Synergism Between Circulating Tumor Necrosis Factor Receptor 2 and HbA_{1c} in Determining Renal Decline During 5–18 Years of Follow-up in Patients With Type 1 Diabetes and Proteinuria

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OBJECTIVE

We studied the serum concentration of tumor necrosis factor receptor 2 (TNFR2) and the rate of renal decline, a measure of the intensity of the disease process leading to end-stage renal disease (ESRD).

RESEARCH DESIGN AND METHODS

A cohort of 349 type 1 diabetic patients with proteinuria was followed for 5–18 years. Serum TNFR2, glycated hemoglobin A_{1c} (HbA_{1c}), and other characteristics were measured at enrollment. We used a novel analytic approach, a joint longitudinal-survival model, fitted to serial estimates of glomerular filtration rate (eGFR) based on serum creatinine (median seven per patient) and time to onset of ESRD (112 patients) to estimate the rate of renal decline (eGFR loss).

RESULTS

At enrollment, all patients had chronic kidney disease stage 1–3. The mean (±SD) rate of eGFR loss during 5–18 years of follow-up was −5.2 (±4.9) mL/min/1.73 m^2/year. Serum TNFR2 was the strongest determinant of renal decline and ESRD risk (C-index 0.79). The rate of eGFR loss became steeper with rising concentration of TNFR2, and elevated HbA_{1c} augmented the strength of this association (P = 0.030 for interaction). In patients with HbA_{1c} ≥10.1% (87 mmol/mol), the difference in the rate of eGFR loss between the first and fourth quartiles of TNFR2 was 5.4 mL/min/1.73 m^2/year, whereas it was only 1.9 in those with HbA_{1c} <7.9% (63 mmol/mol).

CONCLUSIONS

Circulating TNFR2 is a major determinant of renal decline in patients with type 1 diabetes and proteinuria. Elevated HbA_{1c} magnifies its effect. Although the mechanisms of this synergism are unknown, our findings allow us to stratify patients according to risk of ESRD.

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We recently reported that concentrations of circulating tumor necrosis factor receptors 1 and 2 (TNFR1 and TNFR2) are strong predictors of future progression to chronic kidney disease (CKD) ≥3 in patients with type 1 diabetes and microalbuminuria (1). These biomarkers also predict the onset of end-stage renal disease (ESRD) in patients with type 2 diabetes (2). Their effects are equivalent and are independent of traditional clinical characteristics measured at the beginning of follow-up such as estimated glomerular filtration rate (eGFR), urinary albumin/creatinine ratio (ACR), and glycated hemoglobin A_{1c} (HbA_{1c}). Here we seek to examine the association of the concentration of circulating TNFR2 with risk of ESRD in a different study group, namely patients with type 1 diabetes and persistent proteinuria, part of a previously described Joslin Proteinuria Cohort (3). In contrast to the previous studies where threshold-based outcomes such as CKD ≥3 or ESRD were used, in this study we used rate of renal decline as the quantitative measure of intensity of disease process leading to ESRD (4). Steeper rate of renal decline results in shorter time to onset of ESRD. This quantitative approach helps us to overcome problems associated with variable renal function at entry and variable duration of follow-up in our cohort. Furthermore, it is suitable for exploration of TNFR2 interactions with other risk factors.

ESRD develops in ~40% of type 1 diabetic patients with proteinuria after 15 years of follow-up (3). As we recently demonstrated, the process of renal function loss leading to ESRD is approximately linear and can be expressed as a constant rate of renal decline, or eGFR slope (4). Thus, if the slope is known, we can estimate the time to ESRD, conditionally on the level of renal function at the beginning of the follow-up (4). In this study of the association between circulating TNFR2 and the rate of renal decline, we used serial creatinine–based eGFR obtained during follow-up together with information about time of onset of ESRD. These two types of information (longitudinal eGFR data and time to ESRD) were combined in a joint longitudinal-survival model to estimate the rate of renal decline (eGFR loss) and time to ESRD, taking into account variable eGFR at entry, variable duration of follow-up, and variable number of eGFR estimates during follow-up (5–8).

RESEARCH DESIGN AND METHODS

Study Group

The study group has been described previously (3). In brief, the Joslin Proteinuria Cohort (n = 423) was ascertained between 1991 and 2004 in the population of adult type 1 diabetic patients receiving long-term care at the Joslin Clinic, Boston, MA (~3,500 adult patients) (3). All patients were Caucasian with urinary albumin excretion within the proteinuria range in at least two out of the three consecutive determinations of the ACR performed at the Joslin Clinic laboratory during a 2-year interval preceding enrollment into the study (3,4). Informed consent procedures and protocols for examinations were approved by the Joslin institutional review board, as were methods for ascertaining dates of onset of ESRD and death. Descriptions of baseline and follow-up examinations, definitions of ESRD, time of its onset, and natural history of ESRD in this cohort have been reported previously (3,4). Out of 423 patients enrolled in the Joslin Proteinuria Cohort, 349 patients were in CKD stage 1–3 at enrollment. These comprise the current study group, which was followed through 2009.

