Soluble TWEAK and PTX3 in Nondialysis CKD Patients: Impact on Endothelial Dysfunction and Cardiovascular Outcomes

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Summary

Background and objectives Chronic kidney disease (CKD) conveys high mortality rates. Soluble TNF-like weak inducer of apoptosis (sTWEAK) and long pentraxin 3 (PTX3) are predictors of mortality in dialysis patients and determinants of endothelial dysfunction. Now, we hypothesize that both sTWEAK and PTX3 act as biomarkers of cardiovascular outcomes in nondialysis CKD patients.

Design, setting, participants, & measurements Cross-sectional analysis in which flow-mediated dilation (FMD) and intima-media thickness (IMT) were assessed in 257 nondialysis stage 1 to 5 CKD patients (mean age, 52 ± 12 years; 130 men), together with biochemical measurements and sTWEAK and PTX3 assessments. Patients were followed for cardiovascular outcomes.

Results PTX3 and IMT increased, whereas FMD and sTWEAK decreased across CKD stages ($P < 0.001$ for all). Both PTX3 and sTWEAK appeared as strong determinants of FMD in multivariate analysis. The univariate associations of sTWEAK and PTX3 with IMT were dependent on estimated GFR. After a median of 39 months (range, 2 to 43 months), 22 fatal and 57 nonfatal cardiovascular events occurred. In a Cox model excluding PTX3, decreasing sTWEAK concentration was associated with increased risk of cardiovascular events independently of basic confounders (age, gender, estimated GFR, C reactive protein, diabetes, and cardiovascular comorbidity) and FMD. In a model excluding sTWEAK, circulating levels of PTX3 were directly associated with cardiovascular outcomes independently of basic confounders, but this association was lost after adjustment for FMD.

Conclusions Both PTX3 and sTWEAK levels associated with the endothelial dysfunction observed with progressive kidney failure. Additionally, both biomarkers impacted the predictability of cardiovascular outcomes.

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Introduction

Chronic kidney disease (CKD) patients die at a markedly accelerated rate, principally from cardiovascular disease (CVD) (1). Progression toward ESRD exposes patients to increased risk of developing premature vascular disease and cardiovascular morbidity, thus contributing to exceedingly high mortality rates (2). In fact, CVD and death are more likely outcomes in subjects with CKD than progression to ESRD and subsequent initiation of renal replacement therapy (3). The mechanisms for the elevated CVD risk in CKD are complex and may involve changes in both the heart and vasculature already at early stages. Of these, endothelial dysfunction increases in prevalence as renal function declines and is considered a prodromal phase in the atherosclerosis that precedes cardiovascular complications (4,5). The etiology of endothelial dysfunction in CKD is likely multifaceted, involving the dysregulation of various pathways.

One of these pathways could be mediated by the TNF-like weak inducer of apoptosis (TWEAK, TNFSF12), a ubiquitously expressed type II transmembrane glycoprotein of the TNF superfamily that circulates in plasma as a soluble form (sTWEAK) with a molecular mass of 18 kD (6). Binding of TWEAK to its receptor, Fn14, mediates multiple biologic effects such as cellular growth, proliferation and migration, osteoclastogenesis, angiogenesis, and apoptosis (6). We have shown that sTWEAK plasma levels in nondiabetic nondialysis CKD stages decrease with progressive loss in kidney function and associate with the aggravation of the endothelial function (7). Furthermore, sTWEAK and IL-6 showed additive effects on

mortality prediction in patients undergoing hemodialysis (8). It is, however, unknown whether sTWEAK levels are effective predictors of (cardiovascular) outcomes in early and moderate nondialysis CKD stages.

