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CLINICAL RESEARCH
Renal disease

Pregnancy-associated plasma protein-A is an independent short-time predictor of mortality in patients on maintenance haemodialysis

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Aims Mortality of maintenance haemodialysis (HD) patients is very high due to polymorbidity, mostly from metabolic and cardiovascular disease. In order to identify patients with high risk for life-threatening complications, reliable prognostic markers would be helpful. Pregnancy-associated plasma protein-A (PAPP-A) has been shown to predict cardiovascular events and death in patients with stable coronary artery disease as well as in acute coronary syndrome in patients with normal renal function. It was the aim of this study to evaluate PAPP-A as a marker for death in patients on maintenance HD.

Methods and results PAPP-A serum levels were measured in 170 patients participating in the *monitor!* trial, a prospective dynamic dialysis cohort multicenter study in Switzerland. Patients were followed up for a median time of 17 months after measuring PAPP-A, and evaluated for death of any cause. Survivors and non-survivors were compared with regard to baseline PAPP-A concentrations. A multivariate logistic regression analysis for death was performed including PAPP-A, age, sex, number of comorbidities, dialysis vintage, Kt/V, IL-6, C-reactive protein, parathyroid hormone (PTH), $Ca \times PO_4$ product, and total serum cholesterol. A cut-off value for PAPP-A was calculated for discrimination between patients with low and high mortality risk, respectively. A total of 23 deaths occurred during follow-up, equalling an incidence rate of 0.1. Baseline median PAPP-A levels were 40% higher in non-survivors vs. survivors ($P = 0.023$). In a multivariate analysis, only PAPP-A, age, and $Ca \times PO_4$ product were independent predictors of mortality. A cut-off value of 24 mIU/L discriminates significantly ($P = 0.015$) between patients at low or high risk for death with a negative predictive value of 91%.

Conclusion PAPP-A is a novel and independent short-time predictor of mortality in a maintenance HD population. The pathogenetic relevance of PAPP-A, particularly in the development of cardiovascular disease, remains to be further elucidated.

Keywords Pregnancy associated protein A (PAPP-A) • Chronic renal failure • Haemodialysis • Cardio-renal syndrome • Cardiovascular mortality • Prognosis

Introduction

Haemodialysis (HD) treatment is associated with a high morbidity and mortality, mainly due to cardiovascular complications.[1] It would be desirable to have simple screening tools available to assess an individual patient's risk with regard to the occurrence of medical complications including death. Pregnancy-associated plasma protein-A (PAPP-A) is a high-molecular-weight,

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zinc-binding matrix metalloproteinase belonging to the metzincin superfamily of metalloproteinases[2] and was originally identified in the plasma of pregnant women.[3] It is widely used to exclude foetal trisomy in the first trimester of gestation. PAPP-A was also found to be abundantly expressed in eroded and ruptured vascular plaques, but is only minimally expressed in stable plaques.[4] In addition, PAPP-A has been identified as an independent predictor of cardiovascular events and death in patients with stable coronary artery disease as well as in acute coronary syndrome (ACS).[5–7] Only limited data are available on PAPP-A in patients with chronic renal failure and dialysis. PAPP-A levels are elevated in chronic HD patients and transiently increase during HD sessions.[8,9] The aim of this study was to assess the prognostic value of PAPP-A as a predictor of mortality in patients on maintenance HD, and to compare it to other prognostic risk markers and parameters of HD quality.

Methods

Patient population and study design

The study population consists of 170 prevalent maintenance HD patients participating in the *monitor!* trial, a prospective dynamic multicentre HD cohort study, including participants from seven hospital-based dialysis units in Switzerland. All patients who had undergone HD treatment for more than 3 months and who gave their written informed consent were included into the study. The only exclusion criterion was a predictable short-term drop-out, such as terminal illness or planned change of renal replacement modality. The study protocol has been approved by the institutional review boards of the respective centres, and all participants gave written informed consent. A baseline assessment including measurement of PAPP-A was performed at time of inclusion into the *monitor!* cohort. The cohort was established between autumn of 2006 and summer of 2007. In the present analysis, all patients with a baseline PAPP-A measurement available (98% of the cohort) are included.

