Soluble TWEAK predicts major adverse cardiovascular events in CKD patients: a sub-study of NEFRONA

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Summary

Background and objectives: Soluble TWEAK (sTWEAK) concentrations have been associated with the presence of chronic kidney disease (CKD) and cardiovascular disease (CVD). Now, we hypothesized that sTWEAK levels may relate to the increased prevalence of atherosclerotic plaques, vascular calcification and cardiovascular outcomes observed in patients with CKD.

Design, setting, participants and measurements: A multicenter cross-sectional study was conducted in 1058 patients with CKD stages III-VD (mean age, 58±13, 665 men) but without any history of CVD from the NEFRONA study (a study design to assess the prevalence of surrogate markers of CVD). Ankle-brachial index (ABI) and B mode ultrasound was performed to detect the presence of carotid and/or femoral atherosclerotic plaques, together with biochemical measurements and sTWEAK assessment. Patients were followed for cardiovascular outcomes (follow-up period of 3.13±1.15 years).

Results: sTWEAK serum levels were reduced in parallel with a reduction in estimated glomerular filtration rate. sTWEAK concentrations were independently and negatively associated with carotid intima/media thickness. Moreover, sTWEAK levels were reduced in patients with carotid atherosclerotic plaques but not in those with femoral plaques. After adjustment by confounders, the OR for presenting carotid atherosclerotic plaques in patients in lower vs higher tertile of sTWEAK was 3.80 [2.36-5.15 (IQR); p<0.001]. Furthermore, sTWEAK levels were reduced in patients with calcified carotid atherosclerotic plaques. The OR for presenting calcified carotid plaques was 2.48 (1.51-4.07; p<0.001) after multivariable adjustment. Finally, after the follow-up, 41 fatal and 68 nonfatal cardiovascular events occurred. In a Cox model after controlling for potential confounding factors, patients in the lower tertile of sTWEAK concentrations had an increased risk of fatal and nonfatal cardiovascular events [HR: 2.64 (1.48-4.68); p<0.005] and cardiovascular mortality [HR: 3.06 (1.14-8.238); p<0.05].

Conclusions: Diminished sTWEAK levels are associated with the presence of carotid atherosclerotic plaques in CKD patients. Additionally, sTWEAK impacted the predictability of cardiovascular morbi-mortality.

Introduction

Chronic kidney disease has become recognized as a key independent factor for several health outcomes including cardiovascular disease (CVD) (1). In fact, compared to the age adjusted CVD mortality in the general population, CKD patients die at accelerated rate, 15 to 30 times higher than non-CKD subjects (2). The mechanisms for the elevated CVD risk in CKD patients are complex and may implicate changes in both the vasculature and heart. Most cardiovascular (CV) events appear to be from sudden deaths related to possible heart failure or arrhythmias (3-4). In addition, the presence of accelerated atherosclerosis in CKD could play a role in the higher cardiovascular mortality. Atherosclerosis is a multifactorial disease characterized by chronic inflammation and excessive cell proliferation. *In vivo* and *in vitro* studies have elucidated molecular and cellular pathways of inflammation that promote atherosclerosis. Unveiling the role of several cytokines as inflammatory messengers provided a potential mechanism whereby risk factors for atherosclerosis can alter arterial wall, and produce a systemic response that favors atherothrombotic events (5). Of these, tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a type II transmembrame glycoprotein that participates, through its sole receptor fibroblast growth factor inducible 14 (Fn14), in several cellular responses associated with atherosclerosis (6). In fact, different experimental studies have demonstrated a key role of TWEAK/Fn14 axis in atherosclerotic plaque development, progression and rupture (7-9). In addition, specific gain or loss-of-function phenotypes have demonstrated the role of TWEAK and Fn14 in the development of abdominal aortic aneurysms, stroke, myocardial infarction and heart failure, among others (10-13).

As other member of the TNF superfamily, TWEAK can be proteolytically processed by furin, leading to the release of an 18 kDa soluble form (sTWEAK) (14). The use of sTWEAK as a potential biomarker of atherosclerosis was described in 2007, analyzing the secretome from human carotid atherosclerotic plaques (15). We demonstrated that sTWEAK is secreted in lower amount from human carotid atherosclerotic plaques than in healthy arteries (15). After that,

the association of sTWEAK with CVD or CVD-related diseases has been extensively studied. Thus, sTWEAK concentrations are diminished in subjects with coronary artery disease (16), abdominal aortic aneurysm (17), systolic heart failure (18), type II diabetes (19) and CKD (20). The pathological effects observed in different animals model induced by TWEAK are mediated by binding to its receptor Fn14. Fn14 is almost absent in healthy tissues but is highly upregulated in human atherosclerotic plaques (21). Binding of sTWEAK with Fn14 in pathological tissues could be responsible to the reduction observed in CKD patients with vascular affectations.