Longitudinal Observation of Renal Decline

In addition to collecting and storing baseline and follow-up serum samples, we retrieved 4,097 serum creatinine measurements performed in the Joslin Clinic laboratory for routine visits during follow-up. Throughout the period of study, they were assayed by Jaffe's modified picrate method on a Ciba Corning Express Plus Chemistry Analyzer (Medfield, MA). For 1,113 of these measurements (27%), serum obtained from the same blood draw as the sample submitted for assay in the Joslin Clinic laboratory was also submitted to the Advanced Research and Diagnostic Laboratory at the University of Minnesota, where creatinine was measured with the Roche enzymatic assay (product 11775685) Q8 on a Roche/Hitache Mod P analyzer (Indianapolis, IN), calibrated to be traceable to an isotope dilution mass spectrometry reference assay. These duplicate measurements were used to develop a conversion formula to calibrate the other Joslin Clinic laboratory results to the isotope dilution mass spectrometry traceable method, as previously described (4). eGFR was estimated using calibrated serum creatinine measurements and the CKD-EPI formula (9).

Biomarker Assays

Biomarkers were measured in serum samples collected at the enrollment into the study and stored at −80°C. TNFR2 was measured with an ELISA assay (catalog no. DRT200; R&D Systems, Minneapolis, MN) as described earlier (2). In our previous study, we found that storage time did not affect TNFR2 concentration (10). Because serum concentrations of TNFR1 and TNFR2 are highly correlated and provide redundant information regarding prediction of CKD 3 or ESRD, only the latter was measured in the current study. Urinary albumin concentration was measured at the Joslin Clinic laboratory by immunonephelometry on a BN100 instrument with N Albumin kits (Behring, Somerville, NJ) and urinary creatinine concentration with Jaffe's modified picrate method on a Ciba Corning Express Plus Chemistry Analyzer, as previously described (4). HbA_{1c} was measured at the Joslin Clinic laboratory in the 1990s with Bio-Rad HPLC analyzer (Bio-Rad Laboratories, Hercules, CA) and in 2001–2004 with Tosoh 2+2 HPLC analyzer (Tosoh Bioscience, South San Francisco, CA). Both methods were standardized to Diabetes Control and Complications Trial (DCCT) and were consistent throughout the study period.

Statistical Analysis

First, we studied the association of serum TNFR2 with traditional clinical risk factors and eGFR at study entry. We used Spearman correlation or Wilcoxon test for continuous and categorical variables, respectively. We also estimated cumulative incidence of ESRD during follow-up in quartiles of TNFR2 and tested differences between quartiles with a log-rank test. For descriptive purpose, we estimated renal function change as regression slope fitted to each patient's longitudinal eGFR data and expressed it in mL/min/1.73 m^2/year.

Second, for measuring the strength of a covariate's association with renal function changes during follow-up, we applied a joint longitudinal-survival model (described in detail in the Supplementary

Data). In brief, this model overcomes the bias that would be present in overall estimates of the slopes in the cohort due to the truncated follow-up observations from patients with rapid eGFR loss (11). It uses both the longitudinal eGFR data and observed times to ESRD for the whole study group to obtain unbiased estimates of individual rates of renal decline (eGFR loss) (5–8,11). This is a novel approach to estimate the impact of a covariate (such as TNFR2) on the rate of eGFR loss, the most important manifestation of the underlying disease process leading to ESRD. The approach also imputes a value of eGFR at zero follow-up time (intercept) and a time to ESRD (whether observed or not). The method is an extension of mixed-effects models and involves two levels of data. First, longitudinal eGFR data and observed times of onset of ESRD serve to estimate patient-specific regression parameters. On the second level, statistical inferences are drawn about the magnitudes of the covariate associations with the rate of eGFR loss. To minimize confusion between the least squares and joint model parameters, we refer to the least squares regression estimate as a "slope of eGFR" and joint model estimate as a "rate of renal decline." Similarly, we refer to the observed eGFR at entry to the study as "entry eGFR" and refer to the joint model intercept as "imputed baseline eGFR."

