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# Serum Amyloid-A Rather Than C-Reactive Protein Is a Better Predictor of Mortality in Hemodialysis Patients

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The most frequent cause of death in hemodialysis patients is cardiovascular disease with chronic inflammation being an epidemiologically proved risk factor. Many studies have shown C-reactive protein (CRP) as the strongest predictor of long-term mortality of hemodialysis patients, while other reports have indicated acute phase proteins as potential predictors of the mortality. The present study therefore aimed to evaluate the prevalence of chronic inflammation in hemodialysis patients and the role of acute phase proteins together with lipids and divalent ions for predicting mortality in hemodialysis patients. Chronic inflammation was defined, based on the serum level of high sensitive CRP > 8.4 mg/L and/or serum amyloid-A (SAA) > 8.9 mg/L. Acute phase proteins are defined as one whose plasma concentration increase (positive) or decreases (negative) by at least 25% during inflammation. High sensitive CRP and SAA were positive acute phase proteins measured, while albumin and fetuin-A, a calcification inhibitor, were selected as negative acute phase proteins. This prospective 36-month follow-up study included 130 patients (60 males and 70 females, aged 55.1 ± 12.9 years) maintained by hemodialysis for 107.2 ± 54.72 months at a Nephrology Clinic in Belgrade. The prevalence of chronic inflammation was 35.4% (46 patients). During the follow-up period, 24 patients (18.5%) died and 2 patients received transplants. In multivariate analysis, potential independent predictors of mortality in hemodialysis patients are hyperphosphatemia, hypoalbuminemia, and high SAA. Considering that assays for SAA are widely used, we propose that SAA is the best predictor for outcomes of end-stage renal disease. ——— acute phase protein; serum amyloid-A; cardiovascular disease; chronic inflammation; dialysis mortality.
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Although improvements in renal care and dialysis technology have provided an increase in life expectancy of maintained dialysis patients over the last 40 years, the majority of them die within 5 years of therapy commencement. Nowadays, cardiovascular disease (CVD) has replaced both electrolyte complications and acute infections as the main cause of morbidity and mortality (Pecoits-Filho et al. 2002; Stenvinkel 2006). For patients with end-stage renal disease (ESRD), the annual CVD mortality is 10-20 fold higher than for the general population, even when adjusted for age, gender, race and the presence of diabetes mellitus (Foley et al. 1998).

While data from Atherosclerosis Risk in Communities (ARIC) suggest that both traditional and non-traditional cardiovascular risks are relevant in ESRD patients, many studies have indicated that novel risk factors are far more common in this population than in the general population (Cheung et al. 2000; Zoccali et al. 2005). Among these new risk factors, which include oxidative stress and hyperhomocysteinemia, persistent inflammation has attracted much interest. The concept of microinflammation and inflammatory markers as predictors of mortality in dialysis patients are currently hot topics in the nephrology literature (Stenvinkel 2006).

Since the first report by Bergstrom and colleagues (1995) of an association between increased mortality and C-reactive protein (CRP), a marker of inflammation and positive acute phase protein (plasma concentration increase by at least 25% during inflammation) many studies have concluded the same in ESRD (Owen and Lowrie 1998; Iseki et al. 1999; Zimmermann 1999) and chronic kidney disease populations (Menon et al. 2005). Conclusions regarding other positive, such as serum amyloid-A (SAA) and haptoglobin, and negative acute phase proteins (plasma concentration decreases by at least 25% during inflammation) as predictors of dialysis patients' overall and CVD mortality, are scarce. One example is fetuin-A, a negative acute phase protein and calcification inhibitor (Schafer et al. 2003), which has been tagged as a strong predictor of high mortality in dialysis patients (Wang et al. 2005; Honda et al.

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2006). Recently, Fukomoto et al. (2007) pointed the high serum CRP as a predictive indicator for renal failure in patients with acute heart failure.

The aims of the present study were to evaluate the prevalence of inflammation in our hemodialysis unit and the role of positive (CRP, SAA, and haptoglobin) and negative (fetuin-A, albumin, and transferrin) acute phase proteins in the prediction of mortality in hemodialysis patients along with age, hemodialysis duration, dialysis adequacy, divalent ions and lipids.