The NEFRONA study was an observational multicenter prospective study designed to evaluate the prevalence and evolution of subclinical atheromatosis in CKD patients, as well as the contribution of vascular imaging for a more precise cardiovascular risk assessment (22). The aims of the study are multiple: first, we wanted to confirm and extend the usefulness of sTWEAK as a biomarker of the presence of atherosclerotic plaques in CKD patients. Second, we wanted to test the ability of circulating sTWEAK concentrations to predict vascular calcification. Finally, we wanted to know the impact of sTWEAK on cardiovascular outcomes in the NEFRONA population.

Materials and Methods

Subjects

NEFRONA study is a 4-year prospective multicenter cohort study aimed to assess the predictive value of non-invasive techniques for CVD events and mortality in patients with CKD (22). In that study, 2445 CKD subjects (18 and 74 years of age were eligible if they had CKD stage 3 or higher as defined by current guidelines (eGFR lower than 60 mL/min/1.73 m^2 estimated using the 4-variable Modification of Diet in Renal Disease (MDRD) equation) were enrolled from October 2010 to June 2012. Five hundred and fifty nine control subjects with and MDRD over 60 ml/min/1.73 m^2 were also recruited.

One thousand and fifty eight subjects from the NEFRONA study with available serum sample were included in the study. Subjects were enrolled within 69 Spanish primary care centres distributed in 38 different regions from Spain. The exclusion criteria were previous CV events, active infections (HIV, tuberculosis), pregnancy, having received any organ transplantation, and having a life expectancy of <1 year. Each local ethics committee approved the study, and subjects were included after providing informed consent.

Clinical and biochemical data

At recruitment, the subjects were asked to complete a questionnaire including clinical history of diabetes, hypertension and dyslipidemia; CV risk factors (such as smoking habit) and medication use.

The ultrasound explorations (carotid and femoral) were performed according to a standardized protocol by three itinerant teams belonging to the UDETMA (Unit for Detection and Treatment of Atherothrombotic Diseases, Hospital Universitari Arnau de Vilanova, Lleida, Spain). The itinerant teams also collected the anthropometric parameters as well as blood samples. Blood samples were processed immediately after extraction and storage at -20º C. After that, samples were sent and stored within 24 h at the centralized biobank of the Spanish Network for Nephrological Research (REDinRen).

Biochemical parameters were obtained from a routine fasting blood

test. Serum 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 7.3 and 8.5%, respectively.

Carotid and femoral ultrasound

B-mode ultrasound of the carotid and femoral arteries was performed using the Vivid BT09 apparatus (General Electric instrument) equipped with a 6-13 MHz broadband linear array probe. The analysis of the presence of atheromatous plaques was performed by a single reader in a blinded fashion, using the semi-automatic software EchoPAC Dimension (General Electric Healthcare). To assess the quality of the reading and the intraobserver reliability, a sample of 20 individuals was measured 3–5 times on different days. A kappa coefficient of 1 was obtained, indicating excellent intraobserver reliability.

Ultrasound imaging was performed for both carotid arteries with the subjects in a supine position and the head turned 45° contralateral to the side of the probe. The presence of atheromatous plaques at each site was defined as carotid intima-media thickness (cIMT) ≥1.5 mm protruding into the lumen, which is the criterion given in the ASE Consensus Statement (23) and the Mannheim CIMT Consensus (24). This criterion was used in the ARIC study (25) and in the Framingham Offspring Study (26), two important Studies showing the predictive value of atheromatous plaques in CV risk.

B-mode ultrasound imaging was performed in the right and left femoral arteries. Subjects were examined in a supine position, and the presence of atheromatous plaques was explored in the common and superficial femoral arteries.

Statistical Analyses

All of the statistical analyses were performed using the SPSS 11.0 (SPSS, Chicago, IL) statistical package. Non-normally 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 X^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 ABI and c-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

Soluble TWEAK concentrations in CKD patients

The demographic and clinical characteristics of the studied population are summarized in Table 1. There were significant differences among the different CKD stages with regard to age, gender, body mass index and cardiovascular risk factors. Confirming previous data in smaller populations, across increasing CDK stages, gradual decreases in sTWEAK concentrations were observed (Fig. 1A, $p<0.001$). The lowest sTWEAK level was found in patients undergoing dialysis (Table 1). No difference in sTWEAK concentrations was observed in CKD patients with diabetes, hypertension or dyslipemia compared with those without these cardiovascular risk factors. However, current smokers (N=605) presented a small reduction in sTWEAK concentrations [372 (246-557) *vs* 412 (267-595) pg/mL; $p<0.05$]. No differences were found according to the prescription of anti-hypertensive drugs, statins or anti-diabetic treatments.