To assess performance of TNFR2 and other covariates in predicting time to ESRD, we calculated C-index (12) using a score derived from the log times to event imputed by the joint model ($X\beta$) with a SAS macro developed in the Mayo Clinic (13). To test interactions between TNFR2 and another risk factor, we used the joint model to compare the association of TNFR2 with renal outcomes across strata formed by the quartiles of the interacting variable using a likelihood ratio test.

Statistical significance was set at a P value <0.05. Analyses were performed in SAS for Windows, version 9.3 (SAS Institute, Cary, NC).

RESULTS

Clinical Characteristics of the Study Group

This study includes 349 patients with proteinuria and CKD stage 1–3 who were enrolled into the Joslin Proteinuria Cohort (3). Their characteristics at entry are summarized in Table 1. Median age was 38 years, diabetes duration 24 years, and BMI 25 kg/m^2. By design, their urinary albumin excretion was within the proteinuric range, and median entry eGFR was 81 mL/min/1.73 m^2 (55, 104). Systolic and diastolic blood pressures were close to their therapeutic target (medians 130 and 78 mmHg, respectively). ACE inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) were already prescribed for 69% of them. This proportion is somewhat lower than is currently the case but reflects the fact that use of these medications was not widespread in the early 1990s when a large proportion of the study cohort entered follow-up. Glycemic control was predominantly poor; median HbA_{1c} was 8.9% (74 mmol/mol). The median of serum concentration of TNFR2 (25th and 75th percentiles) was 4,415 pg/mL (3,497, 5,777) at entry.

Renal function changes during 5–18 years of follow-up were evaluated with the 4,097 creatinine determinations (median seven per patient) and assigned eGFR = 10 mL/min/1.73 m^2 at onset of ESRD in 111 patients. Using least square regression mean ($\pm$SD), eGFR slope was -5.9 (± 8.2) mL/min/1.73 m^2/year. Using the joint modeling, mean rate of renal decline was -5.2 (± 4.9) mL/min/1.73 m^2/year. The incidence rate of ESRD was 3.9 per 100 person-years, and mortality rate unrelated to ESRD was 0.9 per 100 person-years.

Association of Serum Levels of TNFR2 With Clinical Characteristics and Risk of ESRD

Neither HbA_{1c} nor blood pressure varied with increasing concentration of TNFR2; Spearman correlation coefficients were -0.01 ($P = 0.87$) and 0.10 ($P = 0.06$), respectively. The TNFR2 concentration was significantly ($P = 0.006$) lower in the patients without renoprotective treatment (medians: 3,883 vs. 4,574 pg/mL) for reasons that are obscure because their physicians had no knowledge of their low TNFR2. ACR at entry increased ($r = 0.36$, $P < 0.001$) whereas entry eGFR decreased ($r = -0.70$, $P < 0.001$) with increasing concentration of TNFR2.

Serum TNFR2 was strongly associated with risk of ESRD. Cumulative incidence of ESRD in quartiles of serum TNFR2 concentration is depicted in Fig. 1. After 12 years of follow-up, the risk was 14,

Table 1—Clinical characteristics of the 349 patients in the Joslin Proteinuria Cohort in CKD stages 1–3 at the study entry

Baseline characteristics	
Female	45.0 (157)
Age (years)	38 (32, 43)
Diabetes duration (years)	24 (19, 31)
BMI (kg/m^2)	25.1 (22.7, 28.6)
Systolic blood pressure (mmHg)	130 (120, 142)
Diastolic blood pressure (mmHg)	78 (70, 84)
Total cholesterol (mg/dL)	5.3 (4.6, 6.2)
Smoking	23.8 (83)
Renoprotective treatment at baseline	68.8 (240)
Serum creatinine (mg/dL)	1.06 (0.82, 1.38)
eGFR at entry (mL/min/1.73 m^2)	81 (55, 104)
ACR (mg/g)	771 (471, 1,377)
HbA_{1c} (%; mmol/mol)	8.9 (7.9, 10.1); 74 (63, 87)
Serum TNFR2 (pg/mL)	4,415 (3,497, 5,777)
Follow-up characteristics	
Length of follow-up (years)	7.0 (5.2, 11.2)
Creatinine determinations per person (n)	7 (3, 17)
eGFR slope (mean $\pm$ SD, in mL/min/1.73 m^2/year)	-5.9 ± 8.2
Rate of eGFR loss (mean $\pm$ SD, in mL/min/1.73 m^2/year)	-5.2 ± 4.9
Incidence rate of ESRD*	3.9 (111)
Mortality unrelated to ESRD*	0.9 (25)

Data are median (25th, 75th percentile) or percent (n) unless otherwise indicated. *Data are incidence rate per 100 person-years (number of events).

