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Elevation of circulating TNF receptors 1 and 2 increases the risk of end-stage renal disease in American Indians with type 2 diabetes

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Abstract

In Caucasians with type 2 diabetes, circulating TNF receptors 1 (TNFR1) and 2 (TNFR2) predict end-stage renal disease (ESRD). Here we examined this relationship in a longitudinal cohort study of American Indians with type 2 diabetes with measured glomerular filtration rate (mGFR, iothalamate) and urinary albumin-to-creatinine ratio. ESRD was defined as dialysis, kidney transplant, or death attributed to diabetic kidney disease. Age-gender-adjusted incidence rates and incidence rate ratios of ESRD were computed by Mantel-Haenszel stratification. The hazard ratio of ESRD was assessed per interquartile range increase in the distribution of each TNFR after adjusting for baseline age, gender, mean blood pressure, HbA1c, albumin-to-creatinine ratio, and mGFR. Among the 193 participants, 62 developed ESRD and 25 died without ESRD during a median follow-up of 9.5 years. The age-gender-adjusted incidence rate ratio of ESRD was higher among participants in the highest vs. lowest quartile for TNFR1 (6.6, 95% CI 3.3–13.3) or TNFR2 (8.8, 95% CI 4.3–18.0). In the fully adjusted model, the risk of ESRD per interquartile range increase was 1.6 times (95% CI 1.1–2.2) as high for TNFR1 and 1.7 times (95% CI 1.2–2.3) as high for TNFR2. Thus, elevated serum concentrations of TNFR1 or TNFR2 are associated with increased risk of ESRD in American Indians with type 2 diabetes after accounting for traditional risk factors including albumin-to-creatinine ratio and mGFR

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DISCLOSURE

A.S.K. and M.A.N are co-inventors in a patent application on TNFRs, which is assigned to Joslin Diabetes Center, and is licensed to Argutus Medical/EKF Diagnostics.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Inflammatory processes play an important role in the pathophysiology of diabetic kidney disease. Inflammatory cells have been observed in kidney tissue biopsies from streptozocin-treated diabetic rats[1] and patients with various degrees of diabetic glomerulosclerosis.[2] Moreover, inflammatory markers, including tumor necrosis factor (TNFα) and its receptors TNFR1 and TNFR2, are associated with progression of diabetic kidney disease. TNFα is the main ligand for both TNFR1 and TNFR2. Depending on a number of local factors and the activation of the two receptors, TNFα induces different and sometimes contrasting effects.[3] These effects have been observed mostly in vitro and may not be specific to a particular cause of renal disease. Activated TNFR1 is the primary receptor mediating tissue injury through proinflammatory signals and/or cell death, whereas TNFR2 may promote cell migration, regeneration and proliferation and regulates TNFR1 induced apoptosis.[4] In addition, TNFR2 may have a synergistic effect with TNFR1 by ligand passing, a process in which TNFR2-bound TNFα increases the local TNFα concentration in the vicinity of TNFR1.[5] TNFR1 and TNF-related apoptosis-inducing ligand (TRAIL) have been implicated in pancreatic β-cell destruction associated with type 1 diabetes;[6,7] TRAIL correlates positively with body fat and serum LDL cholesterol in elderly subjects,[8] and was shown to induce insulin resistance.[9,10] TRAIL gene deletion in ApoE−/− mice fed a high-fat diet resulted in increased systemic inflammation, diabetes, accelerated atherosclerosis,[11] suggesting that TRAIL may play a role in the development of diabetes and its macro- and micro-vascular complications.

Circulating levels of TNFRs have recently emerged as very robust and independent predictors of the progression of diabetic kidney disease in the Joslin Kidney Study. [12–14] In that study, elevated concentrations of circulating TNFR1 and 2 were strongly associated with subsequent end-stage renal disease (ESRD) in predominantly Caucasian subjects with type 2 diabetes after accounting for several risk factors for kidney disease present at baseline, including HbA1c, albuminuria, glomerular filtration rate (GFR), free and bound TNFα, markers of endothelial dysfunction and markers of systemic inflammation.[13]

To evaluate the role of circulating TNFRs in a population at high risk of renal function decline, we examined the relationship between serum concentrations of TNFR1 and TNFR2 and progression to ESRD in American Indians with type 2 diabetes.