Univariate associates of ABI and IMT are given in Table 2. ABI was negatively associated with eGFR, c-IMT, total cholesterol, LDL-c, and glucose concentrations. In addition, c-IMT was positively associated with age, BMI, SBP, eGFR, triglycerides, glucose and hs-CRP concentrations, and negatively correlated with DBP, HDL-c, ABI and sTWEAK levels. To clarify whether sTWEAK is an independent predictor of c-IMT in the population studied, multiple regression analysis was performed. Variables included in the analysis were age, gender, SBP, DBP, cholesterol, LDL-c, HDL-c, triglycerides, glucose, eGFR and hs-PCR levels as well as sTWEAK concentrations were included in the model. In such model, age ($\beta=0.478$; $p<0.001$), gender (men/women; $\beta=-0.100$; $p<0.01$), HDL-c ($\beta=-0.095$; $p<0.01$), glucose ($\beta=0.064$; $p<0.05$), eGFR ($\beta=0.118$; $p<0.001$), SBP ($\beta=0.068$; $p<0.05$), as well as sTWEAK ($\beta=-0.110$; $p<0.001$) were independently associated with c-IMT.

sTWEAK as a predictor of carotid atherosclerotic plaques

Subjects with atherosclerotic plaques (N=729) showed a reduction in sTWEAK concentrations compared with those without atherosclerotic

plaques (N=329) [357 (244-517) vs 490 (296-652) pg/mL; median (IQR); p<0.001]. When we analyzed sTWEAK serum levels according with the vascular territory affected (femoral or carotid arteries), only patients with carotid or both carotid and femoral plaques showed lower sTWEAK levels compared with those without atherosclerotic plaques (Fig. 1B). The presence of femoral atherosclerotic plaques did not modify sTWEAK concentrations in our patient population (Fig. 1B). Multivariable logistic regression analysis including age, gender, BMI, smoker status, cardiovascular factors, cardiovascular treatments, eGFR and hs-PCR concentrations as well as sTWEAK levels was performed to assess predictors of the presence or absence of carotid atherosclerotic plaques in our studied population. Of these variables, age, current smokers, eGFR, and low sTWEAK levels were independent predictors for the presence of carotid atherosclerotic plaques (Table 3). In addition, we analyzed sTWEAK serum levels according with the presence of calcified carotid plaques. Patients with calcified carotid atherosclerotic plaques showed a reduction in sTWEAK levels (Fig. 1C) compared to patients in which the plaques were not calcified. After multivariable logistic analysis, age, eGFR, hs-CRP>3 mg/L and low sTWEAK concentrations were independent predictors for the presence of calcified carotid atherosclerotic plaques (Table 3).

sTWEAK and cardiovascular outcomes

Cardiovascular outcomes were determined from the day of examination onward, with a mean of follow-up period of 3.13±1.15 years. Eighty-nine patients died, 41 (46%) of which were due to CVD-related disease such as myocardial infarction (N=11), thrombotic stroke (N=6), sudden death (N=12), mesenteric infarction (N=7), or other CVD-related causes (N=5). sTWEAK levels was reduced in patients suffering a fatal CV event compared with those free for fatal CV event [231 (174-354) vs 395 (261-577) pg/mL; median (IQR); p<0.001]. Kaplan-Meier curves showed a significant association of lowest sTWEAK concentrations with worse survival (Log-Rank=15.6; p<0.001; Fig. 2A). In univariate Cox

analysis, the predictors for time-to-cardiovascular death were presence of diabetes, eGFR and low sTWEAK concentrations (Table 4). Multivariate Cox was used to study the impact of these variables considering additional adjustment for age, gender, eGFR, smoker status, cardiovascular risk factors, hs-CRP and sTWEAK levels. In this first model, presence of diabetes, eGFR and low sTWEAK concentrations persisted as predictors of cardiovascular death (Table 4). In addition, in a second model after adjustment for cardiovascular treatments, only eGFR and low TWEAK levels persisted as independent predictors of cardiovascular death (Table 4).

The same analysis was done for total mortality. Crude analysis showed that age, presence of diabetes, eGFR and low sTWEAK were predictors of total mortality. After adjustment for age, gender, eGFR, smoker status, cardiovascular risk factors, hs-CRP and sTWEAK levels, only age, presence of diabetes and eGFR levels persisted as predictors of total mortality (Table 4). Similar results were obtained after inclusion of cardiovascular treatments in the adjusted model (Table 4).