Methods

All blood samples were drawn prior to start of dialysis sessions. Electrolytes, creatinine, urea, cholesterol levels, and parathyroid hormone (PTH) were measured locally in each dialysis centre. Blood samples for interleukin-6 (IL-6) and PAPP-A from all participating sites were analysed in a central laboratory (University Hospital Zurich). Samples for PAPP-A measurements were centrifuged and kept frozen at $-20^{\circ}C$ until analysis. PAPP-A concentration was measured immunometrically by TRACE (Time Resolved Amplified Cryptate Emission) on a Kryptor® analyser from Brahms AG Hennigsdorf, Germany, with a coefficient of variation of 0.036. The assay is based on two monoclonal antibodies that recognize the complexed heterotetrameric PAPP-A/proMBP (proform of eosinophil major basic protein), originally developed to measure PAPP-A in pregnancy. To determine whether PAPP-A concentrations are affected by HD, its levels were measured in nine patients prior to and after a 4 h HD session.

Endpoints

The primary objective of this analysis was to examine the predictive value of serum PAPP-A concentrations for all-cause mortality in maintenance HD patients. In addition, PAPP-A was to be assessed for prediction of cardiovascular mortality and morbidity as secondary outcomes. The following events were labelled as cardiovascular: unstable angina, myocardial infarction, stroke, or arterial revascularization procedures (PTCA, peripheral angioplasty of native vessels, and arterio-venous fistulas). Medical charts of all participants were reviewed in April 2008 by one investigator (Y.S.) for the occurrence of medical complications, interventions, hospitalizations, and death. As no patient prematurely terminated HD treatment, all deaths occurring in the study population during reported follow-up were accounted for. All events were analysed regarding their relation to cardiovascular causes and to time of follow-up within the study cohort. A comorbidity score adapted according to Davies *et al.*[10] was used to characterize patients with regard to possible confounding factors for mortality.

Statistics

Because of skewed distribution of most parameters examined, descriptive statistics are given in terms of medians (interquartile range, IQR; range), counts, and percentages, respectively. Differences between survivors and non-survivors were investigated by Mann–Whitney *U* test in continuous variables and Fisher's exact test in categorical variables. Bivariate correlation analysis was performed according to Spearman (Spearman's rho). Multivariate logistic regression (method: enter) was used to predict death as a dependent variable with PAPP-A as an independent variable, and age, sex, number of comorbidities, time on HD, C-reactive protein, IL-6, PTH, calcium × phosphate product ($Ca \times PO_4$), and total serum cholesterol as covariates. A cut-off value (*C*) for PAPP-A was calculated according to Jacobson and Truax[11] regarding the best sensitivity and specificity for prediction of mortality: $C = (SD_0 \times M_1 + SD_1 \times M_0)/(SD_0 + SD_1)$, whereas M_0 and SD_0 = mean and standard deviation of PAPP-A for patients alive, M_1 and SD_1 = mean and SD of PAPP-A for patients who died during the study. A Kaplan–Meier survival analysis was calculated stratified to PAPP-A cut-off value. The C-statistic for PAPP-A is reported as a measure of its discriminative power. All statistical tests are two-sided and $P \leq 0.05$ was considered to be significant. All analyses were carried out with SPSS for Windows, release 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Pregnancy-associated plasma protein-A measurements were available from 170 of 174 patients of the overall *monitor!* study cohort (98%). Patient characteristics are shown in *Table 1*. Among the total of 170 patients, 59 were women (35%), the median age was 70 years, and median treatment time on HD was 71 months. During the 17.5 months median follow-up time, 23 patients died (13.5%), equivalent to an incidence rate of 0.1 per patient-year. In addition, four cardiovascular events not resulting in death occurred. Non-survivors were significantly older and had slightly more comorbidities than survivors. In contrast, no differences in sex, dialysis vintage, dialysis efficacy (measured as Kt/V), and follow-up time were detectable between groups. Similarly, survivors and non-survivors were statistically comparable with regard to classical risk markers, such as C-reactive protein and IL-6, as well as with regard to parameters of disturbances in mineral metabolism, such as PTH and $Ca \times PO_4$ product. In contrast, baseline serum PAPP-A concentration was 40% higher in non-survivors ($P = 0.008$ for difference vs. 'survivors'). In a bivariate analysis, PAPP-A was related to dialysis vintage ($r = 0.213$, $P = 0.006$), serum PTH ($r = 0.153$, $P = 0.046$), and number of hospitalizations ($r = 0.165$, $P = 0.031$). In contrast, no