RESULTS

The study included 193 subjects with type 2 diabetes, followed for a median of 9.5 years (interquartile range 7.1–11.6 years). During follow-up, 62 (32%) of the participants developed ESRD and 25 (13%) died from natural causes other than diabetic kidney disease without progressing to ESRD. Baseline characteristics of the cohort are summarized in Table 1. At baseline, 127 subjects (65.8%) were receiving glucose lowering medicines and 12 (6.2%) were receiving antihypertensive medicines. 61 participants (32%) had normal ACR, 72 (37%) had moderate albuminuria, and 60 (31%) had severe albuminuria; mGFR was ≥60 ml/min in 171 participants (89%). The frequency distributions of serum concentrations of TNFR1 and TNFR2 at baseline are shown in Figure 1. Median serum concentrations of the receptors were 2833 pg/ml and 4835 pg/ml, respectively, and both distributions were skewed.

Serum concentrations of TNFR1 and TNFR2 correlated strongly with each other (r=0.78) and moderately with mGFR, cystatin C, ACR, age, MAP, and duration of diabetes; they did not correlate significantly with HbA1c, BMI (Table 2). The correlations between the serum concentrations of the receptors and ACR are shown in Figure 2 (r=0.37, $P<0.001$ for TNFR1, r=0.42, $P<0.001$ for TNFR2).

Unadjusted and age-sex-adjusted incidence rates of ESRD are shown in Table 3 by quartiles of TNFR1 and TNFR2. Compared with the lowest quartile of TNFR1, the age-sex-adjusted incidence rate of ESRD was significantly higher among subjects in the third and fourth quartiles of TNFR1 (IRR for third quartile =2.4, 95% CI 1.01–5.7; IRR for fourth quartile =6.6, 95% CI 3.3–13.3). The age-sex-adjusted incidence of ESRD was significantly higher among subjects in the third and fourth quartiles of TNFR2 versus the lowest quartile (IRR for third quartile= 2.8, 95% CI 1.2–6.4; IRR for fourth quartile=8.8, 95% CI 4.3–18.0).

Figure 3 shows the cumulative incidence of ESRD at 10 years of follow-up, when 48 of the 62 cases of ESRD had occurred, according to the level of albuminuria and TNFRs. The highest quartile of each TNFR is compared with the lowest three quartiles combined. Among participants with severe albuminuria, the cumulative incidence of ESRD at 10 years of follow-up was 96.2% in those in the highest TNFR1 quartile at baseline and 44.6% in those in lower TNFR1 quartiles ($p<0.001$). Similarly, for TNFR2 the cumulative incidence of ESRD was 88.7% and 47.3%, respectively ($p<0.001$). Among participants without severe albuminuria, the 10-year cumulative incidence of ESRD was 14.4% and 6.1% in the highest and lower TNFR1 quartiles, respectively (p=0.51), and 26.9% and 4.7% in the highest and lower TNFR2 quartiles, respectively (p=0.049).

In the Cox regression analysis, 26 participants were censored at the time of death (25 deaths due to natural causes and 1 death due to injury of external cause) and 105 were administratively censored at the end of follow-up (December 31, 2013). Unadjusted hazard ratio for ESRD per interquartile range increase of TNFR1 was 2.5 (95% CI 2.1–3.1) and for TNFR2 was 2.5 (95% CI 2.1–3.0). Adjusted for age, sex, HbA1c, MAP, ACR and mGFR, the incidence of ESRD was 1.6 times (95% CI 1.1–2.2) as high per interquartile range increase in the distribution of TNFR1 and 1.7 times (95% CI 1.2–2.3) as high per interquartile range increase in the distribution of TNFR2. The univariate and multivariate models are shown in Table 4.

For the Cox regression model that included baseline clinical covariates alone (i.e., age, sex, HbA1c, MAP, and ACR), the C-index for predicting ESRD was 0.858. The C-index for clinical covariates plus mGFR was 0.880; for clinical covariates plus TNFR1 0.873; and for clinical covariates plus TNFR2 0.879; each of these markers significantly increased the C-index for predicting ESRD ($P<0.001$). After additional adjustment for mGFR, the C-index for predicting ESRD increased from 0.880 to 0.887 for TNFR1 ($P=0.006$) and to 0.888 for TNFR2 ($P=0.002$, Table 5).

DISCUSSION

Circulating TNFR1 and TNFR2 were strongly associated with risk of progression to ESRD in American Indians with type 2 diabetes and mostly preserved kidney function. These associations were present after accounting for the effects of clinically recognized risk factors, including HbA1c, blood pressure, ACR, and mGFR. In Cox regression models adjusting for the traditional clinical covariates, the hazard ratio for ESRD increased nearly 2 times per interquartile range increase in the distribution of TNFR1 or TNFR2. Both receptors enhanced the discrimination of the survival models for ESRD beyond that achievable by the clinically recognized risk factors when examined using the C-index. These findings suggest that either receptor may be used as an early predictor of ESRD.