Due to the limited number of fatal cardiovascular events registered, we also analyzed the impact of sTWEAK concentrations on the prediction of CV event for a composite of fatal and nonfatal CV event (n=109). During the follow-up period, 68 addition nonfatal cardiovascular events were registered as follow: transient ischemic attack (N=4), unstable angina (N=16), myocardial infarction (N=12), intermittent claudication (N=11), aortic aneurysm (N=2), stroke (N=16), and others CV event (N=7). sTWEAK concentrations were reduced in CKD patients suffering a non-fatal CV event compared with those without CV event [275 (191-428) vs 403 (263-586) pg/mL; median (IQR); $p<0.001$]. The figure 2B displays the estimated cumulative evidence of major CV event. When CKD patients were grouped according to sTWEAK levels tertiles, the probability of developing a CV event increased in the lowest tertile of sTWEAK concentrations group [HR= 3.74 (95% CI, 2.18 to 6.42); $p<0.001$]. Cox-proportional Hazards model was performed to study the impact of traditional confounding factors on the probability of developing a CV event. The first model included age, gender, smoker status, traditional

cardiovascular risk factors, eGFR, and hs-CRP levels as well as sTWEAK concentrations. After adjustment, eGFR, hs-CRP levels >3mg/L and low sTWEAK levels were associated with an increase in the risk of developing a CV event (Table 4). These associations persisted in a second model including cardiovascular treatments (Table 4).

Discussion

In this work, we investigated sTWEAK serum levels as predictors of cardiovascular outcomes in a CKD population with or without cardiovascular risk factors but without any history of CVD. This population included CKD patients from several primary care centers in a daily clinical practice, with or without concomitant medication, with the aim to have a representative Spanish population. sTWEAK was originally identified as a soluble protein that was secreted in lower amounts from atherosclerotic plaques than from healthy arteries (16). Previously (20) and in this study, the decline in eGFR was related with a gradual diminution in sTWEAK concentrations. In addition, sTWEAK was also negatively and independently associated with c-IMT. This data are in agreement with previous studies in which sTWEAK was negatively associated with c-IMT in asymptomatic subjects (16) or in non-dialysis CKD patients (27-28). However, the association between c-IMT and sTWEAK was lost and, indeed, positive association has been reported (29). These contradictory data could be due to the presence of different therapy regimens used in those patients (30) and to the high inter-operator variability of the ultrasound technique.

An important finding from our study is the difference of sTWEAK concentrations observed depending on the vascular territory affected. Thus, sTWEAK was reduced in CKD patients with carotid plaques compared with those with femoral atherosclerotic plaques or without plaques. In addition, in a multivariable logistic regression analysis, only age, smoking, eGFR and sTWEAK concentrations were independent predictors for the presence of carotid atherosclerotic plaques. These results are in agreement with previous data from subjects free for clinical cardiovascular disease (30). Femoral plaques have an increment in their fibrotic content compared with carotid atherosclerotic plaques. In addition, carotid plaque are characterized by a more inflammatory cell content that femoral arteries (31). Differences in sTWEAK concentrations between carotid and femoral plaque could be related to differences in cellular composition. More importantly, sTWEAK was also related to the presence of vascular calcification. Vascular calcification is a highly prevalent

phenotype that is identified frequently in patients with CKD (32). In addition, vascular calcification is both a risk factor and contributor to morbidity and mortality in CKD patients (33). In this sense, sTWEAK was and independent predictor of both cardiovascular mortality and first cardiovascular events in our studied population. These data are consistent with previous survival data from patients with stable heart failure (34) and cardiovascular outcomes in non-dialysis CKD (35). Interestingly, we have observed that sTWEAK was not associated with total mortality. This data could indicate that sTWEAK is a good biomarker for cardiovascular prognosis but not for other pathologies.

Strengths of this study are the large sample size of CKD patients included in our study, and the fact that the vascular exploration was performed by the same itinerant team and evaluated by a single reader, minimizing the variability associated with the technique. Furthermore, the assessment of plaque presence also in the femoral territories adds additional information to the analysis. In addition, this is the first time that sTWEAK levels are associated with the presence of cardiovascular events and cardiovascular mortality in a general CKD population. However, some limitations of our study should be highlighted for a correct interpretation of the implications of our findings. There is an intentional bias, because only patients with no history of cardiovascular events were included, as the study was aimed to primary prevention of cardiovascular events. This bias was necessary, but its consequences have to be considered when interpreting the results. A relatively low number of cardiovascular deaths were reported during the follow-up, which might limit the statistical power of our analysis.