## Materials and Methods

*Patients*

A prospective follow-up study of 130 prevalent patients in a single dialysis unit was performed over 36 months. Patients with diabetes mellitus, acute inflammatory disease and those less than 3 months on hemodialysis were not included in the study.

The causes of ESRD in the hemodialysis patients were chronic glomerulonephritis ($n$ = 22), chronic pyelonephritis ($n$ = 16), nephrosclerosis ($n$ = 22), polycystic kidney disease ($n$ = 18), systemic diseases ($n$ = 18), urological malformation ($n$ = 24) and unknown ($n$ = 10).

All patients were dialyzed thrice weekly (each time 4-5 hours) with a bicarbonate-based solution using polyamide and polysulphone low-flux or high-flux membranes. Clinical and immunological observations, as well as laboratory parameters, were monitored when participants entered the study.

All patients gave informed consent prior to their enrolment in the study, which was planned according to the ethical guidelines following the Declaration of Helsinki. The institutional review committee approved our study protocol thereby following local biomedical research regulations.

*Laboratory methods*

A blood sample was drawn from each patient before the beginning of the second weekly hemodialysis session in order to measure the following parameters: complete blood count (CBC), serum concentrations of uric acid, creatinine, albumin, calcium, phosphate, cholesterol, triglycerides, high sensitive CRP (hs-CRP), haptoglobin, transferrin, SAA and fetuin-A. Hematological profiles were determined using a LH 750 hematology analyzer (Beckman Coulter Inc., California, USA). Creatinine, uric acid and albumin were analyzed employing routine methods (Olympus System Reagents using an Olympus analyzer AU 2700, Hamburg, Germany). hs-CRP, haptoglobin and SAA were measured using immunonephelometric assays (Dade-Behring, BN II, Marburg, Germany). Serum fetuin-A was determined with ELISA (Epitope Diagnostics, Inc., San Diego, California, USA). The reference ranges for acute phase reactants were: transferrin (2.00-3.60 g/L), haptoglobin (0.30-2.00 g/L), hs-CRP (0.00-3.00 mg/L), SAA (0.00-6.8 mg/L) and fetuin A (350-950 mg/L).

The patient database included demographic (sex and age), clinical (underlying disease, hemodialysis duration), hematological and biochemical variables.

Blood pressure was measured before dialysis. The mean value of all recordings taken during the month before the study was considered as representative of the blood pressure of each patient. The mean arterial blood pressure (MAP) was calculated as diastolic blood pressure plus 1/3 of the pulse pressure.

*Hemodialysis adequacy*

At the point of study entry Kt/V, normalized protein catabolic rate (nPCR) and residual renal function were determined (Blake and Daugirdas 1996).

*Statistics*

Differences in continuous variables between the analysed groups were tested using the Student's t-test for normally distributed variables. Because the distributions of hs-CRP and SAA were skewed, logarithmic transformation of the values was performed before comparisons were made. Group differences for categorical variables were examined by the $\chi^2$-test.

Variables for inflammatory markers (hs-CRP and SAA) as well as for albumin were divided into high-risk and low-risk fractions according to quartile values in the all examined patients. High-risk values defined as the higher quartile in the patients group were > 8.4 mg/L and > 8.9 mg/L for hs-CRP and SAA, respectively. High-risk values for albumin defined as the lower quartile in the group of patients were < 37 g/L. Low-risk values for hs-CRP and SAA were ≤ 8.4 mg/L and ≤ 8.9 mg/L, respectively and for albumin ≥ 37 g/L. Chronic inflammation was defined, in group of patients with concentrations of hsCRP > 8.4 mg/L and/or concentrations of SAA > 8.9 mg/L.

In order to analyze the risk of death, Kaplan-Meier curves and univariate survival analysis with the Cox proportional hazard model were obtained. The primary dependent variable was the time to death measured in months. Variables that were significant in uni-variate analysis were entered into forward stepwise Cox proportional hazard model to determine the adjusted hazard ratio (HR). The final model only included those variables that emerged as statistically significant.

