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Recent last 3 years in General Hospital Dubrovnik has been seen slightly declaine in number of patients under blood transfusion therapy because of respectful and strong medical documented indications for blood transfusion and using alternatives as erythropoetin (EPO)in clinical patient treatment.

Table 2:

Year	No.of patients under blood transf. therapy	No.(%)of patients with informed consent for transf.th.	No.(%)of patients without informed consent for transf.th.
2007	2 216	1 551 (70%)	665 (30%)
2008	1 935	1 470 (76%)	465 (24%)
2009	1 516	1 213 (80%)	303 (20%)
Total	5 667	4 234 (75%)	1 433 (25 %)

One urological patient who was member of Jehovah"s Witnesses refused blood transfusion therapy and two terminally ill persons rejected treatment. Others were in medical or surgical emergency and did not have the opportunity to consent the recipient of a blood transfusion.

Conclusions: Informed consent for derivatives of human plasma as IVIG, albumin recombinant products (rFVIIa, rFVIIIa) was not used. Consent has been remained effective during the course of treatment.

Every new hospitalisation obtained consents for transfusion therapy because of posibility diferent benefit/risk ratio for the patient. It is not recomended to give consent for covering all situations during different treatment program because of unexpected changes. There is also possibility to refuse a patient access to his health record if it is considered to be harmful to the patient. Although transfusion medicine in Croatia last 10 years has passed through lot of regulations and legal requirements, informed consent for transfusion therapy has not been obligatory yet. It has been still in state of recommendation.

P-0110

PRE-TRANSFUSION TESTING OF BLOOD EXAMPLES AT WU TRANSFFUSIOLOGY AT GENERAL HOSPITAL IN STIP REPUBLIC OF MACEDONIA

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Aim: To present the technical and the organizational procedures which are used at RE of Transfusion for procuring safe blood transfusion.

Material and methods: The safety of blood transfusion starts with an indication of the need of blood transfusion and filling in of the request which consists of: name and surname, date of birth, unique birth number, hospital identification number of the patient and signature and seal of the doctor which fills in the request, type and quantity of the blood component, diagnosis, short anamnesis, date, hour, institution, the unit where the request comes from and the degree of emergency. The next step is proper blood drawing from the patient, signing the blood sample with information from the patient, evidence number or barcode, date, hour and a signature of the health worker. This blood sample is submitted to the RE of Transfusion. The transfusion worker accepts the request and the blood sample, writes the date, the hour of acceptance and puts its own signature. If the blood sample is not urgent, it is kept at +4°C. ABO and RhD blood type of the blood sample of the patient is determined with two techniques and two different series monoclonal reagents. The techniques which are used are disc and microgel-agglutinative techniques on an adequate card. The determination of the blood subtype of A and AB is done if necessary. For determination of the RhD phenotype, a monoclonal anti-D reagent is used. The result is signed by the transfusion worker and the transfusiologist independently from one another. The test for detecting anti-erythrocyte antibodies is compulsorily done as an additional part of the test of compatibility. The above mentioned is conveyed with microgel method on an adequate card. Once again the blood type is checked and the Rh factor of the patient or the donor. A documentation is being filled in with the name and surname of the patient, year of birth, unit, blood type and Rh factor, date and hour of issuing, evidence number of the blood components and the transfusion worker and the transfusiologist put their signatures.

Results: In the last five years at the Clinical hospital in Stip, 12,569 transfusions of erythrocyte concentrate are being done. Only 10 (0.08%) post-transfusion unwanted reactions are detected. Not so heavy non-haemolitic febrile reactions are detected with 4 (0.032%) of the patients, and allergic post-transfusion reactions with 6 (0.048%) of the patients. Other difficulties and late reactions are not detected.

Conclusions: The use of pre-transfusion techniques and procedures in the modern transfusion unquestionably lead to safe blood transfusion and blood components.