We have analyzed sTWEAK serum levels and different concentrations of this protein have been published depending of the source analyzed (plasma, serum or urine) (36). Reagents to measure sTWEAK concentrations are commercially available for research purposes only. We need a standardization of the available kits to obtain consensual normal ranges of sTWEAK levels (36). Finally, in a very complex disease such as CVD, a single biomarker could be not sufficient to detect unstable atherosclerotic plaques. sTWEAK levels could add

information to know cardiovascular risk factors and contribute to a better prediction of cardiovascular events.

In conclusion, reduced sTWEAK serum levels are associated with the presence of carotid atherosclerotic plaques in CKD patients. In addition, sTWEAK levels were significantly and independently related with cardiovascular outcomes in CKD patients.

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Figure Legends

Figure 1: sTWEK levels, atherosclerotic burden and vascular calcification in CKD patients without any history of CVD.

A) Box plots showing the reduction in sTWEAK serum concentrations [median (IQR)] in parallel with the reduction in eGFR. * p<0.001 *vs* CKD III; † p<0.001 vs CKD IV-V.

B) Box plots showing sTWEAK levels [median (IQR)] according to the vascular territory affected. No plaques, N=329; Carotid plaques, N=193; Femoral plaques, N=116; Both, N=420. *p < 0.001 vs no plaques.

C) Blox plots showing sTWEAK levels [median (IQR)] according to the presence of calcified carotid atherosclerotic plaque. No, N=900; Calcified carotid plaques; N=158.

Figure 2: Impact of sTWEAK in cardiovascular outcomes

A) Kaplan-Meier curve of cardiovascular mortality risk in CKD patients according with their sTWEAK levels.

B) Kaplan-Meier plot of cumulative probability of a first major CV event when CKD patients were grouped according to their sTWEAK concentration tertiles.

Low sTWEAK levels were defined as sTWEAK concentration below the 33rd percentile (<289 pg/mL) and high sTWEAK as its concentration above the 66th percentile (>465 pg/mL).

Table 1.- Baseline demographic and biochemical parameters.

	All patients (N=1058)	CKD III (N=435)	CKD IV-V (N=377)	CKD 5D (N=246)	p
Age, years	58±13	61±11	58±12	53±14	<0.001
Male, % (N)	62.8 (665)	70.8	58.9	54.9	<0.001
BMI, Kg/m^2	28 (25-32)	29 (26-32)	28 (25-32)	27 (23-32)	<0.001
SBP, mmHg	140 (129-156)	142 (130-156)	143 (130-159)	137 (123-153)	<0.001
DBP, mmHg	81 (75-89)	81 (75-89)	81 (74-88)	80 (70-90)	0.102
Cholesterol, mg/dL	178±40	187±37	177±38	163±42	<0.001
LDL-cholesterol, mg/dL	101±34	110±32	100±34	87±32	<0.001
HDL-cholesterol, mg/dL	46 (38-57)	47 (39-56)	47 (37-59)	44 (36-54)	<0.05
Triglycerides, mg/dL	126 (92-177)	125 (96-175)	127 (92-185)	124 (82-176)	0.814
Glucose, mg/dL	99 (88-118)	103 (92-125)	98 (89-112)	91 (82-113)	<0.001
Hs-CRP, mg/L	1.88 (0.92-4.7)	1.83 (0.92-4.07)	1.68 (0.82-4.22)	2.44 (1.14-7.16)	<0.005
eGFR, mL/min/1.73 m^2	26 (12-40)	43 (36-50)	21 (15-25)	6.6 (5.2-9.1)	<0.001
ABI	1.04 (0.95-1.15)	1.00 (0.93-1.12)	1.04 (0.95-1.15)	1.07 (0.96-1.25)	<0.001
c-IMT, mm	0.71 (0.62-0.82)	0.75 (0.66-0.84)	0.68 (0.59-0.81)	0.69 (0.60-0.79)	<0.001
Smoker, % (N)	57.2 (605)	60.9 (265)	55.7 (210)	52.8 (130)	0.095
Dyslipemia, % (N)	64.6 (684)	71.7 (312)	69.0 (260)	45.5 (112)	<0.001
Medical Treatment, % (N)	57.0 (603)	57.7 (251)	67.1 (253)	40.2 (99)	
Diabetes Mellitus, % (N)	29.0 (307)	32.2 (140)	30.0 (113)	22.0 (54)	<0.05
Medical Treatment, % (N)	27.9 (295)	31.9 (139)	28.6 (108)	19.5 (48)	
Hypertension, % (N)	92.1 (974)	91.5 (398)	96.0 (362)	87.0 (214)	<0.001
Medical Treatment, % (N)	89.2 (944)	92.6 (403)	95.8 (361)	73.2 (180)	
Familial CVD, % (N)	8.5 (90)	9.4 (41)	9.0 (34)	6.1 (15)	0.296
sTWEAK, pg/mL	389 (254-576)	459 (323-631)	405 (286-549)	214 (115-346)	<0.001

Results expressed as media ± SD or median (IQR). BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C reactive protein; eGFR: estimated glomerular filtration rate; ABI: Anklebrachial index; c-IMT: carotid intima/media thickness.

