record the POCT colour reaction.

Point of Care Testing

0940

FROM FUJI DRI-CHEM 4000I TO FUJI DRI-CHEM 7000I

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Background. This evaluation's aim is to examine the analytical performances and the practicability of the FUJI DRI-CHEM series (FDC4000i and FDC7000i, Fujifilm Corporation), compact instrumentations with ready-to-use reagents that use dry chemistry to analyze a wide range of general chemistry parameters, helping users in their choice of suitable tests.
Methods. The analysis was performed on 75 samples and the results were compared with those obtained on Modular Analytics DP (Roche Diagnostic GmbH).
Results. The precision evaluated on 2 control levels (Liquid Unassayed Multiqual, Bio-Rad Laboratories) underlines CV included between 0.00% (electrolytes, Mg) and 15.68% (P)
According to Passing-Bablok, the analysis of the comparison with Modular highlighted:
Constant and proportional bias: Cl= 103 (89 : 117), Ca= 2.27 (1.89: 2.65), CRE= 341 (21:661), GLU= 9.5 (4.4: 13.70), P= 1.57 (0.7:2.44);
As to practicability, both FDC are fast and easy to use and the loading of primary tubes with barcode is important for the operator's safety, sample traceability and for avoiding potential errors.
The TAT can vary according to the slide loading order in the cartridge ranging from 11':49'' to 9':43'' for 16 tests, using a volume of 250 μl of sample. Time is important for FDC7000i: the addition of samples in STAT and the loading of 5 samples simultaneously (supporting 50 tips and 20 dilution cups), allow better instrument efficiency and lower manual work by the operator.
Conclusions. Steps forward have been made with FDC solutions that becomes an excellent candidate for POCT.

0941

EFFECT OF MARKED FLUCTUATIONS IN HAEMATOCRIT ON POINT OF CARE BLOOD GLUCOSE SYSTEMS

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Background. Marked fluctuations in Haematocrit are complications of Critical Illness, Trauma and Surgery resulting from blood loss, fluid resuscitation, blood transfusion, inappropriate fluid therapy or severe dehydration. Haematocrit values outside the manufacturer's specified limits are contraindications to the use of POC blood glucose test systems. Within this study Roche Accuchek Inform II, Nova Stat Strip and Abbott PXP were critically compared to reference Roche Cobas B221 blood gas methodology.
Methods. 30 random heparinised venous blood samples were obtained from patients with Haematocrit values outside the manufacturer specified limits.
Results. Haematocrit values 18.64 ±5.16 (below manufacturers specified limits) mean blood glucose 7.8 ± 2.77 mmol/l cobas b 221, 8.14 ± 2.87 mmol/l Accu-Chek Inform II (positive bias 0.34 ± 0.19), 8.07 ± 2.75 mmol/l Nova StatStrip (positive bias 0.27 ± 0.16) 8.12 ± 3.17 mmol/l Medisense PXP (positive bias 0.44 ± 0.15).
Haematocrit values 72.52 ± 8.35 (above manufacturer specified limits) mean blood glucose 8.56 ± 3.24 mmol/l cobas b 221, 8.01 ± 3.06 mmol/l Accuchek Inform II (negative bias -0.55 ± 0.25), 8.12 ± 3.17 Nova StatStrip (negative bias -0.44 ± 0.15) and 8.00 ± 3.02 mmol/l Medisense PXP (negative bias -0.56 ± 0.34).
Conclusions. All POC systems demonstrated a negative bias with elevated haematocrit and positive bias with decreased haematocrit. Bland Altman analysis showed similar mean and bias derivation for all POC systems. This would not preclude their use in those clinical areas where this may occur, but does require effective education and training of all personnel.

Point of Care Testing

0942

CLINICAL EVALUATION OF POC BLOOD GLUCOSE TEST SYSTEMS IN PATIENTS RECEIVING TREATMENTS THAT CONTAIN, OR ARE METABOLISED TO, MALTOSE

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Background. Determination of glucose in patients receiving parenteral treatments that contain, or are metabolised to, maltose has been problematic due to maltose interference with POC chemistries It is important to determine the effectiveness of POC systems in the presence of maltose or maltose derivatives. Within this study Roche Accuchek Inform II, Nova Stat Strip and Abbott PXP were compared to reference Roche Cobas B221 blood gas methodology.
Methods. 50 paired venous samples were obtained from renal patients, whose treatment involved maltose-containing solutions.
Results. Blood glucose: 8.51 ± 4.50 mmol/l cobas b221, 8.52 ± 4.52 mmol/l Accuchek Inform II (mean bias 0.01± 0.24, limits of agreement -0.46 – 0.48), 8.49 ± 4.50 mmol/l Nova StatStrip (mean bias 0.004 ± 0.21, limits of agreement -0.41 – 0.42) and 8.56 ± 4.48 mmol/l Medisense PXP (mean bias 0.05 ± 0.34, limits of agreement -0.48 – 0.58)
Spearman Rank Correlation/Regression: Accuchek Inform II r^2=0.997, slope 1.005, intercept -0.028, Nova StatStrip r^2= 0.996, slope 0.999, intercept -0.008, Medisense PXP r^2= 0.994, slope 0.994, intercept 0.100.
Paired student T test: p =0.989 Accu-Chek Inform II, p=0.985 Nova Stat Strip and p=0.954 Medisense PXP.
Conclusions. Spearman Rank Correlation/Regression demonstrates that Accuchek Inform II, Nova StatStrip and Medisense PXP significantly correlate with reference methodology across the working. Paired Student T test demonstrates no statistically significant difference between reference and POC data. This study confirms that the performance of the Accuchek Inform II, Nova StatStrip and Abbott Medisense PXP systems are not adversely affected by the presence of maltose containing solutions.

0943

CLINICAL EFFECT OF HPERNATRAEMIA AND HYPONATRAEMIA ON POINT OF CARE BLOOD GLUCOSE SYSTEMS

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Background. Hyper and Hyponatraemia are not uncommon electrolyte disturbances in critical illness and across clinical disciplines and pathologies within hospitalised patients. Severe dehydration resulting in poor peripheral blood circulation or aggressive fluid resuscitation leading to oedema and fluid overload result in marked electrlolyte disturbances and are contraindications to the use of POC blood glucose test systems. Within this study Roche Accuchek Inform II, Nova Stat Strip and Abbott PXP were critically analysed compared to reference Roche Cobas B221 blood gas methodology.
Methods. 52 hyponatraemic and 50 hypernatraemic patients (normal range 134 – 144 mmol/l) were critically analysed for whole blood glucose using random arterial blood samples.
Results. Hypernatraemic subset (151.94 + 4.70 mmol/l), in comparison to reference b221 Accuchek Inform II mean bias = -0.17 + 0.29; limits of agreement = -0.74 – -0.40), Nova StatStrip mean bias = -0.03 + 0.29; limits of agreement = -0.60 – 0.54) and Medisense PXP mean bias = 0.02 + 0.39; limits of agreement = -0.74 – 0.78). Hyponatraemic subset (128.95 + 4.14 mmol/l), in comparison to reference b221 Accuchek Inform II mean bias = -0.05 + 0.22; limits of agreement = -0.48– -0.38), Nova StatStrip mean bias = 0.04 + 0.23; limits of agreement = -0.42 – 0.49) and Medisense PXP mean bias = 0.18 + 0.27; limits of agreement = -0.35 – 0.71.
Conclusions. Within this study, minimal scatter with no significant demonstrable bias was evident in both hyper and hypernatraemic subsets iwith all POC systems tested, which would not preclude their routine use in clinical practice.

Point of Care Testing

0944

CLINICAL EVALUATION OF SIEMENS DCA VANTAGE POCT SYSTEM COMPARED TO REFERENCE HPLC METHODOLOGY - CAN IT BE IMPLEMENTED AS PART OF A SINGLE VISIT SETTING IN THE ASSESSMENT OF THE DIABETIC PATIENT?

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Background. HbA1c is used for patient education/ counselling, feedback re diabetic control, improved patient motivation, and to effectively monitor management. Measurement should be accurate and precise and provided rapidly in a same-visit setting. To achieve this goal in the clinical setting POC solutions may provide this adjunct to care.
Siemens DCA Vantage was evaluated compared to Reference HPLC methodology in order to ascertain whether it provided the level of accuracy and precision for incorporation into a POC care pathway for Diabetic assessment.
Methods. 1. Precision of DCA Vantage determined using bi-level Quality Control Media (10 replicates).
2.Correlation: 50 random whole blood samples, were analysed for HbA1c using the DCA and compared to HPLC methodology.
Results. Precision: Quality Control Media Normal : NGSP Mean 5.42 % SD 0.06 CV% 1.17, IFCC Mean 36.1 mmol/mol SD 0.88 CV% 2.43. Quality Control Media Abnormal; NGSP Mean 10.44 % SD 0.13 CV% 1.29, IFCC Mean 90.4 mmol/mol, SD 1.34, CV% 1.49.
Correlation: HbA1c NGSP r^2=0.975, slope 0.94 intercept 0.27. Bland Altman analysis: Mean difference -0.20 ± 0.35 % with 95 % confidence interval –0.89 – 0.48%.HbA1c IFCC r^2=0.970, slope 0.93 intercept 2.37.Bland Altman analysis: Mean difference -2.42 ± 4.25 mmol/mol with 95 % confidence interval -10.76 – 5.91 mmol/mol.
Conclusions. DCA showed excellent precision with CV < 2.5 %.Correlation compared to HPLC was excellent for both NGSP and IFCC. Bland Altman demonstrated minimal scatter for both HbA1C % and mmol/mol. Pilot study suggests DCA can be used to effectively implement a same- visit setting.

0945

HUMAN-FACTORS ENGINEERING ENHANCES NEW POINT-OF-CARE BLOOD-GAS AND ELECTROLYTE ANALYZER

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Background. A project team from Roche Diagnostics Graz GmbH paid special attention to human-factors engineering during the development of the *cobas b* 123, a dedicated point-of-care analyzer for blood gases, electrolytes, metabolites, hemoglobin derivates, and bilirubin. One of the main goals of this process was to design the system to be as user-friendly as possible, while maintaining its high performance and versatility. The *cobas b* 123 is designed to be accessible to minimally trained users. Meeting their demands, especially in a critical care environment, requires a high standard of usability therefore the system must be intuitive to use.
Methods. Early in the development, the team initiated a human-factors engineering process, starting by thoroughly analyzing major user tasks (such as routine measurements or exchanging consumables), which led to the development of usability specifications.
Results. These specifications were then verified in serial tests with health care professionals in several countries. Beyond fulfilling the regulatory demands of IEC 60601-1-6, ease of use was the primary goal of the usability process.
Conclusions. The project team is convinced that the cobas b 123 achieves this goal, and that the new system is an innovative, no-maintenance blood-gas and electrolyte analyzer that, due to the fact that it is so easy to use, requires minimal operator training.

Point of Care Testing

0946

EVALUATION OF FOUR HOSPITAL USE POINT-OF-CARE GLUCOSE METERS IN AN INTENSIVE CARE UNIT

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Background. Implementation of tight glycemic control (TGM) in intensive care patients require frequent analysis of blood glucose. This can be achieved by accurate point-of-care (POC) hospital use glucose meters (GM). In this study four hospital use POC-GM were evaluated in intensive care unit patients.

Methods. All patients (n=80) requiring TGM were included. For each patient on the basis of their stay 3 to 6 measurements (n=394) were performed. Blood glucose was determined by Roche Accu-Check Inform II System, HemoCue Glu201DM, Nova StatStrip and Abbott Precision Xceed Pro (PXP). The glucose results from these POC-GM were compared with the results obtained from the central laboratory method, Beckman Coulter DxC 800. The criterions (95% values within the range) described in ISO 15197 and in TNO quality guideline (PG/TG/2001.045) were applied in the comparisons. All measurements were performed in the same heparinized arterial whole blood sample.

Results. According to the ISO 15197, 98.7% of the measured values by Roche fulfilled the criterion, whereas for HemoCue, Nova and Abbott these values were 94.9%, 94.7%, 97.7%, respectively. According to the TNO quality guideline the percentages by Roche, HemoCue, Nova and Abbott were 96.1%, 91%, 81.8%, 94.2%, respectively.

Conclusions. Twoof the most common hospital use POC-GM fulfilled ISO 15197, whereas only one fulfilled the TNO quality guideline in intensive care unit patients. This study contributes to the discussion on the necessity for guidelines describing the requirements for hospital use POC-GM.

0947

COMPARISON OF HBA1C ANALYZERS: VARIANT TURBO ® AND POINT OF CARE ANALYZERS (DCA SYSTEM, AFINION™, IN2IT™).

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Background. Point-of-care instruments for the measurement of hemoglobinA1c (HbA1c) may improve the glycemic control of people with diabetes by providing a rapid result if the performance of the instruments used is acceptable. The purpose of this study was to evaluate the performance and agreement among HbA1c values measured using selected analyzers of point of careand Variant Turbo ®.

Methods. HbA1c determined using DCA Vantage™, In2it and Afinion™ were compared with Variant II Turbo. Precision and method comparisons with Passing and Bablock regression were evaluated according to CLSI recommendations and kappa statistics.

Results. At HbA1c levels of 4.5 and 14.9%, total coefficients of variation (CV) for the In2it were 3.0 to 3.8%, for the DCA Vantage were 2.4 to 2.8% and for an Afinion™ were 1.4 to 1.7% depending on the level number of the controls. . The correlation coefficients of HbA1c were 0.97 for In2it vs. Variant II Turbo, 0.98 for DCA Vantage vs. 0.98 for Afinion™vs. Variant II Turbo. Kappa agreement statistics for the three diabetic control group HbA(1c) values of "less than 6.5%," "6.5%-7.5%," and "greater than 7.5%" for In2it vs. Variant II Turbo, Vantage vs.Variant II Turbo, and Afinion™ vs. Variant II Turbo were 1.000, 1.000, and 0.911, respectively.

Conclusions. These analyzers of point of care would appear to be a satisfactory analytical system for HbA1c testing in POC sites that could be controlled by central laboratories.

Point of Care Testing

0948

COMPARISON OF THREE HEMOGLOBIN A$_{1c}$ POINT OF CARE ANALYSERS WITH A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) REFERENCE METHODS

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Background. Interest in hemoglobin A$_{1c}$ point of care analysers to provide rapid turnaround results in diabetic care units isincreasing. To cope with the associated rise in assay requests, three point of care instruments were assessed.
Methods. A total of 56 total blood samples were analysed. The performance of three point of care instruments: DCA 2000+ (Bayer), Afinion (Axis-Shield) and A1CNow (Bayer) was compared to that of the HPLC reference method (Adams HA816 Menarini).
Results. The Hb A$_{1c}$ values of the studied samples ranged from 3.6 to 13.36 %. Good agreement with the reference method was observed for the three instruments: HbA$_{1c}$ [DCA 2000] = 0,89xHbA$_{1c}$ [reference]+1,05. Passing Bablok; r=0.993; n=30; slope =0,894 (95%CI 0,859 to 0,945); Intercept=1,05 (95%CI 0,71 to 1,28). HbA$_{1c}$ [Afinion] = 0,83xHbA$_{1c}$ [reference]+1,70. Passing Bablok; r=0.997; n=28; slope =0,831 (95%CI 0,808 to 0,857); Intercept=1,70 (95%CI 1,51 to 1,86). HbA$_{1c}$ [A1CNow] = 1,00xHb A$_{1c}$ [reference]+0,40. Passing Bablok; r=0.975; n=26; slope =1,000 (95%CI 0,923 to 1,111); Intercept=0,40 (95%CI -0,25 to 0,92). Bland Altman means differences: DCA 2000: 0,208 (95%CI 0,0791 to 0,336). Afinion: 0,35 (95%CI 0,183 to 0,517). A1CNow: 0,419 (95%CI 0,25 to 0,588)
Conclusions. Agreement with the reference method was good for the three instruments (r>0,9), however we observed constant and proportional differences in DCA 2000 and Afinion, so we chose A1CNow asthe most appropriated Hb A$_{1c}$ point of care analyser.

0949

IMPLEMENTATION OF A POINT-OF-CARE QUALITY CONTROL FOR BLOOD GLUCOSE MEASUREMENT

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Background. Point of care testing (POCT) is related to each laboratory testing outside clinical laboratories. It is very difficult to supervise all POCT instruments in an extensive medical centre with about 7.500 employees and 100.000 hospitalized patients per year. Furthermore, quality control of POCT is usually underestimated amongst medical personnel, as they are oblivious of performing internal and external quality control.
Methods. We formed a POCT-workgroup lead by laboratory personnel to organize a preliminary quality control of glucometers in medical wards within six clinics. We delivered a control serum sample with known glucose concentration of 14.4 mmol/L to 27 departments.
Results. We obtained77 glucose results with average and SD of 14.52±2.635 mmol/L, and 18.1% CV. All results were statistically elaborated within five groups regarding the instrument manufacturer. The less scattered results were in the Hemocue group (n=22) with average and SD of 14.20±0.463 mmol/L and 3.3% CV. The highest variability was observed in the Lifescan group (n= 11) with 23.4% CV. Average and SD values of glucose measurements in Abbott, Bayer, and Roche group were 18.12±0.750 mmol/L, 13.00±0.964 mmol/L, and 13.60±0.893 mmol/L, respectively. All glucose measurements showed variability below 20% except for one in Lifescan (5.8 mmol/L) and one in the Bayer group (16.6 mmol/L).
Conclusions. This preliminary POCT quality control was positively accepted in all medical wards. Therefore, a successful implementation of laboratory supervision of POCT instruments in an extensive medical centre is possible with a recurrent programme of quality control at least three times per year.

Point of Care Testing

0950

MULTI POINT OF CARE INSTRUMENT EVALUATION FOR ASSESSMENT OF BIOCHEMICAL PARAMETERS IN ARV CLINICS IN SOUTH AFRICA

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Background. South Africa has the largest prevalence of HIV infected individuals in the world. Provision of adequate anti-retroviral (ARV) therapy to this population is a primary concern. The introduction of point of care testing to ARV clinic sites is hoped to fast track initiation of patients on ARVs and to allow for earlier recognition of adverse effects such as dyslipidaemia, renal and hepatic dysfunction. We evaluated six point of care (POC) instruments for placement in ARV clinics. The following analytes were evaluated total cholesterol, HDL cholesterol, triglycerides, glucose, HbA1C,creatinine, AST, ALT and lactate

Methods. Comparisons with the central laboratory analyser were performed as well as precision, accuracy and interference studies. A weighted scoring system was developed by the authors to evaluate the instruments in terms of analytical performance, cost, ease of use and other operational characteristics.

Results. Our results show that analytical performance of the POC analysers were generally similar however there were significant differences in operational characteristics and ease of use. Bias for the different analytes when compared to the laboratory analyser ranged from -27% to 14%. Total allowable errors for the different analytes on the point of care instruments were acceptable at medical decision limits with the exception HDL cholesterol.

Conclusions. An important consideration regarding the utilization of POC instruments is whether the same clinical reference ranges, decision limits and standards of analytical performance can or should be applied. This study has led to the development of a POC analyser scoring system that incorporates analytical and operational characteristics that may be utilised by other centres.

0951

WHAT ARE THE „TRUE" PLASMA GLUCOSE REFERENCE RANGES?

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Background.POCT analyzers measuring glucose concentration in whole blood specimens are used to fast determination of blood glucose. The glucose level decreases during preanalytical phase as glucose is metabolized by blood cells.We studied changes in glucose concentrations over time in whole blood and corresponding plasma specimens to ascertain the degree of decrease in glucose concentration to model common practice of blood sampling and sending specimens before analysis.

Methods. Glucose was measured in 100 random K_3EDTA pair samples: whole blood specimens tested by Roche Reflotron analyzer and plasma samples tested by hexokinase method using Roche Integra 800 at time 0, 120, 240 and 360 minutes after blood sampling.

Results. Using Integra 800, median decrease of plasma glucose concentration compared to baseline (time 0) was 0.51 mmol/l within 2 hours after blood sampling, 1.02 mmol/l within 0-4th hours and 1.57 mmol/l within 0-6th hours. Median decrease of blood glucose concentration measured by Reflotron was 0.37 mmol/l in 2 hours, 0.81 mmol/l in 4 hours and 1.27 mmol/l in 6 hours. The concentration of glucose in plasma was in average 0.67 mmol/l higher than concentration in whole blood.

Conclusions. Decrease in concentration of glucose during preanalytical phase can lead to false decrease ofglycemic value. Therefore, early transport of sample to laboratory is essential. Our results imply clinical significance of these findings since – in a real life situation – the time gap elapsed between blood sampling and analysis may take rather a couple of hours, typically 2-4 hours in our settings.

Point of Care Testing

0952

GLUCOTROL-WB: A HIGHLY COMMUTABLE WHOLE BLOOD QUALITY CONTROL FOR GLUCOSE POINT-OF-CARE TESTING

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Background. Glucose point-of-care testing (POCT) is mainly performed on fresh whole blood. Whole blood is not suitable as a Quality Control (QC) for glucose analysis due to glycolysis. In currently used QCs the loss of glucose is prevented by changing the characteristics of blood or by using a plasma, serum or aqueous matrix.
Glucose measurements are based on different methods with its specific preanalytical and analytical factors. Due to the matrix alterations of existing QCs there is a noncommutability of the QCs with different glucose POCT devices.
GlucoTrol-WB is a glucose QC that is compatible with all glucose POCT devices, with minimal matrix effects.
Methods. The cell fraction and glucose spiked plasma fraction of whole blood were separately stored in a two compartment container. Prior to use, the two fractions were completely mixed to reconstitute a whole blood sample.
The commutability was tested on 22 different glucose POCT devices and compared to human EDTA blood and to existing glucose QCs.
Results. For a normal glucose level the CV of human blood was 12%, CV of GlucoTrol-WB was 14%, CVs of existing glucose QCs varied from 17% to 36% with errors on a few glucose POCT devices.
For a low and high glucose level the CVs of GlucoTrol-WB were 22% and 14%, CVs of human whole blood were 25% and 14%.
Conclusions. GlucoTrol-WBis a highly commutable whole blood glucose QC with a low imprecision compared to existing glucose POCT QCs and most comparable to fresh human whole blood.

0953

ACCURACY OF THE PRECISION® POINT-OF-CARE KETONE TEST EXAMINED BY LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTROMETRY (LC-MS/MS) IN THE SAME FINGERSTICK SAMPLE

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Background. The Precision® (Abbott Diabetes Care) point-of-care biosensor test strips are widely used by patients with diabetes and clinical laboratories for measurement of plasma β-hydroxybutyrate (β-HB) concentrations in capillary blood samples obtained by fingerstick. In the literature, this procedure has been validated only against the enzymatic determination of β-HB in venous plasma, i.e. the method to which the Precision® has been calibrated.
Methods. In this study, the Precision® Xceed was compared to a methodologically different and superior procedure: determination of β-HB by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in capillary blood spots. Blood spots were obtained from the same fingerstick sample from out of which Precision® measurements were performed. Linearity was tested by adding varying amounts of standard to an EDTA venous whole blood matrix.
Results. The Precision® was in good agreement with LC-MS/MS within the measuring range 0.0-6.0 mmol/L (Passing and Bablok regression: slope = 1.20 and no significant intercept, R=0.97, n=59). Surprisingly, the Precision® showed non-linearity and full saturation at concentrations above 6.0 mmol/L, which were confirmed by a standard addition experiment. Results obtained at the saturation level varied between 3.0 and 6.5 mmol/L.
Conclusions. The Precision® β-HB test strips demonstrate good comparison with LC-MS/MS. Inter-individual variation around the saturation level, however, is large. Therefore, we advise to report readings above 3.0 as >3.0 mmol/L. The test is valid for use in the clinically relevant range of 0.0-3.0 mmol/L.

Point of Care Testing

0954

COMPARISON OF EUROPIUM-DOPED NANOPARTICLES AND COLLOIDAL GOLD AS LABELS IN LATERAL FLOW BIOAFFINITY ASSAY

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Background. Lateral flow (LF) -based bioaffinity assays are currently among the most common formats of point-of-care testing (POCT). Since appearing in the 80's they have established their status in routine POCT. The simplicity of the procedure makes LF-tests feasible for various POCT-applications. However, the analytical performance is often impaired by the common properties of the test format, including detection Methods. Hereby, we examine an alternative fluorescence-based detection system and its capability to improve LF-assay performance.

Methods. A model bioaffinity assay was constructed to compare the performance of europium-doped nanoparticles (EU-np) and colloidal gold labels in lateral flow format. Biotinylated BSA was chosen as model analyte to avoid the effects of low-affinity binding. Thus, streptavidin was used as immobilized and labelled binder. Dose-response curves obtained with both label technologies were compared in terms of analytical sensitivity and dynamic range.

Results. The lowest detectable analyte amount was 14-fold lower with Eu-fluorescence measurement than with the reflectance-based measurement of colloidal gold. Due to the high specific activity of Eu-np the detection of low analyte levels was significantly more robust with fluorescence measurement than with reflectance based scanning of colloidal gold. The sensitivity of reflectance-based detection of colloidal gold was equal to visual examination. However, the scanning was prone to errors caused by scratches and dust.

Conclusions. In this model assay, we demonstrate that high specific activity of lanthanide-doped nanoparticles can be utilized in development of high-sensitivity bioaffinity assays. Within defined analyte range both of the label technologies can be measured for quantitative Results.

0955

NEW REDUCED PHENOLPHTHALEIN REAGENT FOR DETECTION OF A BLOOD CONTAMINATION ON GLUCOSE METERS

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Background. This study was to develop the new reduced phenolphthalein(RP) reagent with high efficiency and introduced this reagent for detections of the blood contamination on glucose meters in hospital and primary care unit.

Methods. The RP reagent was prepared and optimized. Sensitivity, specificity, precisions, and stability of developed RP reagent were investigated using fresh blood and dried blood samples. The blood detection results were compared among using the new RP reagent, the original method, and a commercial test kit. The Chi-square statistical method was used for data analysis. The new RP kit was designed and used for occult blood contamination on glucose meters in 15 hospitals and 74 primary care units at Phichit and UttaraditProvinces. The users satisfy on the new RP test kit was surveyed by using questionnaires.

Results. The optimal RP reagent composed of phenolphthalein; potassium hydroxide; water; and zinc power with the ratios were 1:10:30:10. The reflux reaction was ranged 45 -60 minutes. The reagent was treated with ethanol and kept at refrigerator until using. Glucose meters exhibited blood contamination 55% (59/108). The users had satisfied on the new RP test kits and the overall of product satisfactions was good.

Conclusions. The new RP reagent represented good precisions, stability and high sensitivity, and specificity. The new RP test kits could be used conveniently for occult blood contamination detection on medical devices by good satisfactions of users. Blood contamination frequencies still found on glucose meters, therefore the medical personnel should be avoid for the biohazard control.

Point of Care Testing

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ADVANCED REAL-TIME PCR ASSAY CONCEPT USING SWITCHABLE LUMINESCENT REPORTER SYSTEM

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Background. Nucleic acid amplification assays has been used increasingly for the diagnosis of pathogenic bacterial and viral infections. Rapid and easy-to-use point-of-care PCR assays would diminish the time from sampling to therapy leading to improved prevention of transmission of infectious diseases like chlamydia and lower the healthcare costs.
Methods. Homogenous real-time PCR assay concept using lanthanide chelate complementation reporter technology was developed for the detection of *Chlamydia trachomatis* in urine samples and the performance of the new assay was compared to the commercial TaqMan-based real-time PCR assay. In the developed PCR assay the hybridization of two non-fluorescent oligonucleotide probes to the adjacent positions of the amplified complementary target strand lead to the formation of highly fluorescent lanthanide chelate complex due to the self assembly of the reporter molecules.
Results. Signal-to-background ratio maximum of 300 in the homogenous real-time PCR assay was measured due to the very low background fluorescence and high specific signal level. The positive results in the real-time PCR was detected about 7 amplification cycles earlier with the new PCR assay concept compared to the TaqMan PCR assay.
Conclusions. We have developed homogenous real-time PCR assay concept that shows outstanding signal to background discrimination and enables the detection of the target DNA at the earlier amplification cycle in the real-time PCR compared to commonly used method. In the next step the *C. trachomatis* PCR assay will be integrated into automated DNA amplification and time-resolved detection device using ready-to-use dry-reagent reaction chips for the point-of-care application.

0957

NOVEL LANTHANIDE NANOPARTICLE LABEL-BASED LATERAL FLOW IMMUNOASSAY FOR DETECTION OF CARDIAC TROPONIN I

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Background. Point-of-care (POC) testing of cardiac troponin I (cTnI) is required to ensure rapid further treatment of the patient when myocardial infarction is suspected. Lateral flow (LF) assays are typical and common POC tests, whose analytical performance, however, is often impaired by the low sensitivity of the labels traditionally used. Therefore high-sensitive europium(III) chelate doped nanoparticles were evaluated as a label in LF assay for detection of cTnI.
Methods. Eu(III) nanoparticles coated with anti-cTnI antibody were first pre-incubated with the analyte after which the label-analyte solution was drawn into the LF strip. Strip consisted of sample pad, adsorbent pad and analytical nitrocellulose where test line (anti-cTnI antibodies) and control line (purified cTnI) were printed. Eu(III) chelate fluorescence was measured in a scanning, time-resolved mode. Dose-response curve was analyzed in terms of detection limit and dynamic range. Performance of the LF-based assay was compared with highly-sensitive microtiter well-based cTnI assay which relies on the same binder antibodies and label technology.
Results. For the LF-based cTnI assay detection limit and dynamic range were <0.01 ng/ml and 0.05-5 ng/ml, respectively, which are reasonable close to the performance of microtiter well-based cTnI assay (0.005 ng/ml and 0.01-10 ng/ml, respectively). Intra-assay variation between replicates was higher in lateral flow assay (up to 30 %) compared to microtiter well-based assay (less than 15%).
Conclusions. A quantitative lanthanide nanoparticle label-based cTnI LF assay was developed. Its performance is close to the performance of the highly-sensitive microtiter well-based cTnI assay and comparable with commercial POC cTnI assays.

Point of Care Testing

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TIME-RESOLVED IMMUNOFLUOROMETRIC ASSAYS IN A FUNCTIONAL MICROFLUIDIC CARTRIDGE

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Background. One attractive technological approach for point-of-care testing comprises self-contained microfluidic cartridges featuring add-only-sample principle. We have developed a generic cartridge for heterogeneous immunofluorometric assays especially considering aspects of the solid phase coating and use of nanoparticle labels.

Methods. Various streptavidin-coating methods were studied to create an optimal binding surface for biotinylated capture antibodies in a reaction chamber of an injection moulded polystyrene cartridge. A syringe pump was used for actuation of fluids in the assay, and fluorescence from bound antibody-coated europium-dyed particles was measured in a time-resolved manner. Cardiac troponin I assay was used to study the performance of the cartridge system.

Results. Modifications improved the density of the streptavidin surface up to 20-fold compared to regular passive streptavidin coating. A 15-minute assay with troponin I concentrations ranging from 0,05 to 5 ng/ml provided a standard curve with a regression coefficient of 0,986 when performed conveniently at room temperature without shaking. Nanoparticle labels enabled high signal levels and potential for a very sensitive assay as analytical sensitivity of 3,1 pg/ml was reached with the microtiter plate format using conditions simulating the cartridge assay. Optimal performance of the label technology was still partially hidden under non-specific binding in the cartridge assay.

Conclusions. The microfluidic cartridge was shown to be a feasible platform for heterogeneous immunoassays. The cartridge features integrated systems for mixing the sample to the assay buffer and sample volume metering and we are further developing the cartridge for example to enable the automation of the system.

0959

MOBILE LABORATORY UNIT. A NEW APPROACH DECISIONS THERAPEUTIC IN CARDIAC SURGERY AND TRANSPLANTATION

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Background. The application specific programs based on the use of POCT technology can bring significant benefits patients demanding analytical response immediately. One area that can benefit from the implementation of these programs is the surgical area.

Methods. Implementing a mobile laboratory unit at strategic points of care population at high risk of bleeding and blood product consumption, such as patients with heart disease or liver disease undergoing conventional surgery or orthotopic transplantation. The implementation involved the device Mov1Lab®, manufactured by Roche Diagnostic, Barcelona, Spain (glucose, sodium, potassium, chloride, calcium ion, urea, bilirubin, blood gases, oximetry, lactate, WBC count and thromboelastometry). Program impact was evaluated by comparing the months of September to March 2010 with the same period last year.

Results. The program was implemented in, cardiac surgeries, 248 (CS), 12 heart transplants (TXC) and 36 liver transplantation (LT). The percentage of patients transfused was 5% and 77% CC LT vs. 20% and 96% the previous period. The ratios of blood transfusion have been reduced considerably: packed red blood cells THX 64% and 19% in CC, plasma units by 89% LT and 43% in CC and units of platelets in 59% LT and 37 in CC. Reduced ICU stay (6.6 days to 5.7), the number of reoperations for bleeding (16 vs. 1), patients ventilated at 24 hours (17.3% to 14%), readmission to ICU from plant (7.6% to 1.9%) and renal failure acute (34% to 14%) among others.

Conclusions. Addresses issues of high strategic impact and that are related to patient safety, quality assistance, costs and the role that laboratory professionals should play in the new care scenarios that are being raised in our hospital.

Point of Care Testing

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EVALUATION OF THREE POINT OF CARE LACTATE METHODS FOR ASSESSMENT OF WHOLE BLOOD LACTATE IN AN OBSTETRICS SETTING

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Background. Measurement of whole blood lactate, the major end product of anaerobic metabolism, has been shown to be a reliable alternative to foetal scalp pH as an indicator of the development of metabolic acidosis. The aim of this study was to assess the accuracy and performance of two established Point-of-Care (POC) Lactate methods, Lactate Pro (Arkray) and Gem 4000 (IL) alongside a new POC device, StatStrip Lactate (Nova Biomedical).
Methods. The performance of the POC Lactate methods was assessed with umbilical cord blood and the results were compared to the laboratory analyser results (Cobas 6000, Roche Diagnostics). Precision and method correlation using linear regression analysis was performed.
Results. The three POC methods showed acceptable precision at a lactate level of 3.2 mmol/L (Lactate Pro 4.72%, StatStrip 4.69% Gem 4000 3.08%). The three methods also showed good correlation with the laboratory method, Lactate Pro r=0.973, y=0.725x+0.55 StatStrip Lactate r=0.989, y=0.931x+0.18 and GEM 4000 r=0.980, y=0,779x+0.12. Of the three POC methods the accuracy of StatStrip Lactate was closer to the laboratory method with a mean % bias -3.82 compared to a mean % bias of −17.90 for Lactate Pro and −20.03% for GEM 4000.
Conclusions. The three POC devices showed good analytical performance for measurement of lactate in umbilical cord blood. The accuracy of StatStrip Lactate was closer to the laboratory method with a minimal mean % bias compared to the other two Methods. Further study is needed to derive cut-off levels for the three methods for obstetric use.

0961

THE MEASURING SYSTEM OF THE BIOCHEMICAL TEST BY A VERY SMALL AMOUNT BLOOD COLLECTING OF A FINGERTIP

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Background. The monitor by the laboratory study about the metabolic syndrome is important to the prevention of life-style related diseases. The particularly important testing is a lipid test, diabetes test, liver function test respectively. The finger collecting blood that there are few times and limitation of the place is useful to perform a medical examination in the inhabitants widely.
Methods. We used finger collecting blood test kit. Well kit (PSI, Tokyo, Japan).Operation of this kit adds about 65µl of blood l which collected blood from the finger to the dilution buffer solution. This buffer contains two internal standards. It isolates blood cells and dilution plasma by filtration with the mixed solution. A measurement item is AST, ALT, GGT, triglyceride, HDL cholesterol, LDL cholesterol, creatinine, glucose. It measured obtained dilution plasma using a biochemistry automated analyzer and commercial testing reagents. The measurement condition gained quantity of sample volume as compared with normal serum measurement conditions is measured.
Results. With this testing system, examined the assay properties about a lipid analyses, diabetes testing, liver function tests associated with the metabolic syndrome. The assay properties of eight items and the correlation coefficient of the plasma between this system measurement were good with correlation coefficient 0.876-0.991, and the hematocrit was 0.956, too.
Conclusions. We are simple and easy and can examine testing associated with the metabolic syndrome by using an examination of this finger system and are useful in the medical examination of personal health care.

Point of Care Testing

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NATRIURETIC PEPTIDE MEASUREMENT ON THE POC PLATAFORM TRIAGE

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Background. The routine method that is used in our Hospital for the determination of NTproBNP is an immunoassay performed in a MODULAR ANALYTICS E170 (Roche Diagnostics). Results to Coronary and Heart Failure offices is delayed .To cope with the associated turnaround time and workflow in this offices, POCT was considered.
Methods. A method comparison study between TRIAGE meter (Izasa) tests for NTproBNP and E170 was performed using 160 patient samples from coronary office using whole blood and serum specimens respectively obtained from the same venopuncture.
Results. Comparison Plot has been prepared and also a visual line of best fit, no discrepant results were identified and confirm that data cover the entire working range. Bland-Altman plot was also performed. Kolmogorov-Smirnov analysis showed non normality. Passing-Bablok regression analysis was performed. Agreement with the automated E170 NTproBNP was good: NTproBNP[POCT] =1,418x NTproBNP[Automated]+1,014 ; CI for b [0,953 to 1,082]; CI for a [-43,238 to 44,205] (Passing Bablok; ma68= 675,317; medians difference= 119; r=0,989**; n=80). No proportional or constant bias was observed. Wilcoxon Signed Rank Test (Z=-0,158; p=0,874).
Conclusions. We conclude that the Triage assay for NTproBNP can be used in clinical practice to aid clinicians to safety evaluate patients in our institution.

0963

LACTATE AND IN-HOSPITAL MORTALITY IN SEPTIC PATIENTS

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Background. The aim of our study was to evaluate the relationship between the first hospital lactate measurement and in-hospital mortality in patients with severe sepsis in the North Estonia Medical Centre.
Methods. The retrospective data of 253 patients with severe sepsis, first lactate results and clinical diagnosis during 2009 – 2010 were obtained from HIS. The arterial blood lactate measurement was done by blood gas analyzer ABL825. The sensitivity and specificity for lactate were calculated, the receiver operator characteristic (ROC) curve was constructed.
Results. The patients were devide in two groups: survivors and non-survivors (45%). To avoid the potential effect of bias in survival to the increased blood lactate, we exluded data 24 patients from survivors group. They first lactate level was >4.0 mmol/L and second was >10% lower. Compared with the survivors, the non-survivors had significantly higher lactate levels: 4,7 ± 3,9 vs 2,2 ± 1,4 (P<0,001). Mortality was also higher in patients with lactate levels of 3.5 mmol/L or higher in non-survivors group.The sensitivity and specificity according to lactate levels below or above the cut-off threshold of 2.0; 3,0; 3,5; 4,0 or 5,0 mmol/L were calculated. ROC curve analysis suggests a lactate concentration of 3.5 mmol/l as the optimal cut-off value for mortality prediction.
Conclusions. In this study a lactate concentration > 3.5 mmol/l can be used by clinicians to identify patients with severe sepsis at higher risk for mortality.

Point of Care Testing

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LABORATORY SUPERVISION OF POC-URINALYSIS IN THE GENERAL MEDICAL PRACTICE

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Background. Urinalysis at the general medical practice (GMP) is routinely performed by dipstick testing without supervision from the laboratory. Quality of results, therefore, is questionable, but could theoretically be improved by applying information technology. We investigated the quality of urinalysis and introduced a POCT-management system to improve urinalysis at the GMP.
Methods. A questionnaire on (pre)analytical variables was sent to 78 GMPs (116 GPs) to evaluate current methods and quality of urinalysis. Additionally, semi-automatic urine-dipstick analysers were placed in two GMPs and users were trained to laboratory standards. Analysers were connected to the LIS using a POCT managementsystem (Meditrack™; POCcelerator™). Barcode labels with patient data were printed through this connection. Urine was analysed after scanning the barcode and Meditrack™ sent the results including patient and GP data to POCcelerator™ which integrated them into the LIS.
Results. Quality of POC-urinalysis differed largely between practices. Striking results were the acceptance of urine-samples in non-hospital containers (62% of the respondents), no mixing of urine before analysis (72%), and wrong timing of reading the dipstick (45%). Moreover, nearly all dipsticks were visually read. After creating an order, results are reported from the LIS to the hospital and GP information systems within 15 minutes. GP's were very satisfied with the laboratory controlled POC urinalysis.
Conclusions. The quality of POC-urinalysis at the GMP can be improved at several points. Laboratory control of POC-urinalysis is technically possible, thereby enhancing the quality of urinalysis through standardisation of equipment, methods and training.

0965

IMPACT OF ANALYTICAL PERFORMANCE OF POINT OF CARE (POCT) BLOOD GLUCOSE METERS ON APPLICATION OF A ´TIGHT GLYCAEMIC CONTROL´ (TGC) PROTOCOL IN AN INTENSIVE CARE UNIT SETTING

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Glycaemic control influences outcomes in a variety of clinical conditions. Many intensive care (ICU) units implement a TGC program using POCT meters. The decision to initiate or alter insulin infusion is based on POCT Results. With specific cutoff points for treatment, the analytical performance of the measurement device can have a major influence. We validated 2 BG meters in the laboratory to confirm ISO 15197 standards and compared 841 paired results in an ICU setting using the Bath Infusion Protocol (BIP). Both meters had CVs of 1.6 - 3% across the relevent range of BG readings, meter 1 was subject to a number of analytical interferences while meter 2 was unaffected. Both meters compared well to laboratory methods (meter 1, r = 0.998, meter 2, r = 0.995) at BG levels between 2.5 to 30mmol/L. Finger prick samples were taken using standard techniques and measured on meter 1 (standard) and meter 2 (test) in random order. Meter 1 results were used for clinical care purposes as per usual practice and meter 2 results were used to test whether a different decision would have been made in the TGC protocol had meter 2 results been used. Despite the satisfactory performance and excellent agreement between both meters in the laboratory evaluation, >60% of measurements differed by >5% and almost 30% by >10% in clinical use. Simulation studies indicate that a 5% difference in BG led to an incorrect insulin dosage in 8 -23% of cases and a 10% difference in 16 - 45% of cases. In this study, on 149 occasions (20%) meter 2 results would have altered the decision category in the BIP. Blood glucose < 4mmol/L was found in 0.9% (meter1) and 1.8% (meter2).Freedom from analytical interferences may be more important that analytical accuracy and precision in a clinical setting.

0966

INTERFERENTS IN GLUCOSE DETERMINATION DO NOT INFLUENCE THE HOSPITAL POC GLUCOSE METER STATSTRIP IN ACCURACY AND PRECISION OF BLOOD GLUCOSE MEASUREMENT

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Background . Critical care settings require accurate and precise glucose determinations to avoid potentially fatal errors with insulin dosing according to false Results. Particularly in hospitalized patients, blood hematocrit value as well as interfering substances may have an impact on glucose measurements. This multi-center study investigated the impact of interferents on the accuracy of the StatStrip Glucose Meter.
Methods. Glucose from whole blood patient specimens was determined using StatStrip in comparison to six commonly used glucose meters. The corresponding plasma samples were measured with a central laboratory analyzer. Accuracy interference studies were performed using acetaminophen, ascorbate, and maltose monohydrate at different glucose levels. Hematocrit interference was tested using 3 glucose concentrations over a 25-65% hematocrit range. The results were compared to acceptance criteria defined by ISO 15197 guideline.
Results. Neither hematocrit variation nor any of the interfering substance tested affected the accuracy of StatStrip. The accuracy of the comparators was affected by low and high hematocrit levels and the presence of ascorbate. Maltose interfered significantly with the accuracy of three commonly used meters. One meter was affected by acetaminophen.
Conclusions. The improved accuracy of StatStrip POC glucose meter was substantiated in all four clinical sites indicating that this meter provides an improved level of clinical accuracy and reliability for managing glucose levels in hospitalized patients. In this analytical assessment, interfering substances commonly present in hospitalized patients compromised the accuracy and specificity of six glucose meters commonly used in Germany.

0967

HEMOLYSATE GLUCOSE CONCENTRATIONS MEASUREMENTS – ARE THEY SUSCEPTIBLE TO INTERFERENCES?

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Background. We evaluated the effect of plasma total cholesterol (TC) and triglycerides (TG), and hematocrit (HCT) values on glucose measurements in blood hemolysate.
Methods. Glucose was measured using the HemoCue analyzer in 162 EDTA blood samples in groups: A. TC <7.0 mmol/L and TG <4 mmol/L; B. TC >7.0 mmol/L and/or TG >4.0 mmol/L; C. TG >4.0 mmol/L irrespective of TC. Comparative measurements were performed in plasma using GOD method on Modular P analyzer. In HCT effect studies 135 glucose assays were performed in 27 batches of secondary blood samples with HCT from 20% to 60%, prepared by adding or removing defined aliquots of plasma.
Results. HemoCue analyzer yielded significantly higher glucose concentrations than Modular P in group A (119.7 vs 104.4 mg/dL) and lower values in group B (88.2 vs 93,2 mg/dL) and group C (95.6 vs 103.3 mg/dL) (p<0.005). In trivariate regression model, TG concentration had the highest impact on magnitude of inter-method difference ($r^2 = 0.3$), TC and glucose concentration impact was equal and significant ($r^2 < 0.07$). In consensus error grid analysis 155 (95.7%) results were in zone A, 5 (3.1%) results in zone B and 2 (1.2%) results in zone C. The linear relationship and significant correlation (R from 0.9 to 0.998) between HCT and glucose and significant (p<0.0001) relative decrease in glucose level amounting to 0.73% per 1% increase in HCT were found.
Conclusions. High plasma TG and high HCT values decrease glucose concentrations measured in hemolysate presumably due to reduced its water content.

Point of Care Testing

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SOFTWARE SUPPORTING THE ACCREDITATION OF POINT-OF-CARETESTING (POCT) SERVICES ACCORDING TOISO-15189 AND ISO-22870 AND THE SUPERVISION OF ITS QUALITY INDICATORS

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Background. The purpose of this investigation was the development of Software-tools, supporting the implementation of a Point-of-Care Testing (PoCT) Quality-Management System according to ISO-15189 and ISO-22870 and the supervision of its Quality Indicators.

Methods. An Internet-based application was developed that allows for controlled accessibility for eligible PoCT users (or potential ones). The technical contentsof the system are complying with internationally recognized Guidelines.

Results. The system provides on-line guidance, for a Standard Operating Procedure approach, based upon quantifiable Quality Indicators, concerning the following relevant issues:

· Feasibility of a PoCT-service, as an effective response to a valid and continuing Clinical need.
· Continuity of care for in-, out-, and home-care patients.
· Multi-disciplinary approach of relevant clinical-governance issues.
· Active involvement of local Hospital-laboratory in the PoCT-Services.
· Training, updating and monitoring of all staff involved in the POCT-Services.
· Robustness, workload, throughputandmaintenance requirements of the equipment.
· QC-material and reagents lot-number and expiration date monitoring.
· Accuracy, traceability and consistency of the Results.
· QC-results documentation, statistics and adverse incidents reporting.

Specific assistance-data have been included for 24 frequently measured parameters (metabolites, electrolytes, coagulation etc.), and means for the insertion of procedural information, concerning additional analytes, are also provided.The PoCT-Service Quality indicators comprise of, among others, the number of unidentifiable samples, expired material quantities, confidence-intervals (Levey-Jennings graphs) for all parameters, inoperative equipment log-book etc.

Conclusions. The software is presently tested, under virtual PoCT-Service conditions, for bugs and drawbacks, regarding workflow, resources-utilization,and expected medical benefits.

0969

PREDICTING THE DEVELOPMENT OF IN VITRO DIAGNOSTICS POINT OF CARE TESTING TECHNOLOGIES BY EVALUATING RELEVANT INDUSTRIAL PROPERTY DOCUMENTS

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Background. Industrial Property (IP) legislation regulates the protection of innovation, and facilitates the cooperation of Industry and Academia. However, IP-Documents (Patents) are often disregarded, as a valuable source of technical knowledge, as well as a market-trend prediction instrument. The purpose of this investigation was,by evaluating relevant Patent Documents, to attempt mapping the development of in vitro Diagnostics (IVD) Point-of-Care Testing (PoCT) Technologies, and to predict, consequently,the main IVD-PoCT market-trends, for the next 5-10 years.
Methods. We have searchedfivemajor subcategories of IVD-PoCT, by employing the on-lineesp@cenet search-engine of the European Patent Office.
Results. We have retrieved and evaluated 137 relevant Patent Documents. The most promising-ones are presented here:

• Lateral-Flow (15)	RapidPathogen Screening Inc.	USA (3)
• Immunoassays (17)	Nanogen Inc.	USA (1)
	Citizen Watch Co LTD.	USA (1)
• Molecular Diagnostics (15)	Abbott Point of CareInc.	USA (1)
	Cornell Res. Foundation Inc,	USA (1)
• Fluorescence (18)	Koninkl. Philips Electronics NV	NL (1)
• Electrochemical (13)	Univ. Columbia	USA (1)

The most promising and reliable emerging Technologies, revealed by the performed search, comprise of, first, combined lateral-flowimmunoassays and color tagged nucleic-acid sequences, second, dielectrophoretic separation methods on active electronic matrix-devices and magnetic immunoassays, third, non-isothermal, integrated Nucleic-Acid Test (NAT)-cartridges, fourth, methods based on evanescentillumination (excitation) and fluorescence, and finally, active CMOS-sensor arraysfor electrochemical detection.
Conclusions. Our results are comparable with the estimations of well-known IVD-market Research Institutions, and they corroborate, both, the technical significance and the market-predictive potential of Patent-Literature.

0970

CLINICAL EVALUATION OF A NEW POINT OF CARE CREATININE-METER IN A PEDIATRIC NEUROONCOLOGICAL OUTPATIENT CLINIC

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Background. Routine testing of Creatinine (Crea) levels is obligatory in neuro-oncology patients, before they are cleared for planned Radiological Examinations (CT/MRI) or before a new chemotherapy block. Routinely the serum creatinine analysis on a routine laboratory analyzer requires a TAT of approx. 69 mins thus leading to long waiting times for patients which cannot be tolerated in pediatric patients. In order to overcome this disadvantage a POCT for Crea, e.g. StatSensor Creatinine (Nova Biomedical) was evaluated. The aim of the present study was to assess the performance of the new POCT device in a pediatric neuro-oncological outpatient ward.
Methods. Accuracy (bias) of the new POC creatinine-meter was compared to a routine analyzer (Vitros 5.1, Ortho Clinical Diagnostics). For method comparison Passing-Bablok Regression Analysis and Students T-test were applied. Additionally the effect on logistics of the outpatient-ward investigated (questionnaire for patient's satisfaction and staff stress).
Results. The Nova StatSensor Creatinine-meter correlated very well with the reference method in pediatric blood specimens (r^2=0,918; y=0,936x+0,03) within the reference range (limit of detection: 27μmol/L) and for Crea concentrations above 88 μmol/L.
Conclusions. Nova StatSensor Creatinine showed good clinical accuracy and performance for measuring and monitoring Crea levels in the patients of a neuro-oncological outpatient ward and is a rapid alternative to the routine analyzer. The routine use of the creatinine meter could greatly improve patient satisfaction and because of the immediate availability of the results (30 seconds) the number of patients that could be treated per day could be increased significantly.

0971

CONTROLLED TRIAL BETWEEN MEASUREMENT OF GLOMERULAR FILTRATION RATE ON VENOUS BLOOD (AUTOMATED LABORATORY) AND CAPILLARY BLOOD (BEDSIDE SYSTEM)*

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Background. Medical imaging departments require a measurement of creatinine/eGFR that is fast and reliable in order to shorten waiting time in outpatient clinics and Emergency Departments and to reduce the risk of contrast medium associated adverse reactions. The serum creatinine analysis on a routine laboratory analyser can have a turn around time of about 60 minutes, which can lead to a delay in patient processing. Recently a Point of Care (POC) device for the measurement of creatinine/eGFR, StatSensor Creatinine (Nova Biomedical) has been introduced, which provides the opportunity to rapidly assess kidney status in capillary or venous blood. The aim of this study was to assess the performance of the new POCT device in a Radiology setting.
Methods. The performance of StatSensor Creatinine (enzymatic method) was assessed with capillary blood and compared to the results of venous blood routinely tested using LX20, (Beckman-Coulter) and RXL (Siemens) laboratory analysers. Concordance of StatSensor Creatinine results compared to the laboratory methods was assessed around radiology decision making levels. For method comparison linear regression analysis was performed.
Results. StatSensor Creatinine capillary results showed a good correlation with the laboratory analyser venous results (r²=0.908, y=0,972x+4.544). StatSensor Creatinine also showed good linearity in the range 27-1056 µmol/L and good concordance (>95%) based on a creatinine decision-making level of 130 µmol/L
Conclusions. StatSensor Creatinine showed good accuracy and performance for measuring and monitoring Creatinine/eGFR levels in Radiology patients and is a rapid alternative to the routine laboratory analyser.

0972

PROFESSIONAL GLUCOSE POINT OF CARE TESTING USING MULTI-WAY BIOSENSORS

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Background. In general POCT methods are rapid, however, not generally of high quality. Thus, an improvement is eligible. By using multi-way biosensors we have been successful in significant improvement of quality in POCT analysis.
Methods. Glucose oxidase and lactate oxidase multi-way biosensors have been used for the instruments GLUKOMETER PRO and LAC PRO.
Results. The biosensors placed inside the portable instruments are the heart of the POCT instruments. Size and weight of the instrument and the sample matrix (whole blood) are adapted to the purpose. An internal cleaning system is rinsing the biosensor after a whole blood sample. The technical parameter are highlighted by very low response time (5 sec), long lifetime of the multi-way biosensor (30 days / up to 1000 samples) and internal control of the biosensor. Based on the multi-way biosensor the analytical quality is comparable to laboratory instruments: Interassay CV at 5.5 mM is ≤ 5%; linearity in the concentration range from 0.5 mM to 33.3 mM glucose was excellent (day 30: (0.9282x + 5,0949)mg, R²=0.9998); no significant influence of potential interfering substances, no crossing-over between low and high concentrations; agreement with the hexokinase method (Dimension/DADE Behring) between 2.1 mM and 49.9 mM (n=104) was very good: y = (0,9512x + 0,4687) mM, R²= 0.9858.
Conclusions. We succeded in the combination of the advantages of both laboratory analyzers and patient self monitoring instruments. Thus, innovative GLUKOMETER PRO and LAC PRO bring lab quality out of the lab into the POCT area.

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PRENATAL DIAGNOSIS OF FETAL RHD STATUS BY MOLECULAR ANALYSIS OF MATERNAL PLASMA WITH REAL-TIME PCR ASSAY

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Background. The discovery of circulating cell-free fetal nucleicacids in maternal plasma has opened up new possibilities forno-ninvasive prenatal diagnosis fetal RhD status.

Methods. The fetal Rh genotyping was performed by the use of TaqMan@real-time PCR (Model 7300, Applied Biosystems). DNA was extracted commercial set MagMAX ™ Total DNA Isolation Kit (Manufacture of the USA) on magnetic spheres.

Results. For the detection of fetal RhD status we used uniplex RhD exon 7 genotyping TaqMan system, which consisted of amplification forward primer, reverse primer and dual-labeled fluorescent probe, TAMRA [6 carboxytetramethylrhodamine] were the fluorescent reporter dye and quencher dye. Among 65 plasma samples, 45 were in their first trimester of pregnancy, 17 were from the second trimester and 3 were from the third trimester.

Among 65 RhD-negative women the results from 20 samples diagnosed Rh negative factor and 45 cases - Rh positive factor. One pregnant women Rh negative has high title antibodies, but the fetus were examined Rh-negative and were comfirmed by the serological analysis on cord blood after delivery.

Conclusions. To assist in the management of pregnancy when an RhD negative woman is pregnantwith an RhD positive fetus, reliable and accurate noninvasive diagnostic methods topredict the fetal RhD type will be of great clinical value.

0974

ANTENATAL SCREENING FOR DOWN'S SYNDROME: ANALYSIS OF THE MULTIPLES OF THE MEDIAN (MOM) TRUNCATION LIMITS FOR THE FIRST TRIMESTER MARKERS

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Background. There is not a general consensus about the truncation limits of the MoM used for the first trimester markers to fit a Gaussian distribution. The truncation limits recommended in the protocol of antenatal screening implemented in Catalonia are 0.21-4.99 MoM for PAPP-A and free β-hCG. The aim of this study was to evaluate the results obtained following this protocol in order to establish future recommendations concerning truncation limits.

Material and Methods. A total of 14720 pregnancies attended during 2009 in 11 centres in Catalonia were evaluated. PAPP-A and free β-hCG were measured using two different automated immunoassays, Siemens® Immulite 2000 or Perkin Elmer® Delfia Xpress.

Results. 167 of 14720 PAPP-A MoM (1.1%) were ≤ 0.20. Of these, 76 generated a positive risk for T21, T18 o T13; in the remaining 91, in spite of a negative risk, 2 cases of affected T21 were registered. A total of 14512 PAPP-A MoM (98.6%) were between 0.21-4.99. No affected case was registered for PAPP-A MoM > 5 (0.3%).

55 of 14720 free β-hCG MoM (0.4%) were ≤ 0.20. Of these, 12 generated a positive risk for T21, T18 o T13; in the remaining 43 with negative risk no abnormalities were found. A total of 14574 free β-hCG MoM (99%) were between 0.21-4.99. No affected case was registered for β-hCG MoM > 5 (0.6%).

Conclusions. If we had recommended additional studies for PAPP-A MoM ≤ 0.20 we would have detected 2 more cases of T21 with an increase of 0.62% of invasive procedures.

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CALCULATION OF UNCERTAINTY FOR GLUCOSE: ANALYTICAL ERROR MAY AFFECT THE DIAGNOSIS OF GESTATIONAL!

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Background. The effect of uncertainty of measurement in the glucose were investigated forgestational diabetes at screening test results

Materials and Methods. 50 g glucosechallengetest (GCT)results were evaluated effect of uncertainty of measurement in the glucose analysis retrospectively between September 2009-August2010

Results. The mean age of 937 participations were 28±5 years old(17 to 43) who were tested GCT and OGTT(Oral glucose tolerance test) in the Gulhane School of Medicineof MedicalBiochemistryLaboratories participations(16.1% CI15.1to17.1%) were found 50gGCT'spositive(≥ 140).According tothe Eurachem/CITACGuideCG4 the uncertainty of measurement in the glucose was calculated 7.02% and 7.54% (mean 7.26%).

According to the value of uncertainty inthe measurementof glucose, pregnants who 50gGCT positive (≥ 140) were 213(22% CI21.3 to 24.1%) assumingto falselowmeasurement and 101(10.7% CI10.1 to 11.4%) assumingto falsehighmeasurement.

Conclusions. The uncertainty of measurement in the glucose was found to bewithin acceptable limitsaccording toFraserandCLIA in the clinical laboratory

Thefalsehighmeasurement observed 26% (CI 21 to 30.9%) false-positive results while lowfalsemeasurementobserved 41%(CI, 35.5 to 46.4%) offalse-negative Results. Such asGCTandOGTT results, within specificlimits (the values arewithinuncertainty measurement) isin thedirection ofbeingmore carefulfollow-upshould alertthe clinician.

0976

INCREASED HE4 LEVELS IN PREGNANCY ARE INDEPENDENT OF ACTUAL TESTOSTERONE LEVELS

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Background. Human epididymis protein 4(HE4) is expressed in some normal tissues and tumors. Currently HE4 is recommended as a tumor marker for the detection of ovarian cancer.

HE4 levels, however, are less known in various physiological conditions including pregnancy. Our first aim was to explore association of HE4 with gestational age.

Speculatively, as HE4 was originally described in human epididymis, its levels should be also higher in a milieu with higher testosterone levels. This situation may occur in pregnant women bearing a male fetus. Our second aim was to compare HE4 levels of pregnant women with male and with female fetuses.

Methods. Blood was taken from 41 pregnant women and 28 healthy fertile women. Serum HE4 and testosterone levels were measured, compared between pregnant and control groups and related to gestational age (25-40 weeks) and fetal gender (19 male /22 female).

Results. HE4 levels were higher in pregnant women than in controls (42.0±7.2 vs. 27.9±7.1 ng/ml, $p<0.001$). No association between HE4 and GA was detected. While testosterone levels were also high in pregnant women (0.80±0.46 vs. 0.40±0.16 ng/ml, $p<0.001$), HE4 and testosterone levels were not associated. Fetal gender had no impact on HE4 or testosterone levels.

Conclusions. This study demonstrated high HE4 levels in pregnancy. The origin of high HE4 is probably independent from testosterone production. As ovaries are the major source of testosterone in women, the independence of HE4 from testosterone may also indicate that HE4 levels are not affected by testosterone production of normal or malignant ovarian cells.

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SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (SUPAR) LEVELS IN PREGNANCY AND PREECLAMPSIA

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Background. Pregnancy is characterized by an immune compromised status in order to prevent the rejection of fetus. In pre-eclampsia (PE) immune tolerance during pregnancy is deficient and a systemic inflammatory response evolves. Soluble urokinase Plasminogen Activator Receptor (suPAR) is a biomarker that is increasingly used to monitor inflammation. We aimed to obtain data on suPAR levels in healthy pregnancy and PE and to compare them to those measured in healthy fertile women.
Methods. We took peripheral blood samples from 41 PE and 62 healthy pregnant women between 26 and 40 week of gestation and determined serum suPAR concentrations with commercially available ELISA kits (A002SuparnosticFlex ELISA assay). As controls, 19 healthy fertile age-matched women were enrolled.
Results. In control women suPAR levels (mean ± SD) were higher compared to those in healthy pregnant women (3.27 ±0.65 versus 2.20±0.58 pg/ml, p<0.001). Importantly, suPAR levels were higher in PE women compared to those in healthy pregnant women (3.43±1.43 pg/ml, p<0.001) and were comparable to those in healthy controls. The impact of PE on suPAR levels remained significant (p<0.0001) when gestational age at blood sampling and maternal age were also taken into account. In PE group these factors were inversely associated with gestational age and maternal age (p=0.02 and 0.04), respectively.
Conclusions. suPAR levels fit well into the widely accepted concept of immune tolerance in healthy pregnancy and also to the increased inflammatory response in PE. It might be a useful tool to monitor immune status during pregnancy.

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COMPARISON OF THE BECKMAN COULTER ACCESS® CMV IGG AND CMV IGM ASSAYS TO THE ROCHE ELECSYS CMV IGG AND CMV IGM ASSAYS IN ROUTINE LABORATORIES

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Background. The human cytomegalovirus (CMV) is a serious concern for both immunosuppressed populations and women of child-bearing age.
Methods. 500 samples (from transplant or immunosuppressed patients and from pregnant women) were tested for CMV IgG and CMV IgM with the Elecsys assays run on the Roche Modular E170 platform and with Access assays run on the Beckman Coulter UniCel DxI 800 platform. Samples showing discrepant CMV IgG results were tested with the Vidas* CMV IgG assay. Samples showing discrepant CMV IgM results were further tested with CMV IgG avidity methods (Liaison, Vidas and in-house avidity). Concordance was calculated before and after resolution.
Results. The total, positive, and negative agreement of Access CMV IgG vs. Elecsys CMV IgG were 98.4%, 99.1% and 97.4%, respectively. After resolution, the total, positive, and negative agreement of Access CMV IgG vs. consensus method were 100%. The total positive and negative agreement of Access CMV IgM vs. Elecsys CMV IgM were 92.8%, 33.3% (2/6 – not statistically significant) and 95.7%, respectively. Because the CMV IgG avidity index was high (i.e., the CMV IgM detected was not linked with a primary infection and can be considered as negative), the negative agreement after resolution was 94.2% for Access CMV IgM and 96.6% for Elecsys CMV IgM.
Conclusions. Based on these data, Access CMV IgG demonstrated better performance (relative sensitivity and specificity after consensus) than Elecsys CMV IgG and slightly lower relative specificity for CMV IgM (according to IgG avidity index).
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EVALUATION OF INFLAMMATORY PROCESS IN PREECLAMPTIC PATIENTS

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Background. Preeclampsia is characterized by hypertension and proteinuria that begins in the second half of pregnancy. Endothelial dysfunction and trophoblastic hipoperfusion seen in preeclampsia suggested to be part of an increased maternal inflammatory response to pregnancy. In this study, we aimed to evaluate some inflammatory markers in preeclamptic and normotensive pregnants.

Methods. The study included 36 cases with mild preeclampsia, 36 cases with severe preeclampsia and 33 cases of normotensive pregnant. Serum Amyloid A (SAA) and high sensitive C reactive protein (hsCRP) were measured by enzyme linked immunosorbent assays, serum procalcitonin was measured by enzyme linked fluorescent immunassay. Mean arterial pressure (MAP) was used as an indicator of the severity of the disease.

Results. In severe preeclampsia group, serum amyloid A, procalcitonin, and hsCRP levels were significantly higher than mild preeclamptic and normotensive groups. SAA and hsCRP levels were higher in mild preeclamptic group when compared with normotensive pregnant but no significant difference was found in procalcitonin between these groups. There were significant correlations betweeen SAA, hsCRP, procalcitonin and MAP.

Conclusions. Our study suggest that inflammatory reactions are closely associated with preeclampsia.

0980

SERUM LEVELS OF LEPTIN AND RESISTIN IN MACROSOMIC NEWBORN: RELATIONSHIP TO ANTHROPOMETRIC PARAMETERS

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Background. Various studies have demonstrated an increased risk for adult diseases in macrosomic newborns. Leptin and resistin are adipokines secreted by human adipocytes and mononuclear cells, which have been postulated to play roles in the regulation of energy metabolism during pregnancy.

Methods. This case-control study included 82 newborns (40 macrosomic: 24 female and 16 male, and 42 no macrosomic: 20 female and 22 male), whose birth weights exceed 90th percentile for gestational age and gender are termed macrosomic.Soluble resistin and leptin were determined by ELISA kits (ALPCO). The body composition, anthropometric measures and skin fold thicknesses (i.e., biceps, triceps, subescapular and suprailiac) were obtained on the left side of the body by using a Lange® calliper,and following the procedures recommended by anthropometric indicators measurement guide(Cogill, Bruce, 2003). Statistical analysis was performed using the PASW Statistics v18.0.

Results. In this study group subcutaneuos body fat distribution was different between sexes, and showedhigher (p=0.0027) leptin levels in macrosomic newborn than no macrosomic newborn and these were significantly correlated with body fat mass (r=0,261), fat percentage (r=0.279) and skin fold thicknesses (r=0.349 to r=0.2665), p<0.05.

Conclusions. These results suggest that the increase of body fat mass possibly will affect leptin production in macrosomic newborns as an adaptive mechanism, but an altered functional response of adipocytes will be investigated.

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FETAL SCALP BLOOD SAMPLING, HOW TO RECOGNIZE AN UNRELIABLE TEST RESULT? A DESCRIPTIVE STUDY OF 633 SAMPLES

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Background. Fetal scalp blood sampling (FBS) in labour aims to detect fetal acidosis. Since it is often used as the reference test in clinical decision making, it is of utmost importance that results are reliable. No studies have been presented on FBS validation yet. The aim of our study is to determine the number of unreliable FBS results by evaluating pCO_2 in relation to pH.
Methods. Data were used from two previous studies and one cohort. Singleton foetuses in vertex position of labouring women at a gestational age of $\geq$ 36 weeks in which a FBS was taken were included. Blood gas analysis was performed using the iSTAT1®analyser. Blood gas results were validated using arterial umbilical cord blood pH corrected pCO_2 percentiles, as recently described by others. In addition, discrepancies between FBS and arterial umbilical blood gas results were investigated.
Results. 633 FBS results of 477 foetuses were obtained. Median values for pH and pCO_2 were 7.32 and 5.85 kPa respectively. One out of seven FBS pCO_2 results was outside the normal limits when using arterial cord blood reference ranges. Putative discrepancies between arterial umbilical cord blood and FBS blood gas results were found in 6% of cases.
Conclusions. According to arterial umbilical cord pH corrected pCO2 limits, 14% of the FBS results could be invalid. Putative discrepancies between FBS and arterial umbilical cord blood gas analysis were detected in 6% of cases. Like in umbilical cord blood samples, pCO2 could be a promising parameter to recognize erroneous FBS Results.

0982

THE EFFECT OF NICOTINE REPLACEMENT THERAPIES (NRT) ON SECOND TRIMESTER MATERNAL SERUM SCREENING MARKER MULTIPLE OF THE MEDIANS (MOMS)

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Background. With the use of InhibinA in the quadruple test for Down's syndrome screening the need for accurate smoking information has become paramount as it has a major impact on InhibinA levels. There has been an increase in the use of NRT's in pregnant women. We investigate the effect of NRTs and compare them with the effect of smoking.
Methods. Beckman Coulter Access® II was used to measure InhibinA, and PerkinElmer AutoDELFIA to measure Alpha-fetoprotein (AFP), Intact human chorionic gonadotrophin (hCG) and Unconjugated Estriol (uE3). PerkinElmer Lifecycle software was used to calculate the MoM values. Estimates of the median effect from 71 NRT samples were obtained for each marker, together with 95% confidence intervals. Corresponding estimates for smokers were obtained from Newcastle submissions to DQASS, based on 5483 InhibinA samples, 8080 AFP samples, 8102 hCG samples and 8089 uE3 samples.
Results. Estimates (with 95% confidence intervals) of the median MoM, for the different markers, in women using NRT are: InhibinA 1.26 (1.16, 1.36); AFP 0.99 (0.92, 1.06); hCG 0.81 (0.73, 0.9); uE3 0.97 (0.91, 1.03). The corresponding results for smokers are: InhibinA 1.540 (1.521, 1.558); AFP 1.025 (1.017, 1.033); hCG 0.743 (0.734, 0.752); uE3 0.957 (0.951, 0.963).
Conclusions. We conclude that the effects of NRT on hCG and InhibinA are less than the respective effects in smokers. There is no evidence of an effect on AFP and uE3. We would suggest the introduction of separate correction factors for hCG and InhibinA in women on NRT in the risk calculation software.

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PERFORMANCE EVALUATION OF THE ROCHE ELECSYS PAPP-A AND FREE BETA HCG SCREENING ASSAYS

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Background. Routine first trimester Down syndrome screening supports an earlier decision-making process and helps to ensure safer pregnancies. We evaluated the performance characteristics of Roche Elecsys screening assays pregnancy-associated plasma protein-A (PAPP-A) and maternal serum free beta-human chorionic gonadotropin (beta-hCG).
Methods. PAPP-A and free beta hCG concentrations were measured by electrochemiluminescent (ECL) technology on Roche Elecsys analyzer. Verification of performance for precision was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) Document EP15-A2. Participation in the United Kingdom National External Quality Assessment Service (UKNEQAS) scheme for First Trimester Downs Syndrome Screening on monthly basis allowed us to monitor long-term analytical bias and risks calculations for trisomy 21 and 18/13 using SsdwLab 5.0.9 software approved by the Fetal Medicine Foundation.
Results. The within-laboratory precision obtained for PAPP-A assay was 2.0% and 2.1% in the low and high concentration level, respectively and 2.7% and 3.3% for free beta hCG measurements, respectively. Expanded measurement uncertainties were ±8.6% and ±7.8% (k=2 representing a confidence level of 95%) in the low and high concentration level for PAPP-A assay and ±6.4% and ±6.8% for free beta hCG measurements, respectively. The long-term results in the UKNEQAS scheme showed acceptable performance with the analytical bias from the method mean within 10% for both assays.
Conclusions. The Roche Elecsys serum PAPP-A and free beta hCG assays ensure reliable analytical results with method target values that fall within Fetal Medicine Foundation accreditation targets.

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PREGNANCY LEVELS OF IRON, TOTAL IRON-BINDING CAPACITY, TRANSFERIN AND FERRITIN

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Background. Serum iron, ferritin, transferin and total iron-binding capacity (TIBC) were used to evaluate the body's iron levels and stores during normal pregnancy.
Methods. Iron status parameters were measured in sera of 43 pregnant women in 1st, 2nd and 3rd trimester, before (38th week of gestation) and after (6.5th weeks) delivery and in 40 samples of non-pregnant women. The iron status was compared using ANOVA and relationship between iron status parameters was tested by using nonparametric Spearman's correlation method.
Results. Iron levels were tenable till third trimester, and was also lower after delivery compared to 1st trimester (p<0.016, p<0.0026, respectively), probably because of prophylactic iron supplementation during the whole pregnancy. Accordingly, serum transferin levels and TIBC increased gradually from second towards third trimester and pre-term period (transferin:p< 0.023, p<0.0001, p<0.00001, respectively and TIBC: all three p<0.0001) compared to values in non-pregnant women. Ferritin levels progressively fell during the second, third trimester and in pre-term period and was significantly lower than in non-prenant women (p<0.0028, p<0,0009, p<0.0267, respectively). Ferritin values correlated with iron levels during all periods of pregnancy. In the pre-term period and after delivery ferritin levels had a significant inverse correlation with transferin (pre-term: ρ= -0.514, p=0.012; after delivery: ρ -0.400, p< 0.05) and marginally significant with TIBC levels (ρ= -0.372, p=0.088).
Conclusion Iron status change throughout the normal pregnancy evidenced higher sensibility of transferin and ferritin in recognizing iron deficiency, which is often a cause of pregnancy-related anemia, condition frequently associated with the risk of preterm delivery.

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EVALUATION OF SPOT URINE PROTEIN-CREATININE RATIO TO PREDICT SIGNIFICANT PROTEINURIA DURING PREGNANCY

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Background. To assess the diagnostic performance of the spot urine protein/creatinine (P/C) ratio to predict the absence or presence of significant proteinuria (³300 mg per 24 hours) among outpatient pregnant women with suspected or previous diagnosis of preeclampsia.
Methods. P/C ratio was calculated in 106 single voided urine samples, obtained after the completion of the 24-hour collection, from 66 outpatient pregnant women admitted to the Maternal Fetal Care Unit to follow-up of hypertension gestational. ROC curves analysis was used to evaluate the diagnostic performance and to determinate the best cutoff to predict the absence or presence of significant proteinuria.
Results. Significant proteinuria on 24 hour collection urine was identified in 31 urines from 22 pregnant women. ROC curves analysis revealed an AUC for spot P/C ratio of 0,838, greater than urine dipstick (0,629). No single P/C ratio cutoff was appropriate to rule-out or predict significant proteinuria; however, use of dipstick and spot urine P/C ratio, with two cutoffs, 120 mg/g to predict the absence of significant proteinuria and 240 mg/g to confirm it, clasiffied correctly 44,3% of urines and avoided the collection of 24 hours urine in 51% of the cases.
Conclusions. Spot urine P/C ratio, in conjunction with dipstick urianalysis, is a useful test in the initial screen for rule-out and predict significant proteinuria in outpatient pregnant women with hypertensive pregnancy or preeclampsia, but it should not be used as an alternative to 24-hour total protein evaluation in midrange P/C ratio, requiring a full 24-hour urine for accurate Results.

