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hsCRP AND ALL CAUSE MORTALITY IN HEMODIALYSED PATIENTS

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ABSTRACT

Introduction: High sensitivity CRP (hsCRP) has been used as a marker of low intensity chronic inflammation in many different pathologic conditions.

Aim of The Study: The aim of this study was to elucidate the value of hsCPR as a marker of one year and two years all cause mortality in patients on chronic hemodialysis treatment.

Patients, Methods and Study Design: All 60 patients (31 men and 29 women) from Department of Hemodialysis were included in study. Four consecutive measurements of serum hsCRP were done: at the beginning of the study, after two weeks, 6 and 9 months. Immunoturbidimetric method on Olympus (now Beckman Coulter) AU400 automated biochemistry analyzer was used.

The lowest of four hsCRP values was considered as the "basal hsCRP value" for any individual patient.

Based on their basal hsCRP values all patients were divided in three groups:

1. basal hsCRP value up to 1 mg/L (n = 26, 8 men and 18 women; age = 57±12 years; duration of hemodialysis = 8±7 years);
2. basal hsCRP value from 1 to 3 mg/L (n = 18, 12 men and 6 women; age = 62±10 years; duration of hemodialysis = 7±6 years);
3. basal hsCRP value above 3 mg/L (n = 16, 11 men and 5 women; age = 66±9 years; duration of hemodialysis = 9±7 years).

The all cause mortality rate was calculated after one and two years since the last hsCRP measurement. Student's t-test, Chi-Square test and Fisher's exact test were used for statistical calculations, as appropriate.

Results: There was no statistically significant difference in the duration of hemodialysis between all three groups ($p > 0,05$). There was no statistically significant difference in the age between the first and second group as well as the second and third group. Patients from the first group were significantly younger than those from the third group ($p < 0,025$) therefore these two groups were not compared.

One year after the last hsCRP measurement, the mortality rates were: 3,8% for the first group, 5,6% for the second group and 37,5% for the third group.

There was no statistically significant difference in the mortality rate between first and second group one year after the last hsCRP measurement. At the same time, the difference in the mortality rate between the second and third group was statistically significant ($p < 0,025$).

Two years after the last hsCRP measurement the mortality rates were: 3,8% for the first group, 27,8% for the second group and 37,5% for the third group.

Statistically, there was no significant difference in the mortality rate between the second and third group. However, the difference between the first and second group was statistically significant ($p < 0,025$).

Almost all patients that died during the study period were older than 60 years.

Conclusion: High hsCRP values, above 3mg/L, could be considered as a marker for prediction of one year all cause mortality among elderly patients on chronic hemodialysis treatment, while values

between 1 and 3mg/L have a higher association with two years all cause mortality when the age of the patients and duration of hemodialysis were standardized.

Very low mortality rate within the two years study period among patients with basal hsCRP lower than 1mg/L confirms that these are “safe hsCRP values” associated with very low risk of all cause mortality independently of age, duration of hemodialysis and comorbidities.

Keywords: hsCPR, all cause mortality, elderly, hemodialysis

INTRODUCTION

C-reactive protein (CRP) is a widely recognized indicator of inflammation as a nonspecific acute-phase protein. Historically, it was first observed in 1930 as a substance in the serum of individuals with *Pneumococcus* infections that formed a precipitate when mixed with the C-polysaccharide coat of *Streptococcus pneumoniae*ⁱ. In response of infection or tissue injury, CRP synthesis occurs in hepatocytes. Once synthesized, CRP binds to different foreign substances, thus activating the classic complement system, and promoting opsonisation and fagocytosis of bonded substances. Mild inflammation and viral infections generally cause CRP concentration to rise from 10 – 50mg/L. Concentrations from 50 – 200mg/L are seen in cases of active inflammation and bacterial infection. In patients with severe infection or trauma serum CRP concentration can increase to more than 200mg/Lⁱⁱ.

As it became clear that all stages, i.e. initiation, growth and complication of atherosclerotic plaque might be considered as an inflammatory response to injury, CRP in its reference values (up to 10mg/L) arose as an independent marker of increased risk of nonfatal and fatal cardiovascular disease. It was recommended that CRP should be measured in metabolically stable patients, without obvious infection or inflammation. Two assays, averaged, fasting or no fasting, optimally two weeks apart, should be performed, and results over 10mg/L discardedⁱⁱⁱ. For this purpose high sensitivity methods for CRP measurement were developed (hsCRP) and within reference range of up to 10mg/L three independent ranges were identified: less than 1,0mg/L – low coronary risk; from 1,0 to 3,0mg/L – average risk; above 3,0mg/L – high risk. Values of CRP persistently higher than 1,0mg/L but still lower than 10,0mg/L, measured with hsCRP methods, were considered as a marker for chronic low intensity inflammation.

Except with cardiovascular disease, chronic low intensity inflammation, identified with elevated hsCPR, is known to be associated with many other pathologic conditions as various malignancies or chronic obstructive pulmonary disease, that contribute to increase mortality rate in general population^{iv}. Thus, hsCRP is often referred as a “marker for all cause mortality”.