30, 35, and 88% in the first through fourth quartiles, respectively ($P < 0.001$).

Univariate Joint Longitudinal-Survival Analysis of Serum Level of TNFR2 and Other Covariates on Rate of Renal Decline

Subsequently, we used a joint longitudinal-survival model to examine the associations of clinical covariates at entry with the rate of eGFR loss and with the imputed baseline eGFR and time to ESRD (Table 2). In this analysis, serum TNFR2 was transformed in percentile ranks and scaled to one quartile units, as in our previous publications (1,2), and ACR was log transformed, such that 1 unit change corresponds to doubling of its value. The remaining variables were modeled on natural scale.

In univariate analysis, serum concentration of TNFR2 had a strong impact on the rate of renal decline. A one quartile increase in TNFR2 was associated with a 1.3 mL/min/1.73 m^2/year steeper rate of eGFR loss and a 17.4 mL/min/1.73 m^2 lower imputed baseline eGFR ($P <$ 0.001 for each). Together, the lower baseline and steeper rate of eGFR loss shortened the imputed time to ESRD by 38.4% ($P < 0.001$). Doubling of ACR was associated with a 1.7 mL/min/1.73 m^2/year steeper rate of eGFR loss and a 6.9 mL/min/1.73 m^2 lower imputed baseline eGFR ($P < 0.001$ for each). Together, the lower baseline and steeper rate of eGFR loss shortened the imputed time to ESRD by 29.0% ($P < 0.001$). A 1% (11 mmol/mol) increase in HbA_{1c} was associated with a 1.0 mL/min/1.73 m^2/year steeper rate of eGFR loss ($P <$ 0.001) but was not associated with imputed baseline eGFR. The result was a 10.4% shorter imputed time to ESRD ($P < 0.001$).

Several other covariates were significant in univariate analysis but with lesser magnitudes of association. A counterintuitive effect of diabetes duration was observed on eGFR loss. A 10-year increase in duration of diabetes was associated with a 0.8 mL/min/1.73 m^2/year ($P = 0.024$) reduction in the rate of eGFR loss, an 8.6 mL/min/1.73 m^2 lower imputed baseline eGFR ($P < 0.001$), and no reduction in imputed time to ESRD. A 1 mmol/L increase in total serum cholesterol level was associated with a 1.0 mL/min/1.73 m^2 faster rate of eGFR loss ($P < 0.001$), no change in imputed baseline eGFR, and 8.1% reduction in imputed time to ESRD ($P =$ 0.026). Diastolic (but not systolic) blood pressure was also associated with a slightly steeper rate of eGFR loss ($P =$ 0.014) but not with the imputed baseline eGFR or time to ESRD. Renoprotective treatment was associated with diminished rate of eGFR loss ($P =$ 0.021) but with significantly lower imputed baseline eGFR ($P < 0.001$). These associations did not change the imputed time to ESRD. Other covariates measured at enrollment such as sex, age, BMI, and smoking did not have a significant impact on the rate of renal decline during follow-up (data not shown).

To help understand the results of the analyses described above, we use a simplified example with TNFR2 dichotomized by its median value. Conceptually, the model is illustrated in Fig. 2. It illustrates relationships between imputed baseline eGFR, rate of renal decline, and imputed time to ESRD. The average rate of eGFR loss in patients below median is 4.1 mL/min/1.73 m^2/year, and imputed baseline eGFR and time to ESRD are 96.4 mL/min/1.73 m^2 and 20.2 years, respectively. In patients with TNFR2 above median, the rate of eGFR loss is steeper (6.2 mL/min/1.73 m^2/year), their imputed baseline eGFR lower (62.3 mL/min/1.73 m^2), and their imputed time to ESRD substantially shorter (8.2 years). The differences in the joint model trajectories for patients above versus below the median TNFR2 are indicated in the figure with thin black lines. They are as follows: rate of eGFR loss steeper by 2.1 mL/min/1.73 m^2/year, imputed baseline eGFR lower by 34.1 mL/min/1.73 m^2 ($P < 0.001$), and imputed time to ESRD shortened