Our findings confirm the important role of circulating levels of TNFRs as predictors of risk of ESRD as previously reported in 410 predominantly Caucasian subjects with type 2 diabetes participating in the Joslin Kidney Study.[13] There were some differences, however, between the results obtained in these two studies. First, the 10-year cumulative risk of ESRD among participants in the 4th (highest) quartiles of TNFR1 and TNFR2 was higher in Pima Indians than in Caucasians with type 2 diabetes and severe albuminuria (96% and 89% in Pima Indians and 75% and 73% in Caucasians, respectively). This risk was also different in subjects in quartiles 1–3 combined (45% and 47% in Pima Indians and 16% and 20% in Caucasians, respectively). Second, in subjects without severe albuminuria, although the 10-year cumulative risk of ESRD according to quartiles of serum TNFRs was very similar in both populations and was higher in the 4th quartile than in quartiles 1–3 combined, at 12-years follow-up the cumulative risk in the Pima Indians converged for the highest and lower quartiles of TNFR1.

The absolute concentrations of circulating TNFRs in the Pima Indians were almost twice as high as those in the Joslin Kidney Study participants. Previous studies show that obesity is associated with macrophage accumulation and increased TNF mRNA expression in adipose tissue[15,16] and severely obese individuals (BMI~40kg/m^2) have much higher TNFR concentrations than those who are lean.[17] These observations suggest that a higher degree of obesity among the Pima Indians may be responsible for their higher TNFR concentrations, although other factors might also be involved. Serum concentrations of TNFRs were not correlated with BMI or HbA1c, because these variables were narrowly distributed, with most participants having high BMI and high HbA1c. The differences in the distribution of circulating TNFRs in Pima Indians and Caucasians indicate that the risk of ESRD is not associated with a specific threshold of circulating TNFRs that is consistent across populations. Factors unrelated to risk of kidney disease may also influence the concentrations of circulating TNFRs, suggesting that population-specific risk assessment may be needed to identify subjects at high or low risk of ESRD in type 2 diabetes. Such a requirement could diminish the clinical value of these measures as biomarkers of diabetic kidney disease.

Circulating TNFR1 and TNFR2 were highly correlated with each other in the present study, as in the Joslin Study. Moreover, the associations of both receptors with diabetic kidney disease progression were equivalent. Similarities in the generation of the soluble forms of

these receptors may explain the tight correlation. It remains to be elucidated whether alterations of the soluble form generation may be responsible for increase in TNFRs in circulation and whether this increase contributes to kidney injury. The 55-kDa TNFR1 and 75-kDa TNFR2 are cell membrane bound receptors involved in key aspects of the immune response. TNFR1 and TNFR2 are released into the extracellular space via inducible cleavage of TNFR ectodomain by ADAM17.[18] An additional mechanism of the soluble form generation, the constitutive release of TNFR1 within exosome-like vesicles, was described by Levine et al. [19] The presence of circulating TNFR1 in the exosomal fraction was confirmed in the Joslin Kidney Study subjects with diabetic kidney disease, and the presence of TNFR2 in the exosomes and correlation of exosomal TNFRs with their respective protein expression in leukocytes was also demonstrated.[14,20] Exposure of kidney organ culture to TNFRs increases tubular apoptosis,[21] and development of fibrosis is delayed in TNFR-deficient murine models of tubulointerstitial injury.[22] Nevertheless, a particular role of TNFRs in diabetic kidney disease has not yet been established, but the strong association between circulating TNFRs and risk of ESRD argue for development of a diagnostic test to identify subjects at risk of ESRD in diabetes.

Since Hasegawa et al. first demonstrated the role of TNFα pathway in the experimental model of diabetic nephropathy,[1] a number of reports have pointed to potential involvement of the TNFα pathway in diabetic kidney disease.[23,24] Experimental studies demonstrated that TNFα-mediated mechanisms may result in vasoconstriction leading to GFR decline, in the disruption of the glomerular barrier resulting in increased permeability to albumin, and in the recruitment of inflammatory cells into the kidney.[25] TNFα is assumed to mediate these actions via its two TNF receptors; nevertheless most of those experimental studies did not investigate in greater detail whether TNFRs mediated actually those biological effects of TNFα. In humans, TNFα level is associated with diabetic nephropathy,[25,26] but these associations are weaker than for TNFRs.[12–14]

We are uncertain whether TNFRs are associated with progression to ESRD in a non-diabetic population. Circulating TNFRs were previously shown to associate with renal function and albuminuria in subjects without diabetes, but those studies were mainly cross-sectional or focused on more advanced stages of chronic kidney disease.[27–30] In addition, TNFR1 and TNFR2 were also implicated in the development of specific non-diabetic kidney diseases such as kidney allograft rejection, immune-complex mediated glomerulonephritis, lupus nephritis, hepatitis C virus-associated glomerulonephritis, obstructive renal injury, and ANCA-associated vasculitis.[31–36] Whether TNFRs are also implicated in the progression of these kidney diseases to ESRD, however, is not known.

