Plasma Pentraxin 3 in Patients with Chronic Kidney Disease: Associations with Renal Function, Protein-Energy Wasting, Cardiovascular Disease, and Mortality

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Background and Objectives: Plasma protein pentraxin 3 concentrations are elevated in a wide range of diseased states. However, no study has evaluated protein pentraxin 3 in patients with chronic kidney disease.

Design, Setting, Participants, & Measurements: Plasma protein pentraxin 3 concentrations were analyzed in relation to GFR, inflammation, cardiovascular disease, and protein-energy wasting in 71 patients with stages 3 to 4 chronic kidney disease, 276 patients with stage 5 chronic kidney disease, and 61 control subjects. Survival (5 yr) in patients with stage 5 chronic kidney disease was analyzed in relation to protein pentraxin 3 levels.

Results: Both patient groups with chronic kidney disease had higher protein pentraxin 3 concentrations than control subjects, with the highest concentration in patients with stage 5 chronic kidney disease. In all patients with chronic kidney disease, protein pentraxin 3 correlated negatively with GFR and positively with inflammatory markers. Patients with protein-energy wasting, inflammation, and cardiovascular disease had higher concentrations of protein pentraxin 3 than their counterparts. Patients with high protein pentraxin 3 levels had higher all-cause and cardiovascular mortality. After adjustment for age, gender, C-reactive protein, and cardiovascular disease, all-cause mortality was still significantly higher in patients with high protein pentraxin 3. Finally, protein pentraxin 3 showed a predictive value of mortality similar to that of IL-6 and better than C-reactive protein.

Conclusion: Plasma protein pentraxin 3 increases as GFR declines and is associated with the presence of cardiovascular disease and protein-energy wasting. Furthermore, in patients with chronic kidney disease, elevated protein pentraxin 3 predicted all-cause mortality.

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A constellation of protein-energy wasting (PEW), chronic low-grade inflammation, and cardiovascular disease (CVD) are present in a large proportion of patients with advanced chronic kidney disease (CKD) (1), and each of these risk factors independently predicts outcome in these patients (2–4). The causes of inflammation in patients with CKD are probably multifactorial (5,6). To date, C-reactive protein (CRP) is the most common biomarker to assess the inflammatory status. However, it is not know yet whether CRP is only a marker of inflammation (7) or a direct mediator of vascular disease (8).

Pentraxins are a superfamily of evolutionarily conserved proteins characterized by a cyclic multimeric structure (9). On the basis of the primary structure of the subunit, the pentraxins are divided into two groups: Short pentraxins (*e.g.*, CRP, serum amyloid P) and long pentraxins. The prototype protein of the long pentraxin group is pentraxin 3 (PTX3). Whereas CRP and serum amyloid P are produced primarily in the liver in response to IL-6 (10), PTX3 is produced by a variety of tissues and cells and in particular by innate immunity cells in response to proinflammatory signals and endothelial cells (11–13). Because of this extrahepatic synthesis and in contrast to CRP, PTX3 levels are believed to be a true independent indicator of disease activity produced at sites of inflammation (14).

Previous studies in nonrenal patients aimed at assessing the usefulness of PTX3 in diverse human pathologic conditions with an inflammatory component, such as angina pectoris, myocardial infarction, rheumatoid arthritis, and psoriasis (15–20). In these studies, it was hypothesized that PTX3, unlike CRP, may represent a rapid marker for primary local activation of innate immunity and inflammation and an indicator of disease activity. Indeed, in some clinical studies, correlation between levels of PTX3 and CRP was weak and even nonsignif-

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icant (17,18). A second characteristic emerging from studies of blood levels of PTX3 in murine and human pathology is the rapidity by which it increases, as compared with CRP and consistent with its original identification as an immediate early gene (17,21).

The production of PTX3 in human renal epithelial cells was recently reported (22), together with an increased PTX3 expression in mesangial cells of renal biopsies obtained from patients with IgA glomerulonephritis (23), suggesting a role in the innate immune response and inflammatory reactions in the kidney. The bigger molecular size for PTX3 (40.6 kD) compared with CRP (21.5 kD) may suggest increased retention in uremia. We recently reported on high plasma PTX3 levels in dialysis patients with CKD (24). However, no studies have analyzed the potential prognostic value of PTX3 in patients with CKD. PTX3 may amplify the inflammatory response after being produced both in peripheral tissues and in the kidney; it may also play an important role in the atherogenic process present in CKD. Taking into consideration the possible clinical implications of PTX3 levels in CKD, we studied this novel molecule in relation to degree of renal function, inflammation, PEW, and signs of CVD in a cross-sectional cohort of patients with various degrees of CKD severity. In addition, to explore the etiologic role of PTX3 in inflammation-mediated mortality in CKD, we assessed survival in a group of patients who had stage 5 CKD and were prospectively followed for 5 yr.