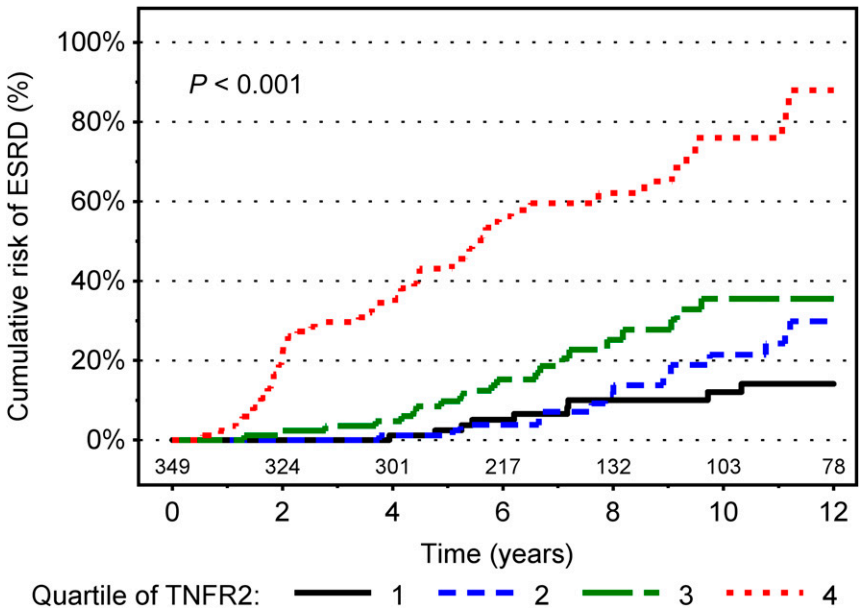

Figure 1—Cumulative incidence of ESRD in quartiles of serum TNFR2 concentration. Numbers of patients at risk are provided inside cumulative risk plot. Quartile boundaries for serum concentration of TNFR2 are provided in Table 1. *P* value is from trend test (log-rank) across quartiles.

Table 2—Estimates from univariate (*top*) and multivariate (*bottom*) joint models of the effects of TNFR2 and clinical characteristics on three components of renal decline: rate of eGFR loss (expressed in mL/min/1.73 m^2/year), eGFR at baseline (mL/min/1.73 m^2), and time to ESRD (percent change of time to ESRD)

	Association with rate of eGFR loss		Association with imputed baseline eGFR		Association with time to ESRD	
Covariates	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
Univariate models						
Serum TNFR2 (one quartile increase)	**−1.3 (−1.8, −0.8)**	**<0.001**	−17.4 (−19.3, −15.5)	<0.001	−38.4% (−42.7, −33.8)	<0.001
ACR (doubling)	**−1.7 (−2.1, −1.2)**	**<0.001**	−6.9 (−9.5, −4.3)	<0.001	−29.0% (−34.6, −22.9)	<0.001
HbA_{1c} (1% or 10.9 mmol/mol increase)	**−1.0 (−1.3, −0.7)**	**<0.001**	0.9 (−0.9, 2.7)	0.32	−10.4% (−15.5, −5.1)	<0.001
Diabetes duration (10 year increase)	**0.8 (0.1, 1.4)**	**0.024**	−8.6 (−12.1, −5.1)	<0.001	−8.8% (−19.2, 3.0)	0.137
Total cholesterol (1 mmol/L increase)	**−1.0 (−1.4, −0.6)**	**<0.001**	0.9 (−1.4, 3.2)	0.44	−8.1% (−14.7, −1.0)	0.026
Diastolic blood pressure (10 mmHg increase)	**−0.7 (−1.3, −0.1)**	**0.014**	1.3 (−1.8, 4.4)	0.40	−4.3% (−13.9, 6.2)	0.40
Renoprotective treatment (yes vs. no)	**1.4 (0.2, 2.6)**	**0.021**	−10.9 (−17.3, −4.4)	0.001	−7.4% (−27.6, 12.8)	0.47
Multivariate model						
Serum TNFR2 (one quartile increase)	**−0.8 (−1.3, −0.4)**	**<0.001**	−17.2 (−19.3, −15.2)	<0.001	−34.6% (−39.3, −29.8)	<0.001
ACR (doubling)	**−1.3 (−1.7, −0.8)**	**<0.001**	−0.4 (−2.5, 1.7)	0.69	−15.0% (−21.1, −8.8)	<0.001
HbA_{1c} (1% or 10.9 mmol/mol increase)	**−1.0 (−1.3, −0.7)**	**<0.001**	1.1 (−0.2, 2.4)	0.090	−9.6% (−13.7, −5.6)	<0.001

Data are point estimates (95% CIs). The primary outcome in the study, the rate of eGFR loss, is in boldface.

by 59.3%, i.e., −0.9 in log(time) ($P < 0.001$). In analyses from Table 2, the predictors are modeled as continuous variables (except renoprotection at baseline).