Strengths of the study include measurement of GFR to account for differences in baseline kidney function. In addition, the study has excellent follow-up and was conducted in a population with a high baseline GFR. Indeed, 67 (34.7 %) subjects had hyperfiltration, defined by an mGFR ≥154 ml/min, a value two standard deviations above the mean mGFR for Pima Indians with normal glucose tolerance. Limitations include the small study size, and the arbitrary distribution of circulating TNFRs into quartiles, with the most significant differences in risk of ESRD observed between the 4th quartile and quartiles 1–3 combined. This dose-response relationship needs to be investigated further to identify the best

diagnostic criteria to predict risk of ESRD. The potential impact of TNFα on ESRD was not explored in this study due to insufficient serum sample volume for measuring free and total TNFα, and the effect of TNFRs on cardiovascular mortality was not evaluated due to the small number of cardiovascular deaths in this study population. Moderate associations of circulating TNFRs with cardiovascular mortality (but weaker than for progression to ESRD) were suggested elsewhere.[13,37]

In conclusion, elevated serum concentrations of TNFR1 or TNFR2 are associated with an increased risk of ESRD in American Indians with type 2 diabetes after accounting for traditional risk factors including ACR and GFR. Absolute concentrations of these receptors in the serum are substantially higher than in a Caucasian type 2 diabetes population, suggesting that population-specific risk assessment may be needed to identify subjects with type 2 diabetes who are at high or low risk of ESRD.

MATERIALS AND METHODS

Study participants

Between 1965 and 2007, American Indians from the Gila River Indian Community participated in a longitudinal study of diabetes and its complications. Each member of this community who was at least 5 years old was invited to have a research examination approximately every 2 years. Diabetes was diagnosed by a 2-hour post-load plasma glucose concentration ≥200 mg/dl (11.1 mmol/l) at these biennial examinations, or when the diagnosis was documented in the medical record. For the present study, we selected participants from this longitudinal population-based study who had type 2 diabetes and also participated in longitudinal studies of kidney function that included measurements of glomerular filtration rate (mGFR) by the urinary clearance of iothalamate.[38,39]

Laboratory measurements

All urine and serum samples were stored at −80°C until assay. Urinary albumin was measured by nephelometric immunoassay, and concentrations below the threshold detected by the assay (6.8 mg/l) were set to this value in the analyses. Urinary albumin excretion was estimated by computing the urinary albumin-to-creatinine ratio (ACR) in units of mg/g. ACR was considered normal if <30 mg/g, moderate if ≥30 mg/g but <300 mg/g, and severe if ≥300 mg/g.[40]

Urinary clearance of non-radioactive iothalamate was estimated by the average of four timed urine collections, bracketed by the collection of blood samples, made at 20-min intervals after a water load and a 60-minute equilibration period. A high performance liquid chromatography system with a sensitive ultraviolet light detector was used to assay iothalamate at 236 nm (Instrumentation Shimadzu #6A, www.shimadzu.com).[41] Serum levels of TNFRs were measured in samples collected from the eligible participants between July 1989 and December 2001. Measurements were performed by ELISA in Dr. A. Krolewski's laboratory, Joslin Diabetes Center, Boston, MA, according to the same protocol used in the Joslin Kidney Study.[13] Intra-assay coefficient of variation (CV) for mGFR was 1.1%, and for TNFR1 and TNFR2 were <5%; the inter-assay CVs were 2.9%, 16%, and 5%,

respectively. Reproducibility of the TNFR assays was assessed by intra-class correlation of measurements from 21 duplicate samples blinded to the performance laboratory. The intra-class correlation for TNFR1 was 0.80 and for TNFR2 was 0.97, reflecting good agreement.

Body mass index (BMI) was defined as weight divided by the square of height (kg/m^2). Mean arterial pressure was calculated as MAP = 2/3 diastolic arterial pressure + 1/3 systolic arterial pressure.

The study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. Each subject gave informed consent at each renal clearance study.