Materials and Methods

The study protocol was approved by the Ethics Committee of Karolinska University Hospital Huddinge (Stockholm, Sweden), and informed consent was obtained from each patient and control subject.

Patients

In this study, *post hoc* analyses were done in 347 patients with CKD (mean age 55 ± 13 yr) by retrospectively pooling data from two ongoing prospective cohorts of patients with stages 3 to 4 CKD and stage 5 CKD, respectively (1,25), using the staging classification recommended by the Kidney Disease Outcomes Quality Initiative guidelines (26). Of these, 276 consecutive patients with stage 5 CKD (160 men; age 53 ± 12 yr [range 19 to 70 yr]) were enrolled at a time point when renal replacement therapy was planned to be initiated with a median GFR of 7 ml/min per 1.73 m^2 (range 3 to 14 ml/min per 1.73 m^2). Seventy-one patients (49 men; age 59 ± 14 yr [range 27 to 80 yr]) with stages 3 to 4 CKD (median GFR 30 ml/min per 1.73 m^2; range 15 to 52 ml/min per 1.73 m^2) were prevalent patients who were recruited from the hospital renal outpatient clinic since 2001. The patient groups with stages 3 to 4 CKD and stage 5 CKD were observed alongside each other. The exclusion criteria were age <18 or >70 yr (in patients with stage 5 CKD) or 80 yr (in patients with stages 3 to 4 CKD); clinical signs of acute infection, active vasculitis, or liver disease at the time of evaluation; or unwillingness to participate in the study.

The causes of CKD were chronic glomerulonephritis in 82 (24%) patients, diabetic nephropathy in 99 (29%) patients, polycystic kidney disease in 41 (11%) patients, interstitial nephritis in six (2%) patients, nephrosclerosis in 23 (7%) patients, and other or unknown in 96 (27%) patients. Presence of clinical CVD was defined by medical history and clinical symptoms and/or findings of cardiac, cerebrovascular (stroke), and/or peripheral vascular disease. A total of 125 (36%) patients had a clinical history or signs of cardiovascular, cerebrovascular, and/or peripheral vascular disease at the start of the study. Most patients were on antihypertensive medications as well as other commonly used drugs in patients with CKD, such as phosphate and potassium binders; diuretics; erythropoiesis-stimulating agents; iron substitution; lipid-lowering medication; and vitamins B, C, and D supplementation.

Control Subjects

A population-based randomly selected group of 61 control subjects (42 men; age 59 ± 14 yr [range 28 to 80 yr] were used for comparative analyses of biochemical and metabolic parameters. The control group was a population-based group that comprised individuals who accepted to participate, as volunteers, in response to an invitation sent to 1000 randomly selected individuals in the Stockholm region by Statistics Sweden. The control subjects were investigated according to a similar protocol and simultaneously as the patients. No other exclusion criteria than unwillingness to participate in the study were applied in the selection of the control group.

Biochemical Methods

After an overnight fast, venous blood samples were drawn and stored at −80°C for biochemical analyses. Plasma PTX3 concentration was measured by using a novel commercially available ELISA kit (Perseus Proteomics, Tokyo, Japan), as well as the serum concentrations of vascular cellular adhesion molecule-1 (VCAM-1; R&D System, Minneapolis, MN). The serum levels of IL-6 and TNF-α were quantified on the Immulite automatic analyzer (Diagnostic Products Corp., Los Angeles, CA). Serum cholesterol and triglyceride levels were analyzed by means of standard enzymatic procedures (Roche Diagnostics, Mannheim, Germany). HDL cholesterol level was determined after precipitation of apolipoprotein B–containing lipoproteins by using phosphotungstic acid. Serum albumin (bromcresol purple method), CRP, creatinine, urea, and hemoglobin and urinary creatinine and urea were determined by routine procedures at the Department of Clinical Chemistry, Karolinska University Hospital Huddinge. GFR (corrected for body surface area) was estimated as the mean of urea and creatinine clearance from 24-h urinary samples (in the patients with stage 5 CKD) or using iohexol clearance (in the patients with stage 3 to 4 CKD and the control subjects).

Nutritional Status

Nutritional status was assessed by subjective global assessment (SGA) (27) at the time of inclusion, concurrent with the drawing of blood samples. SGA included six subjective assessments; three based on the patient's history of weight loss, incidence of anorexia, and incidence of vomiting; and three based on the physician's grading of muscle wasting, presence of edema, and loss of subcutaneous fat. On the basis of these assessments, each patient was given a score of 1 to 4, indicating normal, mild, moderate, or severe malnutrition, respectively. Malnutrition was defined as SGA score of 2 to 4, and patients who met these criteria were grouped together as malnourished patients. Body mass index was calculated as weight in kilograms divided by height in meters squared.