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POSSIBILITIES OF LONG-TERM STORAGE OF BIOCHEMICAL SCREENING MARKERS OF INBORN ERRORS OF DEVELOPMENT AND POSSIBLE INFLUENCE OF STORAGE ON REASSESSMENT OF DOWN SYNDROME RISK

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Backround. The study deals with possibilities of long-term storage of samples for the purpose of assessment of biochemical markers (AFP, HCG, free βHCG and PAPP-A) of inborn errors of development stability, and with the aim of assessment of Down syndrome risk under the influence of the markers prospective change.
Methods. We used two ways of the storage - in primary serum separation tubes SST with gel and separated serum storage in polypropylene tubes. Measurement of serum concentrations of AFP, HCG, free βHCG and PAPP-A we used the analyzer COBAS E411. Assessment of Down syndrome risk were proceed by Alpha software.
Results. The study of 120 samples demonstrated the possibility of long-term storage merely with free βHCG, HCG and PAPP-A for the period of 30 weeks under the temperature of -20°C and only in serum separation gel tubes SST. AFP cannot be stored for a long time. It appears improper to store separated serum in polypropylene tubes. Reassessment of long-term stored AFP samples or separated serum samples stored in polypropylene tubes can provide falsely negative results with assessment of potential risk of Down syndrome. Long-term storage of samples in primary gel tubes SST under -20°C does not demonstrate any hemolysis.
Conclusions. Long-term storage of samples for mentioned purpose is possible only for free βHCG, HCG and PAPP-A and can be practically used in screening of Down syndrome for the period of 9 weeks or 30 weeks in primary serum separation gel tubes SST and kept under -20oC.

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REFERENCE VALUES OF FETAL ERYTHROCYTES IN MATERNAL BLOOD DURING PREGNANCY, ESTABLISHED USING FLOW CYTOMETRY

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Background. The Kleihauer-Betke (K-B) assay is still widely used for quantifying fetomaternal hemorrhage (FMH). The K-B assay has inherent inaccuracies which are largely eliminated by flow cytometry. Although flow cytometry has superior analytical performance, surprisingly few papers reported reference ranges in pregnant women. The aim of our study was to assess the fetal RBC count in maternal blood during uncomplicated pregnancies. We used a flow cytometric method on a routine hematology analyzer, permitting FMH assays on a 24/7 basis.
Methods. Pregnant women were recruited through midwives and obstetricians; pregnancies with complications and high-risk pregnancies were excluded. All laboratories used the FMH QuikQuant kit (Trillium Diagnostics, Brewer, ME, USA) on a CELL-DYN Sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). Raw data were collected in FSC format and analyzed using flow cytometric software by two observers. Standard statistical methods were used. The 95% percentile reference range was estimated according to CLSI.
Results. In total 238 samples were suited for statistical analysis. Gestational ages ranged from 21.6 to 41 (mean 32.0) weeks and fetal RBC count from 0.00 to 0.50 (mean 0.0466) %. There was no significant correlation between fetal RBC count and gestational age ($r = -0.096$, $P=0.141$). ANOVA analysis also did not show a relationship ($P = 0.666$). The reference range for fetal RBC was 0.00 – 0.125% (90% confidence limits of the upper reference value 0.115 – 0.145%).
Conclusions. The fetal RBC count in maternal blood shows no correlation with gestational age. The reference values during pregnancy are < 0.125%.

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PRENATAL BIOCHEMICAL SCREENING IN THE 1ST(FREE BETA-HCG, PAPP-A) AND THE 2ND(TOTAL BETA-HCG, A-FETOPROTEIN, FREE ESTRIOL) TRIMESTERS OF PREGNANCY WITH IMMULITE 2000, ARCHITECT I2000, AND SOFTWARE PRISCA

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Background. Authors present a large laboratory's experience in use of some commercial reagents for prenatal biochemical screening and calculation an assay-dependent median values for weeks of gestation.
Methods. The 1st and 2nd trimester screenings are carried out with Immulite 2000, Siemens (free beta-hCG, PAPP-A, free estriol), Architect i2000, Abbott (hCG, AFP), and software PRISCA, Siemens. Data refer to presumably Moscow (Russia) region population. Total samples number for both 1st and 2nd trimester screenings is more than 7000/year. Used cut off value in Down syndrome screening was 1:250, % positive results was about 5,4%, detection level was > 70%.
Results. Free beta-hCG lot 250-265 (median ng/ml; n) – 9w (72.9; 151), 10w (58,8; 989), 11w (51.05; 3190),12w (44.35; 3142), 13w (36.9; 1343). PAPP-A, lot 401- 405 (median mU/ml; n) – 9w (0.87; 36), 10w (1.4; 365), 11w (2.14; 1293), 12w (3.2; 1310), 13w (4.24; 613). Total beta-hCG lot 82900-89907JN00 (median mU/ml, n) – 14w (51217; 271), 15w (41313; 622), 16w (33690; 1658), 17w (28312; 2256),18w (25934; 1733), 19w (24574; 1089), 20w (23112; 508), 21w (22886; 211). AFP lot 76503-88512LF00 (median U/ml; n) – 14w (25; 162), 15w (29; 348), 16w (33; 946), 17w (37; 1367),18w (40; 1050), 19w (45; 651), 20w (53; 300), 21w (62; 125). Estriol free lot 407-412 (median nmol/l, n) – 14w (5.1; 241), 15w (7.6; 517), 16w (11.0; 1463), 17w (13.0; 2067),18w (17.0; 1587), 19w (21.0; 1028), 20w (24.0; 448), 21w (27.0; 211).

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LEVELS OF ALPHA-FETOPROTEIN DURING PREGNANCY IN NORMALSTATEIN RUSSIAN POPULATION WITH TWO-SITE MONOCLONAL ELISA

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Background. Alpha-fetoprotein (AFP) is a protein normally produced by the fetal liver.AFP crosses the placenta into the mother's blood. Abnormallevels of maternal serum AFP may signal a problem with the fetus. For this reason, the determination of normal range limits is actually.

Methods. Serum concentration of AFP was measured using a monoclonal two-site ELISA (DS-EIA-AFP).

Results. Maternal serum AFP values from 804 unaffected, singleton white pregnancies (Central Russia and Volgo-Viatsky Region, mean age 27 years, range from 16 to 40 years) were ranged according to the gestational week from 8 to 36. Medians and multiples of medians (MoM) were calculated for every group. Normal range limits (0.5MOM-2.5MOM) for gestational weeks 14 to 21 are presented: 14 gestational weekmedian 25.24IU/ml normal range from 13 to 63IU/ml; 15 gestational weekmedian 32.59IU/ml normal range from 16 to 81IU/ml; 16 gestational weekmedian 36.78IU/ml normal range from 18 to 92IU/ml; 17 gestational weekmedian 37.47IU/ml normal range from 19 to 94IU/ml; 18 gestational weekmedian 38.25IU/ml normal range from 19 to 96IU/ml; 19 gestational weekmedian 50.70IU/ml normal range from 25 to 127IU/ml; 20 gestational weekmedian 58.19IU/ml normal range from 29 to 145IU/ml; 21 gestational weekmedian 53.51IU/ml normal range from 27 to 134IU/ml.

Conclusions. We defined normal limits of maternal serum AFP in Central Russia population with DS-EIA-AFP. We concluded that the definition of abnormal range of AFP for the identification of cases at pregnancy risk will probably only be possible at 14-21 weeks.

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A COMPARISON OF RAPOD AMNIOTIC FLUID MARKERS IN THE PREDICTION OF MICROBIAL INVASION OF THE UTERINE CAVITY

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Background. Bacterial infection of amniotic cavity (chorioamnionitis) is a significant couse of perinatal morbidity and mortality. Our objective was to analyze the value of amniotic fluid test for detection of microbial invasion of the uterine cavity.

Methods. Amniotic fluid was obtained by amniocentesis from one hundred fifteen consecutive patients, from 16 to 18 gestational weeks. Fluid was analyzed for leukocyte count,glucose level,lactate dehydrogenase level (LDH), and Gram stain. Cultures for aerobes and anaerobes were performed. Sensitivity, specificity, and positive and negative predictive value were calculated for LDH, leukocyte count, glucose, and Gram stain in the prediction of positive amniotic fluid culture. Receiver-operator characteristic curve analysis,t test, and nonparametric test (Mann Whitney U-test) were used.

Results. The prevalence of positive amniotic fluid cultures was 13% (14 of 115).The median LDH level (414 U/L) was significantly greater for women with a positive amniotic fluid culture than for those with a negative culture (median LDH 143 U/L;p<0,01). Critical values of LDH > or = 205 U/L, leukocite count > or = 10 cells/mm (10x10(6)/L) and glucose < or = 1,15 mmol/l were selected for optimal performance in prediction of a positive amniotic fluid culture. LDH level had the best sensitivity (71%) in contrast to leukocyte count (51%), glucose (65%) and positive Gram stain (36%).

Conclusions. Amniotic fluid LDH level has diagnostic value in prediction of a positive amniotic fluid culture. Lactate dehydrogenase is a readily available,inexpensive, rapid amniotic fluid marker that can be measured in any hospital laboratory.

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CORRECTION FACTORS FOR ETHNIC GROUPS APPLIED IN FIRST TRIMESTER PRENATAL SCREENING

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Background. The recent incorporation of first trimester prenatal screening for aneuploidies detection in clinical laboratories in Catalonia, as well as the demographic changes in the last years has let to us to *review* the correction factors for the different ethnic groups we have in our population.
Material and Methods. We have retrospectively evaluated the results obtained in 30500 pregnant women in Catalonia, attended in 2010 in 11 different clinical laboratories, using two different commercial kits (Siemens® Immulite 2000 and Perkin Elmer®) for PAPP-A and fβhCG levels determination.
Corrected MoMs for PAPP-A, fβhCG and NT were calculated in the total group, and in subgroups according the following ethnic differences: Caucasian, black, maghribian and asiatic women. Calculations were performed globally and for each laboratory to detect differences attributable to possible different correction factors applied.
Results. The median of MoMs in all the cases was 0.98 for PAPP-A (0.99 for Caucasian, 0.93 for black, 0.96 for maghribian and 1.13 for asiatic women), 1.04 for fβhCG (1.03 for Caucasian, 1.05 for black, 1.02 for maghribian and 1.22 for asiatic women). The analysis of the different laboratories showed different values in few of them. All the medians of NT values expressed as MoMs were lower or equal than 0.95 in both analysis (global an ethnic groups)
Conclusions. Revaluation of correction factor for fβhCG and or PAPP-A values in maghribian, black and asiatic women has to be considered.All laboratories need to unify the correction factors to lower results variability.The low level of NT observed in all the analysis can imply that the curve introduced in our calculation programs needs to be changed in our geographic context.

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RELATIONSHIP OF SOLUBLE RESISTIN LEVELS, GLUCOSE AND INFLAMMATION MARKERS WITH ADIPOSITY AND THE 45T>GAND 276G>T *ADIPOQ* POLYMORPHISMS IN MEXICAN PREGNANT WOMEN

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Background. Human pregnancy is characterized by a progressivechange in body fat mass, whereas soluble resistin is produced in human placenta and adipose tissue that have profound effects on inflammation and glucose metabolism. Adiponectin is a functional opponent of resistin. *ADIPOQ* gene presents 45T>G and 276G>T single nucleotide polymorphisms (SNPs), however the functional effects are undefined.
Methods. In this cross-sectional study 340 Mexican pregnant women classified by trimester were included. Resistin was determined by ELISA (R & D Systems Inc.; USA). The body composition, glucose and inflammation markers were measured by routine Methods. The genotypes were characterized using the PCR-RFLP technique. Statistical analysis was performed using the PASW Statistics v18.0.
Results. The adiposity in pregnant women showed positive correlation with erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCrp), glucose and white blood cells (WBC) [r=0.125 to 0.302]; and glucose with sResistin [r=0.294], and hsCrp [r=0.139]; p<0.05. Genotype distributions were in Hardy-Weinberg equilibrium for both SNPs. In this study group the ADIPOQ SNPs showed the follow distribution, 45T>G: T/T, T/G, and G/G genotypes frequencies were 67.2%, 31.3%, and 1.5%, respectively; and 276G/T: G/G, G/T, and T/T genotypes frequencies were 55.2%, 39.5%, and 5.3%, respectively. Individuals carrying out the allele 276T showed a tendency to have higher levels of sResistin, WBC, glucose and ESR, and lower adiposity and hsCrp, than individuals with 276G allele.
Conclusions. This study suggests that SNP 276G>T *ADIPOQ* possibly will affect the accumulation of fat mass during pregnancy.

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IMPACT OF AGE ON LEVEL OF CYSTATIN C IN WOMEN WITH PIH

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Background. Pregnancy can induce anatomical and functional changes of the kidneys, hypertension and other complications. The aim of this study was to determine the age impact on levels of cystatin C in women with PIH (pregnancy induced hypertension) to defining its role in complicated pregnancies. The investigation was performed according to the Declaration of Helsinki and all subjects gave informed written consent for participation. A total of 33 pregnant women in the third trimester with PIH were divided in the age group: group I – 7 pregnant women were in the age-group of 20–24.9 years, group II-7 pregnant women were in the age group of 25–29.9 years, group III 14 pregnant women were in the age-group of 30–34.9 years and group IV–5 pregnant women were in the age-group >35 years.

Methods. Serum cystatin C was determined by the PENIA (Particle-Enhanced Nephelometric Immuno-Assay) method, using the Dade Behring tests, on a Behring Nephelometer II (DADE Behring, Marburg, Germany). Results were statistically analyzed using the ANOVA.

Results. The mean serum cystatin C in total pregnant women with PIH was 1.45±0.43 mg/L and increasing gradually with age: it was 1.08±0.21 mg/L in the group I, 1.40±0.31 mg/L in the group II, 1.56±0.37 mg/L in the group III and 1.76±0.65 mg/L in the group IV. We found statistical significant difference between age group I and III as well as I and IV (p <0.05).

Conclusions. We conclude that Cystatin C depending of age in pregnancy women with PIH.

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BIOCHEMICAL MARKERS IN UMBILICAL CORD BLOOD AS PREDICTORS OF PERINATAL COMPLICATIONS

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Background. During pregnancy inflammatory,metabolic and immunologic disorders that affect differently the fetus,are known. These could be early disorders:abortion,intrauterine growth retardation(IGR),low birth weight and neonatal death; or late disorders: cardiovascular and metabolic disease in adults.

To analyze different biochemical parameters(BP) in maternal venous blood(MVB) and umbilical cord blood(UCB) of newborn from healthy mothers and mothers with basal pathologies and associated to gestation that allow the early detection of perinatal complications.

Materials and Methods. Samples from MVB(173) and UCB(173) were analyzed. Delivery was by cesarean. Mothers and newborn were classifiedcontrols(C-n=64) and pathological(P-n=109). Maternal pathologies: diabetes,hypertension,anti-phospholipid syndrome,hyper/hypotiroidism,intrahepatic-cholestasis and genital infections. Pathological newborn:IGR and/or fetal distress.

BP:Glucose,urea,creatinine,uricacid,bilirubin,proteins,albumin,transaminases(ALT/AST),alkaline-phosphatase,gammaglutamylt ranspeptidase(GGT),creatinkinase,lactatedehydrogenase(LD),iron,calcium,phosphorus,magnesium,sodium,potassium(K),chlori ne,cholesterol,triglycerides,hsCRP were determined by recommended methods-Roche autoanalizer.Student and Mann Witney tests were applied, p<0.05.

Results.

- Pnewborn from P mothers showed significant decrease: in gestation weeks(GW) and newborn weight(NW) with respect to C newborn from C mothers (p:0,001;0.01, respectively); significant increases in K,AST,LD,GGT (p:0,005;0,03;0,03;0,02;respect ively).

- P mothers related to C mothers showed significant increase in hsCRP(p:0,02).

Conclusions. In P newborn from P mothers with respect to C, the decrease in GW and NW would be related to IGR that accompany these pathologies; increases in K,AST,LD,GGT would be related to cellular destruction associated to maternal pathologies and deficit in pulmonary development by IGR, respectively.

The increase of hsCRP from P mothers with respect to C mothers would be associated to an inflammatory process.

A future study with a greater number of samples and analysis of each maternal pathology,in order to obtain early markers of neonatal damage, is proposed.

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ENDOTHELIUM DYSFUNCTION AND DYSLIPIDEMIA AS RISK FACTORS OF FETOPLACENTAL INSUFFICIENCY IN PREGNANCY WITH ADIPOSITY

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Background. The aim of this study is to investigate features of lipid metabolism and endothelium function at pregnant women with adiposity.
Methods. 90 women were examined at the third trimester of pregnancy. There were 35 patients with adiposity of 1st degree (Quetelet index (QI)=30.0-34.9 kg/m^2) aged 25.4±7.6 yrs in the first group, 35 ones with adiposity of 2nd-3d degree (QI=35-40 kg/m^2) aged 26.7±6.1 yrs in the second group. The control group was consisted of 20 pregnant with normal weight body (QI=18.5-24.9 kg/m^2) aged 24.2±5.4 yrs.
The lipid status was evaluated by determination of cholesterol, triglycerides, high-density and low-density lipoproteins (HDL, LDL) blood levels with spectrophotometry. Serum concentration of apolipoproteins A-1 and B, homocysteine was determined by means of immunoturbidimetry. The content of endothelin-1, nitric oxide products (nitrites, nitrates) was measured by ELISA. Statistical analysis was made with Mann-Whitney U-test, analyzed parameters were presented as median and range.
Results. At the first and second groups it was detected the high levels of cholesterol, LDL, triglyceride versus controls (p˂0.01). Endothelin-1 level was 5.34[2.85] and 5.94[3.04] pg/ml accordingly, what exceeded control level in 4.0 and 4.4 times accordingly (p˂0.001). The highest content of endothelin-1 was revealed in patients with adiposity in development of fetoplacental insufficiency. Homocysteine concentration exceeded controls by 117.0 and 200.0 % in these groups accordingly. Such changes were obtained against background of marked reduction of nitric oxide content.
Conclusions. Thus, endothelium dysfunction and atherogenic dislipidemia contribute to the formation of fetoplacental insufficiency at pregnancy with adiposity.

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SECOND TRIMESTER MATERNAL SERUM SCREENING FOR DOWN'S SYNDROME: TOTAL HCG, α-FETOPROTEIN AND UNCONJUGATED ESTRIOL (TRIPLE TEST)

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Background. The triple screening test is performed between the 14th and 22nd week of pregnancy. This test is recommended for women who have a family history of birth defects, are 35 years or older, used possible harmful medications or drugs during pregnancy, have diabetes and use insulin, had a viral infectious during pregnancy.
Methods. These results represent two years study of screening for genetic disorders (trisomy 21, trisomy 18 or another tip of chromosome abnormality). Triple screening test is a combination of maternal age, weight, ethnicity, gestation of pregnancy, biparietal diameter (BPD) and serum total HCG, AFP and unconjugated estriol. We analysed 104 serum samples from pregnant women in second trimester by Immulite 1000 (Siemens, risk assessment software PRISCA 4). The distribution of estimated risks for trisomy 21 was determined and sensitivity and false-positive rate for a risk cut-off 1 in 250 were calculated.
Results. We analysed 104 pregnant women. The average maternal age was 29 (range 16-43) years and in 15 (14%) the age was 35 years or more, the average gestation at screening was 15.5 (14-22) weeks and average of BPD was 35 (range 29-56) mm. The estimated risk for trisomy 21 based on mentioned parameters was 1 in 250 or greater in 2.9% (3 of 104) of pregnancies.
Conclusions. Our result of screening for chromosome abnormality by measuring of fetal BPD and maternal biochemical parameters are similar to those reported in literature.

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INVESTIGATION OF NORMALHCGRANGES DURING PREGNANCY IN RUSSIAN POPULATION WITH TWO–SITE MONOCLONAL ELISA

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Background. Level of human chorionic gonadotropin (HCG) is an important marker for prenatal screening.
Methods. Serum concentration of HCG was measured using a monoclonal two-site ELISA (DS-EIA-GONADOTROPIN-HCG).
Results. 817 samples of serum from white women (Central Russia and Volgo-Viatsky Region, mean age 27 years, range from 16 to 40 years) with normal pregnancy have been measured. Results of analysis were ranged according to the gestational week. Median, 2.5th and 97.5th percentile were calculated for every group: 1-3 gestational weeks: median 7740mIU/ml, normal range from 50 to 15000mIU/ml; 3-4 gestational weeks: median 20100mIU/ml, normal range from 1200 to 30000mIU/ml; 4-5 gestational weeks median 33800mIU/ml, normal range from 2500 to 85000mIU/ml; 5-6 gestational weeks: median 67200mIU/ml, normal range from 8500 to 155000mIU/ml; 6-7 gestational weeks median 108100mIU/ml, normal range from 12000 to 215000mIU/ml; 7-8 gestational weeks median 132900mIU/ml, normal range from 35000 to 255000mIU/ml; 8-9 gestational weeks: median 149200mIU/ml, normal range from 40000 to 280000mIU/ml; 9-10 gestational weeks: median 137800mIU/ml, normal range from 35000 to 250000mIU/ml; 10-11 gestational weeks: median 114600mIU/ml, normal range from 30000 to 205000mIU/ml; 11-12 gestational weeks: median 100600mIU/ml, normal range from 16000 to 195000mIU/ml; 13-14 gestational weeks: median 60400mIU/ml, normal range from 16000 to 165000mIU/ml; 15-16 gestational weeks: median 35000mIU/ml, normal range from 9500 to 100000mIU/ml; 17-21 gestational weeks: median 30100 mIU/ml, normal range from 7000 to 75000mIU/ml.
Conclusions. We defined normal limits of serum HCG in Central Russia population with "DS-EIA-GONADOTROPIN-HCG". It is necessary for estimation of abnormal pregnancy risk.

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THE USE OF CELL POPULATION DATA FOR THE DIAGNOSIS OF NEONATAL SEPSIS

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Background. Larger, immature neutrophils pour in the bloodstream during neonatal sepsis. Current technology allows to assess the neutrophil volume distribution, an index that has previously been used to detect acute bacterial infections in the adult. We have studied automated neutrophil volume and its distribution as a screening tool for late onset sepsis in very low birth weight neonates.
Patients and Methods. Consecutive very low birth weight symptomatic neonates were screened for sepsis using complete blood count (CBC), absolute neutrophil count (ANC), immature /total (I/T) ratio, C-reactive protein (CRP). Neutrophil volume (NeV) and neutrophil volume distribution width (NDW) were determined both in infants with suspected sepsis and in a group of controls matched for gender, birth weight and gestational age who were not symptomatic for sepsis. Blood culture was used as the gold standard for infection. Receiver operator curves, area under the curve, sensitivity, specificity, positive and negative predictive values were calculated for each test.
Results. We enrolled 120 neonates with suspected sepsis and 60 asymptomatic infants. NeV proved to be a useful diagnostic test for sepsis on a single determination (sensitivity= 95% ; specificity=88%; cut off =148 arbitrary units) performing better than CRP (sensitivity= 65%; specificity=96%; cut off =0.9 mg/dl), white blood cells count, ANC and I/T ratio. NDW was of poor value (sensitivity=80% specificity=52% cut-off=27.5). When CRP and NeV were considered together, sensitivity was unchanged while specificity rose to 97%. NeV was positive in 1 out 60 asymptomatic infants.
Conclusions. NeV is a reliable and inexpensive adjunct to the current screening tests for neonatal late-onset sepsis.

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IMPACT OF DIFFERENT CLASSIFICATIONS ON THE PREVALENCE OF GESTATIONAL DIABETES MELLITUS

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Background. There are different recommendations (WHO, ADA, and HAPO) on how to diagnose gestational diabetes mellitus (GDM) and there is so far no worldwide consensus, which cut-offs should be used.
Aim. To assess, how the application of different recommendation will affect the prevalence of GDM.
Methods. Data from a cohort of women routinely having an oral 75g oral glucose tolerance test done during pregnancy was analyzed. Glucose was tested on Roche Integra at baseline, 60min, and 120min. GDM was defined according to the recommendations of WHO (glucose after 120min $\geq$7.8 mmol/L), ADA ($\geq$5.3 mmol/L baseline, $\geq$10 mmol/L 60min, $\geq$8.6 mmol/L 120min; at least 2 criteria have to be met for GDM-diagnosis) and HAPO-based consensus conference ($\geq$5.1 mmol/L baseline, $\geq$10 mmol/L 60min, $\geq$8.5 mmol/L 120min; at least 1 criterion has to be met for GDM-diagnosis).
Results. From 770 women, 171 (age 31.2 ± 5.1 years, 26 ± 2.8 weeks pregnant) met inclusion criteria. A significant proportion of women were excluded due to protocol violation (n=599; reasons: wrong window of testing, incomplete sampling, use of sucrose instead of glucose). When applying the WHO guidelines, 15 women (8.3%) were diagnosed with GDM. According to the ADA guidelines, only 3 women (1.7%) had GDM. Finally, when applying the new HAPO recommendations, 17 women (8.8%) had GDM (p=0.02 vs. cases identified by ADA-criteria). In a substantial proportion of the cases (53.3%), GDM according to HAPO could be diagnosed with the baseline value.
Conclusions. Compared to the formerly used recommendations, implementation of HAPO-based consensus conference recommendations will lead to an increase of GDM prevalence in Switzerland. At the moment , there is poor adherence to protocols.

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EVALUATION OF A POINT OF CARE TEST FOR MEASUREMENT OF BETA-HCG IN SERUM

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Background. The panel for emergency markers in clinical chemistry includes the measurement of beta-HCG. To date, only few devices for point-of care (POCT) determinations of this marker are available.
Aim. To characterize the analytical characteristics of the AQT90 Flex Beta-HCG Test (Radiometer, Copenhagen), which can be measured in a POCT setting.
Methods. We assessed imprecision, analytical sensitivity, linearity, carry-over, minimum sample volume needed and performed a method comparison in routine samples also employing VIDAS beta-HCG test (Biomerieux, Geneve) and the Architect Beta-HCG test (Abbott, Baar).
Results. Coefficients of variation (CV's) were (n=20) were 9.7% at a level of 6 U/L and 3.3% at a level of 369 U/L. Analytical sensitivity was 2 U/L. The test has a measurement range up to 5000 U/L and did not provide substantial differences between expected and observed values in linearity on dilution studies. No carry-over could be detected. Because of an absent level detection, a minimum amount of at least 150 microliter in the specified sample tube is needed to provide reliable Results. The method comparison revealed the following linear regression equations: VIDAS Beta-HCG = 0.87 *AQT90 Flex Beta-HCG – 115 U/L ($R2=$ 0.97); Architect Beta-HCG= = 0.95 *AQT90 Flex Beta-HCG – 332 U/L ($R2=$ 0.99).
Conclusions. The AQT90 Flex Beta-HCG test provides a feasible and accurate method for rapid point of care assessment of Beta-HCG concentrations in serum.

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ROLE OF ANTI-B2- GLYCOPROTEIN I ANTIBODIES IN RECURRENT EARLY PREGNANCY LOSS

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Background. Antiphospholipid (APL) antibodies have been known to be associated with poor pregnancy outcome. Among which anti-beta-2-Glycoprotein I antibodies have been shown to be most specific for thrombosis in patients with antiphopholipid syndrome suffering from Recurrent pregnancy loss RPL.
Methods. The present study was carried out in El Shatby Maternity University Hospital to study the levels of Anti-β2-Glycoprotein I (b2-GPI) antibodies (IgG, IgM) and Antiphospholipid (APL) antibodies (IgG, IgM)antibodies in normal pregnant females (control group 22),at first trimester and in those with recurrent early pregnancy losses within first trimester (cases group 62), in order to evaluate and compare the role of each antibody in recurrent early pregnancy loss.
Results. The present study showed that the cases group had a significant higher concentration of anti-β2-glycoprotein 1 IgG(7.88+/-6.71 U/ml (P=0.009*) as well as APL IgM antibodies(12.3+/-6.46 U/ml(P<0.001*) than the control group (5.48 U/ml ,6.83 U/ml respectively).According to the statistical ROC Curve, APL IgM showed significantly highest specificity and sensitivity followed by anti-β2-GP1 IgG antibodies (area= 0.727, 0.687 which were statistically significant (P = 0.000, P= 0.009 respectively), followed by anti-β2-GP1 IgM, then APL IgG antibodies (area = 0.585, 0.526 respectively which were not statically significant (P= 0.238, 0.714 respectively).
Conclusions. Anti-b2-GP1 IgG and APL IgM are better diagnostic markers for recurrent pregnancy loss compared to Anti-b2-GP1 IgM and APL IgG. Anti-b2-GP1 IgG antibodies and APL IgM were significantly higher in women with recurrent pregnancy loss than in control group.

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ASYMMETRIC DIMETHYLARGININE (ADMA), IN PREGNANCIES WITH SMALL FOR GESTATIONAL AGE BABIES, AND IN PREECLAMPSIA

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Backgound. Asymmetric dimethylarginine (ADMA)is an endogenous competitive inhibitor of nitric oxide synthase. In many studies, elevated ADMA levels have been reported in preeclampsia (PE) compared to normal pregnancies, but not in pregnancies with small for gestational age (SGA) babies.
Patients and Methods. ADMA concentrations in a) 42 normal pregnancies, b) 5 pregnancies that developed early PE, c) 8 pregnancies that developed late PE and d) 12 pregnancies that delivered a SGA baby, were determined retrospectively in the first (11.57 to 13.57 weeks), second (20.29 – 23.43 weeks) and third (28.0 – 34.71 weeks) trimester of pregnancy. ADMA was measured with commercial kit: ADMA-ELISA (*DLD*, Hambugr, Germany).
Results. Mean (±SD) concentrations of ADMA (μmol/L) in normal pregnancies were: 0.49±0.14; 0.51±0.13; 0.55±0.17 in the three trimesters respectively. ADMA concentrations in pregnancies with SGA infants were significantly lower in each trimester compared to normal pregnancies: (0.40±0.08, P=0,03 1st trim**;** 0.41±0.10, P=0,03 2nd trim;0.45±0.09, P=0,04 3rd trim). Although ADMA concentrations in both early (0.57±0.04; 0.56±0.05; 0.68±0.15) and late PE pregnancies (0.53±0.15; 0.61±0.20; 0.52±0.08) were higher than in normal pregnancies, in all three trimesters, this difference was not statistically significant. 2nd trimester ADMA concentration in normal pregnancies correlated significantly with maternal weight. (r=0.44; p<0.01).
Conclusions. Maternal serum ADMA concentration remains unchanged throughout normal pregnancy. An important finding of our study is that pregnancies that gave birth to SGA infants had significantly lower ADMA levels in all trimesters of pregnancy. We didn't find significantly elevated ADMA concentrations in PE pregnancies in any trimester.

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CORD BLOOD LEVELS OF BIOMARKERS IN EARLY NEONATAL SEPSIS

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Background. Early neonatal sepsis is a major cause of morbidity and mortality in the neonatal period, and confirmatory diagnosis depends on the results of blood cultures. Therefore,it is necessary to have a biochemical marker that allows early diagnosis.The aim of this study was to investigate whethercord blood (cb) levels of C-Reactive Protein (CRP), Procalcitonin (PCT) and Interleukin-6 (IL6)were useful markers in the diagnosis of early neonatal sepsis.
Methods. Umbilical cord blood samples were obtained from 813 newborns (454 men, 647 women; age: 39 [37-40] weeks), and we established two groups; group 1 (G1): with suspected infection and associated risk factors requested a blood culture, and group 2 (G2):infants without known risk factors as reference group.We studied the relationship of biomarkers between these two groups, both in blood of newborns and cord blood; and correlations between these parameters.
Results. There were significant differences between both groups, over neonatal biochemical parameters: CRP (0.0 [0.0-0.63] and 0.2 [0.0-0.7] mg/dL),($p<0.001$); IL6 (44.92 [17.26-184.4] and 35.73 [18.37-86.17]pg/mL), ($p=0.050$); and over cord blood:IL6 (8.29 [2.33-43.29] and 3.405 [1.735-8.178]pg/mL), ($p<0.001$). Correlations were significant between biomarkers in newborn and in cord blood: PCR-PCRcb(r =0.15, $p=0.037$), PCR-PCTcb(r=0.22, $p=0.002$), PCR-IL6cb(r=0.14, $p=0.049$), PCT-PCRcb(r=0.18, $p=0.019$), PCT-PCTcb(r=0.19, $p=0.014$), IL6-IL6cb(r=0.18, $p=0.018$).
Conclusions. CRP and IL-6 were markers that correlated with the presence of risk factors or suspected infection in newborns. Therefore, the determination of these markers in cord blood could be a breakthrough on the diagnosis of early neonatal sepsis and subsequent appropriate treatment.

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FIRST-TRIMESTER RISK CALCULATION FOR TRISOMY 13, 18, AND 21: COMPARISON OF THE SCREENING EFFICIENCY BETWEEN TWO SELF-DEVELOPED PROGRAMS AND ASTRAIA SOFTWARE

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Background. First trimester screening for Trisomy 13, 18, and 21 can be based on maternal age, fetal nuchal translucency thickness (NT), free beta subunit of human chorionic gonadotropin (hCGβ), and pregnancy-associated plasma protein-A (PAPP-A) in maternal plasma. Reliable individual risk calculation is dependant on good estimates of the medians for NT, hCGβ, and PAPP-A in unaffected pregnancies and the means and standard deviations of these parameters in unaffected and affected pregnancies in the risk calculation program.
Methods. We established these estimates from 19594 women with singleton pregnancies and 100 pregnant women carrying fetus affected with trisomy (11 with T13, 23 with T18, and 66 with T21). All measured values were recalculated to a multiple of the median (MoM) and $\log_{10}$ transformed; the mean and standard deviation were calculated for each group. Two risk calculation programs, based on univariate and trivariate normal distributions, were developed to see whether the screening efficacies could be improved by using our own estimates compared with those obtained by the commercial program from Astraia.
Results. At a given risk cut-off value, we observed a slight improvement in detection rate (DR) for T13, T18, and T21 for a slightly higher false-positive rate (FPR) compared with the commercial program. The lower FPR in the commercial program was caused mainly by an inaccuracy in the PAPP-A median.
Conclusions. Center-specific medians are preferable as default medians for NT, hCGβ and PAPP-A in risk calculation programs in order to assure high DRs and low FPRs for all three trisomies at a given risk cut-off.

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MISSED FOLLOW-UP OPPORTUNITIES IN GESTATIONAL DIABETES SCREENING

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Background. Convincing evidence of benefit from large outcome trails has reinforced recommendations to screen all pregnant women for gestational diabetes (GDM). Canadian guidelines, among others, recommend a 2-step screening approach: a 50g glucose challenge test (GCT) followed by a 75g-oral glucose tolerance test (OGTT) in those who meet dysglycemia criteria below the GDM threshold. To assess adherence to this two-step approach, we undertook an audit.
Methods. The audit was conducted at Hamilton Health Sciences (Hamilton, ON, CANADA), a university hospital centre with a catchment of ~2.3million people and ~3000births/year. We audited all GCTs and OGTTs entered into the laboratory information system (January 2007-June 2010). Data were collected for plasma glucose, practitioner-type, age, and date. Practice was compared to Canadian Diabetes Association guidelines.
Results. The audit identified 1026 GCTs. 274 (27%) were positive (43 for GDM and 231 for dysglycemia criteria). Of the 231 women who met dysglycemia criteria, only 87(38%) received the recommended 75g-OGTT. Normal glucose tolerance, impaired glucose tolerance, and GDM were diagnosed in 59 (68%), 19 (22%), and 9 (10%), respectively. Practitioner-type, date in relation to the latest outcome trial showing benefit, age, and borderline results did not effect the probability that the 2-step approach was followed appropriately (c^2, p>0.05).
Conclusions. Adherence to the 2-step screening approach for GDM was low over the 3.5-years. Only 38% of those who were diagnosed as meeting GCT dysglycemia criteria received a follow-up 75g-OGTT. A repeat audit will assess resulting changes to practice. Newer guidelines with a 1-step approach may improve adherence.

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SERUM HIGH SENSITIVE C-REACTIVE PROTEIN LEVEL IN NORMAL PREGNANCY AND HIS ASSOCIATION WITH BODY MASS INDEX

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Background. The aim of this study was to determine the level of high sensitive C-reactive protein (hsCRP) in healthy pregnant women and his association with the body mass index (BMI).
Methods. hsCRP was measured by a immunoturbidimetric method in forty-three healthy pregnant women in the 1st, 2nd and 3rd trimester, before (38 week of gestation) and after (6.5 weeks) delivery.
Results. Geometric mean and 95% confidential intervals were calculated according to gestational age and BMI values. The values of hsCRP increased through pregnancy and decreased after delivery. 1st trimester: 2.67 mg/L (1.96-3.71), 2nd trimester: 2.60 mg/L (1.99-3.39), 3nd trimester: 2.89 mg/L (2.14-3.90), before deliver: 2.50 mg/L (1.91-3.28) and after delivery: 1.48 mg/L (1.07-2.06). The hsCRP values through pregnancy were not significantly different, but after delivery were significantly lower.
Mean values of hsCRP were higher in overweight (BMI 28.4+/-2.52) than in normal weight women (BMI 22.3+/-1.71): 3.05 mg/L (2.52-3.70) vs. 1.97 mg/L (1.65-2.35), p<0.01. The hsCRP values were higher as BMI quartiles increased: Q1 (BMI 17.6-21.9) –hsCRP 1.51 mg/L (1.19-1.93), Q2 (BMI 22.0-24.2) – hsCRP 2.21 mg/L (1.73-2.80), Q3 (BMI 24.3-27.4) – hsCRP 2.88 mg/L (2.14-3.86) and Q4 (27.5-35.5) – hsCRP 3.50 mg/L (2.78-4.42). Post hoc test among quartiles of BMI were significant for Q1xQ2, Q1xQ3 and Q1xQ4. Spearman correlation coefficient between BM and hsCRP (log) were significant: r = 0.294, p<0.001.
Conclusions. The present investigation provides evidences on higher levels of hsCRP in overweight pregnant women and the positive relationship between BMI and hsCRP values in pregnancy.

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A CASE OF A YOUNG WOMAN WITH HAEMOLYTIC ANAEMIA

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Background. A 34-year old woman was referred to the hospital by her general practitioner because of haemolytic anaemia and, since two weeks, complaints of fatigue, mild abdominal pain and irregular vaginal blood loss. Three weeks earlier she suffered from pharyngitis wherefore antibiotic therapy.
Methods. Common laboratory and imaging tests were used. Methaemalbumin was measured spectrophotometrically.
Results. On admission blood analysis showed increased CRP, ESR, LD levels and an increased reticulocyte count. In addition haemoglobin concentration was decreased and haptoglobin was undetectable. The direct Coombs test was negative and erythrocyte morphology was normal. Remarkably, her serum was darkly brown coloured (cola), and turned out to be caused by the presence of methaemalbumin. The next days haemoglobin and LD levels normalised and therefore first diagnosis was transient haemolytic anaemia following infection. In addition, the patient was referred to the gynaecologist because of a positive home pregnancy test. Blood hCG was 89 U/L. Surprisingly, ultrasonography showed an extrauterine gravidity (EUG) in the right fallopian tube and a fluid collection intraperitoneally. Salpingectomy revealed an EUG imbedded in old blood. Laboratory findings normalised two weeks after.
Conclusions. The possibility of an atypical haemorrhagic EUG should be considered when a fertile woman presents with Coombs negative haemolytic anaemia. Detection of methaemalbumin can be a supporting laboratory finding.

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AUTOMATED ASSAYS FOR SVEGF-R1, PLGF, INHIBIN-A AND PAPP-A AS AN AID IN EARLY PREDICTION OF PREECLAMPSIA : PRELIMINARY RESULTS FROM A PROSPECTIVE CLINICAL STUDY IN A GENERAL POPULATION

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Background. The purpose of this study was to assess the utility of free soluble vascular endothelial growth factor 1 (sVEGF-R1), free placental growth factor (PlGF), Inhibin-A and Pregnancy-associated plasma protein A (PAPP-A) levels in early prediction of preeclampsia.
Methods. A multicenter prospective clinical study conducted in a general population of singleton pregnancies. Plasma was collected between 14 weeks' gestation (WG) and 18 WG, from 8516 patients; 139 patients developed preeclampsia (1.6%). A nested case-control study was performed. Preeclamptic patients were compared to a first group of 556 patients randomly selected, and to another group of 556 patients matched to the case group according parity, gestational age at blood sampling and maternal age. Assays for free sVEGF-R1(pg/ml)*, free PlGF(pg/ml)*, Inhibin-A(pg/ml) and PAPP-A(mIU/L)* were performed on a Beckman Coulter UniCel® DxI 800 chemiluminescent immunoassay analyser
Results. Free PlGF (92.7 vs 120.4, $p<0.0001$), PAPP-A (2598.7 vs 3066.4, $p=0.007$) and Inhibin-A (94.1 vs 124.9, $p=0.001$) were significantly different between patients who later developed preeclampsia compared to the randomly selected control group. Free PlGF was significantly different between the case group and the matched controls (92.7 vs 108.9, $p<0.0001$). Free sVEGFR-1 levels were not significantly different between the three groups.
Conclusions. In a general population where the prevalence of preeclampsia is low, free PlGF, Inhibin-A and PAPP-A assessed between 14 and 18 WG appear to be useful markers in differentiating women who later develop preeclampsia from patients without preeclampsia.
Assays are under development and not available for clinical use

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BECKMAN COULTER TOTAL HUMAN CHORIONIC GONADOTROPHIN (HCG) AND INHIBIN A IN SECOND TRIMESTER DOWN'S SCREENING USING A QUADRUPLE TEST: A RETROSPECTIVE COMPARISON WITH TRIPLE TESTING

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Background. Inhibin A, total beta hCG, Alpha-fetoprotein (AFP) and Unconjugated Estriol (uE3) are available on the Beckman Coulter Access® II but free beta hCG, often preferred in Down's screening, is not. The aim of this study was to investigate the effect of substitution of Total for free beta hCG in the quadruple test and the efficacy of this over the Triple test.
Methods. Total beta hCG and Inhibin A were measured on the Beckman Access® 2, PerkinElmer AutoDELFIAwas used to measure AFP, free beta hCG and uE3. Sera from 770 pregnant women were investigated and detection rates (DR) and false positive rates (FPR) compared.
Results. In the quadruple test, for a fixed FPR of 3% the use of total beta hCG gave a DR of 69% whilst the use of free beta hCG gave a DR of 68%. Using a fixed cut off of 1 in 200, DRs are 65% with a FPR of 3% for total and 62% with FPR of 3.1% for free beta hCG .The triple test at a fixed FPR of 3% gave a detection rate of 66%. At a fixed cut
off 1 in 200 the Dr was 65% and FPR 3.8%.
Conclusions. Total beta hCG can be substituted for free beta hCG in the second trimester Downs' screening quadruple test without loss of performance and may give slightly better Results. The addition of Inhibin A as the fourth marker shows an improvement in either DR or FPR over the triple test.

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SCREENING FOR CHROMOSOMAL ABNORMALITIES IN THE FIRST TRIMESTER USING ULTRASOUND AND MATERNAL SERUM BIOCHEMISTRY. A REWIEW OF ONE YEAR PROSPECTIVE EXPERIENCE

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Background. The first-trimester prenatal screening is a combination of tests performed during the first –trimester of pregnancy. This approach calculates the risk of the most frequent chromosomopathies (i.e. Down Syndrome and Edwards Syndrome) through multivariant statistical analysis.
The aim is to offer a non-invasive confirmatory test or voluntary pregnancy termination option to patients with a high risk of chromosomopathies.
Specific aims: To evaluate the accuracy and reliability of one-step multidisciplinary clinical screening for foetal chromosomal anomalies in the first trimester of pregnancy. This approach combines biochemical analysis of maternal serum and foetal ultrasonography supplied by our hospital.
Material and Methods. During one-year study 1904 serum samples from pregnant women have been studied. PAPP-A and beta-HCG have been determined by chemiluminescent immunoassay.
PRISCA 4.0 program has been used to estimate syndrome risks. A cut-off of 1/250 for Down Syndrome and 1/100 for Edwards Syndrome risk has been set up in this study.
Results. Patients studied: 1904.
Down Syndrome: High risk: 57. Low Risk: 1847.
Confirmatory diagnosis: (amniocentesis, karyotype): 6 VP and 51 FP. (S: 85.7%, E: 97.3%, PPV 10.53%, NPV 99.9%).
Edwards Syndrome: High risk: 8. Low risk.
Confirmatory diagnosis: (amniocentesis, karyotype): 2 VP and 6 FP. (S: 100%, E: 99.68, VPP25%, NPV 100%).

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THE CONTRIBUTION OF LYMPHOCYTE POTASSIUM CHANNELS TO ALTERED T-CELL FUNCTIONS IN MOTHER AND NEWBORN

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Potassium channels regulate lymphocyte activation through the modulation of intracellular calcium handling. Alteration of potassium channel activity results in altered T-cell functionality. We collected data regarding T-cell potassium channels in the neonatal period and pregnancy: both states are characterized by impaired T-cell function.

We isolated mononuclear cells from fertile and pregnant women and neonates (n=9 in each group). Using calcium sensitive dyes we sequentially measured intracellular calcium levels of CD4, CD8, Th1 and Th2 cells using flow cytometry during aspecific activation performed with phytohaemagglutinine in the presence or absence of selective inhibitors of potassium channels. The generated fluorescence data were analyzed by our specific programme.

Ca2+ response of Th1, CD4 and CD8 subsets during activation was lower in pregnant women compared to fertile women. In pregnant women, Ca2+ influx was sensitive to potassium channel inhibition in CD8 and Th1, but not in Th2 cells. In neonates, calcium influx kinetics also decreased compared to fertile women. Neonatal lymphocytes were less sensitive to the specific inhibition of potassium channels.

These results indicate a characteristic pattern of Ca2+ influx in activated T-lymphocytes and its sensitivity to potassium channel inhibition. This raises the notion that T-lymphocyte Ca2+ handling may have a role in the characteristic immune status of healthy pregnancy. In neonates, the observed characteristics of potassium channel function may explain the clinical experience why T-lymphocytes' function is impaired compared to that in the adult.

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EVALUATION OF NEW STRATEGY IN THE USE OF FIRST- TRIMESTER COMBINED SCREENING FOR TRISOMY 21,18 AND 13 IN A SPANISH POPULATION

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Background. Nowadays, the first-trimester combined screening for chromosomal abnormalities offers the major advantages and is associated with very high detection rates for chromosomal abnormalities. The objective was to estimate the effectiveness of the first-trimester combined screening test in our population, and to develop a new strategy that increases sensitivity without exceeding the false positive rate (FPR) by 5%.
Methods. The test was evaluated in 14250 pregnant women for two years, calculating the theoretical risk of first trimester combined test between weeks 10-13 of gestation. The following variables were studied: the number and motives for request and invasive techniques, the newborn with chromosomal abnormalities, total births, the variation of biochemical markers along gestational weeks, and the quality of the measurement of biochemical and echographic markers.
Results. We obtained an almost universal coverage of 76.15 % of our population and a reduction of the invasive procedures of the 18%, due to a better prenatal control of the population.The positive tests and those pregnant women with a negative screening test but with multiple of the median (MoM) ≤ 0.26 for pregnancy associated plasma protein-A (PAPP-A), would be selected for invasive procedures, so the sensitivity improves of 64.51% to 71% for Down syndrome and 70% to 80 % for Edwards or Patau syndrome.
Conclusions. Using this new strategy, the first-trimester combined screening sensitivity could be improved without a high increase of invasive techniques (amniocentesis or chorionic villus).

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VARIATIONS AND ACCURACY OF CARBOHYDRATE-DEFICIENT TRANSFERRIN DURING PREGNANCY USING THE HPLC CANDIDATE REFERENCE METHOD

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Background. Contrasting data are available on the diagnostic accuracy of Carbohydrate-Deficient Transferrin (CDT) during pregnancy. These differences may depend in part on how CDT was evaluated and expressed. Here, we report on variations in CDT levels in pregnant women using the HPLC candidate reference method.

Methods. Serum Transferrin, urine and serum Ethyl glucuronide (EtG) and CDT were measured in 64 women, self-reporting as non-alcohol abusers, at different stages of normal pregnancy (gestational weeks: median 28, IQR 8-33). CDT was expressed as percentage of disialotransferrin to total transferrin (%CDT) (cut-off 2.0%).

Results. Recent alcohol consumption was excluded in all women by both undetectable serum and urine EtG. Transferrin was associated with both %CDT (r=0.66; P<0.001) and gestational week (r=0.68; P<0.001). Interestingly, %CDT was highly correlated with gestational week (r=0.77; P<0.001), even after controlling for the effect of transferrin. Moreover, statistically significant differences in %CDT were also evident between women grouped for pregnancy trimester (I: mean 1.01% (SD 0.19); II: 1.30% (SD 0.14); III: 1.53% (SD 0.22); ANOVA P<0.001). Trend analysis confirmed a proportional increase of %CDT along with pregnancy trimesters (P<0.001). Two women at III trimester had %CDT close to the cut-off (1.96 and 2.01%).

Conclusions. %CDT is independently associated with gestational week. Differently from what has been previously reported or expected, the relationship between pregnancy and CDT could be more complex. The diagnostic accuracy of %CDT for detecting alcohol abuse in a legal context may be limited in pregnant women and the effect of gestational age should be considered.

Quality assessment, standardization

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HUMAN URINE QUALITY CONTROL MATERIAL FOR DETERMINATION OF ETHOXYACETIC ACID (BIOMARKER OF ETHYLENE GLYCOL MONOETHYL ETHER EXPOSURE) – PRELIMINARY STUDIES

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Background. Ethoxyacetic acid (EAA) is the metabolite of industrial solvent ethylene glycol monoethyl ether (EGEE).
As EGEE enters to the body not only by inhalation but also via skin, the determination of biomarker exposure in urine is of high relevance. When workplace air alone is monitored, dermal exposure by liquid and vapour is not taken into account and consequently occupational risk might be underestimated.
Methods. Pooled freeze-dried material for quality control was prepared from urine of persons exposed to EGEE.
The concentration level of EAA, homogeneity and stability testing of material were performed by using two GC methodswith MS detection in EI mode: 1. EAA was after acid hydrolysis extracted with ethyl acetate and derivatized with N-tert- butyldimethylsilyl-N-methyltrifluoroacetamide, 2. SPME technique with simultaneous acid hydrolysis with derivatization with ethanol).
Results. Using ANOVA no changes of the concentration values (on statistical level $\alpha=0.05$) of EAA were found: as for six month isochronous stability testing, and for homogeneity testing.
The concentration value of ethoxyacetic acid of freeze-dried urine material was evaluated from the results of two independent methods, homogeneity and stability tests via interactive statistical program IPECA. The value ($c=17.4\pm2.3$ mg.l^{-1}) is unweighted aritmetical average of theaccepted results and its uncertainty is combined uncertainty ($k=2$).
Conclusions. Thecontrol material has the advantage of containing the EGEE metabolite, ethoxyacetic acid, as found in native urine.

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APPLICABILITY OF THE "MODEL FOR QUALITY INDICATORS" IN A CLINICAL LABORATORY

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Background. Quality indicators are a part of laboratory quality systems and are used for different purposes. Standardisation in handling our indicators is desirable. The objective of our study was to assess the applicability of the proposed Model for Quality Indicators (MQI) by IFCCin our laboratory setting.
Methods. The 29 proposed indicators by the IFCC working group on laboratory errors and patient safety (Clinica Chimica Acta 404 /2009; 79-85) were individually evaluated and scored for applicability in our routine. Three different categories were granted to each indicator: indicator already available, data available but not presentable in a automatic way, indicator not applicable. Data retrieval is performed by a business query software (Business Objects) on our LIS system (Cegeka, Lab400)and data are reported/presented as proposed by the MQI.
Results. In our evaluation we report 13 indicators in the category already available (and retrievable in a automatic way). Three indicators were scored "data available but not automatically presentable", e.g. EQA and IQC performance and presence of interpretative comments. We report 13 indicators not applicable which comprise the total testing process (pre-, post- and analytical phase).
Conclusions. The proposed MQI is in our opinion partially applicable in our laboratory setting. Efforts will be made to report in a periodic manner our available results to the organiser via the organised website. If more laboratories participate in this initiative, evaluation criteria for each indicator can be developed and benchmarking will be a valuable tool in quality management and continuous improvement of our activities.

Quality assessment, standardization

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ANALYSIS OF INTERNAL QUALITY ASSESSMENT CVS OF COMMON CHEMISTRY ANALYTES FROM CHINESE CLINICAL LABORATORIES

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Background. To analysis the internal quality assessment CVs data of common chemistry analytes from Chinese clinical laboratories during Augest in 2010.

Methods. Web-base EQA system was used to collect the internal quality control data of common chemistry analytes from 1315 clinical laboratories in China. And the data included current month CV and accumulative CV for two lots. Then Spss13.0 was used to analysis the effective data from 615 laboratories.

Results. Four of the analytes (amino transferase, glucose, total calcium, serum potassium) were chosen to be analyzed according to five allowable imprecision (1/4 TEa, 1/3TEa, optimal allowable imprecision, desirable allowable imprecision and minimum allowable imprecision drived from within-biological variation). Take glucose for example, the number of laboratories from which current month CVs for lot 1 are below the five allowable imprecision are 410,491,180,456,533respectively, 66.67%,79.84%,29.27%,74.15%,86.67% respectively.And the number of laboratories from which accumulative CVs for lot 1 are below the five allowable imprecision are 310,382,114,355,434respectively,60.55%,74.61%,22.27%,69.34%,84.77%, respectively. For lot 2 the number of laboratories from which current month CVs below the five allowable imprecision are 180,216,86,202,232respectively, 70.59%,84.71%,33.73%,79.22%, 90.98% respectively. And the number of laboratories from which accumulative CVs for lot 2 are below the five allowable imprecision are 136,178,50,159,195respectively, 60.99%,79.82%,22.42%,71.30%,87.44%respectively.

Conclusions. By analysis CVs of common chemistry analytes we propose to do more efforts to improve the management of internal quality control of clinical laboratories.

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LACK OF COMMUTABILITY BETWEEN QUALITY CONTROL MATERIALS AND PLASMA SAMPLES IN A TROPONIN I ASSAY

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Background. Checking the commutability between quality control materials (QC) and clinical patient samples is necessary to assess good laboratory practices. This is especially important in certain cases, such as in troponin assays, in which there is a consensus for the imprecision requirement at the decision limit used in the diagnosis of myocardial injury ($CV \le 10\%$) (NACB) and the way to know the CV is usually through QC. Non-commutability may exclude their use.

Methods. Troponin I concentrations were measured with three different reagent lots in the Dimension RxL analyzer (Siemens). Quality control samples (n=60) of three different QC concentrations (20 for each one; ref.B5960-1 Siemens) and 60 samples of three different frozen plasma pools (20 for each one) with the same concentrations were measured. The between-day imprecision was estimated through the coefficient of variation (*CV*). The commutability between QC and plasma samples was assessed by the *F*-Snedecor test.

Results. The three comparisons between the standard deviation of QC and plasma pool samples had a significant difference (p<0,05). In fact, the CV corresponding at 0,26 *µ*g/L was 17% in QC and 9% in plasma samples.

Conclusions. It is well known that it is important to check the commutability between QC and samples to assess good laboratory practice. It is necessary to take into account that if a lack of commutability exists, could imply that this material not be useful to check the QC.

Quality assessment, standardization

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EXTERNAL QUALITY CONTROL ASSESSMENT IN A HOSPITAL CLINICAL CHEMISTRY LABORATORY

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Background. The Clinical Chemistry Laboratory is a public health laboratory in Geneva University Hospital with approximately two thousands beds. Eight labs (clinical chemistry emergency and routine, toxicology emergency and routine, endocrinology, blood gas, biological liquids, molecular clinical chemistry) are parts of it. More than 6 Mio results of patient analyses are generated (nearly 600'000 prescriptions). This sector follows the ISO15189 norm and since 2001 is in compliance with accreditation requirements. Due to Swiss legal obligations, nearly 3500 results (350 parameters) of external quality controls (EQC) are submitted to 12 different European External Quality Coordinators to determine the accuracy.
Methods. Centralised reception and dispatching of the samples are performed. The elaborated results are mostly transmitted on-line to the Coordinators to be integrated in statistics. Returned reports are signed by each head of lab to act for improvements. The encoded evaluations are integrated in an Excel program in order to follow accuracy month by month. Once a year their graphics are submitted to the headmaster of the Department to show the evolution of the parameters. In order to maintain this performance, a biomedical analyst is detached.
Results. A rigorous EQC management helps us to lower the number of unsuccessful Results. 2008 (4.2%), 2009 (3.9%) 2010 (3.4%). 90% of the mistakes were transcription ones.
Conclusions. In order to improve the quality system in our sector a closed management of external quality and internal quality control as well is necessary to maintain the high standards we already have. This allows us to produce very precise and accurate patient Results.

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IMPLEMENTATION OF A STAFF HABILITATION PROCESS IN THE CLINICAL BIOCHEMISTRY LABORATORY OF A FRENCH TERTIARY TEACHING HOPSITAL

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Background. The implementation of an auto-analyser which consolidates workstations, in our Emergency Biochemistry department, led to dissatisfaction and unconfident use by our staff. The lack of documentation at the workstation and insufficiently defined training were identified using the Ishikawa diagram. Our objective was to produce specific accessible documentation and implement a formalized training, in order to allow workers' habilitation as required by the NF-EN-ISO15189.
Methods. Following the Deming's wheel, exact responsibilities and tasks of the workstation, documentations, a formalized teaching program for all new technicians and an evaluation questionnaire (EQ on 45 items) were established by referee biologists and technicians. All technicians completed the EQ. According to results, supplementary training was proposed. The laboratory direction decided to deliver an habilitation which certifies required skills prior to autonomous work.
Results. Heterogeneous EQ scores (31/45 +/- 3.41) reflected the heterogeneity of a non-formalized training. The best scores were obtained by two new technicians who followed the whole habilitation process (35.5 and 36/45) and by one referee (37.5/45). The experience was well-accepted by our staff and recognized as a valorisation of skills. The habilitation process uniformed technical staff training and allowed an equal quality of work, independently of time or technician.
Conclusions. Our experience shows the importance of a formalized training process in a 24/24h opened laboratory with successive work teams. The definition of responsibilities and tasks is an essential work preliminary to the habilitation process. This positive experience initiated in the biochemistry laboratory will soon be extended to all the hospital laboratories.

Quality assessment, standardization

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PRE-ANALYTICAL EFFECTS OF PLASMA SEPARATION TUBES IN THE ROUTINE CLINICAL CHEMISTRY LABORATORY

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Background. This study evaluated the pre-analytical effects of four different separation tubes for the preparation of lithium heparin plasma in comparison to a reference collection procedure.
Methods. Blood was collected from 20 healthy volunteers using four different separation tubes and manually separated plasma as reference. 15 clinical chemistry parameters were determined at 0h, 24h, and 72h. Statistical differences were evaluated at time-point 0h using the Kruskal-Wallis-test and over time using a generalized estimating equation regression model.
Results. Significant differences between the different tubes could be demonstrated for lactate dehydrogenase activity and the hemolysis index results at time-point 0h ($p<0.05$). The regression model revealed significant changes over time for almost every parameter in comparison to the reference tube. The estimated maximum allowable storage time in the primary tube was considerably reduced using separation tubes. Lactate dehydrogenase activity, glucose, and potassium were most affected. The corresponding maximum allowable storage time was reduced to 12-32h, 7-15h, and 10-13h depending on the tube type.
Conclusions. Despite a moderate effect on hemolysis and lactate dehydrogenase activity the examined separation tubes had no significant analytical influence at time-point 0h. However, the results indicate that sample storage beyond 7-24h (at 4°C) is associated with clinically relevant deviations for certain parameters. Therefore, sample storage should be questioned, in particular for parameters susceptible to interference by hemolysis or platelet contamination.

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ELECTRONIC DOCUMENT MANAGEMENT SYSTEM INTRODUCTION INTO LABORATORY PRACTICE

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Background. Our laboratory, Institute of Clinical Chemistry and Biochemistry, was audited for certification of professional activity following the local legislation for medical laboratories. The legislation considers requirements from ISO 15189 standard. All laboratory personnel had spent efforts with great and most heavy work on preparation of documents to successfully passed trough this process. An additional and challenging circumstance was the fact that our Institute is comprised of 14 departments, that are located in four different locations, having all together 130 employees. Therefore we had decided to use an electronic method for the documents management and control respectively.
Methods. We participate in the pilot project at our University Medical Centre of testing a web based document management application. This application is designed to support electronic approval of documents, reading receipts, reviewing, searching, archiving of withdrawn versions. The applicationalso allows us tomanage human resources, conduct on-linemeetings,recordactions(tasks, preventiveandcorrectivemeasures) and processes modelling according to standard BPMN (Business Process Modelling Notation).
Results. 1100 audited documents are on-line available to all employees. The controlled hard copies versions, mainly working instructions, are available only next to the working places. Quality manual written in Extensible Markup Language admits integrationwithanywebbaseddocuments, including ourwebsiteandintranet sites.
Conclusions. Easy and quick access to documentation improves the usefulness of information, save time of documentation management and the most importantly, the electronic document management method allows us only to use the latest updated version of approved documents.

Quality assessment, standardization

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COMPARISON OF SHORT TERM IMPRECISIONS OF RAPIDLAB 400 AND RADIOMETER 800 BLOOD GAS SYSTEMS DERIVED FROM THE STATISTICAL ANALYSIS OF ROUTINE QUALITY CONTROL DATA

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Background. The short term imprecision (STI) is the probably the most important determinant of the utility of laboratory data in the acute care environment. Analytical methods with lower STI will allow the clinician to more rapidly determine patient disposition. We have devised a unique methodology to summarize the STI of quality control (QC) results and have used it to compare two blood gas instruments.

Methods. QC data were collected from a RapidLab 400 (Siemens, Deerfield, IL) operated in a Veterans Administration Hospital intensive care unit, and a Radiometer 800 (Radiometer, Copenhagen, Denmark) operated in a US tertiary care hospital laboratory. Four and eleven months of RapidLab and Radiometer QC data, respectively, were downloaded into Microsoft Access and manipulated with Microsoft Excel to provide consecutive pairs of quality control data. The standard deviation of duplicates (SDD) was calculated for all pairs analyzed within a 12 hours interval and all pairs within a 12 to 24 hour interval. A summary standard deviation was derived from a frequency-weighted average of the SDD.

Results. For potassium, the RapidLab generates results with lower variability compared to the Radiometer; for pCO2, the Radiometer is more precise. The two systems produce pO2, pH, ionized calcium, hemoglobin, glucose and sodium with roughly equivalent STI.

Conclusions. This type of statistical analysis should be made available by the instrument or control manufacturer. It provides information about instrument and reagent stability and permits simple assignment of the statistical quality control limits. Dissemination of instrument STI will improve instrument selection.

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STABILITY OF 6 ANALYTES (PREGNANCY-ASSOCIATED PLASMA PROTEIN-A (PAPP), ANDROSTENEDIONE (AND), VALPROIC ACID (VALP), CARBAMAZEPIN (CARB), TROPONIN I (TPI); FREE PSA (FPSA)) IN SERUM SAMPLES FROZEN ON A SEPARATOR GEL IN PRIMARY GREINER VACUETTE SERUM TUBES

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Background. Using serum specimens frozen in primary gel separator tubes in routine laboratory practice minimizes errors in patient identification, reduces the biohazard risk of aliquoting infectious samples and decreases cost. There are only few studies about analytes stability in these conditions.

Methods. PAPP and AND (Immulite 2000, Siemens), VALP, CARB and TPI (AXSYM, Abbott) ; FPSA (Architect i2000, Abbott) in 10 serum specimens (for each analyte) were measured in duplicate prior to freezing and after freezing of serum at -20oC for 7 days in both the primary gel tube and aliquot in a secondary tube. Ranges of concentration were: PAPP 0,2-18 mU/ml, AND 1,0-25 nmol/l, VALP 25-115 μg/ml, CARB 5–12 μg/ml, TPI 0,3-100 mg/ml, FPSA 0,04-1,65 μg/l.

Results. Minimum and maximum bias between the prior to freezing and the after freezing of serum on a separator gel in primary tubes results were: PAPP -10% /+10%, AND -14% /+ 16%, VALP -3% /+8%, CARB -4%/+ 9%, TPI -11% /+ 2%, FPSA -6% /+2%. Minimum and maximum bias between the prior to freezing in primary gel tube and the after freezing in aliquot into a secondary tube specimen results were: PAPP -5% /+12%, AND -10% /+11%, VALP -4% /+9%, CARB -2% /+7%, TPI -14% /-3%, FPSA -4% /+ 4%.

Conclusions. Results of specimen frozen in primary gel tubes are similar to those frozen in aliquot in secondary tubes. These six analytes can be reported from serum stored frozen in the primary gel tube.

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AUTOMATED IMAGE ACQUISITION, PROCESSING AND ANALYSIS FOR THE STANDARDISATION OF AUTOANTIBODY SCREENING

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Background. The indirect immunofluorescence (IIF) on HEp-2 cells represents a cost-effective multiparametric assay that allows the screening for non-organspecific autoantibodies in a multistep approach for the diagnosis of autoimmune systemic and liver disease. However, the intra- and interlaboratory variability of IIF results may not be satisfactory due toobjective and subjective interference.

Methods. As an important step toward standardisation of autoantibody testing, a novel fully automated IIF-reading systems with pattern recognition software was developed that includes automatic focusing, adjustment of correctimage intensity, quality control of image taking, real-time processing of image data with pattern evaluation, and automated system calibration. This system (AK-LIDES®) was evaluated by a large panel of reference, control, and routine sera (n=1800).

Results. The automated system allows (a) a robust measurement (inter- and intra-assay variance below 20%), (b) an objective discrimination between positive and negative results (total agreement between manual and automated interpretation 97%, less false negative results), (c) a differentiation of the main common pattern (homogeneous, speckled, nucleolar, and centromere staining of nuclei, cytoplasmic), (d) a high sensitive detection of all clinically relevant (such as dsDNA, Sm, U1-RNP, La/SS-B, Ro52, Ro60, CENP-B, Scl70, Ku, Mi2) but also some esoteric (e.g., NuMA, Golgi apparatus) autoantibodies, and (e) an automated documentation and archiving of all relevant data (different kind of reports, pictures).

Conclusions. The system creates the basis for further development of classification algorithms regarding identification and differentiation of a great variety of additional staining patterns including pleomorphic, mixed, and cell-cycle related patterns.

Quality assessment, standardization

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AUTOMATED CELL COUNTING OF BODY FLUIDS: EVALUATION OF THE ABX PENTRA DX 120 COMPARED TO THE MANUAL MICROSCOPE METHOD, THE SIEMENS ADVIA 2120 AND THE SYSMEX XE 2100

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Background. Automation in body fluids cell counting allows better accuracy than manual methods; however, there is still need for standardization of methods and technologies.
The hematology analyser ABX PENTRA DX 120 (HORIBA ABX, Montpellier, France) introduced a Body Fluids (BF) mode for counting White Blood Cells (WBC), polymorphonuclear and mononuclear cells in biological fluids. We performed its evaluation that was compared with two other analysers and the microscopic manual method.
Methods. Horiba ABX PENTRA DX 120 light absorbance/cytochemical system was compared with the optical system Siemens ADVIA 2120, the impedance system Sysmex XE2100 and the microscopic manual counting. The differential counting of polymorphonuclear and mononuclear cells was compared only between ABX and microscope
The analysis was performed on 85 BF samples (15 cerebrospinal, 32 pleural, and 38 ascitic fluids).
Pearson's correlation coefficient (r) was calculated.
Results. Total counted cells varied between 1 and 6244. The WBC correlation coefficients of the microscope manual method were:
• 0.993 with the ABX Pentra DX120,
• 0.992 with the Advia 2120,
• 0.994 with the XE2100.
• The WBC correlation coefficients among instruments were:
• 0.990 (ABX Pentra DX120 vs ADVIA2120),
• 0.992 (ABX Pentra DX120 vs XE2100),
• 0,993 (ADVIA2120 vs XE2100).
The correlation coefficients for polimorphonuclear and mononuclear cells between ABX and microscope were respectively 0.661 and 0.718
Conclusions. These data confirm that BF automatic counting standardization is excellent for WBC and acceptable for polymorphonuclear and mononuclear cells. The new ABX Pentra DX 120 BF counting mode showed analyticcapabilities overlapping with the other technologies tested.

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ASSESSING THE COMMUTABILITY OF INTERNATIONAL STANDARDS USING PROFICIENCY TESTING DATA - A NOVEL APPROACH

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Background. While the importance of comparing "like with like" in the context of immunoassay has been recognized for many years, rigorous procedures for assessing commutability have only recently been developed. Consequently these properties have not yet been assessed for most International Standards. Formal commutability studies require assay of a well-characterized panel of patient sera and the material under investigation at the same time.
Method. A potential surrogate method utilising proficiency testing (PT) data has been developed. Results for pools of patient sera and similar pools containing the International Standard were compared for commutability following an accepted procedure. At least five methods were evaluated for each of the eight Standards tested. For PTH a panel of single patient donations was also used to assess commutability.
Results. Using this method, commutability was confirmed for only three of the eight Standards. Interestingly, two of these (GH IS 98/574 and PTH IS 95/646) are recently introduced recombinant Standards. The third (AFP IS 72/225) is a material which very closely resembles patient sera. The other five (i.e. LH IS 85/552, FSH WHO 94/632, Prolactin IS 84/500, hCG IS 75/589 and CEA IRP 73/601) are all partially purified preparations from human tissue or urine. Using the patient panel for PTH, the results obtained using PT data were confirmed.
Conclusions. The technique developed here requires further formal validation against other accepted procedures but potentially provides a rapid means of assessing commutability which might be helpful in the early evaluation of candidate reference materials.

Quality assessment, standardization

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TURNAROUND TIME IN EMERGENCIES

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Background. The total response time (RT) is an indicator of laboratory quality, which affects diagnosis and treatment. This is defined as the interval between the arrival time of a sample in the laboratory and the time of clinical validation.
Our goal is to evaluate the RT for emergency analytical biochemistry, studying the workflow and the preanalytical, analytical, postanalytical and total phases, in order to plan possible actions for improvement.
Methods. We analyzed the data of the stat request for nine days, and separated in to three periods: 1 (8-10h), 2 (10-12h) y 3 (12-14h), representing different loads. RT was collected (minutes) for each period and phase. The data was processed with Excel 2003.
Results. There were 23.2 samples per day, with 3.7, 8.2 and 11.3 for period 1, 2 and 3 respectively. The number of determinations per sample was 7.8.
46% of samples were tested by six determinations (glucose, urea, creatinine, sodium, potassium and chloride). The total RT average was 23.13, 21.01, 11.24 and 55.35 minutes to preanalytical, analytical, postanalytical and total, respectively. For the periods 1, 2 and 3 this RT was 59.73, 55.78 and 50.54 minutes, respectively.
Conclusions. During the periods when our emergency samples were mixed with routine hospitalization samples (8-10h) and those from Primary Health Care samples (10-12h), the total RT was delayed, mostly at the expense of time spent in the preanalytical phase. We have to see what factors influence this phase and prioritize urgent samples in order to adapt to health care needs.

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EVALUATION OF THE ACCESS® HIV COMBO ASSAY ON THE UNICEL DXI 800 IN A PRIVATE LABORATORY

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Background. The aim of the study was to validate in accordance with ISO15189 Standard, a new assay: Access HIV combo assay (Bio-Rad) on the UniCel® DxI 800 (Beckman Coulter), in terms of specificity, sensitivity, precision, carryover and throughput. It was compared to the routine assays: ADVIA® Centaur HIV Combo (Siemens) and Access HIV-1/2 New (Bio-Rad).
Methods. 503 non-selected serum samples were used for specificity and sensitivity testing. The precision was evaluated by testing 4 negative and 3 positive pools. The carryover study was assessed by testing one very high positive sample and one negative sample.
Results. 497 of the 503 samples were found negative with all assays. The specificity on all assays was 100%. 6 samples were found positive on Access and 5 positive samples on ADVIA Centaur. The discrepant sample was an early HIV-1 seroconversion. The Access sensitivity was 100%.
Intra-assay and inter-assay precisions were found below 10% on both negative and positive samples. We had no effect with the carryover and the throughput of the UniCel DxI 800 was up to 320 tests per hour.
Conclusions. The evaluation of the Access HIV combo assay on the UniCel DxI 800 system in our laboratory showed a superior clinical sensitivity with an early detection of both HIV-1 p24 antigen and HIV antibodies compared to the ADVIA Centaur HIV Combo. The specificity was excellent for both Access and ADVIA Centaur. The excellent results obtained for the precision, carryover and throughput were fully suitable for HIV screening in a large private laboratory.

Quality assessment, standardization

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EVALUATION OF GENERAL IMMUNO ASSAYS OF THE BECKMAN COULTER UNICELL DXI 800 SYSTEM

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Background. Verification on site of immuno assays on the Beckman Coulter Unicell DXI 800 required for the new reference quality Iso 15189.

Methods. The DXI 800 system uses the technology of Beckman Coulter company except for BNP (Alere).The coefficient of variation (CV) was calculated for repeatability and reproducibility that were determined using low, medium and high control levels (Biorad).

We tested TSH, FT3, FT4, Ferritin, B12, Folates, Tn I_C, Myoglobin, BNP, GH, PTH, hCG, and prolactin.

Results. The CV obtained were satisfactory for all analyses when using the SFBC (Société Française de Biologie Clinique) references but not for Ricos/Westgard specifications for FT3 and FT4.

For repeatability, our CV obtained with the Biorad controls (low, medium and high) were very closed to the recommendation by Ricos for FT 3 (3,2 %, 4,1 %, 4,3 % for 4 %) and FT4 (4,0 %, 4,1 %, 2.9 % for 2,9 %).

For reproductibility, our CV obtained for Biorad controls were higher than those proposed by Ricos/Westgard specifications for FT 3 (9,2 %, 7,1 %, 7,1 % for 4 %) and FT4 (8,1 %, 6,5 % , 2,1 % for 2,9 %).

Conclusions. We didn't reach Ricos/Westgard exigencies for both FT3 and FT4 reproductibility suggesting unstability of reagents that would need improvement. All the other assays satisfy both SFBC and Ricos/Westgard references.

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EVALUATION OF GENERAL CHEMISTRY ASSAYS OF THE BECKMAN COULTER DXC 800 CLINICAL CHEMISTRY SYSTEM

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Background. Verification on site of chemistry assays on the Beckman Coulter DXC 800 required for the new reference quality Iso 15189.

Methods. The DXC 800 system uses the technology of Beckman Coulter company.The coefficient of variation (CV) was calculated for repeatability and reproducibility that were determined using low, medium and high control levels (Synchron).

We tested Glucose, Na^+, K^+, Cl^-, CO_2, TP, Creatinine, Bun, Alb, Phos, Tbil, Dbil, ALAT, ASAT, GGT, CPK, LDH, Lipase, Uric Acid, Chol, TG, Trf, Iron, CRP, Hapto, AAG, Ammonium.

