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Circulating TNF receptors predict cardiovascular disease in patients with chronic kidney disease

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Abstract

Cardiovascular disease (CVD) is the main public health problem in patients with chronic kidney disease (CKD); however, there is no established biomarker for predicting CVD morbidity and mortality in CKD. The aim of this study was to evaluate the role of circulating tumor necrosis factor receptors (cTNFRs) in predicting CVD risk in CKD patients.

We prospectively recruited 984 patients with CKD from 11 centers between 2006 and 2012. The levels of cTNFR1 and cTNFR2 were determined by performing an enzyme-linked immunosorbent assay. During the mean follow-up period of 4 years, 36 patients experienced a CVD event. The median serum concentrations of cTNFR1 and cTNFR2 were 2703.4 (225.6–13,057.7) and 5661.0 (634.9–30,599.6) pg/mL, respectively, and the cTNFR1 level was closely correlated with the cTNFR2 level ($r = 0.86$, $P < .0001$). The urinary protein-to-creatinine ratio (UPCR) and estimated glomerular filtration rate (eGFR) were significantly correlated with the cTNFR2 level ($r = 0.21$ for UPCR, $r = -0.67$ for eGFR; $P < .001$ for all). Similar correlations were observed for serum cTNFR1 ($r = 0.21$ for UPCR, $r = -0.75$ for eGFR; $P < .001$ for all). In the Cox proportional hazard analyses, cTNFR1 (hazard ratio [HR] 2.506, 95% confidence interval [CI] 1.186–5.295, $P = .016$) and cTNFR2 (HR 4.156, 95% CI 1.913–9.030, $P < .001$) predicted CVD risk even after adjustment for clinical covariates, such as UPCR, eGFR, and high-sensitivity C-reactive protein. cTNFR1 and 2 are associated with CVD and other risk factors in CKD, independently of eGFR and UPCR. Furthermore, cTNFRs could be relevant predictors of CVD in CKD patients.

Abbreviations: BMI = body mass index, CKD = chronic kidney disease, CVD = cardiovascular disease, DM = diabetes mellitus, ESRD = end-stage renal disease, GFR = glomerular filtration rate, HF = heart failure, HTN = hypertension, MI = myocardial infarction, NRI = net reclassification improvement, TNF-α = tumor necrosis factor-α, TNFR = tumor necrosis factor receptor, UPCR = urinary protein-to-creatinine ratio.

Keywords: cardiovascular disease, chronic kidney disease, circulating TNF receptor 1, circulating TNF receptor 2

1. Introduction

Chronic kidney disease (CKD) affects 10% to 16% of the adult population worldwide, and the prevalence is continuously increasing.[1–3] In accordance with increasing prevalence of CKD, the economic costs resulting from the care of these patients have increase.[4] CKD increases the risks of all-cause mortality and cardiovascular disease (CVD),[2,3,5] which is the leading

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cause of death of CKD patients, irrespective of the stage.[6] Therefore, CVD is one of the main public health problems affecting these patients; however, traditional risk factors, such as old age, hypertension (HTN), and dyslipidemia, cannot sufficiently explain its high incidence.

Recurrent or chronic inflammatory processes are common in individuals with CKD.[7] A role of inflammation has become well established in theories describing the atherosclerotic disease process, and inflammation is common in heart disease and stroke patients.[8]

Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that plays an essential role in mediating inflammatory processes.[9–11] It binds to 2 distinct TNF receptors (TNFRs), TNFR1 and TNFR2, which are the key mediators of TNF signaling.[8] The plasma levels of circulating TNFRs (cTNFRs) are significantly increased in CKD patients, and these levels are closely correlated with kidney function.[12] These biomarkers are also predictive of declining kidney function.[13–16] Further, we have recently reported that cTNFRs may be biomarkers for CKD progression in patients with glomerular nephritis, such as IgA nephropathy and membranous nephropathy, as well as in patients undergoing invasive coronary angiography.[17–19] There has been particular interest in searching for biomarkers that may be predictive of CVD morbidity and mortality in the CKD population. To date, cystatin C,[20,21] uric acid,[22] natriuretic peptides,[23] plasma renin activity,[24] and fibroblast growth factor-23[25] have been investigated as biomarkers for predicting CVD in CKD patients. However, data on the association between TNFRs and CVD in these patients are scarce. Therefore, we aimed to explore the role of serum TNFRs in the occurrence of CVD in CKD patients.

2. Methods

2.1. Study population and data collection

A total of 984 CKD patients were prospectively enrolled from 11 centers in South Korea between 2006 and 2012 (Fig. 1). This study was reviewed and approved by the Institutional Review Board of each center. We conducted the study according to the principles of the Declaration of Helsinki and obtained written informed consent from all of the participants.