Another potential dysregulated pathway in CKD-associated endothelial dysfunction may involve long pentraxin 3 (PTX3), a multimeric mediator that shares structural homology with hepatic short pentraxins such as C-reactive protein (CRP) and serum amyloid P component, but that it is expressed by many cell types, especially in the vasculature (9–12), in response to injury and stress. PTX3 levels are increased in individuals undergoing hemodialysis, contributing to increase both the cardiovascular and mortality risk by pathways independent of its homologous CRP (13,14). In type 2 diabetic individuals with proteinuria but normal renal function, we reported strong independent links between PTX3, endothelial dysfunction, and albuminuria (15). Presently, the association of PTX3 levels with kidney function decline and its potential as surrogate biomarker of endothelial function and outcome in predialysis CKD patients are unknown.

The aims of this study are therefore multiple: first, we wanted to confirm the usefulness of sTWEAK as a biomarker of endothelial function (as assessed by flow-mediated dilation [FMD] assessments) and to study the course of PTX3 levels with endothelial dysfunction and progressive loss in kidney function; second, we wanted to test the ability of circulating sTWEAK and PTX3 to predict cardiovascular outcomes in nondialysis CKD; finally, because both sTWEAK and PTX3 are found present in atheromatous plaques (16,17) and because arterial thickening also occurs in parallel with kidney function loss (18), we wanted to find the relative contribution of these molecules to the variance of arterial thickening as assessed by carotid intima-media thickness (IMT) measurements. We tested this in a large cohort of etiologically diagnosed CKD patients uniformly distributed and balanced across different disease stages.

Materials and Methods

Patients

The ethical committee of Gulhane School of Medicine (Etlik-Ankara, Turkey) approved the study, and informed consent was obtained from each subject. Between January 2005 and July 2009, 711 patients were referred to the Renal Unit of the Gulhane School of Medicine Medical Center, Ankara, Turkey, for the first time because of suspected or manifest renal failure. All patients were diagnosed as having CKD according to their estimated GFR (eGFR) and the presence of kidney injury as defined by National Kidney Foundation Kidney Disease Outcomes Quality Initiative Guidelines (19). To minimize any confounding effects of conditions that may influence endothelial dysfunction, 366 patients who were taking drugs influencing endothelial function were excluded, including angiotensin-converting enzyme inhibitors ($n = 131$), angiotensin receptor blockers ($n = 100$), statins ($n = 76$), erythropoiein ($n = 12$), or supplemental vitamin pills ($n = 47$). Otherwise, other exclusion criteria including acute infections and unwillingness to participate in the study were applied ($n = 21$). Eighty-eight eligible patients dropped out during observation for the following reasons: lost contact or transferred to other dialysis units ($n = 32$), viral hepatitis ($n = 6$), vasculitis ($n = 3$), and withdrew consent ($n = 47$). In total, 257 patients with a mean age of 52 ± 12 years were included in the study.

The etiologies for the CKD are given in Table 1. Forty-four of the patients were on anti-hypertensive therapy (32 patients were treated with calcium channel antagonists, 5 patients were treated with β-blocker agents, 2 patients were treated with α blockers, and 5 patients were treated with loop diuretics). Fifty-eight of the patients were on anti-diabetic therapy (33 patients were treated with oral anti-diabetics and 25 patients were treated with insulin). As soon as diabetic nephropathy was diagnosed, all patients taking oral anti-diabetics were changed to insulin. Forty-nine patients (20%) had a history of CVD as defined by medical history and/or clinical findings at time of en-