Table 2.- Spearman correlations coefficients between c-IMT, ABI, sTWEAK levels, and selected parameters in asymptomatic subjects.

	c-IMT		ABI		sTWEAK	
	r	p	r	p	r	p
Age, years	0.54	<0.001	-0.03	0.33	-0.02	0.43
BMI, kg/m^2	0.21	<0.001	-0.05	0.07	0.03	0.29
SBP, mm Hg	0.18	<0.001	-0.00	0.96	-0.03	0.26
DBP, mm Hg	-0.07	<0.05	0.03	0.39	-0.04	0.17
Cholesterol, mg/dL	-0.02	0.64	-0.14	<0.001	0.13	<0.001
HDL-c, mg/dL	-0.12	<0.001	-0.06	0.06	0.10	<0.01
LDL-c, mg/dL	0.09	0.14	-0.15	<0.001	0.15	<0.001
Triglicerydes, mg/dL	0.07	<0.05	-0.05	0.14	-0.08	<0.05
Glucose, mg/dL	0.22	<0.001	-0.11	<0.001	0.01	0.88
Hs-CRP, mg/L	0.13	<0.001	-0.04	0.21	-0.04	0.23
eGFR, mL/min/1.73m^2	0.22	<0.001	-0.15	<0.001	0.35	<0.001
c-IMT, mm	--------	--------	-0.12	<0.001	-0.10	<0.01
ABI	-0.18	<0.001	--------	--------	-0.05	0.08
sTWEAK, pg/mL	-0.10	<0.01	-0.05	0.08	--------	--------

BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low-density lipoprotein; HDL: high-density lipoprotein; eGFR: estimated glomerular filtration rate; hs-CRP: high-sensitivity C-reactive protein; ABI: Ankle-brachial index; c-IMT: carotid intima/media thickness.

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Table 3.- Multivariate logistic analysis ~~of~~ modeling the presence of carotid or calcified carotid atherosclerotic plaques in the studied population.

	Odds Ratio	Confidence Interval (95%)	p
Carotid Plaques			
Age, years	1.11	1.09-1.12	<0.001
Current Smoker, yes vs no	2.02	1.44-2.82	<0.001
eGFR, mL/min/1.73m^2	0.99	0.98-0.99	<0.05
sTWEAK, lower vs higher tertile	3.48	2.35-5.15	<0.001
Calcified Carotid Plaques			
Age, years	1.07	1.05-1.091	<0.001
eGFR, mL/min/1.73m^2	0.98	0.97-0.99	<0.01
Hs-CRP, >3 mg/L	1.64	1.14-2.36	<0.05
sTWEAK, lower vs higher tertile	2.48	1.51-4.07	<0.001

The analysis included age, gender, smoker status, cardiovascular risk factors, cardiovascular treatments, eGFR and hs-CRP levels as well as sTWEAK concentrations.

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Table 4.- Univariate and multivariate COX regression analysis predicting ~~for~~ cardiovascular outcomes and mortality.