P-0111

STUDY OF BLOOD BANK REFRIGERATORS TEMPERATURE AND ANALYSIS OF FACTORS AFFECTING TEMPERATURE IN IRANIAN BLOOD TRANSFUSION ORGANIZATION (IBTO) CENTERS

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Background and aim: Blood safety depends on temperature control throughout all stages in the cold chain. In fact different factors like: laboratories environment temperature, humidity, range of space between each refrigerator and walls or equipments, and frequency of refrigerator doors opened and the length the refrigerators can affect on refrigerators' operation and temperature.

Methods: Iranian Blood Transfusion Organization (IBTO) presently functioning with 88 centers throughout the country to maintain an effective and safe blood supply. IBTO within a period of 12 months in last year had a total collection rate of 1,825,213 blood units. Some of the IBTO centers are located in the regions where the climates and humidity differ from the other regions and such changes may have significant affects on refrigerators functioning. In order to measure the possible regional affect on each refrigerator's recorded temperature and functioning process since Oct 2008, a survey on 75 refrigerators of seven regional IBTO blood bank centers was carried out. In this survey along with temperatures recording from four shelves in blood bank refrigerator compartments using data loggers, laboratories environment temperature, humidity, range of space between each refrigerator and walls or equipments were also studied.

Results: The results obtained for eighty two blood bank refrigerators from four blood bank regional centers showed that due to various technical problems, only 41 sets of recorded temperatures were acceptable. In this study the averaged temperatures achieved from four shelves of blood bank refrigerators from the top shelve were 5.85, 4.1, 4.26 and 4.42°C respectively. The averaged amount of other factors like: humidity 29.6%, laboratories environment temperature 26.00°C, space distance between each refrigerator and walls 10.2 cm and space between each refrigerator and equipments 8.9 cm. The total time needed for completion of this study in remaining centers including Tehran Blood Transfusion Center is expected to be 5 months following Feb. 2010.

Conclusions: Investigation on the status of 75 blood bank refrigerators for seven IBTO regional blood bank centers is under process and the final results and conclusion will be presented with poster in Berlin. The preliminary studies on the mentioned factors indicates that, by completion of present study with 75 programmed tests in next 4 month, definitely a better idea about the factors affecting refrigerators used in blood bank centers equipped with controlling temperature and humidity versus those not

Table 2: Platelet aggregation

Table 2	Platelet Aggregometry					
	Day 1		Day 5		Day 7	
Aggregation [%] using	MCS+	Trima 5 2	MCS+	Trima 5 1	MCS+	Trima 5
TRAP 6	93 3±6 0* 94 3±4 1**	94 0±3 8* 87 5±11 2**	93 7±9 8* 92 5±5 2**	93 8±8 4* 90 3±5 8**	91 1±5 4* 88 6±6 4**	91 0±5 0* 91 6±5 3**
Arachodonic Acid	92 9±4 1* 35 8±16 3**	95 3±3 6* 49 6±17 4**	92 0±11 0* 42 3±21 5**	94 3±5 8* 57 1±14 0**	89 0±7 6* 42 5±19 4**	91 5±4 8* 56 9±12 9**
Collagen	83 5±8 1* 29 5±29 9**	82 1±19 1* 45 8±30 4**	15 0±13 0* 5 9± 1 1**	36 0±36 7* 5 9± 2 4**	12 0±12 1* 6 3± 1 0**	31 5±35 2* 5 9±0 6**
ADP	57 2±19 8* 13 3±10 6**	72 3±20 1* 19 9±29 3**	22 6±13 5* 1 4± 1 6**	28 1±15 6* 2 4±1 4**	14 1±10 3* 1 7± 0 8**	23 0±16 2* 2 6± 0 9**

after stimulation with ADP. The reversibility of results when diluted in plasma might reflect in vivo conditions and needed to be further investigated. Better knowledge of in vitro plt quality parameters might enable the performance of test systems with higher predictive value of the in vivo plt quality in the routine quality control.