The inclusion criteria were as follows: adult patients (aged ≥19 years), and CKD stage from 1 to 5 (predialysis) based on the estimated glomerular filtration rate (eGFR). Excluded subjects were those who were unable or unwilling to provide written consent, had previously received chronic dialysis or organ transplantation, had heart failure (HF; New York Hear Association class 3 or 4) or liver cirrhosis (Child-Pugh class 2 or 3), or had history of CVD, or were currently pregnant, or had unavailable samples to analysis cTNFR level.

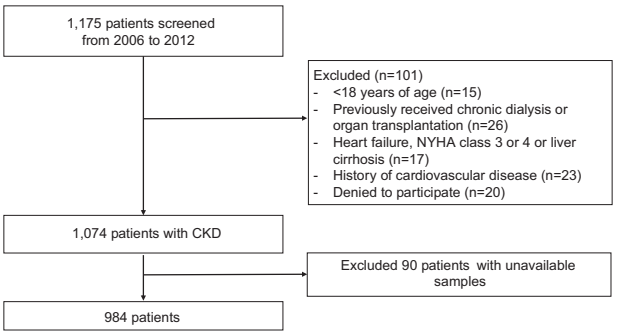

Figure 1. Flow diagram of patient enrollment.

Demographic and laboratory data were recorded at the time of enrollment. Follow-up visits occurred every 6 months, and during each visit, the subjects were evaluated to determine whether they had experienced cardiovascular events or received renal replacement therapy.

The serum creatinine (sCr) level was measured by an assay based on isotope dilution mass spectrometry (IDMS), and the eGFR was calculated using the following IDMS-traceable Modification of Diet in Renal Disease equation: GFR (mL/min/1.73 m^2) = 175 × (sCr) $^{(-1.154)}$ × (age in years)$^{(-0.203)}$ × (0.742, if female). CKD was defined as the presence of kidney damage or decreased kidney function for 3 months or more months. The kidney damage includes albuminuria above albumin-to-creatinine ratio >30 mg/g or urine sediment abnormalities or pathologic abnormalities or structural abnormalities detected by imaging.[6] HTN was defined as a systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or the use of antihypertensive medication. Diabetes mellitus (DM) was defined as a fasting glucose level ≥126 mg/dL, hemoglobin A1c ≥ 6.5%, the use of a glucose-lowering drug, or self-reported diabetes.

2.2. Clinical assessment

The primary endpoint was defined as the first occurrence of either cardiovascular death, nonfatal myocardial infarction (MI), revascularization, or fatal/nonfatal stroke. Cardiovascular death included fatal MI, sudden death, death caused by congestive HF, death attributable to a diagnostic or therapeutic procedure due to coronary artery disease, and death from another coronary cause.[26] The secondary endpoints were defined as all-cause mortality (cardiovascular and noncardiovascular) and end-stage renal disease (ESRD) (with receipt of renal replacement therapy, such as peritoneal dialysis, hemodialysis or kidney transplantation).[26] The date and cause of death were reported within 1 month after the event. For the patients who withdrew from the study, we ascertained mortality data from Statistics Korea.[27]

2.3. Blood sample collection and measurement of TNFRs

Peripheral blood was withdrawn from venous blood vessels and collected in ethylenediamine tetraacetic acid containing tubes. Blood specimens were immediately cooled and centrifuged at 3000 rpm for 10 min, and serum samples were stored at −70 °C until tested. The cTNFR1 and cTNFR2 levels were determined using enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) according to the manufacturer's Instructions. The following quantikine ELISA kits were used: human sTNFRI (catalog number DRT100)/human sTNFRII Quantikine ELISA Kit (catalog number DRT200). The samples were subjected to duplicate and blind testing. Absorbance was measured with an ELISA reader at 450 nm. The minimum detectable concentration was 0.43 to 1.20 pg/mL for sTNFR1and 0.2 to 2.3 pg/mL for sTNFR2.

2.4. Statistical analysis

The data are presented as the mean ± standard deviation or the frequency (count and percentage). The cTNFR levels that were not normally distributed are expressed as the median and interquartile range (IQR) or are presented as log-transformed values. The subjects' characteristics were analyzed using Student *t* test for continuous variables, the chi-square test for categorical variables, or one-way analysis of variance using Scheffe multiple

comparisons for continuous variables among 3 groups. Pearson analysis was performed to analyze the correlations between the log-transformed cTNFR (ln cTNFR) levels and variables. To identify CVD events according to the cTNFR levels, Kaplan–Meier analyses and log-rank tests were performed. Furthermore, multivariate Cox proportional hazards regression analysis using the forward stepwise process was applied to identify associations between the cTNFR levels and CVD events. Analyses were performed using SPSS version 21.0 software (IBM Corporation, Armonk, NY), and statistical significance was defined as $P < .05$.

