Early Progressive Renal Decline Precedes the Onset of Microalbuminuria and Its Progression to Macroalbuminuria

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OBJECTIVE

Progressive decrease in the glomerular filtration rate (GFR), or renal decline, in type 1 diabetes (T1D) is observed in patients with macroalbuminuria. However, it is unknown whether this decline begins during microalbuminuria (MA) or normoalbuminuria (NA).

RESEARCH DESIGN AND METHODS

The study group (second Joslin Kidney Study) comprises patients with T1D and NA (n = 286) or MA (n = 248) who were followed for 4–10 years (median 8 years). Serial measurements (median 6, range 3–16) of serum creatinine and cystatin C were used jointly to estimate GFR (eGFRcr-cys) and assess its trajectories during follow-up.

RESULTS

Renal decline (progressive eGFRcr-cys loss of at least 3.3% per year) occurred in 10% of the NA and 35% of the MA ($P < 0.001$). In both groups, the strongest determinants of renal decline were baseline serum concentrations of uric acid ($P < 0.001$) and tumor necrosis factor receptor 1 or 2 (TNFR-1 or -2, $P < 0.001$). Other significant risk factors included baseline HbA_{1c}, age/diabetes duration, and systolic blood pressure. Relative impacts of these determinants were similar in NA and MA. Renal decline was not associated with sex or baseline serum concentration of TNF-α, IL-6, IL-8, IP-10, MCP-1, VCAM, ICAM, Fas, or FasL.

CONCLUSIONS

Renal decline in T1D begins during NA and it is determined by multiple factors, similar to MA. Thus, this early decline is the primary disease process leading to impaired renal function in T1D. Changes in albumin excretion rate, such as the onset of MA or its progression to macroalbuminuria, are either caused by or develop in parallel to the early renal decline.

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It has generally been assumed that in type 1 diabetes (T1D), increase in urinary albumin excretion rate (AER) precedes the development of impaired renal function and end-stage renal disease (ESRD). In that model, the onset of microalbuminuria (MA) leads to macroalbuminuria, and the latter is followed by progressive glomerular filtration rate (GFR) loss, or renal decline, which eventually leads to ESRD (1). A recently published large follow-up observation of the Diabetes Control and Complications Trial (DCCT)/ Epidemiology of Diabetes Interventions and Complications (EDIC) cohort showed that macroalbuminuria was a strong predictor of the development of chronic impaired renal function (GFR $<$60 mL/min) (2).

This finding, although consistent with the above model of diabetic nephropathy, is open for different interpretations. The authors assumed a priori that abnormalities in AER would precede significant GFR loss and did not test the alternative possibility that so-called early progressive renal decline when GFR falls within the normal range, i.e., from 130 to 60 mL/min, might precede the development of macroalbuminuria. It is also possible that early progressive renal decline and the development of AER abnormalities are two clinical phenotypes that reflect the disease process underlying diabetic nephropathy that eventually results in ESRD. These phenotypes may develop in parallel and would be predictors of each other.

Recently, better tools for tracking GFR trajectories have been developed (3,4). They allow the examination of the above models and, particularly, the establishment of which of the two phenotypes appears first in the clinical manifestation of diabetic nephropathy. Using some of these tools, we already revealed that progressive renal decline commences earlier than previously recognized and is already present in patients with MA (5,6). In our earlier follow-up studies of T1D (the first and second Joslin Kidney Study on the Natural History of Microalbuminuria), progressive renal decline developed in one-third of the patients with MA (5). Furthermore, renal decline was strongly associated with serum concentrations of uric acid and concentrations of tumor necrosis factor receptor 1 or 2 (TNFR-1 or -2) (6,7). Whether the effects of uric acid and the TNFRs are independent is unknown. Furthermore, due to limited follow-up data on patients with normoalbuminuria (NA), we were not able to establish whether renal decline can begin in patients with NA and, if so, whether its determinants are similar as in patients with MA.

To answer these questions, we expanded our previous study in patients with T1D and NA as well as with MA who had been followed with serial measurements of serum creatinine and cystatin C. We combined both markers to derive the most precise indirect measurement of the GFR (eGFRcr-cys) and trace its changes/trajectories during follow-up (4). Clinical characteristics and serum concentrations of markers previously reported in cross-sectional analyses of this cohort (8) were examined as determinants of progressive renal decline.

RESEARCH DESIGN AND METHODS

The Committee on Human Studies of the Joslin Diabetes Center approved the protocol and informed consent procedures for the second Joslin Kidney Study on the Natural History of Microalbuminuria, referred to here as the 2nd JKS. A description of the Joslin Clinic population and the design of the study were reported with results of a cross-sectional examination of the cohort (8). Previous reports of the shorter follow-up of this cohort included only patients with high NA and MA (6,7). The current study includes all patients with baseline NA as well as MA and extends follow-up to median 8 years (25th and 75th percentiles accordingly 6–9 years).