Outcome Ascertainment

Survival was determined only in patients with stage 5 CKD from the day of examination and up to 60 mo, with a mean follow-up period of 26 mo (range 1 to 60 mo). The patients were censored at transplantation or when completing the 5-yr follow-up period, with no loss of follow-up of any patient. Within the follow-up period, 80 (29%) patients died, 48 (60%) of whom died of cardiovascular causes, and 104 (37%) patients received a transplant. Cardiovascular mortality was defined as

death as a result of coronary heart disease, sudden death, stroke, or complicated peripheral vascular disease. Survival analysis was not performed in the cohort of patients with stages 3 to 4 CKD because of the few number of events (only three patients died during the follow-up period).

Statistical Analyses

All values are expressed as means ± SD or median (range), unless otherwise indicated. $P < 0.05$ was considered to be statistically significant. Comparisons between two groups were assessed for continuous variables with the unpaired *t* test, Mann-Whitney test, or χ^2 test, as appropriate. Differences among three groups or more were analyzed by ANOVA using one-way ANOVA or Kruskal-Wallis test, as appropriate, followed by a posttest when ANOVA was significant. Spearman rank correlation was used to determine correlations of PTX3 concentration with other variables. The general linear models procedure with least square means was used to identify significant interactions of age or GFR when PTX3 was assessed in patients with CVD, inflammation, or wasting, respectively, compared with counterparts. For evaluation of the sensitivity and specificity of PTX3, compared with CRP and IL-6, as predictor of mortality, a receiver operator characteristics (ROC) analysis was performed (28), using the statistical software NCSS 2007 and PASS 2005 (Number Cruncher Statistical Systems, Keysville, UT). Survival analyses were made in the patients with stage 5 CKD using the Cox proportional hazard model. The relative risks for mortality were determined by multivariate Cox regression analysis and presented as hazard ratio (HR; 95% confidence intervals [CI]). The statistical analysis was performed using statistical software SAS version 9.1.4 (SAS Institute, Cary, NC).

Results

General Characteristics of Participants and PTX3 Concentrations

The baseline characteristics of the patients with CKD and control subjects investigated are summarized in Table 1. Plasma concentrations of PTX3 showed a non-normal distribution in all three groups and exhibited a significant and gradual increase among the studied groups (*e.g.*, both patients with CKD and stages 3 to 4 CKD showed higher PTX3 values than control individuals), whereas PTX3 concentration was highest for the patients with stage 5 CKD (Table 1). No significant difference was observed with regard to PTX3 levels among male and female patients with CKD (5.11 [0.40 to 57.98] *versus* 4.66 ng/ml [0.52 to 64.28 ng/ml], respectively) or control subjects (1.83 [0.12 to 9.24] *versus* 1.94 ng/ml [0.62 to 6.83 ng/ml], respectively). Plasma concentration of PTX3 did not differ significantly between patients who had stage 5 CKD with and without diabetes (5.67 [0.87 to 36.10] *versus* 5.70 ng/ml [0.96 to 64.28 ng/ml], respectively) as well as between patients who had stages 3 to 4 CKD with and without diabetes (2.04 [0.81 to 6.66] *versus* 2.23 ng/ml [0.40 to 14.95 ng/ml], respectively).

Associations with GFR, Inflammation, Nutritional Status, and Lipid Parameters

In univariate analysis, PTX3 was positively correlated with age in healthy control subjects ($\rho = 0.34$, $P < 0.01$) but not in patients with CKD ($\rho = -0.02$). Moreover, PTX3 was inversely correlated with GFR when all participants were studied to-

Table 1. General characteristics and studied biomarkers in the investigated participants[a]

Parameter	Control ($n = 61$)	CKD 3 to 4 ($n = 71$)	CKD 5 ($n = 276$)	P[b]
Age (yr; mean ± SD)	62 ± 11	59 ± 14	53 ± 12	<0.001
Male (%)	69	69	60	NS
BMI (kg/m^2; mean ± SD)	25.5 ± 3.7	26.8 ± 5.0	24.9 ± 4.4	<0.01
GFR (ml/min per 1.73 m^2; mean ± SD)	86.5 ± 13.7	29.6 ± 8.9	6.4 ± 2.2	<0.001
Diabetes (%)	–	18	31	<0.001
CVD (%)	–	31	37	<0.001
PEW (SGA >1; %)	–	2.8	30	<0.001
Serum albumin (g/dl; mean ± SD)	3.9 ± 0.3	3.7 ± 0.3	3.3 ± 0.6	<0.05
CRP (mg/L)	1.2 (0.2 to 9.1)	2.8 (0.3 to 49.0)	4.9 (0.2 to 218.0)	<0.001
IL-6 (pg/ml)	1.9 (0.4 to 10.0)	2.4 (0.8 to 12.0)	6.7 (0.8 to 120.0)	<0.001
TNF-α (pg/ml)	3.9 (1.3 to 10.6)	8.1 (4.5 to 27.8)	10.3 (3.1 to 240.0)	<0.001
VCAM-1 (ng/ml)	689 (402 to 129)	949 (457 to 1719)	1338 (546 to 4085)	<0.001
ICAM-1 (ng/ml)	229 (109 to 342)	249 (124 to 356)	245 (99 to 1056)	<0.01
Fibrinogen (g/L; mean ± SD)	3.0 ± 0.6	3.8 ± 0.8	5.0 ± 1.5	<0.001
PTX3 (ng/ml)	1.8 (0.1 to 9.2)	2.2 (0.4 to 16.0)	5.7 (0.9 to 64.3)	<0.001
Total cholesterol (mmol/L; mean ± SD)	5.2 ± 0.9	5.3 ± 1.2	5.3 ± 1.6	NS
Triglycerides (mmol/L; mean ± SD)	1.3 ± 0.7	2.1 ± 1.4	2.1 ± 1.2	<0.001
HDL cholesterol (mmol/L; mean ± SD)	1.6 ± 0.5	1.3 ± 0.4	1.3 ± 0.6	<0.001