P-0335

DEXAMETHASONE MOUSE MODEL FOR MONITORING IN VIVO VIABILITY OF STORED HUMAN PLATELET

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Background: Viability of stored human platelets (PLTs) is difficult to predict based on in vitro studies alone. Although Radiolabeled PLTs have been used to assess candidate PLT products for transfusion by infusion into autologous volunteers, such experiments are difficult to perform due to the limited number of qualified laboratories and high cost. An animal model was usually used for evaluating the in vivo recovery and survival of human PLTs before volunteer transfusion. Because human PLTs will be cleared rapidly from circulation of animal, the method allowed prolonging circulation of human PLTs in animal model need to be developed and assessed.
Aim: To establish an mouse model in which can prolong circulation of human PLTs, and tell the difference of in vivo recovery and survival between fresh and stored human PLTs.
Method: To evaluate the effect of Dexamethasone (DEX) on phagocytosis of mouse macrophage, Male 4-week-old KM mice were injected DEX with two kinds of dosage separately. The carbon clearance test was used to determine the phagocytic index of mice. Fresh human PLTs were transfused into DEX treated or control mice through their tail vein. Mice whole blood was collected at 5 min, 2 h, 4 h, 8 h and 24 h after transfusion, and human PLTs in mice whole blood were detected by flow cytometric analysis with an anti-human CD41-fluorescein isothiocyanate(FITC) monoclonal antibody. Recovery and survival of human PLTs either stored at 22° for 5day or at 4° for 1day were compared with human PLTs stored at 22° for 1day by DEX mice.
Result: The difference in corrected phagocytic index was significant between DEX treatment groups and control groups ($P < 0.05$), but there was no significant difference between low and high dosage (30 mg and 60 mg/kg weight) DEX injection groups. ($P > 0.05$; phagocytic index : control 6.69 ± 1.30; low dosage 3.84 ± 0.53; high dosage2.90 ± 0.69). The recovery at 24 h and survival time of fresh human PLTs in DEX mice were much higher than them in control mice (36.62% ± 13.90%, 25.92 ± 9.66 h vs 0.96% ± 0.61%,5.10% ± 0.58 h, $P < 0.01$). Human PLTs stored at 22° for 5 day or at 4° for 1 day presented decreasing in vivo recovery and survival significantly compared with human PLTs stored at 22° for 1 day in DEX mice ($P < 0.05$).

Conclusion: DEX efficiently slowed down the clearance of human PLTs in circulation of mouse. The DEX mouse model was able to tell the differences of recovery and survival time between stored and fresh human PLTs, and could be useful in evaluating the viability of platelets in novel PLT products.
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P-0336

STUDYING THE EFFECT OF PRESTORAGE WASHING (FOR LEUKOREDUCTION) ON THE FUNCTION OF PLATELETS IN PLT BAGS WHICH HAVE KEPT FOR 7 DAYS

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Introduction: The presence of WBCs in platelet component may induce some problems such as alloimmunization against HLA, CMV transmission, febrile non-hemolytic transfusion reactions (FNHTRs) in the patients (recipients). During platelets storage in laboratory, their function may be affected by such factors as cytokine and metabolite release from WBCs and, probably, decrease in pH. Because of these factors PLT shellife has been limited to at most 5 days (depending upon the bag type) in room temperature. The main purpose of carring out this research is washing PLT component for leukoreduction which may help to abate the above concerns.
Materials and methods: This study was on an experimental basis and carried out on the PLT components of random blood donors which were organized into two groups: test (washed products, 20) and control (unwashed products, 10). At the end of day seven of storage all the PLT units were primarily assessed for any bacterial or fungal growth through culture, then WBC count was carried out on the components in two groups. Finally, in order to evaluate platelet function, samples were analyzed in an aggregometer with four agonists, arachidonic acid, ADP, collagen and ristocetin. The obtained data were assessed by t-test.
Results: Statistical analysis indicated that PLT washing had no significant effect on the number of platelets but had caused WBC reduction in washed components ($P < 0.01$). This analysis also showed that there was a significant difference in PLT average reaction with arachidonic acid ($P < 0.01$) and collagen ($P < 0.05$) in two groups, but there appeared no significant difference between PLT average reaction with ADP and ristocetin agonists in two groups ($P > 0.05$).
Conclusion: PLT washing causes WBC reduction in platelet component, and fortunately doesn't have any undesirable on this product, so this protocol may be recommended for increasing PLT shelf life, from 5 days to 7 days.
Keywords: Washed PLT-WBCs-alloimmunization.