Table I Patient characteristics according to survival status

Variable (normal range)	All patients		Survivors[a]	Non-Survivors[a]	*P*-value
	Median (IQR)[a]	Range	Median (IQR)	Median (IQR)	
n	170	—	147	23	—
Sex (male/female), *n*	111/59	—	97/50	14/9	0.643[b]
Age, year	70 (57–77)	28–89	68 (55–76)	77 (72–80)	**0.011**
Follow-up, days	526 (463–553)	35–589	525 (463–553)	526 (498–553)	0.541
Time on HD, days	853 (403–1571)	34–4759	831 (393–1560)	995 (596–1978)	0.557
Comorbidities, *n*	1 (1–2)	0–5	1 (1–2)	2 (1–3)	0.444
PAPP-A, mIU/L	21 (15–26)	7–117	20 (15–25)	28 (19–35)	**0.008**
IL-6, ng/L	5.7 (2.9–11.2)	2–93	5.4 (2.9–10.7)	7.6 (3.8–17.3)	0.279
C-reactive protein, mg/L	4 (2–9)	0–79	5 (2–9)	3 (2-10)	0.563
Cholesterol, mmol/L	4.2 (3.7–4.8)	1.9–7.6	4.2 (3.7–4.8)	4.2 (3.3–4.8)	0.981
PTH, ng/L	210 (126–330)	6–1493	214 (132–331)	173 (95–296)	0.798
$Ca \times PO_4$, $mmol^2/L^2$	3.7 (3.1–4.4)	0.9–7.7	3.7 (3.1–4.3)	3.9 (2.5–5.0)	0.278
Kt/V	1.6 (1.40–1.75)	1–3	1.6 (1.40–1.80)	1.5 (1.30–1.70)	0.819
No. of CV endpoints, *n*	5	—	4	1	—
No. of death, *n*	24	—	0	23	—

[a]Interquartile range: 25th and 75th percentiles.
[b]Fisher's exact test.

Table 2 Multivariate logistic regression model ($n = 170$)

	Odds ratio	95% CI	*P*-value
PAPP-A, mIU/L	**1.048**	**1.009–1.088**	**0.015**
Age, year	**1.077**	**1.015–1.142**	**0.014**
Sex, female	1.449	0.491–4.280	0.502
Comorbidities, *n*	1.339	0.797–2.252	0.270
Time on HD, days	1.000	0.999–1.000	0.655
IL-6, ng/L	1.049	0.991–1.111	0.101
CRP, mg/L	0.999	0.932–1.070	0.967
PTH, ng/L	1.000	0.998–1.002	0.694
$Ca \times PO_4$, $mmol^2/L^2$	**1.692**	**1.009–2.837**	**0.046**
Cholesterol, mmol/L	1.282	0.719–2.285	0.399

Table 3 PAPP-A cut-off and respective number of patients dead below/above cut-off

	Total	Dead
No risk (PAPP-A <24 mIU/L); *n* (%)	112 (66)	10 (9)
At risk (PAPP-A ≥24 mIU/L); *n* (%)	58 (34)	13 (22)

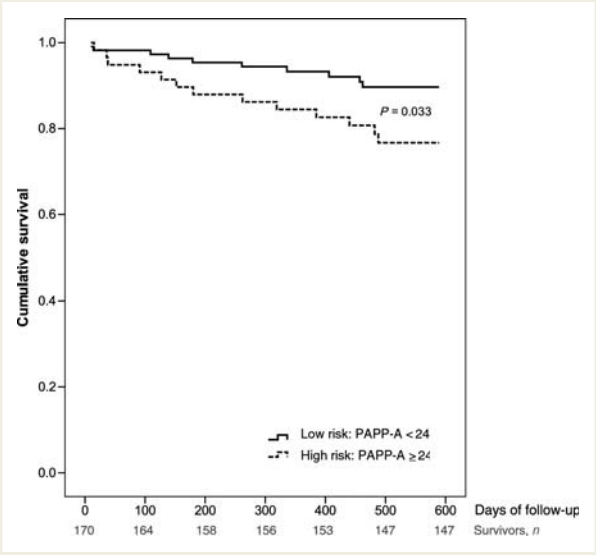

Figure 1 Kaplan–Meier survival curve. The graph illustrates cumulative survival for patients divided up by baseline PAPP-A serum concentrations of either <24 or ≥24 mIU/L ('low' and 'high' risk for mortality, respectively). *P* denotes the statistical significance for the difference between groups with regard to the univariate effect of PAPP-A on survival.

correlation was found with age, number of comorbidities, IL-6, CRP, $Ca \times PO_4$ product, cholesterol or Kt/V (data not shown).