Statistical analyses

Baseline clinical and demographic features are presented as medians (interquartile range). Participants were followed from their first examination with TNFR measurement until December 31, 2013, onset of ESRD, or death, whichever came first. ESRD was defined as initiation of dialysis, kidney transplant, or death from diabetic kidney disease if dialysis or transplantation was refused. Cause of kidney disease was determined by review of medical records and review of available biopsy findings. The concentrations of TNFR1 and TNFR2 were divided into quartiles for the incidence-density and Kaplan-Meier analyses, with the divisions occurring at the 25^{th}, 50^{th}, and 75^{th} percentiles. The incidence rate of diabetic ESRD was computed as the number of new cases of ESRD per 1,000 person-years (pyrs) at risk according to these quartiles.[42] Age- and sex-adjusted incidence rate ratios (IRR) relative to the lowest quartile of each TNFR were computed by an incidence-density adaptation of Mantel-Haenszel stratification which stratifies events and person-years in a time-dependent fashion according to decades of age. This method is robust to sparse data within strata. When the values for age changed during follow-up, person-years for each subject were apportioned to the appropriate new strata. Tests for general association were computed by the Mantel-Haenszel test[43] adapted for person-year denominators[44] and for linear association by the Mantel extension test.[45] The trend test for unadjusted incidence rates across quartiles of TNFRs is based on a weighted regression analysis that changes estimates across the strata.[46] Relationships between baseline characteristics and measures of TNFRs were examined by Spearman's correlations.

Unadjusted cumulative incidence of ESRD as a function of follow-up time, stratified by quartiles of TNFR1 and TNFR2 and by the level of ACR, was estimated by the Kaplan-Meier product-limit method. Differences in cumulative incidence were assessed by the log-rank test. Cox regression analysis was used to estimate the hazard ratio for development of ESRD associated with an interquartile range increase in the distribution of each TNFR after adjusting for known risk factors for ESRD, including baseline age, sex, MAP, HbA1c, ACR, and mGFR. Additional adjustment of the Cox models for diabetes duration, BMI, anti-hypertensive treatment, and glucose-lowering treatment did not change the conclusions of the study, and these variables were therefore not included in the final model. The models assessed the risk of outcome for the difference between the upper 75^{th} and lower 25^{th} percentile as unit of change in the continuous distribution of each TNFR. Adequacy of the fit of each model to individual observations was assessed by inspection of deviance

residuals. Product terms of predictor variables did not significantly improve the regression models and were not included.

C-indexes and the differences in C-indexes were calculated for each predictive model and the 95% CI for the difference in C-indexes was computed based on 1,000 bootstrap samples. Hypothesis testing for the difference between C-indexes was performed by likelihood ratio tests.[47–48] Calculations were performed using SAS software version 9.3 (SAS Institute, Cary, NC). All analyses used only baseline measurements because our primary interest was the clinically-relevant predictive value of TNFRs at a single time point for subsequent development of ESRD.

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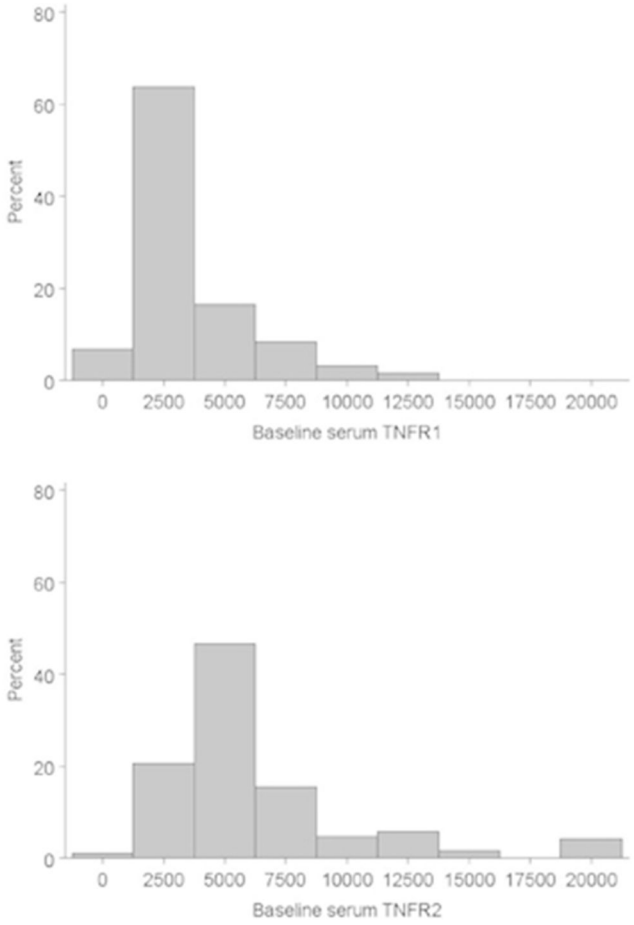

Figure 1.

Frequency distributions of the baseline serum levels of TNFR1 and TNFR2 in Pima Indians with type 2 diabetes.