Results. The CV obtained were satisfactory compared to Beckman Coulter CV specifications but not for all analyses when using the SFBC (société française de biologie clinique) references.

For repeatability, our CV obtained with the low Synchron level were slightly higher than those recommended by SFBC (ex : Creatinine 6,1 vs 4,5 %). We observed similar results with reproducibility (ex : Tbil 10,3 vs 6,8 %).

Conclusions. While some parameters (ALA, CK, K^+ and Ammonium) don't reach the SFBC recommendations, the clinical significance is not affected.

For creatinine, a change for enzymatic creatinine assay is recommended when creatinine < 50 μmol/l.

For total bilirubin, calibration with bilirubin Cal allows a better precision around 300 μmol/l for neonatal screening than low values but those CV are clinically acceptable.

Quality assessment, standardization

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ACCREDITATION OF MEDICAL LABORATORIES IN CROATIA

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Background. Increasing interest for the competent medical laboratories is caused by increasing interest for the safety of patient and for quality of services of medical laboratories. Accreditation is recognized as the best tool to provide the confidence in the results of laboratories´ testing activities.

Methods. In cooperation with the Ministry of Health, Croatian Chamber of Medical Biochemists and Croatian Society of Medical Biochemists the scheme for accreditation of medical laboratories against HRN EN ISO 15189 was established within Croatian Accreditation Agency (HAA). In developing new accreditation scheme the assets of the EU (CARDS 2003) and Sweden (SIS Fund) for building capacity of the Croatian quality infrastructure were used.

Results. The results of activities performed were:

establishment of HAA WG MedLab (participants from HAA staff, Ministry, professional associations, accredited laboratories),

training of assessors, assessments,

five accredited laboratories (two in university hospitals, one in regional hospital, one in Public Health Institute, one private laboratory) in the field of clinical chemistry, laboratory haematology, coagulation, immunology, immunophenotyping, toxicology, molecular diagnostics, human microbiology

3 lead assessors and 22 experts

analysis of nonconformities found during initial and surveillance assessments in laboratories

Conclusions. Accreditation scheme of medical laboratories according to HRN EN ISO 15189 is successfully implemented in Croatia by Croatian Accreditation Agency.

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IDENTIFICATION AND MEASURING OF PRE AND POST-ANALYTICAL QUALITY INDICATORS IN LABORATORY MEDICINE

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Background. Since laboratory results became an important tool to achieve medical diagnosis and treatment evaluation, quality controls during the whole laboratory process is essential. The analytical process includes daily quality management but the pre and post-analytical processes are much more difficult to control. Therefore quality indicators were performed to evaluate pre and post-analytical performance during year 2010.

Methods. A pre-analytical indicator: sample rejection (number of samples rejected/total number of samples received) and three post-analytical indicators: pending results (number of pending results/total number of results), turnaround time (number of results delivered on schedule/total number of results delivered) and modified laboratory results (number of results modified after validation/total number of validated results) were evaluated. Indicators data was extracted from our Laboratory Information System. To estimate the performance of these indicators we applied the useful statistical metric tool Six Sigma. For these complex processes, the acceptable sigma metric should be higher than 3.5.

Results. Sigma metrics: Sample rejection (January-December): 3.8-3.9-3.9-4.1-3.8-3.9-3.9-3.9-3.9-3.9-3.9-3.8; Pending results (January-December): 4.6-4.3-4.3-4.2-4.5-4.4-4.5-4.6-4.6-4.4-4.1-4.4; Turnaround time (January-December): 3.9-3.6-3.4-3.5-3.9-4.2-4.0-3.7-4.0-4.4-3.9-4.4; Modified laboratory results (January-December):4.3-4.1-4.2-4.2-4.4-4.6-4.2-4.3-4.3-4.3-4.3-4.5.

Conclusions. The use of indicators allows the laboratory to regularly evaluate the performance of the pre and post-analytical processes which are, as well as the analytical process, an important key of the essence of patient care.

Quality assessment, standardization

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ESTIMATION AND COMPARISON OF ANALYTICAL UNCERTAINTY OF MEASURE

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Background. We estimated and compared the analytical uncertainty of measureversus theallowable total error for potassium, calcium and AST. This comparison allows the identification of the possible different sources of analytical error and ensures the tests performed completely satisfy their requirements without affecting the clinical interpretation of the Results.
Methods. To estimate the analytical uncertainty, we used three different models. Model 1: we estimated the Bias and the uncertainty associated with the estimation of BIAS from data of a Peer Group (Quality Control Service ROCHE). Model 2: we estimated the Bias and the uncertainty associated with the estimation of BIAS from data obtained from certificates of traceability and uncertainty (ROCHE). Model 3: we estimated the Bias and the uncertainty associated with the estimation of BIAS from data obtained from the EQAs Program (RIQAS). For every model, we estimated the reproducibility within-laboratory standard deviation obtained from the standard deviation of a control sample (Precinorm U, Precipath U ROCHE) during a certain period of time. To choose the allowable total error, we used the analytical quality requirements guidelines. For models 1 and 2, two different concentrations were evaluated. Measurand: potassium-serum-mmol/l, calcium-serum-mg/dl, AST-serum-U/l.
Results. Model 1: potassium: 3.4%-2.1%; calcium: 4.0%-4.9%, AST: 8%-6%. Model 2: potassium: 3.8%-2.3%; calcium: 4.0%-3.7%; AST: 7%-5%. Model 3: potassium: 3.6%; calcium: 4.3%; AST: 13%. Allowable total error: potassium: 5.8% (biological variation); calcium: 10% (CLIA), AST: 20% (CLIA).
Conclusions. The uncertainty calculated by these three models is lower than the allowable total error selected for each measurand.

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INTERNAL QUALITY CONTROL IN THE BIOCHEMICAL LABORATORY

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Background. The analytical quality control entails internal and external control of the quality of work done. Our laboratory has been using the Bio-Rad Unity PC program for all biochemical parameters since 1996. The results appear as tables, histograms and Levey-Jenning's Chart, all of which provide for a detailed and quick results preview as well as ability to process a great deal of data.
Methods. The control material is a product of Bio-Rad. processed on the automatic biochemical analyzer Cobas Integra 400.
Results. In order to be able to see the movement of the coefficient of variation throughout the period of 12 months, the following biochemical parameters were processed on the automatic biochemical analyzer Cobas Integra 400: urea (n=995) CV% ranged from 3,4 to 5,5%, creatinine (n=998) CV% ranged from 2,6 to 6,7%, UA (n=955) CV% ranged from 3,8 to 6,3%, TP (n=1009) CV% ranged from 4,2 to 5,1%,albumun (n=1010) CV% ranged from 3.9 to 5.4%.
Conclusions. All tested parameters in the given time frame ranged within normal deviation as recommended by manufacturer of control material. The clinical biochemical laboratories have a great responsibility for issuing correct results, which will provide for correct diagnoses as well as therapy prescriptions. Therefore, there is no better way in proving the efficiency and accuracy of laboratory than involving the internal quality control of the biochemical analysis.

Quality assessment, standardization

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MINIMAL ANALYTICAL QUALITY SPECIFICATIONS. SPANISH EQAP ORGANIZERS CONSENSUS

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Background. Four Spanish scientific societies organizing external quality assurance programs formed an Interdisciplinary Committee with the aim of defining common minimum quality specifications for clinical test.
Methods. Data obtained from 2005 to 2009 five periods of EQAS programs were compiled in a database. A total of 61 analytes: 24 basic biochemistry, 15 hormones and tumour markers, 14 haematology and coagulation tests, 3 immunologic tests and 5 therapeutic drugs were included.
Calculation Methods. % difference of each result versus target value (in absolute), plotting distribution of the differences, elimination of the 5% of the higher differences, defining the 95 percentile as the candidate minimum quality specification (cMQS). Target value was the overall mean or peer group mean when the results between methods were discrepant. cMQS was compared with mandatory specifications in other countries as Richtline (Grmany) and CLIA (USA). Procedure A: if cMQS was equal or higher than the lower limit of that ones cMQS was accepted as definitive. Procedure B: calculation of probe error value (pEV) increasing cMQS by 0.01%, count the amount of laboratories obtaining 75% of their results within this pEV. When 90% of laboratories have 75% of their results within the pEV this was considered as definitive MQS.
Results. The MQS proposed fell within 4 to 37% and are of similar order than those in Germany or USA for the majority of cases and more restrictive when there are differences.
Conclusions. The model is robust and assures that the majority of laboratories could reach the specification proposed.

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CLINICAL LABORATORY HEMOLYSIS PRACTICES ARE HIGHLY VARIABLE

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Background. Hemolysis is an important clinical laboratory quality attribute that influences test result reliability.
Methods. To establish clinical laboratory practices for hemolysis, we used the College of American Pathologists (CAP) Survey Program to distribute a Q-Probes-type questionnaire to CAP Chemistry Survey participants.
Results. Of 3495 participants sent the questionnaire, 846 (24%) responded. In 71% of laboratories, the hemolysis rate was less than 3.0%, whereas in 5%, it was 6.0% or greater. A visual, an instrument, and combinations of visual and instrument scales were used to identify hemolysis in 41%, 11% and 48% of laboratories, respectively. Forty percent used pictures to aid visual interpretation of hemolysis levels, and 74% used the same hemolysis scales on their primary and secondary chemistry analyzers. In 8% of laboratories all hemolyzed specimens were rejected, in 4% no hemolyzed specimens were rejected, and in 88% of laboratories some specimens were rejected depending on the hemolysis level. Laboratories used 21 different terms and 19 different cutoffs to reject hemolyzed specimens with Slight and Moderate the most common rejected specimen characterizations, used by 30% of participants. Forty-six percent of laboratories did not systematically and regularly monitor the percentage of hemolyzed specimens, and 42% had not taken corrective action yearly to reduce the percentage of hemolyzed specimens. Practice patterns varied among serum potassium, lactate dehydrogenase, and glucose as well as whole blood measurements.
Conclusions. Hemolysis practices are highly variable. Without standard assessment and reporting, clinicians cannot determine the impact of hemolysis on specific laboratory test Results.

Quality assessment, standardization

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ON THE TRAIL OF A USEFUL CONTROL MATERIAL FOR DAILY CV10% VALIDATION OF A TROPONIN T ASSAY

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Background. As troponin assays often have >10% CV at the definitory cut-off (99th percentile), the concentration at CV10% becomes the pragmatic cut-off. Daily compliance with the CV10% rule requires control materials with troponin at or slightly below this cut-off.We evaluated control materials for the 4th generation Troponin T (TnT) assay (Roche) having a CV10% claim at 0.03 µg/L.
Methods. Six commercial control materials were reconstituted according to package inserts, and point assayed for TnT on E1010, E2010, and E170 (Roche). Each material was diluted as recommended by the manufacturers to achieve 0.028 µg/L calculated target concentration for each analyzer. Similarly, a LiHep patient sample was point assayed and diluted with zero LiHep plasma. Preparations were aliquoted and frozen at -20° C. Aliquots were run daily for 20 workdays at best application. For each setting, acceptable measuredconcentrations were 0.022-0.034 µg/L (0.028 µg/L±20%), and CV was SD/mean.
Results. All control materials were initially >0.034 µg/L (0.041 µg/L-1.055 µg/L). Analyzer specific dilution led to acceptable measured concentrations for four materials, whereas two materials had recoveries of 1.96 and 0.76, respectively. CV <10.0% was achieved with one material on E1010, five materials on E2010, and all materials on E170. For all platforms, CV<10.0% was only achieved with PreciControl by Roche, and our in-house prepared plasma.
Conclusions. Assay independent control materials are not all useful for daily CV10% validation of the 4th generation TnT assay. In-house prepared plasma may be preferred. Compliance with the CV10% rule increased with improving analyzer technology.

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ESTIMATION OF POST-MORTEM INTERVAL USING DEVELOPED FORMULAE: IMPROVED USE OF VITREOUS HUMOR ANALYTES

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Background. Vitreous humor is an important investigative fluid in forensic pathology, especially postmortem interval (PMI) determinations. The study objective was to evaluate the utility of vitreous biochemistry in estimating PMI and devise appropriate biochemistry based formulae to aid PMI estimations.
Method. Vitreous humor samples were collected from 61 subjects (Female, 22; Male, 39; Age, 16 - 95 years) with precisely documented time of death (4.5 - 84.3 hours). The supernatant was analyzed for sodium, potassium, chloride, calcium, magnesium, urea, creatinine, glucose and lactate on an LX-20 Analyzer (Beckman-Coulter). Vitreous humor hypoxanthine and xanthine were analyzed using a colorimetric method. The statistical analyses were carried out using linear regression analysis and SPSS for Windows.
Results. It was observed that there was a significant correlation between vitreous potassium ($P<0.0001$), hypoxanthine, ($P<0.0001$), xanthine ($P<0.0001$), lactate ($P<0.0001$), calcium (P <0.01) and PMI. The mean values of vitreous biochemical constituent were used as the dependent variable to calculate the estimated PMI. The resulting formulae, derived from the linear regression equation, for PMI estimation were: for potassium (6.41 (K^+) – 46.25), hypoxanthine (0.32 (Hypoxanthine) – 60.94), xanthine (0.14 (Xanthine) – 50.08), lactate (5.21 (Lactate) – 27.69) and calcium (200 (Ca^{2+}) – 380.4).
Conclusions. On comparing the actual PMI and the calculated PMI, we observed the highest correlation for potassium based formulae ($P< 0.0001$). These results suggest that these analytes are significantly correlated with PMIand the proposed formulae for estimating PMI using other biochemical constituents, in conjunction with vitreous potassium, may enhance PMI estimations.

Quality assessment, standardization

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EXTERNAL QUALITY ASSESSMENT IN THE POST-ANALYTICAL PHASE IN CROATIA

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Background. International standard ISO 15189 promotes the implementation of an effective quality assessment program and recognizes the importance of the post-analytical phase. The sub clause 5.8 is divided into three main parts: the appearance and content of the laboratory findings, validation and the way of reporting laboratory Results. In order to harmonize the way of reporting laboratory results in Croatian medical biochemistry laboratories the Croatian Chamber of Medical Biochemists (CCMB) issued the document based on ISO 15189 requirements, recommendation of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Union of Pure and Applied Chemistry (IUPAC). The implementation of those requirements was evaluated through External quality assessment (EQA) scheme in post-analytical phase.
Methods. EAQ program in Croatia has been continuously performed since 1973 by the Committee for EQA Schemes under the auspices of the Croatian Society of Medical Biochemists but the new scheme in post-analytical phase started in 2009. The comparability analysis of received laboratory reports and the mandatory requirements based on the CCMB requirements was done.
Results. From 210 medical biochemistry laboratories in Croatia 140 sent their laboratory reports (70%). According to the obtained results a small number of laboratories did not use the recommended names, abbreviations, units or recommended reference intervals on their laboratory reports.
Conclusions. Further improving in post-analytical phase especially in harmonization of reporting the patient samples results is a prerequisite for accreditation of medical biochemistry laboratories in Croatia according to ISO 15189.

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COMPARING SERUM AND PLASMA FOR THE MEASUREMENT OF SELECTED ANALYTES BY IMMUNOASSAY BEFORE THE IMPLEMENTATION OF AUTOMATION

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Background. To reduce the pre-analytic handling in the clinical chemistry laboratory it is an obvious advantage to use one sampling tube for both general chemistry assays and immunoassays. In general Li-heparin plasma is used for the general chemistry assays and serum for immunoassays. Patient samples, collected as serum and Li-heparin plasma, for the following immunoassays were compared: TSH, fT4, fT3, vitamin B12, folate, ferritin, FSH, prolactin, progesterone and testosterone.
All assays were run on Architect ci8200 from Abbott Diagnostics. In addition the stability of these analytes in serum and plasma was studied and compared to data from the reagent supplier and related to our own analytical goals.
Methods. Plasma (LH, Sep, Vacuette, Greiner bio-one) and serum (SST, Vacuette, Greiner bio-one) were compared. Stability in the primary tubes at room temperature and in separated serum at 2–8 °Cwas studied for up to 3 days after collection.
Results. The correlation coefficient (r) varied from 0.906 to 1.000 between serum and plasma (n= mean 44) for all the analytes. Plasma results for ferritin, FSH and testosterone showed a small positive bias compared to serum. For ferritin this observation was supported by data from the reagent supplier. Stability was satisfactory during the 3 days after collection, both serum and plasma meeting the analytical goals for all analytes with the exception of folate. Folate in serum showed a decreasing trend below the analytical goal after 3 days at room temperature.
Conclusions. The results show that plasma can replace serum for the analytes measured.

Quality assessment, standardization

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CRITICAL VALUES REPORTING IN 233 SPANISH LABORATORIES

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Background. Detection and reporting of critical values have great implications on patients' safety. The SEQC and SEHH Committees have carried out several surveys in order to evaluate this subject in Spanish laboratories.
Methods. Surveys were distributed among laboratories participating in the External Quality Assessment Schemes. Participants were asked to provide information regarding reporting of critical values and their decision limits (Outpatient and Inpatient).
Results. Most laboratories (82,8 %) had their critical values already defined; physicians assumed the responsibility of notifying critical results in 86,7 % of cases; critical results were mainly informed by telephone (93,1 %) and this report was received by a clinician in a 89,3 % of cases; 57,1 % of laboratories further verified that such notifications were received although 85,8% did not employ any indicator to track this process. Delivery term was not taken into account in 55,8 % of laboratories.
Median values obtained for most constituents did not differ and turned out to be similar for outpatient and hospitalization setting. Nevertheless, differences were found for calcium (low critical value) and for creatinine, glucose and urea (high critical value). For potassium, differences were found at both levels. Regarding haematological constituents differences were found for high critical values in case of Leukocytes and Platelets count.
Conclusions. Handling of critical values lacks of standardization and consensus among Spanish laboratories. Suitable strategies should be developed between laboratories and clinicians in order to correctly define and set-up critical values, as their detection conveys urgent medical action.

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REGIONAL PILOT STUDY TO EVALUATE VARIATIONS IN THE GENERAL PRACTITIONERS TEST REQUESTING PATTERNS IN SPAIN

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Background. Requesting patterns for serum chemistry tests ordered (year 2009) by General Practitioners (GPs) to eight laboratories providing services to eight Health Areas (2.014.475 inhabitants) were analyzed using appropriateness indicators, to compare prescription habits.
Methods. Indicators that measured every test request per 1000 inhabitants (22 serum chemistry tests) or the number of tests per related test requested (aspartate aminotransferase/alanin aminotransferase, urea/creatinine, free thyroxine/thyrotropin) by GPs were calculated. The savings generated if each Health Care Department achieved the indicator standard (0.2 for aspartate aminotransferase/alanin aminotransferase, 0.1 for urea/ creatinine and 0.25 for thyroxine/ thyrotropin), were also calculated. Laboratory Information System registers were collected and indicators were calculated automatically in each laboratory using a data warehouse application.
Results. There was a large difference in every 2009 test request per 1000 inhabitants per Areas. The ratio of related tests also showed a great variability (from 0.25 to 1 for aspartate aminotransferase/alanin aminotransferase, 0.20 to 0.97 for urea/creatinine and 0.26 to 1.0 for thyroxine/thyrotropin). The savings generated if each Area had achieved the appropriateness indicator standard was 80967€ for free thyroxine, 18989€ for aspartate aminotransferase and 62678€ for urea.
Conclusions. Considerable variability exists in the use of laboratory tests by GPs in eight Health Areas serving 2,014,475 inhabitants. Appropriateness indicators can be applied across a spectrum of clinical laboratories, being useful for examining requesting patterns. The study highlighted the need to unify demand by optimizing the use of appropriate tests according to concrete clinical needs, through interdepartmental communication and rigorous application of scientific evidence.

Quality assessment, standardization

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TYPES AND FREQUENCIES OF PRE-ANALYTICAL SAMPLE ERRORS IN SPAIN

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Background. To evaluate preanalytical sample errors in seven laboratories attending seven Health Areas (1.642.337 inhabitants).
Methods. An error was defined as a rejected specimen: any blood or urine sample which cannot be successfully tested as it does not meet laboratory acceptability criteria, or if not received. We evaluated preanalytical errors from the tests requested for haematology, coagulation, chemistry, and urine samples (1.819.567 specimens) in one year period (2009) and collected in Primary Health Care Centres. Laboratory Information System registers (codified results that informs an incidence has occurred that prevents sample processing) were collected and indicators calculated automatically through a data warehouse and OLAP cubes software.
Results. The highest percentage of errors occurred in coagulation samples (47%), followed by urine (46%), haematology (6%) and chemistry (1%), corresponding to 345, 334, 42 and 6 preanalytical errors per 10000 samples respectively. The largest proportion of errors was due to sample unavailability. Larges differences in preanalytical errors were observed between Health Areas.
Conclusions. The main cause that prevents sample processing was sample unavailability suggesting that the main defect in specimen collection was related to container preparation prior to phlebotomy. There is a need to plan strategies to improve/automate laboratory sampling procedure. Preanalytical sample errors related to phlebotomist's expertise were much lower. The high incidence and variability of preanalytical errors between Health Departments suggests that there is a need to standardize the drawing practice through interdepartmental communication as a basis to improve patient safety.

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TURNAROUND TIME VARIABILITY ACCORDING TO CUSTOMER TYPE: REGIONAL PILOT STUDY IN SPAIN

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Background. To evaluate turnaround time (TAT) related to customer type in one year period (2009) in eight laboratories attending eight Health Areas (2014475 inhabitants).
Methods. Intern Laboratory Information System (LIS) registers (test register and verification date and time) and daily LIS registers (demographic data and type of test) were used for routine laboratory TAT indicators design, that were referred to key tests (cells blood count, CBC, and serum glucose and thyrotropin) that were verified the phlebotomy day (primary care patients) and/or at 12 a.m. (inpatients). Stat TAT between test register and validation time (minutes) were also referred to key tests (serum troponin and potassium). LIS registers were collected and indicators calculated automatically through a data warehouse and OLAP cubes software.
Results. TAT differences between Areas were observed in inpatients key tests percentage verified at 12 a.m. (from 15% to 99% for CBC, 32% to 99% for glucose and 40% to 99% for thyrotropin), and verified the phlebotomy day in primary care patients (from 12% to 99% for CBC, 30% to 99% for glucose and 6% to 99% for thyrotropin). Stat TAT variability was also showed (from 29 to 59 minutes for potassium, and 33 to 69 for troponin), and was related to hospital size, workload and if request is validated by the laboratory physician.
Conclusions. The study results showed large TAT differences. The various types of customers attended in the laboratories create the need of continuous mapping processes redesign to achieve customer needs.

Quality assessment, standardization

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TYPES AND FREQUENCIES OF PRE-ANALYTICAL SAMPLE ERRORS IN SPAIN

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Background. To evaluate preanalytical sample errors in seven laboratories attending seven Health Areas (1.642.337 inhabitants).
Methods. An error was defined as a rejected specimen: any blood or urine sample which cannot be successfully tested as it does not meet laboratory acceptability criteria, or if not received. We evaluated preanalytical errors from the tests requested for haematology, coagulation, chemistry, and urine samples (1.819.567 specimens) in one year period (2009) and collected in Primary Health Care Centres. Laboratory Information System registers (codified results that informs an incidence has occurred that prevents sample processing) were collected and indicators calculated automatically through a data warehouse and OLAP cubes software.
Results. The highest percentage of errors occurred in coagulation samples (47%), followed by urine (46%), haematology (6%) and chemistry (1%), corresponding to 345, 334, 42 and 6 preanalytical errors per 10000 samples respectively. The largest proportion of errors was due to sample unavailability. Larges differences in preanalytical errors were observed between Health Areas.
Conclusions. The main cause that prevents sample processing was sample unavailability suggesting that the main defect in specimen collection was related to container preparation prior to phlebotomy. There is a need to plan strategies to improve/automate laboratory sampling procedure. Preanalytical sample errors related to phlebotomist's expertise were much lower. The high incidence and variability of preanalytical errors between Health Departments suggests that there is a need to standardize the drawing practice through interdepartmental communication as a basis to improve patient safety.

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GLOBAL TACROLIMUS ASSAY PROFICIENCY STUDY

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Background. Current LC/MSMS and immunoassay test methods used to monitor tacrolimus concentrations in whole blood of allograft recipients are not standardized due to the lack of an internationally recognized tacrolimus reference material and reference method. The aim of this study was to assess the need for tacrolimus assay standardization.
Methods. A 40 member whole blood tacrolimus proficiency panel (2-30 ng/mL) was sent blinded to 23 clinical laboratories in 14 countries to be tested by the following assays: LC/MSMS (n=9), Abbott ARCHITECT (n=17), Siemens/Dade Dimension (n=5) and Microgenics (n=1). Select LC/MSMS laboratories (n=4) also received a calibrator panel (MassTrak kit, Waters Corp.). Test results from each laboratory were compared to the values of the blinded panel members obtained by a validated LC/MSMS method used at St. George's, Univ. of London, which was designated as the provisional reference method.
Results. The range of CVs observed with the tacrolimus proficiency panel was as follows: LC/MSMS 11.4-18.7%; ARCHITECT 3.9-9.5%; Siemens/Dade 5.0-48.1%. The range of historical within-site QC CVs using controls was as follows: LC/MSMS low=3.8-8.9%, medium=2.0-6.0%, high=2.3-6.3%; ARCHITECT low=2.5-9.5%, medium=2.5-8.6%, high=2.9-18.6%; Siemens/Dade low=8.7-23.0%, medium=7.6-13.2%, high=4.4-10.4%. Assay bias observed between 4 LC/MSMS sites was not ameliorated by implementation of a common calibrator set.
Conclusions. The ARCHITECT assay gave better precision than either the LC/MSMS or Siemens/ Dade Dimension assays for the tacrolimus proficiency panel. Use of a common calibrator did not improve agreement between LC/MSMS Methods. Tacrolimus assay standardization is required in order to provide optimized drug dosing and consistent care across transplant centers globally.

Quality assessment, standardization

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EXTERNAL QUALITY ASSESSMENT SCHEMES (EQAS) IN LABORATORY MEDICINE AND ISO 17043: HOW TO COPE WITH IT?

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Background. Participation in accredited EQAS is one of laboratory requisites to implement ISO 15189. EQAS Buenos Aires, with 33 years of activitiy in Latin America, is undergoing the process for accreditation under ISO 17043:2010 as it is considered the international standard for proficiency testing providers competence. Sample homogeneity and stability are analyzed here as they are key technical requisites for a competent EQAS. Target value assignment is considered.
Methods. ProgBA EQAS samples are prepared in-house from human material, covering clinically useful ranges for most of analytes, following recommendations of IUPAC Harmonized Protocol ($3s < \sigma_{dist}$).
Results. Data from sample lots distributed in 2010 are shown. For target value assignment, we compared our own method (trimmed mean of overall data and outliers detection) with that recommended in ISO 13528:2005 (Algorithm A for consensus values). Target value assignment as consensus mean using any method showed no significant differences: e.g. Glucose 5.54 vs 5.55 mmol/L. Homogeneity testing showed acceptable results (requisites applied were historical SD distribution data): Total protein s_{hom}=1.5%, TSH s_{hom}=3%, HCG s_{hom} =5%, E_2 s_{hom} =8%. Stability testing was assessed with short and long term data: Cholesterol s_{stab} = 0.2%, Na s_{stab} = 0.3% (short-term: 12 months); To s_{stab} = 1.5% Csol s_{stab} = 3% (long term: 36 months).
Conclusions. Accreditation for EQAS in laboratory medicine can be achieved through ISO 17043; homogeneity and stability being the key points. Consensus values can be used as target values, especially in immunoassays where no reference methods or reference materials of higher order are available.

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LICENCING OF MEDICAL LABORATORIES IN SLOVENIA

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Background. Accreditation of medical laboratories in Slovenia is not yet implemented. Nevertheless the concept of laboratory accreditation defined by ISO/IEC *as formal recognition that the testing laboratory is competent to carry out specific tests* was implemented by a different model of licencing. In order to assure quality and competence in laboratory medicine the National Bylaw for medical laboratories based on ISO 15189 was adopted
Methods. Laboratory apply for certification of its professional activity (clinical chemistry, microbiology, transfusiology or pathology /cytopatholgy) at Ministry of Health. The complyment to the requirements is verified by commissions appointed by the Ministry. Members of commissions are proffessionals of the scientific fields trained for auditing. In principle a commission consists of three members but in case that laboratories carry out analyses from diffrent fields, a specialist of the relevant field joins the audit. The special fields often overlap and that as a rule provokes disagreement over proffessional competences.
Results. By the end of the 2010 55 out of 102 registred medical biochemistry laboratories were audited, 49 laboratories complied with the requirements and gained the licence, 6 laboratories got a suspens of 6 months due to minor nonconformities.
At microbiology 15 laboratories were audited 3 out of these have to correct deficiences. Transfusiologists audited all 16 centers and ordered suspenses for two, while at pathology/cytopatholgy one out of 19 got a suspens and two were found not complying.
Conclusions. Process has already proved benefits; laboratories have their quality systems set up, are comitted to continous quality improvement which will eventually result in accreditation.

Quality assessment, standardization

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NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY STANDARD REFERENCE MATERIAL 2921 FOR CTNT AND CTNI: ONLY USABLE WHEN SPIKED IN (HEPARIN) PLASMA?

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Background. European Quality Assurance Organizations (EQAS), such as the Dutch SKML, aim to monitor cardiac troponin (cTnT and cTnI) standardization and harmonization. Recently, well characterized NIST standard reference material (SRM) 2921 became available.
Methods. Serum and heparin plasmafrom healthy controls were spiked with NIST SRM 2921 1:400, incubated at 4 and 37°C, and aliquots were collected at 0, 0.5, 2, 6, 24, 48, and 72 h. cTnT and cTnI were investigated using a Cobas 6000 (Roche) and Axsym (Abbott) analyzer, respectively. Additionally, immunoprecipitation followed by Western Blot was performed using the catcher and detector antibodies of both commercial immunoassays.
Results. Overall cTnT recovery for NIST SRM 2921 remained constant (>90%) when incubated in serum or plasma at 4°Cfor 3 days. Western Blot detection showed degradation of cTnT (MW estimated, 40 kD) in serum, but not in plasma, into 29 and 16 kD. Incubation at 37°C resulted in decreased cTnT recovery in serum (59%), not in plasma (>90%), and Western Blot detection confirmed this higher extent of degradation in serum. Moreover, cTnI recovery in serum/plasma remained constant when incubated at 4°C (>90%), but not at 37°C (<60%), and Western Blot detection did not reveal degradation.
Conclusions. We show that cTnT in NIST SRM 2921 standard is susceptible to time-dependent degradation when spiked in serum and stored at 4°C, whereas it is stable in heparin plasma. For cTnI, as measured with the Axsym assay, this seems not the case. Overall, spiked heparin plasma is thus preferable for cTnT and cTnI standardization and harmonization purposes.

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THE USE OF ACCURACY PROFILES COMBINED WITH MEDICALLY RELEVANT ACCEPTANCE LIMITS FOR PATIENT RESULT COMPARISON DURING METHOD VALIDATION OR VERIFICATION

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Background. Method validation or verification is commonly used in the clinical chemistry laboratory, when new or modified methods or instruments are to be implemented. Comparison is performed in order to evaluate consistency in patient Results. Ifinconsistency is found, a correction factor could be applied for adjustment of reference interval, target value, toxic level, therapeutic range or patient Results.
Methods. Data from method verification of selected analytes has been evaluated, with patient samples being analysed in replicate over several days on the evaluated method as well as the method used in routine at the laboratory. Accuracy profiles have been constructed by calculating ß-expectation tolerance intervals in combination with medically relevant analyte dependent acceptance limits.Limitation of the described procedure is stability and sample volume as the same sample is analyzed in replicate over several days.
Results. Accuracy profiles of the selected analytes illustrate outcomes such as acceptable performance and unacceptable bias and or/imprecision of evaluated method versus routine method.
Conclusions. During method validation or verification, accuracy profiles can be usefulfor visualising the performance of evaluated method,in comparison to routine method,with respect to total error taking account imprecision as well as bias. In combination with suitable acceptance limits this approach can be a useful decision tool in the clinical chemistry laboratory setting, for evaluation of acceptable consistency of patient results as well as determining proper action when not achieved.

Quality assessment, standardization

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STABILITY STUDY OF 80 ANALYTES INCLUDING HORMONOLOGY ON HUMAN WHOLE BLOOD: EFFECT OF TEMPERATURE AND DELAY BEFORE/AFTER CENTRIFUGATION ACCORDING TO DIFFERENT COLLECTION TUBES

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Background. Accreditation of laboratories requires pre-analytical process control. We studied subsequent delays before analysis: 2, 4, 6, 24 and 72h, at 4°C and 25°C for 80 analytes including routine biochemistry (36), haematology (14), coagulation (7) and specialized assays: hormonology (23).
Methods. For each analyte, blood specimens from 10 healthy donors were used, collected in plain glass tubes, serum separator tubes (SSTII), lithium heparin, sodium fluoride, sodium citrate and EDTAK3 (Becton Dickinson). The mean from volunteers for each analyte was obtained at each time point and temperature (Tx). The reference value (T0) was measured 30mn after clotting. To detect a significant change, we compared the mean difference (Tx–T0) to goal limits according to international recommendations. The Reference Change Value was calculated using analytical CVa issued from intralaboratory quality control data (RCV=2.77xCVa), or the 0.5 intraindividual CV (CVb) from Ricos database (when too precise method).
Results. Biochemistry assays were all stable after 24h in whole blood, except glucose, potassium, phosphorus and magnesium, lactate and LD. Haematological and coagulation analytes were not affected until 24h except mean corpuscular volume, mean corpuscular haemoglobin and partial thromboplastin time. Hormonological analytes showed a good stability up to 72h except PTH, Insulin, C-Peptid, ACTH, Osteocalcin, C-Telopeptide.
Conclusions. Most of analytes of biochemistry, coagulation and haematology are stable in whole blood 24h and 72h for hormonology. However, some important analytes are affected by delay or temperature storage. In an accreditation context, results from this study could help other labs to manage rejection/acceptance of sample based on specific pre-analytical conditions.

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DEVELOPMENT OF QUALITY CONTROL RULES FOR AN INTERNAL QUALITY PROGRAM BASED IN OPSPECS CHARTS. A TUMOR MARKER PANEL EXPERIENCE

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Background. The OPSpecs (Operating Specifications) charts are powerful graphical tools for planning and designing a quality control (QC) system in biochemical laboratories. This charts allows to develop strategies to increase performance and reduce costs.
Methods. Over 225 days we daily performed measurements of total PSA, free PSA, CA15.3, CA125, CA19.9, CEA, AFP and NSE from "Liquichek Tumor Marker Control" (BioRad Laboratories). The analyzer used was a E170 Module (Roche Diagnostics). Data were processed using the Westgard Advisor module (Unity Real Time. BioRad Laboratories.), which use the OPSpecs charts to generate the internal QC rules for each test, the number of control materials for analytical series (N), the probability of error detection (Ped) and the probability of false rejection (Pfr).
Results. The QC rules obtained are described as follow: . Free PSA: 1-3s/2-2s/R-4s/4-1s/8-X (Sigma=2.43, N=4, Ped>=90%, Pfr=3%). Total PSA: 1-3s (Sigma=5.51, N=2, Ped>=90%, Pfr=0.4%). CA15.3: 1-3S (Sigma=5.29, N=2, Ped>=90%, Pfr=0.4%). CA125: 1-2.5s (Sigma=5.02, N=2, Ped>=90%, Pfr=2.8%). CA19.9: 1-2.5s (Sigma:4.45, N=3, Ped>=90%, Pfr=3.4%). CEA: 1-5s (Sigma=8.96, N=2, Ped>=90%, Pfr<0.01%). AFP: 1-5s (Sigma=9, N=2, Ped>=90%, Pfr<0.01%). NSE: 1-3s/2-2s/R-4s/4-1s/8-X (Sigma=1.75, N=4, Pde>=90%, Pfr=3%).
Conclusions. OPSpecs charts generate efficient internal QC rules, with a high probability of error detection and a minimum probability of false rejection. sigmavalues obtained using theserules arevery god in most cases, ensuringthe reliabilityoftheresultsissued bythe laboratory.

Quality assessment, standardization

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RISK ANALYSIS OF THE PRE-ANALYTICAL PHASE IN A HOSPITAL LABORATORY

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Background. The majority of errors in the laboratory process occur in the pre-analytical phase. The objective of our study was to perform a risk analysis to each step of this process in order to prioritize improvement projects.
Methods. Risk analysis was performed using the Failure Mode and Effects Analysis model (FMEA). For each sub process of the pre-analytical phase all possible risks were identified. Subsequently a score was granted for severity, frequency and probability of detection. Out of these scores risk priority numbers were calculated, which allowed us to order risks according to priority. A set of quality indicators was defined to monitor high risk sub processes.
Results. Highest risk priority numbers were obtained for risks in the following sub processes: patient selection, sample collection and sample transport.
Hemolysis, sample errors such as unsuitable, insufficient, clotted or non received samples and sample misidentifications were chosen as quality indicators that are now continuously registered and monthly reported to several pilot departments that take part in an improvement project.
Conclusions. Risk analysis has shown to be a powerful tool to identify and prioritize most critical risks in our pre-analytical phase and helped us to develop a strategy for continuous reduction of pre-analytical errors.

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QUALITY INDICATORS IN A CLINICAL LABORATORY: FROM CERTIFICATION TO ACCREDITATION

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Background. Quality indicators are important management tools that can be used to evaluate the organization's overall performance and effectiveness, enabling to examine/follow the evolution of the quality of its services.
Methods. To evaluate the results of the Quality Management System in the laboratory, throughout the upgrade from Certification (ISO 9001:2000) to Accreditation (NP EN ISO15189:2007), main quality indicators from Pre-Analytical, Analytical, and Post-Analytical phases was analyzed, from 2002 to 2009.
Results. The waiting time was reduced from 21.2 minutes (2005) to 11.9 minutes (2009); the repetitions of sample collecting decreased from 1.4% (2002) to 0.91% (2009). Repeat testing decreased from 14% (2002) to 1.98% (2009), being responsible for a reduction of 17% in the cost of reagents per analysis. The correct results in the External Quality Assessment Schemes were 90.45% in 2003 and 96.3% in 2009. The imprecision (CV%) decreased since 2003 to 2009, with an achievement of target values of 72.8% and 79.7%, respectively. In 2009, 77% of Total Error determination had an evaluation between Good and Excellent. The errors detected after the emission of the results decreased from 0.15% (2003) to 0.064% (2009). During this period the laboratory users increased over 60%, from 36 640 Users (2003) to 59,005 (2009) and an increase in the number of analysis / tests from 330 441 to 596 655.
Conclusions. The study provided evidence that the implementation ISO Standards for Quality Systems in Clinical Laboratories (ISO9001 and ISO15189) it's very important to monitor the laboratory's overall contribution to patient care.

Quality assessment, standardization

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STABILITY STUDY OF 80 ANALYTES INCLUDING HORMONOLOGY ON HUMAN WHOLE BLOOD: EFFECT OF TEMPERATURE AND DELAY BEFORE/AFTER CENTRIFUGATION ACCORDING TO DIFFERENT COLLECTION TUBES

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Background. Accreditation of laboratories requires pre-analytical process control. We studied subsequent delays before analysis 2, 4, 6, 24 and 72h, at 4°C and 25°C for 80 analytes including routine biochemistry (36), haematology (14), coagulation (7) and specialized assays: hormonology (23).
Methods. For each analyte, blood specimens from 10 healthy donors were used, collected in plain glass tubes, serum separator tubes (SSTII), lithium heparin, sodium fluoride, sodium citrate and EDTAK3 (Becton Dickinson). The mean from all volunteers for each analyte was obtained at each time point and each temperature (Tx). The reference value (T0) was measured 30mn after clotting. To detect a significant change, we compared the mean difference (Tx–T0) to goal limits according to international recommendations. The Reference Change Value was calculated using analytical CVa issued from intralaboratory quality control data (RCV=2.77xCVa), or the 0.5 intraindividual CV (CVb) from Ricos database (when too precise method).
Results. Biochemistry assays were all stable after 24h in whole blood, except glucose, potassium, phosphorus and magnesium, lactate and LD. Haematological and coagulation analytes were not affected until 24h except mean corpuscular volume, mean corpuscular haemoglobin and partial thromboplastin time. Hormonological analytes showed a good stability up to 72h except PTH, Insulin, C-Peptid, ACTH, Osteocalcin, C-Telopeptide.
Conclusions. Most of analytes of biochemistry, coagulation and haematology are stable in whole blood 24h and 72h for hormonology. However, some important analytes are affected by delay or temperature storage. In an accreditation context, results from this study could help other labs to manage rejection/acceptance of sample based on specific pre-analytical conditions.

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STABILISING LEUKOCYTES ENABLES GLOBAL IMPLEMENTATION OF AN EXTERNAL QUALITY CONTROL FOR HIV-MONITORING

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Background. The diagnosis and antiviral therapy of HIV/AIDS is a major challenge in developing countries. Thus, the accurate determination of CD4+ T-cells is of major importance. Different trials have been started to develop an international external quality control for this immunological measurement. The main problem with implementing interlaboratory tests in countries with resource-limited settings is the insufficient stability of fresh blood samples. For an international distribution, stabilised blood samples are required which can withstand shipment throughout the global, mainly tropical hemisphere. In order to solve this problem we have developed a new method of generating stable leukocytes which are suitable for long-term storage at elevated temperatures.
Methods. Samples of leukocytes with different concentrations of CD4+ T-cells were generated by chemical stabilization. An international survey, mainly in countries within the tropical hemisphere in Africa and Asia, was carried out to demonstrate the quality of stabilised control material.
Results. Stabilised leukocytes are suitable for long-term storage at elevated ambient temperatures. After storage at 40°C for 4 weeks, control samples are still compatible with the common instrumentation and gating strategies of basic flow cytometers. They were used successfully as control material in a multinational pilot survey for flow cytometric CD4 count.
Conclusions. The usage of new stable control material allows the integration of regions with resource-limited settings and enables implementation of an external quality control CD4 measurement as an important step to ensure that patients with HIV/AIDS receive sufficient therapy.

Quality assessment, standardization

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INTER-LABORATORY VARIABILITY OF CURRENT DIAGNOSTIC SYSTEMS BASED ON THE RETROSPECTIVE ANALYSIS OF 21 BIOCHEMISTRY ANALYTES IN 78 EQA SURVEYS

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Background. Quality in medical laboratories is a requirement to certify accurate medical diagnosis and patient healthcare. External quality assessment (EQA) schemes are recognized as an important tool for monitoring quality in medical laboratories. In this objective, ProBioQual, a French association of clinical biologists, provides notably a weekly EQA survey in biochemistry.
Methods. A retrospective analysis of biochemistry EQA surveys in 2008 and 2009 organized by ProBioQual was performed. We focused on 21 common serum analytes (7 electrolytes, 11 substrates, 3 specific proteins) usually determined on chemistry instruments. Groups of peers for the most popular diagnostic systems were constituted. The inter-laboratory variability was assessed using the mean of the coefficient of variation (CV) after discarding outliers. For each analyte, ranks were attributed in each group of peer according to increasing CV.
Results. Seventy eight EQA surveys were analyzed, corresponding to 329486 tests results from 800 participating laboratories. Twelve groups of peer were isolated from 6 commercial companies: Abbott Diagnostics, Beckman Coulter, Roche Diagnostics, Siemens Healthcare Diagnostics, Thermo scientific, Ortho Clinical Diagnostics. No differences were observed in the classification of diagnostic systems based on the ranks sum between 2008 and 2009. In 2009, if only 16 ranks separate the four firsts (82 to 98), the rank sum for the four lasts attain the double from the firsts (151 to197).
Conclusions. Our retrospective analysis of EQA surveysallows comparing performances in daily use of chemistry instruments. Whereas some systems should improve their performances, inter-laboratory variability for the best diagnostic devices is comparable.

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STABILITY OF PATHOGENS IN URINE SAMPLES OVER TIME USING A URINE COLLECTION TUBE CONTAINING A NOVEL MIXTURE OF PRESERVATIVES

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Background. Urinary tract infections are one of the most frequent disorders in primary medical care. Pre-analytical errors including sample collection, transportation to the laboratory and storage are often responsible for false microbiological urine testing.
Materials and Methods. Urine samples (n=25) from healthy volunteers were collected with the new *CCM* collection tube (Greiner Bio-One) containing a novel mixture of preservatives for a better stabilization of the microbiological and stored at room temperature. Samples were spiked with *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Candida albicans*, and were cultivated within 2 hours and again over the time after 24 and 48 hours regarding stability of pathogens.
Results. All spiked 5 pathogens could be isolated in primary urine cultures with CFUs ranging from $10^2 – 10^4$. No significant differences in CFUs (all stayed within 1 log) were observed when results of the primary urine culture obtained within 2 hours were compared to those obtained over the time at 24 and 48 hours indicating high stability of pathogens.
Conclusions. The new Greiner Bio-One CCM urine collection tube is able to keep pathogens responsible for urinary tract infections sufficiently stable for at least 48 hours at room temperature. It therefore seems to be useful in helping to further improve pre-analytics in urine culture testing. A larger study investigating clinical samples isneeded to further prove these findings.

Quality assessment, standardization

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FREE TESTOSTERONE ESTIMATION BY ANALOG RADIOIMMUNOASSAY OR CALCULATED RESULTS. A COMPARISON THROUGH AN EXTERNAL QUALITY ASSESSMENT SCHEME

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Background. Free Testosterone (FT) can be measured by analog RIA (FTA) or calculated by Vermeulen´s formula (FTV) using total testosterone (TT) and SHBG (as recommended by ISSAM). Our objective was to evaluate comparability of FT estimation through EQAS Results.
Methods. Male and female pools were freeze-dried and distributed in an annual round (2009-2010) of ProgBA EQAS. Participants returned FT results by FTA or by calculation (FTC). We also calculated FTV applying Vermeulen´s formula to submitted results of TT and SHBG, in order to check if laboratories reporting FTC applied the correct formula and/or units.
Results. 36% participants informed FTA and 64% FTC. Medians (pg/ml) for female(f) and male(m) pools were: FTAf: 0.56, FTCf: 8.29, FTVf: 10.3; FTAm: 5.38, FTCm: 125 and FTVm: 121. For Analog RIA two reagents were used: DPC CAC and DSL RIA. FTA medians for female and male pools respectively were: DPC CAC: 0.50 and 4.40 pg/ml and DSL RIA: 1.80 and 8.10 pg/ml.
Conclusions. EQAS results reflect the lack of comparability between Methods. There is a significant difference (p<0.05) among results obtained by Analog RIA Free Testosterone and Calculated Free Testosterone. Applying the same formula and units improved calculated results comparability. Both RIA methods showed no concordance with each other. Results show the need of method harmonization to improve usefulness of FT for diagnosis and treatment of hypogonadal men, men with hypertension and men with low total/free testosterone and evaluation of suspected hypogonadal men with low SHBG (obesity, type II diabetes or hypothyroidism).

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PREANALYTIC ASSESSMENT OF SERUM SAMPLE QUALITY BY MEANS OF INTELLIGENT PHOTOGRAPHY

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Background. The majority of errors in the medical laboratory occur within the preanalytical phase. Assessment of sample quality is a crucial procedure in preanalytical handling of a sample.
Aim: To characterize the performance of the newly available system RSA Pro QS 1 (PVT, Waiblingen, Germany) employing intelligent photography (IP) for preanalytical assessment of sample quality.
Methods. Imprecision in judging hemolysis, lipemia and icteria was assessed with 3 different methods [IP, visual assessment (VA), and serum index (SI) measured on Roche Integra 800] and compared to determinations of triglycerides, free hemoglobin and total bilirubin. Reference ranges were determined in 157 subjects, in whom samples were drawn in optimal circumstances. These reference ranges were validated in 2170 samples analyzed in the context of daily clinical routine.
Results. Coefficients of variation (CV's) for IP were 0.2-2.1% (hemolysis), 1.3-12.8% (icteria), and 0.1- 1.2% (lipemia). The respective ranges for serum index measurement were 0.3-2.3% (hemolysis), 0.4-0.5% (icteria), and 0.7-1.3% (lipemia). VA was more imprecise: 3.2-26.9% hemolysis, 8.3-52.3% icteria, 9.9-36.2% lipemia. There were significant correlations between IP and SI measurements as well as with analyte concentrations. The reference cut-offs for IP per definition were at 50% for hemolysis, icteria, and lipemia. The respective values SI measurement were <20 micromol/L (hemolysis),< 35 micromol/L (icteria), <40mg/dL (lipemia).
Conclusions. Intelligent photography (IP) in preanalytics offers a feasible method to characterize hemolysis, icteria and lipemia, which is substantially better than visual inspection of samples. In contrast to SI measurement, IP does not compromise analytical productivity of routine clinical chemistry analyzers.

Quality assessment, standardization

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IFCC WORKING GROUP ON "LABORATORY ERRORS AND PATIENT SAFETY" (WG-LEPS): UPDATING ABOUT THE PROJECT ON QUALITY INDICATORS

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Background. Laboratory Medicine has an important role in the delivery of high-quality care, nevertheless no consensus exists as yet on the use of Quality Indicators (QIs) focussing on all steps of the laboratory total testing process (TTP). IFCC WG-LEPS developed a series of QIs, specifically designed for clinical laboratories with the aim to create a common reporting system and Quality Specifications (QSs) to evaluate laboratories data.

Methods. The WG-LEPS has identified 25 QIs: 16 pertain to the pre-analytic phase, 4 to the intra-analytical, and 5 to the post-analytical. For each QI, have been specified: the measures of information to collect; the steps involved for a uniform data collection; time for data collection and responsibility. A website (www3.centroricercabiomedica.it) has been implemented for allowing the safe collection of data. On the basis of results collected for the period February 2008 to December 2009, preliminary QSs have been calculated. Laboratories data, collected during 2010 year, will be evaluated against the QSs identified.

Results. 39 laboratories around the world were enrolled in the project and a wide range in the results distribution has been obtained. For example, the percentage of requests with clinical question/total number of requests ranged between 9% to 87% and the following QSs have been defined in relation to three performance levels: optimum, > 87%; desirable, 58 – 87%; acceptable, 29 – 57%.

Conclusions. A model of QIs and QSs managed within the framework of a External Quality Assurance Program would allow identification of risks predisposing to errors resulting in patient harm.

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EFFICIENCY OF ANALYTICAL QUALITY CONTROL WITH VARIOUS QUALITY PLANNING TOOLS IN THAI CLINICAL LABORATORY

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Background. Quality control (QC) procedure is one important feature in clinical laboratory. Performing analytical tests with the most effective quality planning (QP) are necessary. Since quality planning plays an important role in the quality control procedure, the quality planning tool must carefully choose. There are various quality planning tools used in Thai clinical laboratory, i.e. sigma metric, Operating Specification (OPSpec) chart and traditional 2 standard deviation (SD) limits.

Methods. Six clinical laboratories with six enzymes tests were selected for this study. Quality planning tools of 2SD limit, sigma metric, OPSpec chart were used. Efficiency of quality control procedure was evaluated by the out-of-control rate.

Results. The average of out-of-control rate detected with the sigma metric, OPSpec chart and traditional 2SD limit were 11.15%, 9.08%, and 28.66 %; respectively. The out-of-control rate by traditional 2SD limit was statistically significant different from those using the sigma metric and OPSpec chart ($p < 0.01$), and no statistically significant difference ($p = 0.35$) between the sigma metric and OPSpec chart. This indicated the capability of quality planning tool, choosing an appropriate one that fitted the types and amount of work must be seriously considered. One must keep in mind that the higher the out-of-control rate might end up with the higher operating cost and time consuming, this might also lead to customer dissatisfaction.

Conclusions. To obtain the quality control procedure that suitable for laboratory test, each laboratory has to do quality planning with the most appropriate tool which may different from laboratory to laboratory.

Quality assessment, standardization

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ASSESSMENT OF QUALITY AND STANDARDIZATION OF SERUM-CALCIUM AND –ALBUMIN MEASUREMENT IN ARGENTINA

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Background. The quality of 3200 Argentinian laboratories is regularly assessed through surveys organized by the Programa de Evaluacion Externa de la Calidad (PEEC) of the Fundación Bioquímica Argentina (FBA). Here, we report on a special study undertaken with a panel of 20 fresh-frozen single donation serum samples in order to get a deeper insight into performance of serum–calcium and –albumin measurement.

Methods. Serum–calcium and –albumin were measured in singlicate by ~300 representative laboratories. Laboratory variation, bias, and total error were evaluated by correlation to and difference from the manufacturer mean of laboratories using homogeneous assays. Assay effects were investigated in groups with n ≥7 participants.

Results. Calcium values ranged from 2.06 to 2.42 mmol/L and albumin values from 33.6 to 44.9 g/L. For calcium, 43 laboratories had *r*-values <0.4; biases ranged from -20% to 40% and 14% of the laboratories had an absolute bias >10%; 97 laboratories exceeded the total error criterium of 10% in ≥5 samples. For albumin, 17 laboratories had *r*-values <0.4; biases ranged from -32% to 39% and 13% of the laboratories had an absolute bias >10%; 86 laboratories exceeded the total error criterium. Laboratories using homogeneous tests performed generally better than the others. Assay effects were moderate for calcium, but significant for albumin.

Conclusions. The study demonstrated a great need to improve the quality and standardization of calcium and albumin tests in the investigated laboratories. Fastest improvement may be achieved by a cooperation between laboratories and manufacturers.

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RECOMMENDATIONS FOR STANDARDISED REPORTING OF PROTEIN ELECTROPHORESIS IN AUSTRALIA AND NEW ZEALAND

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Background. Although protein electrophoresis of serum (SPEP) and urine (UPEP) specimens is a well established laboratory technique, the reporting of results using this important method varies considerably between laboratories. The Australasian Association of Clinical Biochemists recognised a need to adopt a standardised approach to reporting SPEP and UPEP by clinical laboratories.

Methods. A Working Party considered available data including published literature and clinical studies, together with expert opinion in order to establish optimal reporting practices. A position paper was produced which subsequently was revised through a consensus process involving scientists and pathologists with expertise in the field throughout Australia and New Zealand.

Results. Recommendations for standardised reporting of protein electrophoresis have been produced. These cover analytical requirements: detection systems; serum protein and albumin quantitation; fractionation; paraprotein quantitation; urine Bence Jones protein fractionation and quantitation; paraprotein characterisation; cryoglobulin characterisation; and laboratory expertise and staffing. The recommendations also include general interpretive commenting and commenting for samples with paraproteins and small bands together with illustrative examples of reports.

Conclusions. Recommendations are provided for standardised reporting of protein electrophoresis in Australia and New Zealand. It is expected that such standardised reporting formats will reduce both variation between laboratories and the risk of misinterpretation of Results.

Quality assessment, standardization

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ANALYTICAL PERFORMANCE EVALUATION OF THE RESULTS OBTAINED WITH DIASORIN LIAISON

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Background. The purpose of this study was to evaluate the analytical performance of the results, obtained with chemiluminescent immunoassay, using Diasorin LIAISON.
Methods. We evaluated analytical performance parameters of two thyroid hormones and four tumor markers. Reproducibility, repeatability and accuracy were analyzed. Sample carryover was studied. For comparision we performed measurements with the ABBOTT AxSYM.
Results. The study of the imprecision showed coefficients of variation ranging from 0.73 – 4.32% (intraassay) and 1.84 – 8.21% (interassay). Inaccuracy for different parameters was between -13.92% and +12.73%. The metods comparision between Diasorin LIAISON and ABBOTT AxSYM was excellent.
Conclusions. The results obtained with Diasorin LIAISON demonstrated good analytical performances.

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ANALYTICAL PERFORMANCE OF THE RESULTS FOR ZINC PROTOPORPHYRINS

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Background. The purpose of this study was to evaluate the analytical performance of the results for zinc protoporphyrins (ZPP) in venous blood, using ProtoFluor-Z Hematofluorometer (Helena, USA).
Methods. The results for ZPP obtained as ratio between the ZPP fluorescence and the Heme absorbtion. The intra- and interassay variation were determined by repeated measurements of three unselected patient samples with normal and pathological concentrations. For inaccuracy assessment, control from the manufacturer of analyzer were not provided. For statistical evaluation the coefficients of variation (CV) was calculated.
Results. In this study intraassay imprecision CVs for different levels (means: 42.3, 75.4 and 157.4 µmol ZPP/mol Heme) were 2.96%, 5.05% and 3.15% respectively. Interassay imprecision CVs were 4.77%, 9.70% and 6.58% for means: 43.0, 79.7 and 151.6 µmol ZPP/mol Heme.
Conclusions. The evaluation of imprecision revealed acceptable coefficients of variation for intra- and interassay imprecision. The ProtoFluor-Z Hematofluorometer provides quick test results and is easy to handle.

Quality assessment, standardization

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EXTERNAL QUALITY ASSESSMENT IN BACTERIOLOGY ON CLINICAL LABORATORIES

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Background. The Implementation of External Quality Control in the area of bacteriology has become more relevant due to the need to ensure the issuance of timely and reliable reports in the early stages of the disease.

Methods. Wild strains were distributed: *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* collated ATCC strains (*P. aeruginosa* 27853, *S. aureus* 25923 and *E. coli* 25922) to 25 Mexican laboratories. It is considering as a proven report, those with 100% correct genus and species with biochemical justification and 80% correct genus and species, without justification biochemistry. The statistical analysis was performed on 2x2 contingency tables using the EPI INFO 2007.

Results. The accreditation in the identification of *P. aeruginosa* and *S. aureus* was 95.4% (21), and *E. coli* 100% (22). The comparison of these results was not significant ($p > 0.05$). The degree of identification based on biochemical tests to compare the *S. aureus* with a score of 100% in 59% of laboratories (13), and 80% in 36.3% (8) and *E. coli* a grade of 100% in 90.9% (20) laboratories and 80% in 9.09% (2), it was found a statistically significant difference ($p < 0.05$).

Conclusions. The results of the identification of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* were favorable as they are one of the main species isolated, however the justification for its identification based on biochemical tests minimum necessary for this group the laboratories are not solid enough to analyze the degree of identification.

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IMPACT OF A PROGRAM OF EXTERNAL QUALITY CONTROL FOR HEMOGLOBIN

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Background. External Quality Control in the hemoglobin is of great importance because it contributes to the diagnosis and monitoring of treatment of anemia, as well as evaluation of nutritional status of the patient.

Methods. We conducted a longitudinal descriptive study, retrospective and analytical evaluations obtained in the Program for External Quality Mexican (PEEC-UASLP) over a period of six years (2003 to 2008) to hemoglobin in an average of 65 participating laboratories. The cycles of evaluation were three for year, giving each a control sample of commercial whole blood. The rating assigned is based with SDI standard deviation index (value - media laboratories group) / (SD group). Assigning the rating based on the following criteria: "Excellent" between 0.0 and 1.1, "Good" greater 1.1 and less than 1.6 and "Not Acceptable" greater than 1.6. Statistical analysis was performed using EPI INFO 2007 and Office Excel.

Results. Of a total of 382 evaluations for the manual method the average percentage of acceptability was 78% and for the automated method of 83% of a total of 336 evaluations. No statistically significant difference was found according to the type of method used to compare the first against the last year of evaluation, the same way to compare manual vs. automated methods in 2003 and in 2008 ($p > 0.05$).

Conclusions. The PEEC-UASLP has had a major impact on the performance of clinical laboratories because it managed to maintain an adequate percentage of overall acceptability. Therefore, laboratories with reliable performance assessments issued acceptable hemoglobin in the assessment period.

Quality assessment, standardization

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HEMOGLOBIN A$_{1c}$ EXTERNAL QUALITY ASSESSMENT IN CROATIA. A FIVE-YEAR EXPERIENCE

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Background. External quality assessment (EQA) for hemoglobin A$_{1c}$ has been conducted in Croatia since 2005, as a dedicated module within the National EQAS. The aim of this module was not only to improve analytical quality, but also to provide necessary educational tool for local implementation of the global harmonization goals regarding hemoglobin A$_{1c}$ testing.
Methods. Commercially available liophylizates with declared target values according to both IFCC and NGSP reference systems for a wide range of methods/reagents were selected as control materials. Results were evaluated using ERL (European Reference Laboratory for Glycohemoglobin) criteria.
Results. 28 laboratories participated in the first EQA-cycle at the end of 2005. In subsequent years 3, 2, 2 and 2 cycles were conducted, with a continuous increase in the average number of participants/cycle: 29, 34, 36 and 43 (in 2006, 2007, 2008 and 2009, respectively). A continuous improvement in analytical quality has been observed: the proportion of the laboratories reporting results with bad/unacceptable deviations fom target values decreased from 25% at the beginning to 22%, 15%, 11% and 9% in the 2006, 2007, 2008 and 2009, respectively. By the end of 2009 all the methods were anchored to the IFCC-reference system, and reporting units were harmonized to the NGSP/DCCT-equivalents by using appropriate master-equation(s).
Conclusions. These results confirm the validity of sofar implemented EQA for hemoglobin A$_{1c}$ and support further activities aimed to improve quality, harmonization, as well as clinical availability/utilization of hemoglobin A$_{1c}$, as a key laboratory test in the management of diabetes mellitus.

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THE ANALYSIS OF CURRENT CLINICAL CREATININE TESTING QUALITY AND THE STUDY OF THEIR TRACEABILITY

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Background. Because of the significant variation between the results of creatinine of different test methods and different test systems, we intend to trace the results to reference materials and reference test methods for the accuracy of creatinine tests by the cooperation with different institutions.
Methods. By statistics analysis the results of creatinine of institutions which attended the EQA common biochemistry program of CNCCL in 2009, the survey of CAP creatinine tests results and the clinical laboratories in 30 hospitals in Beijing which attended IMEP-17, we complete this study. This program adopts quality control material which has not been modified before but original from clinical daily work. After receiving quality control materials, each laboratory measures the same samples two times a day for sequence five days. The target value of this serum sample is 74.57µmol/L and confirmed by LC-IDMS.
Results. The results of the third time serum creatinine test of CNCCL EQA program were divided into groups for different Methods. the CVs of the results of picric acid method is higher than the enzymatic method in the five lots, the range of concentration is 99µmol/L ~ 344µmol/L and the range of CVs is 3.92% ~ 8.01%. After grouped by testing systems, the CVs ranges of Vitros 250 chem System, BECKSYN CHRON LX20 and DADE BEHRG DIM are 3.61% ~ 5.23%, 3.69% ~ 7.91% and 2.72% ~ 7.42% respectively. The CVs of US 2008 CAP study of creatinine testing of Vitros 250 chem System, BECKSYN CHRON LX20 and DADE BEHRG DIM are 3.5% ~ 5.9%, 1.4% ~ 6.4% and 2.1% ~ 9.8% respectively. In the 30 Beijing laboratories which attended the reference methods target value external comparison program by using fresh frozen serum, the differences of creatinine tests results are remarkable while the minimum value is 66 µmol/L and the maximum value is 105.3µmol/L; the range of test bias variation is 2.18% ~ 38.66%. According to the acceptable range of creatinine which is 74.57µmol/L±15%, only 11 of the 30 institutions can fulfill the application.
Conclusions. The methods of creatinine testing all need to be traced to LC-IDMS.

Quality assessment, standardization

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ACCREDITATION OF NATIONAL EXTERNAL QUALITY ASSESSMENT IN CHINA

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Background. External quality assessment (EQA) or Proficiency testing is a powerful quality assurance tool enabling laboratories to monitor their performance and compare their results with similar laboratories. EQA schemes which are used by laboratory accreditation bodies as part of the process to assess the ability of laboratories to competently perform tests and measurements for which accreditation is held. EQAS complement the on-site laboratory review by technical specialists.
Methods. The Chinese National External Quality Assessment (EQAS) was organized by the National Center for Clinical Laboratory(NCCL), Ministry of Health. There are some 49 clinical laboratory external quality assessment schemes available in the NCCL(www.clinet.com.cn). In China, EQA schemes are accredited against standards developed by the ILAC G-13:2000 as Accreditation Criteria for Providers of Proficiency Testing Schemes (CNAS CL-03) used by the China National Accreditation Service for Conformity Assessment (CNAS) . The CNAS CL-03 accreditation criteria are arranged in two sections. One describes management system requirements. The other section, dealing with the technical requirements.
Results. NCCL has prepared the documents of quality management systemin 2007 according to the ILAC G-13:2000(CNAS CL-03). We passed the on the site inspection from the CNAS, and got the proficiency testing provider accreditation certificate(No. CNAS PT 0016) in the March 10, 2009 and the date of expiry is March 9, 2012. The scope of accreditation includes Complete Blood Cell Count, Routine chemistry, Clinical immunology, PCR - Virology, Clinical microbiology, there are 39 items and/or parameters totally. Accredited EQA schemes will be supervised and inspected on the site from the CNAS in the March 9, 2010.
Conclusions. Substantial progress has been made in accrediting EQA schemes in the Chinese EQAS in the last year. However, it should be pointed out that EQA scheme accreditation is not mandatory. EQAS, a vital component of quality assurance in clinical laboratories, plays an extremely important role in facilitating optimal patient care.

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THE EXTERNAL QUALITY ASSESSMENT IN NEWBORN SCREENING LABORATORIES FOR 10 YEARS IN CHINA

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Background. Newborn screening for congenital conditions is a public health system composed of screening, follow-up, diagnosis management, evaluation, and education. By analyzing the submitted results of newborn screening External Quality Assessment(EQA) program from 2000 to 2009, we could get a whole picture of phenylalanine (Phe) and thyroid stimulating hormone (TSH) testingcompetence in China.
Methods. According to U.S. CDC Newborn Screening Quality Assurance Program and CLIA'88, we established newborn screening EQA program. We distributed five different dry blood spots each time and three times a year for each laboratory. After submitted results statistical analyzing,we demonstrated the laboratories testing competences.
Results. The method principles and ratios for Phe testing were: bacterial inhibition assay (from 58.6% in 2000 to 14.6% in 2009, the same below), fluorescence assay (41.4% to 64.9%) and quantitative enzymatic assay (0% to 12.3%) while for TSH were: DELFIA (44.8% to 58.5%), ELISA(27.6% to 20.7%) and fluorescent enzyme immunoassay (21.6% to 14%). The subgroups latestaverage CVs for Phe were: 13.63% (BIA), 13.95% (fluorescence assay) and 11.7% (quantitative enzymatic)while for TSH were: 17.61% (enzyme-linked immunosorbent assay), 10.99% (time-resolved fluorescence assay) and 12.11% (fluorescent enzyme immunoassay) respectively.Summary passing rates had been increasing for 10 years from the lowest 61.5% and 40.9% to 98.8% and 97.0% for Phe and TSH respectively.
Conclusions. Newborn screening EQA program could contribute to find newborn screening laboratories` current problems and improvethe quality of newborn screening testing.

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COMPETENCY ASSESSMENT OF THE PHLEBOTOMIST IN THAILAND, JAPAN AND OTHER DEVELOPING COUNTRIES

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Background. Quality test results are essential for patient care management. In pre-analytical phase, potential errors in venipunc-ture might be occurred, therefore, the competent phlebotomist plays an important role in the clinical laboratory.
Methods. A universal questionnaire was formulated to assess phlebotomists' competency in various items including general information, venipuncture procedure and a problem solving.
Results. A total of 73 clinical laboratories with 136 participants working in Thailand (n=98), Japan (n=20) and other four develo-ping countries in Asia (n=18) responded this survey. The overall scores of competency assessment were 58.62%, 55.12% and 40.69% for the participants in Japan, Thailand and the developing countries, respectively. Assessed parameters of infection con-trol, tourniquet application, venipuncture technique, order of tube collection and tube mixing technique were observed in the Japan group with the highest score. However, the ability on problem solving was not significantly different with the score of 9.06 ± 1.86, 8.30 ± 0.31 and 7.25 ± 3.89 (mean$\pm$SD) for Japan, Thailand and the developing countries groups, respectively. The assessed pro-blems were penetration through a vein during needle insertion, formation of hematoma, allergies to antiseptic agents, two failed attempts to draw blood, and the occurrence of nerve injury during venipuncture. Supportive indicators for good practices were shown in Japan participants, including an existing of standard operating procedure (94.12%), receiving specific training (100%), reporting an accident event (88.89%) and spending less time for each venipuncture (2.6 ± 0.97 min).
Conclusions. The different levels of competency in venipuncture were demonstrated in the countries studied and a better perfor-mance was observed in the developing country.

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IMPROVEMENT OF THE QUALITY OF THE URINE PARTICLE COUNTING RESULTS BY THE IMPLEMENTATION OF THE STANDARDIZED EXAMINATION PROCEDURES

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Background. The lack of standardization of urine particle counting methods is a common problem in medical laboratories. Among Polish participants of EQA scheme provided by LabQuality (Finland), we performed an educational program, which aim was the implementation of the manual standardized procedures of urine particle counting.
Methods. The program included five surveys from January 2009 to April 2010. Detailed instructions as to how to perform the standardized manual examination were sent to EQA participants and the information about applied methods was collected. The between-laboratory CV for particle counting (leucocytes and erythrocytes) for each survey was calculated. The medians of between-laboratory CVs were compared using the Mann-Whitney test.
Results. The medians of between-laboratory CV obtained by Polish participants were ±71% and ±51% for the urine sediment under a coverslip and for a sediment counting in a chamber method, respectively, and were comparable to those obtained by all participants (±77% and ±54%, respectively). The medians of between-laboratory CV for the standardized manual methods and the automated methods were ±42% and ±39%, respectively. For the non-standardized manual methods the median of between-laboratory CV was ±73% and differed significantly from those obtained by the standardized manual and automated methods (*P*=0,01 and *P*=0,005, respectively).
Only 29% of Polish participants used the standardized methods (16% - manual, 13% - automated methods).
Conclusions. Despite the recommendations of European Urinalysis Guidelines laboratories still apply traditional, semi-quanti-tative manual procedures. The harmonization of the urine particle counting results requires intensive activities to encourage the laboratories to implementation the standardized examination procedures.

Quality assessment, standardization

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COMPARISON OF REFERENCE INTERVALS BETWEEN ROUTINE CHEMISTRY AND DRY CHEMISTRY ANALYTES OF EXTERNAL QAULITY ASSESSMENT PROGRAM

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Background. Biochemistry is largely concerned with the diagnosis and monitoring of a patient `s health as a function of the biochemical analytes typically released into their bloodstream. We compared the analytes upper and lower limits respectively between routine and dry chemistry of External Quality Assessment (EQA) program for studying whether the reference intervals of routine chemistry analytes were equivalent to dry chemistry.
Methods. We collected the submitted results of EQA routine and dry chemistry program, which included reference intervals` information such as the upper and lower limits then, deleted all the data except medical institutions while eliminating all the abnormal values and errors. Data analysis was performed by SPSS 13.0, including 24 analytes.
Results. The numbers of attended laboratories of routine chemistry were much larger than dry chemistry in every analytes. In the comparisons: there were 12 analytes having statistical significant difference between routine and dry chemistry both of upper limits and lower ones (including: serum potassium, sodium, chloride, urea, creatinine, ALT, AST, amylase, creatine kinase, lactate dehydrogenase, γ-GT and bilirubin); only one limits in two, 6 analytes (including: serum total calcium, glucose, uric acid, total protein, albumin and total bilirubin) and neither of them, also 6 analytes (including: serum phosphorus, total cholesterol, triglycerides, alkaline phosphatase, magnesium and iron).
Conclusions. There were more than half numbers of reference limits of analytes between routine and dry chemistry having statistical significant differences, but whether the statistical results having clinical values might be discussed in further study.

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INVESTIGATION AND ANALYSIS OF REFERENCE INTERVALS OF ANALYTES IN DRY CHEMISTRY EXTERNAL QUALITY ASSESSMENT PROGRAM

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Background. Laboratory test results are commonly compared to reference intervals before making physiological assessments. Reference limits and sources of analytes in dry chemistry External Quality Assessment program were described and analyzed forgetting a whole picture of their current status.
Methods. After collecting the submitted results of dry chemistry program, which included reference intervals` information such as the upper and lower limits and sources, we deleted all the data except medical institutions while eliminating all the abnormal values and errors. Data analysis was performed by SPSS 13.0.
Results. The maximum and minimum of reference intervals upper limits of some analytes had great differences among institutions, the same as the lower ones. There were only little differences between medians and meansfor both upper and lower limits of almost all the analytes. While lower limit did not have medical significance, among the submitted data of reference intervals diversity was much higher than others. There were 12 of 25 analytes (12/25) having intersection between upper limits and lower limits but the differences within percentiles (from 5th to 95th) for them were not very significant. The biggest four sources of reference intervals were (descending order): instruction of reagent manufacturer, national guide to clinical laboratory procedures, determined by their own laboratory and instruction of instrument manufacturer.
Conclusions. The reference limits of dry chemistry for each analytes, both upper and lower ones, had big differences among institutions and the sources of them were various thus management should be more standardized.

Quality assessment, standardization

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INVESTIGATION AND ANALYSIS OF REFERENCE INTERVALS OF ANALYTES IN ROUTINE CHEMISTRY EXTERNAL QUALITY ASSESSMENT PROGRAM

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Background. Laboratory test results are commonly compared to reference intervals before making physiological assessments. Reference limits and sources of analytes in routine chemistry External Quality Assessment program were described and analyzed forgetting a whole picture of their current status.

Methods. Collecting submitted results of routine chemistry EQA program then deleting all the data except medical institutions while eliminating all the abnormal values and errors. Data analysis was performed by SPSS 13.0.

Results. The maximum and minimum of reference intervals upper limits had great differences among institutions, the same as the lower ones. There were only little differences between medians and meansfor both upper limits and lower ones of almost all the analytes. While lower limit did not have any medical significance, among the submitted data of reference intervals diversity was much higher than others. Except TBil and ALP, the remaining analytes always had intersection between upper limits and lower limits but the differences within percentiles (from 5th to 95th) for upper or lower limit of each analye were not significant. The biggest three sources of reference intervals were (descending order): national guide to clinical laboratory procedures, instruction of reagent manufacture and determined by their own laboratory.