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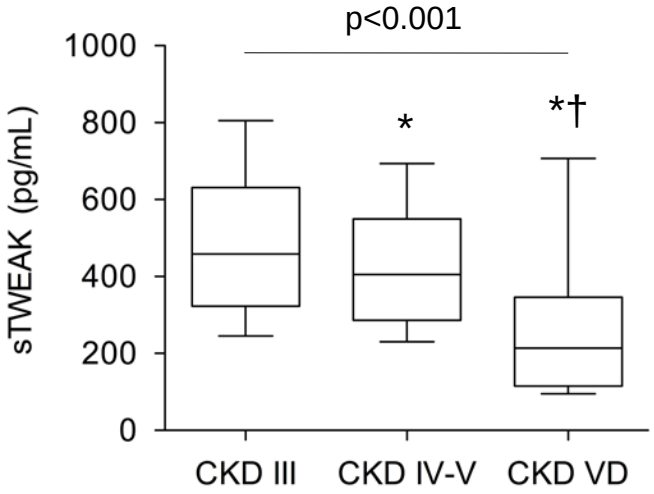
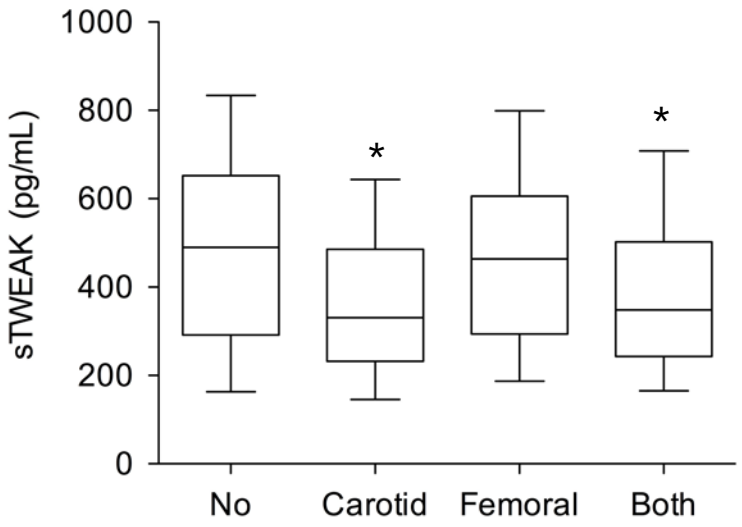
Cardiovascular Mortality						
	Crude		Model 1		Model 2	
Diabetes, yes *vs* no	2.08 (1.39-4.73)	<0.005	2.52 (1.28-4.96)	<0.01	2.08 (0.61-7.12)	0.242
eGFR, mL/min/1.73m^2	0.94 (0.92-0.97)	<0.001	0.95 (0.93-0.98)	<0.001	0.95 (0.93-0.98)	<0.001
sTWEAK, lower vs higher tertile	4.92 (2.03-11.88)	<0.001	3.24 (1.21-8.69)	<0.05	3.06 (1.14-8.24)	<0.05
Total Mortality						
	Crude		Model 1		Model 2	
Age, years	1.03 (1.01-1.06)	<0.005	1.04 (1.02-1.06)	<0.001	1.04 (1.02-1.06)	<0.001
Diabetes, yes *vs* no	1.82 (1.19-2.77)	<0.01	1.87 (1.19-2.95)	<0.01	2.43 (1.09-5.39)	<0.05
eGFR, mL/min/1.73m^2	0.96 (0.94-0.97)	<0.001	0.96 (0.94-0.97)	<0.001	0.96 (0.94-0.98)	<0.001
sTWEAK, lower vs higher tertile	2.12 (1.28-3.50)	<0.005	1.23 (0.72-2.11)	0.449	1.20 (0.70-2.06)	0.500
Fatal and Non Fatal Cardiovascular Events						
	Crude		Model 1		Model 2	
Age, years	1.03 (1.01-1.04)	<0.005	1.02 (1.01-1.04)	<0.05	1.02 (1.00-1.04)	<0.05
Diabetes, yes *vs* no	2.46 (1.69-3.58)	<0.001	2.30 (1.54-3.43)	<0.001	3.00 (1.48-6.06)	<0.001
eGFR, mL/min/1.73m^2	0.97 (0.96-0.99)	<0.001	0.97 (0.96-0.99)	<0.001	0.97 (0.96-0.99)	<0.005
sTWEAK, lower vs higher tertile	3.74 (2.17-6.42)	<0.001	2.63 (1.48-4.68)	<0.001	2.64 (1.48-4.68)	<0.005

The first model included age, gender, smoker status, cardiovascular risk factors, eGFR and hs-CRP levels as well as sTWEAK concentrations. The second model also included cardiovascular treatments.