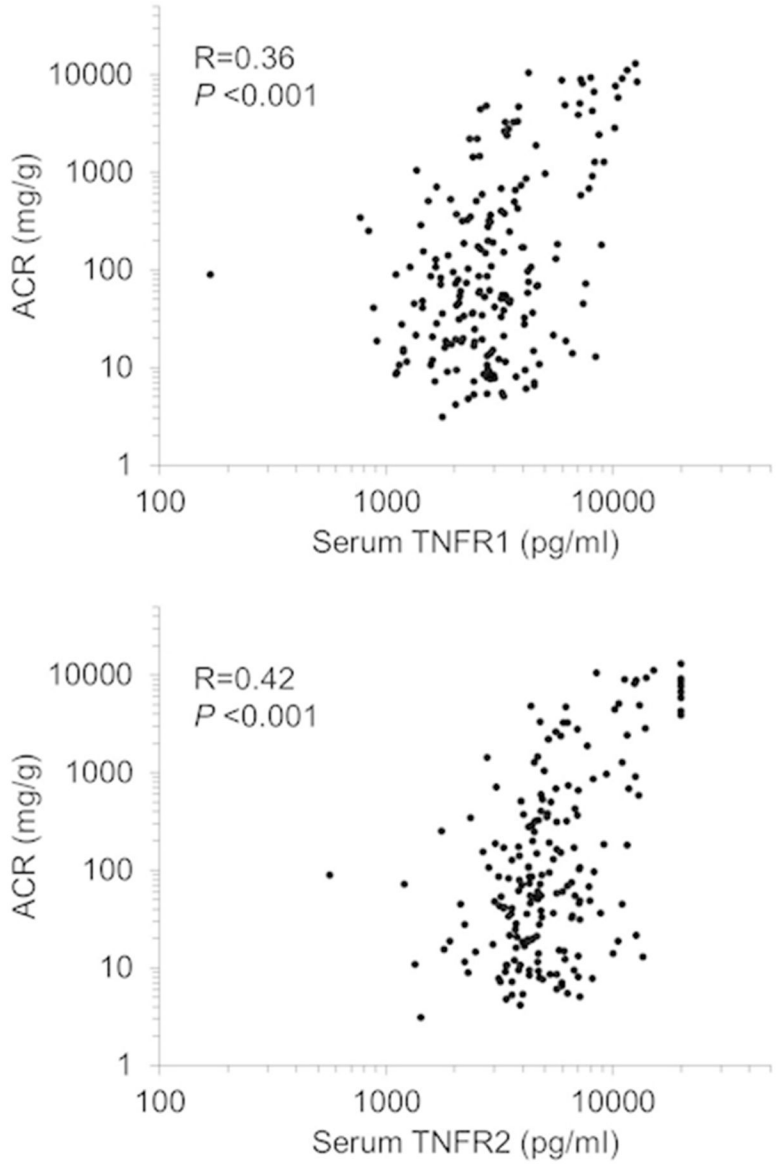

Figure 2.

Relationships of serum concentrations of TNFR1 and TNFR2 with ACR at baseline on logarithmic scales. Spearman's correlations and their corresponding *P*-values are shown on the figure.