Conclusions. The reference limits of routine chemistry, both upper and lower ones, had big differences among institutions and their sources were various. In order to achieve the agreement of clinical test results all the laboratories must share the common reference intervals and management should be more standardized.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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DEVELOPMENT OF A SPECIFIC SOLUBILITY TEST TO IDENTIFY SEPARATELY HAEMOGLOBIN S AND NON-HBS SICKLING HAEMOGLOBINS

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Background. Sickle cell disease (SCD) is prevalent in many countries of the world. Haemoglobin (Hb) electrophoresis is not feasible as a routine diagnostic test for SCD. Modifications of Solubility test during 1970 -1972 failed to distinguish HbS and non-HbS sickling Hb in our laboratory. Hence, this study was initiated to modify solubility reagent to develop a specific Solubility test.
Methods. Diagnosis of SCD was done by Na-metabisulphite Sickling slide test and haemoglobinopathies by Cellulose Acetate Membrane (CAM), Citrate-Agarose Acid electrophoresis & native-PAGE. We modified the Solubility reagent and the test described in literatures in respect to molarity, pH, concentrations of saponin, Na-dithionite, urea and Hb.
Results. We screened 2461 patients by Sickling slide test and 107 SCD cases (4.01%) were diagnosed as SCD. The same cases were also identified positive by our modified Solubility test. Out of these, 40(37.4%) were HbS and 67 were non-HbS sickling Hbs (62.7%). Electrophoretogram of non-HbS sickling Hb and HbS in CAM & native-PAGE were same, but in Citrate-Agarose acid electrophoresis all the 40 cases of HbS & 67 cases of non-HbS could be resolved by slightly lesser migration of non-HbS. Sensitivity and specificity of our developed Solubility test to detect HbS and non-HbS sickling Hb were 100%, in comparison to gold standard Citrate-Agarose acid electrophoresis.
Conclusions. We could successfully modify the Solubility test to distinguish HbS and non-HbS sickling Hb and as a result, the predominance of non-HbS sickling haemoglobinopathies could be reported first time in Vidarbha region of Maharashtra State, India.

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ANALYSIS OF GENE EXPRESSIONS BY QUANTITATIVE RT-PCR OF HLA-B LOCUS IN MELANOMA CELL LINES WITH HLA-B CELL SURFACE DOWNREGULATION

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Seven ESTDAB melanoma cell lines with low surface HLA-B expression measured by flow cytometry were analyzed for transcriptional levels of HLA class I locus B. Real Time PCR in a Roche Light Cycler analyzer was performed and SGRB-green and locus specific primers were used. Specific locus B transcriptional levels ware also studied in 30 PBLs samples which served as controls. All results were referred to G6PDH transcriptional levels, measured with a Roche reference housekeeping gene kit and master plus hybridization probes kit. Melting curve analysis and agarose gel electrophoresis were done to check specific amplification.
All studied cell lines showed specific HLA-B amplification. Mean Locus B specific transcription in PBLs was 29,75 copies of locus B mRNA/copies G6PDH (range 9.82-109). For tumor cell lines the mean transcription was 5,11 copies of locus B mRNA/copies G6PDH (range 0.50-11.43). The results for each cell lines are as follows: FM28, 0.50 locus B/GPDH; E120, 0.56 locus B/GPDH; E135, 2.14 locus B/GPDH; FM3, 6.93 locus B/GPDH; E100, 3.16 locus B/GPDH; E114, 11.43 locus B/GPDH; E125, 11.04 locus B/GPDH. According to these results, FM28 and E120 showed very low locus B transcription which may explain the low surface expression of locus B. In E135, FM3 and E100 cell lines, the reduced transcriptional levels could partially explain the reduced surface expression. In the other cell lines, transcriptional levels were similar to those in PBLs; thus, in this case the low surface HLA-B expression can not be due to transcriptional disregulation.
Therefore, we conclude that low transcription of locus B may be responsible for locus B downregulation in some melanoma cell lines, while in other cells post-transcriptional mechanisms may be involved.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ANALYSIS OF HLA-ABC GENE EXPRESSION IN NORMAL TISSUES BY LOCUS-SPECIFIC QUANTITATIVE RT-PCR

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We developed a novel locus-specific quantitative real-time polymerase chain reaction to determine the locus-specific gene expression of HLA-ABC heavy chain genes. We analyzed peripheral blood leukocytes from 53 healthy controls, 15 colon mucosa 15 larynx mucosa, 15 bladder mucosa, 15 stomach mucosa and 15 normal kidney tissues. Using laser assisted microdissection technique we were able to isolate selected cells without contamination with surrounding stoma cells. Our method has proven to yield specific amplification of each HLA loci and we have obtained data of tissue specific expression of each locus.

The results were expressed as mean ± standard deviation. In the case of peripheral blood leukocytes we obtained the following values: HLA-A/G6PDH=13,9±10,1; HLA-B/G6PDH= 29,6 ± 23,2; HLA-C/G6PDH=17,4 ±17,2. The results in larynx mucosa were: HLA-A/G6PDH= 23±22,8; HLA-B/G6PDH= 19,8±18,6; HLA-C/G6PDH=4,9± 4,3. The results obtained in the colon mucosa were: HLA-A/G6PDH= 16,4±17; HLA-B/G6PDH= 18,6±17,7; HLA-C/G6PDH= 11,2±10,6. In the case of bladder mucosa: HLA-A/G6PDH= 10,1±4,9; HLA-B/G6PDH= 11,3±9,9; HLA-C/G6PDH=6,1±9,7. The results obtained in stomach mucosa were as follows: HLA-A/G6PDH= 1,0±0,5; HLA-B/G6PDH= 2,0±1,6; HLA-C/G6PDH=2,2±1,7. Finally, the results obtained for the normal kidney samples were: HLA-A/G6PDH=2,1±1,2; HLA-B/G6PDH= 2,5±2; HLA-C/G6PDH= 7±7,1.

Larynx, colon and bladder mucosa showed a similar gene expression pattern where transcription levels of HLA-A and HLA-B locus are higher than those of HLA-C locus. This pattern was different in kidney tissue, where HLA-C transcription levels were higher than that of HLA-A and HLA-B locis. Additionally, we found a down regulation of HLA-ABC heavy chain gene transcription in stomach tissue. The biological significance of the observed differences are yet unknown, although it is known that changes in HLA-C locus expression may be involved in the regulation of NK cell function in these tissues.

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ANALYSIS OF TRANSCRIPTIONAL LEVELS OF HLA-B LOCUS IN MELANOMA CELL LINES WITH HLA-B CELL SURFACE DOWNREGULATION

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Seven ESTDAB melanoma cell lines with low surface HLA-B expression measured by flow cytometry were analyzed for transcriptional levels of HLA class I locus B. Real Time PCR in a Roche Light Cycler analyzer was performed and SGRB-green and locus specific primers were used. Specific locus B transcriptional levels ware also studied in 30 PBLs samples which served as controls. All results were referred to G6PDH transcriptional levels, measured with a Roche reference housekeeping gene kit and master plus hybridization probes kit. Melting curve analysis and agarose gel electrophoresis were done to check specific amplification.

All studied cell lines showed specific HLA-B amplification. Mean Locus B specific transcription in PBLs was 29,75 copies of locus B mRNA/copies G6PDH (range 9.82-109). For tumor cell lines the mean transcription was 5,11 copies of locus B mRNA/copies G6PDH (range 0.50-11.43). The results for each cell lines are as follows: FM28, 0.50 locus B/GPDH; E120, 0.56 locus B/GPDH; E135, 2.14 locus B/GPDH; FM3, 6.93 locus B/GPDH; E100, 3.16 locus B/GPDH; E114, 11.43 locus B/GPDH; E125, 11.04 locus B/GPDH. According to these results, FM28 and E120 showed very low locus B transcription which may explain the low surface expression of locus B. In E135, FM3 and E100 cell lines, the reduced transcriptional levels could partially explain the reduced surface expression. In the other cell lines, transcriptional levels were similar to those in PBLs; thus, in this case the low surface HLA-B expression can not be due to transcriptional disregulation.

Therefore, we conclude that low transcription of locus B may be responsible for locus B downregulation in some melanoma cell lines, while in other cells post-transcriptional mechanisms may be involved.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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BIOFUNCTIONALIZED NANOSIZED UPCONVERTING PHOSPHORS FOR BIOANALYTICAL APPLICATIONS

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Background. Photon upconversion technology is a promising alternative to conventional labels in bioanalytical assays, but previously the relatively large size of the submicrometer upconverting phosphor (UCP) particles has caused problems such as nonspecific binding. Smaller sized (<50 nm) nanophosphors (UCNPs) would improve the performance of these labels.
Methods. A synthesis route with organic oils was used to prepare strongly luminescent and highly monodisperse hexagonal $NaYF_4$:Yb^{3+},Er^{3+} particles with spherical morphology and a diameter of only 10–30 nm. Less than 10 nm thick silica layer was polymerized on their surface and streptavidin molecules were conjugated to the functionalized silica via basic conjugation chemistry. UCNPs were used in a heterogeneous binding assay and a homogeneous resonance energy transfer -based assay as donor. Several fluorophores were used as an acceptor. Upconversion luminescence was measured with a plate reader or an imager equipped with an infrared laser diode as excitation source.
Results. Nonspecific binding of the protein coated UCNPs in the heterogeneous assay was hardly detectable with the plate reader and the signal-to-background ratio reached over 3000. The sensitivity of the assay was enhanced at least four-fold by reducing the particle size from ~100 nm to sub-50 nm. In the homogeneous assay maximal signal-to-background ratios were over 70 with every acceptor and donor cross-talk did not cause significant Background.
Conclusions. By reducing the size of the UCPs, the feasibility of these labels in bioanalytical applications is greatly enhanced. Nonspecific binding decreases and increased surface-to-volume ratio enables more efficient energy transfer.

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VARIATION IN FATTY ACID COMPOSITION OF CULTURED NASAL FIBROBLAST DERIVED FROM NASAL MUCOSA AND NASAL POLYPS: ROLE OF CULTURE PASSAGES

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Background. Fibroblasts from asthmatics, especially those with aspirin sensitivity, produce little prostaglandin E_2 and have an abnormal regulation of cyclooxygenase 2. Moreover, cyclooxygenase 2 expression in cultured fibroblasts varies according to the number of culture passages. The purpose of this study is to determine and compare the fatty acid composition in culture airway fibroblasts from non-asthmatics and from aspirin-tolerant and aspirin-intolerant asthmatics.
Methods. Fatty acids composition was analyzed by gas chromatography in fibroblasts obtained from controls and asthma patients with or without aspirin intolerance.
Results. The fatty acids profile changed with the number of culture passages in all patients, regardless of the group; especially that of polyunsaturated fatty acids ω-3 and ω-6, 18- carbon species and 20-carbon species, at passage 6 (a decrease in 18:3n6, $p<0.037$, 18:3n3, $p<0.008$, 20:2n6, $p<0.024$) and passage 8 (an increase in 20:3n3, $p<0.001$, 20:3n6, $p<0.001$, 20:4n6, $p<0.034$). Differences were observed in asthmatics vs non-asthmatics, mainly at passage 8. In asthmatics, the fatty acid contents of 18:0 ($p<0.015$), 18:2n6,20:3n6, 20:4n6,and 22:1n9 ($p<0.001$) increased, while those of 18:3n6,($p<0.001$), 20:2n6 ($p<0.001$), and 24:0 ($p<0.002$) decreased. Differences in the changes in fatty acid composition were mainly detected between non-asthmatics and aspirin-tolerant patients, and also between aspirin-tolerant and intolerant patients at passage 4. At passage 8, there were differences between most of the fatty acids analyzed, especially in 20:3n3, 20:5n3, 22:0, 22:4n6 and 22:6n3 ($p<0.001$) between aspirin-tolerant and aspirin-intolerant patients.
Conclusions. Asthmatic and non-asthmatic fibroblasts show different changes in fatty acid composition at different times of culture.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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HIGH RESOLUTION CAPILLARY ELECTROPORESIS (CZE-HR) FOR SERUM PROTEINS: CLINICAL USES AND REFERENCE VALUES

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Background. High resolution capillary electrophoresis (CZE-HR) for serum proteins allows resolution of many proteins in 8 fractions and their relative quantification. We illustrate CZE-HR electrophoretic patterns in different clinical pathologies and their specific protein values with relevant laboratory data. We establish reference values for each of the 8 protein fractions of CZE-HR SPE electrophoregram.
Methods. This study was conducted on Sebia Capillarys 2 with high resolution buffer. To establish our reference values, we selected patient samples (n=95) analyzed the same day with the capillary Paragon CZE method (Beckman Coulter) and found to be normal. In addition for each sample, the following proteins, alpha-1-acid glycoprotein, alpha-1-antitrypsin, haptoglobin, alpha-2-macroglobulin, complement C3 and C4 were determined by nephelometry; transferrin by turbidimetry and albumin by BCP. All patients with CRP > 8 mg/L were excluded.
Results. Acute inflammatory state is clearly characterized by increased inflammatory proteins. Clear electrophoretic resolution of haptoglobin from alpha-2-macroglobulin allows discrimination between acute inflammatory state, nephrotic syndrome, intravascular haemolysis and in vitro haemolysis. Polyclonal hyper-IgA is clearly identified as well as transferrin increase or decrease. Reference values for CZE-HR SPE fractions were greater than those established for nephelometric/turbidimetric methods except for alpha-1-antitrypsin.
Conclusions. CZE-HR SPE reflects and differentiates more accurately some clinical conditions while allowing resolution and direct measurement of many specific proteins. Availability of this new high resolution technology on a daily basis improves the diagnostic information and clinical usefulness of first line laboratory services.

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EVALUATION OF COMMUN INTERFERENCES ON SERUM PROTEIN HIGH RESOLUTION CAPILLARY ELECTROPHORESIS SYSTEM (CZE-HR) CAPILLARYS 2

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Background. To assess interference of common clinical conditions (hemolysis, lipemia, complement degradation products (C3d), fibrinogen and C-reactive protein (CRP)) and exogenous product (radio-contrast agents) on analysis of specimens by an8 fractions high resolution serum protein capillary electrophoresis (CZE-HR SPE).
Methods. This study was conducted on Sebia Capillarys 2 with high resolution buffer. Samples used to measure hemolysis, lipemia and radio-contrast agents were prepared by adding increasing amounts of hemolysate, Intralipid 10% and Omnipaque™ respectively in serum of patients with normal electrophoretic profile. C3d was evaluated on samples stored at 4°C or at room temperature for 0, 24h, 48h, 72h and 96h. Fibrinogen interference was observed by comparing paired plasma and serum samples. Finally, CRP interference was evaluated on serum from patients with measured CRP ranging from 7 to 567 mg/L.
Results. Hemolysis causes a decrease of haptoglobin with the emergence of a haptoglobin-hemoglobin complex on the cathodic side of the α2-macroglobulin. Free hemoglobin is observed between transferrin and complement. Intralipid and Omnipaque™ interferencesare observed between the α1-acid glycoprotein and α1-antitrypsin. C3d appeared in early gammaglobulin region after 72h at 4°C, and after only 24h at room temperature. Fibrinogen is substantially at the same position as C3d while CRP is beside C3d, on the cathodic side.
Conclusions. The CZE-HR SPE can detect and differentiate intravascular hemolysis from *in vitro* hemolysis, a1-globulins increase from lipid or radiocontrast agent interference, and paraprotein from C3d, fibrinogen and CRP . Adequate knowledge of interfering allows optimal use of this new clinical application.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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A NOVEL ALKALINE TREATMENT METHOD TO REMOVE NEGATIVE INTERFERENCE OF BILIRUBIN IN CREATININE ASSAY IN ICTERIC SERA BY JAFFE'S KINETIC METHOD

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Background. The negative interference of bilirubin in creatinine assay by Jaffe's kinetic method is well documented. The problem has not yet been solved satisfactorily. Hence, we developed a novel alkaline treatment method to process icteric sera.
Methods. Creatinine kit development: We modified completely concentrations of picric acid reagent and sodium hydroxide reagent (final pH 13.05). Alkaline treatment method of icteric sera: Mix 200uL serum to 250uL of sodium hydroxide reagent and keep it for 20 minutes at room temperature. Then mix 250ul of picric acid reagent to start assay. Creatinine assay in icteric sera after bilirubin oxidase treatment: One unit of enzyme was mixed well to 300uL serum and was kept at 37°C for 10 minutes to oxidise bilirubin to biliverdin. Creatinine values in these sera were taken as reference. Bilirubin spectra in icteric sera, after alkaline treatment, after bilirubin oxidase treatment and of bilirubin solution were recorded.
Results. Creatinine assay by our kit gave perfect linear increasing graph. Values in non-icteric sera, after alkaline treatment, were found to be 10% more. Creatinine values in icteric sera after alkaline treatment were 10.7% more (average) as compared to that after bilirubin oxidase treatment. Bilirubin spectra were also different. Bilirubin molecules were never destroyed after alkaline treatment.
Conclusions. Actual creatinine value was found by deducting 10% from experimental values. The great advantages of our method are that no separate reagent is required and its simplicity. Most likely mechanism of abolition of negative interference is due to conformational changes of bilirubin molecules.

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AUTOMATION OF SEVEN NON STANDARD ASSAYS ON KONELAB 20 XT

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Background. Most biochemistry automates are exclusive and do not allow non standard assay development, in particular for clinical chemistry.
Methods. We developed and validated seven assays on the open Konelab 20 XT instrument from ThermoFisher: 4 enzymatic assays without calibration (glucose-6-phosphate-dehydrogenase or G6PD; pyruvate-kinase or PK; aldolase A; angiotensin-converting enzyme or ACE) and 3 non-enzymatic and calibrated assays (total biliary acids or TBA, phospholipids or PL, and fructosamines or Fru), using either commercial kits (G6PD, aldolase, TBA, PL, Fru) or hand made reagents (PK, ACE). G6PD and PK activities are determined in blood essentially from newborns; results are expressed in function of erythrocyte haemoglobin content (also measured on automate) in the search of a genetic defect. Plasma aldolase A is a marker in myositis. Serum ACE is the classical marker of sarcoidosis. Serum TBA and phospholipids are useful in hepatic and metabolic disorders. Plasma Fru is used for diabetes mellitus monitoring in combination with HbA1c.
Results. All the analytic performances of these assays were verified (intra- and inter-day reproducibility, sensitivity and linearity, definition of the analytic domain), and with daily internal quality control. Quality control is run in real time with variable and multiple rules and diagram production. Moreover, all data from specimens and controls are recorded for a long time as specified for the certification ISO 15189.
Conclusions. The Konelab 20 XT is well adapted to non standard assays, also without commercial kits, in particular for developing specialized biochemical assays as necessary in a university hospital.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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SORTING OF CLOSED PRIMARY BLOOD SAMPLE TUBES BY AN AUTOMATIC SORTER MAY CAUSE HAEMOLYSIS

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Background. To increase efficiency of preanalytical operations, sorting systems for blood-sample-tubes are becoming more widespread in the area of clinical laboratory diagnostics. For certified laboratories it is essential to control preanalytical factors caused by different means of transportation and sorting systems. Therefore we analyzed the preanalytic influences of the sorter system HCTS 2000 (M.U.T. Germany), in respect to parameters of haemolysis.
Methods. To assess the degree of haemolysis, parameters were chosen with respect to the literature on the evaluation of similar systems. Parameters were measured at different sorting passages (1x, 5x and 10x; n=18) and different sample-tubes (plasma and serum).
Results. The most significant deviations after automatically sorting were measured for neuron specific enolase (NSE) after 1 cycle. Here an increase of NSE concentration up to 5.29 μg/l could be observed. 5 cycles showed an increase of NSE up to 26,58 μg/l and of potassium up to 0.29 mmol/l. Nearly identical deviations were observed after 10 cycles of sorting.
Conclusions. Sorting systems for blood sample tubes gain popularity. However, blood specimens are particularly sensitive to forces in these systems, which requires specific attention. We could show that one passage through the sorting system causes no preanalytical influences on the measured parameters, accept on the values of NSE. Higher number of cycles with 5 passages have additionally a relevant effect on potassium-levels. Increasing the number of cycles further has no additional effect. In conclusion, the sorting system should not be used when measuring haemolysis-sensitive-parameters, and more then 1 cycle should be avoided to minimize preanalytic failure.

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VALIDATION OF THE NEW LIAISON® XL ANALYSER WITH THYROID AND TUMOUR MARKERS ASSAYS

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Background. The performance of the new LIAISON® XL Analyser was evaluated with high volume immunometric assays to assess its suitability for medium-high size laboratories. 13 assays (3 Thyroid – TSH, FT3 and FT4 - and 10 Tumour Markers – AFP, CA 15-3, CA 19-9, CA 125, CEA, NSE, PSA, fPSA, S100 and TPA-M) were tested for precision, analytical sensitivity and correlation with the LIAISON® Analyser. Throughput and ease of use of LIAISON® XL were also evaluated.
Methods and Results. A panel of samples spanning the assay range was tested in 20 determinations over the calibration time span to determine inter-assay variability and in 20 replicates within the same run to determine intra-assay variability. Inter-assay CVs ranged from 1.5 to 8.3% and intra-assay CVs from 0.7 to 5.4% for all analytes.
Analytical sensitivity was established for each assay according to CLSI EP17.
Dose correlation between the LIAISON® and the LIAISON® XL Analysers was calculated for each assay on a minimum of 160 patient samples spanning the assay range. Regression coefficients between 0.995 and 0.999 were obtained.
Throughput was assessed by running 100 samples of each of the most requested tests for thyroid (TSH and FT4) and tumour markers (PSA, fPSA, CEA). The total 200 thyroid results were obtained in 1h28'; 300 tumour markers results in 2h37'.
Conclusions. The excellent analytical performance of the immunometric assays tested, combined with the high throughput and correlation with the LIAISON® Analyser, makes the LIAISON® XL a reliable platform, well suited for medium to high volume laboratories.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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URINARY TRACT INFECTION IN CHILDREN: EVALUATION OF SYSMEX UF 1000I AS A SCREENING METHOD

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Background. Flow cytometry is increasingly used to screen urine samples for suspected urinary tract infection (UTI). The aim of our study was to evaluate diagnostic accuracy of Sysmex UF 1000i as a rapid screening method for UTI in a paediatric population using urine culture as reference method.

Methods. A total of 360 sterile samples were obtained from children up of 10 years (22.5% younger than one year). All the samples were examined by Sysmex UF1000i a flow cytometer with a specific analitical channel for bacteria counting and cultured onto CLED and CNA agar plates by means of 10 uL loop. For each sample the data of UF1000i analysis (bacteria and white blood cell counting) were compared with urine culture results considered positive as $^3 10^3$ CFU/mL using ROC curve analysis.

Results. 207 samples were negative at culture (57.5%) and 153 were positive (42.5%).Different cut off values for instrumental bacteria count were considered. At a bacteria count > 10/uL UF1000i results were: true positives 152 (42.2%), true negatives 110 (30.5%), false positives 97 (26.9%),false negatives 1 (<1% of all positive samples), thus obtaining a sensitivity of 99.3%, a specificity of 53.6, with AUC = 0.965, NPV = 99.1%, PPV = 61.3 % and an agreement with culture = 74%.

Conclusions. The results suggest that, if specific thresholds for significant bacteria counts are selected in the paediatric population, UF1000i warrants optimal diagnostic performances and provides results in a few minutes with a very low number of false negatives reducing unnecessary testing.

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PROTEIN QUANTIFICATIONIN HUMANSERUM BYFAST CHROMATOGRAPHY AND QUADRUPOL/LINEAR IONTRAP MASSSPECTROMETRY

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Background. Quantitative proteinanalysis in human body fluids by liquid chromatography/tandem mass spectrometry has become important for clinical diagnostics due to its potential of multiplexed testing. In this study we compare differentanalytical strategies for the quantification of C-reactive protein (CRP) and 3 apolipoproteinsin human serumby nanoflowand ultra high performance liquid chromatography(UHPLC) combined with hybrid quadrupole/linear iontrap mass spectrometry (QTrap).

Methods. Serum wasanalyzed by nanoHPLC and analytical UHPLC coupled to a QTRAP 5500 (AB SCIEX, Toronto). To evaluate the influence of high abundant proteinson the protein quantification, samples were optionally depleted (Albumin and IgG). Serum samples were denaturated, tryptically digested and desalted prior to LC-MS/MS analysis. Characteristic CRP and apolipoprotein proteotypic peptideswere quantified using MRM and newly patented MRM[3] approach.

Results. By nanoLC-QTRAP MS 35amol/μl of the CRP peptide standard were detected with signal-to-noise (S/N) ratio of 13.6.S/N value for the UHPLC approachwas 350 amol/L.

Although nanoLC-MS/MS analysis showed higher sensitivity for quantification of peptides, the analytical runs timewas 120 minand depletion of high abundant proteins was needed before analysis. Using UHPLC-QTRAP MSCRPand ApoA1, Apo B100 and Apo E lipoproteinscould simultaneously quantifiedin only 50 μL in an total run time of 5 min without depletion.

Conclusions. Our results show the great potential of the approach for a rapid and simultaneous quantification of proteins in human serum without depletion.

The demonstrated MRM[3] approach showed significant improvement in the specificity and sensitivity of peptide detection.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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THE VALIDATION OF SERUM TESTOSTERON LEVELS WITH LC-MS AND COMPARISION WITH OTHER IMMUNOLOGICAL ASSAYS

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Background. The low levels of testosterone are problematic in immunologıcal assays. Low precision and cross reactivity are major problems. LC-MS was reported to be more sensitive
Methods. LC-MS was main method for testosterone and compared with commercially available Methods.
Results. In LC-MS method for low,moderate and high levels of testosterone between-run and between-day CV for testosterone %6.7 and %9.4, %2.6 and %8.7, %2.8 and %0.3,respectively. Linearity was 0.0205 -16 ng/mL. Recovery was %50 for 0.01 ng/mL and %133 for 0.03testosterone,respectively. CLSI EP 9 was used for method comparision . The correlation value r was 0.9483, 0.9221, 0.9670 for LC-MS and Abbott Architect, LC-MS and Beckman DXI, LC-MS and Roche E170, respectively.
Conclusions. According to our result Roche E170 modular system has a good correlation with LC-MS when compared with other immunological assays.

1093

STABILITY STUDY OF 80 ANALYTES INCLUDING HORMONOLOGY ON HUMAN WHOLE BLOOD: EFFECT OF TEMPERATURE AND DELAY BEFORE/AFTER CENTRIFUGATION ACCORDING TO DIFFERENT COLLECTION TUBES

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Background. Accreditation of laboratories requires pre-analytical process control. We studied subsequent delays before analysis: 2, 4, 6, 24 and 72h, at 4°C and 25°C for 80 analytes including routine biochemistry (36), haematology (14), coagulation (7) and specialized assays : hormonology (23).
Methods. For each analyte, blood specimens from 10 healthy donors were used, collected in plain glass tubes, serum separator tubes (SSTII), lithium heparin, sodium fluoride, sodium citrate and EDTAK3 (Becton Dickinson). The mean from all volunteers for each analyte was obtained at each time point and each temperature (Tx). The reference value (T0) was measured 30mn after clotting. To detect a significant change, we compared the mean difference (Tx–T0) to goal limits according to international recommendations. The Reference Change Value was calculated using analytical CVa issued from intralaboratory quality control data (RCV=2.77xCVa), or the 0.5 intraindividual CV (CVb) from Ricos database (when too precise method).
Results. Biochemistry assays were all stable after 24h in whole blood, except glucose, potassium, phosphorus and magnesium, lactate and LD. Haematological and coagulation analytes were not affected until 24h except mean corpuscular volume, mean corpuscular haemoglobin and partial thromboplastin time. Hormonological analytes showed a good stability up to 72h except PTH, Insulin, C-Peptid, ACTH, Osteocalcin, C-Telopeptide.
Conclusions. Most of analytes of biochemistry, coagulation and haematology are stable in whole blood 24h and 72h for hormonology. However, some important analytes are affected by delay or temperature storage. In an accreditation context, results from this study could help other labs to manage rejection/acceptance of sample based on specific pre-analytical conditions.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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COMPARISON OF THE BD VACUTAINER® RAPID SERUM TUBE WITH A RANGE OF COMMERCIALLY AVAILABLE SERUM SEPARATOR TUBES FOR CLOTTING TIME

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Background. For many diagnostic assays, serum is the preferred supernatant however in situations where rapid results are required its use is precluded because of the required clotting time. If centrifugation is conducted prior to complete clotting it will result in fibrin formation in the serum sample and potentially lead to rejection of the sample. A study was conducted to evaluate the clotting performance of BD Vacutainer® Rapid Serum Tubes (BD RST), which contain a thrombin clot activator, in comparison with a number of other widely available serum separator tubes.

Methods. Blood from 32 apparently healthy adult donors was collected into BD RST, BD Vacutainer® SST™ II *Advance* (BD SST™ II), Greiner Serum Separation & Clot Activator Tube (Greiner) and Terumo Gel & Clot Activator Tube (Terumo). The samples were mixed and the clotting time measured using a stopwatch. The tubes were then centrifuged according to the manufacturers' instructions. Visual observations of sample quality were made (gel barrier formation; presence of fibrin ring; presence of gel globules in the serum).

Results. Mean (SD; minimum; maximum) clotting times in seconds were BD RST: 152 (46; 54; 240), BD SST™ II: 527 (155; 275; 935), Greiner: 673 (146; 275; 946) and Terumo: 780 (105; 520; 962).

Conclusions. All tubes had no gel globules, contained no fibrin and had complete gel barrier formation. The BD Vacutainer® RST tubes had a significantly shorter clotting time than the other tubes in the study, with samples fully clotted and ready for centrifugation within five minutes of collection.

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EVALUATION OF COPPER ASSAY IN SERUM ON THE ABBOTT ARCHITECT ANALYZER

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Background. Copper is an essential element for growth and normal metabolism. Atomic absorption spectrophotometry (AAS) was the method of choice for the copper assay in biological material. Since such an apparatus is not avail able to most laboratories we considered it necessary to compare the results obtained by ASS and by a colorimetric test for the determination of copper in serum.

Methods. We evaluated a direct colorimetric determination of copper on Architect analyzer. Concentration of copper was determined in 41 sample. In a pH 4,7 buffer system, copper is released from its carrier protein, ceruloplasmin, and forms with the specific complexant 3,5-DiBr-PASE 4-(3,5-dibromo-2-pyridilylaso)-N-ethyl-N-(3-sulfopropyl)aniline a stable colured complex. The colour intensity of this complex is proportional to the amount of copper in the sample.

Results. Accuracy and precision were checked and confirmed by use of control serums. Repeatability of serial sampling for colorimetric determination of copper described by coefficient of variation was CV=2,6 % for samples with copper concentration of 12,1 umol/L. . Day by day repeatability was CV=3,7% for a sample with a mean concentration of 20,1umol/L. Regression analysis comparing colorimetric test and AAS method yielded the following equation: y=1,06x – 2,3 and correlation coefficient (r) of 0,97.

Conclusions. The presented results of the analytical evaluation methods for the determination of copper on the Abbott Architect analyzer showed an acceptable accuracy and precision.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ANALYTICAL PERFORMANCE EVALUATION OF THE ROCHE COBAS 8000 ANALYZER SERIES

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Background. The Roche Cobas 8000 is a fully automated, random access analyzer series intended for large volume clinical chemistry laboratories. The analytical performance of such a system was evaluated for 22 general chemistry and three ISE assays and the methods compared with a Roche Modular P analyzer.
Methods. The intra- and inter-day imprecision and accuracy were calculated using low and high concentration serum quality control material and correlations between the methods using patient plasma samples (n ≥100).
Results. The intra- and inter-day imprecision had coefficients of variation ranging from 0.1% (potassium) to 3.5% (creatine kinase MB isoenzyme) and from 0.7% (pancreatic alpha-amylase) to 5.0% (bilirubin total), respectively, while accuracy was between 92% (bilirubin total) and 103% (alanine aminotransferase). Method comparisons showed close agreements between the cobas 8000 and the Modular P systems, with correlation coefficients ≥ 0.987 (chloride), except for sodium (0.955). No significant proportional or constant bias was found. However, for several assays the manufacturer report different cut-offs for interferences by hemolysis, lipemia and icteria for the two analytical systems.
Conclusions. The imprecision and accuracy obtained with the cobas 8000 system met the pre-established acceptance criteria for all 25 assays and methods correlated very well with the Roche Modular P analyzer. Attention must be paid to interferences by hemolysis, lipemia and icteriawhen transferring assays from the Modular P to the cobas 8000 analyzer system.

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PERFORMANCE EVALUATION OF THE NEW VITAL DIAGNOSTICS EON™ 100 CHEMISTRY SYSTEM

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Background. The Vital Eon 100 is a fully automated random access bench-top chemistry system, designed to meet the needs of the small to medium size laboratory, satellite laboratory, or to provide extra capacity to perform low volume and esoteric tests in high volume laboratories. The Eon 100 has a throughput of up to 180 tests with ISE and 150 tests per hour without. Key features include cooled on-board reagent storage, reagent and sample management through bar coding, reusable cuvettes, optimization mapping and low reagent test volumes. The Eon 100 Chemistry System was recently granted 510(k) clearance from the U.S. FDA.
Methods. The analytical performance included assessment of precision, accuracy, linearity and method comparison using approved CLSI protocols EP5, EP6 and EP9. Correlation studies were performed against existing routine Methods. A total of 20 chemistry applications were assessed including ISE analytes Sodium, Potassium and Chloride. In addition, on-board open vial stability was assessed.
Results. For imprecision studies 3 levels of serum were evaluated with within-run CV's ranging between 0.7 and 11% and Total CV's between 1.1 and 11.6%. The method comparison studies displayed slopes between 0.93 and 1.06 with R values between 0.98-1.00. Accuracy was verified using selected trueness controls with acceptable bias criteria being met for each chemistry. Open vial reagent stability ranged from 3 days to 28 days.
Conclusions. The Eon 100 Chemistry System demonstrates excellent reliability and practicability with convenient operation making it an attractive analyzer suitable for routine use in small hospitals and physician office laboratories.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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VITAMIN B6 MEASUREMENT IN WHOLE BLOOD USING LC-MS/MS

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Background. Our aim was to develop a method to measure the concentration of the biologically active form of vitamin B6 (pyridoxal-phosphate, PLP) in heparin whole blood with stable isotope dilution LC-MS/MS and compare this new procedure with an established home-made HPLC method based on derivatization of pyridoxal-phosphate.
Methods. A stable isotope (PLP-D3) was added to the samples, followed by deproteinization with 20% TCA. After centrifugation, 20 μl of the supernatant was injected into the LC-MS/MS. Reversed phase chromatography was performed on a UPLC system, using a Waters™ Symmetry C18 column, with a gradient of 0.1% formic acid in methanol. PLP was measured on a tandem MS with a mass transition of 247.8>149.8 in the positive ion mode with a collision energy of 14 eV.
Results. The chromatographic run lasted 5 minutes. The method was lineair from 0-300 nmol/l. The intra-assay CV was 2.2% and the inter-assay CV was 4.2% at 122 nmol/l. The absolute matrix-effect was 115%. The relative matrix-effect was 95%. The mean recovery was 95% [91%-105%]. The comparison of the LC-MS/MS method with our current HPLC method yielded the following equation: LC-MS/MS=1.14 [1.07-1.21] x HPLC + 6.7 [0.4-11.9] (r^2=0.94).
Conclusions. This LC-MS/MS based method is characterized by simple sample processing and a short run time. The comparison with the current HPLC method is excellent although a significant positive bias was detected. To conclude, the LC-MS/MS method is an appropriate method to determine PLP in whole blood both for clinical routine and research applications.

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CLINICAL RELEVANCE OF DONOR SPECIFIC ANTIBODIES (DSA) DETECTED BY LUMINEX TECHNOLOGY: AN EARLY SYSTEMATIC REVIEW AND META-ANALYSIS OF THE LITERATURE FOR A HOSPITAL-BASED HEALTH TECHNOLOGY ASSESSMENT PROJECT

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Background. Aim of this work is to provide a meta-analysis to evaluate the clinical relevance of donor specific antibodies (DSA) detected by Luminex technology, in order to produce quantitative clinical data for a Hospital-Based Health Technology Assessment project.
Methods. We performed a literature search using the Medline database and critically appraised all relevant articles.
The search terms used were "Luminex","transplantation", and "HLA typing". Studies referring to DSA detected by Luminex on patients undergone transplantations were considered. Articles were subsequently divided into three subgroups: acute rejection AR and 1 year (5 years or more) post transplant graft failures 1GF (5 GF). A meta-analysis was then performed for each group using the odds-ratio (random effects) method in order to compare transplantation outcomes among patients in presence or not of DSA.
Results. 10 trials were selected, collecting a total of 913 patients for the AR group, 266 patients for 1GF and 1846 patients for 5GF. Pooled comparison of study outcomes showed that the incidence of both acute rejection and post transplant graft failure increased in the case of DSA detected by Luminex. Combined odds ratio (confidence interval 95%) for AR, 1GF and 5GF groups were in fact 2.34 (0.59-9.37), 3.41 (1.47-7.88) and 1.85 (1.08-3.17), respectively.
Conclusions. Our meta-analysis confirmed the capability of Luminex technology to provide a clinical support in predicting both acute rejection and late graft failure. We cannot fully exclude the possibility of centre biases in treatments and selection patients and well-designed trials need to carry out in the future.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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EMULSION PCR: A NEW SELECTION STRATEGY IN SYSTEMATIC EVOLUTION OF LIGANDS BY EXPONENTIAL ENRICHMENT

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Background. AnaptamerisashortRNAorDNAoligonucleotidethatbindstodifferentclassesoftargetswithhighaffinityandselectivity, superior funtionally toantibody, which can be selected by systematicevolutionofligandsbyexponentialenrichment(SELEX). The amplificationofthe binding sequences to aptamers isa crucial step involved in SELEX. However, current amplified strategiesfor selectingaptamers from randomDNAlibraries remain undesirable due to their high hybridized by-products. emulsions PCR (ePCR) with thousands and thousands of cell-like compartments, which can inhibit the product–product hybridization in PCR reaction, may be a new selection strategy in SELEX.
Methods. The randomDNA library consisted of a single-stranded 90-mer oligonucleotides was constructed, ePCR were proceeded according to the described methodsand opitimized as follows: the cycle number, annealingtemperature, and DNA polymerase, primers, dNTPs, magnesium chloride and template concentration. Then evaluated the efficiency ofePCR and the normal PCR by polyacrylamide gel electrophoresis and microfluidic chip electrophoresis.
Results. Theamount ofthenormalPCR productsrapidlydeclinedwhenreached atthe peak accompanying with theby-productsincreasedconstantly, while ePCR could improve the forementioned disadvantages notably through maintaining notmorethanatemplate in eachemulsionparticle. The optimal condition of ePCRwere as follow: 25 cycle, 5×10^{-4} pMtemplate concentration, annealingtemperature at 65°C, 0.125U/μlDNApolymerase, 1 μMprimers, 0.4 mM dNTPs and 3mMMgCl$_2$.
Conclusions. EmulsionPCRcouldimprovetheefficiencyofSELEX and be expected to become a new selection strategy in SELEX.

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UTILIZATION OF LIPID ELECTROPHORESIS IMPROVED THE DIAGNOSIS IN A CASE WITH CHYLOPERICARDIUM

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Background. Chylopericardium secondary to open heart surgery is a very rare complication. The diagnosis of chylopericardium rests on the detection of chylous fluid in the pericardium, based on laboratory analysis of lipid contents, cytological findings of a lymphocytic predominance, and negative cultures. Classically, the fluid is *milky-white* with a triglyceride concentration of >500 mg/dL and a ratio of cholesterol to triglycerides <1.0. Nevertheless, the diagnosis has been established in cases with lower levels of triglycerides.
Methods. A 74-year-old man with previous myocardial infarction was accepted for semi-urgent coronary artery bypass grafting (CABG) due to unstable angina pectoris. Postoperativelythe patient developed a never-ending extensive pericardial effusion, which clearly represented a hemodynamic threat.
Results. The fluid was never *milky-white* with surprisingly low levels of triglycerides. Lipoprotein electrophoretic analysis excluded the presence of circulating chylomicrons, but contained a lipoprotein fraction with mobility similar to VLDL-remnants. Analysis of the apolipoprotein content demonstrated greater ratio between apolipoprotein E and apolipoprotein B than in plasma. The triglyceride levels in the pericardial fluid were similar to the cholesterol *levels with a cholesterol/triglyceride ratio was <1.*
Conclusions. These lipid analysis data, combined with the occurrence of negative cultures and an abundance of lymphocytes in the fluid, gave no other plausible explanation for this serious and long-standing complication than chylous involvement. As conservative therapy and a limited surgical procedure failed, the problem was finally resolved by ligation of the thoracic duct and a wider pericardial fenestration, further verifying that the patient suffered from chylous pericardial effusion.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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A NOVEL CONCEPT FOR WALK-AWAY AUTOMATED SAMPLE PREPARATION FOR PHARMACOGENOMIC STUDIES

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The analysis of genetic information continues to gain importance in all aspects of life ranging from individual ancestry research to pharmacogenomic studies in drug development. Here we introduce a novel method of sample collection specifically designed for high DNA yields from saliva samples. The novel saliva stabilization buffer supersedes cooling of samples and stabilizes genomic DNA for months at room temperature. The non-invasive saliva collection method reduces puncturing-associated infection risks and is designed to integrate with automated nucleic acid extraction procedures.
In this study the saliva samples are purified using a fully automated magnetic particle processor for variable sample volumes from 50 to 5000μl. By a unique magnetic separation tool combined with reliable pipetting technology and a newly developed incubator the instrument automates diagnostic extraction protocols for nucleic acids with full in-process control. High and reproducible yields are obtained in extraction protocols of DNA from blood and saliva. As much as 90 % of contained nucleic acid are recovered in the automated process. Here we show that blood and saliva samples lead to comparable amounts and purity of the extracted DNA, as well as to equivalent results in molecular diagnostic downstream analysis. While the extraction of DNA from bloodrequires elaborate logistics, the collection of saliva is easy and free of pain for the participants of studies.

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FAST SEPARATION THE SUBCLASSES OF SERUM HIGH-DENSITY LIPOPROTEIN BY USING A NOVEL MICROFLUIDIC CHIP ELECTROPHORETIC MOTHOD

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Background. It is well-known that high-density lipoprotein (HDL) can be separated into two major subfractions: HDL_2 and HDL_3 on the basis of density by ultracentrifugation. Our previous studies have been reported the separation of serum HDL_2 and HDL_3 by using quartz-based microchip. However, there are still some drawbacks limiting in its wide application.
Methods. We introduced a novel poly (dimethylsiloxane) (PDMS)/glass microchip electrophoretic mothod to fast separation the subclasses of serum HDL.
Results. N-Dodecyl beta-D-maltoside (DDM), sodium dodecylsulfonate (SDS) and hydroxypropylcellulose (HPC) were chose to modify both lipoprotein and the channel surface to reduce lipoprotein adsorption and improve the resolution of lipoprotein separation.Under optimal conditions, HDL_2 and HDL_3were effectively baseline separated and identified by PDMS/glass microchip with high speed and high reproducibility. Relative standard deviation (RSD) values of the migration time and peak areas of HDL_2 and HDL_3 were 2.05% and 2.71%, and 1.98% and 2.89%, respectively. Serum HDL subclasses from 38 healthy subjects and 35 CHDpatients were separated. Two peaks (HDL_2 and HDL_3) were detected in serum samples of healthy ones while HDL_2fractional peaks almost disappeared in patients' entire serum samples.The statistical data reveal that HDL_2 content, other than HDL_3,was significantly lower in CHD patients than healthy subjects ($P < 0.01$).
Conclusions. Our results suggested that PDMS-based microchip for HDL subclasses assay was a simple, highly efficient and novel method as diagnostic tool for CHD risk assessment.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ASSESSMENT OF THE NEW ACCESS® HIV COMBO ASSAY ON A FULLY AUTOMATED BECKMAN COULTER IMMUNOASSAY SYSTEM

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Background. The French Ministry of Health has decided in 2010 that only one Ag/Ab combined assay should be used for routine detection of HIV infection.We present here the evaluation of the new Access HIV combo assay (Bio-Rad) using the UniCel® DxI 800 immunoassay system (Beckman Coulter), a random access equipment associated or not with the fully automated centrifugation (Beckman Coulter Powerprocessor); the results were compared to those obtained with the Vidas® HIV DUO Ultra (bioMérieux).

Methods. 327 routine sera plus 60 HIV positive and 4 seroconversion reference sera were investigated by both assays using routine centrifugation at 3,000g; 263 sera were investigated with Access HIV combo assay after centrifugation at 2,000g on the Powerprocessor and the results compared to Vidas HIV DUO Ultra assay.

Results. There was a 100% concordance between both assays for the first series of experiments; all routine sera were negative; 4 sera of recently infected patients were found positive and 59 out of 60 HIV reference positive sera were found positive (only one positive serum from a newborn was negative with the two assays but the signals were very closed to the cut off values). Out of 263 sera, 260 were found negative and 3 were positive by both assays when investigated on UniCel DxI 800 after Powerprocessor centrifugation and on Vidas after 3,000g centrifugation.

Conclusions. The results of Access HIV combo assay on UniCel DxI 800 immunoassay system are excellent in terms of specificity and sensitivity and concordant with those of Vidas HIV DUO Ultra assay. Of particular interest is the fact that automated centrifugation using the Beckman Coulter Powerprocessor gives a very good specificity.

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SIMULTANEOUS SCREENING OF STEROLS AND FATTY ACIDS IN HUMAN SERUM BY A FAST AND SIMPLE GAS CHROMATOGRAPHY METHOD

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Background. In the last years, many new strategies for the simultaneous analyses of serum lipids have been developed, but the analytical procedures remains tedious and time consuming. We have reported the development of a rapid and sensitive method involving gas chromatography flame ionization detection (GC-FID) that has been validated for specific laboratory screening of the sterol disorders like Smith-Lemli-Opitz syndrome, sitosterolemia, Niemann-Pick type C, cerebrotendinous xanthomatosis and some metabolic fatty acids metabolism defects (Refsum syndrome, adrenoleucodystrophy etc.).

Methods and Results. Sterols and fatty acids can be detected in 50 μl human serum and quantified after a simple liquid-liquid extraction and derivatisation at room temperature to obtain the corresponding trimethylsilyl compounds. The chromatographic separation is performed in a total time of 25 min, using a GC column (100 % crossbond trifluoropropylmethyl polysiloxane, 30-m length, 0.25-mm internal diameter, 0,5μm film thickness) with excellent performance for lipid separation. The limits of detection are satisfactory for screening purposes in children for several substances; including 7-dehydrocholesterol, sitosterol, cholestanol, phytanic acid etc.; the repeatability of concentrations (percent coefficients of variation) are lower than 12% at high and low concentration levels, and accuracy, expressed as % error on the true value, is lower than 12% for all the analytes tested.

Conclusions. GC-FID can be successfully applied for the high-throughput screening of lipid disorders in children.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ROCHE CARDIAC TROPONIN T QUANTITATIVE ASSAY FAILURE DUE TO ANTIBODY INTERFERENCE

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Background. Cardiac troponin is widely used as a specific and sensitive marker of myocardial injury. Measurement of cardiac troponins is achieved by immunoassay which, despite extensive experience with this methodology, is still subject to interfering substances compromising accuracy. We report a case of assay failure, in a specimen from a 61-year-old female, using the CARDIAC Troponin T Quantitative reader (Roche Diagnostics).
Methods. Cardiac troponin T (cTnT) was measured on the Roche CARDIAC Troponin T Quantitative cardiac reader and the Roche Elecsys E170 automated troponin T platform, both methods utilising two murine monoclonal antibodies. The presence of an interfering immunoglobulin G was identified by protein A-Sepharose affinity chromatography.
Results. Repeated attempts at obtaining a cTnT measurement using the Roche CARDIAC Troponin T Quantitative cardiac reader failed, as reflected by the absence of a positive control line on test strips. A control line, however, appeared after removal of immunoglobulins from the specimen, indicating the presence of an interfering immunoglobulin G as the cause for this measurement failure. This autoantibody was not directed against murine immunoglobulin, as evidenced by assay failure in the presence of blocking murine serum, but was most likely directed against cTnT. Further investigation showed no interference by this autoantibody on the Roche Elecsys E170 automated troponin T platform.
Conclusions. To the best of our knowledge, this is the first report of interfering substance-mediated cTnT assay failure on the CARDIAC Troponin T reader platform.

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CREATININE DEIMINASE - BASED ASSAY OF CREATININE REVISITED

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Background. Enzyme-catalyzed hydrolysis of creatinine to N-methylhydantoine and ammonia and the determination of the ammonia formed in a glutamate dehydrogenase assay is the most direct method for the enzymatic determination of creatinine. So far suitable preparations of creatinine deiminase were not available.
Methods. Recombinant creatinine deiminase was produced and a kit "Crea-Direct" was designed. Its performance was tested in routine assays of creatinine with special reference to the concentration range around 1 mg/dl and to interferences.
Results. The gene encoding creatinine deiminase was cloned from *Tissierella creatininii*, genetically engineered and overexpressed in *Escherichia coli*. The enzyme was purified avoiding ammonium sulphate precipitations so that the final preparation was free of detectable ammonia. The yield was in the order of 100 kU of enzyme per 100g of cells. The stability of the enzyme was as follows: no loss of activity in 60% glycerol (v/v) at -20°C in 18 months; loss of less than 10% in 40% ethylene glykol (v/v) at 4°C in three weeks by partial dissociation of the enzyme, then a plateau was reached.
In the designed kit "Crea-Direct" the ammonia formed was determined by following NADPH disappearance in the glutamate dehydrogenase reaction. The assay is of high precision at low creatinine concentrations. Interferences were not observed, a list of the compounds tested will be presented.
Conclusions. The creatinine deiminase-based assay is ready for its introduction in routine determinations of creatinine.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1108

REAGENT ON-BOARD STABILITY STUDY ON THE NEW ECONOMIC RESPONS®910 CLINICAL ANALYZER

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Background. The DiaSys respons®910 system is a compact, economic, fully automated bench-top clinical chemistry analyzer designed for small to mid-size workloads. Key features are the simultaneous 12 wavelengths detection and the economic long term on-board stabilities of reagents on a non-refrigerated reagent rotor tray.

Methods. On-board and calibration stability tests for 11 assays have been carried out on two respons®910 systems in parallel. All reagents, calibrators and controls were from the portfolio of DiaSys Diagnostic Systems GmbH.

Three samples per assay, one within normal range, one pathological sample and one near the upper linearity limit of the assay, were measured twice a week over a total period of nine weeks. Target criterion was the recovery of each assigned target value within ±10% limits.

At daytime the reagents were stored in open vials at ambient temperature on-board the analyzer. Over night the reagent containers were covered by a plastic wafer.

Results. The reagent in-use stability under non-refrigerated conditions is shown for a panel of 11 clinical parameters. All reagents onboard the respons®910 system showed calibration and in-use stabilities comparable to modern analyzers with refrigerated reagent compartments. Examples for the notable in-use stability in weeks (w) are: CHOL (8 w), TRIG (8 w), UA TOOS (6 w), ALT (4 w), ALP (3 w), and even CREA Jaffé (3 w).

Conclusions. The in-use stability study of a representative panel of liquid-stable, ready-to-use IVD reagents on-board the economic DiaSys respons®910 demonstrated the robustness of the system and showed a performance which is fully compliant with the demands of a state-of-the-art clinical laboratory.

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OXYSTEROL PROFILING IN PLASMA AND LIPOPROTEINS BY FAST LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Backround. A number of pathophysiological effects in atherogenesis and inflammation are attributed to oxysterols which can be formed enzymatically and non-enzymatically from cholesterol. The analysis of oxysterols is hampered by their low physiological concentrations and the susceptibility to *in-vitro* autoxidation of cholesterol. The aim of our study was to develop a rapid, high-throughput method for the simultaneous quantification of enzymatically and non-enzymatically derived oxysterols in human plasma and lipoprotein fractions.

Methods. An API 4000® (AB SCIEX) with atmospheric pressure chemical ionization and multiple reaction monitoring (MRM) was applied in positive ion mode. Chromatographic separation of 7 oxysterols was performed by high resolution fast liquid chromatography using a monolithiccolumn RP-18e Chromolith (Merck, Germany) and a Kinetex C-18 UHPLC column (Phenomenex, Germany) at flow rates between 0.5 ml/min and 2.5 ml/min. Protein precipitation with sample dilution, solid-phase extraction (SPE) and liquid-liquid extraction (LLE) were compared to determine oxysterols and cholesterol in plasma and low density lipoprotein (LDL).

Results. Using the monolithic column the simultaneous analysis of cholesterol and the oxysterols 22-R-hydroxycholesterol, cholestanetriol, 7a/β-hydroxycholesterol, 7-ketocholesterol, cholesterol β-epoxide, cholesterol α-epoxide, 4-b-hydroxycholesterol in 5 min at a flow-rate of 1 ml/min was possible. The limit of detection (LOD) was 0.5 ng/mL. The linear range of the method was 3-1000 ng/mL. After hydrolysis simple protein precipitation and dilution of plasma and LDL showed recovery rates ranged between 75-95% compared to SPE.

Conclusions. Fast liquid chromatography combined with tandem mass spectrometry is suited for a rapid profiling of oxysterols and cholesterol in plasma and isolated lipoproteins.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1110

PERFORMANCE EVALUATION OF THE ELECSYS® HGH ASSAY, A NEW REAGENT FOR THE QUANTIFICATION OF HUMAN GROWTH HORMONE

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Background. Measurement of human growth hormone (hGH) in the context of stimulation or suppression tests is a key step in the diagnosis of hGH disorders. We have evaluated the analytical performance of a new hGH reagent from Roche Diagnostics using Modular® Analytics E170 and *cobas*® *e* 411 analyzers.

Results. Within run CVs (21 replicates, 16 samples: 0.2 – 12 ng/mL) ranged from 0.6% to 1.7%. Within laboratory CVs(21 days, 2 series daily, 2 replicates per series, 11 samples: 0.2 – 14 ng/mL)were between 1.7% and 4.1 %. Ring trial: deviations of mean values (3 replicates, 3 days, n=9) from 5 control materials (1.4 – 13 ng/mL) were < 5% from the group mean of the 3 sites involved. The measuring range of the assay was 0.030 – 50.0 ng/mL. Pearson's coefficients of correlation were > 0.98 for method comparison to Access, LIAISON, and Immulite 1000 / xPI and linear regression data (Passing/Bablok) showed slopes from 0.97 to 1.05 and intercepts < 0.1 ng/mL vs. methods from Siemens (range evaluated: 0.1 – 37 ng/mL). Larger deviations were observed in other comparisons (slopes ranged from 0.95 to 1.45). Median HGH peak concentrations were 10.3, 15.7, and 21.4 ng/mLin patients without and 3.9, 4.8, and 2.1 ng/mL in patients with growth hormone growth hormone deficiency after stimulation with arginine, clonidine, and GHRH or GHRH/Arg, respectively

Conclusions. The new reagent allows precise measurement of hGH. It is well suited for routine hGH testing and results are in close agreement to well established reagents.

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DIMENSION VISTA® 1500 THROUGHPUT AND TURNAROUND TIME OPTIMIZATION USING SERVER ASSIGNMENT CAPABILITY

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Background. Modern laboratories must continuously increase productivity and quality of results at reduced cost and with short, consistent turnaround times (TAT). Integration of clinical chemistry and immunoassay analyzers is a common strategy to streamline laboratory operations by reducing space and personnel requirements, and optimizing pre-analytics.

Methods. Enterprise Analysis Corporation (EAC) has developed a specially designed protocol of 200 STAT and routine samplesto evaluate TATs on integrated analyzers and to establish a benchmarking system. We applied this protocol to the integrated Dimension Vista® 1500 system which features independent, simultaneously processing reagent servers with assay mapping according to method complexity and the laboratories' peak workload characteristics.

Results. In two EAC protocol runs employing different test mappings we found that the average analytical TAT for all panels including routine and STAT panels was 18 min (median = 14 min). Average TAT for all STAT panels was 9 min (median = 9.5 min). Mean time to result for panels including a troponin I request was 13 min (median = 12.5 min). The time to completion of the EAC protocol could be reduced from 3 h 21 min to 2 h 30 min by changing the server assignment of only seven assays.

Conclusions. The Dimension Vista® 1500 has proven the announced performance indicators for throughput and TAT which can be largely influenced by individual server assignment. EAC panels should be adapted to their clinical relevance and to the performance and capacity of contemporary analyzers.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1112

URINE PROTEIN EXCRETION (UPE): 24 HOURS OR SINGLE RANDOM SAMPLE. A DIAGNOSTIC DILEMMA?

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Background. Differences between protein to creatinine ratio in random urine and 24 hour are quoted with respect to ethnicity, sex and muscle mass. Efficacy and accuracy of UPE by both methods, at all levels of excretion and filtration ability of the kidney with UPE was evaluated.

Methods. Samples were collected from 170 patients. Urine protein by turbidometry, Creatinine by Jaffe's kinetic; Creatinine clearance and protein to creatinine ratio were calculated. UPE was compared in different classes of proteinuria (I-IV) and filtration rates (GFR= I-V, KDOQI). Bland-Altman analysis was used to compare the two methods and Pearson's correlation at different levels of GFR.

Results. Good correlation between the two methods was noted only when proteinuria is less than 300mg/day (bias= -0.2655; LA= -2.195 to 1.664); 300 -500 mg/day (bias= -0.1624; LA= -0.5663 to 0.2415). At protein excretion >500 mg/day, no consistency in measurement was noted. Comparison using the Pearson's correlation at different GFRs, indicated good correlation only above 60 ml/min GFR (r= 0.9374, p < 0.0001 at GFR >90ml/min, and r = 0.6001,
p= <0.0001 at GFR 60-89 ml/min but not <60 ml/min.

Conclusions. At UPE >500 mg/day, there is a level of disagreement (95% Limit of agreement = -6.370 to 2.711) that includes clinically important discrepancies of up to 3g/day. Protein excretion is also influenced by the low GFR. Hence random urine sample can be used only to "rule out" proteinuria and monitor efficacy. A 24 hour urine specimen is imperative for diagnosis and assessment of the disease.

1113

TECHNICAL PERFORMANCE OF AMYLASE ASSAY RECOMMENDED BY IFCC ON BECKMAN COULTER SYNCHRON SYSTEM

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Background. Commonly used Methods for testing amylase activity require standardization in clinical laboratories. In this study, we validate performance of a new amylase assay on Beckman Coulter analyzer, based on IFCC reference method.

Methods. Amylase levels were measured by IFCC method (AMY7) and saccharogenic method (AMY) on Beckman Coulter SYNCHRON System with serum, urine, and quality control (QC) samples.

Results. The precision study with the BioRad QC serum samples showed the coefficient of variations (CVs) of between-run were 1.5%, 2.1%, and 1.0% at the means of 44, 136, and 290 U/L, respectively. The precision of AMY7 at low amylase level (CV, 1.5%) is better than AMY (CV, 4.5%). The linearity of AMY7 was up to 1,200 U/L. The interference study showed no significant interference for bilirubin (up to 30 mg/dL) and lipid (up to 1,000 mg/dL), and there was about 15% increase in lower amylase level for hemoglobin at 400 mg/dL. We also compared the methods of AMY7 and AMY with patient samples. The Deming regression equations were: AMY7 = 0.809 x AMY + 5.7 with r = 0.991 (serum) and AMY7 = 0.946 x AMY + 7.6 with r = 0.967 (urine). This indicates that the serum amylase level by AMY7 is about 20% lower than AMY; however, for QC serum samples from BioRad and Beckman, the results were opposite. This needs further investigation for potential interference.

Conclusions. The new Beckman Coulter IFCC amylase assay is a standard, precise, and accurate method for amylase testing in the clinical laboratories.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1114

A GAGE R&R STUDY ON THE QUANTITATION OF AVIDIN ON LATEX BY CAPILLARY ELECTROPHORESIS

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Background. Biotinylated haptens or proteins have been coupled to avidin-immobilized latex particles to produce conjugates for immunoassays. The amount of avidin on the latex and hence the biotin-binding capacity can be determined by capillary electro-phoresis with a laser induced fluorescence detection (CE-LIF), using a fixed amount of a biotin-fluorescein label. We used a Gage R&R study to assess the variability of the CE-LIF method in measuring avidin on the latex particle.
Methods. The Gage R&R study consisted of four experiments in four days, with four lots of latex-avidin samples at three dilutions each. Each latex dilution from each particle lot was assayed daily for avidin and biotin-binding capacity by CE-LIF in duplicate on a Beckman Coulter ProteomeLab™ PA800 System. Statistical analysis was performed with Minitab˚ software.
Results. The CE-LIF method exhibited a total assay imprecision of ≤4.2% for each lot of latex-avidin over four days. One lot showed substantially higher biotin-binding capacity as compared to the other three lots (380 vs. ~257 ng biotin/mg particle). The mean binding capacity was 287ng/mg. The difference in the biotin-binding capacity among latex-avidin lots contributed 97.5% of the total measurement variation, while assay repeatability and reproducibility contributed 2.5%. The X-Bar chart revealed a clear distinction of the difference of the latex lots in biotinbinding.
Conclusions. The Gage R&R results indicated repeatability, reproducibility and reliability of the CE-LIF method for the quantitation of avidin on the latex particle.
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QUANTIFICATION OF BLOOD FOLATE FORMS USING STABLE-ISOTOPE DILUTION ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Background. Low folate intake is associated with increased risk of neural tube defects, cardiovascular diseases, cognitive dys-function, and cancer. Changes in blood folate forms might change the balance between DNA methylation and DNA synthesis.
Methods. We describe a stable-isotope dilution ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the quantification of 5-methyltetrahydrofolate (5-methylTHF), 5-formylTHF, 5,10-methenylTHF, and folic acid in whole blood hemolysates (200 μL whole blood, 1:11 dilution in 10 g/L ascorbic acid solution (pH 4.0, containing 0.2% Triton X-100, 1 h incubation at 37°C)). The method includes an Oasis MAX solid-phase extraction procedure. Folates were eluted by methanol containing 1% formic acid. The eluates were taken to dryness and resuspended in H_2O/methanol (60:40, v/v) containing 0.1% for-mic acid and 1 g/L ascorbic acid. Samples were separated using an Acquity UPLC HSS T3 column within 2.5 min with a gradient of aqueous acetic acid (pH 2.636) and methanol.
Results. The method was linear over a broad range (0.2-100 nmol/L) with r² ≥ 0.999. In whole blood, limits of detection were 0.17 nmol/L for 5-methylTHF, 0.12 nmol/L for 5-formylTHF, 0.40 nmol/L for 5,10-methenylTHF, and 0.15 nmol/L for folic acid. Intraassay CVs were between 2.3-10.0%with a mean recovery between 98.8% and 101.2% for the different folate forms.
Conclusions. Compared to earlier LC-MS/MS procedures, our UPLC-MS/MS method has better sensitivity and selectivity for the quantification of folates. The fast sample preparation and analysis allows the accomplishment of large-scale clinical studies.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1116

STANDARDIZED HIGH THROUGHPUT LC-MS/MS STEROID HORMONE QUANTIFICATION FOR CLINICAL METABOLOMICS

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Background. Mass spectrometry based clinical metabolomics needs standardization of analytical assays to improve future inter-laboratory comparability and to become a successful established technology in clinical routine laboratories such as for therapeutic drug monitoring (TDM) over the past 10 years. Steroid hormones are highly interesting endogenous metabolites in clinical metabolomics today. Standardized determination of concentrations of steroid hormones in serum may aid in improvements in the clinical environment, e.g. in vitro fertilisation, diagnosis and treatment of steroid-related diseases in children and adults.

Methods. We will present a newly developed high throughput and standardized steroid LC-MS/MS based assay intending the quantitative determination of 16 steroids in human serum samples for clinical application.

Results. The steroid metabolites are testosterone, progesterone, cortisol, estradiol (E2), DHEAS androstenedione, 17-OH Progesterone, corticosterone, 11-Deoxycortisol, estrone (E1), DHEA, 11-Deoxycorticosterone, aldosterone, cortisone, etiocholanolone, androsterone. The assay includes standardized sample preparation and LC-MS/MS analysis in 96-well plate format. The sample preparation, needed to clean up and pre-concentrate the sample, is performed by a solid phase extraction (SPE) procedure in 96-well plate format. $500 \mu L$ serum sample volume is needed. Two different elution steps are necessary (first fraction: all steroids except DHEAS, second fraction: DHEAS) for highly pure extraction fractions resulting in minimal matrix effects and improved accuracy. As the result there are two subsequent LC-MS/MS runs with a total run time of 22 minutes.

Conclusions. Validation data of the assay for human serum will be presented including inter-laboratory comparisons demonstrating the precision, accuracy, stability, and reproducibility of the newly developed standardized assay.

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QUANTIFICATION OF THE FABRY MARKER LYSOGB3 BY TANDEM MASS SPECTROMETRY

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Background. The lysosomal storage disease Morbus Fabry is a hereditary metabolic disorder with low prevalence and late clinical manifestation. It is caused by a defect in the α-galactosidase gene, leading to accumulation of the glycolipid globotriaosylceramide (Gb3). Gb3 is used for monitoring of enzyme replacement therapy (ERT), but suffers from low sensitivity. Recently, globotriaosylsphingosine (lysoGb3) was introduced as a promising new marker with significantly better sensitivity.

Methods. Protein precipitation and glycolipid extraction from EDTA plasma was performed using acetone/methanol. Samples were analysed by UPLC-MS/MS in MRM mode. Calibration was achieved by standard addition, and controls were prepared from pools. Since no internal standard (ISTD) is available, a derivative of lysoGb3 was synthesized.

Results. The literature HPLC-FLD-method is laborious, including a derivatization step. Our LC-MS method requires less sample preparation and has a short instrument cycle time <3 min. The ISTD showed almost identical extraction, elution and fragmentation behaviour as lysoGb3. Preliminary data confirmed better discrimination between Fabry patients and controls than Gb3. In a subset of 48 male and female controls and Fabry patients (receiving ERT), lysoGb3 sensitivity was 83% for lysoGb3 and only 42% for Gb3. Preliminary evaluation was acceptable (imprecision 8-15%, inaccuracy 5%, linear range 2 decades). Analytical sensitivity and low range imprecision are currently limited by ISTD purity. Successful purification was achieved (analytical scale), and experiments with the purified ISTD are in progress.

Conclusions. The new LC-MS assay for the Fabry marker lysoGb3 shows good performance, and requires less sample preparation and analysis time compared to HPLC.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1118

VALIDATION OF A FAST HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY ASSAY FOR THE SIMULTANEOUS MEASUREMENT OF CYCLOSPORIN A, TACROLIMUS AND RAPAMYCIN IN WHOLE BLOOD

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Background. Therapeutic drug monitoring of immunosuppressants such as cyclosporin A (CsA), tacrolimus and rapamycin is necessary after organ transplantation due to their narrow therapeutic range.

Methods. The immunosuppressants as well as the internal standards cyclosporin D (CsD) and ascomycin were measured using a Quattro micro tandem mass spectrometer (Micromass) operated in multiple reaction mode using the following transitions: m/z1234.0 > 1216,8 (CsD), m/z1219.9 > 1202.7 (CsA), m/z931.5 > 864.4 (rapamycin), m/z821.4 > 768.3 (tacrolimus) and m/z809.4 > 756.4 (ascomycin).

Results. The retention times were 1.55 min for CsD, 1.52 min for CsA, 1.45 min for rapamycin, 1.44 min for tacrolimus and 1.42 min for ascomycin, respectively. Cycle time was 3 min, injection to injection. The detection limit were 6.2 μg/l for CsA, 0.6 μg/l for tacrolimus and 0.8 μg/l for rapamycin and the calibration curves were linear up to 1500 μg/l for CsA, 36.0 μg/l for tacrolimus and 44.6 μg/l for rapamycin. The intraassay and the interassay imprecision of the method investigated for a low, a medium and a high analyte concentration were between 2.7% and 4.4% for CsA, 7.5% and 9.7% for tacrolimus and 11.3% and 11.1% for rapamycin, respectively. Recoveries were 101.1% ± 3.9% for CsA, 97.0% ± 12.2% for tacrolimus and 103.4% ± 12.2% for rapamycin. Samples exhibited stability for at least 4 week at 4°C.

Conclusions. We validate a mass spectrometric assay which is suitable for a fast, precise, economical and simultaneous measurement of immunosuppressants in whole blood.

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ANALYTICAL PERFORMANCE OF IMMUNOASSAYS ON COBAS® 8000 PLATFORM, DEMONSTRATED AT 4 EUROPEAN SITES

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Background. The *cobas*®8000 modular analyzer series is the newest member of the Roche *cobas*® modular platform family integrating both clinical chemistry and immunoassay testing. It is designed to meet the needs of high volume laboratories to deliver high quality results fast and efficiently from a single patient sample. We demonstrated this seamless consolidation throughout an extensive multicentre study and report here on the results generated for immunoassays.

Methods. At four study sites, different configurations of the photometric modules *cobas c* 701, *cobas c* 702, *cobas c* 502 were combined with one of the two ISE modules and with one or more cobas *e* 602 modules.

The experiments were designed to allow focus on data generated, using request patterns and samples from the daily routine. Within laboratory precision on 21 days was calculated from daily QC processed throughout the evaluation period at two sites. Test results and sampling patterns from the routine laboratory analyzers were electronically captured and the samples were then reprocessed on the *cobas*® 8000 platform at four sites. Results were evaluated using the Bablok/Passing regression procedure.

Results. 86% of CVs at all tested concentration levels were ≤ 4%. With few exceptions, method comparisons generated for 27 assays from randomly selected routine samples yielded slopes and intercepts within the ranges (slope 0.90 – 1.00, relative intercept 90 – 110%) expected for comparisons performed using standard batch testing protocols.

Conclusions. High quality immunoassay results are generated on the *cobas*® 8000 platform.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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EVALUATION OF THE THERMO SCIENTIFIC INDIKO CLINICAL CHEMISTRY ANALYZER

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Thermo Scientific Indiko is a new compact clinical chemistry analyzer, especially suitable for small laboratories or as a back-up analyzer for bigger ones. It is intended for colorimetric and turbidimetric assays as well for electrolytes employing ISE technology. Throughput of the analyzer is maximum 200 photometric tests/hour. The Indiko analyzer is a complete system including the instrument, system reagents, calibrators and controls as well the CE marked applications.
In this study we have evaluated the performance of two enzyme assays, ALT (IFCC) and GGT (IFCC) and two substrate assays, Creatinine (Enzymatic) and Glucose (HK). We have performed a precision study as well as linearity and method comparison studies for all these Methods. The method comparison study was performed using Thermo Scientific Konelab clinical chemistry analyzer as a comparison system.
Precision study was made with three different analyte concentrations.
Observed CV% for repeatability (21 days, n= 84) was 0.6% – 0.8% for ALT, 0.6% – 0.9% for GGT, 0.5% – 1.5% for Creatinine and 0.6% – 1.0% for Glucose. Observed CV% for total imprecision (21 days, n=84) was 1.4% – 1.8% for ALT, 1.1% – 1.6% for GGT, 1.7% – 2.7% for Creatinine and 1.5% – 1.8% for Glucose.
In the comparison studies the observed results showed excellent correlation for all the tested analytes between the evaluated and the existing routine analyzer. The results in total demonstrate that the Indiko is a precise and reliable analyzer for routine biochemistry tests.

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EVALUATION OF ROCHE C-REACTIVE PROTEIN (CRPL3) GEN.3 ON ROCHE COBAS PLATFORM

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Background. Our objective was to validate the analytical performance of the Roche C-Reactive Protein (CRPL3) Gen.3 reagent before replacing the existing Roche C-Reactive Protein (CRPLX) Latex reagent used in our laboratory. According to the manufacturer, CRPL3 is a modification of CRPLX that provides a broader analytical measuring range (0.3 – 350 mg/L as opposed to 1.0 – 250 mg/L).
Methods. The analytical performance of CRPL3 was assessed for imprecision, linearity, analytical sensitivity, carry-over and method comparison. Imprecision was studied by measuring two levels of QC material (Roche CRP T Control N and Precipath Protein) over five days in accordance to CLSI EP5-A2 guidelines. Linearity was determined by measuring five serial dilutions of a patient sample at concentrations that span the assay measuring range in triplicates.Limit of blank and limit of detection were determined using the CLSI EP17 protocol. Carryover was evaluated by analysing two samples with low and high concentrations in the following sequence: $L_1 L_2 L_3 H_1 H_2 L_4 L_5$. Method comparison was done against CRPLX reagent using 91 serum samples.
Results. The total within-laboratory CV were 2.9% at 3 mg/L and 3.1% at 51 mg/L. Linearity was verified with recoveries of 84-100%. Limit of detection was determined as 0.3 mg/L and no significant carry-over was observed. Method comparison yielded a Passing/Bablok equation of y = 0.98x − 0.31.
Conclusions. The CRPL3 reagent showed satisfactory performance in analytical studies. Besides its routine function to detect inflammation,this reagent is also suitable for risk assessment of cardiovascular diseasebecause of improved sensitivity.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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FIRST SYSTEM FOR FULLY AUTOMATIC PRE-ANALYTICAL PROCESSING OF FECAL SAMPLES

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Background. Discovery of important diagnostic markers in feces such as calprotectin for IBD has lead to increased number of fecal samples requiring more efficient system for sample processing.

We have invented a fully automatic system for pre-analytical processing of stool samples, which increases extraction efficiency, improves quality and reproducibility of processed samples and improves the working environment.

Results. The system *integrates several modules necessary for efficient sample processing:*

- Decapping
- Pipetting- and Pick & place arm
- Barcode reader
- Balance
- Ultrasonic station
- Centrifuge

The system performs: registration and weighing, addition of appropriate buffer volume, homogenization, centrifugation and transferring of the supernatant to a secondary tube(s) for subsequent analysis or storage.

The instrument has been verified at the Karolinska University Laboratory and shows good correlation when compared with manual extraction procedure. Moreover, the system offers high quality of processed samples and reproducible results with no carry-over.

Conclusions. The new automatic system offers following improvements compared to the manual Methods.

- Sonification of samples results in faster extraction process and improved extraction efficiency.
- Higher and more consistent quality of the extracted samples.
- Increases the throughput capacity and the sample traceability and reduces the need of personnel resources.
- Eliminates manual handling of the samples resulting in a morehygienic and safer working environment.
- The automatic processing system is cost-effective already when performing 5000 – 6000 tests/year.

Furthermore, this system will create possibilities to detect new diagnostic biomarkers in stool samples e.g. various cancer- and inflammatory markers.

1123

PROTEOMICS: A POWERFUL TOOL TO DEEPEN THE MOLECULAR MECHANISMS OF ISCHEMIC STROKE

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Background. Ischemic stroke (IS) is characterized by the sudden loss of blood supply to an area of the brain originated by an occlusion of a cerebral vessel. In industrialized countries,strokeconstitutes the main cause of death and long-term disability in adults. Its consequences impose a burden on patients and society in terms of cost of care and lost of productivity. Currently, the diagnosis relies on neurological assessment of the patient and neuroimaging techniques. A clinical goal for decades has been the identification of biomarkers of impending stroke in asymptomatic subjects and prognosis in IS patients. Proteomics is a powerful tool for novel drug targets identification andnovel prognosis/diagnosis biomarkers discovering in body fluids like plasma or urine, which would be useful for early diagnosis of IS.

Methods. 5 IS patients and 5 healthy volunteers (controls) were studied. Plasma samples were depleted using a MARS Hu-14 column (Agilent Technologies). The proteomic analysis was performed by two-dimensional differential in gel electrophoresis (2D-DIGE) to identify proteins involved in the disease process.

Results. 40 spots with altered expression levels (average ratio≥1,5) were found after analysis of spot patterns by Decyder Image Analysis Software (GE Healthcare). At the moment, more than 10 differential expressed proteins have been identified by mass spectrometry.