The results are expressed as arithmetic mean (X) ± standard deviation (S.D.) for normally distributed variables and as geometrical mean and 95% confidence intervals (CI) (Bland and Altman 1996) for hs-CRP and SAA. HRs are presented with 95% CI. A two-tailed $p$-value of less than 0.05 was considered significant. All calculations were performed using SPSS software.

## Results

The characteristics of the 130 hemodialysis patients at the beginning of the follow-up period are shown in Table 1.

During the 36-month period 24 patients (18.5%) died and 2 patients received transplants. The cause of death was a fatal cardiovascular event (heart failure, arrhythmia, myocardial infarction, thrombotic and hemorrhagic stroke) in 18 (75.1%), malignancy in 2 (8.3%) and unknown in 4 (16.6%) patients.

The deceased patients were significantly older, had significantly higher leukocyte and platelet counts, higher phosphate concentrations and calcium-phosphorus (Ca × P) products but lower Kt/V. The duration on dialysis therapy, MAP values, gender distribution and hemoglobin, creatinine, uric acid, calcium and lipid serum concentrations were similar for both groups.

The serum concentrations of inflammatory markers, SAA ($p < 0.01$) and hs-CRP ($p < 0.01$), were higher in deceased patients compared with those that survived. The

Table 1. Demographic and clinical characteristics of the patients and a comparison between surviving and deceased patients during the 36-month study period.

| | Total patient group ($n$ = 130) | Surviving patients ($n$ = 106) | Deceased patients ($n$ = 24) |
|---|---|---|---|
| Age (years) | 55.12 ± 12.99 | 53.8 ± 13.4 | 61.8 ± 10.0[a] |
| Gender (M/F) | 60/70 | 50/56 | 10/14 |
| MAP (mmHg) | 93.31 ± 11.87 | 92.66 ± 11.92 | 95.80 ± 12.85 |
| Hemodialysis duration (months) | 107.17 ± 54.72 | 105.0 ± 59.4 | 119.3 ± 85.2 |
| Kt/V | 1.42 ± 0.21 | 1.45 ± 0.20 | 1.31 ± 0.19[a] |
| Hemoglobin (g/L) | 108.08 ± 14.08 | 109.3 ± 14.5 | 104.8 ± 10.9 |
| Leukocytes ($10^9$/L) | 6.37 ± 1.82 | 6.10 ± 1.73 | 7.61 ± 1.75[a] |
| Platelets ($10^9$/L) | 189.92 ± 69.07 | 177.88 ± 56.63 | 233.82 ± 98.49[a] |
| Creatinine ($\mu$mol/L) | 871.36 ± 165.67 | 876.6 ± 170.0 | 819.5 ± 143.5 |
| Uric acid ($\mu$mol/L) | 337.98 ± 59.74 | 337.02 ± 61.20 | 348.36 ± 57.34 |
| Calcium (mmol/L) | 2.26 ± 0.21 | 2.24 ± 0.22 | 2.35 ± 0.17 |
| Phosphate (mmol/L) | 1.42 ± 0.41 | 1.36 ± 0.33 | 1.68 ± 0.64[a] |
| Ca × P ($mmol^2/L^2$) | 3.24 ± 1.07 | 3.02 ± 0.88 | 3.98 ± 1.55 [a] |
| Cholesterol (mmol/L) | 4.81 ± 1.09 | 4.76 ± 1.06 | 5.05 ± 1.24 |
| Tryglicerides (mmol/L) | 2.31 ± 1.44 | 2.29 ± 1.45 | 2.40 ± 1.52 |
| hs-CRP (mg/L)* | 2.79 (1.97 - 3.96) | 2.29 (1.57 - 3.36) | 6.05 (2.68 - 13.65)[a] |
| SAA (mg/L)* | 6.46 (4.69 - 8.91) | 5.27 (3.98 - 6.98) | 18.13 (4.75 - 69.13)[a] |
| Haptoglobin (g/L) | 1.27 ± 0.57 | 1.19 ± 0.51 | 1.61 ± 0.72 |
| Albumin (g/L) | 39.19 ± 4.49 | 39.7 ± 4.15 | 36.4 ± 5.3[a] |
| Transferrin (g/L) | 2.11 ± 0.70 | 2.1 ± 0.72 | 1.95 ± 0.67 |
| Fetuin-A (mg/L) | 185.4 ± 59.2 | 189.7 ± 62.5 | 166.8 ± 47.1 |