Hemodialysis is a lifesaving treatment for the patients with end-stage renal disease. Despite technological advances in hemodialysis in past few decades, all cause mortality rate in patients on chronic hemodialysis treatment remains high. Although cardiovascular disease is a major cause of death among chronically hemodialysed patients, it accounts for approximately only 45% of all cause mortality. That is why the aim of this study was to elucidate the value of hsCPR as a marker of short term all cause mortality in patients on chronic hemodialysis treatment.

PATIENTS, METHODS AND STUDY DESIGN

All patients from Department of Hemodialysis in Military Hospital in Skopje were enrolled in the study. No exclusion criteria were established. The only inclusion criterion was: treatment by chronic hemodialysis (1.Monday, Wednesday and Friday or 2.Tuesday, Thursday and Saturday). Data about comorbidities were not taken.

In the beginning there were 61 patients included in study. During the study period one patient continued her treatment in another clinic and she was excluded from the final report where there were total of 60 patients (31 men and 29 women).

Blood was drawn on Wednesday from the first group and on Thursday from the second group, immediately before hemodialysis. From half of the patients, blood was drawn in the morning (about 7:30 am), and from others in the early afternoon (about 1:30 pm), according to the data from literature that there is no diurnal variation in plasma CRP concentration^v and that fasting or nonfasting samples are suitable for analysis. Anyway, from patients from whom blood was drawn at about 7:30 am, fasting blood samples were obtained, while others were advised to have only light early breakfast to avoid gross lipemia.

For blood collection serum monovettes from Sarstedt were used with standard preanalytic procedure.

The study started in May 2007. Four consecutive measurements of serum hsCRP were done: at the beginning of the study, after two weeks, 6 and 9 months. The first, the third and the fourth measurement were done in blood samples drawn for regular routine laboratory analysis of hemodialysed patients. The second blood sample was drawn to meet recommendation for hsCRP measurement: two measurements, two weeks apartⁱⁱⁱ.

After centrifugation, tests were run in fresh serum samples, without delay. Analysis was done by immunoturbidimetric method on Olympus (now Beckman Coulter) AU400 automated biochemistry analyzer. The high sensitivity application was used with linearity from 0,08 – 80,00mg/L and lowest detectable level of 0,02mg/L.

The lowest of four hsCRP values was considered as the “basal hsCRP value” for any individual patient.

The all cause mortality rate was calculated after one and two years since the last hsCRP measurement.

Student's t-test, Chi-Square test and Fisher's exact test were used for statistical calculations, as appropriate. Differences between groups where p value was lower than 0,05 were considered as statistically significant.

RESULTS AND DISCUSSION

As stated in the description of study design, hsCRP was measured in fresh serum samples i.e. in few different runs. That is why a test of between-run precision was performed by running aliquots of four frozen serum samples with different CRP concentrations lower than 10mg/L in 12 days. The results from this test are summarized in Table1.

Table 1: Between-run precision of method for hsCRP measurement

hsCRP (mg/L) n = 12	CV (%)
0,48	8,3
1,39	3,6
2,37	3,0
4,92	2,4

The results presented in Table1 show an excellent between-run precision that allows comparison of results from different runs even for the lowest CRP concentrations.

There are few different approaches for determining individual's basal hsCRP value: 1.multiple blood sampling^{vi}, 2.three measurements at monthly intervals provided that there is not intercurrent infection^{vii}, 3.two independent measurements three months apart^{viii} or 4.two measurements, averaged, fasting or no fasting, optimally two weeks apart, values above 10mg/L discardedⁱⁱⁱ.

For study presented in this paper four consecutive measurements of hsCRP were done: at the beginning of the study, after two weeks, 6 and 9 months. Because there were no data about comorbidities, serial hsCRP measurements were preferred instead of only two measurements two weeks apart. For study design like this it was the best way to determine the basal hsCRP value for every individual patient: the lowest of four hsCRP values was considered as the "basal hsCRP". At this point it is important to stress that for some of the patients included in the presented study the lowest hsCRP value was not that from the first two measurements. This finding favors serial multiple sampling instead of "two measurements, two weeks apart" approach.

Based on their basal hsCRP values all patients were divided in three groups.

The first group consists from patients with basal hsCRP value up to 1mg/L (n = 26, 8 men and 18 women). Mean age of the patients from this group was 57±12 years, range from 34 – 77 years. Mean duration of hemodialysis for the patients from this group was 8±7 years, range from 2 – 28 years.

The second group consists from patients with basal hsCRP value from 1 to 3mg/L (n = 18, 12 men and 6 women). Mean age of the patients from this group was 62±10 years, range from 44 – 79 years. Mean duration of hemodialysis for these patients was 7±6 years, range 1 – 19 years.

For the third group basal hsCRP value was above 3mg/L (n = 16, 11 men and 5 women). Mean age was 66±9 years, with range from 53 – 78 years and duration of hemodialysis was 9±7 years, with range from 2 – 29 years.

First of all, the difference in duration of hemodialysis between groups was tested. For this purpose Student's t-test (two-sample, equal variance; two-tailed distribution) was used. No statistically significant difference in the duration of hemodialysis between all three groups was found ($p > 0,05$).