Conclusions. Our results provide additional information to increase the knowledge of physiology and etiology of IS, and the identification of these proteins could allow us to findnovel potential biomarkers and therapeutic targets to assist in the development of rapid diagnostic tests.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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DETECTION AND CHARACTERIZATION OF MONOCLONAL PROTEINS IN SERUM: IMMUNOFIXATION ELECTROPHORESIS (IFE) OR IMMUNOSUBTRACTION ELECTROPHORESIS (ISE)

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The aim of study was to compare the immunotyping ability of suspected monoclonal protein with immunofixation electrophoresis (IFE) and immunosubtraction electrophoresis (ISE). It is important to use a sensitive method to detect monoclonal protein (M-protein) because asymptomatic patientswith detected M-protein develop multiple myeloma or similar myeloproliferative disorder at the rate of about 1-2% a year.

Electrophoresis of serum proteins was performed in all samples by capillary zone electrophoresis (CZE) method using Capillarys 2 Sebia system. Concentration of total proteins was measured on Olympus AU 2700and concentration of immunoglobulin G, immunoglobulin A and immunoglobulin M on Roche Cobas 6000 by turbidimetric procedure. IFE was performed on the Sebia Hydrasys using Hydragel 2 IF gels. ISE was performed on Capillarys 2. IFE is based on detecting appearance of a monoclonal band after agarose gel electrophoresis and immunoprecipitation. The principle of ISE involves comparison of electrophoretic reference pattern with specific electrophoretic patterns obtained after immunoadsorption with specific antibody bound to Sepharose beads. Our study included 40 patients with suspected M-protein on CZE. The results obtained by both methods were equal in 38 patients: 34 M-proteins, 2 biclonal gammopaties, 1 hypogammaglobulinemia and 1 polyclonal hypergammaglobulinemia. For the remaining two samples, immunosubtraction results were unclear so they were confirmed by immunofixation electrophoresis to be normal and polyclonal fraction of IgG, respectively.

The immunosubtraction electrophoresis is a good alternative for M-protein immunotyping. The method is fully automated but it seems that well trained laboratory professionals are necessary for result interpretation.

1125

AN EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE AU5800® CLINICAL CHEMISTRY SYSTEM USING A PANEL OF AU REAGENTS*

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Background. The Beckman Coulter AU5800 Clinical Chemistry System is designed for ultra high-throughput laboratories. A single-photometric unit of the AU5800 has a throughput of 2000 tests/hour with four connected units completing 8000 tests/hour. The reactant volume of the AU5800 is 90μL to 287μL with sample volumes as low as 1μL.

Methods. 26 currently available AU reagents were evaluated as part of this study. These reagents were used to evaluate precision, linearity, and method comparison.

Results. Precision studies were carried out over a period of 20 days using a protocol based on the CLSI guideline EP5-A2. Typical results were: Calcium Arsenazo 0.5% CV and 0.6% CV for the low and high concentration pools, respectively; and AST 0.8% CV and 1.9% CV for the low and high concentration pools, respectively. Method Comparison studies were carried out using a minimum of 100 serum samples spanning the dynamic range based on CLSI guideline EP9-A2. Results were calculated using Deming regression analysis. The following data were generated from the method comparison carried out versus an AU2700 for AST: number of samples (N) = 127; Slope = 1.021; Intercept = -1.157U/L; R = 0.9997; standard error estimate (Sy,x) = 4.973, and for Calcium Arsenazo N = 142; Slope = 0.996, Intercept = -0.0143mmol/L; R = 0.9995; Sy,x = 0.0238.

Conclusions. The evaluation of the analytical performance of the AU5800 demonstrated substantial equivalence to its predecessor.

*The AU5800 is currently only available in limited geographies. Not currently available in the United States or Canada.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1126

PLASMA HOMOCYSTEINE DETERMINATION:COMPARISON OF ABBOTT AUTOMATED IMMUNOASSAY AND THE DIAZYME ENZYMATIC METHOD

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Background. Elevated levels of homocysteine are associated with many diseases. Most commonly, measurement of homocysteine concentration is used as an independent risk factor for cardiovascular diseases, but many neurological disorders, as Alzheimer`s disease and Parkinson`s, problem with the osteoporosis, Dawn syndrome, eclampsia and many others are involved. Because of that, many methods for homocysteine determination are not surprising. In this work we evaluated results of homocysteine determination by two Methods. immunoassay and enzymatic.

Methods. Homocysteine was determined in plasma EDTA samples, with fluorescence polarization immunoassay technique on an Abbott Homocysteine IMx Analyzer (Abbott Diagnostics, Abbott Park,) and with Diazyme enzymatic method (Diazyme Laboratories, San Diego) on Olympus AU400. Imprecision, recovery test and method comparison were evaluated.

Results. Imprecision was determined by using commercially quality control material. Three concentration levels for FPIA method (6,27; 11,1; 22,7 µmol/l) and two for enzymatic method (7,38; 11,32 µmol/l) were used.Coefficients of variation for FPIA method ranged from 3,41 % to 5,52 % and for enzymatic method were 3,14% for low and 2,46% for medium concentration. Recovery test ranged from o,9988 to 1.0249 for FPIA method and from 0,9595 to 1.0456 for enzymatic method. Correlation coefficient wereR^2= 0,970(y=0,856 x+0,733).

Conclusions. Homocysteine level was determined using the FPIA method on IMx Analyzer and Diazyme enzymatic method. Both methods showed acceptable efficacy, good precision and accuracy, and revealed good correlation.

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HIGHLY SENSITIVE DETECTION OF BACTERIA USING NOVEL "EUKARYOTE-MADE"*TAQ* POLYMERASE

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Background. Concerning the detection of a broad range of bacteria by PCR, the main problem is the achievement of a high sensitivity along with a clear negative control. Over the years, this has been an unsolved problem because commercial *Taq* polymerases are contaminated with trace amounts of bacterial DNA as a result of its incomplete purification. Therefore, reliable and efficient decontamination methods need to be developed.

Methods. To achieve the production of *Taq* polymerase free from bacterial DNA contamination, we newly developed "eukaryote-made" *Taq* polymerase, which means the recombinant *Taq* polymerase manufactured using eukaryotic host cells. As eukaryotic cells, we utilized yeast (*Saccharomyces cerevisiae*) and plant (Tobacco-cultured BY-2 cells).

Results. We checked the nonexistence of contaminating bacterial DNA in "yeast-made" and "plant-made" *Taq* polymerase using bacterial universal primers. No contaminating bacterial DNA was detected even after 100 cycles of PCR amplification, which indicates a highly sensitive detection of bacteria without any false-positive results becomes feasible using "eukaryote-made" *Taq* polymerase.

As a practical example, we performed highly sensitive PCR assay (60 cycles) for bacterial contamination of household water using bacterial universal primers. Unlike conventional "bacteria-made" *Taq* polymerase, "eukaryote-made" *Taq* polymerase does not generate false-positive bands at all, so very low abundance bacteria could be detected.

Conclusions. We demonstrated that the "eukaryote-made" *Taq* polymerase solves the problem of contaminating bacterial DNA in conventional "bacteria-made" *Taq* polymerase. Using "eukaryote-made" *Taq* polymerase, highly sensitive detection of bacteria becomes feasible in practice for large fields. This achievement would enable the development of a wide range of powerful applications.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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QUANTITATIVE MULTIANALYTE IMMUNOASSAY USING UPCONVERTING PHOSPHORS AS LABELS

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Background. Multianalyte assays enable development of rapid and cost-effective tests. Material fluorescence background and sample autofluorescence conventionally limiting assay sensitivities can be avoided by using upconverting phosphors (UCPs) as labels. Feasibility of UCPs to multianalyte immunoassay in an array-in-well platform was studied.
Methods. Prostate specific antigen (PSA), cystatin C (CysC), and thyroid stimulating hormone (TSH) were used as model analytes and anti-mouse antibody as a control. Antibody spots for each analyte were contact printed to a 96-format microtiter well creating a four-spot array. Different analyte concentrations (PSA 0–200 ng/mL, CysC 0–100 ng/mL, TSH 0–500 ng/mL) in spiked buffer were bound to the spots and detected with biotinylated antibodies in combination with streptavidin-coated UCPs. The array was imaged with a CCD-based anti-Stokes photoluminescence imager and analyzed with ImageJ software. The array wells were also scanned with an anti-Stokes photoluminescence plate reader using 7x7 raster.
Results. Quantitative results for each analyte were obtained. The analytical sensitivities in buffer were in clinically relevant range-being 0.1 ng/mL for PSA, 0.2 ng/mL for CysC and 0.05ng/mL for TSH. The linear range of the assay was approximately 2 orders of magnitude.
Conclusions. Quantitative multianalyte immunoassay was demonstrated by using UCPs in protein array platform. The array-in-well format enabled the use of commonly available laboratory instrumentation and both the imager and the plate reader were feasible for the measurement of the array. Non-specific binding of the label was the limiting factor of the assay sensitivity. The array has potential for higher multiplexing.

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PERFORMANCE EVALUATION OF THE HEMATOLOGY ANALYZER SYSMEX XE-5000 FOR WHITE BLOOD CELL ANALYSIS IN CEREBROSPINAL FLUID

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Background. The improved body fluid mode of the hematology analyzer Sysmex XE-5000 has been announced to be particularly suitable to enumerate and differentiate white blood cells (WBC) in cerebrospinal fluid (CSF).
Methods. 425 routinely collected consecutive CSF samples were included in the study. For comparison of total WBC counts the results of routine chamber counts were grouped into 0-5 (n=330), >5-10 (n=36), >10-50 (n=39), >50-200 (n=15), and >200 (n=5) WBC/μl. Microscopic differential counts were performed from cytospins in 276 samples. According to the content of polymorphonuclear cells (PMN) results were grouped into 0-25% (n=263), >25-50% (n=7), >50-75% (n=3), and >75-100% (n=3) of WBC. Corresponding results of the XE-5000 were matched to these particular count categories.
Results. Total WBC counts: the proportions of samples correctly classified by the XE-5000 were 88%, 47%, 72%, 93%, and 100%, respectively. After combining the two lowest count categories to one range of 0-10 WBC/μl matches increased to 95%. PMN counts: From the group of 0-25% PMN 37% of samples were misclassified by the XE-5000, and more than half of automated counts even exceeded 75% PMN. Conversely, for samples with microscopic PMN counts of more than 25% there was a trend for underestimation by the XE-5000.
Conclusions. The Sysmex hematology analyzer XE-5000 yields valid total CSF cell counts and may be considered as proper alternative to the traditional chamber method. However, the XE-5000 often fails in providing correct PMN counts, and is therefore not a suitable alternative for manual differential cytologic work-up.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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PERFORMANCE AND PRACTABILITY EVALUATION OF RADIOMETER'S NEXT-GENERATION ABL 90®. A COMPACT ANALYZER

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Background. Radiometer ABL 90 Flex® blood gas analyzer is the latest analyzer added to Radiometer's blood gas portfolio, offering speed and high throughput in a compact instrument. ABL 90® is a test-select analyzer that measures 17 parameters, including blood gases, electrolytes, metabolites and co-oximetry, from just 65 μl of whole heparinised blood in 35 seconds. It was evaluated in our laboratory to assess its correlation with the current analyzers in use (ABL 705®, 735®) and to investigate its ease of use in a point-of-care testing (POCT).
Methods. All gases arriving at the laboratory were simultaneously measured on both ABL 700® for daily practice and on ABL 90® for the evaluation. Approximately 520 gases were tested, number varying according to the parameters. So, pH, pCO2, pO2, sO2 were compared on all three analyzers and haemoglobin, K^+, Na^+, Ca^{++}, lactate, only between ABL 90® and ABL 735® (co-oximetry). The statistical analysis used SPSS software.
Results. Regression equations were calculated and near the diagonal. Correlation coefficients were excellent, between 0.999 and 0.997, except for sO2 (0.93, in acceptable limits). Difference diagrams (Bland and Altman) always showed a mean of differences in the acceptability. For example, mean pH 90 vs 735 was – 0.04. For all parameters, out of limits points (> ± 2SD) were lower than 2.5 %.
Conclusions. ABL 90® performance is excellent, both for laboratory and POCT. No maintenance, cartridge operation, easy replacement and automatic quality management save time, and reduce errors. It was intuitively simple and easy to use.

1131

EVALUATION OF IMPAIRED FASTING GLUCOSE WITH COLORIMETRY IN RESOURCE POOR SETTINGS

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Background. Impaired fasting glucose (IFG) has been described as a prediabetic state. Early intervention is important in preventing diabetes and cardiovascular disease. Quantifying plasma glucose with precision and accuracy is essential in its diagnosis. A significant proportion of clinical chemistry laboratories in resource poor countries still make use of colorimeters. This study was designed to investigate the agreement between colorimetry and spectrophometry in the diagnosis of IFG.
Methods. The fasting plasma samples of 40 patients diagnosed with impaired fasting glucose attending the out-patient departments of University College Hospital, Ibadan, Nigeria on the basis of fasting plasma glucose between 100 and 126mg/dl were analysed using glucose oxidase method and read with UNICO 2100 spectrophotometer and LAB -TECH Digital Colorimeter. The tests were run in duplicates. Data obtained for the glucose values were subjected to Regression and Bland-Altman Analysis.
Results. 1. LAB -TECH Digital Colorimeter C.V 10%; UNICO 2100 spectrophotometer C.V 5.6%; 2. Regression analysis: Correlation coefficient $r^2 = 0.243$, slope = 0.653 with intercept 42.68.; 3.Bland-Altman analysis: Mean difference between methods was -4.53± 7.83 mg/dl with 95% confidence interval of -20.19-11.13 mg/dl.
Conclusions. Co-efficient of variation of the colorimetry is higher than that of the spectrophotometry assay. Also the Bland-Altman analysis 95% interval is too wide for the impaired fasting glucose range. Considering the economic and health burden of diabetes and comorbidities resource poor settings should improve on laboratory practice. This will reduce health costs and the prevalence of diabetes in the future.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ENRICHMENT OF AMYLOID-β-PEPTIDES FROM CEREBROSPINAL FLUID USING SINGLE SOLID PHASE EXTRACTION BEADS AND SUBSEQUENT MASS SPECTROMETRY ANALYSIS

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Background. Clinical diagnostics of Alzheimer's disease (AD) focus on the determination of relative ratios of amyloid-β-peptides (Aβ-peptides) Aβ1-42 and Aβ1-40 in cerebrospinal fluid (CSF). Enrichment of such low abundance peptides on solid phase extraction (SPE) beads in combination with mass spectrometry (MS) provides a promising technique to detect biomarkers in body fluids.
Methods. Miniaturisation of the SPE technique allowed capturing and subsequent analysis of the secreted Aβ-peptides from body fluids. Salt load and high abundant liquor proteins were depleted while simultaneously Aβ-peptides where enriched from cerebrospinal fluid on one single SPE bead. The SPE bead was transferred to a matrix-assisted laser desorption/ionization (MALDI) target, peptides were directly eluted with 2,5-Dihydroxyacetophenone matrix and subsequently analysed on a Bruker Ultraflex MALDI-TOF/TOF with high confidence. CSF from non-demented controls spiked with different Aβ-peptides was tested against AD patient liquor.
Results. Spiked CSF samples allowed an efficient enrichment procedure with subsequent MALDI analysis within a few minutes. Aβ-peptide concentrations comparably with literature values ($<1 \mu$M) could be detected in sample volumes less than 50 μl. Peptides from patients with high unspecific protein background (blood-brain barrier defects) could still be detected. AD patient samples were tested, peak intensities/areas were determined and compared with the clinical Results.
Conclusions. The single bead technique enabled a fast and sensitive analysis of Aβ-peptides in low sample volume even against high protein Background.

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DEVELOPMENT OF A NEW METHOD FOR DETECTION OF AMYLOID-β-PEPTIDES IN CEREBROSPINAL FLUID OF ALZHEIMER PATIENTS

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Background. Alzheimer's disease (AD) is the most common neurodegenerative disorder. One hallmark of AD is the formation of amyloid plaques by accumulation of amyloid-β-peptides (Ab-peptides). Recent studies have shown that the ratio rather than the absolute concentration of the Aβ peptides 40 and 42 in cerebrospinal fluid (CSF) of AD patients significantly differs from non-demented controls.
Methods. We established a competitive assay for antibody based detection and quantitative evaluation of the Aβ40- and Aβ42-peptides using Surface Plasmon Resonance (SPR). Synthetic Aβ-peptides immobilized on a Biacore sensor chip were detected by injection of monoclonal antibodies highly specific against Aβ40- or Aβ42-peptides.
Results. A solution competition assay was established allowing detection of synthetic Aβ-peptides with high accuracy and reproducibility. In a second step we monitored the Aβ-peptide status in CSF from patients. CSF from AD patients showed a significant lower ratio of Aβ42- to Aβ40-peptides in comparison to patients with other neurodegenerative diseases.
Conclusions. We present a novel method for SPR based detection of Ab-peptides, with potential use as a diagnostic tool for early onset AD as well as for monitoring the biochemical effects of drugs on Aβ formation. Early diagnosis is particularly important in AD since the majority of (disease modifying) drugs are most effective in an early stage of Aβ aggregation and less effective in later stages with severe plaque pathology and neurodegeneration.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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EVALUATION OF THE THERMO SCIENTIFIC INDIKO CLINICAL CHEMISTRY ANALYZER IN LIPID TESTS

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Thermo Scientific Indiko is a new bench-top clinical chemistry analyzer developed and manufactured by Thermo Fisher Scientific for colorimetric and turbidimetric tests as well for electrolyte measurements. Throughput of the analyzer is up to 200 photometric tests/hour. The Indiko analyzer is a complete system including the instrument, system reagents, calibrators and controls as well the CE marked applications.

In this study we have evaluated the performance of the lipid assays, Cholesterol, Triglycerides, and direct methods for HDL and LDL Cholesterol. We have measured both repeatability (within-run precision) and total precision. The method comparison study was performed using Thermo Scientific Konelab clinical chemistry analyzer as a comparison system and for HDL and LDL Cholesterol additionally a reference laboratory method.

Precision study was made with three different analyte concentrations.

Observed CV% for repeatability (21 days, n= 84) was 0.7% – 0.9% for Cholesterol, 1.3% – 1.8% for Triglycerides, 0.7% – 1.2% for HDL Cholesterol and 0.8% – 1.3% for LDL Cholesterol. Observed CV% for Total imprecision (21 days, n=84) was 1.1% – 1.4% for Cholesterol, 2.3% – 2.6% for Triglycerides, 1.3% – 2.4% for HDL Cholesterol and 2.8% – 4.1% for LDL Cholesterol.

In the comparison study the observed results showed excellent correlation for all the tested analytes between the evaluated and the existing routine analyzer as well in the reference method.

The results in total demonstrate that the Indiko is a precise and reliable analyzer for the routine lipid tests.

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LANTHANIDE CHELATE COMPLEMENTATION FOR HIGHLY SENSITIVE PROTEIN DETECTION

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Background. The sensitivity offluorescence-based bioanalytical applications is commonly limited by background fluorescence arising from autofluorescence and non-specific binding of the fluorescent labels. A novel lanthanide-based label technology eliminates the background problems related especially to homogeneous assays. Luminescence is produced only when two non-luminescent moieties (lanthanide ion carrier and light-harvesting antenna) are brought together via specific binding events while all other labeled components remain non-detectable. Autofluorescence is avoided with time-resolved measurement. We have now studied the applicability of this homogeneous assay format to protein detection.

Methods. Human α-thrombin standard (0–50 nM) was mixed with two different thrombin-binding aptamers (5 nM each), from which one was labeled with a carrier chelate coordinating a water molecule-quenched Eu^{3+} ion and the other with a light-harvesting antenna. Formation of the luminescent complex was assisted by six-base long complementary oligonucleotide sequences linked at the end of the aptamers. Time-resolved luminescence was measured after 5-minute incubation.

Results. The detection limit for α-thrombin was 50 pM (corresponds to 3 fmol) and the linear range extended up to 10 nM (over 2 orders of magnitude). The highest observed signal-to-background ratio was over 60, which can be even improved when the length of the complementary oligonucleotides in the two aptamers are shortened.

Conclusions. In conventional fluorescence resonance energy transfer-based assays the donor cross-talk and the reabsorption of donor emission compromise the performance. The novel lanthanide chelate complementation-based technology eliminates these limitations resulting in a highly sensitive, rapid high-throughput method for protein detection.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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ANALYSIS OF THC AND THC-COOH IN PLASMA AND URINE USING ONLINE EXTRACTION MASS SPECTROMETRY

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Background. Tetrahydrocannabinol (THC) is the most abundant component in *Cannabis Sativa* plant. After assumption, THC is rapidly metabolized to THC-COOH, conjugated with glucuronic acid and excreted in urine. LC-MS/MS is a useful tool to establish the assumption of *Cannabis* by the assessment of THC and/or THC-COOH in plasma and urine. Aim of this work was to develop a mass spectrometry method for the analysis of THC and THC-COOH in human plasma and urine performing on-line sample purification.

Methods. An online extraction TurboFlow™ system (Thermo Scientific) was used, chromatographic separation was performed with a C18 column using water and methanol as mobile phases. The detector was a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific) working in APCI +/- mode; for each compound three SRM transitions were monitored. Each run takes about 10 min per sample.

$10 \mu l$ of the samples were analyzed, plasma was injected without any sample preparation while urine have been hydrolyzed with basic treatment (NaOH) before the analysis.

Results. The use of a TurboFlow system coupled with tandem mass spectrometry allowed the specific and sensitive analysis of THC and THC-COOH in biological matrices. The calibration curves for all the analytes considered were linear over the concentration range 5-100ng/ml and the LOQ was 5ng/ml.

Conclusions. The method enables the toxicologist to assess the presence of THC and THC-COOH in plasma and urine with sensitivity and specificity. Since no sample preparation is required, as consequence, significant time is saved in the absence of SPE or liquid/liquid sample preparation.

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ANALYSIS OF COCAINE, AMPHETAMINE, MORPHINE, METHADONE AND BUPRENORPHINE IN HUMAN HAIR BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

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Background. Hair is a biological matrix widely used in toxicological analysis to establish chronic abuse of substances. To avoid possible interferences, it is necessary to quantify both parent drugs and metabolites. Aim of this work was to develop a method for the analysis of several drugs of abuse and their metabolites in human hair by using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods. Hair samples (20-50 mg) were provided from 100 drug addicted subjects, washed with solvents and cut. The samples were subjected to acid hydrolysis, spiked with internal standards, diluted 1:10 and analysed. Chromatographic separation was performed with a C18 column. The detector was a triple quadrupole TSQ Vantage mass spectrometer.

Results. Inten minute analysis, amphetamine methamphetamine, MDA, MDMA, MDEA, MBDB, cocaine, ecgonine methyl ester, benzoyl ecgnonine, cocaethylene, heroine, morphine, 6-monoacetyl morphine, codeine, methadone, EDDP, buprenorphine and norbuprenorphine can be detected. Calibration curveswere linear over the range 0.016 – 33.3 ng/mg. Limits of quantification were between 0.016 ng/mg and 0.066 ng/mg depending on the compound. These quantification ranges are consistent with concentrations expected in hair samples of addicted subjects. Matrix effect was not detected in real hair samples analysis. Specificity of the method was verified analysing 50 negative hair samples among 100 positive samples, no false positive were found among negative samples.

Conclusions. The use of LC-MS/MS method allowed the specific and sensitive analysis of various common drugs of abuse and their metabolites in hair. Since no complex sample extraction was required, significant time and cost are saved.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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THYROID SCREENING TESTS: COMPARISON AND VALIDATION OF THE REFERENCE VALUES BETWEEN METHODS

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Background. Thyroid screening tests result a significant part of the daily routine for a clinical laboratory. Their use need clear settings of reference values, in order to achieve the differential diagnosis of clinical situations, which should be reconsidered in every change of methodology.

Methods. We investigated the correlation of TSH, TT4, FT4, TT3, TG, Anti-TG and Anti-TPO in 50 patient samples between Beckman/DXi 800 and our former suppliers ABBOTT/Architect i2000 and Siemens/Immulite 2500 in order to validate the recommended reference values.

Results. There was good correlation for TSH (ARCH=1.081xDXi, r=0.995), T4 (ARCH=0.562xDXi, r=0.911), FT4 (ARCH=0.788xDXi, r=0.917), and TT3 (ARCH=0.812xDXi, r=0.920) between DXi 800 and Architect i2000. Moreover the recommended reference values were in consistency with the resulted correlation.

The comparison between DXi 800 and Immulite 2500 for TG, Anti-TG & Anti-TPO showed also acceptable correlation (TG:IMM=1.335xDXi+0.542, r=0.915, Anti-TG:IMM=0.895xDXi+93.6, r=0.917, Anti-TPO:IMM=2.120xDXi-26.6, r=0.973). However, in this case, there was a significant difference between the resulted values, especially for the Anti-TG & Anti-TPO determinations where the Dxi 800 is able to detect much lower concentrations of antibodies (0.3 vs 20 and 0.1vs 10 IU/ml).

Conclusions. Conclusively, even if there is statistically good correlation between methods, it is necessary to perform the clinical evaluation of the recommended reference values.

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COMPARISON STUDY OF TUMOR MARKERS IMMUNOASSAYS AND VALIDATION OF THE RECOMMENDED REFERENCE VALUES

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Background. The evaluation of the correlation between different analytical methods is of grate clinical importance, because their results must be comparable and lead to the same clinical assessment. The analytical performance of the assays, the correlation studies and the recommended reference values have to be reconsidered by each laboratory, in any methodology change.

Methods. To this aim, fifty patient groups were assayed for seven of the most common tumor markers, like Ca 125, Ca 15-3, Ca 19-9, CEA, FPSA, PSA and AFP in two different immunoassay analyzers: Siemens Immulite 2500 and Beckman Dxi800 were used for the first two, Abbott Architect i2000 and Beckman Dxi800 for the following five.

Results. Comparison of the results obtained for the first two antigens (Ca 125, Ca 15-3), showed high correlation between Siemens Immulite 2500 and Beckman Dxi800 (r=0.987 and r=0.955 respectively), with the values of Siemens Immulite being lower in the case of Ca 125 (slope=0.7234) but much higher for Ca 15-3 (slope=2.0086). The correlation of AFP, CEA and, PSA between Abbott Architect and Beckman Dxi800 was excellent with similar values (AFP: slope=0.9242, r=0.998, CEA: slope=1.0772, r=0.984 and PSA: slope=0.9905, r=0.994). For the last two markers (FPSA and AFP), the correlation was found acceptable (r=0.914 and r=0.881) with Abbott Architect giving slightly higher values (slope=1.2824 and slope=1.3537 respectively).

Conclusions. The comparison of the tumor markers assays showed good correlation between the immunoassay analyzers used in the study and no serious deviations in clinical assessment between them was observed.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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NEW AUTOMATED OPERATING PROCEDURE FOR THE ASSESSMENT, TRANSFER AND FINAL RESULT DESCRIPTION OF GENOTYPING ANALYSIS ELIMINATES HUMAN ERROR AND INCREASES ACCURACY

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Background. Manually assessing genotyping results and entering the data into the laboratory computer system may generate inaccurate reporting of analysis Results. To decrease the risk of mistakes our policy has been to make the manual assessment independently by two people followed by an additional check of the data by a third person. This routine is time-consuming and demands the work of at least two people. To reduce processing time and minimize the risk for inaccuracies, we have developed a system with automatic assessment, electronic transmission of results from the instruments to the laboratory system, and automatically generated results comments.
Methods. Genotyping of SNPs is carried out on a LightCycler 1.5 (Roche Applied Sciences) or 7500 Fast Real-Time PCR System (Applied Biosystems). For each run a technical assessment of the analysis is done, and automatic assessment is performed by the software affiliated with the LightCycler (Software 4.1) or the 7500 equipment (SDS software v. 1.4), respectively. The results are electronically transferred via a MultiCalc program and generate automatic results comments in our laboratory computer system (FlexLab, Tieto Enator).
Results. Since the new routine using automatic assessment, electronic result transmission and automatically generated responses has been applied, no incorrect responses have been discovered or reported. Furthermore, the processing time has decreased, and the working time is reduced as only one person is now required for the procedure.
Conclusions. We consider that the new routine has minimized the risk for inaccurate result interpretation and reporting and has thereby increased patient safety.

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SETTING-UP A LOW-COST UV/VIS/NIR MICROSCOPE-SPECTROPHOTOMETER TO SUPPORT MEDICAL DECISION MAKING AND DOCUMENTATION

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Background. A microscope-spectrophotometer combines an optical microscope and a highly sensitive spectrophotometer, as an add-on component,measuring the intensity of light versus its wavelength. The purpose of the project was the development of a low-cost UV-VIS-NIR microscope-spectrophotometer to support Medical Decision Making and Documentation.
Methods. We have assembled together a typical over 20 years old Zeiss-Jena Laboval 30-G062 optical microscope, with a modern USB-connectedHR4000CG composite-grating Ocean-Optics spectrometer, using a HC-1 variable-blazed grating, and a Toshiba TCD1304AP linear Charge-Coupled Device (CCD)-array, providing for full spectral output (200-1100 nm), and finally, with a Bresser eyepiece-insertable PC-USB digital imaging camera.
Results. The condenser-lens light, or that from an external-source, is focused onto the sample that absorbs, emits (fluorescence), or reflects some wavelengths better than others, depending upon the sample's biochemical structure, staining or tagging. Most of this light is reflected through the microscope's bending-mirror, into the oculars and/or the digital imaging camera. Part of it is collected by an optical-fiber probe, proximal to the bending-mirror, and is guided into the spectrophotometer's aperture. Thus, the spectrophotometer's aperture is virtually overlaid on the sample, which can easily be appropriately positioned, for spectra-uptake. The light is then analyzed by the optical grating and it its spectral-intensity is measured on the CCD-detector. The data processing, display and documentation is performed by, both, Original Equipment Manufacturer (OEM) and custom-made software.
Conclusions. The presented approach offers low-cost upgrading of existing microscopes. The system is being presently laboratory-assessed for NAD(P)H skin-autofluorescence, immunofluorescence and IR-absorption based subcutaneous tissue-O_2 detection.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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COMPARISON OF THE BD VACUTAINER® RAPID SERUM TUBE WITH BD HEMOGARD™ CLOSURE WITH THE BD VACUTAINER® PST™ II TUBE FOR A RANGE OF CHEMISTRY ANALYTES

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Background. While turnaround time is often a barometer of laboratory performance, sample integrity is vital to ensure accurate test results and quality patient care. The BD Vacutainer® Rapid Serum Tube with BD Hemogard™ Closure (BD RST) promotes rapid clotting and a high-quality serum sample by minimizing fibrin formation post centrifugation.

Methods. The clinical performance of the BD RST (serum) was compared with the BD Vacutainer® PST™II tube (BD PST™II) (plasma) for 29 routine chemistry analytes and 3 select analytes to determine whether the BD RST could provide a suitable alternative to the BD PST II. Blood samples were collected from 40 adult subjects and serum/plasma was tested on the Roche Integra® 800 and Olympus AU5200™/AU5400™ at initial time. Data were analyzed to determine mean biases and confidence limits for each tube type for each analyte.

Results. Clinical criteria for the bias were calculated for each analyte, that is, the maximum allowable difference in test Results. Following data review, all analyte results were determined to be within the clinical criteria and considered acceptable, except Ca, GGT, and K on the Roche platform: Analyte, Clinical Criteria, Mean bias [95% confidence limits]; Ca, ± 0.075 mmol/L, 0.092 [0.08, 0.105]; GGT, ±10%, -12.9%[-15.8%, -10.2%]; K,0.3 mmol/L, 0.314[0.245, 0.384].

Conclusions. Based on the study results, the BD RST demonstrated clinically acceptable performance when compared to the BD PST II for the chemistry analytes evaluated, except Ca, GGT, and K. These may be attributed to serum/plasma differences and/ or interactions with instrument/reagent systems.

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DETERMINATION OF TEICOPLANIN BY LC/MS/MS – A FEASIBILITY STUDY

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Background. Teicoplanin has been monitored in our laboratory by TDx FPIA in the past. As the service for TDx systems will be discontinued, an alternative method is needed.

Our aim was to establish a fast, robust and reliable method to quantify teicolanin by HTLC™/MS/MS.We developed a two dimensional LC/MS/MS method and compared it to the TDx FPIA assay.

Methods. For MS analysis different compounds of teicoplanin were detected by six double charged MRM $949\rightarrow316$ Da (A_{2_4}, A_{2_5}), $957\rightarrow316$ Da ($A_{2\_1}+NH^{4+}$), $941\rightarrow316$ Da (A_{2_1}), $942\rightarrow316$ Da (A_{2_2}, A_{2_3}), $933\rightarrow302$ Da (R_{5_3}, R_{5_4}) and $955\rightarrow330$ Da (R_{5_1}, R_{5_2}) as single ion or summed ions. Ascomycin was used as internal standard. Serum samples were separated by two dimensional HPLC after protein precipitation.

Results. A fast LC/MS/MS method was developed. However, using commercial standards and controls a problem was identified. Controls had a medium accuracy of 138 %. As well method comparison resulted in a significant bias between the TDx FPIA and the mass spectrometric method. The Passin-Bablock fit for 15 patients resulted in a proportional bias of 1.5.

Conclusions. At current the correlation of the mass spectrometric method to the TDx FPIA is dissatisfying. To approach the bias alternative commercial standards may need to be assessed in the future. Additionally, a more specific internal standard may improve the Results. Due to variable cross-reactivity of the various teicoplanin compounds in the FPIA assay, the assay may render an inadequate reference method for LCMS.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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DETERMINARION OF INDAPAMIDE IN HUMAN WHOLE BLOOD BY A VALIDATED LC-MS/MS METHOD

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Background. Development and validation of a LC-MS/MS determination of indapamide (IND) was performed with the aim to be applied for pharmacokinetic analysis and therapeutic drug monitoring.
Methods. IND and glibenclamide (GBC, internal standard) were extracted from human whole blood with tert-butylmethylether. Chromatographic separation was performed on a C18 analytical column with isocratic elution, utilizing a mobile phase consisting of methanol and ammonium acetate buffer. Positive-ion electrospray ionization and selected reaction monitoring were used to follow the predominant transitions: collision energy (CE)=14 at m/z 366□132 for IND, and CE=14 at m/z 494□369 for GBC.
Results. Selectivity was assessed with 6 different individual sources of human plasma, and confirmed with matrix effect (ME) averaging 90-95% for IND, 89-94% for GBC, and relative ME of 100-101% for IND. Accuracy and precision (within-run and between-runs) were all within 10%. Extraction recoveries averaged 63-71% (IND) and 75-79% (GBC); linearity range was 0.197÷78.88 μg/L, R^2>0.99. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 96 h at 8ºC, short-term stability at ambient temperature was proven for 12 h in the dark and for 4 h at daylight; stock solution stability and long term stability in plasma - for 99 days at -20ºC. With run time of less than 2.0 min, a throughput of over 200 samples per working day can be achieved.
Conclusions. The method was validated according to FDA requirements and allows the accurate and precise determination of IND in human whole blood.

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COMBINED DETERMINATION OF VITAMIN B1 AND B6 WITH HPLC

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Background. Determination of vitamin B1 and B6 is normally performed by two separate HPLC methods, differing in deproteinization, derivatization and mobile fase. Integration of both assays results in a considerable gain of time. An integrated method is developed by Instruchemie (Delfzijl, the Netherlands) and tested within the Medical Centre Alkmaar.
Methods. EDTA-blood is deproteinized. The supernatant is split in two aliquots followed by a (different) derivatization step for B1 and B6. Afterwards the two aliquots are combined again. The resulting sample is separated on a Polaris C18A column with an isocratic gradient. B1 and B6 are determined with fluorescence detection.
Results. Results of the combined method are compared with the separate methods that are now in use in the Medical Centre Alkmaar (Passing Bablok). CV's of different levels are determined, as is the sensitivity for both vitamines. Time gain was determined by comparing 50 patient samples with the new integrated method and the two separate Methods.
Conclusions. The correlation of vitamin B1 and B6 using the new integrated method in comparison with the separate methods is good (R>0,95 and >0,89). The bias is dependent on the calibrator used. CV's are comparable with the separate methods (intra-assay <5%). Sensitivity of the B6 is comparable with the separate method, whereas the B1 has a slightly less sensitivity without consequences for clinical practice. Time gain is considerable: 50 patient samples needed two times 3-4 hours against 2 hours with the combined method and only one HPLC run is necessary.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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A RATE ASSAY METHOD FOR DETERMINATION OF ACID PHOSPHATASE ACTIVITY

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Background. Acid phosphatase (AcP) known as diagnostic markers of prostate and bone diseases has its optimum pH in acidic region. For the determination of its enzymatic activity, 4-nitrophenyl phosphate(4-NP-P) is usually used as a substrate. Although a rate assay method is preferable for the determination of its activity, a troublesome end-point assay method is applied owing to lack of dissociation of 4-nitrophenol (4-NP) in acidic region. Here we propose a rate assay method to determine AcP activity.

Methods. Basic alpha-cyclodextrin (CD$^+$)was synthesized by the reaction of alpha-cyclodextrin with 2-chlorotriethylammonium chloride. AcP was added to the assay mixture (pH, 5.4)containing 4-NP-P and CD$^+$, and the increase in the absorbance caused by AcP at 420 nm was continuously monitored.

Results. Prior to the assay, CD$^+$was observed to lower pKa of 4-NP, which suggested that CD$^+$ promoted the dissociation of guest 4-NP at acidic pH. Correspondingly, the increase in the absorbance wascontinuously monitored in the process of time successfully.A dilution test showed a good linearity over a wide range of AcP activity from 0 to 300 U/L (r=0.999, n=44). CV's for 10-300 U/L specimens were satisfactory for clinical analysis: 1-3% (within-run, n=40), 1-4% (between-run, n=40). This method was confirmed sufficiently accurate by the recovery tests. Typical coexistents in serum did not significantly interfere with the method. This method was found easy, rapid and reliable.

Conclusions. Rate assay of AcP with 4-NP-P as a substrate was made possible by adding CD$^+$ to the assay mixture.

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NOVEL LIGAND FOR NEAR VISIBLE WAVELENGTH EXCITATION OF EUROPIUM(III) FLUORESCENCE

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Background. Lanthanide labels enable highly sensitive detection in immunoassays and other ligand binding assays. A major drawback with these labels is that they normally require xenon flash or nitrogen laser based UV-excitation. A novel ligand was studied to efficiently excite europium(III) at near visible wavelengths and utilize co-fluorescence phenomenon to enable solid-state component based detection.

Methods. Synthesized ligand (9-ethyl-3,6-bis(5',5',5'-trifluoro-4',4'-difluoro-1',3'-dioxopentyl)carbazole) was utilized in enhancement solution with europium(III). Characterization was performed by measuring time-resolved excitation and emission spectrum as well as fluorescence signal intensities. Co-fluorescence phenomenon was studied by adding gadolinium(III) or yttrium(III) to the solution together with europium(III) and observing their effect on the fluorescence intensity. Results were compared to commercial Wallac DELFIA enhancement solution (DES).

Results. Excitation spectra showed two peaks at 330 and 380 nm wavelengths. Commercial DES has one excitation peak in 340 nm. Excitation efficiency of the new ligand with 380 nm excitation was 84 % compared to 340 nm excitation. Using 365 nm excitation the novel ligand produced over two fold higher time-resolved signal with europium(III) compared to DES. Utilizing co-fluorescence the signal was further increased almost three fold. The novel ligand and DES displayed similar sensitities in low picomolar concentrations while dynamic range was over four orders of magnitude.

Conclusions. The novel chelate enables sensitive detection of europium(III) ions in solution using near visible excitation and emission intensity can be increased with co-fluorescence phenomenon. These aspects are crucial when developing UV-LED and photodiode based instrumentation.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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EVALUATION OF A NEW AUTOMATED CAPILLARYS ELECTROPHORESIS METHOD FOR THE DETECTION THE QUANTIFICATION OF THE HEMOGLOBIN PATTERNS

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Background. In this study has been evaluated a new capillarys electrophoresis (CE) method using Capillarys 2 flex piercing system (Sebia), which automatically provides the separation and the quantification of the normal hemoglobins and the detection of the major hemoglobin variants directly from whole blood sample.

Methods. 488 samples tested from routine analysis (HPLC Adams A1C HA-8160 Menarini for the quantification; Hydrasys Sebia electrophoresis on alkaline/acidic agarose gel for the identification of haemoglobin variants) were assayed on CE.
The evaluation was done as agreement in quantification of hemoglobins, as well as in identification of correct haemoglobin patterns.

Results. Passing and Bablok regression (slope, intercept confidence interval (CI) 95%):
HbA2 CE=1.00HPLC -0.1 (CI95% from 1 to 1.05, from -0.24 to -0.10), n=451, range 0.1-5.8.%);
HbF CE=1.31HPLC-0.75 (CI95% from 1.25 to 1.36, from -0.85 to -0.65); n=90, range 0.30-17.4%;
Hbvariants CE=1.10HPLC-3.24 (CI 95% from 1.08 to 1.12, from -3.92 to -1.98), n=59, range 8.4-93.80);
HbS<40% CE=1.00HPLC-0.00 (CI 95% from -2.27 to 1.28; from -0.96 to 1.07), n=27.
Bias in HbA2 quantification, statistically different and not proportional, is not clinically relevant; while HbF bias is ascribable to a lower sensitivity of CE.
Differently from routine methods,in 2 samples (double heterozigotes) CE allowed the separation and quantification of HbS, HbC and HbA2 and in 6 samples provided correct quantifications of HbA2 indicating an alpha-thalassemia pattern.

Conclusions. CE Sebia alone provides accurate haemoglobin separation and quantification, avoiding pre treatment samples and the use of two different semi-automated methods, thus saving resources and time.

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COMPARISON OF FIVE DIFFERENT PLASMA HOMOCYSTEINE ASSAYS

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Background. The increasing interest in homocysteine has been paralleled by the development of a variety of laboratory tests on semi- and fully automated instruments. Here we compared four different analytical methods with the HPLC reference method.

Methods. Homocysteine concentrations of 145 unselected patients were determined using: 1) AxSYM and 2) IMx (Abbott Diagnostics); 3) ADVIA Centaur (Siemens Healthcare Diagnostics); 4) enzymatic kit (Axis-Shield Diagnostics) on ADVIA 2400; 5) Homocysteine kit by HPLC (Bio-Rad).

Results. Homocysteine measured by different methods was respectively (median, IQR, min-max): 13.6 μmol/l (9.9-21.5, 4.6-113.0) [AxSYM], 19.6 μmol/l (10.1-29.3, 4.1-83.8) [Imx], 15.4 μmol/l (11.8-26.0, 6.5-93.0) [ADVIA Centaur], 12.8 μmol/l (9.5-19.6, 3.7-90.0) [Axis-Shield], 11.2 μmol/l (7.5-17.6, 1.8-74.6) [HPLC]. Regression slopes (Passing-Bablok regression) and bias (Bland-Altman analysis) observed by comparing test methods with the reference HPLC method were respectively: 1.23 (95%IC 1.15-1.32) and 4.0 (95% limits of agreement from -8.1 to 16.1) [AxSYM], 1.26 (95%IC 1.19-1.33) and 6.2 (95% diff. from -2.2 to 14.5) [Imx], 1.39 (95%IC 1.24-1.56) and 6.4 (95% diff. from -5.8 to 18.6) [ADVIA Centaur], 1.21 (95%IC 1.12-1.31) and 3.0 (95% diff. from -5.1 to 11.0) [Axis-Shield]. By applying a cut-off of 15 µmol/l, the prevalence of positive subjects was respectively 31.7% with HPLC, 43.4% (concordant items with HPLC 86.7%, Cohen's k=0.72) [AxSYM], 55.6% (conc. 88.9%, k=0.78) [Imx], 53.1% (conc. 77.6%, k=0.56) [ADVIA Centaur], 35.0% (conc. 93.5%, k=0.85) [Axis-Shield].

Conclusions. Among the methods investigated the Axis-Shield kit displayed a good agreement with the HPLC reference method, possibly representing an alternative for high workload laboratories.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

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FLUORESCENCE-QUENCHING-BASED CASPASE-3 ACTIVITY ASSAY USING UPCONVERTING NANOPARTICLE REPORTERS

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Background. Caspase proteases are key mediators in apoptosis and thus of great interest in pharmaceutical industry. Enzyme-activity assays are commonly employed in the screening of protease inhibitors that are potential drug candidates. The problem of conventional homogeneous assay principles relying on fluorescence resonance energy transfer (FRET) and a labeled substrate is the autofluorescence originating from biological material. This can be eliminated by using upconverting phosphors (UCPs) that emit visible light upon excitation at near-infrared.

Methods. To circumvent the inherent impossibility to fully quench the luminescence of the ~80-nm sized streptavidin coated UCPs, a biotinylated and dual-labeled caspase-3-specific substrate peptide was used. The method utilized intramolecular energy transfer to quench the upconversion-FRET-excited Alexa Fluor 680 (AF680) acceptor emission with a Black Hole Quencher (BHQ-3) in an intact peptide. The optimal UCP, substrate and enzyme concentrations were determined before applying the method for the detection of a known caspase-3 inhibitor (Z-DEVD-FMK).

Results. The optimal substrate amount was 0.3 pmol/μg of UCP with all tested UCP-concentrations. The maximum signal-to-noise ratio (46) was achieved at 40 ng enzyme per pmol substrate and the maximum signal was obtained around 4 hour incubation of the enzyme reaction, but for practical reasons the parameters were set to 6 ng enzyme/pmol substrate and 3 hours. The IC_{50} value of the final inhibitor titration was around 1 nM.

Conclusions. We have demonstrated the applicability of UCPs on this fluorescence-quenching-based homogeneous enzyme-activity assay for the detection of caspase-3 inhibitors. This method enables inexpensive instrumentation and total elimination of autofluorescence.

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CELL TYPE SPECIFICITY OF INFLAMMATORY SECRETOME PROFILES OF PRIMARY HUMAN ENDOTHELIAL AND DENDRITIC CELLS

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Background. Inflammation may be mediated by a variety of cells including immune cells, stroma cells and epithelial cells. Despite a comprehensive knowledge of many inflammatory cytokines, the identity of the main contributing cell types remains unknown. Therefore, we performed a comparative secretome profiling study of inflammatory activated primary human dendritic and endothelial cells.

Methods. Dendritic cells were generated from human peripheral blood monocytes and stimulated with lipopolysaccharide. Endothelial cells were isolated from umbilical veins and stimulated with Interleukin 1-beta. Cell supernatants were isolated and processed by in-gel-tryptic-digest for subsequent nano liquid chromatography and mass spectrometric analysis. Data was analysed with the aid of the CPL/MUW database.

Results. 452 proteins were identified in the secreted protein fraction of mature dendritic cells. 138 of those are genuinely secreted proteins, while 36 were found to be induced upon LPS treatment. 735 proteins were identified in the secreted protein fraction of the endothelial cells. 191 of them are classified as genuinely secreted, while 30 were triggered upon Interleukin-1beta treatment. Of interest, as revealed by data comparison 15 proteins, including tumor necrosis factor alpha, Interleukin-6 and Interleukin-8, were induced in both dendritic cells and endothelial cells.

Conclusions. The present data demonstrate that several known inflammatory cytokines were secreted by both cell types, whereas a substantial part of the induced proteins was characteristic for the respective cell type. Based on these data cell type-specific inflammation-associated signatures might be established, which could be used for the determination of the inflammatory status, directly out of peripheral blood.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1152

INFLAMMATORY ACTIVATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS IS ACCOMPANIED BY AN ALTERED GROWTH FACTORS SECRETION PROFILE

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Background. Inflammatory activation of cells is known to induce the secretion of cytokines and chemokines. Growth factors may support the survival of the inflammatory stimulated cells. We, therefore wondered, whether and how the secretion of growth factors is affected by inflammatory activation of primary human endothelial cells.

Methods. Endothelial cells were isolated from umbilical veins, kept untreated and, alternatively, stimulated with Interleukin 1-beta or Tumor necrosis factor alpha. Cell supernatants were isolated and processed for subsequent nano liquid chromatography and mass spectrometric analysis of tryptic peptides. Growth factor properties were assigned to secreted proteins according to the gene ontology (www.uniprot.org). Data were analysed with the aid of the CPL/MUW database.

Results. 534 (735/701) proteins including 10 (17/9) growth factors were identified in the secreted protein fraction of untreated (Interleukin 1-beta/TNF-alpha treated) endothelial cells. Data analysis after treatment with Interleukin 1-beta (TNF-alpha) revealed that 4 (1) growth factors remained unchanged whereas 4 (7) were down- and 13 (5) up-regulated. In total we found 25 up-regulated growth factors stimulated by Interleukin 1-beta whereas 15 of those were also up-regulated through TNF-alpha stimulation. Hence all proteins induced by TNF-alpha were induced as well by Interleukin 1-beta.

Conclusions. The inflammatory activation of endothelial cells by two different inflammatory cytokines resulted in a similar induction of cytokine secretion but unexpectedly a different profile of growth factor secretion. The comprehension of a relation of growth factor secretion with inflammatory activation might improve our understanding of pathophysiologic mechanisms.

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NEW BLOODADDITIVE FOR GLUCOSEAND ROUTINE CLINICAL CHEMISTRY TESTING

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Background. Measurement of glucose and routine biochemical testing are important simultaneously to perform in urgent cases. This study was focused on the development of a new effective blood additive for preserving glucose level as an antiglycolytic agent, which did not affect other routine biochemical tests.

Methods. Fasting blood samples from 15 healthy volunteers and 11 diabetic patients were used in this study. Efficiencies of preserving glucose concentrations among sodium fluoride (NaF), lithium heparin (LH), glyceraldehydes (GA), tris-bromoacetate (BA), D-mannose (MA), and LH plus various antiglycolytic agents were compared. The most effective racing mixes were selected and then their ratios were optimized. Partial clotting and hemolysis were observed in plasma samples. Concentrations of measured analytes in 15 biochemical tests were compared. The ANOVA statistical method was used for data analysis.

Results. LH plus MA, LH plus GA and LH plus BA represented longer-sustaining glucose levels for 8 hours and did not significantly differ from NaF ($p>0.05$). Slight hemolysis occurred in plasma samples obtained from a combination of LH and BA after 4 hours. The racing mix of LH, GA and MA was the most effective preparation for preserving plasma glucose at room temperature for up to 8 hours and did not interfere with 15 biochemical tests. The optimized ratios of LH, GA, and MA were approximately 3:10:1.

Conclusions. LH plus glyceraldehydes and D-mannose was the most effective agent for simultaneously measuring glucose and performing routine chemistry testing.

Technology, instrumentation and methods, metabolomics (proteomics, mass spectrometry)

1154

PERFROMANCEEVALUATIONOF ROCHE COBAS C311 ANALYZER

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Background. A comprehensive Performance evaluation done before Roche C311 was served in lab
Methods. Specimens from healthy people and clinical patients, Calibrators and quality control products of ROCHE were selected. Internal and external quality control were run to validate accuracy.The CV% of within-run and between-run were calculated to validate precision. Representative tests were selected to perform carryover. Mixed serum was runto validate common intereferents. Specimens from healthy individualswere run on to validate reference ranges. Three levels of analytes are used in validation of AMR (analytic measurement range). CRR (clinical reportable range) was established.Comparison study was performed between C311 and Vitros 250 we used before to evaluate the effect of instrument changeover to patient's Results.
Results. All results of quality controls were in acceptable ranges. Good accuracy was considered. The CV% of within-run and between-run of all parameters were under or similar to CV% provided by ROCHE lab. Carryover results show:Creatinine: 0%;Glucose: 0.02%; ALT: 0%; Na: 0.01%. High bilirubin and lipid had no effect on others blood tests. All validated reference interval provided by ROCHE Reference Manual were acceptable. AMRwe validated were within the analytical range of ROCHE. According to validated AMR and decision of lab director, CRR was established. Comparison studies between the Vitros 250 and Cobas C311 showed following test had significant difference: ALP (Decrease 11.55%), ALT (Decrease 68.76%) AMY-P(Decrease133.74%),AST(Decrease26.25.55%),CK(Increase33.68%),CKMB(Increase18.67%),LDH(Decrease206.09%),LIP(Decrease294.92%).
Conclusions. All items we evaluated are consistent with the performance characteristics of manufacturer and could be used in lab.

1155

DETERMINATION OF REDUCED AND OXIDIZED GLUTATHIONE BY ISOTOPE DILUTION LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Background. Glutathione (GSH) is the most abundant and important antioxidant in liver. We aimed to establish a method to simultaneously measure GSH and glutathione disulfide (GSSG), and to further investigate the effects of carbon monoxide (CO) on bile excretion and its underlying mechanism.
Methods. A liquid chromatography/positive electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) was used for separation and quantitation of GSH and GSSG, with Q1 to Q3 308.4/162.3 for GSH, 613.7/354.8 for GSSG, and 311.4/181.8 for the internal standard, $^{13}C_2$,^{15}N-labeled GSH. Sprague-Dawley rats were treated with dichloromethane (DCM), which was used as CO pro-drug, or corn oil as the control group. Bile samples were collected hourly and measured GSH and GSSG.
Results. The detection limit for GSH and GSSG were 5 ng/mL and 50 ng/mL, respectively. The linear range was up to 5000 ng/mL. Intra-assay imprecision (CV %) for GSH and GSSG were < 2.4% and <4.9%, respectively. The average recovery for GSH and GSSG were 105% and 114%, respectively. At the fourth hour after DCM administration, bile output increased 11.8 folds with biliary total GSH concentration increased 2.2 folds.
Conclusions. We concluded that the tandem mass spectrometry method for the measurement of GSH and GSSG is accurate and sensitive. Our data indicated that CO administration increased bile output through stimulation of biliary GSH excretion.

Toxicology

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RAPID SIMULTANEOUS NEAR PATIENT SCREENING OF DRUGS OF ABUSE IN DIFFERENT MATRICES WITH THE FULLY AUTOMATED EVIDENCE MULTISTAT ANALYSER

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Background. Evidence biochip array technology performs simultaneous detection of multiple analytes from a single sample and is applicable to a range of analysers. With this technology the assay kits for drug of abuse testing are matrix dedicated. The reported fully automated analyser Evidence Multistat allows near patient testing reducing the time to analysis and provides laboratory standard Results. The applicability of this system to the multiplex determination of amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine metabolite (benzoylecgonine), methadone, opiates and PCP is reported.
Methods. Simultaneous competitive immunoassays are employed for the detection of drugs of abuse. With this system, two individual biochips and all the required reagents are provided in a cartridge. Sample is manually added to the sample well on the cartridge, which is then inserted onto the analyser, the rest of the process isfully automated.
Results. Test results were produced within 25 minutes of sampling. For urine screening, the assay ranges for all assays are applicable for current SAMHSA recommended cut-offs. Total imprecision is typically<15% and all assays showed agreement >90% to the Evidence analyser and GC/MS. For the screening of whole blood, the limit of detection is<10% of the assay range. Inter-assay imprecision is typically<15% and all assays showed agreement typically >95% to the existing Evidence system.
Conclusions. Data indicates applicability of biochip array technology to the point of application screening of drugs of abuse in human urine and whole blood with the Evidence Multistat analyser. This enables STAT testing, reduces the time to analysis and provides rapid laboratory standard Results.

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DEVELOPMENT OF A HIGHLY SENSITIVE POLYCLONAL ANTIBODY FOR THE DETERMINATION OF LIDOCAINE IN BIOLOGICAL FLUIDS

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Background. Lidocaine was originally developed as a local anesthetic, but also possesses antiarrhythmicproperties, particularly against ventricular arrhythmics. It is widely used in the treatment of post-myocardial infarction. The toxic side effects of lidocaine, hypertension, CNS depression and convulsions, appear to be avoidable if the blood levels do not exceed 5µg/ml. We report the development of a sensitive polyclonal antibody to lidocaine for use in the development of immunoassays, fortherapeutic drug monitoring applications.
Methods. A novel hapten to lidocaine was synthetized and conjugated directly to bovine thyroglobulin (BTG) as carrier. The resulting immunogen was administered to adult sheep and a lidocaine specific polyclonal antiserum was generated.A microtitre plate was coated with Ig fraction of the antiserum produced. The coated plate was simultaneously incubated with lidocaine at 100ng/ml and the lidocaine hapten conjugated to horseradish peroxidase. The lidocaine competed with lidocaine HRP for the binding sites on the immobilized antibody. Absorbance at 450nm was measured in the absence and presence of lidocaine.
Results. 100ng/ml of lidocaine produced 95% displacement of absorbance.
Conclusions. These results indicate that the polyclonal antibody generated is highly sensitive and suitable for development of immunoassays for the determination of lidocaine in biological samples.

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DEVELOPMENT OF A HIGHLY SENSITIVE BIOCHIP ARRAY-BASED IMMUNOASSAY FOR DETECTION OF ZALEPLON IN BIOLOGICAL FLUIDS

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Background. The pyrazolopyrimidine zaleplon is a short-acting, benzodiazepine-like sedative/hypnotic used in the treatment of insomnia. Following oral administration it is rapidly adsorbed, the blood concentration peaking after approximately one hour. Zaleplon is rapidly metabolised to 5-oxozaleplon, 5-oxo-N-desethylzaleplon and 5-oxozaleplon glucuronide. Because of its rapid action and short half-life, zaleplon is increasingly being used in drug-facilited crimes (e.g. robbery, sexual assault, mugging etc.) and recreational abuse. Hence methods are required for the detection of zaleplon in biological fluids. The aim of this study was to develop a sensitive biochip immunoassay capable of detecting and determining low levels of zaleplon in biological samples.
Methods. An immunogen comprising, the zaleplon hapten conjugated to bovine thyroglobulin (BTG), was administred to adult sheep for production of polyclonal antiserum. The resulting antiserum was used in the development of the biochip assay for the detection and the quantification of zaleplon. The competitive chemiluminescent biochip assay was applied to the semi-automated analyser Evidence Investigator.
Results. The specificity of the assay, expressed as % cross-reactivity was 100% for zaleplon and the sensitivity value, expressed as IC50, was 0.27ng/ml.
Conclusions. Results indicate that this zaleplon biochip immunoassay is highly sensitive and it can be used to monitor the use or misuse of this compound.

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DEVELOPMENT OF A HIGHLY SENSITIVE, GENERIC BIOCHIP ARRAY-BASED IMMUNOASSAY FOR DETECTION OF ZOLPIDEM AND ITS MAJOR METABOLITE IN BIOLOGICAL FLUIDS

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Background. Zolpidem, an imidazopyridine is a non-benzodiazepines prescription drug used to treat insomnia.Reports dealing with "drug-facilitated crimes"(robbery, mugging, sexual assault) related to benzodiazepines and benzodiazepines-like hypnotics are tending to increase because of their short half-life, their rapid excretion and/or relatively long delay between the intake and urinary sampling. Zolpidem is metabolised to 4-[3-(2-N, N-dimethylamino-2-oxoethyl)-6-methylimidazo[1,2-a]pyridin-2-yl]benzoic acid (80%) and to a lesser extent to 3-(2-N,N-dimethylamino-2-oxoethyl)-2-(4-methylphenyl)imidazo[1,2-a]pyridin-6-yl carboxylic acid. Due to the rapid and varied inter-individual metabolism of zolpidem, the development of screening tests which detect zolpidem and its main metabolite would enable the detection of the drug beyond approximately 8-24 hours. The aim of this study was to develop asensitive biochip assay capable of detecting and determing low levels of zolpidem and its major metabolite zolpidem carboxylic acid in biological samples.
Methods. Immunogen comprising zolpidem-hapten conjugated to bovine thyroglobulin(BTG) was administered to adult sheep and target-specific polyclonal antisera for the detection for zolpidem and zolpidem carboxylic acidwas generated. The resulting antibody was used in the development of the biochip assay for the determination of zolpidem and zolpidem carboxylic acid metabolite.
Results. The specificity of the assay, expressed as %cross-reactivity was 100%(zolpidem) and 71%(metabolite). The sensitivity values, expressed as IC50, were 0.75ng/ml for zolpidem and 1.056ng/ml for the metabolite.
Conclusions. Results indicate that the developed biochip assay for the detection of zolpidem and the metabolite is a very applicable analytical tool to monitor the use or misuse of this compound with an extended detection window.

Toxicology

1160

DETECTION OF MORPHINE AND CODEINE IN HUMAN TEETH, IN COMPARISON WITH SIMULTANEOUS BLOOD, URINE AND BILE SAMPLES BY GC-MS IN CORPSES REFERRED TO ESFAHAN FORENSIC MEDICINE CENTER IN 2010

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Background. In the last 2 decades, measurement of drug concentration in matrices other than blood and urine gained increasing importance. Indeed we could identify opioids in teeth from human remains of individuals suspicious for drug overdose or chronic consumption. The aim of present study was identification of morphine and codeine in human teeth of drug overdose mortalities comparing with their blood, urine and bile samples using thin layer chromatography (TLC) and gaschromatography-massspectrometry (GC-MS).
Methods. 30 Positive cases(case group) were those who had positive history or signs of drug abuse with positive result of each of their blood, urine or bile specimens and 30 negative cases (control group) were those without the above condition. The teeth were pulverized and after extraction and derivatization, the analyte separation was achieved on a fused silica capillary column and determined in the selected ion monitoring (SIM) mode. Data were analyzed with SPSS using t-test and chi-square test.
Results. The test was positive for morphine in 60% and for codeine in 46.6% of the case group. In control group, just one case was positive for morphine that showed a significant difference between the two groups ($p<0.001$). In case group, all positive tests of teeth were also positive for urine and of course in more accordance with bile tests.
Conclusions. This method is sensitive enough for determination of morphine and codeine in tooth as a non-invasive biological matrix for both clinical and forensic purposes.

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BIOMARKERS AS A TRACER IN FOLLOW-UP OF THERAPY EFFECT ON PATIENTS WITH RHEUMATOID ARTHRIT

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Toxicology

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FALSE BENZODIAZEPINES RESULTS IN A RAPID IMMUNOCHROMATOGRAPHY TEST FOR URINARY DRUG DETECTION IN TOBACCO SMOKERS

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Background. This study aimed to evaluate the interference of tobacco smoke on immunochromatography assay for urinary benzodiazepines detection and the interest of two biological markers and smoking status parameters as predictive factors of false Results.

Methods. Our study included 256 voluntary subjects (143 passive smokersand 113 smokers). Cotinine was measured by homogenous immunoenzymatic assay and SCN- by selective electrode. Urinary drugs were detected by immunochromatographic assay. A positive result is completed by an analytical method with an immunometric assay.

Results. False positive results for benzodiazepines are significantly more frequent in smokers compared with passive smokers (90.2% *Vs* 22.4%, p< 10^{-3}).

For smokers, the number of cigarettes was significantly higher in subjects with falsely positive results for benzodiazepines compared with subjects with negative results (32 ± 11 *Vs* 20 ± 10; p= 0.04). Between these two groups, we established a significant difference for urinary cotinine (345 ± 211 *Vs* 117 ± 54 μg/μmol; p<10^{-3}) and for plasma SCN- (101.6 ± 3.4 *Vs* 98.8 ± 2.1μmol/L; p= 10^{-3}).

Urinary cotinine and consumption duration present the highest values of AUC of the ROC curves. The cut-off of 167.6μg/μmol and 10.5 years were found as predictive factors of false positive Results.

Conclusions. tobacco smoke interferes with immunochromatographic assay of urinary drug detection; therefore, all subjects must be questioned about their smoking status to avoid such false results during results interpretation.

Toxicology

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DECENTRALIZED MONITORING AND RISK ASSESSMENT OF HEPATOTOXIN PRODUCERS IN DRINKING WATER RESERVOIRS BY QPCR

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Background. Hepatotoxic cyanobacterial blooms are a serious risk to public health worldwide. Fast and reliable methods are needed to evaluate the risk associated with the blooms and to identify possible poisoning sources. We have developed a dry reagent -based, simple and rapid quantitative PCR method for the detection of *Anabaena*, *Microcystis* and *Planktothrix*, potential producers of the hepatotoxin microcystin.
Methods. Water samples from drinking water reservoirs were analyzed on automated qPCR platforms using polypropylene chips containing all PCR reagents in pre-dried form. Genus-specific target copy numbers were calculated and results compared with ELISA toxin analyses.
Results. Environmental samples showed positive correlation between microcystin synthetase B gene copy numbers and toxin concentrations. The method's analytical sensitivity was 5 target copies per reaction, and the dynamic range spanned from $5 \cdot 10^0$ to $1 \cdot 10^7$ copies per reaction.
Conclusions. The positive correlation between target gene copy numbers and toxin present in the water supported the use of quantitative PCR in drinking water monitoring. Suitability to decentralized testing was readily demonstrated. No training in molecular biology nor specialized facilities are required to perform the analysis. Complete quantitative analysis results are available 1.5 hours after sample collection, enabling rapid preventative actions to minimize the health risk of hepatotoxic cyanobacteria.

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DEVELOPMENT OF A BIOCHIP ARRAY-BASED IMMUNOASSAY FOR THE DETERMINATION OF ZOPICLONE AND ITS METABOLITES IN BIOLOGICAL FLUIDS

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Background. Zopiclone is a non-benzodiazepine hypnotic from pyrazolopyridine class and is indicated for the short-term treatment of insomnia. Zopiclone is extensively metabolised in the liver via decarboxylation, demethylation, and side chain oxidation. The major metabolites are zopiclone N-oxide and N-desmethyl zopiclone. Aproximately 50% of the dose is converted to other inactive metabolites via decarboxylation. The aim of this study was to develop a generic and highly sensitive biochip assay allowing the stereospecific detection of very low amounts of (+)-and (-) enanthiomers of zopiclone and its major metabolites in biological fluids without sample pretreatment.
Methods. Polyclonal antisera were generated from adult sheep, immunized with zopiclone-hapten conjugated to bovine thyroglobulin (BTG) as carrier. The competitive chemiluminescent biochip assay was applied to the semi-automated analyser Evidence Investigator.
Results. The assay showed a sensitivity value, expressed as IC50, of 0.72ng/ml for zopiclone. The assay specificity, expressed as % cross-reactivity, was 100% for zopiclone, 112% for N-desmethyl zopiclone and 55% for zopiclone N-oxide.
Conclusions. Data indicate suitability of the biochip assay for the sensitive determination of zopiclone and its metabolites in biological samples.

Toxicology

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DEVELOPMENT OF A HIGH SENSITIVITY BIOCHIP ARRAY FOR THE SIMULTANEOUS SCREENING OF TRICYCLIC ANTIDEPRESSANTS, BUPRENORPHINE AND DRUGS OF ABUSE

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Background. More commonly in the workplace employers are administrating drug screening of urine, blood and saliva samples for monitoring of safety, absenteeism and low productivity. Hair testing for drugs of abuse offers a non-invasive screen with the possibility of drug detection months after substance abuse. This eliminates the need for random testing, however requires a very sensitive immunoassay.
Biochip array technology(BAT) is used to perform simultaneous immunoreactions for detection of multiple analytes from a single sample. We report the initial performance evaluation of a high sensitivity biochip array enabling the simultaneous measurement of amphetamine, methamphetamine, barbiturates, benzodiazepines, methadone, opiates, PCP, cannabinoids, tricyclic antidepressants, buprenorphine, MDMA and the cocaine metabolite benzoylecgonine.
Methods. Simultaneous competitive chemiluminescent immunoassays are employed. The biochip (9mm x 9mm) represents the solid phase and the vessel where the immunoreactions take place. Sample and reagents are added to each biochip and incubated under controlled conditions. The assays were applied to the semi-automated Evidence Investigator analyser. The system incorporates the software to process, report and archive the data generated.
Results. 702 test results are produced within 165 minutes of sampling. Assay ranges for assays are applicable for current SAMH-SA recommended cut-offs for drugs of abuse in hair where quoted. Analytical sensitivity ranged from 0.004ng/ml for cannabinoids assay to 2.6ng/ml for methamphetamine assay. The imprecision is typically<15%.
Conclusions. Data from this initial performance evaluation indicates the BAT is applicable to the high sensitive screening of drugs of abuse, which is of interest for applications to different matrices (hair, blood, urine).

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APPLICATION OF AN EVIDENCE BIOCHIP ARRAY TO THE SIMULTANEOUS DETERMINATION OF TRICYCLIC ANTIDEPRESSANTS, BUPRENORPHINE, MDMA AND OTHER DRUGS OF ABUSE IN BLOOD

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Background. Simultaneous screening of tricyclic antidepressants(TCAs), buprenorphine and drugs of abuse is relevant for applications in therapeutic drug monitoring, toxicology, forensic settings. Evidence biochip array technology provides a platform for the simultaneous determination of multiple analytes from a single sample, which leads to an increase in the results output and a reduction in the consumption of sample/reagents. We report the applicability of this technology to the simultaneous determination of these compounds in blood with a matrix dedicated biochip array kit.
Methods. Competitive chemiluminescent simultaneous immunoassays are applied for the determination. The core of this technology is the biochip (9mm x 9mm), which represents the chemically activated solid phase where the ligands are immobilised and stabilised defining microarrays of test sites and also the vessel where the reactions take place. The assays were applied to the automated analyser Evidence. The system incorporates the software to process, report and archive the data generated.
Results. The TCA assay detects approximately 14 compounds in this class including desipramine, nortryptiline and trimipramine(206%, 100% and 238% respectively). The sensitivity value of the TCAs assay was 2.04ng/ml, the buprenorphine assay 0.03ng/ml and the MDMA assay 1.31ng/ml. The sensitivity values for the drug of abuse assays ranged from 0.07ng/ml(oxazepam) to 13.19ng/ml (methamphetamine). The intra-assay and total precision, expressed as %CV were<17.7% and<20% respectively for all the assays
Conclusions. Data show applicability of biochip array technology to the simultaneous determination of TCAs, buprenorphine and drugs of abuse in blood. This is of value for applications in therapeutic drug monitoring, toxicology, forensic settings**.**

Toxicology

1167

APPLICATION OF AN EVIDENCE BIOCHIP ARRAY TO THE SIMULTANEOUS DETERMINATION OF TRICYCLIC ANTIDEPRESSANTS, BUPRENORPHINE, MDMA AND OTHER DRUGS OF ABUSE IN URINE

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Background. Simultaneous screening of tricyclic antidepressants(TCAs), buprenorphine, MDMA and other drugs of abuse is relevant for applications in therapeutic drug monitoring, toxicology, forensic settings. Evidence biochip array technology provides a platform for the simultaneous determination of multiple analytes from a single sample, which leads to an increase in the results output and a reduction in the consumption of sample/reagents. We report the applicability of this technology to the simultaneous determination of these compounds in urine with a matrix dedicated biochip array kit.
Methods. Competitive chemiluminescent simultaneous immunoassays are applied for the determination. The core of this technology is the biochip(9mm x 9mm), which represents the chemically activated solid phase where the ligands are immobilised and stabilised defining microarrays of test sites and also the vessel where the reactions take place. The assays were applied to the fully-automated Evidence analyser. The system incorporates the software to process, report and archive the data generated.
Results. The TCA assay detects approximately 12 compounds in this class including amitryptiline(%cross-reactivity 72%), desipramine(%cross-reactivity 131%), nortryptiline(%cross-reactivity 100%) and trimipramine(% cross-reactivity 375%). The sensitivity of the TCAs assay was 3.9ng/ml.The buprenorphine assay exhibited a sensitivity value of 0.04ng/ml and the MDMA assay 7.06ng/ml.The intra-assay precision and inter-assay precision, expressed as %CV, were<13% and <19% respectively for all the assays.
Conclusions. Data show applicability of biochip array technology to the simultaneous determination of TCAs, buprenorphine, MDMA and other drugs of abuse in urine. This is of value for applications in therapeutic drug monitoring, toxicology, forensic settings.

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HEROIN DEPENDENTS ARE INSULIN RESISTANT

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Background. Fasting insulin levels, HOMA-IR and HOMA-%B are precise methods for determining insulin resistance and can be used in assessing the insulin sensitivity in heroin dependents (HD). Heroin dependents are expected to be insulin resistant.
To determine the heroin influence on insulin sensitivity in male heroin dependents compared to control healthy male (K).
Materials. Fasting insulin levels (I), HOMA-IR, HOMA-%B, fasting glucose levels (gl) and C-peptide (C) were determined in both groups, group K with mean age (28.1±4.2 yr) and mean BMI (22.8±2.5 kg/m2), not different compared to group HD with mean age (27.9±5.4 yr) and BMI (22.3±3.1 kg/m2),which were HCV negative.
Methods. I and C were determined with hemiluminiscent method on Immunology Analyzer Immulate 2000, HOMA-IR was calculated [(FIxFgl)/22.5], as well as HOMA-%B [(20xFI)/(Fgl-3.5)].
Results. I values in HD group were 15.24±27.6 IU/L, significantly higher compared to K (4.58±3.23) (p<0.035). Glucose levels were not significantly different between HD (5.0±0.83 mmol/l) and K (4.93±0.45 mmol/l) (p>0.05). HOMA-IR in HD (2.52±3.29) and HOMA-%B (111.56±58.14) were significantly higher compared to the correspondent values in K (1.02±0.8) (p<0.016) and (68.83±46.57) (p<0.05). I values correlated highly significantly positively with HOMA-IR, HOMA-%B and C-peptide (p<0.0001) in HD.
Conclusions. HD were characterized with significantly higher I, HOMA-IR, HOMA-%B values, which correlated highly significantly between themselves, confirming insulin resistance in HD.

Toxicology

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DETERMINATION OF TETRAHYDROCANNABINOL IN WHOLE BLOOD USING BIOCHIP ARRAY TECHNOLOGY: ANALYTICAL PARAMETERS COMPARISON WITH OTHER IMMUNOASSAY TECHNIQUE

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Background. Evidence biochip array technology provides a platform for the simultaneous screening of drugs of abuse from a single whole blood sample. Miniaturised simultaneous chemiluminescent immunoassays are employed with this technology. This leads to an increase of results output and a reduction of sample/reagent consumption. Abuse of cannabinoids is a significant problem and the availability of sensitive and reliable immunoassays for their determination is relevant. We report the comparison of analytical parameters of the THC biochip array-based immunoassay with a commercially available ELISA.
Methods. The analytical parameters considered for comparison were sensitivity, limit of detection and intra-assay precision. The biochip assay for amphetamines was applied to the Evidence Investigator analyser. Both methodologies –biochip array technology and ELISA were carried out according to the manufacturers' instructions.
Results. The analytical sensitivity of the THC using the biochip-array assay was 2.72 ng/ml and the limit of detection in whole blood was 7.34ng/ml. The intra-assay precision of this technology was %CV <11%.Analysis with the ELISA showed sensitivity of 12.98ng/ml, limit of detection at 9.11ng/ml and intra-assay precision exceeded 17% (17-20%).
Conclusions. The results indicate superior sensitivity and reproducibility in the detection of THC using the Evidence biochip technology when compared to an ELISA technique.