To identify the level of statistical power, we computed post hoc power analysis using Gpower version 3.1 software (Franz, Universitat Kiel, Germany) and XLSTAT version 2016.3 (Addinsoft, New York, NY) software for the Cox proportional hazard model because this is end of the study.[28,29] The statistical power in this study was at least 98% for a given sample size (n = 984), effect size (0.02, using the estimates from this study), and alpha level (0.05). All elements used in the calculation are conservative value such as sample size and effect size with small convention value.

To evaluate the model performance according to the contributions of cTNFRs to the prediction of CVD events, *c* statistics was calculated using the area under the receiver-operating curve. For the statistical significance and 95% confidence intervals (CIs) for *c* statistics, Mann–Whitney contrast test was used. In addition, the continuous net reclassification improvement (cNRI) was computed using logistic regression models with the function *improveprob* in R. The NRI quantifies the increase in predicted risk categories for events and the decrease in risk categories for nonevents with the addition of a new marker.[30,31] Data were analyzed by using SAS 9.4 for Windows software (SAS Institute, Cary, NC) and R software version 3.2.3 (Comprehensive R Archive Network: http://cran.r-project.org). In all analyses, $P < .05$ was considered to be statistically significant.

3. Results

3.1. Baseline characteristics

Between 2006 and 2012, we prospectively recruited 984 CKD patients from 11 centers. The baseline characteristics of the study participants are presented in Table 1. Among a total of 984 patients, 56.1% were men, and the mean age was 49.7 ± 15.8 years. A total of 267 participants (27.1%) had DM, and 527 (53.6%) had HTN. The mean eGFR level was 50.8 ± 31.6 mL/min/1.73 m^2, the urinary protein-to-creatinine ratio (UPCR) was 2.2 ± 2.6 g/g creatinine, and the high-sensitivity C-reactive protein (hs-CRP) level was 0.7 ± 2.4 mg/dL. The median cTNFR1 and cTNFR2 levels were 2703.4 pg/mL (IQR 225.6–13,057.7 pg/mL) and 5661.0 pg/mL (IQR 634.9–30,599.6 pg/mL), respectively. The group of patients in the highest tertile of ln cTNFRs had higher percentages of older individuals, males, and DM patients compared with those in the lowest tertile (Table 2). They also had a higher serum hs-CRP level and more advanced CKD based on both the lower eGFR and higher UPCR.

3.2. Associations of clinical parameters with cTNFR levels

Risk factors for CVD or CKD were studied by examining their correlations with each other and with the ln cTNFR levels (Table 3). A significant negative correlation between the eGFR and ln cTNFR2 level was observed (Pearson correlation coefficient $[r] = -0.67$, $P < .001$ for ln cTNFR2). Age, SBP, low-density lipoprotein (LDL), body mass index (BMI), proteinuria (UPCR), and the hs-CRP level were positively correlated

Table 1

Baseline characteristics.

Characteristic	
Age, y*	49.7 ± 15.8
Sex, male (n, %)	552 (56.1%)
hs-CRP, mg/dL*	0.7 ± 2.4
cTNFR1, pg/mL†	2703.4 (225.6–13,057.7)
cTNFR2, pg/mL†	5661.0 (634.9–30,599.6)
eGFR, mL/min/1.73 m^{2*}	50.8 ± 31.6
eGFR categories	
>90	139 (14.4%)
60–90	178 (18.4%)
30–60	301 (31.1%)
15–30	330 (34.1%)
<15	19 (2.0%)
UPCR, g/g cr*	2.2 ± 2.6
Albumin, g/dL*	3.9 ± 0.6
LDL cholesterol, mg/dL*	95.8 ± 58.1
BMI, kg/m^{2*}	24.0 ± 3.7
SBP, mm Hg*	128.2 ± 16.8
Hypertension (n, %)	527 (53.6%)
Diabetes (n, %)	267 (27.1%)

BMI = body mass index, eGFR = estimated glomerular filtration rate, LDL = low-density lipoprotein, SBP = systolic blood pressure, UPCR = urine protein-to-creatinine ratio.
* Data are expressed as the mean ± standard deviation or the median (25th and 75th percentiles).
† Data are expressed as the median and interquartile range (IQR).