Multivariate Joint Longitudinal-Survival Analysis of Serum Level of TNFR2 and Other Covariates on Rate of Renal Decline

In multivariate analysis, only three covariates were significantly associated with the rate of renal decline (see lower part of Table 2). The changes of the rate of eGFR loss associated with TNFR2, ACR, and HbA_{1c} were −0.8, −1.3, and −1.0 mL/min/1.73 m^2/year, respectively, and all were significant at $P < 0.001$. TNFR2 was the only one of the three that remained significantly associated with imputed baseline eGFR. The coefficient for its association remained unchanged in comparison with the univariate model (−17.2 mL/min/1.73 m^2, $P < 0.001$). The strong association of TNFR2 with the rate of eGFR loss and imputed baseline eGFR produced the largest change in imputed time to ESRD, a reduction by 34.6% ($P < 0.001$). This was more than twice the magnitude of the associations of ACR and HbA_{1c} with imputed time to ESRD: reductions of 15.0 and 9.6%, respectively ($P < 0.001$ for each).

In addition to the analyses on the determinants of the rate of renal decline described above, we compared the predictive performances of TNFR2, ACR, and HbA_{1c} for discrimination between

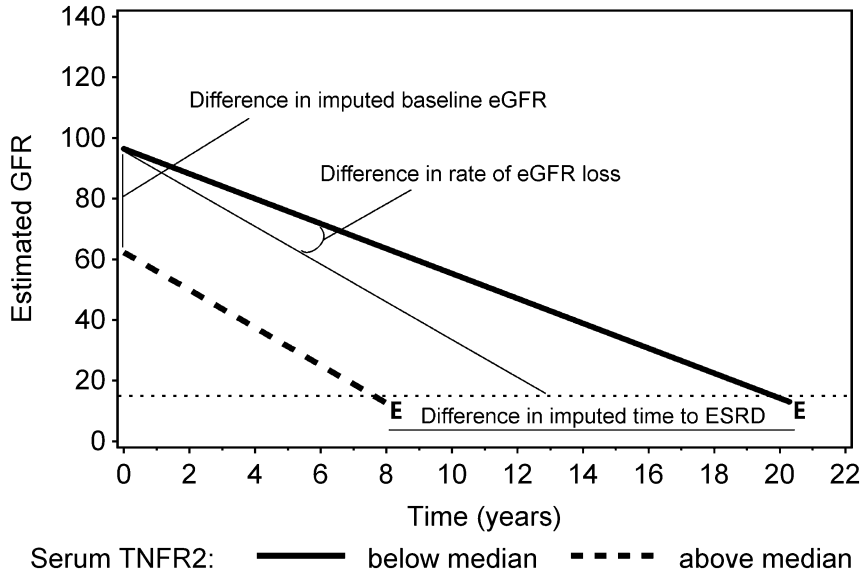

Figure 2—Illustration of joint longitudinal-survival model parameters. Thick lines depict imputed eGFR trajectories, and thin solid lines indicate a covariate's (TNFR2) associations with imputed baseline eGFR, rate of renal decline, and imputed time to ESRD. Thin interrupted line indicates CKD stage 5. E, imputed time of ESRD.

patients who developed and did not develop ESRD during 5–18 years of follow-up. In this analysis, we used the C-index derived from the time to ESRD component of the joint model. The highest discrimination in predicting time to ESRD was provided by the serum concentration of TNFR2; the C-index from a single-variable model was 0.79 (95% CI 0.75, 0.83). The index was 0.72 (0.66, 0.77) for ACR and 0.62 (0.57, 0.68) for HbA_{1c}. In the multivariate joint model presented in the lower portion of Table 2, TNFR2, ACR, and HbA_{1c} together had a C-index 0.86 (0.84, 0.89). The results of this analysis were comparable with our previous findings regarding determinants of progression to ESRD in type 2 diabetes (2).

Search for Modifiers of the Association of TNFR2 With the Rate of eGFR Loss

To evaluate whether the association of serum TNFR2 with the rate of eGFR loss depended upon the value of eGFR at entry, we tested the association for heterogeneity across strata of the study group defined by CKD stage. Similarly, we tested for its heterogeneity across quartiles of other covariates (HbA_{1c} and ACR). The only statistically significant interaction (P = 0.030) was that between TNFR2 and HbA_{1c}. This is illustrated in Fig. 3, where the study group was subdivided according to quartiles of TNFR2 and quartiles of HbA_{1c}. In the first quartile of HbA_{1c}, the relationship between TNFR2 and the rate eGFR loss was weak; the difference in mean rates of eGFR loss between first and fourth quartiles of TNFR2 was only 1.9 mL/min/1.73 m^2/year. This difference increased in three successive quartiles to 3.0, 5.4, and 5.4 mL/min/1.73 m^2/year, respectively. Expressed as linear trend across TNFR2 quartiles, the coefficients were 0.8 (P = 0.157), 1.1 (P = 0.051), 1.7 (P = 0.001), and 1.9 ($P < 0.001$) in the first, second, third, and fourth quartiles of HbA_{1c}, respectively. Equivalent results were obtained when we examined the effect of HbA_{1c} within quartiles of TNFR2 (reading down the columns in Fig. 2 rather than across the rows).