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DETERMINATION OF OPIATE IN WHOLE BLOOD USING EVIDENCE BIOCHIP ARRAY TECHNOLOGY: ANALYTICAL PARAMETERS COMPARISON WITH OTHER IMMUNOASSAY TECHNIQUE

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Background. Evidence biochip array technology provides a platform for the simultaneous screening of drugs of abuse from a single whole blood sample. Miniaturised simultaneous chemiluminescent immunoassays are employed with this technology. This leads to an increase of results output and a reduction of sample/reagent consumption. Abuse of opiate is a significant problem and the availability of reliable immunoassays for the determination is relevant. We report the comparison of analytical parameters of the opiate biochip array-based immunoassay with a commercially available ELISA.
Methods. The analytical parameters considered for comparison were limit of detection and intra-assay precision. The biochip assay for opiate was applied to the Evidence Investigator analyser. Both methodologies –biochip array technology and ELISA were carried out according to the manufacturers' instructions.
Results. The limit of detection of the opiate biochip-array assay was 1.64 ng/ml. The intra-assay precisionof this technology was %CV<20%.Analysis with the ELISA showed limit of detection at 1.01ng/ml and intra assay precision exceeded 27% (27-30%).
Conclusions. The results indicate superior reproducibility in the detection of opiate using the Evidence biochip technology when compared to an ELISA technique.

Toxicology

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DETERMINATION OF AMPHETAMINE IN WHOLE BLOOD USING EVIDENCE BIOCHIP ARRAY TECHNOLOGY: ANALYTICAL PARAMETERS COMPARISON WITH OTHER IMMUNOASSAY TECHNIQUE

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Background. Evidence biochip array technology provides a platform for the simultaneous screening of drugs of abuse from a single whole blood sample. Miniaturised simultaneous chemiluminescent immunoassays are employed with this technology. This leads to an increase of results output and a reduction of sample/reagent consumption. Abuse of amphetamines is a significant problem and the availability of reliable immunoassays for the determination is relevant. We report the comparison of analytical parameters of the amphetamine biochip array-based immunoassay with a commercially available ELISA.
Methods. The analytical parameters considered for comparison were sensitivity, limit of detection and intra-assay precision. The biochip assay for amphetamines was applied to the Evidence Investigator analyser. Both methodologies –biochip array technology and ELISA were carried out according to the manufacturers' instructions.
Results. The analytical sensitivity of the amphetamine biochip-array assay was 3.12ng/ml and the limit of detection in whole blood was 5.68 ng/ml. The intra-assay precision of this technology was %CV <12%.Analysis with the ELISA showed sensitivity of 0.72ng/ml, limit of detection at 3.84ng/ml and intra- assay precision exceeded 20% (21-24%).
Conclusions. The results indicate superior reproducibility in the detection of amphetamine using the Evidence biochip technology when compared to an ELISA technique.

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A FAST SCREENING PROCEDURE FOR THE SIMULTANEOUS DETECTION OF MEPHEDRONE AND KETAMINE WITH TANDEM MASS SPECTROMETRY

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Background. Mephedrone is a designer drug of the amphetamine family. It could not be detected with amphetamine immunoassay reagent. ELISA for ketamine could not be fully automated. An efficient alternative procedure is desirable.
Methods. Urine samples, calibrators (containing amphetamine, methamphetamine, MDA, MDMA, MDEA, p-chloroamphetamine, ephedrine, mephedrone, ketamine, norketamine, dehydronorketamine), and controls were spiked with internal standard (norketamine-d$_4$, MDA-d$_5$). The mixtures were alkalinized and extracted with ethyl acetate by vortex and sat still for 5 min. The upper organic layer was aspirated for analysis. Extracted samples were monitored with ESI tandem mass spectrometry without chromatographic column. Mass detector was operated in positive Multiple Reaction Monitoring mode. Two transitions for each drug were monitored. Data were collected for one minute per sample. A total of 11 drugs were simultaneously analyzed. Mobile phase constitutes of 0.1 % formic acid in 50 % methanol. The flow rate is 0.2 mL per minute. Total analysis time is 1.8 min/sample.
Results. Limit of detection and limit of quantification were less than 1 ng/mL and 10 ng/mL, respectively. Within run precision (%CV) and bias at 3 different concentrations were less than 6.4% and 17.0%, respectively. Correlation coefficients (R^2) for the calibration curves were all greater than 0.999. A group of 200 samples were screened with this procedure and results compared with GC/MS, no discrepancy of results were observed.
Conclusions. A screening procedure for multi-analytes analysis based on quick extraction and tandem mass spectrometry is fast, accurate and efficient. It is a good alternative for the immunoassays.

Toxicology

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DEVELOPMENT OF A ONE MINUTE LC-MS/MS METHOD FOR IMMUNOSUPPRESSANTS

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Background. An increasing number of immunosuppressant determinations are performed with tandem mass spectrometry due to the higher sensitivity and specificity compared to immunoassays. In order to add to the advantages of the MS/MS analysis we developed a very fast and automated method with an injection cycle of one minute.
Methods. We achieved the short injection cycle with a set-up of two isocratic pumps, a 6-port switch valve, a trap and analytical column. This set-up allows for the parallel flow of the two mobile phases thus washing the trap column and loading the MS with the matrix-free analytes at the same time. A complete cycle takes 60 seconds. The sample preparation consists of a single protein precipitation step and can be done automatically with microtitre plates (MTPs).
Results. The method has been thoroughly validated in terms of performance parameters such as precision, accuracy, LOQ, matrix effects and interferences of therapeutic drugs. Intra- and inter-assay precision values have been found to be below 7% in the low concentration range. 83 NEQAS samples were successfully analysed. Experiments with phospholipids and >50 drugs showed no interferences. The extent of matrix effects has been determined by means of different experiments which indicated negligible impacts on Results.
Conclusions. Using this robust method in a high throughput laboratory enables technicians to save up to 80% of manual sample preparation time when MTPs and a liquid handler are employed. Furthermore, the short injection cycle allows a quick response time and can save, in certain scenarios, one mass spectrometer, thereby lowering costs significantly.

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CHOLINESTERASE ACTIVITY IN SCHIZOPHRENIC PATIENTS

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Background. The objective of the present study was to assess the cholinesterase activity in schizophrenic patients.
Methods. This study included 60patients with chronic schizophrenia according DSM-IV criteria from Psychiatric departmentof the University Hospital of Monastir (43 males and 17 females, mean age = 37.52 ± 10.01 years) and 30healthy controls (27 males and 3 females, mean age = 26.43 ± 5.81 years).
Cholinesterase activity was determined by enzymatic method on Integra 400 plus™ analyzer (Roche Diagnostics).
Results. No significant difference was observedbetweenschizophrenic patients and controls(mean = 8913 U/L in patients versus 8612 U/L in controls; p = 0.45).
In patients,cholinesterase activity was higher in female than male without significant difference (mean = 9314 U/L in female versus 8754 U/L in male; p = 0.41). In the controls we found an increase only in the male.
A significant difference was noted between female patients and controls (mean = 9314 U/L in patients versus 7386U/L in controls; p = 0.02).
Cholinesterase activity was significantly higher in patients treated by antipsychotic than those treated by anticholinergic drugs (mean = 10099 U/L Vs 8319 U/L; p = 0.02).
No significant difference was noted accordingto the tabagic status.
Conclusions. In the schizophrenia, cholinesterase activity is not affected but its increase in female patients and those treated by antipsychotics requires more investigations.

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EFFECT OF OPIUM ADDICTION ON SOME SERUM PARAMETERS IN RABBIT

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Background. In traditional medicine, opium has been considered as a remedy for many disorders. This belief along with use of opium as recreation can lead to addiction. Addiction is one of the most important issues of the 21st century that received more attention all over in the world.
Methods. New Zealand white rabbits (20 males and 20 females) were randomly divided into two groups (control and addicted group). Addicted and control group received opium and distilled water separately by gavage. Rabbits were weighed at the beginning and the end of the experiment. Blood samples also were taken from ear veins at the beginning and at the end of the experiment. Serum samples were stored at -20 C until biochemical analysis. Various parameters such as Fasting blood glucose(FBS), total cholesterol, high density lipoproteins–cholesterol (HDL-c), low density lipoproteins–cholesterol (LDL-c),triglycerides (TGs), sodium (Na$^+$), potassium(K$^+$), calcium (Ca^{2+}), phosphor (P), serum total protein(Pr), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase(ALP), amylase, Creatine phosphokinase (CPK), uric acid (UA), creatinine(Cr) and urea (Ur) were measured in the serum of both groups. Paired t-test was used for data analysis.
Results. Addicted male and female rabbits showed higher serum FBS, AST, ALT, LDL, TG, CPK, K+ and Cr values in compare to the control group.
Conclusions. Our findings show that opium addiction in rabbit has many biological consequences.

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EFFECT OF OPIUM ADDICTION ON T4, T3 AND TSH IN MALE AND FEMALE RATS

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Background. Opium addiction is a serious social problem in Iran. Beside of social effects, opium addiction has various physiologic consequences. Thyroid hormones have direct effect on metabolism and at present study the effect of opium addiction on thyroid stimulating hormone (TSH), Triiodothyronine(T3) and thyroxin(T4) was evaluated.
Methods. 60 male and female rats (weighting 250-300g) were randomly divided into control and addicted groups. Addicted groups were received opium solution by gavage for 60 days, at the end of study blood samples were taken from all groups and T4, T3 and TSH of addicted group were compared with control groups. Paired t-test was used for data analysis.
Results. In male and female addicted rats T4 and TSH decreased but T3 did not alter significantly.
Conclusions. Our findings show that opium addiction in rats has many various problem on Thyroid hormones.

Toxicology

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SERUM TOXICOLOGY ASSAYS ON SYNCHRON UNICEL DXC 800 SYSTEM FOR ROUTINE TESTING OF BARBITURATES, BENZODIAZEPINS AND TRICYCLIC ANTIDEPRESSANTS

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Background. Evaluation of three new serum toxicology assays for quantification of barbiturates, benzodiazepins and tricyclic antidepressants on Synchron UniCel DxC 800 (Beckman Coulter) using serum samples of hospitalised patients.
Methods. The performance of three serum toxicology assays (Thermo Fisher) on a UniCel DxC 800 system (Beckman Coulter) were compared to barbiturates and benzodiazepins (Thermo Fisher reagents on Roche Modular system) and tricyclic antidepressants to AxSYM (Abbott).
Results. Inter-assay variation coefficient for barbiturates, benzodiazepins and tricyclic antidepressants as determined with commercially available controls were 4.67 %, 2.13 %, 1.87 % at 1.72 μg/mL, 191 ng/mL and 356 ng/mL, respectively (n=15). Concordance analysis for barbiturates (n=34) and benzodiazepins (n=96) showed identical results for both assays. All results were confirmed by chromatographic analysis. In contrast for tricyclic antidepressants (n=37) 4 discordant results were identified. In these samples the DxC 800 results were negative, however, the AxSYM determined positive Results. All DxC 800 negative results were confirmed by chromatography.
Conclusions. All three serum toxicity assays for barbiturates, benzodiazepins and tricyclic antidepressants on UniCel DxC 800 exhibit excellent performance for both routine and STAT analysis and reliable Results.

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TOXIC EFFECTS OF ETHANOL ON INFLAMMATORY BRAIN DAMAGE AND THE ROLE OF ADHESION MOLECULES IN ANIMAL MODEL WITH ROLE OF GRAPE POLYPHENOLS

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Background. Ethanol is a small molecule that has a negative impact on human health and moderate to heavy doses of alcohol has deleterious effects and especially on brain and its different compartments along with adhesion molecules that plays an important role in the pathogenesis of several diseases and modulate various functions.
Methods. In the present study with male wistar rat model, we investigated the role of natural antioxidants like grape polyphenols and Vit E in combating the ROS balance in alcoholism and along with the expression of different adhesion molecules for a dose of 12g /Kg b.w/day of ethanol for a period of 3 months.
Results. Ethanol intoxication up regulates COX-2, iNOS levels and p38 MAPK pathways, and increases cell by Caspase-3 and Caspase-8 assay .Chronic ethanol treatment also alters intercellular adhesion molecules expression in brain tissues of rats with duration of ethanol exposure. Polyphenols an ubiquitous groups of plant metabolites and are an integral part of both human and animal diets shows protective role.
Conclusions. We conclude that chronic ethanol exposure leads to inflammatory brain damage with alteration of different adhesion molecules and grape polyphenols plays the protective role.

Toxicology

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CEDIA TACROLIMUS APPLICATIONS FOR THE ORTHO CLINICAL DIAGNOSTICS VITROS SYSTEMS

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Background. Tacrolimus (FK506) is an antibiotic with potent immunosuppressive function as prescribed for patients with kidney and liver transplantation. Monitoring Tacrolimus is important for effective use of the drug in preventing renal or liver allograph rejection. The Ortho Clinical Diagnostics VITROS 5600 Integrated System and VITROS 5,1 FS Chemistry System are new applications for the CEDIA Tacrolimus Assay.

Methods. The CEDIA assay uses the bacterial enzyme b-Galactosidase; the amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.The CEDIA Tacrolimus application on the VITROS 5600 and 5,1 FS Systems was evaluated for precision and accuracy.

Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. Within run CVs were 14.1, 6.5, and 5.3% and total CVs were 19.4, 11.0, and 9.8% at 6.65, 13.9, and 18.9 ng/mL on the VITROS 5600 System. Within run CVs were 11.5, 6.5, and 5.9% and total CVs were 21.4, 9.2, and 9.8% at 6.74, 14.0, and 19.1 ng/mL on the VITROS 5,1 FS System. Agreement with the predicate Hitachi 917 was good using patient samples spanning the reportable range:

5600 = 1.02 (Hitachi 917) + 1.0 with a correlation coefficient of 0.984

5,1 FS = 1.00 (Hitachi 917) + 1.1 with a correlation coefficient of 0.975

Conclusions. We conclude that the performance of the CEDIA Tacrolimus Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

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CEDIA CYCLOSPORINE APPLICATIONS FOR THE ORTHO CLINICAL DIAGNOSTICS VITROS SYSTEMS

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Background. Cyclosporine is a cyclic undecapeptide with potent immunosuppressive function in reducing the incidence of tissue rejection following organ transplantation. As cyclosporine therapy has a narrow range for optimum safety and efficacy, monitoring is essential. The Ortho Clinical Diagnostics VITROS 5600 Integrated System and VITROS 5,1 FS Chemistry System are new applications for the CEDIA Cyclosporine Assay.

Methods. The CEDIA assay uses the bacterial enzyme b-Galactosidase; the amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.The CEDIA Cyclosporine application on the VITROS 5600 and 5,1 FS Systems was evaluated for precision and accuracy.

Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. Within run CVs were 7.0, 1.3 and 1.1%; total CVs were 9.7, 2.8 and 2.5% at 49, 188 and 302 ng/mL on the VITROS 5600 System. Within run CVs were 9.4, 2.5 and 1.3% and total CVs were 13.0, 3.9 and 3.2% at 52, 201 and 323 ng/mL on the VITROS 5,1 FS System. Agreement with the predicate analyzer was good using patient samples spanning the reportable range:

5600 = 1.07 (Hitachi 911) + 3.8 with a correlation coefficient of 0.994

5,1 FS = 1.08 (Hitachi 911) - 1.4 with a correlation coefficient of 0.995

Conclusions. We conclude that the performance of the CEDIA Cyclosporine Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

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CEDIA MYCOPHENOLIC ACID APPLICATIONS FOR THE ORTHO CLINICAL DIAGNOSTICS VITROS SYSTEMS

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Background. Mycophenolic Acid (MPA), metabolized from pro-drug mycophenolate mofetil or mycophenolate sodium is widely used for the prevention of rejection in patients receiving renal, heart, or liver transplants. The Ortho Clinical Diagnostics VITROS 5600 Integrated System and VITROS 5,1 FS Chemistry System are new applications for the CEDIA Mycophenolic Acid Assay.
Methods. The CEDIA assay uses the bacterial enzyme b-Galactosidase; the amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.The CEDIA Mycophenolic Acid application on the VITROS 5600 and 5,1 FS Systems was evaluated for precision and accuracy.
Results. Tests for within-run and total precision (N=80 per level) were run over 20 days. Within run CVs were 2.0, 1.0, and 0.9% and total CVs were 5.9, 2.8, and 2.3% at 1.1, 3.4, and 7.0 μg/mL on the VITROS 5600 System. Within run CVs were 2.0, 1.2, and 0.7% and total CVs were 12.0, 6.0, and 4.4% at 1.1, 3.3, and 6.9 μg/mL on the VITROS 5,1 FS System. Agreement with the predicate Hitachi 917 was good using patient samples spanning the reportable range:
5600 = 1.04 (Hitachi 917) + 0.2 with a correlation coefficient of 0.998
5,1 FS = 1.03 (Hitachi 917) + 0.3 with a correlation coefficient of 0.998
Conclusions. We conclude that the performance of the CEDIA Mycophenolic Acid Assay on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System warrants their introduction in clinical practice.

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DEVELOPMENT OF A MONOCLONAL ANTIBODY FOR THE DETECTION OF MEPERIDINE AND ITS METABOLITE NORMEPERIDINE

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Background. Meperidine was first introduced in the 1930s as an analgesic, producing effects that are similar to morphine. It is currently used for pre-anaesthesia and the relief of moderate to severe pain. The metabolite normeperidine is approximately half as potent as meperidine, but it has twice the CNS stimulation effects. The society of Forensic Toxicologists (SOFT) recommends meperidine and normeperidine as target analytes for meperidine. We report the development of a monoclonal antibody to meperidine and normeperidine which is of value in developing more effective immunoassays for detecting these compounds and for applications to different settings.
Methods. Sheep were immunized with normeperidine conjugated via an amino group to a carrier protein bovine thyroglobulin (BTG). Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of antibody using competitive ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA.
Results. For a calibration range of 0-50ng/ml the sensitivity expressed as IC50 was 0.7ng/ml for normeperidine and 1.7ng/ml for meperidine. This clone showed >40% cross reactivity between normeperidine and meperidine.
Conclusions. Data indicate that the monoclonal antibody generated is suitable for the development of immunoassays for the determination of meperidine and normeperidine in test samples.

Toxicology

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DEVELOPMENT OF A GENERIC MONOCLONAL ANTIBODY AGAINST BARBITURATES

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Background. Barbiturates are a class of compounds derivatised from barbituric acid. They are central nervous system (CNS) depressants and can be used as sedatives, hypnotics, anaesthetics and anti-epileptic drugs. Immunoassays enabling generic determination of barbiturates are of interest to monitor their use/misuse for applications in therapeutic, forensic and toxicology settings.The aim of this work was to produce a generic monoclonal antibody presenting a broad specificity profile, which will be of value in the development of immunoassays for the detection of barbiturates.
Methods. Sheep were immunized with the barbiturate secobarbital, conjugated to bovine thyroglobulin (BTG) as carrier. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of generic antibody using competitive ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA.
Results. Initial evaluation showed an IC50 of 0.868ng/ml for phenobarbital with %CVs <5% for all standards (4, 2, 1, 0.5, 0.125, 0.063 and 0 ng/ml). The cross- reactivity of the antibody was 638% for alphenal, 313% for secobarbital, 225% for aprobarbital, 97% for butabarbital, 74% for amobarbital, 54% for pentobarbital and 14% for barbital.
Conclusions. This generic monoclonal antibody exhibits high sensitivity and specificity for a wide range of barbiturates. This is of value in developing effective immunoassays applicable to therapeutic, forensic and toxicology fields.

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DEVELOPMENT OF A RAPID GC METHOD FOR THE MEASUREMENT OF GAMMA-HYDROXYBUTYRIC ACID (GHB) IN ANTE- AND POST-MORTEM SAMPLES

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Background. In recent times, Gamma- Hydroxybutyric acid (GHB) and its precursor molecule, gamma butyrolactone (GBL), have gained popularity as recreational drugs. These controlled compounds have been implicated in drug-facilitated sexual assault and death by accidental overdose. As a consequence, the number of clinical and forensic requests for GHB toxicology has increased. We present here an efficient assay for the measurement of GHB in blood samples.
Methods. A gas chromatography-flame ionisation detection (GC-FID) method was established to measure GHB in blood indirectly following acid conversion to GBL and extraction into dichloromethane. Chromatographic separation was performed on a ZB-WAX+ column at 50ºC (ramp 50°C/min to 150ºC) with FID detection at 240ºC in <10 minutes. Calibration was established (n = 3) using drug-free blood spiked with GBL and alpha-methylene-gamma-butyrolactone (internal standard). Linearity, LOD, LOQ, efficiency of GHB conversion to GBL and percentage recovery was assessed.
Results. The assay was linear over the range 5-200 mg/L (R = 1) and adequately detected concentrations of GBL with a LOD of 2.5 mg/L (CV 2.3%) and LOQ of 5 mg/L (CV 4.9%). GHB was efficiently converted to GBL using sulphuric acid with preliminary data demonstrating percentage recoveries of 84% and 90% for 25 and 100 mg/L GHB standards, respectively.
Conclusions. The GC-FID method presented is a rapid screening method for measurement of GHB and GBL in ante- and post-mortem samples. It is readily accessible for use in both clinical and forensic toxicology laboratories.

Toxicology

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DEVELOPMENT OF A LC-MS/MS METHOD FOR MEASUREMENT OF NEW PSYCHOACTIVE COMPOUNDS *LEGAL HIGHS* IN THE CLINICAL LABORATORY

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Background. The new psychoactive compounds Methylenedioxy-2-aminoindane (MDAI) and Cathinone (+ derivatives) have been associated with euphoria, paranoia, arrhythmia and death. In April 2010 the UK government scheduled Cathinone (+ derivatives) as Class B drugs; however, MDAI is still legal. Potential for increasing use of these drugs may be mirrored by requests for analysis. Consequently, we developed a method for measurement of such compounds.

Methods. A LC-MS/MS method was established on the Waters® Xevo™ TQ MS ACQUITY UPLC® System to measure Cathinone, Methylone, Ethylone, Butylone Methylenedioxypyrovalerone (MDPV) and MDAI. Calibration was achieved (n = 8) using drug-free blood spiked with these compounds and quetiapine (internal standard). Liquid-liquid extracts were injected onto a C8 column and chromatographic separation was performed using gradient elution (methanol/water/0.05% formic acid) with MS/MS detection. Linearity, assay imprecision, LOD, LOQ (CV <20%) and efficiency of recovery for each compound was determined.

Results. Linearity was observed over the range 0.005-0.500 mg/L for each compound: Cathinone (R = 0.997), Methylone (R = 0.936), Ethylone, Butylone, MDPV and MDAI (R = 0.999). Intra- and inter-assay imprecision at 0.01 mg/L was <20% and <28% for all compounds, respectively. LOD were: 0.03 mg/L (Cathinone), 0.001 mg/L (Methylone, Ethylone, Butylone, MDPV, MDAI). LOQ were: 0.01 mg/L (MDPV, Butylone), 0.02 mg/L (Methylone, MDAI), 0.05 mg/L (Cathinone, Ethylone). Preliminary recoveries were: Cathinone (89%), Methylone (66%), Ethylone (61%), Butylone (63%), MDPV (87%), MDAI (65%).

Conclusions. In the absence of commercial immunoassays for detection of these novel compounds, we have developed an effective technique for their measurement.

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EFFECTS OF THREE ANTIDEPRESSANT TREATMENTS ON PARAOXONASE ACTIVITY

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Background. Our study aims to investigate the in vitro inhibitory effects of three antidepressants imipramine, amitriptyline and fluoxetine on the human serum paraoxonase activity (PON1).

Materials and Methods. Plasma from healthy volunteers was spiked with antidepressant drugs. Pure working solutions of drugs were half diluted with plasma to obtain the desired concentrations which cover the therapeutic margin. PON1 was tested in triplicate after incubation at 37°C in the presence of different drugs every 30 minutes during two hours, by kinetic method.

Results. We found that tricyclic antidepressants inhibited significantly PON1, while fluoxetine had no effect. The inhibition percentage of imipramine was 15,6% at 100 μg/L after one hour incubation (131 ± 1 IU/L Vs 155 ± 2 IU/L; $p < 0,001$). At 350 μg/L, the inhibition was of 19,2% after one hour of incubation (125 ± 1 IU/L Vs 155 ± 2 IU/L; $p < 0,001$) and 20,2% after two hours.

The inhibitory effect of amitriptyline was higher and appeared earlier : 26% after 30 minutes at 125 μg/L (117 ± 2 IU/L Vs 159 ± 2 IU/L; $p < 0,001$). At 250 μg/L, the inhibition was of 36,5% after 30 minutes (100 ± 4 IU/L Vs 159 ± 2 IU/L; $p < 0,001$) and 34,5% after two hours.

Conclusions. Two tricyclic antidepressants had an inhibitory effect on PON1. This effect is concentration-dependent and doesn't seem to be related to the time of incubation. Amitriptyline showed a higher inhibitory potency than imipramine. Fluoxetine had no effect.

Toxicology

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CYTOTOXIC EFFECTS OF "INDOLE-3-ACETIC ACID AND TURNIP PEROXIDASE" AND " INDOLE-3-ACETIC ACID AND HORSERADISH PEROXIDASE" SYSTEMS ON HUMAN COLON CANCER HT2G CELLS

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Background. To clarify the mechanism of cellular injury induced by indole-3-acetic acid (IAA) - turnip peroxidase (TRP) and IAA - horseradish peroxidase systems, we studied their effects on human colon cancer HT2g cells.
Methods. First, HT2g cells derived from human colon cancer were cultured in RPIM supplemented with FBS and penicillin-streptomycin in CO_2 at 37°C. Then, cells were exposed to 80 μmol/L IAA - 1.2 μg/ml TRP or 80 μmol/L IAA - 1.2 μg/ml HRP for different times. The cytotoxic effects were estimated by measuring thiobarbituric acid-reactive substances (TBARS).
Results. Results showed that the TBARS levels in HT2g cells increased after IAA-TRP and IAA-HRP treatment, in a time-dependent manner. These finding showed that IAA-TRP exerted a stronger effect. After exposure to IAA-TRP for 3 days, the contact of TBARS (90.30 ± 15.66 μmol/L/mg protein) increased compared to control group (20.80 ± 0.33 μmol/L/mg protein).
Conclusions. The combination of IAA with TRP or HRP can increase the TBARS contact in human colon cancer HT2g cells; therefore they have favorable effects in ameliorating cancer.

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THE COMPARISON OF "INDOLE-3-ACETIC ACID/TURNIP PEROXIDASE" AND " INDOLE-3-ACETIC ACID/ HORSERADISH PEROXIDASE" SYSTEMS ON HUMAN COLON CANCER HT2G CELLS VIABILITY

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Background. The effects of indole-3-acetic acid (IAA) - turnip peroxidase (TRP) and IAA - horseradish peroxidase systems were studied on human colon cancer HT2g cells viability.
Methods. First, HT2g cells derived from human colon cancer were cultured in RPIM supplemented with FBS and penicillin-streptomycin in CO_2 at 37°C. Then, cells were exposed to 80 μmol/L IAA - 1.2 μg/ml TRP or 80 μmol/L IAA - 1.2 μg/ml HRP for different times. 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to detect cell viability.
Results. Results showed that both IAA-TRP and IAA-HRP decreased the viability of human colon cancer cellsin a time-dependent manner, and IAA-TRP exerted a stronger effect. After exposure to IAA-TRP for 72hr, the viability rate decreased to 80%.
Conclusions. These findings indicated that combination of IAA with TRP or HRP can reduce the viability of on human colon cancer HT2g cells in vitro.

Toxicology

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MANGANESE INTOXICATION: CASE REPORT

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Background. Manganeseisan essential trace elementthat requiresthepresenceasa nutrient. Itis important toconsideroccupational poisoning, calledmanganese, whosesymptomsreminiscent ofParkinsondisease. Dystoniaandmovement disordershave also be-enreportedin patientsreceivingtotalparenteralnutrition (TPN)for a long time, associated abnormalitiesinMR images.
Casereport. 73 year old manwas referred for evaluation ofabnormal movements.
Personal Background. Gastric adenocarcinomatreatedwithchemotherapyandsubtotalgastrectomysurgerywithsepticshock. Presentedsepticshockwith multiple organ failure, remainedlogged210 daysinICUreceiving TPN for amonth.
Currentdisease: typechoreicmovementsin the headandneckandleft hemibodythatincrease.
Investigations: MRI: T1-weightedhyperintensityinbasal gangliainT2hypointensityintheputamennucleus.
ClinicalTrial: Radiological findingssuggestamanganesedeposit, after TPN with 300mg/day, coinciding withmildliver failureinthe ICU.
Manganesedeterminationin bloodbyAASgraphite furnaceprovedtotalof 34mg/L, higher than the reference value.
Conclusions. Parenteral exposure to Mn with impaired excretory bladder has been the cause of poisoning, suspected by the radiological images. Parkinsonian type symptoms made suspect this poisoning whose incidence is often underestimated. Despite the normal dose of Mn contained in the NPT could be toxic concentration by altering homeostatic and a possible contamination in TPN. In the patient's evolution has been an improvement in their movements by getting to walk with a walker. Mn concentrations remain slightly elevated, despite the time elapsed since TPN.

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VALIDATION OF AN LC-MS/MS ASSAY FOR METHOTREXATE WITH GOOD COMPARISON TO FPIA

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Background. Methotrexate is an antimetabolite used to treat a variety of cancers and autoimmune disorders. During therapy, serum concentrations are useful in assessing whether the drug is being adequately cleared; many clinical protocols confirm that serum methotrexate is below a set threshold (often <0.1 µmol/L after 72 hr) for patient safety. One of the most common assays used to measure low methotrexate concentrations, the Abbott TDx/FLx fluorescence polarization immunoassay (FPIA) is soon to be discontinued. Several other commercial immunoassays have unacceptably high metabolite cross-reactivity at low methotrexate concentrations, thus the loss of the FPIA creates a serious difficulty for clinical laboratories. Our laboratory has developed and validated a liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay for serum methotrexate that shows good agreement with FPIA Results.
Methods and Results. The LC-MS/MS method requires 100 µL serum, and was validated from 0.05 – 0.8 µmol/L. Within- and between-run imprecision studies showed ≤3.5% CV across this concentration range. Extraction efficiencies for methotrexate and the D_3-methotrexate internal standard averaged 59.4% and 63.4% respectively. Comparison to FPIA showed a linear regression equation y = 1.036x – 0.0003, R^2 = 0.994; importantly, there was excellent agreement of points near the 0.1 µmol/L clinical cutoff, with ≤0.01 µmol/L absolute difference in values for all samples (range <0.05 – 0.28 µmol/L).
Conclusions. The methotrexate assay described here is sufficiently robust for clinical laboratory use. It compares well to the FPIA assay currently used to assess drug clearance, allowing replacement of the FPIA test without disruption of clinical service or protocol decision points.

Toxicology

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DEVELOPMENT OF A METHOD TO MEASURE PLASMA SORAFENIB CONCENTRATION BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Background. The multikinase inhibitor Sorafenib is therapeutically used in various malignancies, including renal cell and hepatocellular carcinoma. The need of sorafenib to be orally administered for prolonged periods, the wide interindividual variability, the influence of host and environmental factors, severe toxicity, particularly in patients with renal and hepatic diseases, and costs require a therapeutic monitoring of the drug, useful in tailoring personalized protocols to ensure an optimal exposure to sorafenib.
Methods. 50µl of plasma and 100µl of water:acetonitrile (60:40) containing 500ng/ml of the internal standard ([2H3, 15N] sorafenib tosylate) were deproteinized with 700µl of acetonitrile. The LC/MS/MS system consisted of an Agilent 6410 triple-quadrupole mass spectrometer equipped with ESI interface and an HPLC Agilent 1290 Infinity. Analytes were separated at 50°C on a Zorbax Eclipse XDB-C18 column 2.1mm x 50mm, 1.8µm, using gradient elution of ammonium formate 5mM, formic acid 0.01% in water (A) and formic acid 0.01% in acetonitrile (B). Monitored MRM transitions (ESI+, deltaEMV 400V, fragmentor 130, CE 34) were respectively 465.1>252.1 (sorafenib) and 469.1>256 (IS).
Results. Recovery and repeatability at 50, 500 and 5000 ng/ml were respectively 99.9%, 95.3%, 93.7% and 5.8%, 1.1% and 1.8%. Calibration (24 indipendent samples, 8 levels and 3 replicates) was linear within the range 50-10000ng/ml (weighted regression 1/x; R2=0,9998). At LOQ (50 ng/ml, n=10) RSD was <6%. No interference peak was observed in blank samples.
Conclusions. The sensitive and fast LC/MS/MS method developed provides a useful tool for pharmacokinetic investigations as well as for therapeutic drug monitoring of sorafenib.

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CONFIRMATORY ANALYSIS FOR DRUGS OF ABUSE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY– TANDEM MASS SPECTROMETRY (LC/MS/MS)

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Background. Illicit drug-induced alterations and the application of administrative or penal sanctions rely on drug blood concentrations. Here we developed a LC/MS/MS confirmatory method to quantify blood levels of drugs of abuse.
Methods. Cannabinoids (THC e THC-COOH): 500µl of sample mixed with 20µl of d3-deuterated analogues 500ng/ml, 1 ml of water and 200µl of acetic acid 10% (v/v), after liquid-liquid extraction with 5 ml of hexane:ethyl acetate (90:10), were dissolved in 300µl of water:acetonitrile (1:1). Opioids (morphine, codeine, 6-MAM) and stimulants (cocaine, BEG): 500µl of sample mixed with 50µl of d3-deuterated analogues 500ng/ml and 400µl of acetonitrile, after solid phase extraction, were dissolved in 200µl of acetonitrile:buffer A (ammonium formate 5mM, formic acid 0.01% in water) (1:9), then diluted with 200µl of buffer A. The LC/MS/MS system consisted of an Agilent 6410 triple-quadrupole mass spectrometer with ESI interface and an HPLC Agilent 1290 Infinity. Analytes were separated at 50°C on a Zorbax Eclipse XDB-C18 column 2.1mm x 50mm, 1.8µm, using gradient elution of buffer A and buffer B (formic acid 0.01% in acetonitrile). Two MRM transitions for each substance were monitored.
Results. Linearity ranges (ng/ml) investigated were (6-8 levels, 3 replicates): THC (1-50, r2=0.991), THC-COOH (1-50, r2=0.998), morphine (1-500, r2=0.999), codeine (1-500, r2=0.998), 6-MAM (1-500, r2=0.998), cocaine (1-500, r2=0.996), BEG (1-500, r2=0.999), using weighted regression when needed. At LOQ (1ng/ml, n=10) RSD was <15%.
Conclusions. Presented LC/MS/MS methods allow confirmation and quantitation of drugs of abuse in blood and may be used both in forensic and traffic medicine.

Toxicology

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IMPACT OF CHRONIC LEAD EXPOSURE ON CERTAIN ENZYMATIC ANTIOXIDANTS

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Background. Lead poisoning remains a major problem in India due to the lack of awareness of its ill effects among the clinical community. The present study was designed to determine the impact of chronic lead exposure on selected oxidative stress parameters.

Methods. A total of 250 subjects of either sexes ranging in age 20 to 70 years, drawn from Jaipur and adjoining areas, were recruited to determine Blood Lead Level (BLL), δ-ALAD, Malondialdehyde (MDA), Glutathione (GSH) and Glutathione Peroxidase (GPx) in blood and Catalase (CAT) , & Superoxide Dismutase (SOD) in RBCs.

Results. Mean blood lead levels of subjects was found to be 15.16 ±11.82 μg/dl. On the basis of BLLs, subjects were categorized into two groups: Group I (BLL <10 μg/dL) and Group II (BLL ≥10 μg /dL) having BLLs 3.47±2.58 μg/dl and 23.092±8.60 μg/dl ,respectively. δ-ALAD, GPx , SOD & CAT activity was significantly lower (P<0.001), while MDA level was significantly higher (P<0.01) in Group II .Further BLLs were showed a negative association with δ-ALAD (r=-0.425), SOD (0.699) ,CAT (r=-0.119) and GPx (r=-0.065) and a positive correlations with MDA (r=0.803, P<0.001).

Conclusions. Chronic lead exposure affects prooxidant-antioxidant equilibrium leading to cellular oxidative stress. Further, the strong association between BLL and δ-ALAD supports.

Traceability and standardisation

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COMPARABILITY OF RESULTS WITHIN A FAMILY OF ANALYZERS AND ESTIMATION OF BIAS (TRUENESS) BY COMPARISON TO REFERENCE METHOD TARGET VALUES

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Background. Assay accuracy depends on bias (trueness, measurement error). Comparison of field methods to recognized reference methods of highest metrological order that provide target values representing "scientific truth" yield the best estimate of bias. All members of a family of analyzers should produce comparable Results.
Methods. Samples for six routine assays (Na, K, Cl, Glucose, BUN, and Creatinine) with reference method target values were tested using Abbott ARCHITECT cSystems (c4000, c8000, c16000) field Methods. Six samples for each analyte were tested in triplicate for three days (n = 9). Mean concentrations for all analyzers were compared to reference method target values to assess bias and results from all analyzers were compared to each other to evaluate comparability.
Results. Average bias for five analytes was less than +/- 1.0% of reference method target values. Average bias for alkaline picrate/Jaffe creatinine ranged from -0.35% to +7.25% compared to ID-GC/MS. Results from all three systems are comparable and Sigma metrics are typically > 6.
Conclusions. Trueness (bias) for six routine assays for a family of automated clinical chemistry analyzers compared very well to reference method target values. Proficiency testing often uses "peer group grading" instead of determining how closely field method results match "scientific truth" determined using recognized reference Methods. Results for the same samples tested by any member of the ARCHITECT cSystems are comparable. Comparability of patient test results generated by all members of a family of analyzers is necessary for transferability of patient information as through electronic medical records.

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COMMUTABILITY STUDY ON ERM-DA472/IFCC, C-REACTIVE PROTEIN IN HUMAN SERUM

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Background. C-reactive protein (CRP) is one of the major acute-phase proteins in humans. Its concentration in serum increases rapidly by several orders of magnitude in the very early stages of infection. Modestly increased CRP concentrations are associated with long-term risk of cardiovascular disease. Therefore there are two types of assays for CRP: for measurements at high concentration and for measurements at low concentration (high sensitivity CRP assays).
In 2009 IRMM has released, in collaboration with the IFCC working group for the standardisation of plasma proteins, ERM-DA472/IFCC, a certified reference material (CRM) which is used as calibrant for commercially available immunoassays. The commutability should be demonstrated for both the undiluted and the diluted CRM.
Methods. Commutability studies have been performed using seven assays (five of them being high sensitivity assays), all employing antibodies from different sources. Triplicate measurements were performed on 30 serum samples, a 1/10 dilution of ERM-DA472/IFCC and the undiluted reference material, in one analytical run.
The commutability of the material was assessed according CLSI guideline C53-P by verifying that the means of the measurement results for the CRM and the 1/10 dilution were within the 95 % prediction interval of the Deming regressions for the serum samples.
Results. ERM-DA472/IFCC and its 1/10 dilution are commutable for all the assays.
Conclusions. This study was able to demonstrate the commutability of ERM-DA472/IFCC for all the methods tested, whether used as such or diluted 10 times. The CRM is also suitable for the calibration of high sensitivity CRP assays.

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IS THE ACCURACY OF SERUM ALBUMIN MEASUREMENTS SUITABLE FOR CLINICAL APPLICATION OF THE TEST?

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Background. Albumin is the major protein in plasma and the determination of its concentrations is used for the prognostic assessment of several diseases. Using the information on the biological variation of the analyte, the degree of albumin measurement uncertainty for clinical laboratories should stay within ±3.90% or ±5.85% (desirable or minimum quality level, respectively). We checked the performance of the albumin immunoturbidimetric assay (Tina-quant Albumin Gen. 2, Roche Diagnostics), carried out on the Cobas c 501 platform.

Methods. To estimate the uncertainty of albumin measurement, we employed the imprecision and bias data obtained by measurements of IRMM ERM-DA 470k/IFCC Human Serum Proteins reference material. The material was measured in duplicate for three consecutive days, in two identical experiments carried out 5 months apart, using the analytical system in accordance with the manufacturer's instructions.

Results. The relative standard uncertainty (u_c) of imprecision was 1.88%; the bias u_c, derived from the difference (-6.18%) between the obtained mean of the means (34.9 g/L) and the target value (37.2 g/L), the bias variability (0.95%) and the u_c of the certified value of reference material (1.61%), was 6.42%. Thesegave a combined u_c of 6.69%.

Conclusions. As the requested analytical quality for albumin measurement in serum is high, the performance of field methods should be extremely good to permit their application in clinical setting. Our results seem to demonstrate that, at least for some commercial assays, problems with standardization of these measurements still persist, even if highly specific and relatively expensive immunochemical methods are used.

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EVALUATION OF COMMUTABILITY OF THE ERM-DA 470K REFERENCE MATERIAL FOR TWO ALBUMIN ASSAYS USING IMMUNOCHEMICAL PRINCIPLES

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Background. To standardize the measurement of human serum proteins, a new reference material (RM)(IRMM ERM-DA 470k/IFCC) has recently been released. We evaluatedthe commutability of this RM for two immunochemical albumin methods based on turbidimetry (Roche Cobas Tina-quant Gen. 2) and nephelometry (Delta Radim) principles.

Methods. We measured albumin concentrations on the two systems in20 human serum samples (albumin concentrations, 16.3 to 42.3 g/L) and in the RM in duplicate in two different runs on the same day. Biological samples were collected, aliquoted and stored(£24 h) at +4°C until their use. Manufacturer's control materials were used to validate analytical runs. The RM commutability was estimated from data of regression analysis using the 95% prediction interval (95PI) and multiples of the standard error of regression ($S_{y\text{-}x}$), according to the CLSI C53-A document.

Results. The RM results were contained within the 95PIbasedon the results for the native clinical samples. In addition, using an acceptance criterion for commutability of ±2 times the experimental $S_{y\text{-}x}$ (±3.135), the relative residual for the RM (-2.107) was within the acceptable range. Albumin values for RM on both analytical systems (34.6 g/L for turbidimetry and 34.2 g/L for nephelometry) were, however, markedly lower than expected (certified value±uncertainty, 37.2±1.2 g/L).

Conclusions. The new RM is commutable between the evaluated immunochemical assays and can be used as a basis to maintain their traceability to higher-order references. However, some inconsistency in the assignment of values to the manufacturer's working calibrators is likely and should be verified.

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MEASUREMENT UNCERTAINTY FOR ROUTINE MEASUREMENT PROCEDURES IN CLINICAL CHEMISTRY LABORATORY ACCREDITED ACCORDING TO ISO 15189

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Background. Medical laboratories accredited according to ISO 15189 have to meet standards of traceability and measurement uncertainty (MU). Since MU encompasses all random and non-corrected systematic errors, we evaluated whether the obtained MU for routine diagnostics tests meet "fitness for clinical purpose" by comparison with desirable analytical quality goals based on Stockholm Statements.
Methods. The uncertainty components we use are uncertainties related to 1-calibrator, 2-within-laboratory precision and 3-trueness estimates based on the results of external quality assessment (EQA). Measurement uncertainties of routine quantitative biochemical assays are estimated on the basis of "Guide to the Expression of Uncertainty in Measurement".
Results. Biochemical analysis of metabolites, enzymes, proteins and electrolytes are performed on Olympus AU 600 multiparametric chemistry analyzer (Beckman Coulter Inc., USA). Traceability of analytical measurement procedures is achieved through a manufacturer's reference materials (calibrators) or reference Methods. Analyzer–based calibrations are routinely performed for compensation of systematic effects. Estimates of within-laboratory precision are provided by internal quality control data in two concentration levels while participation in EQA allowed us to monitor long-term analytical bias.
Conclusions. The obtained measurement uncertainties show that Institute of Clinical Chemistry and Laboratory Medicine of Clinical Hospital Merkur accredited according to ISO 15189 produces test results that are within the desirable analytical goals defined by European recommendations. According to our experience the GUM uncertainty should be applied to measurements in laboratory medicine as one of the prerequisites to fulfil the long-term clinical goal which is to compare test results produced by any laboratory at any time.

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STATISTICAL TEST FOR EQUIVALENCE IN ANALYSIS OF METHOD COMPARISON EXPERIMENTS. APPLICATION IN COMPARISON OF AMH ASSAYS

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Background. A considerable part of method comparison and bias estimation experiments are performed to confirm a distinct relationship between two Methods. Usually, the traditional null hypothesis of bias=0 is tested in statistical analysis and failure to reject the hypothesis (p>0.05) leads to the conclusion that both methods are equivalent. Because "absence of evidence is not evidence of absence", this approach is inappropriate.
Methods. Statistical testing of equivalence was performed after transforming new data according to a previously determined relationship. Reversed null and alternative hypotheses must be formulated incorporating user-defined thresholds for maximal allowed biases. - Method comparison experiments were performed on two manual AMH assays (GEN II vs. DSL) whereby a slope/conversion factor of 1.42 was known from previous validation. In a new study an additional 196 samples were measured and this factor was applied. Then, equivalence of methods was assessed by proving the null hypothesis i.e. that 95% confidence interval of bias at decision limits (5, 15 pmol/L) is within range defined by ±10% of these values.
Results. For both decision limits, null hypotheses were rejected. Equivalence of AMH concentrations measured by both assays was concluded with p<0.05.
Conclusions. Statistical equivalence testing, known from pharmaceutical research (bioequivalence), is appropriate if a known relationship between two assays can be proven. In fact, this is a standard situation which occurs when laboratories compare their existing method with a new one for the same parameter. The methodology is also applicable for statistical analysis of a wide range of experiments (carry-over, robustness, commutability).

Traceability and standardisation

1200

RESEARCH OF METROLOGICAL TRACEABILITY FOR HIGH DENSITY LIPOPROTEIN CHOLESTEROL

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Background. Traceability is a unique tool to improve standardization in laboratory medicine. Joint Committee for Traceability in Laboratory Medicine(JCTLM)recommended reference measurement procedures for high density lipoprotein cholesterol (HDL-C) is ultracentrifugation reference method (UC), and the Food and Drug Administration(FDA) has recognized the value of Cholesterol Reference Method Laboratory Network(CRMLN) certification program and encourages manufacturers to certify their products through the CRMLN.

Methods. According to *HDL cholesterol certification protocol for manufacturers* and EP9-A2 by National Committee for Clinical Laboratory Standards, the direct comparison for HDL-C between UC and Maker analytical system was performed through using fresh human specimens which covered the National Cholesterol Education Program(NCEP) medical decision points.

Results. Linear regression of the results between UC and Maker analytical system was excellent : y=0.9046x+3.0939, and r^2=0.993. Through Regression prediction,bias at 39.3mg/dl was 1.8%, 57.4mg/dl and 4.4%. Average bias was -2.8%,and average absolute bias Among-run CV and total error was 3.1% 0.4% 3.6% respectively. T-test for mean percent bias between two methods showed that t-value was 0.87(T=2.03, a=0.05, degrees of freedom=39). There was no within-method outliers and between-method outliers, and all results were accorded with statistical criteria used for certification by CRMLN.

Conclusions. The effective traceability for Maker HDL-C analytical system was established to ultracentrifugation reference method by CDC. Maker analytical system has demonstrated the ability to meet the NCEP performance criteria for accuracy and precision and the practical requirements from medicine labs.

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EVALUATION OF HOMOGENEITY OF CREATINE KINASE SAMPLES IN RELA 2008

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Background. Complying with In-vitro-diagnostic Medical Devices Directive1998, traceability of calibration materials and control materials has to be assured by reference measurement procedures and reference materials of higher order. Reference measurement (calibration) laboratories have to participate in External Quality assessment scheme for Reference laboratories in laboratory medicine(RELA).We had taken part in the activity of RELA 2008 to validate the competent of our reference labs .Here we evaluated the homogeneity of creatine kinase samples of RELA 2008.

Methods. According to The guideline of China National Accreditation Service for Conformity Assessment (CNAS)-GL03 and regulations from State Food and Drug Administration (SFDA) we evaluated and calculated the homogeneity of creatine kinase samples in RELA 2008 through Hitachi 7180 antomatic analyzer. In addition, we analyzed reference measurement data of creatine kinase from different global reference labs in RELA network of DGKL, and evaluated the homogeinety of creatine kinase samples. At last through above-mentioned two approaches we assessed our assay behave and competence in the activity of RELA 2008.

Results. Data showed that the creatine kinase samples were inhomogeneous.The coefficient of variation (CV)of bottle-to-bottle of A samples was 6.265% , and that of B samples was 4.642%. The coefficient of variation of creatine kinase reference measurement from different reference labs was greater than 3.00%.

Conclusions. The creatine kinase samples were inhomogeneous and the difference of bottle-to-bottle was comparatively large, which possibly affected the quality of external quality assessment of creatine kinase in RELA.

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TRACEABILITY OF ARCHITECT ENZYME ASSAYS TO IFCC REFERENCE METHOD

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Background. The ARCHITECT clinical chemistry systems enzyme assays determine catalytic concentrations by measuring the rate of substrate conversion using a constant calibration factor (k-factor). Goal of the study is to show metrological traceability by using k-factors optimized for agreement of measurement results to the published (AST, LDH, AMY) or proposed (Alkaline Phosphatase, AP) IFCC primary reference measurement procedures (RM).
Methods. In study phase 1, first estimates of optimal k-factors were derived from testing aliquots of three frozen serum pool panels characterized by manual RM on 2 ARCHITECT ci8200 systems.k-factors were adjusted for AST, LDH, AP and AMY assays. Confirmation of k-factors was done in phase 2, in which frozen aliquots of individual serum samples were tested on 2 ARCHITECT ci8200 systems and were compared with the automated RM.
Results. In phase 1 ARCHITECT enzyme assay results of the 3 frozen serum pools were on average 2,5% higher for AST and -7.2% lower for LDH and 8% for AP and AMY compared to the RM. In the phase 2 confirmation runs using the optimized k-factors ARCHITECT results of individual serum samples were highly correlated to RM (R>0.99) with an average bias of -4.7%, 1.4% , 2.6% and 4.7% for AST, LDH, AP and AMY respectively.
Conclusions. By using serum pools with certified enzyme catalytic activity concentration determined by IFCC primary reference methods, alignment of the ARCHITECT k-factor to the IFCC reference methods is possible allowing traceability to the highest metrological level.

1203

STANDARDIZATION OF CREATININE MEASUREMENT IN URUGUAYAN CLINICAL LABORATORIES. A REGIONAL COLLABORATION

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Background. Following the recommendation of the National Kidney Disease Education Program to use MDRD equation to assess the Glomerular Filtration Rate, the National Renal Healthcare Program in Uruguay includes a Standardization Program of Creatinine Measurement in Uruguayan clinical laboratories. This standardization program was established in cooperation between the EQAS of Uruguay (CECC) and the Laboratory for Reference and Standardization in Clinical Biochemistry (LARESBIC) of the Argentine Biochemical Foundation.
Methods. Five levels of creatinine concentration in frozen serum ranging 41.2 to 222.1μmol/L were analyzed by 102 laboratories by triplicate during three days. An enzymatic method (Crea Plus, Roche Diagnostics) performed manually; calibrated with SRM 967 (NIST) was used as reference. Total CV; Bias%, Total Error (as Bias%+1.65CV) and Regression analysis was calculated. By inverting regression line a correction factor for bias was calculated. Results were compared against Biological Variation criteria.
Results. Through levels, total CV ranged 6.8% to 3% while meeting Minimum/Desirable criteria 14.7%-67%/8.8%-49%. Abs(Bias%) ranged from 18% to 5.6% while meeting Minimun/Desirable criteria 10.8% to 56.9%/5.9%-41.2%. Total Error(%) ranged 29.2%-10.6% while meeting Minimum/Desirable criteria 3%-58.8%/1%-36.3%. From regression, slope average 0.958 and ranged 0.624 – 1.1 while average ordinate was 6.36 μmol/L ranging -6.18 to 41.5 μmol. Average r was 0.985 ranging 0.09981-0.9996. Correction reduce an average of 36.3% in Total Error although was not maintained in subsequent experiment.
Conclusions. The experience shows the real state of analytical performance and that the model could be use to standardize. Laboratories should implement systematic analytical quality control to be able to maintain standardization.

Traceability and standardisation

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HARMONIZATION FOR MEASURANDS THAT DO NOT HAVE A REFERENCE MEASUREMENT PROCEDURE

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Background. Standardized (traceable to a reference measurement procedure) or harmonized (traceable to a reference material) results from clinical laboratory procedures enable effective use of clinical guidelines for patient management.

Methods. The American Association for Clinical Chemistry (AACC) convened an international conference in 2010 to address how to improve harmonization of laboratory results for measurands, for example prostate specific antigen, that do not have reference measurement procedures.

Results. A limitation for such measurands has been inadequate attention to the commutability of reference materials, and lack of a systematic approach. An infrastructure consisting of a harmonization oversight group (HOG), specialty work groups (SWG), and harmonization work groups (HWG) will be created and may be housed by a existing organization. The HOG will coordinate all activity and will solicit and receive input from clinical practice and laboratory organizations, government or regulatory agencies, journal editors, and research organizations. Structured checklists will be used to prioritize measurands and secure funding based on input and analysis by a SWG formed to address a specific measurand. A HWG will be formed to manage the technical implementation of a harmonization process for a specific prioritized measurand. Procedures that include individual clinical sample panels, commutable reference materials and manufacturers internal controls will be developed. The procedures developed will lead to JCTLM listing to enable manufacturers to implement harmonization in conformance to regulatory requirements.

Conclusions. The AACC is committed to supporting further development of the infrastructure and technical operations needed to support harmonization for this category of measurands.

1205

ALKALINE PHOSPHATASE (ALP): CURRENT ROUTINE PROCEDURE FROM ROCHE DIAGNOSTICS CORRELATES VERY WELL WITH THE IFCC PROPOSAL FOR A PRIMARY REFERENCE MEASUREMENT PROCEDURE

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Background. IFCC is proposing currently a primary reference measurement procedure for ALP (EC 3.1.3.1).

Methods. We have already implemented in our reference laboratory the proposed IFCC primary reference measurement procedure (IFCC-PRMP) for ALP, and we have assigned reference measurement values to pooled human sera. An automated version of the proposed IFCC-PRMP with self prepared reagent solutions and six self-prepared calibration materials consisting of pooled human sera was adapted to a KONELAB 30i instrument from Thermo. This automated procedure (x) for ALP was used for a method comparison versus the current routine procedure from Roche Diagnostics (y) with C.f.a.s. (lot 153316) as calibrator on a Hitachi 917.

Results. The catalytic concentrations for ALP in self-prepared calibrators ranged from 73 U/L to 641 U/L, and the corresponding combined expanded measurement uncertainties were less 3,1 %. Samples from 200 adult patients were investigated simultaneously with the two procedures. The measurement results of x-values ranged from 35 U/L to 747 U/L. The regression line according to Passing/Bablok was $y = 0,919\,x - 0,2$. The 95 % confidence interval of the slope was 0,911 to 0,929. The intercept did not differ significantly ($p < 0,05$) from zero. The relative residues were distributed randomly around the regression line. The 95 % tolerance range was approximately ± 9 %.

Conclusions. The current routine procedure from Roche Diagnostics requires only adjustment of the ALP concentration of C.f.a.s. by approximately 8 % in order to achieve very good traceability of ALP concentrations in samples from patients to those obtained by use of the proposed IFCC-PRMP.

Traceability and standardisation

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EXTERNAL QUALITY ASSESSMENT SCHEME (EQAS) FOR THE URINARY SEDIMENT: STUDY OF CLINICAL CASES

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Background. The study of clinical cases was introduced in the EQA scheme, managed since 2001 by the Centre of Biomedical Research (CRB), in 2007 (2 surveys/year). Each clinical case consists in a brief clinical history, some key laboratory data and 4 phase-contrast microscopy images of particles found in the urine sediment of the patient presented. Participants must identify the particles shown and choose one possible clinical diagnosis among those proposed. The images are presented in CRB web site, where the participants submit their answers and download the reports containing a judgement and score for the identification of the particles and clinical association and a nephrologist's exhaustive comment. For clinical diagnosis the answer is evaluated only if all 4 particles are correctly identified.

Methods. The participants' answers of the 8 presented clinical cases.

Results. (= n. of participants with access to diagnosis/total, % of correct answers)**:***Acute nephritic syndrome:* 168/325, 86,9%; *Ureteric stone*: 125/310, 95.2%; *Urine contamination from genital secretions:* 251/326, 77.3%; *Nephrotic syndrome:* 257/310, 93,4%; *Acute tubular necrosis due to urate nephropathy:*175/284, 73.7%; *Microscopic isolated haematuria of likely glomerular origin:* 113/285, 97.3%*; Urine contamination from vaginal secretions due to protozoa:* 232/274, 97.8%;*Acute renal failure due to acute rhabdomyolysis:* 160/268, 98.1%.

Conclusions. The high rate of correct answers shows a good knowledge of clinical association for 6 out 8 clinical cases.

The introduction of clinical cases encourages the participants to express interpretative comments on the reports, which is aimed at improving the co-operation between specialists of laboratory medicine and clinicians.

Traceability and standardisation

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A COMPARATIVE STUDY BETWEEN TWO START REAGENT SOLUTIONS IN LDHPRIMARY REFERENCE MEASUREMENT PROCEDURE

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Background. There are two Lactate Dehydrogenase (LDH) primary reference measurement procedures (PRMPs), one is IFCC PRMP-2002, and the other is IFCC PRMP-2002 with the start reagent solution being modified according to IFCC advice. The aim of this study is to address whether the modified start reagent solution had effect on the results ofLDHPRMP.

Methods. Two start reagent solutions were prepared by using β-NADlithium salt and lithium hydroxide, and their effect on accuracy of LDHPRMPwas studied byusing the samples of RELA 2009and certifiedreference material ERM-AD453/IFCC. Also, the start reagent solutionswere incubated at 37°Cwater bath for a different period of 0 to 120 minutes, and then the possible effect of 37°C treatment to the start reagent solutions was evaluated. In addition, MAKER LDH Assay kit and calibrator were evaluated against IFCC PRMP-2002. FollowingCLSI EP9-A2 principles, multiple correlation analyses were carried out by using Microsoft Excel 2003.

Results. With both start reagent solutions, the measurement bias of samples fromERM-AD453/IFCC and RELA 2009 were lower than 1.00%.Forstart reagentsolution with β-NADlithium saltthe measurementresultsdecreased as 37°Cincubationtime increased;while it is opposite for the start reagent solutionwith lithium hydroxide. The correlation between two start reagentsolutions is: y=0.9974x-2.6512 (R^2=0.9999). The correlation between MAKER LDH Assay and the PRMP is: y=0.992x-1.0343 (R^2=0.9998).

Conclusions. In LDH reference measurement procedure(IFCC PRMP-2002),there was no significant difference between the performances of two start reagent solutions that includesβ-NAD lithium salt or lithium hydroxide. At the same time, the traceabilityof Maker LDHAssaywas also verified.

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Traceability and standardisation

1208

EVALUATION OF THE UNCERTAINTY OF LONG-TERM STABILITY ON JAPANESE CERTIFIED REFERENCE STANDARD FOR ENZYMES

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Background. JCCLS CRM-001, Japanese Committee for Clinical Laboratory Standards certified reference standard for enzymes, being composed of 7 kindsenzymesobtained from human genes except LD (erythrocytes) in a matrix of bovine serum albumin, has been widely used in Japan. ISO Guide 34 requires producers to include contributions of possible instability to the overall uncertainty of certified reference material. Uncertainties of the long-term stability for 5 years of CRM-001 in each enzyme of the past two lots were evaluated according to the literature of TPJ. Linsinger et.al.

Methods. Every 6 month for each enzyme stored at -20 °C, 10 measurements (duplicate in 5 vials) were conducted. Uncertainty of the long-term stability (Ults) was estimated.

Results. All enzymes were stable under given storage condition without any loss of the activity. Relative uncertainties of Ults were from 0.4(GGT) to 0.7%(LD) (Average:0.5%) in lot4 and from 0.3(LD) to 0.5%(ALP) (Average:0.4%)in lot5, respectively. These values were comparable to those of the materials certified by IRMM. The values of Ults in 5 years measurements were lower than those in 3 years as expected.

Conclusions. Three lots of JCCLS CRM-001 in lyophilized formulation have been manufactured sofar.Though advantageous in comprising 7 human enzymes all in one vial, CRM-001 has been limitedly used only in Japan because of the lack of the assigned values based on IFCC reference procedures .This stable composition with the values of IFCC reference method would bring new aspect and contribute to global standardization for enzymes in clinical field.

Transfusion medicine

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EVALUATION OF LEUKOCYTE REDUCTION FILTERS FOR PLATELET TRANSFUSION: COMPARISON BETWEEN BIOP-PLUS AND PLX8

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Background. Leukocyte reduction filter is widely used for platelet transfusion therapy, and effective leukocyte removal is mandatory for transfusion safety. We evaluated both the performance of leukocyte reduction filters for platelets and the effects of filtration on platelet functions.
Methods. A total of 100 pooled products (8 platelet concentrates were randomly pooled for each product) were enrolled in this study: 50 products were filtered by BioP-plus (Fresenius Kabi AG, Homburg, Germany) and 50 products by PLX8 (Pall Corporation, East Hills, NY, USA). The Characteristics of the leukocyte reduction filters were evaluated including leukocyte reduction, platelet recovery, and filtration time. Platelet aggregation responses to thrombin receptor activation peptide (TRAP) stimulation were compared in pre- and post-filtration products by using an impedance aggregometry (Multiplate Analyzer, Dynabyte Medical, Munich, Germany).
Results. Leukocyte counts after filtration were uniformly less than 8.3×10^5 in all the products except for 1 pooled product in PLX8. Leukocyte reduction was 99.09% for BioP-plus and 99.71% for PLX8, and platelet recovery was 84.17% for BioP-plus and 86.74% for PLX8. The filtration time of BioP-plus was shorter than that of PLX8. Platelet aggregation responses after filtration tend to decreased before filtration in both filters. The mean values of area under the curve in post-filtration products were 46.56 for BioP-plus and 51.92 for PLX8 showing no difference between the 2 filters.
Conclusions. Both BioP-plus and PLX8 bedside filters for platelets perform well with effective leukocyte reduction and satisfactory platelet recovery. Platelet functions were decreased after filtration procedure with these filters.

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RETROSPECTIVE COMPARISON OF THE TRANSFUSION OF BLOOD PRODUCTS IN THE HOSPITALS OF THE ELBLANDKLINIKEN-GROUP FROM 2005 TO 2010

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Background. In this survey the transfusion of blood products during the years 2005 to 2010 in the hospitals of the Elblandkliniken-Group is investigated. The Elblandkliniken-Group is a clinical complex with four hospitals and about 1.200 beds and consists of clinics for Internal Medicine, Surgery, Orthopaedics and Traumatology, Gynaecology and Obstetrics, Paediatrics, Psychiatry, Ophthalmology, Oto-Rhino-Laryngology and Urology.
Methods. The clinics of the four hospitals of the Elblandkliniken-Group were combined to the following three medical disciplines:
a) predominantly internal working disciplines [Internal Medicine;Paediatrics, Psychiatry, Ophthalmology, Oto-Rhino-Laryngology];
b) predominantly surgical working disciplines [Surgery, Orthopaedics and Traumatology, Gynaecology and Obstetrics,Urology] and
c) intensive care.
The transfusion of the following blood products was statistically evaluated for the years 2005 to 2010: heterologous and autologous red blood cells (RBCs); heterologous and autologous fresh frozen plasma (FFP); platelets; prothrombine complex (PPSB), Antithrombin III (ATIII) and Fibrinogen.
Results. An increase in the transfusion of heterologous RBCs can be observed in all three disciplines from 2005 to 2010. The transfusion of autologous RBCs decreased continuously during this period. In contrast the transfusion of platelets increased in all three medical disciplines. While transfusions of PPSB and Fibrinogen increased, the number of transfusions of ATIII decreased strongly.
Conclusions. The results of this investigation reflect on the one hand the new Guidelines in transfusion medicine. On the other hand the results provide as well information about the modified and new implemented methods of treatment in most disciplines of the hospitals of the Elblandkliniken-Group.

Transfusion medicine

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EVALUATION OF TWO CMV IGG ANTIBODY ASSAYS (ABBOTT ARCHITECT CMV IGG TEST, BIOTEST CMV IGG TEST) IN A TRANSFUSION MEDICINE SETTING

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Background. Assessment of the performance characteristics of two fully automated CMV antibody tests, the Abbott ARCHITECT CMV IgG assay (chemiluminescent microparticle immunoassay [CMIA]) and the Biotest CMV IgG assay (ELISA) in a transfusion medicine laboratory.
Methods. Samples from blood donors were tested by both assays. Samples with discrepant results between both assays were subject to supplemental testing for anti-CMV IgG by two further commercial assays, IgM and CMV DNA by PCR.
Results. Of 4941 samples, 362 (7.3 %) were positive in both assays, 4496 (91.0 %) negative and 83 (1.7 %) samples were discrepant. Supplemental testing for IgG was possible in 82/83 discrepant samples: of 37 samples positive only in the ELISA, 33 were thereafter considered false positive, in two them no definite classification was possible, in two further, CMV DNA was detectable. In 45 samples positive only by CMIA, 7 were false positive, in 22 samples, definite classification was not possible and 16 were confirmed to be true positive. In none of 76 indeterminate samples (7 not tested, including one PCR positive sample) anti-CMV IgM was detectable. The calculated specificity for ELISA and CMIA were 99.27% and 99.84 %, respectively
Conclusions. The specificity of the CMIA is slightly better compared to the ELISA. The detection of CMV DNA in samples positive in the ELISA only remains unclear: in case of an early CMV infection with weak IgG antibodies, anti-CMV IgM typically should be detectable, in case of reactivation of a CMV infection, detection of IgG antibodies should not fail in all other IgG assays.

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RIBOFLAVIN AND IMPACT ON QUALITY OF INACTIVATED BLOOD COMPONENTS

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Background. Riboflavin as a non-toxic compound is used in the process of inactivation of infectious agents in blood components, what can lead to a reduction in the risk of transmission of pathogens in blood products and blood components.
Aim. To evaluate the effect of riboflavin on the quality of platelet concentrates (PC) and plasma (FFP) using a system to inactivate pathogens Mirasol PRT (pathogen Reduction Technology)
Methods. The study used 18 PC and 29 FFP. We have analyzed in PC- pH and the average number of platelets. And for FFP were taken into consideration- activity of factor VIII and the amount of protein.
Results. In 18 platelet concentrates we have noticed: the pH value of 7.14 (SD = ± 0.076) at 0 day to 6.49 (SD = ± 0.3) on day 7 and the number of platelets from 4.06 (SD = ± 0.76) at first day to 3.51 (SD = ± 0.76) on the last day of storage. However, in 29 FFP we have observed: 95% (SD = ± 23.4) activity of factor VIII before inactivation and 70% (SD = ±21.8) after inactivation and 64.25 g / l (SD = ± 3.6) amounts of protein before the trial to 53.9 g / l (SD = ± 3.4) after the trial.
Conclusions. Riboflavin as a natural, non-toxic and non-mutagenic occurring compound in the blood does not affect the quality of inactivated blood components. That fact may be important in the current transfusion medicine because it can contribute to a safer use of blood components and blood products.

Transfusion medicine

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HBS AG, ANTI HCV, ANTI HIV, TPHA AT THE BLOOD DONORS IN KOSOVO

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Background. Blood donation besides saving lives, under special circumstances might be fatal to a patient. Many infectious diseases can be transmitted through blood transfusion viral, bacterial or parasitic diseases. The determining of these parameters is obligatory in blood donors.
Methods. Samples were analyzed via Elisa method on the Abott Tecan, Abott Axsym apparatus.
Results. In the period 2001 – 2009, were tested 114092 blood samples of blood donors, from which 113638 males, and 454 females, of the age range 18 to 60,of different nationalities and social structures. HBsAg positive donors (twice confirmed) were 3469, namely 3.04%. From them 3066 were male (2.69%) and 403 female (0.35%).2001 – 3.93%, 2002 – 5.41%, 2003 – 3.40%;2004 – 2.94%, 2005 – 2.77%, 2006 – 2.64%;2007 – 2.18%, 2008 – 2.06%, 2009 – 2.07%.Anti HCV positive donors were 233, namely 0.20%. From them 191 were male (0.17%) and 42 females (0.03%).TPHA positive were 78, namely 0.07%. From them 64 were male (0.06%) and 14 female (0.01%). Anti HIV positive was only one case in 2002, female. Statistical methods, T-Test have depicted differences in transmissible diseases incidence between these years and based on trend measurements it seems that the presence of HBSAg,HVC,TPHA,HIV positive has a reducing tendency.
Conclusions. In the early post-war years, the number of patients with positive markers in Kosovo was high, while in the last years it has dropped significantly and has a reducing trend .All this as a result of people hard living conditions in Kosovo.

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BACTERIAL SCREENING OF PLATELET CONCENTRATES USING A NOVEL FLOW CYTOMETRIC ASSAY (BACTIFLOW): RESULTS OF A MULTICENTER STUDY

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Background. Bacterial contamination of platelet concentrates (PCs) still represents an ongoing risk. As a result of septic complications, particularly observed with older PCs, the shelf life has been reduced to four days in Germany. In the present study, a novel flow cytometry-based rapid bacterial screening method (BactiFlow, BF) was implemented as a routine in-process control to extend the shelf life of PCs. It was further evaluated in two other German blood services.
Methods. 1834 apheresis-derived PCs were tested using the BF to detect and count bacteria based on esterase activity in viable cells. The BacT/Alert culture system served as reference method. The BF assay was further compared to an FDA-licensed commercially available immunoassay (Pan Genera Detection Technology) with inoculated PCsin an interlaboratory comparison.
Results. Six of the 1834 PCs tested were positive only in culture and identified as *Propionibacterium* species (n = 3) or *Staphylococcus* species (n = 3). However, corresponding bacterial titers were below the BF detection limit (<150 CFU/mL) and had no transfusion relevance. Two PCs were positive for *Staphylococcus aureus* or *Streptococcus mitis*by BF and culture,all remaining specimens were tested negative. Results of the interlaboratory comparison revealed that the BF assay detected all samples correctly (positive: 12/12, negative: 8/8);the PGD test only detected fourof the positive samples.
Conclusions. Bacterial testing of PCs was successfully implemented and the established algorithm proved efficient. The BF assay is the first rapid screening method which is suitable for a routine application combined with a high sensitivity.

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TWO-THIRD OF HUNGARIAN PATIENTS ATTENDING OSTEOPOROSIS AND NEPHROLOGY OUTPATIENT CLINICS HAVE LOW VITAMIN D LEVELS

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Background. Recent surveys indicated that a major part of general population have vitamin D deficiency. In order to elucidate the extent of this problem in Hungary we retrospectively analyzed data on vitamin D levels in patients treated at outpatient clinics of osteoporosis and nephrology at SemmelweisUniversity, Budapest.

Methods. Between March and December, 2009, serum vitamin D3 levels were measured with Elecsys 25-OH vitamin D3 test in 2987 patients (2395 women and 592 men, aged 0 – 91 years). We tested the association of D3 levels with patients' age, gender and month of blood sampling.

Results. The average (±SD) vitamin D3 levels were 27.0±11.3 ng/ml. Sixty-six % and 8% of patients exhibited moderate and severe vitamin D deficiency (defined as vitamin D3 levels under 30 and 15 ng/ml), respectively. Female gender and advanced age were independent risk factors for low vitamin D levels (p < 0.0001 for each). Of note, lowest and highest levels were measured in December and August (21.99±9.66 and 32.49±10.10 ng/ml, respectively) and in women ≥80 years of age (21.17±10.9 ng/ml). Severe vitamin D deficiency was present in 31% of this population.

Conclusions. This study supports a high prevalence of vitamin D deficiency in high-risk populations treated at university hospitals. Screening protocols for vitamin D status are highly needed for general Hungarian population.

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VITAMINS A, E, B12 AND FOLATE LEVELS IN DIFFERENT TYPES OF GLAUCOMA

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Background. The relationship between the increase in intraocular pressure and nutritional and environmental features in patients affected with glaucoma could suggest a role of oxidative stress in the etiology of this pathology. To address this hypothesis, we will determine the levels of the antioxidative vitamins A, B12, E and folate in 48 patients affected with primary open-angle glaucoma (POAG) and 13 patients with normal tension glaucoma (NTG). Results were compared with the data obtained from 78 healthy controls (C).

Methods. Patients were evaluated and selected in the Ophthalmology Department. Serum samples were collected. Vitamins A and E were determined by High Pressure Liquid Chromatography (Bio-Rad). Vitamin B12 and folate were determined by solid-phase competitive chemoluminiscence immunoassay (Architect, Abbott). ANOVA test was used for statistical treatment of data.

Results. The levels were as follows: vitamin A (POAG: 39.7+/-13.2 ug/dL; NTG: 30.4+/-15.8 ug/dL; C: 37.9+/-12.0 ug/dL) (p=0.061); vitamin E (POAG: 1050.4+/-330.5 ug/dL; NTG: 739.9+/-288.0 ug/dL; C: 1008.7+/-232.5 ug/dL) (p=0.001); vitamin B12 (POAG: 404.3+/-198.2 pg/mL; NTG: 471.7+/-177.6 pg/mL; C: 425.7+/-137.7 pg/mL) (p=0.385) and folate (POAG: 6.7+/-3.6 pg/mL; NTG: 5.9+/-3.4 pg/mL; C: 5.7+/-2.8 pg/mL) (p=0.173).

Conclusions. Both pathologies show important differences in the homeostasis of the different vitamins. Patients with POAG show a significant increase in vitamins A and E levels when compared with NTG patients. Indeed, this patients (NTG) show even lower levels of both vitamins than healthy controls. Vitamin deficiency in NTG patients could trigger this pathology trough an increase in oxidative stress.

Vitamins

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THE CONCENTRATION OF VITAMIN D$_3$ IN RELATIONSHIP TO BONE MINERAL DENSITY OF POSTMENOPAUSAL WOMEN IN THE REGION OF EAST SLOVAKIA

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Background. Osteoporosis is a chronic, progressive bone disease in which bone resorption exceeds bone formation, leading to a reduction in bone microarchitecture. The incidence of osteoporosis increases with age and occurs most frequently in postmenopausal women. Many studies have investigated the effect of vitamin D$_3$ and calcium supplements on osteoporosis and fracture risk in postmenopausal women.

Methods. The aim of this paper was to reveal the concentration of vitamin D$_3$ in postmenopausal women (n=100) in the East Slovakia region accordig to bone mineral density (BMD).

Results. The average value ofvitaminD3was28.65µg/L(minimum value 10.68µg/L, maximum value 60.20µg/L). The average value ofbonemineraldensity(BMD) was0.78g/cm^2(minimum value 0.37 g/cm^2, maximum value of 1,10 g/cm^2).

Conclusions. In conclusion, we may state that it is necessary to monitor the selected parameters including osteomalaciaandbone resorptionmarkers within the screening and prevention of osteoporosis.