Table 2

Clinical parameters according to circulating tumor necrosis factor receptor s and circulating tumor necrosis factor receptor 2 concentrations.

	Ln cTNFR1 T1	Ln cTNFR1 T2	Ln cTNFR1 T3	P
Range, pg/mL	<7.226	7.226–8.115	≥8.116	
n	329	328	327	
Age, y	40.1 ± 14.7	53.2 ± 14.0	55.9 ± 13.9	<.001
Sex, male (n, %)	142 (43.2)	198 (60.4)	212 (64.8)	<.001
hs-CRP, mg/dL*	0.6 ± 1.6	0.5 ± 1.1	0.9 ± 3.4	.059
eGFR, mL/min/1.73 m^{2*}	80.9 ± 27.0	46.1 ± 24.7	25.7 ± 10.2	<.001
UPCR, g/g cr*	1.6 ± 2.3	2.3 ± 2.9	2.7 ± 2.6	<.001
Albumin, g/dL*	4.0 ± 0.6	3.8 ± 0.7	3.8 ± 0.6	<.001
LDL cholesterol, mg/dL*	86.0 ± 66.6	105.2 ± 62.5	97.1 ± 41.6	.001
BMI, kg/m^{2*}	23.1 ± 3.4	24.4 ± 3.7	24.3 ± 3.8	<.001
SBP, mm Hg*	125.0 ± 15.1	128.7 ± 17.3	131.1 ± 17.3	<.001
Hypertension (n, %)	218 (66.3)	183 (55.8)	126 (38.5)	<.001
Diabetes (n, %)	21 (6.4)	92 (28.0)	154 (47.1)	<.001
	Ln cTNFR2 T1	**Ln cTNFR2 T2**	**Ln cTNFR2 T3**	**P**
Range, pg/mL	<8.068	8.068–8.811	≥8.811	
n	326	326	328	
Age, y*	40.8 ± 15.1	51.4 ± 13.7	56.8 ± 14.0	<.001
Sex, male (n, %)	147 (44.7)	202 (61.8)	203 (61.9)	<.001
hs-CRP, mg/dL*	0.6 ± 1.6	0.4 ± 1.1	1.0 ± 3.4	.013
eGFR, mL/min/1.73 m^{2*}	76.6 ± 28.4	47.3 ± 27.4	28.6 ± 15.6	<.001
UPCR (g/g creatinine)*	1.5 ± 2.1	2.3 ± 2.9	2.8 ± 2.7	<.001
Albumin, g/dL*	4.0 ± 0.6	3.8 ± 0.7	3.8 ± 0.6	<.001
LDL cholesterol, mg/dL*	88.1 ± 64.3	101.4 ± 63.0	98.0 ± 44.5	.024
BMI, kg/m^{2*}	23.2 ± 3.4	24.3 ± 3.5	24.4 ± 3.9	<.001
SBP, mm Hg*	126.2 ± 16.4	128.0 ± 16.8	130.5 ± 17.0	.004
Hypertension, (n, %)	234 (71.1)	156 (47.7)	137 (41.8)	<.001
Diabetes (n, %)	25 (7.6)	85 (26.0)	157 (47.9)	<.001

BMI = body mass index, CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, LDL = low-density lipoprotein, SBP = systolic blood pressure, UPCR = urine protein-to-creatinine ratio.
* Data are expressed as the mean ± standard deviation or the median (25th and 75th percentiles). The chi-squared test and analysis of variance was used.

Table 3

Correlation coefficients between various clinical parameters in CKD patients.

	Ln cTNFR1	Age	SBP	eGFR	LDL	BMI	UPCR	hs-CRP
Ln cTNFR2	0.86*	0.43*	0.11†	−0.67*	0.09†	0.14*	0.21*	0.10†
Ln cTNFR1	1.00	0.43*	0.17*	−0.75*	0.12†	0.16*	0.21*	0.06
Age		1.00	0.15*	−0.51*	0.15*	0.25*	0.15*	0.05
SBP			1.00	−0.15*	0.08†	0.25*	0.19*	−0.02
eGFR				1.00	−0.01	−0.19*	0.05	−0.03
LDL					1.00	0.04	0.31*	−0.02
BMI						1.00	0.01	0.03
UPCR							1.00	−0.04
hs-CRP								1.00

BMI = body mass index, eGFR = estimated glomerular filtration rate, LDL = low-density lipoprotein, Ln cTNFR = log-transformed circulating tumor necrosis factor receptor, UPCR = urine protein-to-creatinine ratio.

† $P < .001$.