[a]BMI, body mass index; CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; ICAM-1, intercellular adhesion molecule-1; PEW, protein-energy wasting; PTX3, pentraxin 3; SGA, subjective global assessment; VCAM-1, vascular cellular adhesion molecule-1.

[b]Significantly different ($P < 0.05$) as assessed by ANOVA or χ^2 test.

gether ($\rho = -0.56$; $P < 0.0001$; Figure 1), but this association remained significant only in the patients with CKD ($\rho = -0.45$, $P < 0.0001$) and not in the control group ($\rho = -0.12$).

In patients with CKD (Table 2), plasma PTX3 concentration was positively correlated with the plasma levels of CRP, IL-6 and TNF-α, fibrinogen, and soluble VCAM-1 (sVCAM-1). When patients and control subjects were analyzed together, PTX3 showed the strongest positive correlations with fibrinogen ($\rho = 52$, $P < 0.0001$) and sVCAM-1 ($\rho = 59$, $P < 0.0001$; Figure 2) as well as soluble intercellular adhesion molecule-1 ($\rho = 0.12$; $P = 0.02$).

In patients with CKD (Table 2), negative correlations were observed for PTX3 with serum albumin, body mass index, and hemoglobin levels. No association was found with lipid parameters (cholesterol, triglycerides, and HDL cholesterol).

Table 2. Spearman rank correlation coefficients between plasma PTX3 and relevant parameters in 347 patients with CKD

Parameter	ρ	P
Age (yr)	−0.02	NS
GFR (ml/min per 1.73 m^2)	−0.42	<0.0001
Hemoglobin (g/L)	−0.32	<0.0001
Serum albumin	−0.44	<0.0001
CRP (mg/L)	0.36	<0.0001
TNF-α (pg/ml)	0.14	<0.05
IL-6 (pg/ml)	0.46	<0.0001
VCAM-1 (ng/ml)	0.49	<0.0001
Fibrinogen (g/L)	0.39	<0.0001
Total cholesterol	−0.08	NS
HDL cholesterol	0.05	NS
Triglycerides	−0.09	NS

PTX3 in Relation to the PEW, Inflammation, and Atherosclerosis

As shown in Figure 3, patients who had CKD and presented with clinical signs of CVD (5.58 ng/ml [0.75 to 64.28 ng/ml]) had higher PTX3 levels than those without signs of CVD (4.32 ng/ml [0.40 to 54.93 ng/ml]; $P < 0.001$). Furthermore, patients with CKD with signs of inflammation (defined as CRP ≥10 mg/L) also showed higher PTX3 levels than patients who had inflammation with CKD (7.17 [1.35 to 64.3] *versus* 4.18 ng/ml [0.40 to 64.20 ng/ml]; $P < 0.001$). Finally, patients with CKD and signs of PEW (defined as SGA >1) showed higher PTX3 levels than patients without PEW (7.59 [1.7 to 64.21] *versus* 4.17 ng/ml [0.40 to 64.30 ng/ml]; $P < 0.001$). Because the patients with stages 3 to 4 CKD were older than the patients with stage 5 CKD, a further analysis using general linear models was performed to adjust for the influence of GFR and age. We found that the PTX3 concentrations remained significantly elevated in the patients who had inflammation and PEW or presented CVD as compared with their counterparts. The interactions between GFR and age and these comorbidities were not statistically significant. The presence of PEW, inflammation, and CVD has additive effects on plasma PTX3 concentration, because PTX3 levels gradually increased ($P < 0.001$) when none (3.86 ng/ml [0.4 to 54.9 ng/ml]), one (4.27 ng/ml [0.75 to 57.98 ng/ml]), two (6.73 ng/ml [1.87 to 64.28 ng/ml]), or three (8.69 ng/ml [2.22 to 45.26 ng/ml]) of these comorbid conditions were present.