Table 1. Demographic and clinical characteristics of the study groups

eGFR (ml/min per 1.73 m^2)	≥90 (Stage 1) ($n = 44$)	60 to 89 (Stage 2) ($n = 53$)	30 to 59 (Stage 3) ($n = 57$)	15 to 29 (Stage 4) ($n = 49$)	<15 (Stage 5) ($n = 54$)
Age (years)	50 (28 to 71)	55 (30 to 69)	51 (29 to 71)	54 (31 to 71)	49 (28 to 71)
Sex (M/F)	22/22	28/25	28/29	26/23	26/28
Body mass index (kg/m^2)	26.6 ± 2.6	26.5 ± 3.1	25.9 ± 2.5	25.9 ± 2.9	25.3 ± 2.7
History of CVD (n)	11	5	9	9	15
Etiology of CKD (n)					
diabetes	7	13	13	13	12
glomerulonephritis	10	8	9	6	10
hypertension	4	10	12	7	11
ADPKD	2	4	1	1	3
reflux nephropathy	1	1	2	1	2
unknown	20	17	20	21	16
Smoking, current (n)	20	25	25	19	24
Follow-up (months)	39 (25 to 42)	33 (7 to 42)	39 (5 to 42)	36 (3 to 42)	39 (2 to 43)
Deaths (n)	—	—	—	1	1
Cardiovascular death (n)	—	5	7	2	8
Nonfatal cardiovascular event (n)	3	11	9	12	22

rollment. Of these 49 patients, 6 had cerebrovascular disease (stroke), 35 had cardiovascular disease (acute myocardial infarction, angina pectoris, or had undergone coronary artery bypass surgery); 6 had a history of peripheral ischemic atherosclerotic vascular disease, and 2 had a history of an aortic aneurysm. Smoking habits were recorded: 113 patients were former or current smokers and 144 were nonsmokers.

Patients were classified with respect to eGFR levels from stages 1 to 5 as determined by Kidney Disease Outcomes Quality Initiative (Table 1), according to the simplified version of the Modification of Diet in Renal Disease formula as defined by Levey *et al.* (20). Patients were followed for time-to-event analysis of cardiovascular outcomes until cardiovascular event or death, whichever came first. Information regarding median follow-up and events recorded in each CKD stage is detailed in Table 1.

Laboratory Measurements

All samples were obtained from patients and controls in the morning after 12 hours of fasting for measurement of serum albumin, hemoglobin, total serum cholesterol (TC), triglyceride (TG), HDL, and LDL cholesterol. Total plasma cholesterol, TG, and HDL cholesterol were measured by enzymatic colorimetric method with an Olympus AU 600 autoanalyzer using reagents from Olympus Diagnostics (Hamburg, Germany). LDL cholesterol was calculated by the Friedewald's formula. For the measurement of high sensitivity CRP (hsCRP), serum samples were diluted with a ratio of 1/101 with the diluents solution. Calibrators, kit controls, and serum samples were all added on each microwell with an incubation period of 30 minutes. After three washing intervals, 100 μl enzyme conjugate (peroxidase labeled anti-CRP) was added on each microwell for an additional 15-minute incubation at room temperature in the dark. The reaction was stopped with a stop solution, and photometric measurement was performed at the 450-nm wavelength. The amount of serum samples was calculated as milligrams per liter with a graphic that was made by noting the absorbance levels of the calibrators.

Plasma sTWEAK and PTX3 Measurements

Plasma PTX-3 concentration was measured *a posteriori* and in duplicate from frozen samples using a commercially available ELISA kit (Perseus Proteomics). The PTX3 ELISA system has a detection limit of 0.1 ng/ml, with intra-assay and interassay coefficients of variation of 5%. Similarly, plasma concentrations of sTWEAK were determined in duplicate with commercially available ELISA kits (Bender MedSystems, Vienna, Austria). The minimum detectable level of sTWEAK was 10 pg/ml. Intra- and interassay coefficients of variation were 7.9 and 9.2%, respectively.

Vascular Assessment

Vascular assessments were performed subsequent to blood extraction. Arterial BP was measured by a physician in the morning three consecutive times after a 15-minute resting period, and mean values were calculated for systolic and diastolic BP in all patients.