* $P < .0001$.

with the ln cTNFR2 level (Table 3), but the degree of correlation was less than that for the eGFR ($r = 0.43$ for age, $r = 0.11$ for SBP, $r = 0.13$ for BMI, $r = 0.21$ for UPCR, and $r = 0.10$ for hs-CRP; for all $P < .05$). Similar results were observed for serum cTNFR1 except hs-CRP. There was no significant correlation of the cTNFR1 level with the hs-CRP level. To identify the relationship between the cTNFRs and CVD, we divided the patients according to CVD status. The median ln cTNFR1 and 2 levels were higher in the patients with CVD than in those without CVD (ln cTNFR1: positive CVD group vs. negative CVD group, 8.3 vs 7.7 pg/mL, P for trend $< .001$; cTNFR2: 9.0 vs 8.5 pg/mL). In addition, more of the participants with CVD were elderly, male and had advanced CKD, diabetes than those without CVD (table S1, http://links.lww.com/MD/B691).

3.3. Prediction of CVD based on cTNFR levels

A total of 36 (3.7%) patients experienced CVD during the median follow-up period of 3.4 ± 2.0 years. The impact of ln cTNFR expression on CVD was evaluated using Kaplan–Meier analysis (Fig. 2). The results showed a significant difference between the ln cTNFR levels and that they were associated with CVD. The CVD-free rate was significantly lower among the patients in the highest tertile of ln cTNFR1 ($P < .001$, log-rank test). A similar relationship was observed for cTNFR2.

Based on the univariate Cox regression analysis results, stepwise multivariate regression analysis was then performed to examine the predictive potential of cTNFRs for CVD (Table 4).

The results of this analysis revealed that the ln cTNFR2 level was an independent predictor of CVD in the CKD patients, even after adjustments for age, sex, inflammation (hs-CRP), and CVD risk factors, such as the BMI, SBP, DM, UPCR, eGFR, and LDL (ln cTNFR2: hazard ratio [HR] 4.16, 95% CI 1.59–9.03, $P < .001$). Similar result was observed for ln cTFNR1 level. However, the strength of predictive value for CVD was lower than that of ln TNFR2. Figure 3 shows the HRs for CVD according to the spline ln TNFR2 level adjusted for CVD risk factors. As the ln TNFR2 level increased, the HR for CVD increased steeply.

To elucidate the potential of cTNFR2 to serve as a reliable predictor of CVD, we conducted receiver operating characteristic curve and NRI analyses (table S2, http://links.lww.com/MD/B691). The incremental predictive values of hs-CRP and cTNFR2 for CVD showed improvement in discrimination according to the c statistic, although this improvement was not statistically significant. After adjustments for traditional risk factors, including age, sex, SBP, BMI, and DM, the inclusion of hs-CRP and cTNFR2 increased the area under the curve (from 0.829 to 0.846; $P = .134$) for the prediction of CVD occurrence. The NRI showed significant improvement in discrimination (NRI = 534%, CI 19.2–87.5, $P = .002$).

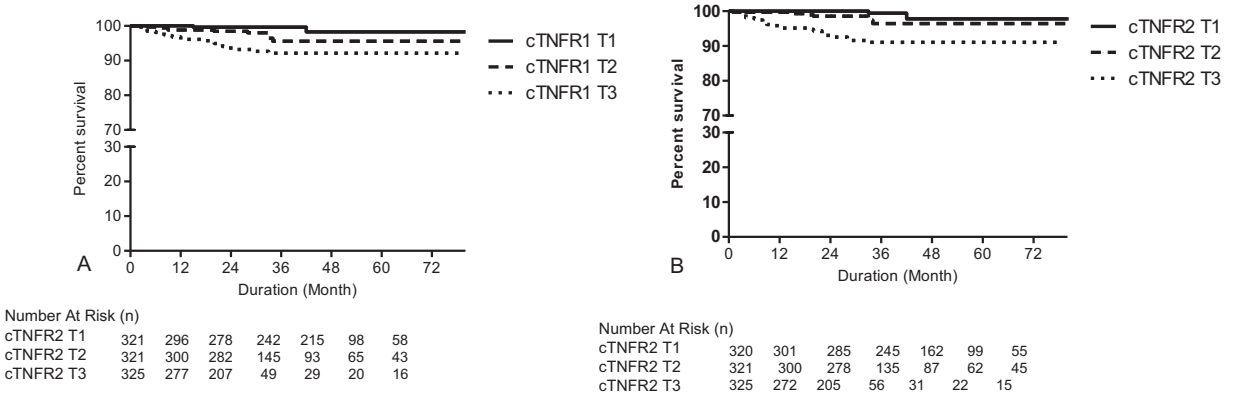

Figure 2. Circulating tumor necrosis factor receptors (cTNFRs) and event-free survival. Patients were stratified by cTNFR1 (A) and cTNFR2 (B). Kaplan–Meier analysis and the log-rank test revealed a significant difference in event-free survival between the groups.