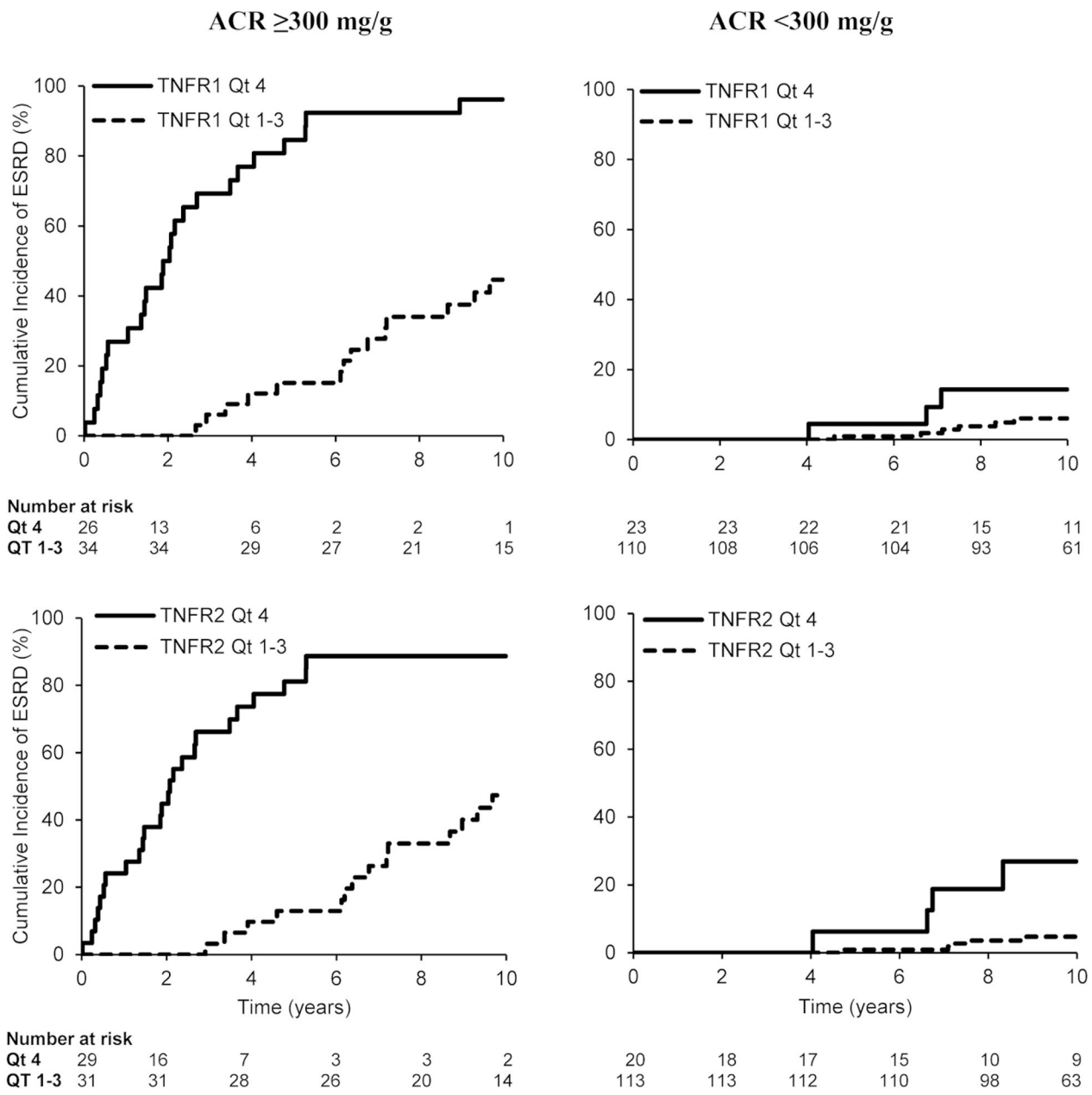

Figure 3.

Cumulative incidence of diabetic end-stage renal disease during 10 years of follow-up, when 48 of the 62 events occurred, according to quartiles of TNFR1 and TNFR2 at baseline and albuminuria status. Cut-points for the 25th, 50th, and 75th percentiles of TNFRs distributions are presented in Table 3. Numbers of participants at risk at the end of each 2-year interval are indicated along the x-axes. ACR=albumin/creatinine ratio, Qt=quartile, TNFR=tumor necrosis factor receptor.

Table 1

Baseline characteristics of Pima Indians with type 2 diabetes.

Baseline characteristic	Median (interquartile range)
n (% male)	193 (29)
Age (years)	46 (39–53)
Diabetes duration (years)	14 (11–19)
BMI (kg/m^2)	33 (29–39)
HbA1c (%)*	9.6 (7.7–11.1)
MAP (mmHg)	93 (87–99)
mGFR (ml/min)	133 (100–171)
mGFR (ml/min/1.73 m^2)	120 (88–149)
Cystatin C (mg/l)	0.97 (0.87–1.10)
Serum creatinine (μmol/l)	57 (48–74)
ACR (mg/g)	72 (19–493)
TNFR1 (pg/ml)	2833 (2081–4092)
TNFR2 (pg/ml)	4835 (3875–6997)
Glucose-lowering treatment (%)	66
Hypertension treatment (%)	6

*HbA1c in IFCC units (mmol/mol)=81.4 (60.7–97.8).

TNFR=tumor necrosis factor receptor, mGFR=iothalamate glomerular filtration rate, ACR=urinary albumin-to-creatinine ratio, BMI=body mass index, MAP=mean arterial pressure. Cystatin C values are IFCC standardized. Serum creatinine values are IDMS standardized.

Table 2

Spearman's correlations for comparisons between TNFRs and baseline measurements. For each comparison, the *P*-values are shown below the correlation coefficients.