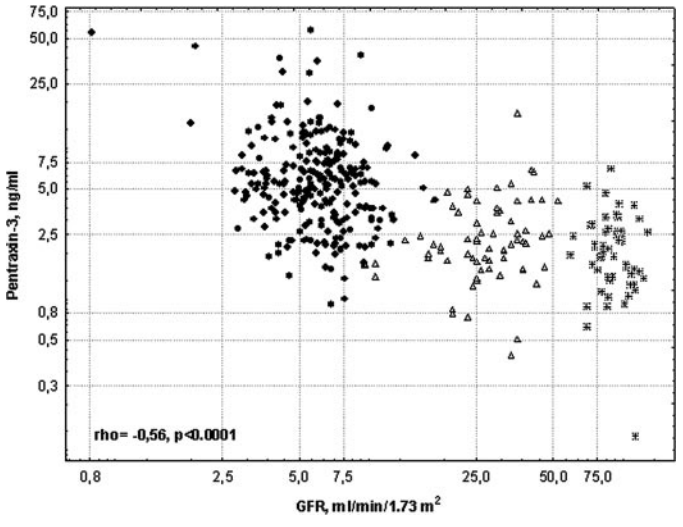

Figure 1. Correlation between plasma pentraxin-3 (PTX3) concentration and GFR among 276 patients with stage 5 chronic kidney disease (CKD; ●), 71 patients with stages 3 to 4 CKD (△), and 61 control subjects (+). $\rho = -0.56$, $P < 0.0001$.

PTX3 Levels and Mortality

Nonsurvivors as compared with survivors showed significantly higher concentrations of PTX3 (7.07 [0.87 to 57.98] *versus* 5.16 ng/ml [0.96 to 64.28 ng/ml]; $P < 0.0001$). Survival analysis was performed comparing patients within the highest PTX3 tertile with patients within the other two tertiles. Whereas 43% of the patients within the upper PTX3 tertile died, only 22% of the patients did so in the group with low PTX3 (lower two tertiles combined; $P < 0.001$). The percentages of patients in each group who died from CVD were 25 and 13%, respectively ($P < 0.01$).

In a nonadjusted analyses, patients with higher PTX3 had a higher all-cause (HR 1.92; 95% CI 1.24 to 2.97; $P = 0.003$) and cardiovascular (HR 1.83; 95% CI 1.05 to 3.21; $P = 0.03$) mortality rate than patients with lower PTX3 concentrations. After adjustment for age, gender, CRP, and CVD, the high PTX3 showed a significant association with all-cause mortality (HR 1.73; 95% CI 1.1 to 2.8; $P = 0.02$; Table 3). However, although the patients in the higher tertile also tended to be associated with high cardiovascular mortality (HR 1.31; 95% CI 0.72 to 2.41), this association did not reach statistical significance.

As a next step, we performed a comparison analysis using the ROC curves of common inflammatory markers, including PTX3, CRP, and IL-6, with regard to their predictive power *versus* a 5-yr all-cause mortality in patients with stage 5 CKD (Figure 4). Although the three biomarkers significantly ($P < 0.0001$) predicted all-cause mortality in this population, the area under the curve (15) for PTX3 (AUC ± SEM = 0.65 ± 0.03) was similar to that for IL-6 (AUC = 0.67 ± 0.03). Both were better