Continuous variables are presented as mean value ± 1SD, whereas categorical variables are presented as absolute frequencies

* For hs-CRP and SAA the geometrical mean and 95% CI for the mean are presented.

[a] Significantly different from surviving patients by the Student-$t$ test, ($p < 0.05$)

concentration of serum albumin was lower among patients that died compared with those that survived ($p < 0.01$). The concentrations of serum haptoglobin, transferrin and fetuin A were not significantly different between the groups (Table 1).

According to results shown on Table 1, we segregated the patients according to quartile values and examined the prevalence of high-risk fractions in both groups. High-risk values defined as the higher quartile in the patients group were > 8.4 mg/L and > 8.9 mg/L for hs-CRP and SAA, respectively. High-risk values for albumin defined as the lower quartile in the group of patients were < 37 g/L. Comparisons of the prevalence of high-risk values for hs-CRP, SAA and albumin in patients that survived or died revealed a significantly greater prevalence of high hs-CRP values (19.6% vs. 50%, $p = 0.002$) and SAA (19.6% vs. 54.5%, $p = 0.006$) in the deceased patients. In addition, the latter group indicated a higher prevalence of low albumin concentrations, but was beyond the limit of statistical significance (24% vs. 36.4%, $p = 0.348$).

The prevalence of patients with chronic inflammation was 35.4% (hsCRP > 8.4 mg/L and/or SAA > 8.9 mg/L).

HRs and the corresponding 95% CI calculated by the univariate Cox proportional hazard model are presented in Table 2. Potential predictors of mortality were: age, leukocyte number, hsCRP, SAA, haptoglobin, albumin and phosphate. Higher levels of hs-CRP, SAA, haptoglobin, phosphate and high leukocyte number were shown to be associated with shorter survival time ($p = 0.002$, $p = 0.003$, $p = 0.026$, and $p = 0.026$, respectively). Increasing of life time for one year there was a higher risk of death during hemodialysis (HR = 1.004; 95% CI 1.002-1.007; $p = 0.041$). As expected, univariate Cox models confirmed the correlation between shorter survival in relation to lower albumin concentration ($p = 0.013$).

All significant predictors of mortality (age, leukocyte number, hsCRP, SAA, haptoglobin, albumin and phosphate) were subjected to a forward stepwise selection process. In

Table 2. Predictors of mortality using the univariate Cox proportional hazard model.

| Variable | Overall causes of Mortality | |
|---|---|---|
| | Hazard ratio (95% CI) | *p* |
| Age (years) | 1.004 (1.002 - 1.007) | 0.041 |
| Gender (M/F) | 0.554 (0.167 - 1.839) | ns |
| MAP (mmHg) | 1.018 (0.971 - 1.068) | ns |
| Dialysis duration (months) | 1.003 (0.995 - 1.011) | ns |
| Kt/V | 0.040 (0.002 - 1.055) | ns |
| Hemoglobin (g/L) | 0.978 (0.934 - 1.025) | ns |
| Leukocytes ($10^9$/L) | 1.373 (1.056 - 1.785) | 0.026 |
| Platelets ($10^9$/L) | 1.005 (0.999 - 1.011) | ns |
| Creatinine ($\mu$mol/L) | 0.998 (0.995 - 1.002) | ns |
| Uric acid ($\mu$mol/L) | 1.008 ( 0.998 - 1.018) | ns |
| Calcium (mmol/L) | 8.449 (0.589 - 121.133) | ns |
| Phosphate (mmol/L) | 3.129 (1.133 - 9.149) | 0.028 |
| Calcium × phosphate ($mmol^2/L^2$) | 1.688 (0.953 - 2.990) | ns |
| Cholesterol (mmol/L) | 1.188 (0.722 - 1.954) | ns |
| Triglycerides (mmol/L) | 1.027 (0.710 - 1.486) | ns |
| hs-CRP* (mg/L) | 4.943 (1.794 - 13.629) | 0.002 |
| SSA* (mg/L) | 3.095 (1.471 - 6.514) | 0.003 |
| Haptoglobin (g/L) | 2.563 (1.121 - 5.860) | 0.026 |
| Albumin (g/L) | 0.891 (0.814 - 0.976) | 0.013 |
| Transferrin (g/L) | 0.642 (0.254 - 1.622) | ns |
| Fetuin-A (mg/L) | 0.992 (0.980 - 1.005) | ns |