	TNFR1	TNFR2	Age	DM Duration	mGFR	Cystatin C	ACR	HbA1c	BMI	MAP
TNFR1	**1**	0.78	0.29	0.27	−0.48	0.52	0.36	−0.13	0.06	0.27
		<.001	<.001	0.001	<.001	<.001	<.001	0.08	0.41	0.0002
TNFR2	0.78	**1**	0.33	0.29	−0.49	0.63	0.42	−0.11	0.05	0.33
	<.001		<.001	<.001	<.001	<.001	<.001	0.15	0.48	<.001

TNFR=Tumor necrosis factor receptor, DM=diabetes, mGFR= measured glomerular filtration rate, ACR=urinary albumin-to-creatinine ratio, BMI= body mass index, MAP=mean arterial pressure.

Table 3

Unadjusted, and age-sex-adjusted incidence rate ratio of end-stage renal disease (ESRD) by quartiles of TNFR1 and TNFR2 distribution. TNFR=tumor necrosis factor receptor.

	Quartile1		Quartile2		Quartile3		Quartile4	
	Unadjusted incidence of ESRD (cases/100 pyrs)**							
	Cases/pyrs	**Rate**	**Cases/pyrs**	**Rate**	**Cases/pyrs**	**Rate**	**Cases/pyrs**	**Rate**
TNFR1*	8/5754	1.4	10/555	1.8	16/479	3.3	28/287	9.8
TNFR2*	7/590	1.2	9/549	1.6	17/500	3.4	29/257	11.3
	Age-sex-adjusted incidence rate ratio for ESRD (95% CI)							
TNFR1*	Ref	1.2 (0.5, 3.1)	2.4 (1.01, 5.7)		6.6 (3.3, 13.3)			
TNFR2*	Ref	1.3 (0.5, 3.3)	2.8 (1.2, 6.4)		8.8 (4.3, 18.0)			

*Quartile boundaries for TNFR1 are: 25th percentile - 2081pg/ml, 50th percentile - 2833pg/ml and 75th percentile – 4092pg/ml and for TNFR2: 25th percentile - 3875pg/ml, 50th percentile - 4835pg/ml and 75th percentile – 6997pg/ml, respectively.

**P <0.001 for trend test of incidence rates.

Table 4

Univariate and multivariate adjusted Cox proportional hazard models (HR and 95% CI) for the risk of ESRD associated with TNFR1 and TNFR2 in Pima Indians with type 2 diabetes. The unit of change is the difference between the 75th and 25th percentiles in the distribution of each TNFR as continuous variable.

Baseline variables	Univariate	Multivariate	
		TNFR1	TNFR2
Age (per 5 years)	1.1 (1.0, 1.2)	0.8 (0.7, 0.98)	0.9 (0.7, 0.99)
Sex (females=0, males=1)	1.7 (1.03, 2.9)	1.6 (0.9, 3.0)	1.7 (0.9, 3.1)
MAP (per 5 mmHg)	1.3 (1.2, 1.5)	1.03 (0.9, 1.2)	1.0 (0.9, 1.2)
HbA1c (per 1%)	1.1 (1.004, 1.3)	1.2 (1.03, 1.4)	1.2 (1.04, 1.4)
Log2 (ACR)	1.8 (1.6, 2.0)	1.5 (1.3, 1.7)	1.5 (1.3, 1.7)
Log2 (mGFR)	4.0 (3.0, 5.3)	2.3 (1.5, 3.6)	2.1 (1.4, 3.3)
TNFR1 (per IQR)	2.5 (2.1, 3.1)	1.6 (1.1, 2.2)	-
TNFR2 (per IQR)	2.5 (2.1, 3.0)	-	1.7 (1.2, 2.3)

HR = hazard ratio, CI= confidence intervals, IQR = interquartile range.

* ACR and mGFR are expressed as the logarithm base 2 (log2) to reflect the association with ESRD corresponding to a two-fold increase in ACR and decrease in mGFR, respectively. Effect measures are expressed as the HRs for an increase per specified unit in the distribution of each covariate except for mGFR.

Table 5

C-indices, differences in C-indices, and *P*-values for the likelihood ratio tests for the differences in the Cox proportional hazards models with and without the biomarker information.

	C-index			
Biomarker	**Covariates only***	**Biomarker + covariates**	**Difference in C-index (95% CI)**	**Likelihood Ratio *P*-value**
mGFR	0.858	0.880	0.021 (0.002, 0.055)	<0.001
TNFR1	0.858	0.873	0.015 (0.0001, 0.042)	<0.001
TNFR2	0.858	0.879	0.021 (0.002, 0.052)	<0.001
	Covariates+mGFR			
TNFR1	0.880	0.887	0.007 (-0.002, 0.022)	0.006
TNFR2	0.880	0.888	0.009 (-0.002, 0.029)	0.002

*Including age, sex, HbA1c, MAP, and ACR