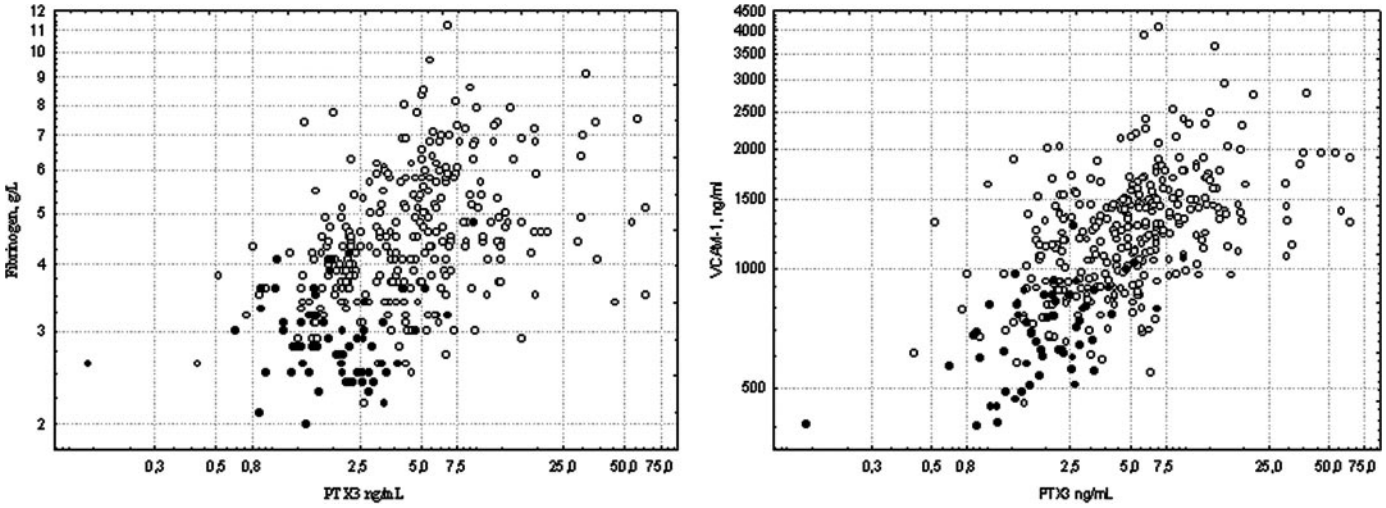

Figure 2. Correlation of PTX3 concentration with fibrinogen (A; $\rho = 0.52$, $P < 0.0001$) and soluble vascular adhesion molecule-1 (sVCAM-1; B; $\rho = 0.59$, $P < 0.0001$) among 347 patients with CKD (○) and 61 control subjects (●).

predictors than CRP (AUC = 0.62 ± 0.03), although no significant differences were found among these comparisons.

Discussion

Extending our previous report (24) on increased plasma PTX3 levels in dialysis patients, this study documents high concentrations of PTX3 in nondialyzed patients with CKD. Increases in PTX3 levels were most pronounced in patients with CVD and showed a strong negative association with GFR. Furthermore, this study showed novel associations of PTX3 with inflammatory and nutritional markers as well as an association between high levels of PTX3 and mortality in these patients.

PTX3 and Renal Function

Some studies have shown that plasma levels of PTX3 are elevated in a number of human disorders, such as myocardial infarction (17), rheumatoid arthritis (20), and sepsis (18). This study shows that PTX3 levels are markedly increased also in patients with CKD.

A novel finding is the strong negative correlation between PTX3 levels and GFR. Although we have not followed PTX3 longitudinally, the gradual increase of PTX3 concomitant to the decline in GFR could be explained by an inadequate clearance because PTX3 is a large molecular weight substance (molecular weight 40.6 KD) characterized by a multimeric, usually pentameric, structure, but it could also be explained by an enhanced synthesis/release upon stimulation in peripheral tissues (12,13) and also perhaps in the remaining functioning kidney (22,23).

In agreement with previous studies (6), the levels of inflammatory markers such as CRP, TNF-α, IL-6, and VCAM-1 are markedly increased in our patients with CKD, and we report that this is also the case for PTX3 levels, showing a strong association with sVCAM-1, which, like PTX3 (29,30), is mainly released from endothelial cells (31). It was recently shown that PTX3 (but not CRP or serum amyloid P) amplifies tissue factor expression in endothelial cells that are exposed to inflammatory cytokines (29). These observations and the presence of PTX3 during the early phases of inflammation offer the biologic plausibility for a role of PTX3 in the modulation of endothelial cell procoagulant activity and would explain the strong positive association with fibrinogen levels in our study. Moreover, fibrinogen is an independent predictor of death in hemodialysis patients (32), and elevations in procoagulant biomarkers including fibrinogen are associated with renal insufficiency and may be one important mediator to increased cardiovascular risk (33). Although inflammation and coagulation are indeed closely interlinked, the intermediate pathways remain to be clarified. During the onset of inflammation, molecules that induce the procoagulant signal that is responsible for the local fibrin deposition are generated. It is possible that PTX3, once generated by or in the proximity of the perturbed endothelial cells, might potentiate the expression of tissue factor, which is required for thrombogenesis and vascular ischemia to occur (29).

PTX3 in Relation to PEW, Inflammation, and CVD

Inflammation, PEW, and atherosclerosis often coexist among patients with CKD, and each of these risk factors independently predicts outcome in these patients, while having additive effects on survival (27,34,35). In our study, elevated plasma concentrations of PTX3 were found in patients who had stage 5 CKD and PEW, inflammation, and signs of CVD. Furthermore, PTX3 levels were incrementally higher when inflammation, PEW, and CVD existed together.

It is of interest to mention that whereas some previous clinical studies found a weak or NS correlation between levels of PTX3 and CRP (17,18), we found a positive and strong correlation among these parameters, although lower in power than that observed for IL-6 and sVCAM-1. It was previously shown that proinflammatory cytokines can upregulate the expression of PTX3 from endothelial cells and macrophages (14,36). The chronically elevated cytokine levels in patients with CKD