Table 4

Hazard ratios of ln circulating tumor necrosis factor receptors with clinical endpoints in CKD patients.

	Ln cTNFR1 HR (95% CI)	P	Ln cTNFR2 HR (95% CI)	P
CVD				
Model 1*	3.477 (1.802–6.709)	<.001	4.988 (2.553–9.744)	<.001
Model 2†	3.066 (1.568–5.996)	.001	4.540 (2.290–9.000)	<.001
Model 3‡	2.506 (1.186–5.295)	.016	4.156 (1.913–9.030)	<.001
All-cause mortality				
Model 1*	2.161 (0.856–5.452)	.103	8.138 (2.895–22.873)	<.001
Model 2†	1.810 (0.710–4.612)	.214	7.774 (2.757–21.923)	<.001
Model 3‡	0.870 (0.245–3.094)	.830	7.448 (2.319–23.925)	.001
ESRD				
Model 1*	9.101 (6.764–12.246)	<.001	6.832 (5.220–8.942)	<.001
Model 2†	10.102 (7.137–14.298)	<.001	6.378 (4.811–8.454)	<.001
Model 3‡	5.927 (3.412–10.296)	<.001	3.297 (2.192–4.959)	<.001

BMI = body mass index, CI = confidence interval, eGFR = estimated glomerular filtration rate, ESRD = end-stage renal disease, HR = hazard ratio, LDL = low-density lipoprotein, Ln cTNFR = log-transformed circulating tumor necrosis factor receptor, UPCR = urine protein-to-creatinine ratio.

* Model 1 was adjusted for age and sex.

† Model 2 was adjusted for age, sex, and inflammation (hs-CRP).

‡ Model 3 was adjusted for age, sex, inflammation (hs-CRP), and CVD risk factors (the BMI, SBP, DM, UPCR, eGFR, and LDL).

3.4. Prediction of all-cause mortality and renal outcome based on cTNFR levels

We also explored the impacts of the cTNFR levels on all-cause mortality and ESRD as a secondary outcome (Table 4). ESRD developed in 174 patients (17.7%), and 19 deaths (1.9%) occurred during the follow-up period. The ln TNFR2 level at baseline was significantly predictive of not only all-cause mortality (HR 7.45, 95% CI 2.32–23.93, $P=.001$) but also ESRD (HR 3.30, 95% CI 2.19–4.96, $P<.001$). In contrast with ln cTNFR2, the ln cTNFR1 level was not significantly predictive of all-cause mortality.

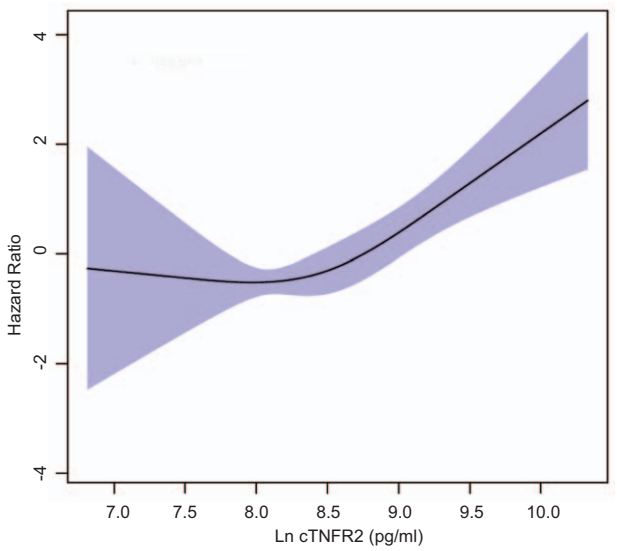

Figure 3. Multivariate Cox proportional hazard ratio for cardiovascular disease according to spline log-transformed circulating tumor necrosis factor receptor 2 (cTNFR2). The solid-black curve was obtained by multivariate Cox analysis according to the spline log-transformed cTNFR2 (ln cTNFR2) level adjusted for age, sex, systolic blood pressure, body mass index, and diabetes.