Endothelium-dependent vasodilatation (FMD) and endothelium-independent vasodilatation (nitroglycerine-mediated dilatation [NMD]) of the brachial artery were assessed noninvasively, using high-resolution ultrasound (21). Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories, Bothell, WA) with a 12-Mhz probe. The subjects remained at rest in the supine position for ≥15 minutes before the examination started. The subject's arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2 to 4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single two-dimensional frames. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. A pneumatic tourniquet was inflated to 200 mmHg with obliteration of the radial pulse. After 5 minutes, the cuff was deflated. Flow measurements were made 60 seconds after deflation. After a further 15 minutes, measurements were repeated, and again 3 minutes after administration of oral sublingual glyceryl trinitrate (400 μg). The maximum FMD and NMD dilation diameters were calculated as the average of the three consecutive maximum diameter measurements. The FMD and NMD were calculated as the percent change in diameter compared with baseline resting diameters.

IMT was assessed in all subjects, Briefly, a high-resolution B-mode ultrasound of the common carotid arteries with scanning of the longitudinal axis until the bifurcation and of the transversal axis was performed using an instrument generating a wide band ultrasonic pulse with a middle frequency of 12 MHz (ATL 5000; Advanced Technology Laboratories). For each carotid artery, two longitudinal measurements were obtained by rotating the vessels at 180° increments along their axis. All patients and controls were blindly examined by one experienced operator (the intraoperator variability was 4%). IMT was measured at 1 cm proximal to the bifurcation on each side.

Statistical Analyses

All of the statistical analyses were performed using the SPSS 11.0 (SPSS, Chicago, IL) statistical package. Nonnormally distributed variables were expressed as median (range), and normally distributed variables were expressed as mean ± SD. Between-group comparisons were assessed for nominal variables with the χ^2 test and by Kruskal-Wallis test (ANOVA). Spearman's rank correlation was used to determine correlations between variables. Stepwise multivariate regression analysis was used to assess the predictors for FMD and IMT levels. Time-to-event analysis of cardiovascular outcomes was done using the Cox proportional hazards model, including adjustment for potential confounding factors. Data are presented in the form of hazard ratios and 95% confidence intervals.

Results

The demographic and clinical characteristics of the study groups are given in Table 1. There were no statistically significant differences among the different CKD stages with regard to age, gender, body mass index, history of CVD, etiology of CKD, and smoking status.

Biochemical and vascular assessments are given in Table 2. Serum albumin, calcium, sTWEAK, and FMD levels gradually decreased across increasing CKD stages, whereas serum phosphate, intact parathyroid hormone, hsCRP, PTX levels, and IMT values increased. Box plots showing the decrease of TWEAK and increase of PTX3 levels in parallel with the reduction in eGFR are given in Figure 1. Neither PTX3 (7.8 ± 7.3 *versus* 7.9 ± 8.2 ng/ml; $n = 49/208$) nor sTWEAK (239 ± 99 *versus* 255 ± 107 pg/ml; $n = 49/208$) concentrations varied between patients with or without previous CVD. However, PTX3 was higher (12.4 ± 8.0 *versus* 6.5 ± 5.9 ng/ml; $P < 0.001$; $n = 58/199$) and TWEAK was lower (220 ± 99 *versus* 261 ± 106 pg/ml; $n = 49/208$) in patients with comorbid diabetes.

Univariate and multivariate associates of FMD and IMT are given in Table 3. Significant determinants of FMD were sTWEAK, hsCRP, PTX3, NMD, systolic BP, diabetes, albumin, iPTH, and eGFR. Significant determinants on IMT were only iPTH and eGFR. Because it could be argued that these biomarkers may be collinear with CRP determinations, we repeated the models excluding CRP measurements. Results remained the same (data not shown). Additionally, we repeated the models excluding diabetics; the results remained the same (data not shown).

Cardiovascular outcomes were determined from the day of examination onward, with a mean follow-up period of 39 months (range, 2 to 43 months). Twenty-four patients died; 22 died from cardiovascular causes and 2 died from malignancies. Causes of cardiovascular death were coronary heart disease ($n = 13$), sudden death ($n = 4$), stroke ($n = 3$), or complicated peripheral vascular disease ($n = 2$). Because only 24 fatal events were registered, we did not study multivariate Cox adjustment because of the likelihood of model overfitting. During the follow-up period, 57 additional nonfatal cardiovascular events were registered as follows: stroke ($n = 13$); myocardial infarction or related ($n = 33$); peripheral vascular disease ($n = 7$), and aortic aneurysm ($n = 4$).