P-0337

USE OF LEUCODEPLETED RED BLOOD CELL CONCENTRATES-OUR 10TH YEAR EXPERIENCE

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Introduction: The removal of the leucocites with the help of filtration improves the quality of red blood cell concentrates, with which the risk of apperance of post-transfusion non haemolitic febrile reactions, aloimunisation, immunosupression, post operative infections, virus contamination (CMV, HTLV), bacteria contamination has been minimized.
Aim: To show the benefit from the use of red blood cell concentrates, poor with leucocites produced with the help of filtration in pacients chronically dependable from transfusion, treated in Daily transfusion hospital.
Methods: In the last ten years in our Daily hospital 816 patients from Stip and from the municipalites from the eastern part of R.Macedonia, have been treated and they have needed blood transfusion once to twice per month, suffering from Talassemia major, Sy.myelodisplasticum, Leucosis lymphatica chr. and malignant disease post operatively, chemio-

therapy and radiotherapy. The blood units (450 ml) were donated by voluntary repeated blood donors and the some ones were separared in period of 10-12 h. The obtained red blood cell concentrates has been filtrated in period of 2-3 days and has been immediately used after this process. Filters for preparation of leucodeplated red blood cell concentrates have been used, produsedby the firms TERUMO-IMUGARD III-RC and PALL-PURECELL RN. The procedure has been done manualy by gravitation. The filtration time is from 18-20 min. The blood samples for analyses have been taken from the system before and after the filter and have been made at the Clinical chematologic laboratory. The following chematologic parameters have been determined: number of Er, Le, Tr and level of Hb and Hct.

Results: With the use of these filters, red blood cell concentrates poor with leucocites for about 99-99.9% have been produced with which post-transfusion side effects of allogenic leucocites have been prevented. The therapy effect is also important because the number of Er, Hb and Hct remain almost unchanged in the unit of filtrated blood.

Conclusion: The use of leucodeplated red blood cell concentrates improves the quality and safety of blood transfusion in patients chronically dependable from transfusion which makes their lives longer.

P-0338

FACTORS INFLUENCING THE RELIABILITY OF CD62P MEASUREMENT IN STORED PLATELETS

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Background: P-selectin (CD62P) is a degranulation/activation marker that is predominantly stored in platelet α-granules. Increasing CD62P expression with time of storage is an indicator of the platelet storage lesion. Flow cytometric CD62P analysis is usually included in any panel of tests that reflect platelet quality. Importantly, although production and storage practices may be similar, CD62 levels reports in the literature or in multi-centre studies may vary widely; however, the contributing variables are not well understood. We observed inconsistency in CD62P levels when data acquisition from a stained sample is delayed. Because the source of antibody, anticoagulant, and brand of instrument may affect the CD62P signal, we conducted a study (i) to identify reagents, antibody and storage conditions that provide accurate CD62P measurement; (ii) to determine the length of storage time within which delayed sample acquisition can be performed without risk of losing the CD62P signal; and (iii) to determine the impact of anticoagulant and fixation on the CD62P signal.

Material and methods: Platelet concentrates were produced by the buffy coat method and analysed directly from the storage bag. Three clones of phycoerythrin-labelled CD62P antibody were compared: Thromb/6 (Immunotech), AC 1.2 and AK-4 (BD Biosciences). Platelets were incubated for 30 min with a saturating concentration of antibody and the incubation was terminated with 1% methanol-free formaldehyde (MFF). Sample acquisition was performed immediately after the fixation step on a FACS Canto-II flow cytometer (BD Biosciences). After acquisition, flow tubes were stored at 4°C in the dark and reanalysed after 2 h, 24 h, 5 days and 8 days. CD62P signal stability over time was compared to signal stability of CD42 and CD63 antibodies (Immunotech).