3.5. Subgroup analyses according to the baseline characteristics

Next, we performed subgroup analysis to confirm the predictive value of cTNFR2 in the different subgroups. The association of cTNFR2 with CVD risk persisted in subgroup analyses according to the categories of age, sex, proteinuria, the eGFR, and evidence of DM, even after adjusting for the covariates. The cTNFR2 level was found to be an independent predictor of CVD in all age (age >65 years: HR 5.69; 95% CI 1.46–22.12, age <65 years: HR 3.37; 95% CI 1.11–10.24; $P=.033$), males (HR 5.37; 95% CI 1.93–14.95; $P=.001$), the lower proteinuria group (UPCR < 3.5 g/g cr) (HR 2.64; 95% CI 1.08–6.42; $P=.033$), the lower eGFR group (eGFR < 30 mL/min/1.73 m^2) (HR 3.96; 95% CI 1.44–10.91; $P=.0008$), and the DM group (HR 7.725; 95% CI 2.28–23.08; $P=.001$) (Fig. 4). No significant interactions were detected among the subgroups except DM (for all interactions, $P>.05$). We also performed subgroup analysis to confirm the predictive value of cTNFR1 in the different subgroups (age, sex, proteinuria, the eGFR, and evidence of DM). However, there was no significant association between cTNFR1 and CVD in any subgroup analysis (Supplementary Fig. 1, http://links.lww.com/MD/B691).

4. Discussion

We found a strong association of cTNFRs with CVD risk in a large, multicenter prospective CKD cohort. Furthermore, the association of the cTNFR2 level at the time of initial diagnosis remained significant after adjusting for CVD risk factors and was stronger in the participants who were male or had advanced CKD or DM.

Although associations between renal dysfunction and cTNFRs have been described in the elderly,[15] diabetes patients,[13,14] and individuals with glomerulonephritis,[16,19] there is a paucity of data on the correlation between CVD and cTNFRs in CKD patients. To our knowledge, the present study is the first prospective, multicenter study to report a relationship between CVD and the cTNFR levels in these patients.

TNF-α is a functional, 26 kDa, homotrimeric, transmembrane protein and is a dualistic cytokine with proinflammatory and

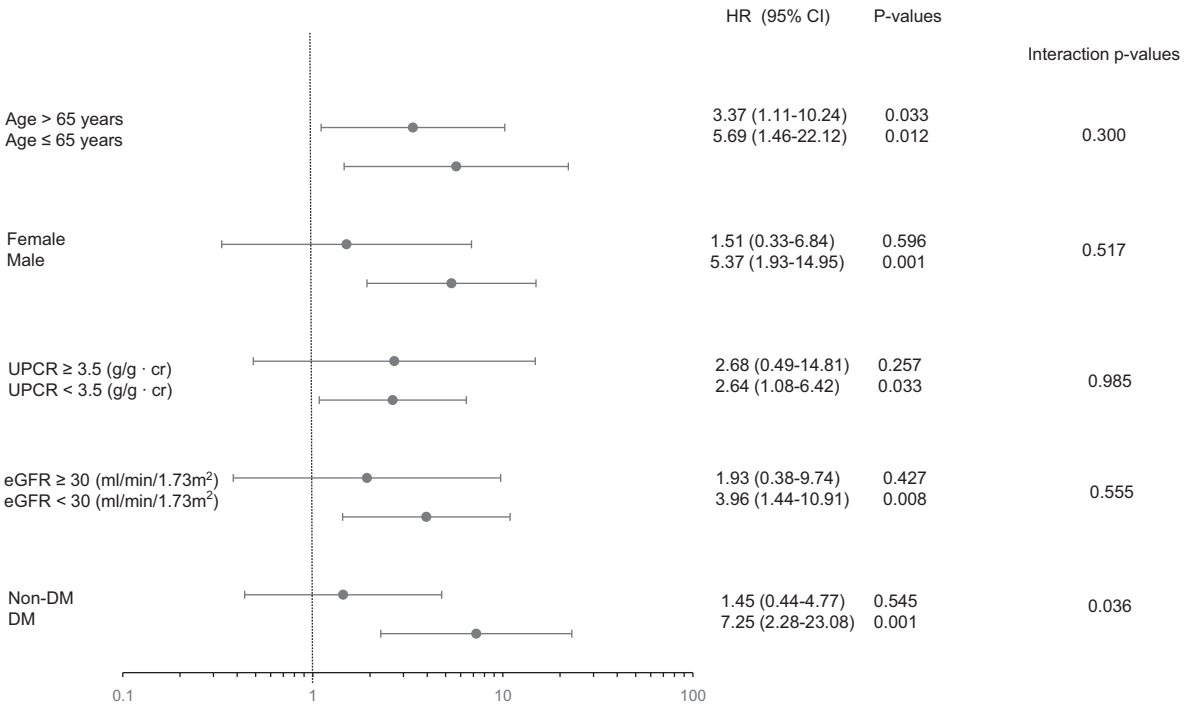

Figure 4. Subgroup analysis of the risk of cardiovascular disease in chronic kidney disease patients with respect to log-transformed circulating tumor necrosis factor receptor 2. The adjusted covariates included age, sex, high-sensitivity C-reactive protein, body mass index, systolic blood pressure, proteinuria, the estimated glomerular filtration rate, and diabetes.