Study Population

Participants in the 2nd JKS were recruited from among patients attending the Joslin Clinic, a major center for treatment of patients with diabetes. During the screening period, 1 January 2003 through 31 December 2006, patients scheduled for an appointment at the Joslin Clinic were evaluated for eligibility in the clinical data system. Eligibility criteria included residence in New England, T1D diagnosed before age 40 years, current age 18–64 years, and diabetes duration 3–39 years. The archived clinical laboratory results during the 2-year pre-enrollment interval were searched for measurements of albumin-to-creatinine ratio (ACR) in urine specimens.

In the Joslin Clinic, laboratory albumin concentration in spot urine was measured by immunonephelometry on a BN Prospec System Nephelometer (Dade Behring, Inc., Newark, DE) with N Albumin kits, with intra-assay and interassay coefficients of variation 4 and 6%, respectively. Creatinine measurements in urine were assayed by Jaffe's modified picrate method on a Ciba Corning Express Plus Chemistry Analyzer (inter- and intra-assay coefficients of variation 3 and 5%, respectively). Individual values for ACR (measured in milligrams per gram) were converted to AERs (in micrograms per minute) by the previously published formula log(AER) = 0.44 + (0.85)log(ACR) – (0.13)sex, where sex is assigned a value of 1 for female patients and 0 for male patients (9).

Patients were not eligible for enrollment if they had macroalbuminuria (median pre-enrollment AER $\geq$300 μg/min), were on dialysis, had received a renal transplant, or had a history of HIV or hepatitis C infection. Among the 4,000 residents of New England with T1D who attended the Joslin Clinic during the screening period, we identified 2,667 eligible patients: 2,007 with NA (pre-enrollment median AER $<$30 μg/min) and 660 with MA (pre-enrollment median AER 30–299 μg/min). Ninety-five percent were white.

Enrollment and Examination

The study aimed to enroll eligible patients with MA and a similar number of eligible patients from the much larger pool of patients with NA. Furthermore, since the number of eligible NA patients with a pre-enrollment AER $<$15 μg/min was much larger than the number with an AER 15–29 μg/min, we sought to enroll only one patient with an AER $<$15 μg/min for each patient enrolled with an AER 15–29 μg/min. Study recruiters approached patients who had been identified as eligible when they arrived

for a scheduled appointment at the clinic. After informing patients about the study and obtaining their written consent to participate, the recruiter *1*) administered a structured interview that solicited the history of diabetes and its treatment, other health problems, and medications; *2*) measured blood pressure; and *3*) obtained specimens of blood and urine for laboratory determinations. Measurements of AER at enrollment examination were combined with AER measurements from the preceding 2-year pre-enrollment interval, and new medians were determined to represent the baseline AER values. For all other characteristics, baseline values are those obtained at enrollment examination. As of the end of 2006, we had examined 304 patients with MA and 363 patients with NA. Patients recruited into the 2nd JKS during a second phase (2007–2010) are not included in this report.

Follow-up Examinations

Enrolled participants were followed until 2013, with a goal to obtain blood and urine specimens at least every 2 years. Collection of research specimens occurred during patients' routine clinic visits. Although most patients had two or more visits per year, the research specimens were collected less frequently, on average 1.5 years apart. Patients with less frequent visits to the clinic or those who stopped coming to the clinic were examined at their homes. Specimens obtained at baseline and during follow-up were stored at −80°C for later analysis.

Determinations of AER performed during routine clinic visits and for research purposes were combined and used to evaluate changes in AER over time (all measurements were performed in the Joslin Clinic laboratory as described above). We considered three 2-year intervals: the baseline interval and the first and second 2-year intervals after enrollment. In patients with NA during the baseline interval, a median AER >30 μg/min in either 2-year follow-up interval constituted the onset of MA. In patients with MA during the baseline interval, a halving of baseline AER constituted regression of MA and the development of macroalbuminuria (>300 μg/min) in either follow-up interval constituted progression.

Laboratory Measurements

Serum Markers

Protocols for measuring concentration of TNF-α (free) and its receptors (TNFR-1 and -2) and other markers such as IL-6, IL-8, IP-10, MCP-1, VCAM, ICAM, Fas, and FasL as well as uric acid were described previously (6,8).

Estimation of GFRcr-cys

From 2009 to 2013, creatinine and cystatin C were assayed in stored baseline and follow-up serum samples in the Advanced Research and Diagnostic Laboratory at the University of Minnesota. Protocols used for creatinine measurements yielded values traceable to isotope dilution mass spectrometry reference measurement procedures, and, for the cystatin C, values were adjusted to be traceable to ERM-DA471/IFCC international reference material as described previously (10). The eGFRcr-cys was estimated with the recently published combined creatinine-cystatin C Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (4).