Results: CD62P signal for all three antibody clones was the same at time 0. After 30 min there was a notable decrease in signal obtained with Thromb/6 (25%), whereas signal obtained with AC 1.2 remained stable for up to 5 days. There was no difference between CD62P expression on platelets diluted with PBS and platelets fixed with 1% MFF at time 0; however, fixation did not stop signal deterioration over time for Thromb/6. If platelets were incubated with EDTA prior to antibody incubation, CD62P signal was not stable for any of the CD62P clones and continued to decrease. Expression of the pan-marker, CD42 and activation-dependent marker, CD63 on the same platelet population was stable for 7 days.

Conclusions: We observed that CD62P expression on the platelet surface was accurately detected by all antibody clones used in this investigation if acquisition was performed immediately after staining. For delayed acquisition, only AC 1.2 resulted in a stable signal. When platelets were subjected to an additional incubation with EDTA, the CD62P signal was not stable. These observations need to be considered when comparing CD62P expression levels between laboratories.

P-0339

EVALUATION OF BLOOD BANKED FIBRIN GLUE – VERSUS PLATELET GLUE AS LOCAL HAEMOSTATIC AGENTS IN ACUTE LEUKEMIC PATIENTS FOLLOWING DENTAL EXTRACTION

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Background: Most signs and symptoms of Acute leukemia are due to bone marrow failure secondary to infiltration & post – chemotherapy leads to susceptibility to infections & bleeding due to thrombocytopenia.

Aim: The aim of the present study is to compare clinically between the effect of using blood banked platelet glue (platelet rich plasma with fibrinogen added to human thrombin) and blood banked fibrin glue (fibrinogen and human thrombin) as a local haemostatic agents and healing promoter after dental extraction in acute leukemic patients suffering from thrombocytopenia .

Method and materials: This study was conducted on 42 patients (32 males and 10 females) with age ranging from 15-45 years old divided into two groups, the control group consist of seven patients and the study group fourteen patients divided into two subgroups each consist of seven patients . The control group patients received platelet transfusion 1 h before extraction to elevate platelet count to control the postoperative bleeding, while in the study subgroups blood banked FG or blood banked PG were used as a local haemostatic agents to control post operative bleeding .

Results: Post extraction bleeding was managed and coagulation achieved within 15 sec after the application of fibrin glue or platelet glue on top of extraction sockets for the study group and with platelet transfusion for the control group . During the 3 months of postoperative clinical follow up, it was evident that the use of local haemostatic used in this study helped in the process of healing of oral sockets. The result were comparable in both groups as regards to the small sized sockets, however, better haemostasis and healing results were achieved with platelet glue and fibrin glue when applied to the sockets of the posterior teeth The post extraction complication including pain, infection and delayed healing were overcome in this study clinical observation revealed excelled results in the study subgroups as compared with the control group.

Conclusion and Recommendations: Blood banked fibrin glue and blood banked platelet are follows:

1. The material is low cost than other commercially available sealants and platelet transfusion procedures and it appeared to be an excellent haemostatic agent.
2. A maximum bonding strength at 3 min.
3. As it made from the human blood it limited the inflammatory response.
4. Non-toxic, non-mutagenic, and non-carcinogenic.

We concluded that the action of blood banked FG, and blood banked PG as a local haemostatic agent is very important also its sealant effect and prevents complications showed significant acceleration of soft tissue healing in leukemic patients. Presence of growth factors in the platelet glue may be the cause of promoting soft tissue healing.

We advise using human thrombin of instead of bovine thrombin to minimize the possibility of complication.

P-0340

QUALITY CONTROL OF BUFFY COAT PLATELET CONCENTRATES

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Introduction: Platelet concentration (PC) preparation is a method stipulated by a large number of variables which either individually or jointly might have a significant effect on the quality of the final product.