A multivariate logistic regression analysis for death was performed with 159 patients (94% of the observed cohort, representing 21 deaths) of which complete data were available for the computed variables. The model includes PAPP-A, age, sex, number of comorbidities, dialysis vintage, IL-6, C-reactive protein, PTH, $Ca \times PO_4$ product, and total serum cholesterol. Of these variables, only PAPP-A, age, and $Ca \times PO_4$ product were independent predictors for death from any cause (*Table 2*). For IL-6, only a trend towards a correlation between elevated serum levels and mortality was observed. All other variables did not show a significant odds ratio in the model predicting outcome. Also, PAPP-A remained significant if Kt/V was included as additional parameter into the model ($P = 0.01$, 95% CI 1.014–1.111; $n = 110$ patients, 17 deaths), suggesting that its levels are not merely reflective of dialysis quality.

Owing to the lack of reference ranges as risk score of mortality, we calculated a cut-off value for PAPP-A regarding the best sensitivity and specificity for prediction of mortality (*Table 3*) according to Jacobsen and Truax.[11] A cut-off value of 24 mIU/L discriminated significantly ($P = 0.015$) between patients at low or high risk for death. In the low-risk group (PAPP-A <24 mIU/L), 10 deaths occurred among 112 patients (9%), in comparison to 13 deaths among 58 patients (22%) in the high-risk group (PAPP-A ≥24 mIU/L). This translated into a sensitivity of 57%, a specificity of 69%, a positive predictive value of 22%, and a negative predictive value of 91% for the chosen cut-off of 24 mIU/L. When PAPP-A was computed as dichotomous variable with the cut-off value of 24 mIU/L, an odds ratio of 3.2 for death resulted ($P = 0.04$, 95% CI 1.056–9.577); the C-statistic amounts to 0.67 ($P = 0.007$, 95% CI 0.53–0.83), which indicates a sufficient discriminative power. A Kaplan–Meier survival analysis of patients stratified into two groups according to their PAPP-A cut-off value below or above 24 mIU/L, respectively, is shown in *Figure 1*.

To determine alterations of PAPP-A levels during dialysis, its levels were measured in nine patients each prior to and after a 4 h HD session. Average serum concentrations before and after HD were 29.8 ± 9 and 28.1 ± 8, respectively ($P = 0.389$).

Discussion

In this Swiss cohort of maintenance HD patients, PAPP-A was identified as an independent short-time predictor of mortality. Patients with a PAPP-A level of ≥24 mIU/L had an odds ratio of 3.2 to die within 1.5 years of follow-up compared with patients with lower PAPP-A values. In contrast, patients with a PAPP-A level of <24 mIU/L had a likelihood of 91% to be alive at the end of the observation period. To our best knowledge, this is the first study having assessed PAPP-A as a prognostic factor in maintenance HD patients.

Whether PAPP-A is merely a risk marker or causally involved in the pathogenesis of cardiovascular and non-CV disorders leading do death remains open for debate. Only a limited number of clinical studies are available to resolve this issue. So far, PAPP-A was found to be elevated in patients with coronary artery disease,[5–7] suggesting its potential relevance in the development of cardiovascular pathology. In a non-dialysis population with ACS and an average PAPP-A concentration of 14.2 mIU/L, PAPP-A levels