The same Student's t-test was used for testing the difference in the age between groups. No statistically significant difference in the age between the first and second group was found as well as between the second and third group. Patients from the first group were significantly younger than those from the third group ($p < 0,025$), therefore these two groups were not compared.

The mortality rate was calculated as a percentage of deaths at a specific point of time. These results are summarized in Table 2.

Table 2: Mortality rate in patients on hemodialysis

	Mortality rate 1 year after the last hsCRP measurement (%)	Mortality rate 2 years after the last hsCRP measurement (%)
1. group: basal hsCRP <1mg/L	3,8	3,8
2. group: basal hsCRP 1-3mg/L	5,6	27,8**
3. group: basal hsCRP >3mg/L	37,5*	37,5

*statistically significant compared with second group ($p < 0,025$)

**statistically significant compared with first group ($p < 0,025$)

One year after the last hsCRP measurement, i.e. in March 2009, the mortality rates were: 3,8% for the first group, 5,6% for the second group and 37,5% for the third group. More precise: from the first group a 67-years old woman, 7 years on hemodialysis, died in June 2008; from the second group a 66-years old man, 19 years on hemodialysis, died in February 2008; from the third group a 59-years old woman, 15 years on hemodialysis, died in October 2007, a 56-years old woman, 29 years on hemodialysis, 76-years old man, 19 years on hemodialysis and 75-years old woman, 3 years on hemodialysis died in February 2008, a 78-years old man, 2 years on hemodialysis died in May 2008 and a 78-years old woman, 2 years on hemodialysis died in December 2008. Almost all of the patients that died during the first year after the last hsCRP measurement were older than 60 years.

Because two expected frequencies were below 2, Fisher's exact test was used^{ix} for comparison of difference in the mortality rate between the first and the second group one year after the last hsCRP measurement. No statistically significant difference in the mortality rate between these two groups was found ($p > 0,05$).

For testing the difference in the mortality rate between the second and the third group one year after the last hsCRP measurement Chi-Square test was used, and it was found that the difference was statistically significant ($p < 0,025$).

Two years after the last hsCRP measurement the mortality rates were: 3,8% for the first group, 27,8% for the second group and 37,5% for the third group. More precise: there were no new deaths within the first and the third group. From the second group a 69-years old man, 5 years on hemodialysis, died in May 2009, a 66-years old woman, 8 years on hemodialysis, died in July 2009, a 72-years old man,

14 years on hemodialysis died in January 2010 and a 79-years old man, 2 years on hemodialysis, died in March 2010. All of the patients that died during the second year after the last hsCRP measurement were older than 60 years.

Using Chi-Square test it was found that two years after the last hsCRP measurement there was no statistically significant difference in the mortality rate between the second and the third group. However, the difference between the first and the second group was statistically significant ($p < 0,025$).

The predictive value of CRP for cardiovascular and all cause mortality in hemodialysed patients was studied either for CRP alone^x or in combination with other biochemical markers^{xii xiii}. In a similar study like this one presented here, serum CRP concentration above 6,2mg/L was found as a strong predictor of overall and cardiovascular mortality in patients with end-stage renal disease^{xiv}.

Results from our study clearly associate high basal hsCRP, above 3mg/L, with very high risk of one year all cause mortality in elderly patients on hemodialysis, while basal hsCRP from 1 – 3mg/L is associated with higher two years mortality rate in these patients. Very low mortality rate within the two years study period among patients with basal hsCRP lower than 1mg/L confirms that these are “safe hsCRP values” associated with very low risk of all cause mortality independently of age, duration of hemodialysis and comorbidities.

Small number of patients included in the presented study can be considered as its major weakness. Anyway, in this occasion I think that it is important to stress the fact that all patients from Hemodialysis Department associated with laboratory where hsCRP was run were included, without any exclusion made by author. The only exclusion was one patient that left this Department of Hemodialysis and continued her treatment in another clinic.

Because of small number of patients included, it was not possible to test statistically the differences between men and women. Further studies, with higher number of patients should be done to test if there is a sex difference in predictive value of hsCRP for all cause mortality in hemodialysed patients.

Lack of data about comorbidities could be considered as another disadvantage of this study, but it was in accordance with study design. As stated before, the aim of this study was to elucidate the value of hsCRP for all cause mortality independently of comorbidities or reason for death (accidents excluded).

CONCLUSION

High basal hsCRP values, above 3mg/L, could be considered as a marker for prediction of one year all cause mortality among elderly patients on chronic hemodialysis treatment, while values between 1 and 3mg/L have higher association with two years all cause mortality among them.

This study provides important data about basal hsCRP values below 1mg/L that can be marked as values of very low risk for all cause mortality among chronically hemodialysed patients of different age, duration of hemodialysis and comorbidities.

In this context, and as a final conclusion, it seems to be useful to run serial hsCRP measurements as a part of regular medical check for patients on hemodialysis. Serial high hsCRP serum concentrations in

these patients, especially in elderly ones, could be considered as an early sign for increased risk of all cause mortality.

Patients with serial hsCRP values above 3mg/L will need further diagnostic procedures and appropriate medical treatment.

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