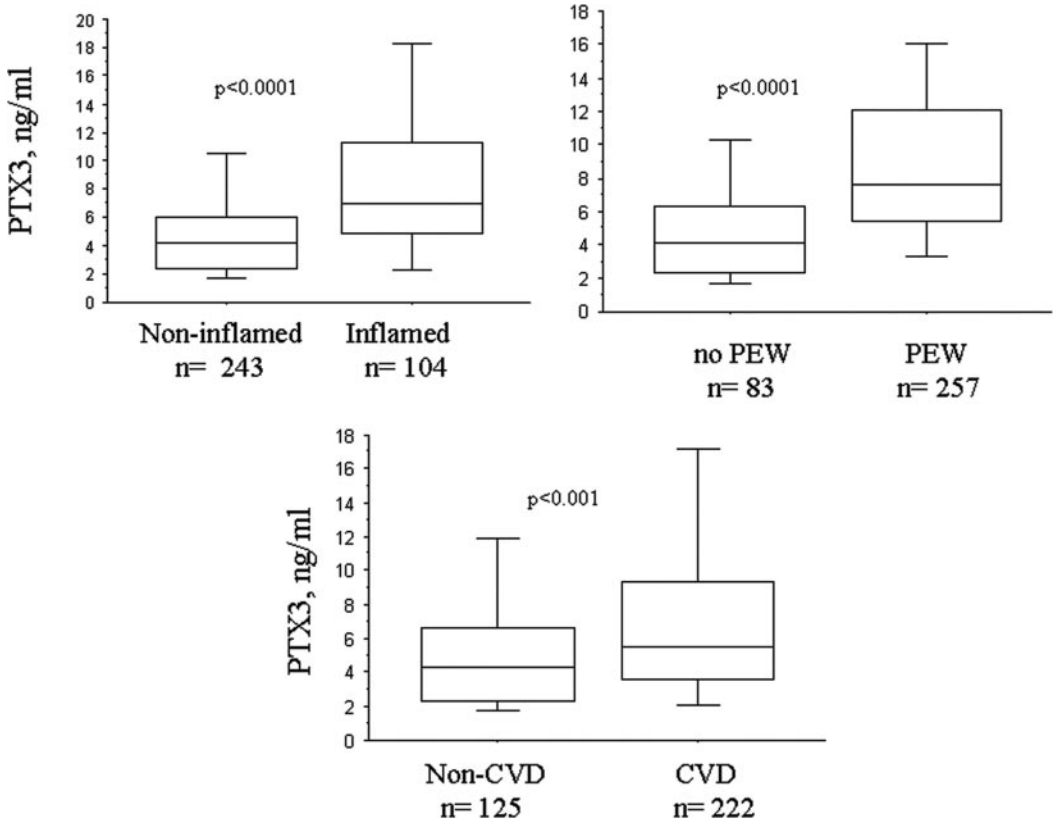

Figure 3. Plasma PTX3 concentrations in relation to the presence of protein-energy wasting (PEW; subjective global assessment >1), inflammation (C-reactive protein [CRP] ≥10 mg/ml), and cardiovascular disease (CVD) in 347 patients with CKD.

(37,38) are likely to be at least partially responsible for PTX3 induction, and the retention of these and other toxins might altogether contribute to the overall inflammatory effect reflected by CRP values. Therefore, this study suggests that PTX3 may provide additional prognostic information to that obtained from CRP (short pentraxin) and that PTX3 may play an important active role in the inflammatory and atherogenic processes in patients with CKD.

PTX3 and Survival

Another novel finding from our study is the predictive value of PTX3 on 5-yr all-cause mortality, independent of CRP levels, clinical CVD, and demographics. In another study, PTX3 levels, measured within the first day from the onset of the myocardial infarction along with other markers including CRP, emerged as the only independent predictor of mortality in the first 3 mo (16). It is plausible that PTX3 may have an additional explanatory power for outcome because of residual confounding that is not captured by standard inflammation markers, such as CRP and IL-6. This may be supported by the findings that CRP did not predict mortality in our multivariate analysis, although both CRP and IL-6 were associated with mortality in univariate analyses and both predicted mortality in multivariate analysis models not including PTX3. The hypothesis that PTX3 levels might more acutely and rapidly reflect the risk for CVD in patients with CKD than other inflammatory markers including CRP cannot be covered in this cross-sectional analysis and

Table 3. Cox proportional HR for all-cause mortality[a]

Variable	HR	95% CI	*P*
Age (≥55 *versus* <55 yr)	1.04	1.01 to 1.07	<0.01
Gender (male *versus* female)	0.94	0.59 to 1.52	NS
CVD (yes *versus* no)	1.72	1.05 to 2.83	0.03
CRP ≥10 mg/L (yes *versus* no)	0.91	0.56 to 1.46	NS
PTX3 (high tertile *versus* low two tertiles)	1.73	1.08 to 2.79	0.02

[a]The model was adjusted for age, gender, inflammation (CRP ≥10 mg/L), and CVD in 276 patients with stage 5 CKD. The two lower tertiles of PTX3 were grouped together as a reference. The likelihood ratio = 29.6; $P = 0.0001$. CI, confidence interval; HR, hazard ratio.