The predictors for time-to-cardiovascular event ($n = 79$, including a composite of fatal and nonfatal) were studied by univariate and multivariate COX analysis (Table 4). In univariate COX, sTWEAK, PTX3, FMD, and hsCRP were significant predictors of outcome. Multivariate COX was used to study the impact of these variables in pairs or together, considering the additional adjustment for age, gender, eGFR, diabetes, and cardiovascular comorbidity. We first studied sTWEAK levels (Table 4), whereby decreasing sTWEAK concentration was associated with increased risk of cardiovascular events independently of basic confounders (model 1) and FMD (model 2). On the other hand, circulating levels of PTX3 (Table 4) were directly associated with cardiovascular outcomes, independent of confounders and its analogous hsCRP (model 1), but this association was lost after adjustment for FMD (model 2).

Discussion

This observational cohort study provides a comprehensive overview of the evolution of sTWEAK and PTX3 levels in patients with progressive kidney failure. PTX3 was initially described as an early marker of innate immunity and inflammatory responses (22), being highly expressed in atherosclerotic lesions and in patients with unstable angina pectoris (12,23,24). Additionally, it was recently shown that HDLs specifically upregulate PTX3 expression through the PI3K/Akt pathway without affecting its homologous CRP (25). Studies in gene-modified mice showed that PTX3 has complex, nonredundant functions *in vivo*, ranging from the assembly of a hyaluronic acid–rich extracellular matrix and female fertility to innate immunity against diverse microorganisms (10,26). Along this line, our study showed the possible role of PTX3 on local vascular health in nondialysis CKD as assessed by FMD (9). Expanding our previous observations on PTX3 and mortality prediction in incident and prevalent dialysis populations (13,14), increased PTX3 levels predicted cardiovascular outcomes in our study, independently of traditional risk factors and of its homologous CRP. The role of PTX3 as a marker of endothelial function in CKD can be supported by the fact that multivariate adjustment for FMD made the predictive performance of PTX3 disappear in our COX analysis. PTX3 has also been detected in atherosclerotic lesions and has been implicated on neointimal thickening plaques (17,27,28). However, the recent observation that double knockout mice lacking ApoE and PTX3 showed an increment in aortic lesion size and a higher inflammatory response compared with ApoE knockout mice expressing PTX3 (29) has lead to hypotheses that PTX3 may be a failed compensatory mechanism to endothelial damage. In this regard, neither PTX3 nor sTWEAK levels explained the IMT variance in multivariate analysis, despite both molecules being present in atheromatous plaques (16,17). Recent studies have suggested that, in nondialysis CKD patients, other etiologies than atherosclerosis (such as fluid overload) may be more important in determining IMT levels (18). This is supported by reports in healthy individuals indicating that wall shear stress may be an important determinant of IMT (30).

sTWEAK was originally identified as a soluble protein that *ex vivo* was released in lower amounts from injured vessels than from healthy vessels (16). In previous observations (7) and in this study, the decline in eGFR was accompanied by gradual reductions both in sTWEAK plasma levels and FMD, both assessments being strongly interrelated and likely suggesting a link between sTWEAK and endothelial dysfunction in CKD patients. Now we add to this evidence that reduced FMD and decreased sTWEAK levels are independent predictors of future cardiovascular outcomes in nondialysis CKD patients. This observation is consistent with recent survival data from patients with stable heart failure (31). As expected from the progressive reduction in sTWEAK with decreasing eGFR, sTWEAK is low in hemodialysis patients (8). In such patients, we reported, however, that increased sTWEAK associated with a higher probability of death (8). A possible explanation for these contradictory findings may lie within the existence of competing risks (or the so-called reverse epidemiology phenomenon) in CKD patients, whereby the presence of certain risk factors in uremia, mainly inflammation and protein-energy wasting, overrides and reverses the natural course and actions of other risk biomarkers (32,33). The pathologic effects of TWEAK observed in animal experimental models (34–36) are mediated by the