immunoregulatory functions.[32,33] Microinflammation driven by TNF-α is directly involved in the pathogenesis and progression of CKD. The TNF-α pathway activates cellular damage and apoptosis, recruits inflammatory cells, and causes tubulointerstitial changes.[18,34] TNF-α increases albumin permeability, and its inhibition in a diabetic animal model has been shown to decrease albuminuria.[35] These functions are relayed by TNFRs, which exist in soluble or membrane-bound forms,[36,37] and cTNFR1 and cTNFR2 are expressed in glomerular and tubular cells after renal injury.[34] In our study, elevated cTNFR levels were implicated in the deterioration of renal function, along with a decreased eGFR and increased proteinuria (UPCR) and ESRD.

In addition, elevated cTNFR levels, and particularly an increase in cTNFR2, at the time of diagnosis was strongly correlated with DM. These findings can be explained as follows. First, TNF-α is a mediator of obesity-related insulin resistance,[38] and the atherosclerotic process, which involves macrophages, endothelial cells, and smooth muscle cells,[39] but it is also derived from adipose tissue.[40] Second, hyperglycemia has been suggested to affect the levels of oxidative stress,[41] and oxidative stress also increases TNF-α activity, and specifically the activity of TNFR2.[42]

Previous studies have assessed the associations between cTNFRs and left ventricular mass,[43] carotid atherosclerosis,[44] coronary heart disease,[45,46] MI,[47] and HF.[48] In addition, several experimental studies have demonstrated a role of TNF signaling in myocardial cell apoptosis, leading to ischemic reperfusion injury, left ventricular dysfunction, and HF.[48,49] TNF-α plays an important role in cardiac toxicity through reactive oxygen species and mitogen-activated protein kinase pathways, as well as through interactions with the renin–angiotensin system in experimental settings.[43,50,51] Elevated cTNFR levels may also be reflective of inflammatory mechanisms involved in subclinical atherosclerotic diseases.[43] In accordance with previous studies, cTNFR2 was found to be an independent risk factor for CVD in our study and to be predictive of CVD, especially in the participants with a lower proteinuria level and advanced CKD and DM. These results can be explained by 2 hypotheses. The first hypothesis is that an elevated cTNFR2 level is a manifestation of the disease process, particularly in the high-risk group, but not in the lower proteinuria group. The second hypothesis is that an elevated cTNFR2 level contributes to the development of CVD. Interestingly, in our study, cTNFR2 had a much more pronounced and significant effect on CVD and mortality than cTNFR1. This difference may be attributed to the distinct natures of cTNFR1 and 2. Indeed, cTNFR1 is abundantly expressed in all nucleated cells, but cTNFR2 expression is restricted mainly to endothelial cells and leukocytes, although its distribution varies between normal and diseased tissues.[52,53] Each receptor may play a distinct role in inflammation and apoptosis, but they also cooperate to regulate many of their downstream effects.[20,54,55] The origins and roles of cTNFRs, however, are still a matter of debate. Further pathogenic studies of cTNFR2 with respect to inflammation and adverse cardiac events are needed. In addition, this study evaluated the incremental predictive values of cTNFRs for CVD, and cTNFR2 showed improvement over traditional risk factors in predicting CVD.

The strengths of our study were the large, multicenter, prospective cohort, and the assessment of detailed participant characteristics focusing on CVD risk factors. The limitation was the lack of information on medications, long-term outcomes, and cTNFR levels during the follow-up period. In addition, we only analyzed the cTNFR levels at the time of enrollment; however, our findings demonstrate that a single cTNFR measurement provides information on the future risk of CVD. Further, we did

not measure any other cardiac biomarkers, such as troponin and natriuretic peptides, except for hs-CRP in this population. Thus, we could not compare the predictive roles of cTNFRs with other established predictors for CVD.

In conclusion, cTNFRs were shown to be associated with CVD, independent of age, sex, inflammatory markers, and other CVD risk factors, in the CKD patients. The predictive effect of cTNFR2 was pronounced in the elderly (>65 years), male, advanced CKD, and DM patients. Accordingly, this study may provide a noninvasive method of CVD prediction and renal function assessment in CKD patients. Further investigations are needed to establish the use of cTNFRs as biomarkers for CVD prediction in the CKD population.

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