All variables were entered as continuous expect gender variable which was entered as categorical (men was coded with 1 and women was coded as 0).

Hazard ratio - Exp(B), 95% CI - 95% confidence interval of Exp(B), *p*-significance of coefficient

*hs-CRP and SAA concentrations were included in the analysis as continuous variables after their logarithmic transformation in order to obtain a near normal distribution.

Table 3. Predictors of mortality using the multivariate Cox proportional hazard model.

| Variable | $\beta$ | Hazard ratio (CI) | *p* |
|---|---|---|---|
| Albumin (g/L) | –0.143 | 0.867 (1.002 - 1.007) | 0.03 |
| Phosphate (mmol/L) | 2.321 | 10.183 (1.671 - 62.045) | 0.012 |
| SAA* (mg/L) | 1.831 | 6.240 (1.664 - 23.403) | 0.007 |

Age, leukocyte number, hsCRP, SAA, haptoglobin, albumin and phosphate were entered into forward stepwise Cox proportional hazard model. All variables were entered as continuous.

SAA concentrations was included in the analysis as continuous variables after their logarithmic transformation in order to obtain a near normal distribution

Hazard ratio - Exp(B), 95% CI - 95% confidence interval of Exp(B), *p*-significance of coefficient

multivariate analysis higher SAA and phosphate as well as lower albumin concentration were found to be significantly associated with shorter survival time (Table 3). In the multivariate Cox proportional hazard model each unit increase in log SAA and phosphate were significantly associated with higher mortality (HR = 6.240; 95% CI 1.664-23.403 and HR = 10.183; 95% CI 1.671-62.045, respectively). However, each unit decrease in albumin was significantly associated with a lower risk of survival (HR = 0.867; 95% CI 1.002-1.007).

In order to analyze the Kaplan-Meier curves for significant inflammatory predictors of mortality, we segregated

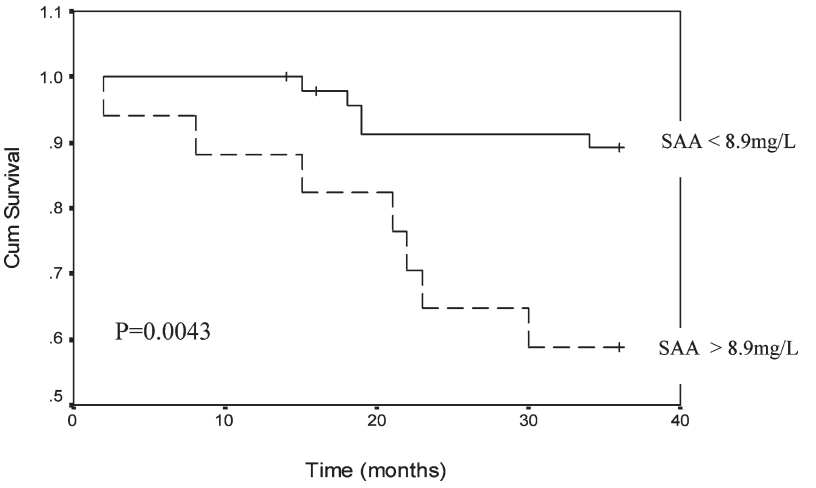


Fig. 1. Kaplan-Meier survival curves. Shown is cumulative survival of patients (Cum Survival). Patients with higher SAA (> 8.9 mg/L) showed a significantly reduced survival rate ($p = 0.0043$).