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PREVALENCE OF VITAMIN D INADEQUACY AMONG THE ADULT POPULATION IN A NORTHERN ITALIAN AREA

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Background. Vitamin D inadequacy, as defined by low serum 25-hydroxyvitamin D [25(OH)D], is commonly recognized worldwide and affects all age groups. Inadequacy includes deficiency, as a 25(OH)D level up to 50 nmol/L, and insufficiency, as from 51 to 74 nmol/L. The study aim was to investigate vitamin D status of an adult population living in a Northern Italian area.

Methods. The evaluation was conducted over the past three years, among the outpatients accessing for Vitamin D testing to Laboratory Medicine of Treviso Hospital, Italy (45° 40' latitude N), by selection of serum 25(OH)D first result. The study included 7545 adults (1449 men and 6096 women, aging from 18 to 104 years). 25(OH)D analysis was performed by chemiluminescence immunoassay with the automated Liaison analyzer (Diasorin, Saluggia, Italy).

Results. The overall mean serum 25(OH)D concentration was 47.7 nmol/L. The vitamin D deficiency prevalence was 55.7% for patients aged 18-49, 56% for 50-69 and 67.9% for ≥70. The vitamin D insufficiency prevalence was 25.9%, 27.2% and 18%, respectively. Vitamin D inadequacy (as deficiency and insufficiency) involved 80.5%, 82.6% and 85.3% of females and 84.7%, 86.4% and 88.5% of males, respectively for the defined age groups. Overall mean serum 25(OH)D had seasonal variation, the highest level (64.5 nmol/L) in August and the lowest (35.1 nmol/L) in April.

Conclusions. The resulted high prevalence of vitamin D inadequacy suggests the extension of vitamin D status evaluation, to recognize hypovitaminosis and related diseases. The availability of automated 25(OH)D assay may easily support this investigation.

Vitamins

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VITAMIN D CONCENTRATIONS IN NORTHEASTERN ITALY: A STUDY IN A FREE-LIVING POPULATION

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Background. Vitamin D, 25(OH)D, levels are affected by both geographic and seasonal variations. Aim of this study was to measure Vitamin D in dwellers of the city of Verona (Italy) and to assess the associations of 25(OH)D concentrations with sun exposure.
Methods. 492 subjects (305 females, 187 males) aged 26-79 years, among the participants to an epidemiological survey, were examined. Subjects with disorders affecting Vitamin D or taking 25(OH)D were excluded. A questionnaire about sunlight exposure was submitted. Blood samples were taken at the end of the summer (November). 25(OH)D concentrations were measured by Liaison (DiaSorin). According to recent reports 25(OH)D deficiency was defined as < 50 nmol/L and insufficiency in the range 50 - 75 nmol/L.
Results. Overall, 56% of participants showed Vitamin D deficiency, 28% insufficiency and only 16% had sufficient levels. Vitamin D deficiency was significantly more common among women (88.7%) than men (3.7%). Values are reported as mean and 5th- 95th percentiles (in parentheses): entire population 52.6 (15.8 – 109.8) nmol/L; women 50.5 (16.2 – 107) nmol/L; men 55.7 (15.6 - 110) nmol/L. Serum concentrations were significantly associated ($p < 0.001$) with self-reported sun exposure: exposure (n = 249) = 58.5 (21.3-112.6) nmol/L, no exposure (n=120) = 39.7 (14.1 –83.7) nmol/L.
Conclusions. Women living in Verona have a high prevalence of vitamin D deficiency even at the end of the sunny season. Our study confirms that serum 25(OH)D concentrations are influenced by sun exposure.

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FOLATE, VITAMIN B$_{12}$ AND HOMOCYSTEINE LEVELS IN HEALTHY YOUNG ADULTS IN SARAJEVO, BOSNIA AND HERZEGOVINA

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Background: The aim of the present work was to investigate relationship between folate, vitamine B12 and homocysteine in the serum and plasma of healthy young population in Sarajevo, Bosnia and Herzegovina.
Materials and Methods. Our study included 72 participants (mean age 24.8±2.6 years) of both sexes (35% man and 65% woman). Venous blood samples were collected and analyzed at the UniversityClinicalCenter in Sarajevo. Plasma concentrations of total homocysteine were determined by fluorescence polarization immunoassay (AxSYM® Homocysteine, Abbot, USA), and serum concentrations of active B12 (holotranscobalamin, holoTC) were determined by microparticle enzyme immunoassay (AxSYM® Active-B12, Abbot, USA). Serum folate levels were determined using ion capture technology (AxSYM® Folate, Abbot, USA).
Results. Mean levels of folate, holoTC and total homocysteine were 8.42±4.44 ng/mL, 45.3±21.0 pmol/L and 9.48±2.03 µmol/L, respectively. In the whole group, folate but not holoTC was found to be significant predictor of total homocysteine (p=0,003 and p=0,087, respectively). When divided in subgroups of low normal folate (3.10-4.70 ng/mL), medium normal (4.75-14.0 ng/mL) and high normal (14.05-17.00 ng/mL), none of the two vitamins was useful predictor of total homocysteine in any of subgroups. Still, significant (p<0.05) difference was found for total homocysteine in low normal vs. high normal folate subgroup (10.90±1.40 vs. 6.97±0.74 µmol/L).
Conclusions. Our results confirm that folate is useful predictor of total homocysteine even in healthy subjects.

Vitamins

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CORRELATION OF METHYLENETETRAHYDROFOLATE REDUCTASE MUTATIONS WITH HOMOCYSTEINE METABOLISM IN HEALTHY LEBANESE ADULTS

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Background. Common mutation in methylenetetrahydrofolate reductase (MTHFR), an enzyme required for efficient homocysteine metabolism, results in a thermolabile enzyme with reduced activity. The goal of this study is to correlate MTHFR gene polymorphism (C677T and A1298C) with homocysteine, folate, and vitamin B12 in healthy Lebanese.
Methods. Biochemical analysis were done in serum of 233 individuals (male/female =109/124) using Abbott Axsym for homocysteine and Roche Modular analytics for folate and Vitamin B12. MTHFR was assayed by PCR using DNA from EDTA blood.
Results. 17 individuals were wild for both. The distribution of the combination C677T/A1298C was: 66 wild/hetero, 21 wild/homo, 1 homo/homo, 22 homo/wild, 4 homo/hetero, 47 hetero/hetero, 53 hetero/wild, and 1 hetero/homo. The proportion of subjects with abnormal serum folate (<4.2 ng/ml)was 7.3%, vitamin B12 (<243 pg/ml) 37.8%, and homocysteine (>15 umol/L) 22.3% with the majority of the later subjects being males (n=39; 75%). Body mass index (BMI) >25 Kg/m^2 was found in 39.5% (male/female =63/29). The homo/wild group had the highest mean homocysteine (21.9 umol/L; range 8.4-63.1); however, without signifcant differences between groups. The same applies to folate, vitamin B12, and BMI. There was a significant negative correlation between homocysteine and folate (r= -0.277; p=0.000), vitamin B12 (r= -0.249; p=0.000), and combination of the two (r= -0.277; p=0.000).
Conclusions. Increased homocysteine was not related to MTHFR polymorphism in Lebanese. However, enhancing vitamin B12 and folate status may significantly decrease homocysteine.

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SERUM FOLIC ACID LEVEL AND HEPATOTOXICITY OF METHOTREXATE IN RHEUMATOID ARTHRITIS

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Background. Methotrexate (MTX) inhibits the activity of dihydrofolate reductase, resulting in a decreased supply of folats. Therapy with MTX can induce a lot of side effects, one of which is liver dysfunction with increases in aminotransferases. The aim of this study, therefore was to evaluate the incidence of MTX induced hepatotoxicity and its risk factors among rheumatoid arthritis (RA) patients.
Methods. This study described 42 patients with RA who received 7.5-10.0 mg MTX weekly, more than one year. We undertaken to determine serum ALT activities and folic acid levels in samples 30 patients with folate supplementation therapy and 12 patients without it. Folic acid was measured by an immunochemical technique, and ALT using standard biochemical Methods.
Results. The results showed elevated ALT levels in approximately 18.8 % (6) of patients from group with supplementation therapy and 58.3% (7) of patients without folic therapy. The highest level of ALT was 96 IU/L. In first group 59.4% (19) of patients had elevated levels of serum folic acid (>20.0 ng/mL). In second group 91.6% (11) of patients had low levels of serum folic acid (< 3.0 ng/mL).
Conclusions. Hepatotoxicity is a common complication of long term treatment with MTX. It is associated with mild liver enzyme elevation and related to the duration of therapy. Supplementation therapy with folate is associated with a reduced incidence of serum transaminase elevation. Thus, folate supplementation may prevent hepatotoxicity in patients taking MTX.

Vitamins

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ABBOTT ARCHITECT 25-OH VITAMIN D ASSAY COMPARED AGAINST LC-MS/MS

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Background. There is an urgent need for reliable automated immunoassays due to the increasing demand of vitamin D (25(OH)D) testing. The new ARCHITECT 25-OH Vitamin D assay (Abbott) was compared against LC-MS/MS in a teaching hospital with an annual workload of ~50,000 samples.
Methods. The precision of the ARCHITECT assay was determined across the dynamic range using assay controls and samples pools in a 5 day protocol. Correlation with LC-MS/MS was evaluated on 300 specimens and 40 DEQAS samples. All samples were tested twice in separated runs on both systems. Recovery of 25(OH)D2 on the ARCHITECT assay was determined.
Results. The inter-assay precision (%CV) was 7.8%, 3.7% and 3.1% for the Low, Medium and High assay controls, respectively and 4.5% , 3.5%, and 4.7% for low, medium and high sample pools. The functional sensitivity (10 %CV) was determined as 10 nmol/L (4 ng/mL). The mean recovery of 25(OH)D2 was 76.7% (95% CI: 58.2% to 95.2%). Passing-Bablok Correlation with local LC-MS/MS was 0.98 x LC-MS/MS + 2.29 (Pearson correlation = 0.86). On 40 DEQAS samples the ARCHITECT results were in good agreement with the LC-MS/MS (proportional bias 0.96). The ARCHITECT assay and LC-MS/MS showed high reproducibility with slopes of 1.01 and 1.02 (r=0.99 and 0.98).
Conclusions. The fully automated ARCHITECT 25-OH Vitamin D assay exhibits excellent precision and correlates well with LC-MS/MS. The assay is suitable for monitoring patients taking exogenous vitamin D providing appropriate clinical vigilance is applied for patients under vitamin D2 supplementation.

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VITAMIN B1 AND B6 ANALYSIS: COMPARISON OF METHODS BETWEEN LABORATORIES ENROLLED IN AN EXTERNAL QUALITY ASSURANCE PROGRAM

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Background. The Royal College of Pathologists (RCPA) Quality Assurance Program (QAP) offers a monthly proficiency testing program for vitamins A, B1, B6, β-carotene, C and E to laboratories worldwide. A review of results submitted for the whole blood vitamin B1/B6 sub-program revealed a wide dispersion, particularly for vitamin B1. This observation led to the development of a methodology questionnaire for participants. Here we describe the survey results for vitamins B1 and B6.
Methods. A detailed questionnaire was sent to the 12 participating laboratories in 2010. Eleven of these laboratories where returning QAP results for vitamin B1 (thiamine pyrophosphate) and five laboratories where returning results for vitamin B6 (pyridoxal-5-phosphate).
Results. Nine of 12 laboratories completed the questionnaire. Most (8/9) respondents provided a clinical service for vitamin B1 and B6 utilising HPLC with fluorescent detection. For vitamin B1, 75% of respondents used a commercial assay kit whilst 25% ran in-house methods; whole blood was the matrix for all. For vitamin B6 only three respondents reported measuring whole blood vitamin B6. Sample preparation for both vitamins incorporated protein precipitation and derivatisation. An internal standard was reported to be added during sample preparation by one survey respondent; this was to the only combined vitamin B1/B6 assay.
Conclusions. The outstanding result of this survey is the absence of an internal standard to what are relatively complicated chromatographic Methods. The Vitamins Working Party strongly recommends that companies and laboratories with vitamin B1 and B6 assays review their method/s to include an internal standard into the procedure.

Vitamins

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USEFULNESS OF SERUM HOLOTRANSCOBALAMIN II AND/OR VITAMIN B12 IN DIAGNOSIS OF VITAMIN B12 DEFICIENCY

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Background. Vitamin B deficiency is still a widely spread health problem. It may take up to 20 years until clinical symptoms of vitamin B12 deficiency become overt. As some of this symptoms like neurological disorders already might be irreversible at time of diagnosis, efficient laboratory screening tests for vitamin B12 deficiency are necessary.
Methods. We investigated 996 healthy individuals with normal renal function (GFRmayo >60ml/min/1.73m²) and no vitamin supplementation in measuring vitamin B12 and holotranscobalamin II (HTC) to evaluate which of these parameters are prone to be used as an early laboratory marker for vitamin B12 deficiency. Reference values were 216ng/ml for vitamin B12 and 43ng/ml for HTC; methylmalonic acid (MMA) values >0.3ng/ml as external validation criterion for vitamin B12 depletion and the cut-off for folic acid were >3.5ng/ml.
Results. Among all subjects 88 (8.8%)showed avitamin B 12 deficiency. In receiver operating characteristic curve (ROC) analysis correlation of HTC and vitamin B12 with vitamin B12 deficiency was moderate and the areas under the curve (AUC) were significantly different for HTC compared to vitamin B12 in subject with vitamin B12 deficiency (AUC: 0.76 versus AUC: 0.71; p=0.0308). The areas under the curve were not statistically different for HTC compared to combined HTC and vitamin B12, indicating no additional information in measuring vitamin B12 in addition to HTC alone.
Conclusions. HTC is a more reliable indicator than total serum vitamin B12 in this healthy population with normal renal function and should be used to screen for vitamin B12 deficiency.

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ELECSYS® VITAMIN D (25-OH) TOTAL: FIRST EVALUATION RESULTS OF THE EARLY ADOPTER PROGRAM

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Background. The assessment ofVitamin D sufficiency plays a major role in the diagnosis of bone metabolism disorders and is increasingly an important diagnostic factor in many other diseases.Aim of the present study was to evaluate the technical performance of a newly developed Elecsys® Vitamin D assay.
Methods. The Elecsys® Vitamin D (25-OH) Total assay is a fully automated competitive protein binding assay based on the biotin-streptavidin technology and calibrated against the LC-MS-MS.
Results. Within-run and between-run imprecision coefficients of variation (CV) were determined between 1.9% - 5.4% (range: 15.0 – 55.2 ng/mL), and 2.9% - 11.6% (range: 7.5 – 52.6 ng/mL), respectively. Using the 20% CV criteria the functional sensitivity was found at 4 ng/mL by regression analysis. A method comparison study with the LC-MS-MS using 194 serum samples of the laboratory routine (Vitamin D3 levels: 4 – 66.3 ng/mL) yielded a slope of 1.020 and an intercept of 0.686, respectively according to the Passing/Bablok regression. A Pearson correlation coefficient was determined at r = 0.9204. Finally, the comparability of Vitamin D test results was studied in 50 matched serum and heparin plasma samples (Vitamin D3 levels: 3 – 59 ng/mL). Highly similar results were observed across the studied measuring range. The Passing/Bablok regression yielded the following equation: y = 1.017x + 0.268; r = 0.9925.
Conclusions. Our data confirm an excellent analytical performance of the newly developed Elecsys® Vitamin D (25-OH) Total assay. Therefore it is qualified to be a valuable diagnostic tool in the assessment of Vitamin D sufficiency.

Vitamins

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A RETROSPECTIVE STUDY OF VITAMIN D DEFICIENCY IN SERBIAN POPULATION

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Background. Vitamin D deficiency and insufficiency are present, according to some estimations, in over one billion people all over the world. Up to date, no study was conducted to evaluate the presence of vitamin D deficiency in Serbian population.

Methods. We have searched the database of the laboratory information system and analyzed the data from over 2000 patients whose 25-hydroxyvitamin D_3 (25-OHD$_3$) was determined in the period from November 2008 to December 2010 in the Department of Polyclinic Laboratory Diagnostics of Clinical Center of Serbia. In 978 of these patients, 20–90 years old, besides 25-OHD$_3$, intact parathyroid hormone (iPTH), calcium (Ca), ionized calcium (Ca^{2+}) and inorganic phosphorus (P) were determined. These data were included in subsequent statistical analyses, together with age and gender. 25-OHD$_3$ was determined using Roche electrochemiluminescence immunoassay on analyzer Cobas e601.

Results. Median population values of 25-OHD and iPTH were 14.5 ng/mL and 60 pg/mL, respectively. Examined population was devided into four groups according to 25-OHD$_3$ concentrations (<10, 10-20, 20-30 and >30 ng/mL). ANOVA analyses showed significant decrease in iPTH(P=0.009), Ca (P=0.031) and Ca^{2+} (P=0.037) across the 25-OHD$_3$ concentration categories. Stepwise multiple linear regression analyses indicated independent correlation of iPTH with 25-OHD$_3$ concentration ($β$ = -0.160, P=0.005). Also, one-way ANOVA with Tukey's *post-hoc* test demonstrated that 25-OHD$_3$ concentrations measured in summer were significantly (P=0.020) higher compared to those determined in winter.

Conclusions. More than 74% of the examined population had 25-OHD$_3$ below 20 ng/mL, which is minimal desirable concentration, acompanied with increase in iPTH concentration.

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CONCENTRATION OF UNMETABOLIZED FOLIC ACID IN SERUM AFTER FOLIC ACID SUPPLEMENTATION

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Background. Folic acid (FA) has been considered safe for many decades. Pregnant women and older adults are advised to use FA supplements. There is currently a debate of whether free FA in serum can be harmful.

Methods. We investigated the concentrations of free FA and folate forms in pregnant women (n=61) and elderly people (n=74) with/without FA supplementation at different doses according to the MTHFR C677T genotype.

Results. Median plasma total folate, 5-methyltetrahydrofolate (5-methylTHF), and formylTHF were significantly higher in supplemented (n=25;400 $μg$ FA/day) than in non-supplemented pregnant women. 43.6% of the pregnant women had detectable FA. However, FA in plasma was not predicted by FA supplements. We also tested the effect of 3 weeks supplementation with 5 mg FA or placebo on free FA in serum of older adults. At baseline, 26% had detectable amounts of unmetabolized FA in serum, which was increased after FA intake (n=37; median baseline FA concentration 0.08 vs. 15.3 nmol/L after treatment). In addition, total folate and 5-methylTHF were significantly increased after supplementation. Unmetabolized FA correlated significantly with 5-methylTHF and THF after supplementation. Moreover, the MTHFR C677T polymorphism seemed to have no effect on free FA in non-supplemented people. The effect of the mutation on post-treatment FA is being evaluated.

Conclusions. FA supplementation increased total folate and 5-methylTHF in serum. Unmetabolized FA was present in non-supplemented people and was mostly converted to 5-methylTHF and THF after supplementation. This argues against a harmful effect of FA, but further studies are needed.

Vitamins

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CONCETRATIONS OF VITAMINS A AND E AND TRACE ELEMENT ZN IN DIFFERENT TYPE OF ANIMALS

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World of animals is full of surprise even for routine work in laboratory, for example estimation of vitamins A and E and trace element Zn**.** Different species (mammals, birds, snakes), heterogeneity in enviroment and food reflect in wide spectrum of vitamin needs in animals.
The measuring was carried out by HPLC kit RECIPE, Zn estimated by AAS-F.
Biological material:serum.
We measure very low concetrations (vitamin A-0,13 mg/l or vitamin E-0,33 mg/l) it isn´t error of the instrument but we estimated biological material of elephant. Higher concentrations of these vitamins were estimated in even-toed ungulate of Africa and Asia animals (herbivorous average vitamin A-0,5 mg/l, vitamin E average 2,5 mg/l), concentrations vitamin A-average 0,8 mg/l and vitamin E-average 10 mg/l in wolf and bear are more similar to man.
Concentrations common in hyena vit.A-3,5mg/l and flamingoes vit.E-150 mg/l vit.E would be dangerous for man (vit.A 0,5-1,0 mg/l , vit.E 5-20 mg/l).
The same situation is setting in findings of extreme concetrations of Zn. We estimated 358,4 mmol/l Zn, it is sample of python (man - reference interval lower then 15 mmol/l).
The results are very usefull for breeder in ZOO Garden.

1230

CONCENTRATIONS OF VITAMINS A AND E AND TRACE ELEMENT ZN IN DIFFERENT TYPE OF ANIMALS

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Backround. World of animals is full of surprise even for routine work in laboratory, for example estimation of vitamins A and E and trace element Zn**.** Different species (mammals, birds, snakes), heterogeneity in enviroment and food reflect in wide spectrum of vitamin needs in animals.
Methods. The measuring was carried out by HPLC kit RECIPE, Zn estimated by AAS-F.
Biological material:serum.
Results. We measure very low concetrations (vitamin A-0,13 mg/l or vitamin E-0,33 mg/l) it isn´t error of the instrument but we estimated biological material of elephant. Higher concentrations of these vitamins were estimated in even-toed ungulate of Africa and Asia animals (herbivorous average vitamin A-0,5 mg/l, vitamin E average 2,5 mg/l), concentrations vitamin A-average 0,8 mg/l and vitamin E-average 10 mg/l in wolf and bear are more similar to man.
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Conclusions. The results are very usefull for breeder in ZOO Garden.

Vitamins

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ASSOCIATION OF VITAMIN 25[OH]D3IN YOUNG WOMEN WITH METABOLIC SYNDROME

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Background. The prevalence of vitamin D3 insufficiency is high among obese subjects and low levels of 25[OH]D3 have been associated with increased risk of metabolic syndrome (MetS). We aimed to assess which components of MetS are related with serum concentration of vitamin 25[OH]D3 in young women.
Subjects and Methods. The study group included 98 young women aged 20-40 yrs with excessive body mass (BMI≥25 kg/m2). Fifty nine of them were diagnosed to have Mets based on the definitions of the International Diabetes Federation (2005). Serum was assayed for lipids, glucose, insulin (Architect ci8200, Abbott Diagnostics) and vitamin 25[OH]D3 (Elecsys 2010, Roche Diagnostics GmbH). Insulin resistance was estimated by homeostasis model assessment (HOMA-IR). Blood pressure was measured.
Results. Serum vitamin 25[OH]D3 concentration was lower in women with MetS than in women without MetS (18± 8 ng/mL vs. 24± 13 ng/mL; p=0.01). Vitamin D3 insufficiency (<30 ng/mL) was more prevalent in women presenting with MetS, compared to those who did not achieve the MetS criteria (71% vs. 47%, respectively).When serum concentrations of 25[OH]D3 were categorized in tertiles, there was a decreasing prevalence of MetS in women with increasing concentrations of vitamin D3 (T1 vitamin 25[OH]D3<16 ng/mL – MetS 36%; T3 vitamin 25[OH]D3 >24ng/mL – MetS 22%). Insulin resistance was found in 59% of women with Mets and 25[OH]D3 insufficiency. In MetS inverse correlation of 25[OH]D3 with HOMA-IR (r=-0,31; p<0,01) and insulin was found (r=-0,26; p<0,05).
Conclusions. Insulin resistance was the only component of metabolic syndrome essentially associated with 25[OH]D3 insufficiency in young women.

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VITAMIN D IN PROBLEM ORIENTED REQUEST PANEL "VAGUE COMPLAINTS"

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Background. Vitamin D deficiency can lead to myopathy and osteomalacia. More recently it was brought in relation with many chronic diseases including malignancies, autoimmune diseases, infectious diseases and cardiovascular diseases. In a pilot study conducted in 40 patients with "vague complaints" 18 patients (45%) had a concentration below 50 nmol / L, the recommended level in The Netherlands.
Methods. The GPs in our region agreed to add vitamin D to the problem-oriented request panel "vague complaints". After one year this policy was evaluated.
Results. In 2010, the panel "vague complaints" was applied by GPs in 4148 patients. The average vitamin D concentration was 62 ± 30 nmol / L (range 10-311 nmol / L). In 34% of these patients, vitamin D was below 50 nmol / L. A severe vitamin D deficiency (<20 nmmol / L) was found in 5%. Low vitamin D deficiency was by far the most frequent abnormal result in the request panel "vague complaints". In comparison, the second most frequent abnormal test in this panel was ESR (13%).
Conclusions. A remarkably high percentage of people with vague complaints suffer from a vitamin D deficiency. Adding vitamin D to the problem-oriented request panel "vague complaints" is therefore considered useful. Further research will show whether there is a causal link between a vitamin D deficiency and vague complaints.

Vitamins

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VITAMIN D INTOXICATION: A CLINICAL CASE

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Background. Vitamin D is a fat-soluble vitamin involved in the absorption of calcium and phosphorus in the intestine, contributing to the mineral content in bones and teeth. Administration of high doses for prolonged periods can cause hypercalcemia, leading to kidney failure and renal calcifications.

Clinical case. Male, 6 months old in treatment with Biominol® (a vitamin D supplement), was admitted to the emergency service of this hospital because of a state of decay and irritability. The initial analytical results shows ionic calcium concentration in blood of 2.11 mmol/L (reference values (RV): 1.15 to 1.29 mmol/L), and a plasma total calcium concentration of 22.0 mg/dL (RV: 9 to 11 mg/dL). It was decided to transfer to the Pediatric Intensive Care Unit of the hospital for continuous cardiorespiratory monitoring and was treated with hydration and diuretics to correct hypercalcemia.

In our laboratory, vitamin D2 and D3 were determined by liquid chromatography high resolution (HPLC), referring to the status of body reserves of calcium, and an electrochemiluminescence methodthat measures total vitamin D. The results were vitamin D2 419 ng/mL and total vitamin D 482 ng/mL (RV: 30 to 100ng/mL). It was found that vitamin D intoxication was of exogenous origin, due to increased vitamin D2, and it was confirmed by the medical staff, who verified that the patient was administered an incorrect dosage.

Conclusions. The definitive diagnosis of this patient was severe Hypercalcemia secondary to exogenous vitamin D intoxication, and Nephrocalcinosis secondary to this with normal renal function with hypercalciuria.

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SEASONAL FLUCTUATIONS OF D3(25-OH) VITAMIN LEVEL IN PATIENTS' SERUM IN EAST-TALLINN CENTRAL HOSPITAL

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Background. Physiological level of vitamin D3 in organism depends on its supply through food as well as on its biosynthesis from 7-dehydrocholesterol in skin under influence of ultraviolet in sun rays (UV-B of sunlight, wave length 290-315nm).

Synthesised vitamin D3 is hydroxylated in liver with forming of 25-hydroxyvitamin D3, which is the main circulating metabolite of vitamin D. Despite the fact that biologically active form of vitamin D is $1,25(OH)_2$, which is synthesised in kidneys, detection of 25-OH is used as a method to diagnose hypovitaminosis D.

Aim. Influence of ultraviolet radiation on vitamin D3(25-OH) balance in human body based on data collected from East-Tallinn Central Hospital.

Method. Measuring of 25-hydroxyvitamin D3 was performed using electrochemiluminiscence immunoassay (Roche Diagnostics, Cobas e411). The reference range was from 50 to 80 nmol/l.

Results. The data was obtained from HIS for 2009. The study sample consisted of 515 patients, 78% women, 22% men (28-80 years).Average level of vitamin D3(25-OH) in patients' serum by month: January –38,2nmol/l, February-39,9nmol/l, March-41,6nmol/l, April-49,8nmol/l, May-50,7nmol/l, June-55,6nmol/l, July-70,2nmol/l, August-59,6nmol/l, September-54,5nmol/l, October-53,8nmol/l, November-43,8nmol/l, December-37,1nmol/l.Percentage of patients with level of vitamin D3(25-OH) >50nmol/l by month: January-19%, February-31%, March-33%, April-50%, May-58%, June-59%, July-75%, August-69%, September-67%, October-52%, November-37%, December-21%.

Conclusions. Level of vitamin D3(25-OH) in patients serum in East-Tallinn Central Hospital has an expressed seasonal dependency.

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DETERMINATION OF REFERENCE INTERVALS FOR VITAMINS A AND E IN HEALTHY PEOPLE BY HPLC

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Background. The quantification of vitamin A and E levels is especially important in premature infants, cancer patients, and malabsorption disease or patients with parenteral nutrition.
To establish vitamins A and E reference interval in Toledo health area.
Materials and Methods. We analyzed50serum samples from healthy donors,40 males and10females in range of 22- 61 years, following the pre analytical recommendations for samples manipulation as dark storage and fast centrifugation. The chromatographic analysiswascarriedoutbyHPLC 1200 series (Agilent Technologies) equipped withanEclipseXDB-C18 column andUVlight detector. Methanoland distilled water were used asmobile phases,previously to the chromatographic analysis vitamins were extracted using our protocol.
Results. The data follows normal distribution according to Kolmogorov-Smirnov test. The mean value for vitamin A was 0.61mg/Lwitha standard deviation of0.1619, being thelower limit0.29mg/l(90% CI (0.232 to 0.363)) andtheupper limit0.93mg/l (90% CI (0.867 to 0.998)). The mean value for vitamin E was 18.36withaSDof5.37mg/ml, thelower limitbeing7.84mg/ml(90% CI (5.664 to 10.02)) andthe upper limitis28, 89mg/ml(90% CI (26.715 to 31.071)).
Conclusions. Ourreference intervals are similartothosedescribedinliterature.

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D VITAMIN IN PATIENTS WITH CANCER DISEASES

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Background. The aim of this study was to determine the frequency of D vitamin hypovitaminosis in patients with malign cancer diseases.
Methods. Serum levels of 25-OH vitamin D was measured using 25-hydroxy vitamin D radioimmunoassay (RIA() manufactured by Immunodiagnostic Systems, Ltd., UK in 300 patients with colorectal, lung, prostate and breast (pre- and postmenopausal) cancer. Serum levels were correlated with disease stage.
Results. 83 patients (83%) with colorectal cancer, 50 patients (62.5 %) with breast cancer, 59 patients (73.6%) with lung cancer and 16 patients (40%) with prostate cancer had significantly low serum levels of D vitamin (lower than 40 nmol/L).
Authors have confirmed the correlation between disease stage and serum levels in patients with colorectal lung and premenopausal breast cancer. No correlation was found for postmenopausal breast cancer and for prostate cancer.
Conclusions. Authors found high incidence rate of extreme hypovitaminosis D in cancer patients among Czech population. This rate is significantly higher when comparing literature data. Correlation with disease stage was found.

Vitamins

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METHODOLOGICAL AND CLINICAL EVALUATION OF THE LIAISON AUTOMATED ANALYSER FOR THE QUANTITATIVE DETERMINATION OF 25-OH VITAMIN D

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Background. Meaning of vitamin D (VitD)has been expanded beyond traditional bone health.However, validation of methods is mandatory.
Aim and Methods. To evaluated the performance of the "LIAISON 25-OH Vitamin D TOTAL"by DiaSorin (Italy).
Results. The equivalence between serum and lithium-heparin plasma samples (n=11) did not evidence significant difference. The effect of light and ambient temperature was investigated (n=10 samples): 1 set maintained at room temperature, one on ice, one exposed to direct sunlight and another set covered since 4 hours before assaying (p=ns). Concentrations resulted unaffected from multiple (n=2-5) freeze-thaw cycles.
Fresh samples (time 0) were repeated at different time (1, 6 and 24 h) on aliquots maintained at 4° C (p=ns). However, a 16% (p<0.001) mean loss of the initial value was observed after a 4-month storage period at -20°C. Within run: 7–10.6% and total precision: 3.5–11.5%. Mean (SD) recovery: 105 (7)%. The assay was linear on dilution (r=0.97). Comparison with DiaSorin RIA yielded acceptable correlation (r=0.83) and clinical equivalence (range 6-55 ng/mL).
The assay was evaluated in a general population (age: 60±16 years, 27 males), without observing any difference according to gender. VitD resulted inversely related to age (r=-0.2 p≤0.05) and parathyroid hormone levels (r=-0.35 p≤0.001).Ninety-four subjects (71%) resulted vitamin D-insufficient (<30 ng/mL). Seasonal changes according to exposure to sun light were observed (p≤0.05).
Conclusions. The LIAISONR 25-OH VitD method is reliable. Hypovitaminosis D is common also in subjects without apparent risk factors for vitD deficiency.

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SERUM 25-HYDROXY VITAMIN D AND FRAILTY IN ELDERLY CHINESE

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Background. Vitamin D deficiency causes muscle weakness and bone diseases.This study aimed to establish a method for 25-hydroxyvitamin D (25OHD, D2/D3) measurementusing LC-tandem mass spectrometry (LC-MS/MS), to investigate whether it isrelated to the frailty in elderly chinese.
Methods. 25OHD2/D3 was extracted from serum or plasma with methanol-isopropanol mixture containing hexadeuterated 25OHD3 internal standard. The supernatant was injected into a C18 analytical column, and 25OHD was quantitated by API 5000 mass spectrometer in the positive electrospray ionization mode. Frail status was evaluated according tothe outcome ofweak hand-grip strength, slow walking speed, weight loss, exhaustion, and low physical activity, and was classified as robust, prefrail, or frail.
Results. The intra-assay imprecision (CV%) for 25OHD2 and 25OHD3 was 6.4% and 5.2%, respectively, and inter-assay imprecision 13.2% and3.7%, respectively. Compared withSMR 972 from NIST, the average recoverieswere100% for 25OHD3, and 103% for 25OHD2. 25OHD concentration in elderly patients (mean=26.09, SD=7.87, N=75) were higher than healthy (mean=17.89, SD=5.80, N=60). LC-MS/MS and Elecsys 2010 chemiluminescence immunoassay showed moderate correlation: y=0.9807X+1.471 (r=0.736, N=66). No significant difference was found in 25OHD concentration using three blood collection tubes (SST, EDTA, or HEPARIN)with LC-MS/MS or Elecsys 2010 assay. Serum 25OHD concentration tended to decrease from robust to frailty, but no significant difference was found (N=75).
Conclusions. The established LC-MS/MS method is accurate for 25OHD measurement. Although no significant relation found between 25OHD concentration and frailty, the prevalence of vitamin D insufficiency was high in our population.

Miscellaneous

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PROTECTIVE EFFECTS OF BLUEBERRY ON CCL4 INDUCED ACUTE HEPATOTOXICITY IN RATS

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Background. Flavonoids and phenolic acids which are contents of blueberry fruit, have high level of antioxidant and antiinflammatory effects. We think that this feature of blueberry fruit can reduce inflammation and the level of damage on liver tissue. In our study, we aimed to search the effect of blueberry fruit on acute liver damage which made by CCl_4.

Methods. We applied blueberry fruit as tea and wine, to the rats. Totally 32 rats were divided into four groups (n=8). Groups are arranged as; 1. normal diet; 2. normal diet and liver damage; 3. liver damage and blueberry tea; 4. liver damage and blueberry wine. Following day of the study completion (8th day), we investigated some biochemical (SOD, CAT, TBARS, serum oxidation, TEAC, FRAP) and histopathological parameters on liver tissue and blood samples.

Results. We found both histopathological (lowering of steatosis, ballooning degeneration and lobular necro-inflammation scores and increase of mitosis score) and biochemical (increase in tissue CAT and SOD activity, decrease in TBARS level and serum oxidation) parameters indicates that blueberry tea can reduce the toxic effect of CCl_4 on liver through its phenolic, anthocyanidin and flavonoids contents.

Conclusions. These findings suggest that, its useful effects (hepatoprotective effect, increase of liver regeneration) may improve, when we give blueberry tea for a longer time and higher dose. On the other hand, this study showed that blueberry wine which has higher phenolic activity, has no significant protective effects on CCl_4induced acute liver damage.

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THE EFFECT OF ANKAFERD ON BONE FRACTURE HEALING

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Background. Ankaferd BloodStopper® (ABS) is an herbal extract used as a haemostatic agent. Whether ABS benefit on bone fracture healing process is unknown well.

Methods. The rats were divided into seven groups of 8 each. Group 1: control, group 2: Rats were observed for 7 days after femur fracture. Group 3: Rats were observed for 7 days after applied ABS (0.5 ml) on femur fracture. Group 4: Rats were observed for 21 days after femur fracture. Group 5: Rats were observed for 21 days after applied ABS on femur fracture. Group 6: Rats were observed for 45 days after femur fracture. Group 7: Rats were observed for 45 days after applied ABS on femur fracture. After treatments, blood was taken for biochemical analyses.

Results. No statistical difference was observed for bone morphogenic protein-2 (BMP-2) and fibroblast growt factor-2 (FGF-2), bone formation markers (osteocalcin, alkalinephosphatase) and bone resorption markers (pyridinoline, deoxypyridinoline) studied among the groups. However, BMP-2 and FGF-2 in serum of rats treated with ABS for 45th day were suppressed in the group 7 compared to group 6 (P=0.017, P=0.006 repectively). Deoxypyridinoline in serum of rats treated with ABS for 45th day was higher in the group 7 than in the group 6 (P=0.003).

Conclusions. We concluded that the administration of ABS on fracture healing in the presence of femur fracture decelerates to bone fracture healing. On the other hand, ankaferd has been approved in the management of external hemorrhage and dental surgery bleedings in Turkey.

Miscellaneous

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CHANGES OF C-AMP LEVEL DURING ESTRUS CYCLE IN PINEAL GLAND IN NORMOTENSIVE AND SPONATANEOUS HYPERTENSIVE RATS

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Background. The aim of this study was to determine the basal levels of c-AMP in the pineal gland during the various phases of estrus cycle in normothensive (NTR) Wistar rats and spontaneously hypertensive (SHR) Okamoto and Aoki rats and to describe the pineal gland histological finding.
Methods. A number of 200 female mature rats (100NTR and 100SHR) were investigated, divided in 4 groups: diestrus, proestrus, estrus and metaestrus, determined by microscopic analysis of the vaginal smears. The level of c-AMP (RIA method, Amersham) in the pineal gland was the parameter of its intracellular activity.
Results. In SHR there is a slight shortening of the oestrus cycle. In NTR there was statistically significant increase of the c-AMP level ($p<0.01$) from diestrus (815 ± 30 pmol/gr tissue) proestrus (1240 ± 86 pmol/gr tissue) estrus (1350 ± 102 pmol/gr tissue) and to metaestrus (1580 ± 123 pmol/gr tissue). In SHR c-AMP was found to be 1080 ± 50 pmol/gr tissue in diestrus, 870 ± 55 pmol/gr tissue in proestrus, 970 ± 75 pmol/gr tissue in estrus and 1220 ± 110 pmol/gr tissue in metaestrus, but only in proestrus phase statistically significant decrease was found ($p<0.01$). Between two groups, NTR and SHR, only in proestrus statistically significant difference was found ($p<0.01$). Histological findings in SHR showed the presence of changed pinealocytes with picnotic nucleuses, while the neuroepithelial cells were separated in gland-like islets. In NTR no histological changes were found.
Conclusions. This study indicated significant neurohormonal differences within the cycle phases and between NTR and SHR. The changed adrenal activity in SHR correlated with histological findings in the pineal gland.

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QUANTITATIVE DETERMINATION OF LYSOZYME IN TEARS BY RATE NEPHELOMETRIC ASSAY ON IMMAGE® 800 (BECKMAN COULTER). METHODOLOGY AND CORRELATION STUDY

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Background. The lysozyme, a bactericidal enzyme produced by lacrimal glands, constitutes one of the principal proteins of tears which contribute to protect the cornea and the conjunctiva. Its quantitative determination allows us to explore the lacrimal function in dry eye syndromes and other pathologies of the ocular surface.
Methods. Determination of the lysozyme was carried out by rate nephelometry with Immage®800 (Beckman-Coulter) using human calibrator and specific antibodies on a very small volume of tears collected with capillaries ($21\,\mu l$ of 1/60 prediluted samples). Limit of detection and between-run imprecision were evaluated. Study of correlation was carried out between this technique and two other techniques: electrophoresis on agarose gel pH 9 (n=100) and radial immunodiffusion (n=12).
Results. The average and standard deviation carried out by rate nephelometry on 40 samples with a normal electrophoretic profile gave usual values, closed to those of the literature (1.74 ± 0.20 g/l). This technique presents a very good correlation with radial immunodiffusion ($R^2 = 0.99$) and with electrophoresis techniques ($R^2 = 0.86$).
Conclusions. The quantitative assay of lysozyme in tears by rate nephelometry is a specific and precise method, usable on a very small volume of tears and the determination of the major lacrimal proteins may help with the etiologic diagnosis and the follow-up of different pathologies of the ocular surface.

Miscellaneous

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COMPARABILITY AND IMPRECISION OF EIGHT FREQUENTLY USED COMMERCIALLY AVAILABLE IMMUNOASSAYS FOR THERAPEUTIC DRUG MONITORING

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Background. Goal of this study was to evaluate assay comparability and imprecision of eight immunoassays for therapeutic drug monitoring using six different analyzers from three manufacturers.
Methods. The following drugs were measured using left-over routine samples: Carbamazepine (Carb), digoxin (Dig), phenobarbital (Pheno), phenytoin (Pheny), theophylline (Theo), tobramycin (Tob), vancomycin (Vanc), valproic acid (VPA). Analytical systems were *cobas c* 501 and COBAS INTEGRA® 800 (Roche Diagnostics GmbH), AxSYM® and ARCHITECT® (Abbott Laboratories), Dimension Xpand® and Dimension Vista® (Siemens Healthcare Diagnostics Inc).
Results. Methods were compared by Passing/Bablok regression analysis against COBAS INTEGRA 800 analyzer as an arbitrary standard and within manufacturers. The majority of results had slopes of 1.0±0.15, intercepts <1/10 of low-end therapeutic range, and r>0.97. The observed exceptions were as follows: Carb slope COBAS INTEGRA to Xpand/Vista 1.21/1.19; Dig slope COBAS INTEGRA to ARCHITECT/Vista 1.20/1.16, AxSYM to ARCHITECT 1.20, increased intercepts in five comparison studies; Pheno slope within Abbott 1.17, intercept COBAS INTEGRA to AxSYM 1.17; Tob intercept COBAS INTEGRA and ARCHITECT to AxSYM 0.20/-0.16; Vanc intercept COBAS INTEGRA and Xpand to Vista 1.10/1.09. The following CVs were obtained using 2 replicates in 20 runs on 10 days and the respective test-specific low-level control: Carb 3.0–6.8%, Dig 6.8–9.7%, Pheno 3.0–7.7%, Pheny 2.9–9.1%, Theo 3.0–7.5%, Tob 5.9–10.3%, Vanc 4.0–7.7%, VPA 3.9–5.4%.
Conclusions. With the exception of Carb, Pheno, Vanc, and particularly Dig, a good overall method comparability could be demonstrated indicating an improved assay harmonization for therapeutic drug monitoring.

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ESTIMATION OF ALERT AND CHANGE LIMITS AND ITS APPLICATION IN THE PLAUSIBILITY CONTROL

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Background. In the clinical laboratory one of the most objective ways to perform the final review of patients' results is the use of the *plausibility control.*This study focuses on the estimation of alert and change limits (usually called *deltacheck*), tools commonly applied to detect doubtful results in this process.
Methods. Patients' results from year 2008 of serum creatininium and glucose concentration are used to estimate alert and change limits. Three alert intervals are estimated using the percentiles p5.00 and p95.00, p0.50 and p99.50, and p0.05 and p99.95from patients' Results. Three change limits are also estimated using the percentiles p90,0, p99,0 and p99,9 from relative differences (between a patient's result and the previous result of the same patient). Moreover, alert and change limits are estimated separating data in two subgroups: inpatients and outpatients. To check the reproducibility of the model, data from year 2009 are used.
Results. In serum creatininium concentration, the application of alert and change limits obtained with all data, provide until 53% more of doubtful results in inpatients and until 144% less in outpatients than the application of limits obtained with each subgroup. In serum glucose concentration, this comparison provide until 37% more of doubtful results in inpatients and until 150% less in outpatients.
Conclusions. It should be necessary to use different alert and change limits for inpatients and outpatients, since the application of only ones limits to both subgroups make that the percentage of doubtful results can be overestimated in inpatients and underestimated in outpatients.

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LIPID PROFILE AND SOME ANTROPOMETRIC PARAMETERS IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Background. Polycystic ovarial syndrome (PCOS) is one of the most frequent endocrinological disorders in reproductive period of women and is one of the most frequent reasons for infertility. There is increased risk in these patients for cardiovascular disease, Diabetes, endometrial carcinoma etc.
Methods. In this study evaluation of some antropometric parameters (body mass index-BMI and waist/hip ratio) and lipid profile in patients with PCOS has been made. The study population consisted of 120 patients with PCOS, at the age of 17-39 and age matched control group of 30 healthy women. According to BMI and fasting glucose/insulin ratio patients were classified in four groups: NL (normoinsulinemic lean, BMI from 18 to 25 Kg/m^2), NO (normoinsulinemic obese, BMI > 25 Kg/m^2), HL (hiperinsulinemic lean) and HO (hiperinsulinemic obese). Fasting serum level of insulin was determined with ELISA method. Serum levels of glucose, total lipids, triglycerides, total and LDL and HDL cholesterol were determined with standard enzymatic photometric tests after 12-hours fasting.
Results. In our study increased values for BMI and waist/hip ratio in NO and HO patients were detected in comparison with control group (p<0.01), as well as in comparison with NL and HL patients with PCOS (p<0.01). The results of our study have shown disturbances in lipid profile in patients with PCOS.
Conclusions. Continous evaluation of parameters of lipid profile in patients with PCOS, reduction of body weight, hyperinsulinemia and dislipidemia treatment is necessary for timely prevention and decreasing the risk of development of cardiovascular and cerebrovascular diseases in these patients.

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OXIDIZED DNA IN PATIENTS WITH POST-TRAUMATIC STRESS DISORDER

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Background. Post-traumatic stress disorder (PTSD) is an anxiety disorder developed after a traumatic experience. Considering that free radicals mediate neuronal damage in several psychiatric disorders, we wanted to investigate the possible difference in the concentration of oxidized DNA (8-hydroxy-2`-deoxyguanosine, 8-OHdG) as most significant marker of DNA oxidative damage in PTSD patients in comparison to the healthy control group.
Methods. The study included 47 male PTSD patients with a median age of 42 (32 - 62), and 43 healthy male controls with a median age of 39 years (31 - 58). Oxidized DNA was determined in urine samples of both patients and controls using ELISA kit (Bühlmann, Schönenbuch, Switzerland).
Results. The median value of 8-OHdG in the urine samples of PTSD group was 75 ng/ml (29 - 320) and in the control group 8-OHdG concentration was 87 ng/ml (27,5 - 160).
Mann-Whitney test did not show statistically significant difference between values for 8-OHdG between the studied groups (P=0,726).
Conclusions. Our results show that oxidative damage of DNA molecule in PTSD group did not differ from healthy control group. Considering that literature data suggest that other anxiety disorders like obsessive-compulsive disorder could be related to free radicals influence, our results contribute to the efforts to biologically differentiate PTSD from other anxiety disorders.

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COMPARISON OF TWO DIFFERENT ASSAYS FOR THE QUANTIFICATION OF REGULATORY T CELLS (TREGS) IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE(IBD) – FLUORESCENCE ACTIVATED CELL SORTING (FACS) VS. DNA DEMETHYLATION

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Background. Expression of forkhead box protein P3 (FoxP3) in regulatory T cells (Tregs) is essential for their suppressor activity. Although detailed regulation of FoxP3 expression on both the protein and DNA level is not yet fully understood it was proposed as a surrogate marker for Treg activity indicating the relative strength of the suppressive action on the immune system. In this study, we compared two different assays for the quantification of regulatory T cells based on both immunophenotyping and DNA demethylation at a Treg-specific demethylated region (TSDR) on the *FOXP3* gene.

Methods. Peripheral mononuclear blood cells were isolated by density gradient centrifugation from 21 patients with inflammatory bowel disease. CD4+CD25high FoxP3+ cell ratio (compared to total CD4+ cells) was determined using flow cytometry and fluorescence labelled antibody conjugates. FoxP3-specific DNA demethylation ratio was quantified using quantitative real-time PCR.

Results. Numbers of FoxP3+ events within CD4+ cells did not significantly correlate with the ratio of FoxP3-specific demethylated DNA (p=0.452, Spearman rank correlation). A significant correlation was found for Foxp3+ counts by flow cytometry in relation to the self-reported general well-being of patients with inflammatory bowel disease (p=0.028, Kruskal-Wallis-Test). In contrast, the percentage of FoxP3-specific demethylated DNA did not correlate with the general well-being (p=0.212, Kruskal-Wallis-Test).

Conclusions. These preliminary data indicate that flow cytometric quantification of FoxP3 may correlate better with disease activity in patients with inflammatory bowel disease as compared to an FoxP3-specific demethylation assay. Both FoxP3 assays need to be further evaluated in clinical studies to assess their clinical use.

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HLA DRBI, DQBI AND DQAI ALLELES' FREQUENCIES IN NILO-SAHARAN AND FELLATA POPULATIONS OF SUDAN

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Background. HLA Class II alleles are pivotal for initiation and control of immune responses against intra-cellular parasites, can determine susceptibility/resistance to diseases and can be helpful in transplantation and vaccines development. Little is known about HLA alleles and haplotypes of the Nilo-Saharian and Fellata populations of the Sudan. This study aims to determine the frequencies of HLA class II alleles (DRBI, DQBI, DQAI) in an internally displaced Nilo-Saharan and Fellata populations of the Sudan, mostly from the Masalit (Nilo-Saharan; Zurga) and the Fellata (Bargo/Maba/Wadians) tribes. These groups immigrated to the visceral leishmaniasis endemic areas of eastern Sudan more than five decades ago.

Methods. The alleles were identified using the PCR-Sequencing based Typing (PCR-SBT) technique.

Results. A total of 39 alleles were detected, 21 in DRB1 locus, 11 in DQB1 and 7 in DQA1. Among the 21 DRB1 locus alleles: DRB1*1101, DRB1*0804, DRB1*1001 and DRB1*0301 were the most commonly observed with frequencies of 25.3%, 24.1%, 10.6% and 6.5% respectively. The most predominant alleles at DQB1 locus were DQB1*0501 (39.5%), DQB1*0301 (34.9%), with a less prevalent DQB1*0201 allele (7%). The DRB1*1503 allele which was reported exclusively in groups of African descent could not be detected in the study population. While in the DQA1 locus, DQA1*0501 (39.3%) and DQA1*0102 (35.1%) alleles were the most prevalent followed by DQA1*0101 (16.6%). The highest level of allelic heterogeneity was observed within the allelic groups DRB1*04 (5 alleles) and the DQB1*06 (5 alleles). Other allelic groups showed limited polymorphism (1-2 alleles). DRB1*0804-DQA1*0501-DQB1*0301 and DRB1*1101-DQA1*0102-DQB1*0501 haplotypes were the most prevalent in these ethnic groups of Sudan with frequencies of 34% and 27.7% respectively.

Conclusions. The limited number of alleles reported in these ethnic groups can be explained by the fact that they live in closed communities where consanguineous marriages are the rule. The study ethnic groups appear to share ethnic backgrounds with other populations including, sub-saharan Africans, north- Africans, Arabic-speaking groups and the Amhara.

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A NEW FRUCTOSAMINE REFERENCE RANGE FOR THE PATHCARE PATHOLOGY GROUP, SOMERSET WEST, SOUTH AFRICA

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Background. Fructosamine together with glycated haemoglobin (HbA1c) are used to monitor the state of hyperglycaemia in diabetics. The evolvement of automation in Clinical Chemistry necessitates that each pathology laboratory provides relevant sets of reliable reference values that are population and analyzer/method specific.
Methods. Four hundred and forty two healthy subjects (120 white females, 116 white males, 112 females and 94 males of mixed ancestry origin) visiting the Somerset PathCare laboratory were recruited into this study. Serum fructosamine, random blood glucose, HbA1c, total protein, albumin, and lipid profiles were preformed on all subjects. Nonparametric methods were used to determine the reference values for fructosamine.
Results. Though no significant differences (p = 0.086) were observed between males and females in the total population group, mixed ancestry males had significantly higher fructosamine levels (p = 0.01) than their female counterparts. The reference range of the entire sample was 224 – 294 μmol/L, however it differed in the different population groups (white females = 230 – 289 μmol/L; white males= 226 – 292 μmol/L; mixed ancestry females = 223 – 275 μmol/L; mixed ancestry males = 225 – 301 μmol/L).
Conclusions. The new fructosamine reference range is higher than the one currently in use at PathCare (200 – 285 μmol/L).Our results further strengthen the recommendations by pathology bodies that laboratories must establish reference values that are representative of local populations.

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IDENTIFICATION OF *AEROMONAS* SPECIES BY MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

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Background. To assess the performance of the MALDI-TOF MS for *Aeromonas* strains identification.
Methods. 26 clinical isolates of *Aeromonas,* previously identified by biochemical tests and RFLP of 16S gene, including *A. caviae* (20), *A. hydrophila* (3) and *A. trota* (3) were analyzed. Controls were *A. hydrophila* ATCC7966, *A. caviae* ATCC14486 and *E. coli* ATCC25922. MALDI-TOF analysis were performed in a Bruker Autoflex I MALDI-TOF mass spectrometer with a nitrogen laser (337 nm) operating in linear mode with delayed extraction at 20 kV accelerating voltage. The matrix was alpha-cyano-4-hydroxycinnamic acid. Processing of the data was done using FlexAnalysis and Speclust softwares.
Results. Multiple ions in the 3,000 to 20,000Da mass range were present in the spectra. Clustering of the mass spectral fingerprints showed a clear separation between *E. coli* and the *Aeromonas* group. *Aeromonas* were separated in two principal groups, one containing the *A. trota* strains, and other with two distinct clusters containing the strains of *A. hydrophila* and *A. caviae* respectively.Four mass ions, having an average m/z of 5050.58±1, 6303.28±1, 7206.14±1, and 10302.8±2,were common to all *Aeromonas* species tested, and may be potentially used as genus-specific markers for this bacteria. Mass ions 6085.21±1, 8806.7±1, 9379.51±1, and 9992.86±1were observed only for*A. trota;* while mass ions4447.54±1 and 6022.48±2 only for *A. hydrophila*, and may also be useful for the identification of this *Aeromonas* species.
Conclusions. MALDI-TOF MS analysis is useful for identification of bacteria belonging to *Aeromonas* genus and allows separation of species and strains.

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RELIABILITY OF EVEROLIMUS DETERMINATION ON THE VIVA VITALAB ANALYZER

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Background. Everolimus is a potent immunosuppressive agent with anticarcinogenetic proprieties that is use in many kind of solid organ transplantation and in different oncologic therapies. Until de recent incorporation of the QMS® turbidimetric method, the only technology available for Everolimus monitoring was HPLC-MS. Alternatively, it was a common practice to use a method based on chemioluminiscent technology for rapamicin monitoring (Sirolimus) in the measure of everolimus concentration.
Methods. 45 whole blood samples from stable kidney transplantation patients were studied for everolimus concentrations in two lab analyzers: Viva-Vitalab® (based on QMS turbidimetric assay) and Architect i2000® (based on chemioluminiscent microparticle immunoassay technology (CMIA) for quantitative determination of Sirolimus®). HPLC-MS was also used as reference method.
Results. The functional sensitivity and detection limit for QMS method was 0.79ng/mL and 1.5ng/ml respectively. The total coefficient of variation at a level of 5ng/mL was of 13% and the mean recovery rate, as a measure of inaccuracy, was 94.6%.
When we compared QMS turbidimetric assay results with that obtained with Architect Sirolimus® assay, correlation coefficient was 0.908, at a 95% confidence limit of 0.835-0.949.
When comparing with HPLC-MS, both analysers showed a correlation coefficient higher to 0.8.
Conclusions. Reliability of the QMS turbidimetric method on VIVA-Vitalab analyzer for the quantitative determination of Everolimus is acceptable. From a technical point of view and for practicability purposes, QMS Turbidimetric method can be replaced with Architect Sirolimus assay on the Architect i2000 platform.

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DETECTING PARAPROTEINS WITH MEASUREMENT OF SERUM INDEX ON SIEMENS ANALYSERS DIMENSION

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Background. HIL (hemolysis, icterus, lipemia) represents the measurement of serum index on Siemens analysers Dimension to detect spectral interferences on biochemical tests. Ocasionally we find unusual high values for lipemia whereby the serum is clear. This signals are due to interference of immunoglobulins, mostly paraproteins in the sample. We further tried to evaluate the signals on Dimension Vista and RXL Max instruments and clarify the correlation to serum amyloid A (SAA).
Methods. The measurements of serum index were made automatically in serum samples on Dimension Vista and RXL Max analyser.The results are reported as a three figure number in scales from 1-8 for Vista and 1-6 for RXL Max. The impact of interferences grows with each respective class. The measurements of SAA were made on Siemens BN II nephelometer, the serum protein electrophoresis and immunofixation on Sebia analyser.
Results. In the years 2006-2010 we detected 202 different patients with unusual signal HIL 114-118 on Vista or HIL 114-116 on RXL Max. In 87% a monoclonal peak was found with serum electrophoresis and immunofixation method. In contrary, the signal was present only in 25% of all samples with a monoclonal peak, probably due to formation of immunoglobulin complex, as amyloid or chryoglobuline can be. The concentrations of SAA do not correlate with unusual HIL signals. SAA indicates more the activity of disease.
Conclusions. The laboratory should pay a full attention to unusual lipemia signals as they can be of high importance in undiagnosticed patients with multiple mieloma.

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THE INFLUENCE OF PYOSPERMIA ON BIOCHEMICAL MARKERS OF PROSTATIC FUNCTION AND SPERM QUALITY

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Background. Presence of leukocyte in ejaculate in concentration greater then million/mL, is common finding in infertile men. It is often associated with decreased levels of zinc (Zn), acid phosphatase (AcP) and gamma-glutamyltransferase (GGT), sugesting disturbed function of prostate gland. The aim of study was to evaluate relationship between leukocyte concentration and biochemical markers of prostatic function and theire impact on sperm quality. Concentration of zinc, AcP and GGT were determined in seminal plasma. According to concentration of leukocytes (Le), they were divided into: I group with Le$<1,0x10^6$/mL and II group with Le$\geq1,0x10^6$/mL. Additionally, patients were divided into group A (AcP $>0,96$U/L, Zn$>1,50$mmol/L, Le$<1,0x10^6$/mL) and group B (AcP $\leq0,96$U/L, Zn$\leq1,50$mmol/L, Le$\geq1,0x10^6$/mL).
Results. We found significant differences in all parameters between I and II group in patients with oligoasthenozoospermia
Methods. Semen samples from 348 patients were analyzed according to WHO standards. Based on sperm concentration, motility and normal morphology, patients were divided into normozoospermic and oligoasthenozoospermic groups. (AcP, $p<0,001$; Zn and GGT, $p<0,01$; sperm concentration, $p<0,005$; motility and normal morphology, $p<0,05$). No such differences were observed in patients with normozoospermia. Comparing group A with group B we found significant differences in sperm concentration and motility in all patients.
Conclusions. Deterioration of semen quality may be caused by reactive oxygen species derived from elevated leukocyte count, as well as by reduced protection of spermatozoa against oxygen radicals caused by decrease of GGT activity. Decreased protective role of reduced Zn concentration and decreased proteolytic effect of low AcP activity may contribute to the problem.

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NEW LOWER REFERENCE LIMITS IN HUMAN SEMEN EXAMINATION

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Background. Recently a new edition of the WHO manual (5th Ed) for the examination and processing of human semen has been published establishing new reference values for the semen characteristics. The biggest differences are found in parameters of morphology, sperm concentration, motility and semen volume. The objective of this study was to asses the impact in the implementation of this new values in the results of the semen analysis performed in our laboratory during the years 2005 to 2009.
Methods. A total of 1313 semen samples were collected by masturbation after 2 to 7 days of sexual abstinence. Basic semen analysis was done according the WHO manual (4th Ed) assessing the following parameters: volume, pH, total sperm number and concentration, motility and morphology.
Results. Applying the reference values of the WHO manual (4th Ed), 320(24.5%) samples were considered hypospermic (with less than 2 ml volume); 895(67.9%) normozoospermic ; 89(6.8%) azoospermic; 10(0.8%) cryptozoospermic; 306(23.3%) oligozoospermic; 13(0.9%) polyzoospermic; 731(55.2%) asthenozoospermic; and 398(31.4%) teratozoospermic.
Applying the reference values of the WHO manual (5th Ed), 227(17.3%) samples were considered hipospermic; 964(73.4%) normozoospermic; 89(6.8%) azoospermic; 10(0.8%) cryptozoospermic; 237(18.1%) oligozoospermic; 13(0.9%) polyzoospermic; 517(39.4%) asthenozoospermic; and 33(2.5%) teratozoospermic.
Conclusions. Applying the new lower reference limit values there is a significant decrease in sperm abnormalities found, mainly teratozoospermia, reduced in 28.9%, asthenozoospermia in 15.8%, hypospermia in 7.2% and oligozoospermia in 5.2%.
The most common alteration found using both reference values is asthenozoospermia followed by oligozoospermia (5th Ed) and teratozoospermia (4th Ed.).

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CATALASE GENERATES HYDROXYL RADICALS: A NOVEL SELF PRUNING ACTIVITY WITH POSSIBLE CLINICAL SIGNIFICANCE

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Background. Catalase plays very important role in regulation of hydrogen peroxide metabolism. Altered catalase activity has been observed in many pathological conditions. In mammals the role of catalase needs to be further explored. Its proposed mechanism of action has loopholes and many new findings are still unexplained, thus it is called mysterious enzyme. Based on our experimental findings, we hereby propose the 'Hydroxyl radical generation (HRG) theory' to fill the loopholes.
Methods. All the experiments were performed with Bovine liver catalase in standard conditions. HRG by catalase was measured at different concentrations of hydrogen peroxide with and without urea. Tryptophan and tyrosine fluorescence spectra were recorded during the experiments.
Results. HRs were always thought as products due to impurity of ferrous ion in catalytic reaction or due to denaturation of catalase. Our experimental findings prove that catalase in native form, apart from catalytic reaction, itself generates HRs. This HRG was found to be mainly dependent on substrate (hydrogen peroxide) concentration and was also affected due to change in native structure of catalase. The mechanism of HRG was also proposed and accordingly, this HRG activity seems to be required for self pruning of catalase.
Conclusions. The HRG by catalase is novel mechanism which fills the gaps of unexplained findings. On reaction with these HRs, amino acids of other proteins can undergo stepwise oxidation. This type of modifications has been implicated in cell signaling, aging and apoptosis. Therefore, the role of catalase as oxidant is of relevance in human health and diseases.

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KNOCK-DOWN OF HUMAN XYLOSYLTRANSFERASE EXPRESSION DECREASES COLLAGEN GENE EXPRESSION

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Background. Fibrotic diseases affecting heart, liver, lung or skin often are characterized by increased biosynthesis and subsequent accumulation of proteoglycans in the ECM. Xylosyltransferase I (XT-I) and XT-II were identified to catalyze the rate limiting step in proteoglycan synthesis. Moreover, fibrotic remodelling was shown to be accompanied by increased XT expression.
Methods. Human dermal fibroblasts were treated with sequence specific small interfering RNA targeting XYLT1 or XYLT2 mRNA. XT expression and activity was monitored by quantitative real-time PCR and enzymatic activity assays. Relative mRNA transcript levels were determined for selected collagens, proteoglycan core proteins and enzymes playing key roles in glycosaminoglycan (GAG) biosynthesis. We further quantified GAG composition by reverse-phase high performance liquid chromatography.
Results. We observed a significant decrease in total GAG content. In particular, results revealed decreased chondroitin sulfate and hyaluronic acid concentrations whereas heparan sulfate concentrations remained unaffected. Proteoglycan core protein biosynthesis as well as gene expression of selected glycosyltransferases, was not altered by repression of XT-expression. Interestingly, we observed significantly decreased expression of collagen 2 alpha 1 (COL2α1), COL3α1 and COL5α1 by up to 90% due to XT-I repression. Moreover, suppression of XT-II expression led to a strong downregulation of COL1α1 and COL3α1 transcript levels (at least 0.5-fold).
Conclusions. Our results strengthen the significance of XT expression and activity in active fibrotic ECM remodelling. Moreover, this easily accessible model now opens opportunities to develop strategies to control and inhibit fibrotic ECM remodelling by interfering with XT expression and activity using siRNA, aptamers or inhibitory molecules.

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COMPLETE GENOME ANALYSIS OF *STREPTOCOCCUS GALLOLYTICUS* SUBSP*. GALLOLYTICUS,* AN EMERGING PATHOGEN OF INFECTIVE ENDOCARDITIS

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Background. Infectious endocarditis is a disease with rising incidence and a high degree of mortality and morbidity. PCR analysis of heart valves revealed a significant relevance of *Streptococcus gallolyticus* subsp. *gallolyticus* (Sgg)(former *S. bovis* biotype I) as a causative agent, whereas the underlying pathomechanisms are still unclear.
Methods. The complete genome sequencing of Sgg was performed by using the 454 sequencing platform. GenDB and EDGAR software were used for genomic analysis. PCR analysis was used for the detection of microbial surface component recognizing matrix molecules (MSCRAMMs). Southern blotting was used for plasmid screening.
Results. The genome of Sgg strain BAA-2069 contains a 2,356,444 bp circular DNA molecule with a G+C-content of 37.65% and a 20,765 bp plasmid designated as pSGG1.Bioinformatic analysis predicted 2309 ORFs and the presence of 80 tRNAs and 21 rRNAs in the chromosome. 21 ORFs were detected on the plasmid pSGG1, including tetracycline resistance genes *telL* and *tetM*. Screening of 41 Sgg isolates revealed one plasmid (pSGG2) homologous to pSGG1.
Furthermore, we identified 21 surface proteins of MSCRAMM containing the cell wall-sorting motif LPxTG, which were shown to play a functional role in the pathogenesis. Screening of 41 strains for these MSCRAMMs revealed a heterogeneous distribution correlating with binding characteristics to ECM-molecules.
In addition we conducted a whole genome comparison to recent sequenced Sgg strain UCN34, revealing significant differences.
Conclusions. The analysis of the whole genome sequence of Sgg advanced the understanding of genetic factors concerning pathogenesis and adhesion to ECM of this pathogen.

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THE USE OF CARDIAC BIOMARKERS IN DETECTION OF CARDIOTOXICITY INDUCED BY CONVENTIONAL AND HIGH-DOSE CHEMOTHERAPY FOR ACUTE LEUKEMIA

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Background. Monitoring of chemotherapy-induced cardiotoxicity with multiple biomarkers of cardiac injury – glycogen phosphorylase BB (GPBB), heart-type fatty acid binding protein (H-FABP), cardiac troponins (cTnT, cTnI), creatine kinase MB (CK-MB mass), myoglobin.
Methods. 47 adult acute leukemia patients were studied – 24 patients treated with conventional chemotherapy (CT) containing anthracyclines (ANT, cumulative dose 463.2±114.3mg/m2) and 23 patients treated with high-dose CT (HD-CT) followed by hematopoietic cell transplantation (HCT). Cardiac biomarkers were measured before treatment (before CT/HD-CT), after first and last CT with ANT in the first group; after HD-CT and after HCT in the second group. Values above the reference range recommended by the manufacturers (Randox, Roche) were considered elevated.
Results. Before CT/HD-CT, all biomarkers were below the cut-offs. GPBB increased above the cut-off (7.30µg/L) in 4 (16.7%) patients after first CT and in 5 (20.8%) patients after last CT with ANT. GPBB increased above the cut-off in 5 (21.7%) patients after HD-CT and remained elevated in 5 (21.7%) patients after HCT. CTnI became elevated (above 0.40µg/L) in 2 (8.3%) patients after first and last CT with ANT. Both patients with cTnI positivity had elevated GPBB. Other biomarkers remained below the cut-offs.
Conclusions. Our results suggest that GPBB could become a sensitive biomarker for detection of acute cardiotoxicity associated with conventional and HD-CT for acute leukemia. The predictive value for development of cardiomyopathy in the future is not known. Based on our data, a larger prospective and multicenter study would be most desirable.

Miscellaneous

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FALSELY DECREASED IONIZED CALCIUM RESULTS IN PATIENTS TREATED WITH LEFLUNOMIDE (ARAVA®)

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Background. A 65-year-old kidney transplant recipient received Leflunomide (60 mg/day) for biopsy proven BK virus nephropathy. Unexpected low ionized calcium levels were found. Clinically, the patient did not show signs of hypocalcaemia and serum total calcium, albumin, total protein and PTH levels were normal. Similar results were found for other kidney transplant recipients treated with Leflunomide and assay interference was suspected.
Methods. Ca^{2+} results of different patients taken Leflunomide were measured on three different blood gas analyzers (Siemens) and followed in time. In addition, spiking experiments using different concentrations of the active metabolite of Leflunomide, A771726, were performed.
Results. For the initial patient, Ca^{2+} was below the lower reference value on all our three blood gas analyzers (1.06, 0.66, and 0.76 mmol/L, respectively). The lowest results were found on the analyzer equipped with the oldest Ca^{2+} electrode. These effects were confirmed by analyzing more patients and using an I-Stat point-of-care analyzer. After stopping Leflunomide Ca^{2+} concentrations returned to normal, without an effect on total calcium. Spiking experiments using A771726, in clinically relevant concentrations, confirmed the suspected analytical interference. The effect of Leflunomide on Ca^{2+} results was concentration dependent.
Conclusions. Leflunomide (60 mg/day) interferes with the measurement of ionized calcium and results in falsely decreased concentrations while not effecting the total plasma calcium concentration. This effect is reversible and depends on the age of the Ca^{2+} electrode. Awareness of this effect and the use of total calcium concentrations are important to avoid inappropriate and potentially harmful treatment.

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THE EFFECT OF APRIL-TARGETED RNA INTERFERENCE ON COLORECTAL CANCER IN NUDE MICE IN VIVO

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Background. Colorectal cancer (CRC)is one of the most common cancer, worldwide. APRIL (A proliferation-inducing ligand) can stimulate the growth and proliferation of tumor cells, especially in CRC. In our previous work, we have constructed APRIL siRNA expression plasmids and demonstrated that APRIL siRNA could inhibit the expression of APRIL in a CRC cell-line SW480 in vitro, however, the interfering efficiency of APRIL siRNA in vivo still remains unknown.
Methods. CRC xenografted tumor in BALB/c nude mice were treated with APRIL siRNA as follow: The mice were treated with APRIL siRNA through intratumoral injection when forming a 100-200 mm3 tumor by inoculating SW480 cells subcutaneously. The growth rate, size, weight and inhibitory rate (IR) of tumors were deteced. The level of APRIL was examined by PCR, immunohistochemistry (ICH) and Western Blotting. The expressions of the cytokines related to proliferation (PCNA, Ki67), apoptosis (bcl-2, bcl-xl) and metastasis (MMP-2, MMP-9) were detected by IHC or TUNEL experiments application to the apoptosis.
Results. The level of APRIL, PCNA, Ki67, MMP-2 and MMP-9 decreased markedly in APRIL siRNA groups compared with PBS or control groups, $P < 0.05$. The IR of tumors was (55.1±1.4) % in APRIL siRNA groups. Furthermore, the expression of bcl-2 and bcl-xl in APRIL siRNA groups decreased by (82.6±4.5) % and (79.2±3.5) % compared with control groups, TUNEL results was the same.
Conclusions. CRC xenografted tumors were significantly inhibited in APRIL siRNA groups and it might be thought as one important targeting gene for gene therapy of human CRC.

Miscellaneous

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CHANGES IN SRANKLE, TLR2 AND TNFÎ± IN PATIENTS WITH OSTEOARTHROSIS

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Background. Joint destruction and excessive bone loss in osteoarthrosis(OA) and rheumatoid arthritis are associated with changes in expression of some cell receptors and molecules as well as in sera levels of some cytokines.
Aim. To examine the level of soluble receptor activator of nuclear factor kB ligand (sRANKL) in sera and its expression on neutrophil leukocytes in patient with active and non active osteoarthrosis. In the same patient TLR2 expression on neutrophil leukocytes and TNFα secretion in nonstimulated and stimulated neutrophils to be analized.
Methods. Blood neutrophils were obtained by gradient centrifugation on Histopaque 1083 after dextran sedimentation. Concentrations of sRANKL and TNFα in sera were determined by enzyme-linked immunosorbent assay (ELISA). RANKL and TLR2 expression by neutrophils were analyzed by flowcytometry.
Results. The percentage of RANKL and TLR2 positive blood neutrophils was higher in patients with OA than in healthy donors. The neutrophil leukocytes in patients with ostheoarthosis release greater amount of TNFα compared with healthy controls.
Conclusions The results of this study prove that higher levels of sRANKL might be used as a marker of osteoarthrosis. Neutrophils can participate in inflammatory processes mediated by RANKL, TLR2 and TNFα.

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EVALUATION OF TONOMETROL, A QUALITY CONTROL MATERIAL FOR PO2 IN THE ULTRA LOW RANGE

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Background. Quality control measurements of pO2 in the ultra-low range (2-3 kPa; 15-22 mmHg) tend to have large ranges of variation due to poor oxygen buffering capacity. Also, response greatly differs over instrument types. Tonometrol is a pre-tonometered real hemoglobin-based QC material with blood-like oxygen-buffering. In this study, we evaluate the performance of Tonometrol, a pre-tonometered hemoglobin-based QC material with blood-like oxygen-buffering
Methods. Tonometrol was utilized by the Wales External Quality Assessment Scheme (WEQAS) in their EQA/PT program. Data of over 1400 PT/EQA participants were compared to results of a similar data set using an aqueous proteinated material. Analysis was done both in the peer groups by analyzer and overall.
Results. Within 22 peer-groups, the aqueous QC material yielded a wide variety of mean values (31.5 – 84.8 mmHg) and SD (3.1 – 10.9). Tonometrol had superior commutability in terms of correspondence of target values (12.4-19.3) as well as SD (0.75-4.88). In a joint analysis combining all 22 peer groups, the aqueous QC resulted in a mean of 54.7 mmHg with SD 17.9 while Tonometrol resulted in a mean of 15.5 with SD 3.5.
Conclusions. Instrument performance in the critical low pO2 range can be efficiently validated with Tonometrol. Due to its excellent commutability, the material is very suitable for application in EQA/PT schemes. The oxygen buffering and saturation curve of Tonometrol makes the QC behave very blood-like, simultaneously eliminating the need to apply a special QC mode and lowering the threshold of the verified reportable range.

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DETERMINATION OF GLUTATHIONE DISULFIDE IN HUMAN BLOOD BY AN HPLC WITH FLUORESCENCE DETECTION. SAMPLE PREPARATION IS ESSENTIAL FOR ACCURATE ANALYSIS

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Background. Oxidation of glutathione (GSH) to GSSG is the major problem at measurement of GSSG.
Methods. A method for the accurate measurement of glutathione disulfide (GSSG) by an HPLC with fluorescence detection is described. We have tested different anticoagulant agents, protein precipitants and thiol-masking agents. Temperature, light, pH, dissolved oxygen, solvent, ionic strength can influence an oxidation of GSH and a stability of GSSG too. Short-term and long-term stability of GSSG were studied.
Results. The best results were obtained under these conditions: a citrate (pH 5.9) as an anticoagulant agent, immediate addition of thiol-masking agent (N-ethylmaleimide), trichloroacetic acid with EDTA as a protein precipitant and supernatant was without delay subjected a derivatization procedure with ortho-phthaldialdehyde to form a highly stabile tricyclic isoindole derivative. Tricyclic isoindole derivative is stable on a cooled autosampler (4 °C) for at least 12 h. Analytical performance of this method is satisfactory. The intra- and inter-assay coefficients of variation were below 5 %. The recovery was 98.9 % (CV = 3.7 %). The calibration curve was linear in the whole range tested (1.0-200.0 µmol/L) and the LOD was 5.6 fmol/inject.
Conclusions. Sophisticated sample preparation significantly prevents GSH oxidation to GSSG and stabilizes GSSG.