Table 2. Biochemical and vascular assessment according to CKD stages

	All Patients (n = 257)	Stage 1 (≥90) (n = 44)	Stage 2 (60 to 89) (n = 53)	Stage 3 (30 to 59) (n = 57)	Stage 4 (15 to 29) (n = 49)	Stage 5 (<15) (n = 54)	P
eGFR (ml/min)	44 (2 to 106)	97 (91 to 106)	79 (61 to 89)	47 (30 to 58)	27 (15 to 29)	10 (2 to 14)	<0.001
SBP (mmHg)	133 (110 to 185)	132 (103 to 157)	134 (115 to 163)	135 (110 to 180)	133 (113 to 175)	134 (110 to 185)	0.58
DBP (mmHg)	82 (71 to 95)	82 (71 to 93)	83 (73 to 93)	84 (80 to 95)	85 (71 to 93)	83 (71 to 92)	0.06
Serum albumin (g/dl)	4.0 (3.2 to 4.8)	4.0 (3.4 to 4.6)	3.8 (3.5 to 4.6)	4.3 (3.5 to 4.8)	3.9 (3.5 to 4.6)	3.7 (3.2 to 4.5)	<0.001
Total cholesterol (mg/dl)	201 (157 to 245)	202 (168 to 239)	202 (178 to 243)	201 (179 to 245)	200 (167 to 246)	200 (157 to 254)	0.27
Triglycerides (mg/dl)	149 ± 15	144 ± 14	150 ± 11	151 ± 14	147 ± 13	145 ± 21	0.31
Serum calcium (mg/dl)	8.41 ± 0.57	8.99 ± 0.48	8.68 ± 0.55	8.31 ± 0.42	8.07 ± 0.33	8.10 ± 0.36	<0.001
Serum phosphate (mg/dl)	5.25 ± 1.51	4.23 ± 0.42	4.46 ± 0.91	4.56 ± 0.72	5.94 ± 1.41	6.95 ± 1.52	<0.001
Hemoglobin (g/dl)	11.8 ± 2.3	12.5 ± 2.2	12.0 ± 2.2	11.9 ± 2.1	11.3 ± 2.3	11.2 ± 2.52	0.02
iPTH (pg/ml)	146 ± 82	52 ± 12	75 ± 31	155 ± 44	169 ± 34	262 ± 41	<0.001
hsCRP (mg/l)	15.0 (5.2 to 41)	9.3 (5.2 to 13.6)	12.0 (7 to 16)	18.0 (7 to 34)	23.0 (6.7 to 35)	28.0 (6 to 41)	<0.001
PTX3 (ng/ml)	7.90 ± 8.05	3.26 ± 1.64	5.81 ± 4.79	7.56 ± 6.24	9.24 ± 7.75	12.45 ± 12.41	<0.001
24-hour proteinuria (g/day)	1.65 (0.37 to 5.45)	1.43 (0.38 to 2.45)	1.65 (0.37 to 3.92)	1.71 (0.57 to 5.15)	1.60 (0.48 to 4.39)	1.72 (0.57 to 5.45)	<0.001
IMT (mm)	0.68 ± 0.12	0.63 ± 0.06	0.69 ± 0.07	0.77 ± 0.10	0.86 ± 0.09	0.90 ± 0.10	<0.001
NMD (%)	13.1 (10.0 to 13.8)	13.0 (11.8 to 13.8)	13.0 (12.4 to 13.8)	12.9 (12.0 to 13.8)	13.0 (11.6 to 13.8)	11.9 (10.0 to 13.3)	<0.001
FMD (%)	6.9 (4.0 to 9.9)	8.3 (7.1 to 9.7)	7.2 (6.0 to 8.3)	6.9 (4.8 to 8.2)	6.1 (4.1 to 8.2)	5.2 (4.0 to 7.2)	<0.001
sTWEAK (pg/ml)	245.5 (96.0 to 678.3)	365.8 (156.5 to 678.3)	276.0 (117.5 to 458.1)	253.3 (112.0 to 380.1)	185.1 (96.0 to 412.0)	158.2 (100.0 to 280.1)	<0.001

SBP, systolic BP; DBP, diastolic BP.