above 12.6 mIU/L were associated with a significantly increased odds ratio of 2.44 for death or non-fatal myocardial infarction within 6 months of follow-up.[6] In our own cohort, mean PAPP-A concentrations were clearly higher with 21.9 mIU/L (median 21 mIU/L), suggesting limited renal clearance of the protein in chronic renal failure, and/or increased production due to HD treatment or systemic vascular damage. Endogenous clearance by the kidney may be negligible due to the size and structure of the protein. With regard to the effect of renal replacement therapy, in a group of patients undergoing chronic maintenance HD or haemodiafiltration (HDF), mean pre-treatment PAPP-A levels were reported to be 24.3 mIU/L, and to transiently increase during HDF before returning to baseline levels after a 4 h session.[9] In addition, our own measurements during HD revealed no relevant changes in PAPP-A levels. Thus, it seems unlikely that PAPP-A concentrations are substantially affected by dialysis treatment or haemodialytic clearance. Similarly, PAPP-A is not merely a marker of dialysis efficacy, as no correlation was found with Kt/V in our own series. In conclusion, elevated serum PAPP-A concentrations in our study cohort most probably reflect systemic vasculopathy, which is a well-established complication leading to cardiovascular morbidity and mortality in patients with chronic renal failure.

Pregnancy-associated plasma protein-A is the protease for insulin-like growth factor binding protein-4 (IGFBP-4), therefore responsible for degradation of IGFBP-4, a potent inhibitor of IGF-1 action. PAPP-A is reported to be secreted by activated macrophages in the atherosclerotic plaque. IGF-1 stimulates cell proliferation and differentiation, hence has been considered to be a mediator of plaque growth. However, recent evidence suggests that IGF-1 rather provides protection against ischaemic vascular disease, with reduced IGF-1 levels being associated with carotid and coronary artery disease. Protective action of IGF-1 includes preservation of insulin sensitivity and endothelial and microvascular function. Therefore, increased PAPP-A levels may actually offer protection by enhancing IGF-1 bioavailability as part of a reparative pathway reactive to local vascular damage.[2,12,13] Further research on PAPP-A, especially in the setting of chronic renal failure, will have to be undertaken to better understand its exact role(s) in vascular pathobiology.

Several potential limitations apply to our study. First, the 24 deaths within our study cohort equates to a death rate of only 12 per 100 patient-years, which is somewhat lower than expected in an HD population with a median age of 70 years (mean 66 years).[14,15] This could reflect a healthy population or progress in medical care. Most likely, it was the result of excluding patients with limited lifetime expectancy. Given the rather small number of deaths the amount of controlled variables in the multivariate statistical model may be somewhat excessive. Therefore, the results in logistic regression analyses should be interpreted carefully. Whatever the reason for the low mortality may be, the clinical relevance of elevated PAPP-A concentrations thus becomes even more important, as it helps to identify patients who are otherwise less likely to experience death. Similarly, the median observation time limited to 17 months can be seen as a disadvantage, or, in contrast, as particularly helpful in predicting short-time mortality. End-stage renal disease by itself constitutes a cardiovascular risk factor contributing to the mortality exceeding that of non-dialysis populations with coronary artery syndrome. This may explain the shorter time to death in our study cohort compared with that observed in other patients with elevated PAPP-A levels not being on renal replacement therapy.[7] However, with regard to other aspects, our cohort is quite comparable with other published series, and therefore makes our findings applicable to a general maintenance HD population. The effect of age on outcome is evident and has been shown, as well as for the $Ca \times PO_4$ product, in other studies.[16,17] In addition, and in accordance with the concept of 'reverse epidemiology', classical (cardiovascular) risk factors in patients without end-stage renal disease, such as serum cholesterol, were not associated with higher morbidity in the present analysis.[18] Somewhat surprising, classical markers of chronic inflammation were not predictive of outcome in our cohort.[19] Whereas for IL-6 a trend towards correlation with mortality was found, C-reactive protein was clearly unrelated to prognosis. Again, this may be indicative of a population with a relatively low cardiovascular risk profile. Indeed, the number of (confirmed) cardiovascular events in our study was rather low. Because of this, we refrained from subgroup analyses with stratification by aetiology. Moreover, the primary endpoint of our study was 'mortality from any cause'. With the reason for death not being identifiable in most instances, it was presumed 'cardiovascular' in nature in the absence of other obvious causes.

In conclusion, we have identified PAPP-A as novel and independent short-term predictor of mortality in maintenance HD patients. It may be particularly useful in a population of patients with low–to-intermediate cardiovascular risk. Owing to its potential to identify patients with imminent risk for death, repetitive measurements in annual intervals may be advisable with regard to tailoring an individual's diagnostic and medical management.

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Conflict of interest: none declared.

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