It is important to note that baseline TNFR2 and HbA_{1c} are independent (Spearman correlation coefficient −0.01, P = 0.87). The first, second, and third quartiles of the distribution of TNFR2 in patients with HbA_{1c} <8.9% or <74 mmol/mol (median) were 3,542, 4,439, and 5,725 pg/mL, nearly the same as in patients with HbA_{1c} ≥8.9% or ≥74 mmol/mol (3,481, 4,409, and 5,887 pg/mL).

CONCLUSIONS

The current study showed a strong association between a single baseline measurement of serum concentration of TNFR2 and the future rate of renal function decline in type 1 diabetic patients with proteinuria. It replicates and expands our previous findings of a strong association of circulating TNFRs with the risk of CKD stage 3 in patients with type 1 diabetes and microalbuminuria, and risk of ESRD in patients with type 2 diabetes (1,2). In contrast to the previous studies, this study focused on serum TNFR2 as a determinant of the rate of eGFR loss, the most direct measure available of the intensity of the underlying disease process that leads to ESRD.

Our analytical method, joint longitudinal-survival analysis, estimates the rate of eGFR loss together with two other characteristics of the trajectory of renal decline during follow-up: the intercept (imputed baseline eGFR) and the imputed time to onset of ESRD.

In both univariate and multivariate analyses, the serum concentration of TNFR2 is strongly associated with the rate of eGFR loss, secondarily with the imputed baseline eGFR (a consequence of what damage has already accumulated) and ultimately the imputed time to ESRD. The strength of this association is unchanged by inclusion of ACR and other clinical risk factors in the multivariate model, indicating that its role is independent of them. The mechanisms through which circulating levels of TNFRs might impact renal decline have been discussed in our previous publications (1,2).

Urinary albumin excretion (measured as ACR) is also associated with the rate of eGFR loss, and although that association is attenuated in multivariate analysis with TNFR2, it is not fully accounted for. Regarding the respective roles of serum TNFRs and urinary albumin excretion, several aspects are known. In type 1 diabetic patients, circulating TNFR2 predicts the development of proteinuria,

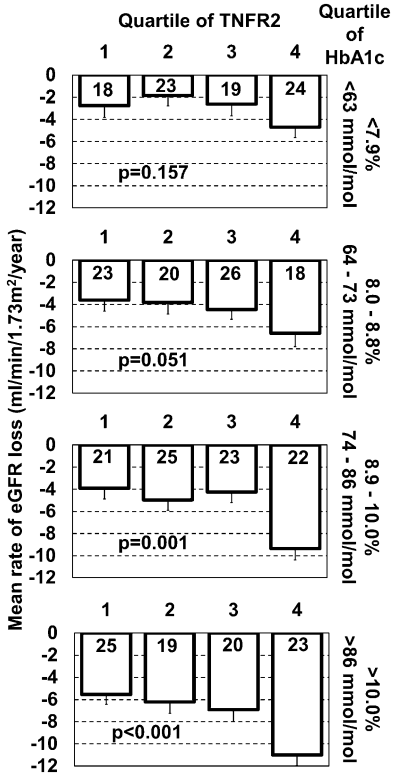

Figure 3—Mean and standard error of the rate of eGFR loss (joint model) according to quartiles of TNFR2 within quartiles of HbA_{1c}. The number in each bar is the number of patients in the subgroup. P values are for a linear trend test across quartiles of TNFR2.

as shown in the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) study (14). Although that study has not yet examined its effect on renal decline, our recent study has shown that TNFR 1 and 2 are major determinants of early renal decline in type 1 diabetic patients with normoalbuminuria, as well as in those with microalbuminuria (15) and those with proteinuria (current study). Thus, one may conclude that a high serum concentration of TNFRs increases both urinary albumin excretion and risk of renal decline. Regarding precedence, early renal decline is demonstrable before the onset of microalbuminuria and its progression to proteinuria (15). Therefore, the level of urinary albumin excretion can be considered more of an intermediate or accompanying phenotype rather than an independent determinant of renal decline.