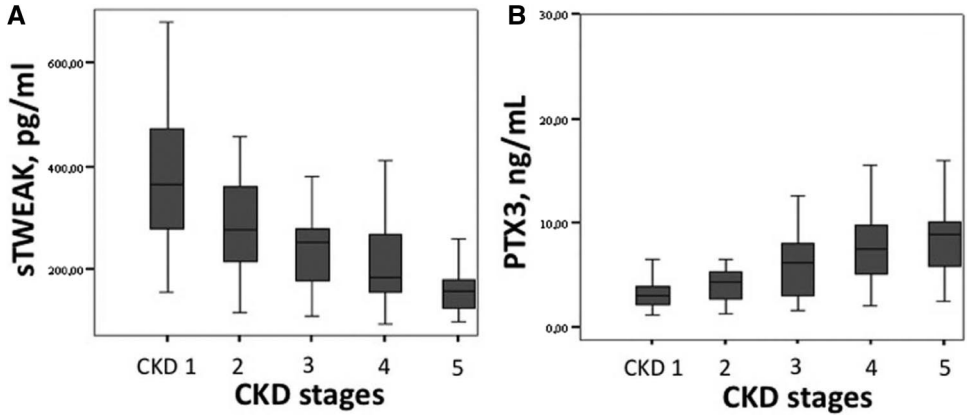

Figure 1. | Box plots showing the decrease in TWEAK (A) and increase in PTX3 (B) levels in parallel with the reduction in eGFR.

Table 3. Univariate and multivariate associates of flow-mediated dilatation (FMD) and intima-media thickness (IMT)

Parameter	FMD		IMT	
	Univariate[a] ρ	Multivariate[b] β (P)	Univariate[a] ρ	Multivariate[c] β (P)
FMD (%)	—	—	−0.64	NS
IMT (mm)	−0.64	NS	—	—
sTWEAK (pg/ml)	0.63	0.10 (0.02)	−0.54	NS
hsCRP (mg/l)	−0.58	−0.10 (0.03)	0.59	NS
PTX3 (ng/ml)	−0.59	−0.09 (0.02)	0.49	NS
NMD (%)	0.43	0.10 (0.007)	−0.29	NS
Age (years)	NS	NS	NS	NS
Gender (men/women)	—	NS	NS	NS
Diabetes (yes/no)	—	−0.11 (0.03)	0.16	NS
History of CVD (yes/no)	—	NS	NS	NS
Smoking (yes/no)	—	NS	NS	NS
Body mass index (kg/m^2)	NS	—	NS	—
SBP (mmHg)	−0.13	−0.07 (0.04)	NS	—
Serum albumin (g/dl)	0.18	0.09 (0.007)	−0.16	—
24-hour proteinuria (mg/day)	NS	NS	0.13	NS
Serum calcium (mg/dl)	0.46	NS	−0.44	NS
Serum phosphate (mg/dl)	−0.61	NS	0.54	NS
iPTH (pg/ml)	−0.79	−0.19 (0.008)	0.69	0.17 (0.04)
eGFR (ml/min)	0.82	0.47 (<0.001)	−0.74	−0.60 (<0.001)

Variables known to influence FMD levels (age, gender, diabetes, history of CVD, smoking, IMT, hsCRP, NMD, SBP, albumin, 24-hour proteinuria, Ca, P, iPTH, and eGFR) and IMT levels (age, gender, diabetes, history of CVD, smoking, FMD, hsCRP, NMD, 24-hour proteinuria, Ca, P, iPTH, and eGFR) were initially included in the multivariate analyses. NS, not significant; SBP, systolic BP.