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ANTI-ANNEXIN V ANTIBODIES AND OXIDATIVE STRESS IN EGYPTIAN PRE-ECLAMPTIC PATIENTS

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Background. Pre-eclampsia (PE) affects more than 4 million pregnant women each year, over 90 % occurring in developing countries. AnnexinV is expressed in placental trophoplasts and has been proposed to maintain blood fluidity.Anti-annexinV antibodies are antiphospholipids able to divert annexinV from the trophoblast cells surface, promoting a pro-coagulant effect. The current study aimed to evaluate the relation of anti-annexinV and oxidative stress to the development of PE in a cohort of pre-eclamptic Egyptian patients.
Methods. 40 female patients in the third trimester of pregnancy with PE (patients group)and 30 pregnant healthy females of comparable age and gestational period(control group) were studied. All subjects were selected to be negative for CRP or rheumatoid factor. Laboratory investigations included routine biochemical tests performed on Konelab analyzer. To evaluate oxidative stress plasma thiobarbituric acid reactive substances (TBARs) as marker of lipid peroxidation were estimated by a colorimetric method. AnnexinV and anti-annexinV were determined using ELISA technique.
Results. TBARs and anti-annexinV were higher in patients groups compared to controls (mean +/- SD: 4.2 +/- 1.6 vs 0.7+/-0.3 umol/L, P=0.000 for TBARs in PE and control groups respectively; 44.8 +/- 10.6 vs 4.2 +/- 1.6 ng/ml for anti-annexinV in PE and control groups respectively, P=0.000). TBARs positively correlated with anti-annexinV (r=0.845, p=0.000) and both of them positively correlated with serum uric acid, urine protein/ creatinine ratio and mean blood pressure.
Conclusions. the results of the present study reflect the close relation of high values of anti-annexinV and oxidative stress with the severity of PE.

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DEVELOPMENT OF AN ULTRA-SENSITIVE HOMOGENEOUS IMMUNOASSAY FOR FERRITIN

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Background. Because of its wide variety of clinical significances of Ferritin both in low and high serum levels (a lower threshold is 12µg/L or below and an upper threshold is 300µg/L or greater), assays for serum Ferritin level are required wide assay range with high sensitivity. Also serum Ferritin levels may increase very high in some disorders such as hemochromatosis, hemosiderosis, porphyria, etc. Thus it is also important for assays for serum Ferritin to have good prozone (high dose hook effect) tolerance for accurate and reliable measurements. We developed anultra-sensitive latex particle-enhanced turbidimetric immunoassay for serum and plasma Ferritinwith the excellent prozone tolerance, and we report its performance characteristics.
Methods. We carried out performance verification study on a Hitachi-917 analyzer.
Results. The lower detection limit was found as~ 1 µg/L and the excellent linearity was observed up to 1000µg/L. No prozone was observed at least up to 60,000µg/L. CVs from within-run imprecision study were below 3% for samples around the lower threshold (10-15µg/L). The highest assay calibrator, patient serum/plasma samples and the WHO Ferritin reference material were tested for dilution linearity and found all parallel over the assay range, indicating commutability of the materials (no matrix effect) and traceability of the assay to the WHO reference material.
Conclusions. The new ultra-sensitive Ferritin assay showed suitable precision, excellent sensitivity and extended linearity for diagnosis of various disorders where Ferritin level is either abnormally low or high. It can give laboratories more economical and flexible approach for Ferritin determination.

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APOLIPOPROTEIN A5 185GG IS IMPORTANTIN LIPOLYSIS

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Background. Hypertriglyceridemia is an independent risk factor for coronary heart disease. According to the previous studies, triglyceride metabolic disorder was correlated with the apolipoprotein A5 (*APOA5*) gene polymorphisms. APOA5 can modulate the triglyceride hydrolysis of lipoprotein lipase (LPL) through direct activation or indirect effects to reduce the plasma triglyceride. However, the mechanism of APOA5 modulation is still unclear. We have identified a *APOA5* c.553G>T polymorphism, which was found in oriental populations only, causes a G185C substitution effect. Moreover, patients with c.553G>T polymorphism tend to suffer from hypertriglyceridemia. Thesephenomenastimulated us that substitution of G185C might results in losing, or at least lowering the function of APOA5. The aim of this study is to assess the importance of glycine at 185th.
Methods. Nineteen mutantsof APOA5 protein were generated by site-direct mutagenesis.The mutant and wild type of *APOA5* plasmid were transformed to *E.coli* BL21 and inducted at 37°C for 3 hr.Proteins produced were purified with nickel magnetic beads.Different APOA5 protein and bovine LPL were added to the VLDL from *apoa5* knockout mice, and the liberated free fatty acids were measured by NEFA kitto assess the LPL activity.
Results. The results showed that the activation ability of LPL by APOA5 mutant-type was lower than that of wild type of APOA5. The decrement range was from 27 to 76% ($p<0.005$).
Conclusions. This might suggest that the importance of APOA5 185GG, any mutation will decrease its LPL activation ability.

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REPRODUCIBILITY OF PHAST SYSTEM SDS-PAGE ELECTROPHORETIC PROFILES OF URINARY PROTEINS

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Background. The quality of home made gradient polyacrilamide gels for separation of urinary proteins was examined by determination of reproducibility of SDS-PAGE electrophoretic profiles of urinary proteins stained with fast silver staining procedure in LKB Phast System Development Unit.

Methods. A standardized original methods were developed for our own production of gradient (4-22%) polyacrilamide gels (43 x 50 x 0,45 mm) and for agarose electrode buffer gels production, for automated Phast System. Electrophoretic separation of urinary proteins from the same urine sample was made on 10 home made gels in Phast System Separation Unit followed by staining of gels in Phast System Development Unit, according to method developed by Melzer and coworkers. The concentration of urinary proteins in urine sample was 510 mg/L. Urinary samples were prepared with 5% SDS-Tris-Borat-EDTA solution. The duration of electrophoretic separation was 40 minutes, followed by 3-hours staining procedure.

Results. Obtained electrophoretic profiles was the same in all 10 gels and was characterized by presence of 18 identical clearly separated fractions, with molecular mass from 12 to 160 kDa, characteristic for mixed proteinuria. The coefficient of variation (CV) of R_f values determined for all 18 separated fractions in all 10 gels varied from 2,38 to 7,24%.

Conclusions. The results have shown that the reproducibility of electrophoretic profiles obtained by home made gradient gels is high and satisfactory. Automated Phast System SDS-PAGE allows obtaining results in a few hours, makes this method appropriate for rapid separation of urinary proteins for diagnosis of renal or systemic disease.

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POST-CENTRIFUGAL STABILITY OF GLUCOSE IN PLASMA AND SERUM GEL SEPARATION TUBES

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Background. Stability of glucose is a general concern in routine laboratories. It is known that prolonged contact with cells decreases glucose very rapidly. However, post-centrifugal stability of glucose is more unclear. In this study we determined the post-centrifugal stability of glucose stored in plasma and serum gel tubes.

Methods. Simultaneously drawn blood samples (n=20) into Terumo® lithium-heparin plasma and serum gel tubes were measured for glucose immediately after centrifugation and after 1h, 3h, 6h, 24h, 48h and 72h storage on gel of these tubes at +24°C and +4°C. Glucose was measured with Roche Modular P800 analyzer (Roche Diagnostics, Germany). Glucose concentrations were classified into three concentration levels, being < 6.0, 6.0-10 and > 10 mmol/l.

Results. Glucose in plasma was stable for 3h when stored at +24°C in gel tubes. After 6h storage significant decrease (-7.4%) was observed at levels below 6 mmol/l (clinical acceptance ± 6%). Glucose in plasma stored at +4°C in gel tubes remained stable for 6h, the maximal average decrease at measured levels was -3.8%. Glucose in serum stored in gel tubes was stable up to 72h at both +24°C and at +4°C, correspondingly, maximal average decreases -4.4% and -2.1% at measured glucose levels were observed.

Conclusions. Our results indicate that glucose has a good post-centrifugal stability up to 72h in serum stored in gel tubes at room or refrigerator temperatures. Instead plasma glucose is very unstable and can be stored in gel tubes before measurements only for 3h at +24°C and for 6h at +4°C.

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INFLUENCE OF LIPEMIA AT IMMUNOTURBIDIMETRIC TRANSFERRIN DETERMINATION

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Background. In our laboratory, transferrin is determined by imunoturbidimetric assay on an analyzer Roche Modular. Lipemia can interfere with this assay by altering light scattering. Manufacturer states that there is no evidence for interference at imunoturbidimetric transferrin determination if lipemia is caused by the addition of lipid emulsion Intralipid to samples up to serum index for lipemia (SI-L) of 500. In our study, we evaluated the interference caused by lipemia in native lipemic samples.
Methods. In 24 native lipemic samples with SI-L from 64 to 442, we measured transferrin and triglycerides. Lipemic samples were than ultracentrifuged (OPTIMA L-80 XP) for 10 minutes at 25000 rpm to remove lipid particles. Transferrin was measured in the ultracentrifuged samples. Transferrin was also measured in some lipemic samples in which lipemia was simulated by the addition of Lipofundin 20% (Braun) up to the concentration of triglycerides of 20.5 mmol/L and SI-L of 518.
Results. In eight native lipemic samples, the difference in transferrin before and after centrifugation was less than 10%, average concentration of triglyceride in this group was 6.7 mmol/L (5.6-9.1). The average concentration of triglyceride in the group where the difference in transferrin before and after ultracentrifugation was greater than 10% was 20.5 mmol/L (10.2-29.6). There was no difference in transferrin concentration in the samples with different concentration of triglycerides because of the addition of Lipofundin.
Conclusions. We may conclude that ultracentrifugation before transferrin determination in lipemic samples is necessary always when concentration of triglycerides is greater than 10 mmol/L and SI-L higher than 80.

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SEXUAL DIMORPHISM IN FOLATE METABOLISM

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Background. Diet, age, genetic variants and sex are major determinants of homocysteine (Hcy) concentration in adults. This study evaluates gender response to folate treatment in a homogeneous age group of young adults. It attempts to avoid differences due to age and dietary factors as contributing factors of changes in Hcy and folate.
Methods. A prospective randomized study was designed comprising 23 men and 26 women (mean age 22.4±1.7; 23.1±2.1, respectively). Subjects were treated with an oral dose of levofolinic acid 5mg/day for 30 days. Plasma Hcy, serum and erythrocyte folate were measured 2 days before starting treatment, days 2,5,10, 30 during treatment and 30 days after the end of treatment. Hcy was analyzed bynephelometric test (BN II®, Siemens) and folates were measured by a radioimmunoassay kit (Solid Phase No Boil Duacont ®, DPC). Student's t-test was achieved for statistical data analysis.
Results. All subjects showed significant variations in Hcy decrease, serum and erythrocyte folate increase when comparing before and after treatment. Higher Hcy levels were found in males every day of analysis (p<0.05). Female group showed higher serum folate concentration, significant differences on days 2, 5 and 10 of treatment (p<0.05). Both groups balanced their serum folate levels in day 30, showing a *plateau* at different timing (earlier in women). Females reached higher erythrocyte folate levels in days 5, 10 and 30 (p<0.05). This variation disappeared 30 days after completion of therapy
Conclusions. Treatment with folinic acid brings new perspectives to the gender differences known about folate and Hcy levels.

Miscellaneous

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ASSOCIATION OF THE -173G>C *MIF* PROMOTER POLYMORPHISM WITH MIF LEVELS AND CLINICAL ACTIVITY IN RHEUMATOID ARTHRITIS IN WESTERN MEXICO

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Background. Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting 1% of the population worldwide. It is characterized by joint destruction and disability. It has been estimated that 60% of the disease susceptibility is owed to genetic factors. Macrophage migration inhibitory factor (MIF) is a potent proinflammatory cytokine that has been postulated to play a role in RA. There is a -173G>C polymorphism on *MIF* gene promoter that has been associated with variations in the transcription rate and susceptibility to inflammatory and autoimmune diseases.The aim was to determine the association of thispolymorphism with RA susceptibility, clinical activity and MIF serum levels in a western Mexican population.

Methods. Genomic DNA was obtained from 204 RA patients and 204 healthy subjects (HS). MIF -173G>C genotypes were determined by PCR-RFLP method. MIF levels were measured in the serum of 54 RA patients and 78 HS by an ELISA assay. Clinical activity of RA was evaluated through DAS28 score.

Results. The HS population was in Hardy-Weinberg equilibrium for the polymorphism. Genotypic and allelic frequencies were similar in both groups. No association of the -173 G>C polymorphism with MIF serum levels or RA activity was found.

Conclusions. The -173 G>C MIF polymorphism is not a susceptibility genetic marker for RA in Western Mexico population and is not associated with MIF serum levels or RA clinical activity. However an haplotype study is required to fully determine *MIF* genetic contribution to RA in Western Mexico.

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ASSOCIATION OF THE -794 CATT$_{5-8}$/-173G>C *MIF* PROMOTER HAPLOTYPE WITH RHEUMATOID ARTHRITIS IN WESTERN MEXICO

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Background. Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovial inflammation of multiple joints, leading to joint destruction and disability. Its aetiology is not completely understood yet but it is recognized that 60% of the disease susceptibility is owed to genetic factors. Macrophage migration inhibitory factor (MIF) is a potent proinflammatory cytokine that has been postulated to play a role in RA. There are two polymorphisms in the *MIF* gene promoter:a -173G>C and a -794CATT tetranucleotide repeat, both are in linkage disequilibrium. The aim was to determine the association of this*MIF* promoter haplotype with RA susceptibility in a western Mexican population.

Methods. Genomic DNA was obtained from 204 RA patients and 204 healthy subjects (HS). MIF -173G>C genotypes were determined by PCR-RFLP method whereas -794CATT genotypes were determined by PCR. Haplotype frequencies were calculated by means of Arlequin software version 3.11. Chi square test was performed to determine the differences between groups, $p < 0.05$ was considered statistically significant.

Results. The HS population was in Hardy-Weinberg equilibrium for both polymorphisms. Genotypic and allelic frequencies were similar in the RA group and the HS group for the -173G>C and -794CATT polymorphisms and there were no statistically significant differences. Significant linkage disequilibrium was detected for both variants in the HS group ($p < 0.0001$), however, there were no statistically significant differences inhaplotypic frequencies between the RA and HS groups.

Conclusions. The -794CATT$_{5-8}$/-173G>C *MIF* promoter haplotype is not a susceptibility genetic marker for RA in Western Mexico population.

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PARABENS AS PRESERVATIVE FOR 24 HOURS URINE SPECIMEN

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Background. Toluene is currently used as a preservative for 24 hours (hr) urine. It is harmful to both the person involved and the environment. This advocated us to seek for other alternatives. Parabens is considered a better substitute because it has been used as an antimicrobial preservative in foods, drugs and cosmetics.To ascertain that parabens can be used as a preservative in 24hr urine for routine chemistry tests (BUN, creatinine, calcium, phosphorus, uric acid, amylase, protein, Na^+, K^+, Cl^-).
Methods. Random urine samples without any preservative were homogenously pooled. This sample was analyzed for routine tests. The results were designated as original values. Each 100 mL of this sample was separated into a bottle containing 5mL toluene or 1mL parabens and left at room temperature up to 72 hours. The samples at 24, 48, and 72 hours were then analyzed. The results from 70 pooled samples were statistically compared levels at each time point to its original values.
Results. Only the level of creatinine from both preservative showed significantly changed after 24 hours ($p<0.05$). While the significant changes after 48 hours ($p<0.05$) was found in the levels of uric acid only from urine sample containing toluene, the changes after 24 hours was found in the levels of Na^+, K^+, andCl^- only from those containing parabens. However, all the changes in every test were still within clinically desirable specifications
Conclusions. Parabens can replace toluene as a urine preservative for routine chemistry tests.

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O-β-N-ACETYLGLUCOSAMINIDASE ON HUMAN ERYTHROCYTES AS BIOMARKER OF OXIDATIVE STRESS IN ERECTILE DISFUNCTION PATIENTS

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Background. Oxidative Stress (OS) plays an important role in pathophysiological process of Erectile Dysfunction (ED). eNOS is an emerging and important actor in promotion and maintenance of erection by phosphorylation of specific site (Ser-117). eNOS inactivation by O-GlcNAcylation at the same site was showed. Consequently ED as well, is a disease where dysfunctional GlcNAcylation seems to have an emerging role. The relation between protein O-GlcNAcylation and O-phosphorylation may influence the stress response pathway; cellular levels of O-GlcNAc, regulated by O-GlcNActransferase (OGT) and O-β-N-AcetylGlucosaminidase (OGA), are considered as OS sensor and are implicated in the aetiology of various diseases.
Human erythrocytes (RBC) are considered as useful model for investigating physiopathological conditions and someglycohydrolases and OGA, present on RBC plasma membrane and cytosol, have been proposed as new, early and sensitive oxidative stress markers.
Methods. To compare the oxidative status of 27 ED patients (36.1±8 years) and 30 matched controls (34.1±7 years), plasma antioxidant total defences (Lag-time, determined by measuring kinetics of Cu-stimulated plasma peroxidation), OGA, cytosolic and membrane Hexosaminidase (Hex-c, Hex) and α-D-Glucosidase (αGLU) activities (by fluorimetric assay) were evaluated.
Results. Compared to controls, ED patients plasma membrane Hex and αGLU activities were significantly higher (p<0.01); Lag-time values (p<0.01), Hex-c and OGA activities (p<0.001) were significantly lower.
Conclusions. Data clearly confirmed: the strong OS in ED patients; the role of OGA and others considered enzymes as early OS biomarkers and suggest, indirectly, a possible alteration of O-GlcNAcylation pattern of erythrocytic proteins.

Miscellaneous

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ASOCIATION OF SOLUBLE CCL2 LEVELS AND INFLAMMATION MARKERS WITH ADIPOSITY AND THE +62G>A 3'UTR *RESISTIN* POLYMORPHISM IN MEXICAN OBESE POPULATION

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Background. In obesity, the accumulation of white adipose tissue (WAT) is the most consistent pathological process.*Resistin (RSTN)* human gen have been implicated in the function of the regulation of the WAT, that presents single nucleotide polymorphism (SNP) G>A, within regulatory region, +62 3'UTR. Most important topics envelop the relationship between monocyte chemoattractant protein-1 (CCL2) with insulin resistance, inflammation, obesity, and associated metabolic disorders.

Methods. In this cross-sectional study, 395 adults from western Mexico were included, and classified as indicated by the World Health Organization (WHO) criteria. Anthropometric measurements, serum glucose and inflammation markers were determined by routine methods, sCCL2 was determined by ELISA method, PCR-RFLP assays was used for detecting SNP of RSTN G>A+62 3'UTR. Statistical analysis was performed using the PASW Statistics v18.0.

Results. Inter-group comparison showed higher levels in obese and/or pre-obese individuals than in lean individuals for glucose, sInsulin, sCCL2, hsPCR, ESR levels $p<0.05$. In this group, correlation was found between levels of sCCL2 with inflammation markers and anthropometrics measures (r=0.251 to 0.159; $p<0.05$).

RSTN polymorphism was kept with the Hardy-Weinberg equilibrium. The G/A genotype frequency in the overweight individuals compared to lean individuals was significantly lower (3.2% vs. 10.2%, p = 0.0414). In obesity study group, we found difference (p=0.008) in CCL2 levels between G/G (280ng/mL±145) compared to G/A (218ng/mL±3.5) genotypes carriers.

Conclusions. We conclude that levels of sCCL2 and inflammation markers reflect adiposity. This study suggests that *RSTN* SNP influences the morbidity development in obesity on the population of West of Mexico.

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SLEPTIN AND SLEPTIN-RECEPTOR IN OBESITY: RELATIONSHIP WITH ADIPOSITY, INFLAMMATION MARKERS AND THE POLYMORPHISMS *LEP* -2548G>A AND *LEPR* Q223R IN MEXICAN-MESTIZO OBESE POPULATION

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Background. *LEP* and *LEPR* present -2548G>A and Q223R transitions respectively that have been associated with leptin levels and obesity. We investigated the relationship of leptin (sLeptin) and leptin-receptor (sLeptin-receptor) levels with adiposity, inflammation markers and those polymorphisms.
Methods. In this cross-sectional study 382 individuals classified by body mass index (BMI) according to World Health Organization criteria were included. sLeptin and sLeptin-receptor were quantified using an ELISA kit. The body composition and inflammation markers were measured by routine Methods. The genotypes were characterized using the PCR-RFLP technique. Statistical analysis was performed with PASW Statistics v18.0.
Results. sLeptin was positively correlated with fat percentage (r=0.650, $p<0.001$) and negatively with sLeptin-receptor (r=-0.609, $p<0.001$). The genotypes frequencies for the two polymorphisms were found to be in Hardy-Weinberg equilibrium [*LEP*: GG=121(31.7%), GA=197(51.6%), AA=64(16.7%); *LEPR*: QQ=104(32.4%), QR=199(51.6%), RR=79(16.0%)].
In the obesity group (BMI≥30 kg/m^2) were observed the follows differences: 1) a tendency of higher sLeptin associated to genotype *LEP* -2548GG (GG=67.9±29.68 ng/ml, GA=57.5±31.26 ng/ml, AA=46.7±32.00 ng/ml) and lower ($p<0.046$) fat percentage associated to genotype *LEP* -2548AA (GG=41.3±5.52%, GA=41.6±5.61%, AA=36.5±6.80%); and 2) association of genotype *LEPR* *223*RR with higher ($p<0.042$) sLeptin (QQ=80.3±33.24 ng/ml, QR= 57.3±29.12 ng/ml, RR= 44.0±28.12 ng/ml), higher ($p<0.035$) basal glucose (QQ=90±8.1 mg/dl, QR=98±15.7 mg/dl, RR=105±18.2 mg/dl), lower ($p<0.045$) sLeptin-receptor (QQ=11.0±2.92 ng/ml, QR= 12.2±3.15 ng/ml, RR= 14.7±4.96 ng/ml), and lower ($p<0.046$) fat percentage (QQ=43.7±4.74%, QR=41.0±5.52%, RR=36.8±6.82%).
Conclusions. *LEP* -2548G>A and *LEPR* Q223R are associated with variations in sLeptin, sLeptin-receptor and fat percentage in Mexican-mestizo obese population.

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CHANGES IN WHOLE BLOOD ANTIOXIDANT AND SERUM MUSCLE ENZYME ACTIVITIES IN POLICE HORSES ON PHYSICALLY DEMANDING DUTY

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Background. Exercise-induced oxidative stress is believed to contribute to muscle fatigue and muscle damage. The study aimed to determine changes in antioxidant and serum muscle enzyme activities in police horses on physically demanding duty. Correlations between antioxidant end muscle enzymes were also determined.
Methods. Fourteen police horses were transported (80 kilometres) to the place of duty were they were patrolling for 6 hours through the hilly woods. Blood samples for determination of whole blood antioxidant enzymes, glutathione peroxidase (GPX) and superoxide dismutase (SOD), and serum muscle enzymes, creatine kinase (CK) and aspartate aminotransferase (AST), were collected five times: before (box) and after (postTr1) transportation, after duty, immediately after transportation back (postTr2) and 24 hours later. Changes of measured parameters (significant difference, $P<0.05$) were assessed by repeated measures ANOVA. Associations between antioxidant and serum muscle enzymes were tested at each sampling time using Pearson's correlation.
Results. Significantly higher AST activities were determined at postTr1, after duty and postTr2 sampling times, in comparison with box values, 276.9 ± 45.9, 287.2 ± 38.7, 303.3 ± 49.3 and 263.4 ± 37.5 U/L, respectively. Comparing to box values, CK activity increased significantly after duty, 165.5 ± 54.2 and 223.6 ± 79.7 U/L, respectively. Despite significant changes, activities of serum muscle enzymes remained within normal range at all sampling times. Antioxidant enzymes activities remained unchanged. No significant correlations between antioxidant and serum muscle enzymes activities were found.
Conclusions. We may conclude that police horses did not develop exercise-induced oxidative stress while being on physically demanding duty.

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EVALUATION OF CIRCULATING ANGIOGENIC FACTOR LEVELS: ENDOSTATIN AND BASIC FIBROBLAST GROWTH FACTOR IN PATIENTS WITH CARCINOMA OF THE BLADDER

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Background. Angiogenesis plays a pivotal role in the pathogenesis and prognosis of bladder malignancy. Both pro-angiogenicand anti-angiogenic factors as basic fibroblast growth factor(b-FGF) andendostatin respectively are secreted by the tumor.
Aim. to evaluate the circulating levels of b-FGF and endostatin in patients with carcinoma of the bladder in diagnosis and correlate their levels with histopathological findings of the tumor.
Methods. Seventy male subjects classified into : 44 patients with carcinoma of the bladder {29 with transitional cell carcinoma (TCC) and 15 patients with squamous cell carcinoma histotypes. 14 patients with benign urological conditions and 12 apparently healthy controls. Routine laboratory investigations : urine examination ,AST,ALT,alkaline phosphatase; urea, and creatinine; and anti-schistosomal antibodies. Specific estimation of serum endostatin (ng/ml) and b-FGF (pg/ml) byELISA. Urine cytology was performed in all patients' groups .
Results. transitional cell carcinoma (TCC) (29/44) versus squamous cell carcinoma (SCC) (15/44) histotypes in cancer bladder group. Staging revealed 50% (22patients) of the cancer bladder cases were superficial (Ta=10, and T1=12). The remainder 50% were invasive (T2=8, T3=7, and T4=7).Significant elevation of both serum endostatin and bFGF in cancer bladder group compared to both benign urological conditions and control mean values($p<0.01$ and <0.01). Early significant elevation of both parameters in Ta histopathological subtype as compared to control group.A crescendo rise in serum endostatin level in the histopathological stages: Ta, T1, T2 and T3. There was a significant positive correlation between serum endostatin and histopathological stage of the bladder biopsy, the highest elevation was recorded in T3 as compared to Ta, T1 and T2 stages.
Conclusion. To conclude, Circulating levels of endostatin and bFGF may be used as non-invasive, reliable angiogenic biomarkers for early prediction of cancer bladder. Monitoring cancer bladder with serial endostatin estimation, could anticipate detection of early metastases; reduce intercystoscopy interval in follow-up of patients.

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FACTITIOUS POTASSIUM RESULTS IN HEMOLYSED SAMPLES: IS NORMOKALEMIA REALLY NORMAL?

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Background. Effect of hemolysis on potassium is well recognised and correction factors may be used to estimate the true potassium concentration. We looked at the trend of true potassium in hemolysed samples and its correlation with free haemoglobin concentration.

Methods. Potassium and corresponding hemolysis index values analyzed in the National University Hospital, Singapore from October to December 2010 were included. 2448 samples had hemolysis index > 0.1g/dL, of which 118 were repeated within 2 hours and were not hemolysed. Repeat potassium concentration within 2 hours was taken as the true value, as a balance between practicality and effects of medical intervention. Correlation between true potassium and hemolysis index was analysed using Microsoft Excel Pearson correlation coefficient calculation and linear regression.

Results. Median initial potassium concentration was 6.1mEq/L(2.3 - 9.0mEq/L). Median haemoglobin concentration was 0.33g/dL (0.101 – 0.924g/dL).

41/62(66.1%) of hemolysed samples >6.0mEq/L were normokalemic and 6/62(9.7%) were hypokalemic after repeating.

3/7(42.9%) of hemolysed normokalemic samples were ≤3.0mEq/L after repeating. 36/45(80.0%) of hemolysed samples 5.1-6.0mEq/L were normokalemic and 3/45(6.7%) were hypokalemic after repeating.

Correlation coefficient R for correction factor of 0.56mEq/L increase in potassium concentration for every haemoglobin concentration increase of 0.1g/dL is improved to 0.77 in samples with Creatinine values below 150mmol/L.

Conclusions. Clinicians tend to repeat hemolyzed potassium earlier if they were severely hemolysed or hyperkalemic. This study underscores that while it's generally not recommended to apply correction factors, it may be attempted in patients with normal creatinine levels. Also, normokalemic hemolysed samples may mask an underlying hypokalemia.

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STANDARD CORRECTION OF POTASSIUM CONCENTRATIONS FOR HEMOLYSIS IS NOT VALID FOR CAPILLARY BLOOD SAMPLES

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Background. Spuriously elevated potassium concentrations through in vitro hemolysis can lead to potassium misinterpretation. Several options to correct results using hemolytic indices (H-index)were described previously. Remarkably, we observed that capillary samples appear to show higher potassium elevations than expected based on H-index. Therefore, the relationship between potassium and H-index was investigated for capillary samples. Elevation of LDH activity through hemolysis was included to study differences between venous and capillary samples for large molecules.

Methods. For potassium, 332,760 venous and 2,607 capillary samples were selected. For LDH, 135,974 venous and 999 capillary samples were included. Venous and capillary samples were differentiated using patient age, as we perform mostly capillary blood sampling in children and venous sampling in adults. Results were obtained using Beckman-Coulter DxC800 analyzers.

Results. The increase in potassium concentration with increasing H-index was considerably higher for capillary samples compared with venous samples. Linear regression revealed a potassium increase of 0.37 mmol/L per increment in H-index for capillary samples, which is significantly higher than the 0.17 mmol/L increase found for venous samples. For LDH, no differences were found between venous and capillary samples.

Conclusions. At identical H-index, capillary samples showedhigher potassium elevations than venous samples. This was not observed for LDH. Capillary blood sampling likely renders erythrocytes more fragile, resulting in leakage of small molecules like potassium, whereas large molecules like hemoglobin and LDH remain intracellular. Consequently, literature based correction factors for potassium are not valid for capillary samples and can lead to misdiagnosis of hypo- and hyperkalemia.

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SERUM LEVELS AND REFERENCE INTERVALS OF LEPTIN, ADIPONECTIN AND RESISTIN IN HEALTHY ADULTS IN NORTH-WEST TURKEY

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Background. Adipokines are derived from adipose tissue and produce important biological actions. The determination of serum levels and reference intervals of adipokines is necessary, asreference intervals are the most widely used medical decision-making tool.

Methods. Serum levels and reference intervals of leptin, resistin and adiponectin were determined by following the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine in 252 (125 males and 127 females) apparently healthy Turkish adults (18-45 years old). Serum levels of adipokines were measured using the ELISA method.

Results. Mean values of serum leptin, adiponectin and resistin levels were 13.3±4.1, 6.5±2.3 or 19.7±3.5 ng/mL, 26.6±2.3, 20.9±57.7or 32.5±5.7 ng/mL and 1.5±0.3, 1.4±0.4 or 1.6±0.5 ng/mL in all subjects, in males or in females, respectively. Serum levels of leptin and adiponectin, but not resistin, in females were higher (p<0.001) than the observed values for males. The reference intervals for females for leptin, adiponectin and resistin were 3.1-40.2 ng/mL, 10.2-57.7 ng/mL, 0.73-2.87ng/mL, respectively and for males, 1.1-17.1 ng/mL, 6.0-40.7 ng/mL, 0.55-2.39 ng/mL. These reference intervals for adipokines were not similar to values suggested by the manufacturers.

Conclusions. These data show that serum levels and reference intervals of adipokines are different in males and females. The adipokine levels obtained in this study were from healthy individuals and the reference values are valid for the population of north-west Turkey and may be compared with values from other regions of Turkey.

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IRON HOMEOSTASIS MARKERS AND C-REACTIVE PROTEIN (CRP) IN PATIENTS WITH STABLE CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Background. The goal of the study was to determine changes in iron homeostasis factors and CRP in patients with stable COPD. ROC analysis was done to investigate the diagnostic efficiency of selected biochemistry analytes in discriminating between COPD patients and healthy individuals.

Methods. The study included 109 COPD patients (FEV_1=41±14%) and 51 healthy examinees (FEV_1=106±15%). Following GOLD guidelines, patients were divided into subgroups II, III, IV. CRP concentration was determined by an immunoturbidimetric method while iron and TIBC concentrations were found using the ferene colorimetric method on a Dimension Xpand Plus analyzer. Ferritin concentration was determined using CMIA technology on an Architect 12000_{sr} immunoanalyzer, TfR concentration by the immunoturbidimetric method on an Olympus AU2700 analyzer and EPO concentration by the ELISA method.

Results. UIBC and TIBC concentrations were significantly lower in COPD patients (p<0.0001, p=0.0002) and in GOLD subgroups (p<0.0001, p=0.001). Iron concentration was significantly higher only in GOLD IV subgroup (p=0.004).
Ferritin and CRP concentrations were twofold higher in patients and all GOLD subgroups (p<0.0001) than in controls.
ROC analysis in COPD patients and control group indicated good discriminatory efficiency of UIBC, ferritin and CRP but did not show good diagnostic accuracy in differentiation between GOLD subgroups.

Conclusions. Iron concentration was significantly higher in the GOLD IV subgroup involving the most severe form of the disease, indicating significant redistribution of iron in severe forms of pulmonary diseases.
CRP concentration was significantly higher in all COPD subgroups and confirmed CRP to be a marker of systemic inflammation in COPD patients.

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ARE GGTP AND URIC ACID COMPONENTS OF PLASMA ANTIOXIDANT CAPACITY?

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Background. Thanks to ability of regeneration reduced glutathione GGTP is a part of antioxidant defense. However, GGTP is oxidative stress inductor, as a result of taking part in diaminoacids synthesis, which reduce ferric ion to ferrous ion taking part in Fenton reaction. Uric acid is strongly reducing agent (electrons donor) and therefore potent antioxidant. In humans, over half the antioxidant capacity of plasma comes from uric acid. However uric acid's synthesis reaction generates free radicals. The aim of this study was to evaluate relationship between FRAP – antioxidant capacity parameter and GGTP activity, and Uric Acid concentrations.

Methods. 46 patients with lever damage were selected. All assays were performed using spectrometric method. on the Maxmat chemistry analyzer. FRAP was measured by Benzie method.

Results. Uric Acid (4,9±1,8 mg/dl)and FRAP (1,06±1,22 mmol/l) were characterized by normal results distribution whereas GGTP activities (67, 23-267 U/l) were analysed using non-parametric test. No significant relationship between GGTP and FRAP was found (Spearman Correlation : p=0,15, r=0,21). Lack of this correlation was observed for normal and increased GGTP results (cut off point – 55 U/l). On the other hand, significant correlation between Uric Acid levels and FRAP was observed (p<0,001 r= 0,81). Moreover, results of multiple linear regression analysis show that correlation between Uric Acid and FRAP is independent from GGTP.

Conclusions. Our results didn't show any relationship between GGTP and antioxidant potential of plasma. Significant correlation between Uric Acid and FRAP found in our study suggests that Uric Acid is an important component of FRAP.

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ECONOMIC VALUE OF AMINO-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE (NT-PROBNP) TEST AT EMERGENCY DEPARTMENT (ED) PATIENTS WITH DYSPNEA IN SPAIN

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Background. The use of NT-proBNP testing for diagnostic of patients with dyspnea and suspected acute heart failure (HF), compared to standard clinical evaluation alone has been studied internationally; we aimed to analyze the efficiency of NT-proBNP use in Spanish Emergency Departments.

Methods. A decision-analytic model was developed to evaluate the clinical and economic outcomes of both diagnostic alternatives. Model's time horizon started at patient ED visits and ended after 60 days of follow-up (differentiating between hospitalized and non-hospitalized patients). Clinical parameters were mainly derived from the PRIDE study and were validated by expert opinion (ED and cardiology doctors). Based on Spanish published data we assumed that 65% of patients with dyspnea had HF. Resource use was obtained through expert opinion and evaluated under a National Healthcare System (NHS) perspective. We considered a 900 pg/ml cut-point for NT-proBNP test (sensitivity of 90% and specificity of 85%). Our model compared final diagnostic result with the initial diagnostic before ED discharge.

Results. Correct diagnostic using NT-proBNP testing was 91.96% of patients (59.09% true positive cases and 32.87% true negative cases) versus 85.53% with the standard clinical evaluation alone (50.79% of true positive cases and 34.74% of true negative cases). Additionally, NT-proBNP testing resulted in less costs (4,045€ versus 5,405€) mainly due to less hospitalizations and a shorter length of stay.

Conclusions. NT-proBNP test is less costly per correctly diagnosed patient than standard clinical evaluation alone in the assessment and management of patients with dyspnea at ED rooms from Spanish NHS perspective.

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THE EFFECT OF HORMONE REPLACEMENT THERAPY ON LIPID PROFILE AND FIBRINOLYTIC ENZYMES IN POSTMENOPAUSAL WOMEN

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Background. Menopause is often accompanied by degenerative processes such as arteriosclerosis that suggest an acceleration of aging triggered by estrogen lack. Therefore, hormone replacement therapy (HRT) has been considered the most suitable treatment for the above symptoms and processes.

Methods. This study was aimed at evaluating the influence of HRT on the lipid profile (total cholesterol, HDL-Ch, LDL-Ch,tryglicerydes) and factor VII of coagulation in postmenopausal women.The total number of 32 women in post-menopause with application of HRT within the period of six months were examined. Lipid concentration was determined with standard colorimetric-spectrophotometric method,and the concentration of factor VII of coagulation with the method of deficiency plasma.

Results. Statistical analisys has shown that HRT applied in post-menopausal women within a six-month period significantly decreased the concentration of LDL-Ch (p<0,001) and factor VII of coagulation but significantly increased the HDL-Ch serum concentration (p<0,001). However, there was no statistical significance in the level of tryglicerides and total cholesterol.

Conclusions. We can conclude that there is a statistical decrease of atherogenic risk factors (LDL-Ch, VII factor of coagulation) as well as significant increase of protective (HDL) in post-menopausal women treated with HRT. This fact justifies the application of this therapy in post-menopausal women.

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EPIDEMIOLOGICAL PROFILE OF HEMOGLOBINOPATHIES FOR MEAUX HOSPITAL (EST - ILE DE FRANCE)

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Background. Hemoglobinopathies, inherited disorders, result from a quantitative deficit (α, β thalassemias) or variants of hemoglobin (Hb). They make them a major public health problem and their detection has important therapeutic consequences early in life. We report here epidemiological data of hemoglobinopathies diagnosed at Meaux hospital between March 2007 and December 2010.

Methods. For each patient, hematological parameters, hemoglobin electrophoresis and iron status were determined and clinical status collected. Electrophoresis was realised on Capillarys® [capillary zone electrophoresis – SEBIA France]. The suspicion of sickle cell disease (SCD) was confirmed by Itano test. Each sample with an abnormal pic unknown by Capillarys® was send to reference center (rare variants).

Results. On 656 hemoglobin electrophoresis, 285 were abnormal and composed of - 28.8% SCD [HbSS (9.2%), HbAS (14.5%), HbSC (2.1%), HbSβ° (0.6%) and rare composites HbS-/+ (K WOOLWICH, D KORLE-BU, HOPE, G GAMMA)], - 3.2% thalassemias, especially β-/+ thal (2.7%), α thal being more difficult to identify, - 11.9% others hemoglobinopathies [PHHF (4.3%), HbCC (1.5%), HbAC (2.7%), HbE (0.6%), Hb Delta (0.9%), Hb D PUNJAB (0.5%) and rare variants (BARTS, NOKO, HOPE, O-Arab)]. Among 285 abnormal hemoglobinopathies, 47 were detected during the determination of the HbA1c on the CLHP automate G8® (TOSOH France), especially HbAS (66%), HbAC (8.5%), HbD PUNJAB (4.3%), HbEE (2.1%), HbD KORLE-BU (2.1%).

Conclusions. SCD which affects patients of Black Africa, Antilles and Reunion is the most hemoglobinopathies, follow-up of patients with β thal trait and HbC. The HbSC observed in West Africa (Mali) is the most frequent association.

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STRESS MARKERS IN BLOOD SERUM OF WEANING PIGLETS AFTER APPLICATIONS OF SAGE ESSENTIAL OIL

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Background. Secondary metabolites reduce the response to stress in animals and emotional stress in human. Physiological parameters reflect important biological and metabolic functions of the body that are sensitive to changes in the environment of animals.

Methods. The aim of the model experiment was to monitor the effect of sage essential oil added into feed in condition of conventional breeding of pigs Slovak White x Pietrain on selected stress biomarkers in blood serum. Weaning piglets were aged 21 days. The experiment piglets were divided into control group (n = 7 pcs) fed ČOS1, ČOS2 with no addition of essential oil, experimental group (n = 11 pcs) in which sage essential oil was applied into feed (0.05%). Four collections were conducted - 0 (21st day), 1st (28th day), 2nd (35th day) and 3rd (42nd day). In the blood serum of weaned piglets selected stress biomarkers were observed - glucose, triglyceride, cholesterol, AST (EC 2.6.1.1, aspartate aminotransferase), ALT (EC 2.6.1.2, alanine aminotransferase).

Results. In the experimental group we found statistically significant changes in glucose concentration between 0 and 3rd collection and between 2nd and and 3rd collection at the level of significance of $P < 0.05$. Statistically significant changes were also monitored in concentration of triglyceride – between 2nd and and 3rd collection, total protein between the 0th and 2nd collection, 1st and 3rd collection, 2nd and and 3rd collection.

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OUR FIRST EXPERIENCE WITH THE NEW CLINICAL CHEMISTRY ANALYZER ILAB ARIES FROM INSTRUMENTATION LABORATORY

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Background. We evaluated ILab Aries – the latest model clinical chemistry analyzer from Instrumentation Laboratory of this class ILab Aries is a completely automatic, random-access computer control, counter top, instrument for chemical and immunoturbidimetric clinical analyses. Throughput of the analyzer is 280 photometric test/h + up to 160 ISE test/h, with comprehensive menu and economic advantages (low reaction volume and not required deionization)

Methods. For the short period of time in our emergency lab we measured both within run and total imprecision as well as performed comparison with patient samples to the data obtained on Olympus AU 400 analyzer. We used for imprecision both control sera and pooled patient samples. We determined several parameters each of them tested for at least 50 samples. The original application protocol including calibration materials were used on both analyzers.

Results. From the statistical evaluation: Sperman's correlation coefficient for the investigated tests (Glu, TP, AST, ALT, Amyl, CK, K, Na) varied from 0,9138 for Glu to 0,9981 for CK ($p < 0,0001$). Bland-Altman plot showed statistically non-significant differences in the Results. According to D'Agostino Pearson test for normal distribution our results show relative SD between 1,44% and 3,91% for the parameters: Glu, CK, CHE, Fe Chol, TP, AST, ALT, Amyl ($p < 0,0001$)

Conclusions. The analytical performance of ILab Aries appears satisfactory compared to Olympus AU 400. ILAb Aries is a precise and reliable analyzer of this class useful for emergency and middle size labs

Miscellaneous

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EFFECTS OF ACTIVIN A ON EXTRACELLULAR MATRIX REMODELLING

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Background. In recent years activin A, a member of the transforming growth factor beta superfamily, was shown to contribute to pathological progression of fibrosis. Tissues which are affected by fibrotic reorganisation of the extracellular matrix (ECM) are hallmarked by an excessive accumulation of ECM components mainly collagens and proteoglycans.
Methods. Primary dermal fibroblasts were cultivated in a three-dimensional collagen gel matrix (3D) as well as in a conventional two-dimensional monolayer (2D). After treatment with activin A (50 ng/mL) for 48 h relative mRNA transcript levels of xylosyl-transferase (XT) isoforms and diverse matrix components were monitored by real-time RT-PCR. XT activity was determined by a radioactive enzyme assay.
Results. mRNA level of the proteoglycan synthesis initiating enzyme XT-I is upregulated up to 4-fold (p<0.001). Expression level of the highly homologous isoform XT-II was slightly upregulated only in 3D cultivation. Correspondingly, total XT enzyme activity was significantly increased up to 2-fold (p<0.001). mRNA expression levels of ECM components like biglycan and collagen type I were strongly upregulated by activin A treatment. For decorin and betaglycan a decreased expression could be detected. For all analysed target genes the mRNA expression profile was found to be comparable for both cultivation Methods.
Conclusions. For the first time our studies identified the pro-fibrotic mediator activin A as a novel activator of XT gene and protein expression. In prospect of further experimental research the supposed pathological effect of elevated xylosyltransferase activity on extracellular remodelling and fibrotic processes needs to be investigated.

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SERUM LEVELS OF CYTOKINES IN DEPRESSED PATIENTS WITH OR WITHOUT SUICIDAL BEHAVIOR

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Background. Patients with depression have been found to exhibit increased peripheral inflammatory cytokines, and several studies indicated that antidepressant decreased the production of IL-6 and TNFα.
Methods. The subjects were depressed patients (N=86) with attempted suicide (N=35, age X=49y, SD= 15,6 , M/F =11/16), those with no suicidal behavior (N=51, age X=51y, SD=12,9 , M/F=34/59), all admitted in the Sveti Ivan Psychiatric Hospital, while the control group was made of voluntary blood donors (N=92, age X=49, SD=11,6, M/F=48/51). Depressed patients were treated with SSRI (36/86) or other tricyclic and heterocyclic antidepressants (50/86). The IL-6 and TNFα concentration was determined using a competitive enzyme immunoassay test (BioSorce, Belgium) on the Elysis Uno immunoanalyser (Human, Germany).
Results. There are no differences in IL-6 (Kruskal-Wallis test, p=0.079) and TNFα (ANOVA, p=0.469) concentration between depressed patients with or without suicidal behaviour and control group.The concentration of IL-6 was significantly higher in depressive suicidal patients without SSRI therapy (median 31 pg/ml, 95%CI 22-88) compared to non suicidal depressed patients either with or without SSRI therapy (median 21 pg/ml, 95%CI 16-3, p=0,046; median 18 pg/ml, 95%CI 12-24, p=0,005, respectively). There are no differences in TNFα concentration regarding antidepressive therapy in those patients.
Conclusions. Translational implication of these findings includes opportunity to identify relevant patients populations and monitor therapeutic efficacy with various antidepressive agents, including SSRIs, tricyclic and heterocyclic antidepressants, at the level of the immune system in addition to behavior.

Miscellaneous

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SERUM TITANIUM CONCENTRATIONS IN ASSESSMENT OF TOTAL HIP ARTHROPLASTY

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Background. Biocompatibility of Ti joined to hardness and lightness, gets to be used in biological prosthesis. It's an ideal material for endosseous implants because it's inert and in living tissue, bone grows on Ti.

Despiteits lowtoxicityhasemerged in recentyears the needtoquantify thelevels ofTiin biological materialsbecause itcanbeusedas a markerofresolutionwearTiimplantsusedinjoint replacements.

Thisinterestismainly due

- Theuseof prosthesesinyoungpeople increasinglydemandsmore activeandlonger-lasting designs.
- The useofsurface texturesinuncementedimplantsexposespatients tohigherareasof interaction.

Methods. Methodof determination: EAAS-GFcorrectionwithlongitudinalZeemaneffect, PerkinElmer4100ZL.

Samples used were specimencollected inatube freeoftraceelementsand frozenat-80°Cuntilprocessing, from 64 patients (32 men and 32 women) from 39 to 86 years, with Total Hip Arthroplasty. We compared preoperative serum Ti concentration with 6 months post operative one.

Results. We found a mean difference between pre and post operative of Ti concentration of 4 mg/L (p=0.018 test Wilcoxon))

Conclusions. These values can be considerate normal release due to exposition to metals. A posterior increased level would be indicative of at least one mode of mechanical dysfunction of the device.

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THE ROLE OF NANOPARTICLES IN CRYOGLOBULINEMIA: A PRELIMINARY STUDY

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Background. Cryoglobulinemia refers to the presence in the blood of antibodies that precipitate, or clump together, under cold conditions. In spite of several past studies on cryoglobulins, no analyses were performed on Nanoparticles possible presence or role on cryoprecipitate. In this abstract, we present our results in the screening of 10 patients affected by membranous and proliferative glomerulonephritis, associated with hepatitis C infection. Peripheral blood specimens were obtained from the patients in vacuum tubes without clot activators for serum separation and in a sterile environment.

Methods. Cryoglobulinemia was confirmed by the detection of protein precipitates in the serum maintained at 4°C during at least 7 days, which dissolved when heated at 37°C. A drop of three times washed cryoprecipitate, placed on a coverslip, was examined with a Quanta 200 ESEM FEG from FEI company, equipped with an EDAX Energy Dispersive X-ray (EDX) system for chemical analysis.

Results. Nanoparticles of Fe, Ni, Zn, Ti, Al and Si were found into IgG-IgM immune complexes. Interestingly Si Nanoparticles were observed in spherical shape coated by cryoglobulins, while other Nanoparticles were observed mainly surrounded by immunoglobulins and not in spherical shape. Kidney biopsy samples coming from pathogenic tissues from all the patients were examined by FEG ESEM and showed correspondence among Results. Confocal microscopy (TCS SP2, Leica Microsystems) immunofluorescence investigations using FITC-conjugated Goat anti-Human IgG+IgM (Jackson ImmunoResearch) on cryoprecipitate confirmed the presence of immunoglobulin complexes.

Conclusions. Early results and Nanoparticles presence correlates with patients medical history, suggesting a possible role of Nanoparticles in crioglobulinemia.

Miscellaneous

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CLONING AND EXPRESSION OF MYCOBACTERIUM TUBERCULOSIS PE5 (RV0285) GENE

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Background. PE5 is a member of the PE protein family whose precise function has not been known yet. There are about 100 members of the PE family proteins in Mycobacterium tuberculosis genome. These glycine- and alanine-rich proteins consist of Proline-Glutamate motifs found near the N-terminus of them. In this study PE5 (Rv0285) gene amplified from DNA of Mycobacterium tuberculosis H37Rv with using specific primers, cloned and the recombinant protein was over expressed in E.coli.
Methods. The gene cloned into specific expression vector containing N-terminal GST.Tag by Ligation independent cloning (LIC) method and the recombinant vectors were transferred into competent E.coli strain TOP10. The positive colonies were screened by colony PCR. The recombinant vectors were isolated and they were transferred into E.coli strain BL21 to monitor its expression using IPTG. Protein analysis was carried out by SDS-PAGE.
Results. The positive colonies were selected after cloning the gene into specific expression vector. The recombinant vectors were transferred into competent E.coli strain BL21 and cells were cultured and induced with IPTG, in order to express the recombinant protein. The cultures were tested for presence of the protein by SDS-PAGE and a specific band was found near 40 kD. Cloning and expression of PE5 protein was confirmed by molecular techniques such as PCR and sequencing which showed exact defined size of the Rv0285.
Conclusions. Our data showed that recombinant PE5 gene cloned in E.coli strain BL21 and the accuracy was confirmed by sequencing and SDS-PAGE of over expressed recombinant protein.

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BIOLOGICAL STRESS DIAGNOSTICS

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Biological stress in humans has assumed epidemic proportions. It is considered to be the primary cause of many behavioral and organic diseases. It affects almost all the physiological functions of the human body including work and mental performance. Yet, its diagnosis, though important, is largely based on behavioral changes. There are only a few metabolic and enzymic changes considered to be the basis of the stress reactions. The evaluation of stress in large human population facing man made or natural disasters is rather difficult. Hence, a comprensive battery of tests consisting of physical, psychological, physiological, biochemical and clinical parameters was evolved to study the stress on soldiers engaged in anti-insurgency operations somewhere in Northern Himalayas.
The battery of tests consisted of Physical parameters like body weight and blood pressure, psychological parameters like self assessment of well being, mental concentration, reaction time, anxiety level and military task performance. The physiological parameters tested were physical work performance and recovery. The biochemical and clinical tests included hematology, electrolytes, urinary steroids, serum LH, FSH, prolactin, estrodiol and Creatine phosphokinase levels.
These tests were able to adequately measure stress on soldiers immediately after the operations. It was also possible to show that post operation rest of seven days was sufficient to provide relief from anti-insurgency stress.
This battery of tests can form a health evaluation package and be utilized to asses the stress status among large human population.

Miscellaneous

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MEASUREMENT OF SERUM DICKKOPF-1 PROTEIN WITH THE NEW ELISA

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Background. Serum Dickkopf-1 (Dkk-1) si a soluble inhibitor of the Wnt signaling pathway which regulates bone remodeling. No valid data on DKK-1serum method for laboratory diagnosis of posteoporosis exist.
Aim. development and validation of the new ELISA test for serum DKK-1 measurements and its testing in individuals with osteoporosis.
Methods. serum samples were used from 34 patients of osteologic out patient centre (11 with osteoporosis, 23 with normal bone density), 64 healthy individuals without signs of diseases. DEXA was made in individuals with osteoporosis. Enzyme-linked immunoabsorbent assays (ELISA) were developed, validated and performed for DKK-1 (specific goat polyclonal anti-human Dkk-1) from serum and PTH, OPG, Calcium, magnesium phosphorus, ALPB, TRAP5b were measured in sera and DPD/creatinin index in urines.
Results. ELISA test for urine DKK-1 measurement had optimal analytic characters (limit of detection 0,01 ug/l,dilutuon linearity recovery 93%, spiking recovery 95%, interassay and interassay CV < 10%). Serum DKK-1 serum values were significant higher in individuals with osteoporosis (4.79 vs. 2.79 ug/l, P<0.01) and test had high diagnostic efficacy (ROC 0.92, 95% CI 0.78-0.99, sensitivity 74, specificity 100%, positive predictive value 100%). Differences were significant after adjustment for age, sex and bone markers. Results were verified with freguency chart (Chi square 18.4, P<0.01).
Conclusions. ELISA test for serum DKK-1 was developed. Serum DKK-1 is promising marker marker for laboratory diagnosis of osteoporosis. Largerstudieswillbecarried outinthe future.

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COMPARISON OF FIVE DIFFERENT SALIVA COLLECTION SYSTEMS

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Background. Interest in using oral fluid as a specimen that can be won easily is increasing. There are different systems existing to collect oral fluid. We wanted to know, if the collected volume of oral fluid depends on the system used.
Methods. The following five collection systems were chosen: Intercept (OraSure Technologies), Quantisal (Immunalysis), Salivette (Sarstedt), Saliva Collection System (Greiner Bio-One) and VersiSAL 1 (Oasis). Oral fluid was collected and measured from 15 volunteers on five days in a row.
Results. The mean values for the effective oral fluid volume were measured with 571,1 mg (Intercept), 1069 mg (Quantisal), 2057,5 (Salivette), 7696,2 (Saliva Collection System) and 1199,2 (VersiSAL 1).
Conclusions. The shown differences in the amount of the collected specimen may be of great influence on the analysis. Some of the described systems are using buffer systems or agents that have positive influence on the amount of oral fluid. Further studies on the preanalytic influence of these agents will be necessary.

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GENETIC PROGRAMMING FOR THE DATA BASED GENERATION OF MEDICAL DIAGNOSIS MODELS

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Background. Medical datasets have been used as benchmark data for a long time in order to analyse and compare machine learning techniques. Roughly speaking, data analysis techniques can be subdivided into two classes: The first class includes methods like linear regression or decision trees and is capable to deliver interpretable prediction models. However, the hypothesis space scanned by these techniques is rather restricted to rather simple and linear models. The second class including nonlinear modelling techniques like artificial neural networks and support vector machines is able to identify highly nonlinear correspondences; however the models are not interpretable by domain experts.
Methods. Genetic programming is a bionically inspired subfield of evolutionary algorithms which becomes more and more popular for data analysis in recent years. Even though genetic programming is a computationally highly expensive technique, it has some certain properties which are very interesting especially for medical data analysis: Genetic programming implicitly identifies the relevant input variables as well as the model structure. Furthermore, genetic programming is able to scan highly complex hypothesis spaces and deliver results in terms of mathematical formulae interpretable by domain experts at the same time.
Results. Results are shown on the basis several benchmark datasets taken from the UCI benchmark repository like the Wisconsin breast cancer or the Thyroid dataset representing 2 and 3-class classification problems. The results achieved by genetic programming are compared to results published in literature.
Conclusions. We conclude that genetic programming based data modelling has some certain properties which make it very well suited for medical data analysis.

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DETERMINATION OF CREATININE IN HUMAN SERUM USING HPLC WITH UV DETECTION: A COMPARISON WITH COMMERCIAL ENZYMATIC AND ROUTINE SPECTROPHOTOMETRIC METHODS

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Background. A sensitive method for the specific determination of creatinine in human serum with high precision and accuracy is described. Results from measurement by high-performance liquid chromatography on 232 serum samples were compared with enzymatic and spectrophotometric (Jaffe) Methods**.**
Methods. Serum samples were deproteinized with ethanol and analyzed by RP-HPLC. For the separation, a reversed phase column MAG 1, 4.6 x 250 mm, Labiospher PSI 100 C18, 5 μm (Labio a.s., Prague, Czech Republic), was used. The mobile phase consisted of 3% ethanol in 25mmol/L NaH_2PO_4 (v/v), pH 6.50. The analytical performance of this method was satisfactory.
Results. Results obtained by chromatographic method correlated with an enzymatic method, as well Jaffe's method. Spectrophotometric method gave almost similar values as HPLC method. But the lowest values at patients with non-insulin dependent diabetes mellitus and with increased bilirubin level were obtained by HPLC. It could be caused by non-specific interferents in the case of Jaffe's method. The highest values were provided by an enzymatic method. HPLC method gave at average much lower values (115.0 vs. 157.5 μmol/L in patients with noninsulin dependent diabetes mellitus; 111.0 vs. 142.3 μmol/L in patients with increased bilirubin level; 130.3 vs. 188.0 μmol/L in patients with cardiovascular diseases; 138.5 vs. 161.2 μmol/L in control group).
Conclusions. It appears that presented method may be useful for the analysis of samples where an enzymatic method do not give reliable Results. Chromatographic method is more selective than spectrophotometric method.

Miscellaneous

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SEROLOGIC EVALUATION OF POSITIVE HIV PATIENT WITH POSITIVE HEPATITIS B AND/OR C MARKER: 2 YEARS REVIEW (2008 – 2010)

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Background. The incidence of HIV is getting increased in last several years. Patients with HIV could get the hepatitis infection or inversely, which could worsen the quality of life patient live with HIV. This study evaluates the serologic marker from patient with positive HIV patient with positive hepatitis B and/or C marker.
Methods. Using retrospective data January 2008 – August 2010 in Dr. Hasan Sadikin Hospital-Bandung, more than 2000 patient have been evaluated for anti HIV, HBsAg and anti HCV. The data divided into 5 groups: anti HIV & anti HCV(+); anti HIV & HBsAg(+); anti HIV, HBsAg, & anti HCV(+); anti HIV(+) & HBsAg(-); anti HIV(+) & anti HCV(-). Anti HIV, HBsAg & anti HCV was examined using ECLIA method (Roche® Cobas Elecsys System).
Results. Over 2000 patient with HIV examination, 994 patients had positive result; In 2008, 8 patient had anti HIV and anti HCV(+); 1 patient had anti HIV(+) and HBsAg(-); 1 patient had anti HIV(+) and anti HCV(-). In 2009, 1 patient had anti HIV and HBsAg(+); 8 patient had anti HIV and anti HCV(+); 1 patient had anti HIV, HBsAg and anti HCV(+). In 2010, 5 patient had anti HIV and anti HCV (+); 1 patient had anti HIV, HBsAg and anti HCV(+).
Conclusions. The leading of infection between HIV and Hepatitis is still unclear, but it is necessary to evaluate the possibility risk of hepatitis infection in HIV patient to improve the quality of life patient live with HIV.

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EVALUATION OF BIOLOGICAL SPECIMEN ACCEPTABILITY IN HIGH-COMPLEX CLINICAL LABORATORY BEFORE AND AFTER IMPLEMENTING AUTOMATED PROCESSING SERUM INDICES

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Background. By continuously monitoring specimen acceptability, collection and transport, problems can be promptly identified and corrected, leading to improved patient care and a reduction in the inconvenience of unnecessary redraw and delays in result reporting. The aim of this study was to identify the unacceptable blood specimens and calculate the specimen rejection rate (SRR) before and after implantation of automated serum indices check.
Methods. This study was conducted between January 2009 and December 2010. The numbers of rejected specimens, location and reason for rejection have been recorded. The ARCHITECT c8000 analyzer (Abbott, USA) assesses the levels of serum indices based on spectral characteristic pattern and mathematical manipulations of absorbance values measured at several specific wavelengths. The SRR was calculated. The target/cut off value for SRR was < 0.5 % as it established by college of American Pathologists (CAP).
Results. The SRR were 0.13% and 0.21% for year 2009 and 2010 respectively. Hemolysis was the most significant reason for rejection with a cumulative rejection rate of 49.3% and 61.4 % for 2009 and 2010 respectively. Adult ICU had the most rejections (23.5%) samples followed by NICU (13.8%), Cardiac ICU (13%), pediatric ICU (10.8%), and long term wards (10.5%) of which 60%, 79%, 84.9%, 36.6 %, and 75% of rejected samples were hemolyzed respectively.
Conclusions. The increase in rejected samples may be due to an improvement in staff awareness on sample rejections aided by automatic sample integrity grading by either chemistry or immunoassay instruments for serum indices.

Miscellaneous

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STABILITY OF ADENOSINE DEAMINASE IN FROZEN POOLED BODY FLUIDS

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Background. Stability of adenosine deaminase (ADA)activity in frozen pooled body fluids was studied. Analytical characteristics of the method for ADA determination were also assessed.

Methods. Pooled body fluids with normal and high ADA activities were prepared and stored freezing. ADA activities in these pooled were determined periodically for 8 weeks by Galanti and Giusti's method, and stability of ADA activity was compared with the criteria of %total error. Linearity, lower detection limit, recovery, and precision of the method were also studied.

Results. Stability of ADA activity in normal and high frozen samples was 30 and 45 days, respectively. Linearity of the method was 250 U/L; lower detection limit was 1.2 U/L, with recovery of 110.9%. Within-run and between-run CVs were 2.09% and 7.27%.

Conclusions. ADA activity in frozen pooled body fluids was stable for at least 30 days of storage. Pooled sample with higher ADA activity was more stable than sample with lower activity. Evaluation of Galanti and Giusti's method for ADA activity determination gave satisfactory Results.

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EFFECTS OF WPC ON THE IMMUNE FUNCTION AND ANTIOXIDANT STATUS IN THE PATIENTS WITH BREAST CANCER

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Background. Recent studies suggest that the imbalance of the antioxidant status and abnormal immune function are involved in breast cancer;therefore, we investigate whether the immune and antioxidant statuses could be improved by the addition of antioxidant additives, such as whey protein concentrate (WPC).

Methods. We analyzed the lymphocyte subpopulations, apoptotic markers, and mitochondria membrane potential by a flow cytometer and the generation of superoxide radical (O_2^-) by ultra-chemiluminescenceto investigate the effects ofWPC on the peripheral blood mononuclear cells (PBMCs)in the 25 breast cancer patients and 25 healthy controls. Additionally, the levels of reduced form glutathione (GSH) and oxidized form glutathione (GSSG) in the erythrocytes were analyzed by a capillary electrophoretic analyzer.

Results. The GSH levels and GSH/GSSG ratios, and the numbers of CD3+ cells, CD4+ cells, and total lymphocytes in the patientswere significantlylower than those in the controls ($p<0.05$ by Student t-test). Conversely, the numbers of CD56+cells and the generationofO_2^-in the patientswere significantly higher than those in the controls ($p<0.05$ by Student t-test). After the supplementationwith WPC,the numbers of total lymphocytes were significantly higher in the controls than those without the supplementation with WPC ($p<0.05$ by Student t-test); however, they were not significantly different in the patients.

Conclusions. These results suggested that the immunological functions might be enhanced by the supplementation with WPC;however, the effects of WPC on the antioxidant statuses were not apparent.Therefore, the precise mechanisms remain to be further explored.

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OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY, INFLAMMATORY PARAMETERS AND BIOMARKERS OF MYOCARDIAL DYSFUNCTION AND DAMAGE AFTER AN IRONMAN RACE

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Background. Although physical exercise is general ly accepted to be protective and reduces the risk of coronary heart disease, acute and strenuous exercise may paradoxically promote inflammatory processes, free-radical formation, and cardiovascular tissue injury (1).

Objective and Methods. to investigate levels of oxidative stress (Reactive Oxygen Species, and Total Antioxidant Capacity, dROMs and OXY-Adsorbent test, respectively, Diacron International, Italy) and inflammation(C reactive protein, CRP, Immulite Medical Systems) and cardiac troponin I (cTnI, ADVIA CP, Siemens) as well as natriuretic peptides (BNP,Triage BNP, Beckman Coulter,Inc; NT-proBNP, Elecsys, Roche), as biomarkers reflecting myocardial stress, in a group of triathletes (n=14; age 37±6 yearsmean±DS; 12 males) before and after an Ironman race (IR).

Results. A significant increase in cTnI (0.005±0.007and 0.093±0.08 mg/L, prerace and postrace; p<0.01), BNP(39±30and 102±51 ng/L; p<0.001), NTproBNP (33±21and 390±196 ng/L; p<0.001) andCRP (0.1±0.15and 0.7±0.7 mg/dl, p<0.01) was observed after the race. No significant changes were foundfor dROMs (277±39and 260±46 AU) and OXY (410±36and 442±55 μmol HClO/ml).

Conclusions. The post-race increase in cTnI and BNP and NT-proBNP, which suggests subclinical cardiac damage and dysfunction, was associated with exercise-induced inflammation but not with elevation in oxidative stress biomarkers. Nonetheless, the lack of oxidative stress increase might represent a protective adaptation and resistance to adverse effects of strenuous bout of exercise in ironman (1).

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CHANGES IN SERUM ERYTHROPOETIN DURING THE TRAINING OF THE ATHLETES (LIVING HIGH - TRAIN MIDDLE/ LOW METHOD)

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Background. The athletes body during the training at high altitude (2400m), because the partial pressure of oxygen is reduced, reacts to hypoxia by increasing the number of erythrocytes. Erythropoietin (EPO) is responsible for this stimulation. Inthe area ofhigh altitude training, wecan finda largenumber ofstudies; however, the results are in manycasescontradictory. Weanalyzedthe impact ofaltitudetrainingon thecontent oferythropoietin.We were interested intheimpact ofmethodslivinghigh-trainlowtomiddle on serum EPOlevel.

Methods. We enrolled13Slovenian cross country ski runners. We divided them in two groups. The blood has been taken seven times upon the protocol. The firstgroup(G2400) wassleepingat an altitudeof2400m, the second group(G1600) wassleepingat an altitudeof1600m.Both groupsperformedthe sametrainingthathasbeenheightenedcharacter. In theG2400 group there were7runner s(averageage22.0years, STD+-2.24) in theG1600 group there were sixrunners(averageage20.8years, STD+-3.19).

Results. The results shows a change inEPOlevelsforseventraining sessionsforboth groups. EPOlevelswere higher in all seven training sessions but statisticallydifferent onlyin thetrainingperiod3 (p =0.027).

Conclusions. EPO concentrations rose at the beginning of training and later byincreasing theintensity oftrainingand time of training graduallydeclined. Allmeasured values werehigher in G2400than theG1600.

Miscellaneous

1305
FALSELY ELEVATED TROPONIN I RESULTS DUE TO EDTA CONTAMINATION

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Background. Potassium ethylenediaminetetraacetic acid (EDTA) is a common anticoagulant used for many laboratory analyses. EDTA contaminationof blood samples is easily recognised by marked hyperkalaemia.However, EDTA contamination leadsto a falsely elevated troponin I results, often is unrecognised.The aimof this study was to validate falsely elevated troponin I due to ethylenediaminetetra-aceticacid (EDTA) contamination

Methods. Specimenwere drawn into serum tubes and performed on the TOSOH AIA assays system with accompanying reagents. Clinician complained that some measuring cTnIvalues do not reflect the symptom,we study a series of factors,exclude mechanical problems ,fibrinogen, heterophilic antibodies,rheumatoidfactor.Later,we began to suspect serum samples to be contaminated. To explore thispossibility, we measured serum EDTA,potassium, calcium, magnesium concentrations and alkalinephosphatase activity in suspected samples.

Results. In seven samples,serum potassium values and EDTA were upper thereference range while calcium ,magnesiumvalues and alkalinephosphatase activity were below thereference range.Repeated drawn blood, above index were normal.we conclude that elevated troponin I samples were due to EDTA contamination

Conclusions. Claim is given in theTOSOH AIA Assays's package insert on thepossible use of EDTA samples, weconfirmedprevious reported. Factitious cTnI caused by EDTAcontamination does occur in routine lab and if unrecognised mayadversely affect patient care and mislead clinician.

1306
N-GLYCOSYLATION OF THE ADHESION RECEPTOR L-SELECTIN

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Background. L-selectin is an adhesion receptor mediating the rolling of leukocytes on endothelial cells, enabling leukocytes to migrate into surrounding tissues during inflammatory processes. L-selectin is a glycoprotein expressedconstitutively on leukocytes, is shed from the cell surface after activation and remains in blood serum in a biologically active form. In this study, the glycan structures within the ligand binding domains of L-selectin were analyzed.

Methods. L-selectin variants with disrupted N-glycosylation sites were expressed in the human cell line HEK293F. After purification, N-glycans were enzymatically released, monosaccharides were analyzed by HPAEC-PAD and the structures were deduced using sequential enzymatic digestions and MALDI-TOF mass spectrometry.The obtained glycosylation profiles were compared to the ones of alpha-1-acid glycoprotein (AGP) which was expressed in the same cell line.

Results. N-glycans of L-selectin were mainly of the diantennary complex type and displayed site heterogeneity at the three positions with the glycans in the first position being least heterogeneous. Glycan structures were highly fucosylated and parts of the antennae were terminating with uncommon N-acetylgalacosamine (GalNAc) units replacing the more common galactose. In addition, novel GalNAc-GalNAc motifs could be described. Parts of these glycans were also found to be sulfated. In comparison, none of these specific features were found in the N-glycan profile of AGP from the same cell line.

Conclusions. N-glycosylation of L-selectin is protein specific, site heterogeneous and contains unusual and sulfated GalNAc residues.

Miscellaneous

1307

THE DIVERSITY OF PLASMID PROFILES IN *ESCHERICHIA COLI* ISOLATED FROM VARIOUS SOURCES IN THAILAND

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Background. An emergence of antimicrobial resistance isa major problem of public health worldwide.*Escherichia coli* could be a reservoir of resistance genes and the potential transferability of the resistance genes among bacteria can happen.
Methods. Two hundred and fifty-eight strains of *Escherichia coli* isolated from various origins including chickens, dogs and cats, humans, vegetables and waters were screened for plasmids. The plasmid-containing strains were tested for antimicrobial susceptibility.
Results. Overall frequency of the isolates containing plasmids was 32.2% with the highest frequency of 55.4% for chicken isolates. The plasmid percentages of the isolates from other sources were 30.4% for edible waters, 16.7% for dogs and cats, 9.4% for fresh vegetables and 8.7% for pet owners. The number of plasmidsin each isolate ranged from one to seven and the variety of plasmid sizes ranged from 1.0 kb to 9.4 kb. More than two plasmids were demonstrated with higher occurrence (29%) in *E. coli* derived from chickens than in the other sources (0-7%). The small-size plasmids of 1.0 to 1.4 kb were found only in chicken isolates with a detection rate 35.5% of positive-plasmid isolates. Upto 96.8% of the chicken isolates harboring plasmids was resistant to tetracycline. The high variety of plasmid profiles with a plasmid sizes ranged approximate 1.0 to 10 kb was revealed in *E. coli* originated from chickens.
Conclusions. The high prevalence of isolates containing plasmids and the high diversity of plasmid profiles in *E. coli* originated from cecal contents of chickens was demonstrated in this study.

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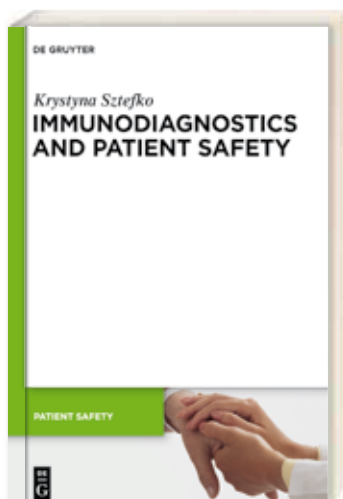
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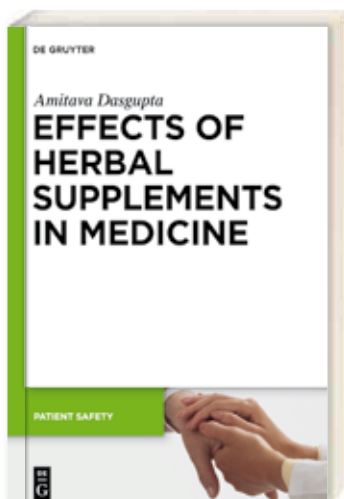
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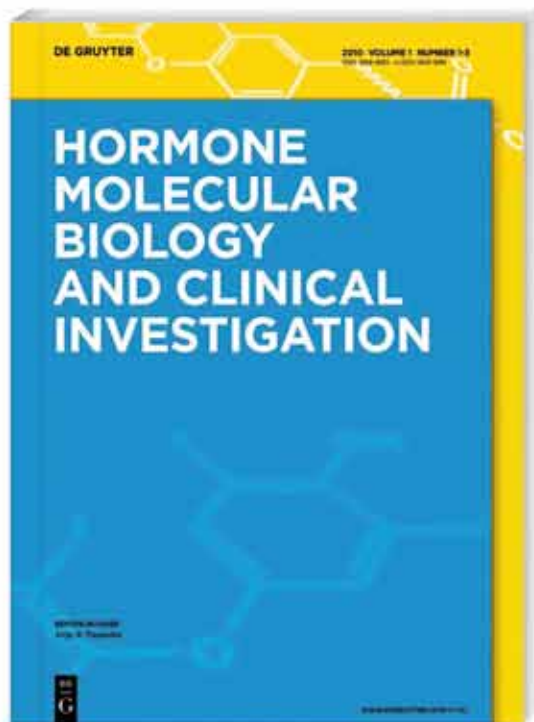**eBook** RRP € 49.95/*US$ 70.00
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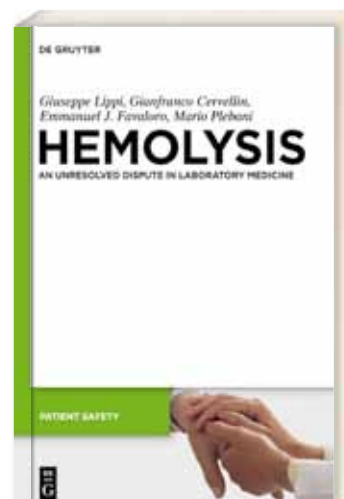
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