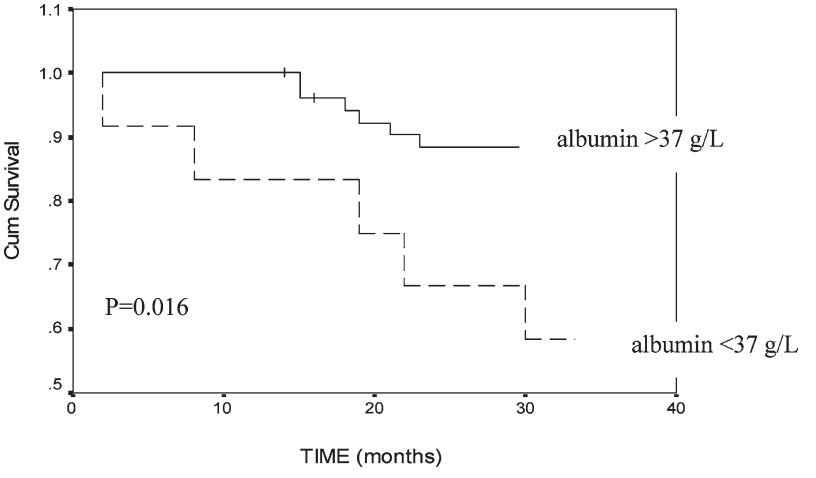


Fig. 2. Kaplan-Meier survival curves. Shown is cumulative survival of patients. Patients with lower albumin (< 37 g/L) showed a significantly reduced survival rate ($p = 0.016$).

the surviving patients according to quartile values. A high-risk value was defined as the highest quartile in the group of patients that survived (> 8.9 mg/L for SSA). High-risk values for albumin defined by the lower quartile in the group of patients that survived were < 37 g/L. The Kaplan-Meier survival estimate in patients according to the SAA and albumin scores are presented in Figs. 1 and 2. Patients with higher SAA (> 8.9 mg/L) had a significantly reduced survival rate ($p = 0.0043$) (Fig. 1). Similarly, patients with lower albumin (< 37 g/L) had a significantly reduced survival rate ($p = 0.016$) (Fig. 2).

## Discussion

In our present study the prevalence of patients with chronic inflammation was 35.4%. The prevalence of inflammation in ESRD patients in the nephrology literature varies from 30 to 75% depending on multiple factors such as residual renal function, geographic and genetic differences, dialysis therapy, co-morbidities as well as the cut-off point used for diagnosing inflammation (hs-CRP) (Stenvinkel and Alvestrand 2002). The reason(s) for the increased prevalence of persistent low-grade inflammation in ESRD patients are multifaceted and include an assortment of factors related to uremia and dialysis. The impaired immune response contributes to low-grade inflammation in ESRD patients (Stenvinkel et al. 2005).

In our present study during the 36-month follow-up period 24 patients died, the majority of them (75.1%) due to a fatal cardiovascular event. Serum concentrations of SAA and hs-CRP were significantly higher in deceased than the surviving patients. In addition, both positive acute phase proteins, along with haptoglobin, were selected as potential predictors of mortality.

SAA and CRP belong to group 3 acute phase proteins whose serum concentrations can dramatically increase (up to several hundred-fold) following acute inflammation. In most studies a parallel increase in both SAA and hs-CRP has been observed, although SAA may be a more sensitive

marker of inflammatory disease (Maury 1985).

Several studies in general population (Albert et al. 2002; Arima et al. 2008) as well as in nephrology literature (Owen and Lowri 1998; Iseki et al. 1999) have indicated that CRP is the most important predictor of cardiovascular mortality. The finding that CRP was a strong predictor of survival has been confirmed in a large Swedish-German and Italian study of 663 dialysis patients (Stenvinkel et al. 2002). However, other studies (Pecoits-Filho 2002; Panichi et al. 2004) reported that IL-6 was a stronger predictor than CRP in the ESRD population.