The effect of HbA_{1c} on the rate of eGFR loss is strong and independent of other clinical risk factors. The fact that, unlike TNFR2, it has no impact on the imputed baseline eGFR suggests that

these two determinants play different roles in the disease process leading to ESRD. One plausible hypothesis accounting for this difference is that HbA_{1c} is an environmental exposure that comes into play only at specific stages of diabetic nephropathy. The decline itself is perhaps a consequence of a genetic susceptibility that is reflected in the serum concentration of TNFR2. An exposure, such as HbA_{1c}, may vary over time, and the single baseline measurement may not reflect well its prior level or that level's influence on baseline eGFR. In contrast, a constitutive susceptibility, such as TNFR2, may be more constant and the baseline eGFR will reflect its prior effect on the disease process. For example, patients with a high serum TNFR2 will have a lower baseline eGFR resulting from the renal decline that took place before the study entry.

Despite different associations with the imputed baseline eGFR, HbA_{1c} and TNFR2 had strong and similar associations with the rate of eGFR loss, and these effects were synergistic. In patients with good and moderate glycemic control, the association of TNFR2 with eGFR loss was much less than in patients with poor glycemic control. By the same token, the association of poor glycemic control with the rate of eGFR loss was blunted in patients with low serum TNFR2. In the remaining patients, i.e., in those with poor glycemic control and high serum levels of TNFR2, eGFR loss was rapid and might lead to ESRD within a short follow-up time. Despite this strong finding, the biological interpretation of this synergism is not clear.

We acknowledge that our study lacks frequent follow-up measurements of clinical covariates. Changes in clinical covariates (such as blood pressure, treatment with renoprotective drugs, and other characteristics) that went unrecognized during follow-up may have contributed to eGFR trajectories and limit the biological interpretation of our findings. Nevertheless, this study provides an assessment that does not need qualification of the predictive ability of a one-time measurement of TNFR2 and HbA_{1c} together for identifying patients at high risk of rapid renal decline and rapid progression to ESRD. Our study design resembles the typical patient visit during which endocrinologists or nephrologists try to predict the future from laboratory results without knowing what pathogenetic mechanisms might be involved or what changes might occur before the outcome (in this case ESRD) is reached many years in the future.

TNFR2 is a strong determinant of renal decline in patients with type 1 diabetes and proteinuria. By combining this measurement with levels of HbA_{1c}, doctors can stratify patients according to the risk of ESRD. Patients at highest risk should be enrolled in therapeutic programs to retard the rapid rate of renal function loss. Although such programs need to be developed, there is some evidence that in patients with proteinuria and high HbA_{1c}, significantly improved glycemic control maintained for 4 or more years can slow the rate of eGFR loss and postpone the onset of ESRD after a several-year lag time (16).

An important strength of our study and its novelty is the focus on the annual rate of eGFR loss as the renal outcome measure. The joint longitudinal-survival model simultaneously uses information about temporal changes in eGFR and the observed times to ESRD to estimate this quantitative outcome. Traditionally, observational studies and clinical trials have used threshold-based outcomes such as time to CKD stage 3, time to a doubling of serum creatinine, or time to onset of ESRD (17,18). Within the typical time horizon of a study (several years), only a fraction, the highest-risk patients or those closest to the threshold, develop the outcome. Such outcomes also pose problems with ascertainment. They depend on measurements of single serum creatinine values, whose assay has considerable random variation. In addition, the precision of measurements of event times is a function of the frequency of study visits and missed appointments. The rate of eGFR loss, on the other hand, is estimated from multiple eGFR observations and is less sensitive to biologic and assay variation in serum creatinine, missed appointments, and frequency of study visits.

There are several shortcomings of our study. First, we used serum creatinine–based estimates of GFR, which are less accurate than direct measurements or estimates based on serum cystatin C. Direct GFR measurements might increase the strength of the associations. Second, we assessed exposure to hyperglycemia by the value of HbA_{1c} at entry to the study only. Similarly, as for other risk markers, we considered only their value at entry. Accounting for possible changes in these covariates before or after the start of follow-up, most likely, might have improved their strength as predictors of the rate of renal decline. Finally, we note that the observed associations do not necessarily imply causation. However, establishing time sequence is the first requisite to hypothesize causality.

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Author Contributions. J.S., J.H.W., and A.S.K. designed the study, supervised data collection, performed data analysis, and wrote the manuscript. M.A.N. and T.G. contributed to research data collection and reviewed the manuscript. M.M. and J.C.M. reviewed and edited the manuscript. A.T.G. supervised the application of the joint longitudinal-survival model and reviewed and edited the manuscript. A.S.K. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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