A


p<0.001
1000
800
600
400
200
0
sTWEAK (pg/mL)
*
*†
CKD III
CKD IV-V
CKD VD

B


1000
800
600
400
200
0
sTWEAK (pg/mL)
*
*
No
Carotid
Femoral
Both

C

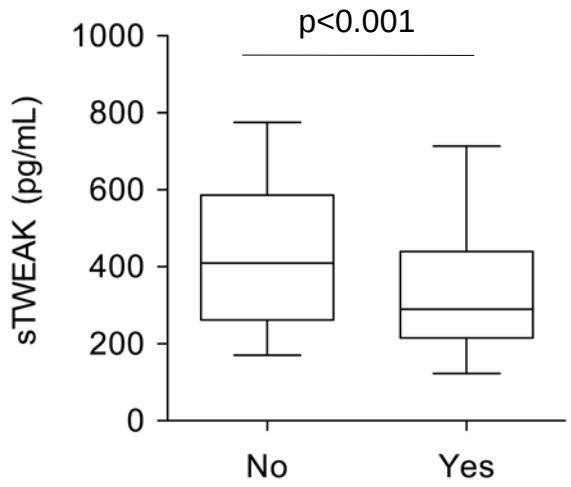
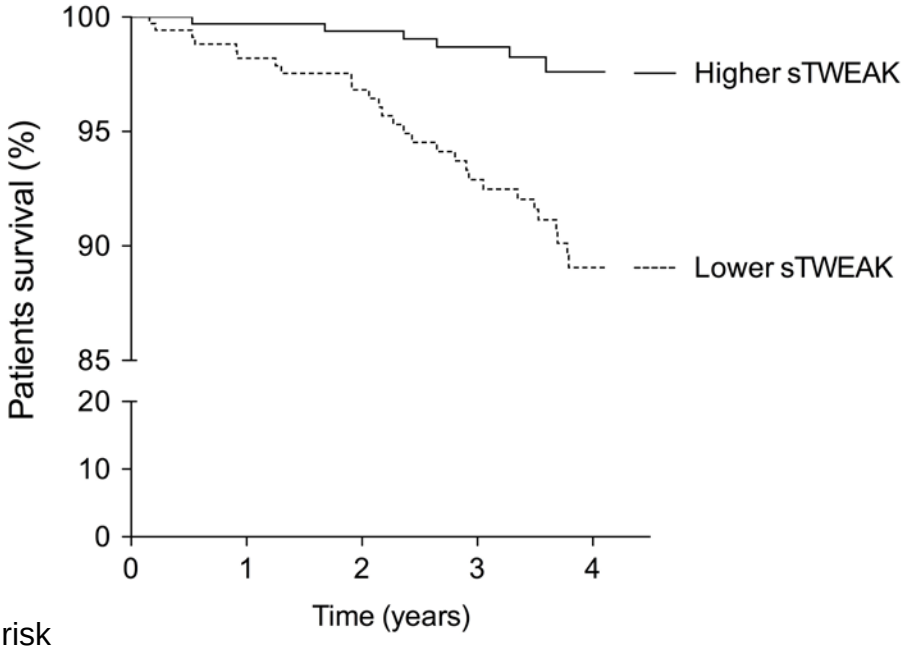

p<0.001
1000
800
600
400
200
0
sTWEAK (pg/mL)
No
Yes

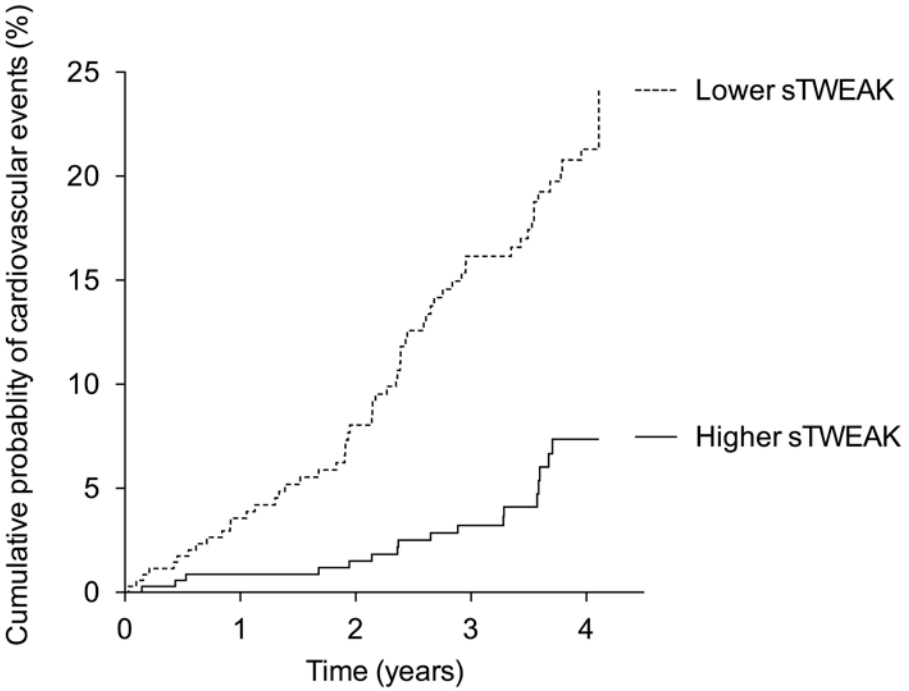
Figure 1

A



Number at risk					
Lower sTWEAK	351	312	259	225	165
Higher sTWEAK	352	335	308	264	124

B



Number at risk					
Lower sTWEAK	351	307	249	208	151
Higher sTWEAK	352	334	306	262	119

Figure 2