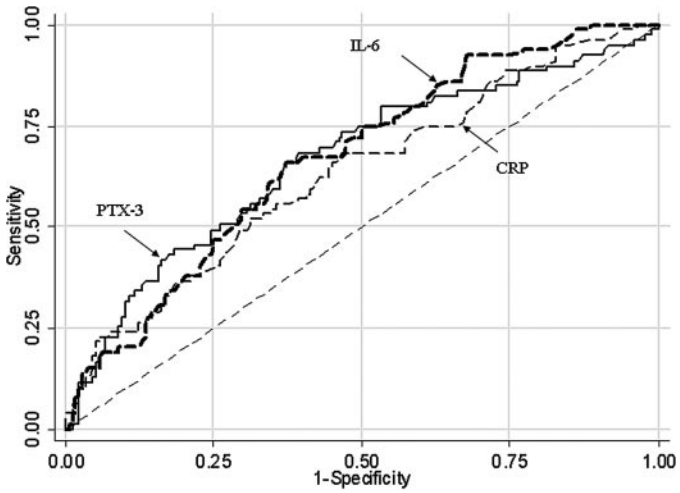

Figure 4. Receiver operating characteristic curves *versus* all-cause mortality generated with PTX3, IL-6, and CRP in 276 patients with stage 5 CKD.

merits further study. Notably, a recent study showed that PTX3 peaked in plasma early, within 6 to 8 h from onset of myocardial infarction, without being correlated to CRP (17). In addition, we recently reported that PTX3 increases rapidly during a hemodialysis session, which marginally affects other inflammation markers (24).

Inflammation is a common feature of CKD (6,39) and an important prognostic and diagnostic tool for ischemic heart disorders as well (40). A recent study described a strong expression of PTX3 in advanced human atherosclerotic lesions by endothelial cells and macrophages, suggesting that PTX3 may be involved in the pathogenesis of atherosclerosis (36). Although our analysis shows the prognostic impact of PTX3 in cardiovascular mortality, we failed to demonstrate an independent role in CVD mortality after adjustment for other known confounders. Although this association showed 31% higher risk in patients with high PTX3 concentrations, it is likely that the reduced number of cardiovascular events in our cohort might have masked such an effect, but it is also plausible that numerous other factors that were present, retained, and exacerbated in the uremic milieu would contribute (41). In this study, because the classification of cardiovascular mortality was based on death certificates, this may also contribute in hiding such effect.

Because PTX3 increases in plasma more rapidly than CRP in response to a stimulus (17,21,24), we evaluated whether PTX could provide better predictive power than CRP. Two recent studies, one using ROC curves (42) and one using multivariate modeling (43), showed that the predictive value of IL-6 levels was higher than that attributable to other molecules studied (including CRP) with regard to all-cause and cardiovascular mortality. Moreover, the predictive power of the combined inflammatory burden of a number of commonly measured cytokines and adhesion molecules was identical to that provided by the sole IL-6 (44). Because PTX3 had a predictive value similar to that of IL-6 and CRP, further studies comparing the predictive value of this novel marker in larger CKD cohorts are warranted. However, measurements of PTX3 in clinical practice to predict outcome does not seem justified, because CRP is a cheaper alternative and more readily available in everyday clinical practice compared with other inflammatory markers (43,45). However, in contrast to CRP, PTX3 is produced at extrahepatic sites and its measurement could have clinical implication in diagnosing the "at site" inflammatory status of the patient, but the potential value of this possibility must await further study.

Limitations

To assess the implications of our study, some shortcomings should be considered. First, the classification of CVD included only patients with clinically significant disease. Therefore, this study cannot show relationships between subclinical early-stage CVD and PTX3. Second, the mortality classification was based on death certificates, which may be biased and inaccurate and lead to misclassification in some cases. Third, because we relied on a single determination of PTX3, we cannot take into account any variation that may have occurred over time. However, this does not seem to be a major limitation because other inflammation markers were measured only once in this study. Finally, it should be pointed out that this is a *post hoc* analysis with increased risk for type 1 error, which may limit the value of the study. Further designed studies to address the clinical implication and pathogenic mechanisms in patients with CKD are warranted to overcome such a limitation induced by *post hoc* analysis.

Conclusions

This study shows that PTX3 levels are increased and associated inversely with GFR in nondialysis patients with CKD. Also, PTX3 levels are more elevated in patients with PEW, inflammation, and clinical signs of CVD. Finally, we demonstrate that PTX3 predicts all-cause CKD mortality independent of CRP and CVD, with a prognostic power similar to IL-6. Further studies are needed to determine whether plasma levels of PTX3 are merely markers of inflammation in patients with CKD or these associations actually reflect a role in the pathogenesis of the atherosclerotic damage, for instance by amplifying the complement and coagulation cascades (29,30).

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Disclosures

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See the related editorial, "Inflammatory Marker Mania in Chronic Kidney Disease: Pentraxins at the Crossroad of Universal Soldiers of Inflammation, on pages 872–875.