In nephrology literature, SAA is a much less common used marker of inflammation than CRP, possibly because SAA assays were not widely accessible in the past. Two investigations compared CRP and SAA as acute-phase proteins in peritoneal dialysis patients (Yuen and Kaysen 1997) and hemodialysis patients in a longitudinal study (Tsirpanlis et al. 2004). Our multivariate analysis found SAA, along with hypoalbuminemia and hyperphosphatemia, to be one of the most powerful predictors of patient death. In contrast, two survival studies indicated that CRP was a better predictor of CV mortality than SAA (Zimmermann et al. 1999; Hung et al. 2005). In those studies, SAA was measured with ELISA. On the other hand, in the present study, we used a nephelometric assay (Ledue et al. 1998). Nephelometric assays for hs-CRP and SAA now provide high analytical quality, enhanced sensitivity, excellent reproducibility and rapid automation. Therefore, they are well suited for routine clinical use and may provide clinical information previously impossible with other assay systems (Ledue et al. 1998).

During inflammation the SAA family can become apolipoproteins (apo) within high-density lipoproteins, synthesized in response to cytokines that are released by activated monocytes/macrophages. While remodeling the lipoprotein particle by relocating apoA-I, apoSAA may significantly modify the protective function of its physiological carrier and open the way for SAA to become a better predictor of risk in atherogenesis than other acute phase proteins (Malle and De Beer 1996).

Our study also revealed significantly lower serum albumin concentrations in deceased patients. In multivariate survival analysis hypoalbuminemia was one of the best predictors of ESRD patient mortality, which confirmed our long-term prospective (Simic-Ogrizovic et al. 2006) and other survival studies (Iseki et al. 1993).

In the Cox survival model fetuin-A was not selected as a predictor of patients' mortality. Some studies have revealed a link between inflammation, coronary calcification and low fetuin-A level in peritoneal (Wang et al. 2001) and hemodialysis (Cozzolino et al. 2006) patients. Honda et al. (2006) found fetuin-A to be one of the best predictors in ESRD patient mortality. Recently, Metry et al. (2008) indicated that a lower serum fetuin-A concentration predicts outcome only in inflamed prevalent hemodialysis patients.

In human hepatocytes IL-1, IL-6 and TNF-$\alpha$ are the most potent stimuli for acute phase protein synthesis. Therefore, pro-inflammatory cytokines will have much better prognostic utility if compared with acute phase response proteins. However, as Zoccali et al. (2004) pointed out, the cost of measuring IL-6 is about four times that of determining hs-CRP. Therefore, hs-CRP measurement is a reasonable, cost-effective option in every day clinical practice. However, in our study SAA was a better predictor of hemodialysis patient outcome than hs-CRP. As assays for SAA are now more practicle, SAA could be more widely used as a predictable inflammatory marker for ESRD outcome in the future.

In nephrology literature (Noordzij et al. 2005; Wald et al. 2008), hypoalbuminemia, and hyperphosphatemia are well known independent predictors of ESRD patient mortality. Our data concur with such large multi-centric studies.

One limitation to our present study is the relatively small number of patients analyzed. Therefore, the proposal that SAA is the best predictor of CVD mortality in hemodialysis patients should be confirmed in a much larger study.

In our present study the prevalence of patients with chronic inflammation was 35.4%. During the 36-month follow-up period 24 patients died, most of them due to fatal cardiovascular events. The serum concentrations of positive acute phase proteins (SAA, hs-CRP and haptoglobin) were significantly higher in deceased than in surviving patients. Each measured positive acute phase protein and hypoalbuminemia appeared as potential predictors of mortality. However, the best predictor was SAA along with hypopalbuminemia and hyperphosphatemia. Bearing in mind that assays for SAA are now more practical, SAA could be broadly used as a predictable inflammatory marker for ESRD outcome in the future.

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