[a]Statistically significant ($P < 0.05$) ρ values as assessed by Spearman Rank's test. The r^2 of the multivariate models were [b]0.75 and [c]0.56.

binding of sTWEAK with its receptor Fn14. Fn14 expression is practically absent in healthy human aortic wall but is highly increased under pathologic conditions (37). A pro-inflammatory environment increases Fn14 expression (35,36) but also allows CD163 to sequester and degrade sTWEAK by acting as a scavenger receptor (38), thus preventing Fn14 binding (39). On the basis of this preceding literature, we speculate that the reduction in sTWEAK concentrations across CKD stages observed in our cross-sectional study could potentially reflect either of these two processes.

Strengths of this study are the relatively large sample size of uniformly distributed etiologically diagnosed CKD patients across the different disease stages, together with the exclusion of drugs that may confound the interpretation of the eGFR–vascular health axis. At the same time, however, and because of these medical exclusions, our data are not necessarily representative of the normal non-dialysis CKD population. This may translate, for instance, into a relatively young cohort and over-representation of certain etiologies. The use of continuous variables reduces residual confounding in our analysis. However, we cannot

Table 4. Univariate and multivariate COX analysis predicting for cardiovascular outcomes

	Crude Analysis		Model 1		Model 2	
sTWEAK						
sTWEAK (pg/ml)	0.99 (0.98 to 0.99)	<0.001	0.99 (0.98 to 0.99)	<0.001	0.99 (0.98 to 0.99)	0.004
FMD (%)	0.55 (0.46 to 0.67)	<0.001	—		0.55 (0.37 to 0.82)	0.004
hsCRP (mg/L)	1.04 (1.02 to 1.07)	<0.001	1.03 (0.99 to 1.07)	0.06	1.03 (0.99 to 1.07)	0.06
PTX3						
PTX3 (ng/ml)	1.06 (1.05 to 1.08)	<0.001	1.03 (1.01 to 1.05)	0.009	1.01 (0.99 to 1.04)	0.2
FMD (%)	0.55 (0.46 to 0.67)	<0.001	—		0.55 (0.36 to 0.82)	0.003
hsCRP (mg/L)	1.04 (1.02 to 1.07)	<0.001	1.02 (0.98 to 1.06)	0.2	1.02 (0.99 to 1.06)	0.2

Represented are hazard ratios (and 95% confidence intervals) in univariate (crude) Cox model and after different adjustments. Models 1 and 2 show different combinations of the variables of interest after adjustment for age (in years), gender (women as reference), eGFR (ml/min), diabetes (absence as reference), and medical history of cardiovascular disease (absence as reference).

exclude the possibility of other unknown confounders. Finally, it can be argued that eGFR is subject to inaccuracies in CKD classification. We included, nonetheless, etiologically diagnosed patients, and eGFR was always treated as a continuous variable in all our analyses.

Ultimately, our interest in these molecular pathway representatives relates to their potential as therapeutic targets (40). In this regard, short-term angiotensin-converting enzyme inhibitor treatment significantly improved endothelial function and normalized both PTX3 and urinary protein excretion in type 2 diabetic proteinuric patients (41). Also, the improvement in FMD after combined therapy with the renin–angiotensin system and calcium channel blockers was independently associated with both PTX3 and sTWEAK normalization in type 2 diabetic hypertensive patients (42). Hence, this study concluded that both PTX3 and sTWEAK levels strongly associate with the endothelial dysfunction typically observed with progressive kidney failure. Additionally, both biomarkers impacted the predictability of cardiovascular outcomes; in the case of PTX3, this was done dependently on FMD levels, and in the case of sTWEAK, it was independent of such vascular derangements. This and other existing evidence may encourage further mechanistic research regarding the potential of these molecules as therapeutic targets in inflammation- or atherosclerosis-related diseases.

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Disclosures

None.

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