Definition of Progressive Renal Decline

Longitudinal measures of log-transformed eGFRcr-cys values were analyzed with linear mixed-effects regression (PROC MIXED, SAS V 9.3; SAS Institute, Cary, NC). This approach takes into account the correlation between follow-up observations from a patient taken at varying intervals. It yields individual-specific slope coefficients that are re-expressed as percent change per year in eGFRcr-cys (6). Progressive renal decline is defined as a negative change equal to or steeper than −3.3% per year, and the patient is referred to as a "decliner." Less steep slopes are defined as stable, and the patients are referred to as "nondecliners." This criterion, an eGFR loss of 3.3% per year or more, has been used in previous reports (5,6) and corresponds to the 2.5th percentile of the distribution of annual renal function loss in a general population (11).

Statistical Analysis

All statistical analyses were performed in SAS V 9.3. Comparisons between those with renal decline and those with stable eGFRcr-cys were made with Wilcoxon rank sum tests for continuous variables and χ^2 test for categorical variables. The risk of renal decline (eGFRcr-cys loss ≥3.3% per year) according to clinical characteristics or baseline serum marker concentrations categorized into quartiles of their distributions or according to other ordinal variables was tested using the Cochran-Armitage trend test. A multiple logistic regression model was used to jointly test the predictors of eGFR decline identified in univariate analysis. *P* values <0.05 were considered significant. The analyses were performed in SAS V 9.3 software.

RESULTS

Progressive Renal Decline in the Study Groups

There were 304 patients with MA and 364 patients with NA enrolled in the 2nd JKS. This study included those with normal renal function at baseline (eGFRcr-cys >60 mL/min) who were followed for 4–10 years: 248 (82%) patients with MA and 286 (79%) with NA. Characteristics of the study groups are summarized in Table 1. In comparison with the NA group, the MA group included more males; had a longer duration of diabetes, higher HbA_{1c}, and higher blood pressure; and was more frequently treated with renoprotective drugs. Whereas the distribution of AER in the NA group was uniform across the normal range (<30 μg/min), the bulk of the distribution in the MA group was in the low abnormal range (25th, 50th, and 75th percentiles were 44, 65, and 116 μg/min, respectively). The distributions of baseline concentrations of serum creatinine and cystatin C and the estimates of eGFRcr-cys were similar in NA and MA.

Duration of follow-up and the available number of measurements of serum creatinine and cystatin C were similar in the two study groups. The annual rate of eGFRcr-cys change was estimated from these serial measurements. The median rate of loss in patients with MA was more rapid than in patients with NA. Renal decline (rate of eGFRcr-cys loss ≥3.3% per year) was present in 10% of the NA group and 35% of the MA group. Impaired renal function (CKD-EPI ≥3)

Table 1—Characteristics of the study groups

	NA	MA
Baseline characteristics	*n* = 286	*n* = 248
Male (%)	43%	62%
Duration of diabetes (years)	20 (14–29)	24 (15–30)
Age (years)	41 (30–48)	42 (34–49)
HbA_{1c} (%)	8.0 (7.4–8.8)	8.3 (7.5–9.3)
HbA_{1c} (mmol/mol)	64 (57–73)	67 (58–78)
Systolic blood pressure (mmHg)	118 (110–127)	122 (114–130)
Diastolic blood pressure (mmHg)	70 (68–76)	72 (70–80)
ACE and ARB Rx	33%	74%
AER (μg/min)	16 (12–22)	65 (44–116)
Serum cystatin C (mg/L)	0.65 (0.58–0.72)	0.66 (0.60–0.78)
Serum creatinine (mg/dL)	0.76 (0.65–0.86)	0.79 (0.69–0.90)
eGFRcr-cys (mL/min)	113 (102–123)	112 (96–122)
Follow-up characteristics		
Duration of follow-up (years)	8.0 (6.0–8.7)	7.9 (5.5–9.2)
Number of serum samples	5 (4–7)	7 (5–9)
eGFRcr-cys loss (% per year)	−1.5 (−2.4 to −0.8)	−2.2 (−4.1 to −1.2)
Renal decline (eGFRcr-cys loss ≥3.3%/year)	**10%**	**35%**
8-year cumulative risk of CKD-EPI ≥3	6%	22%
ESRD (*n*)	1	4

Data are percents or medians (25th–75th percentiles). ARB, angiotensin receptor blocker. Boldface indicates main outcome measurement.

developed in 6% of the NA group and 22% of the MA group, and ESRD developed in one of the former and four of the latter.

The trajectories of renal function changes in the 28 decliners with NA have particular significance because they have not been documented before (Fig. 1). To improve visibility of individual slopes, the 28 patients are stratified at the median baseline eGFRcr-cys (≥105 mL/min in Fig. 1*A* and <105 mL/min in Fig. 1*B*). Two features deserve emphasis. First, the majority of trajectories of eGFRcr-cys loss were approximately linear. Two deviated visibly by accelerating the rate of decline (trajectories in red). These accelerations were accompanied by the onset of MA and macroalbuminuria. One of these patients has reached ESRD and the other soon will. Second, the slopes of eGFRcr-cys loss varied tremendously, ranging from a loss of 3.3 to 21% per year. The fastest decliners, including the two already mentioned, will reach ESRD within 5–15 years, and the slowest might take 25–30 years. The pattern of eGFRcr-cys trajectories in 86 decliners with MA was similar; i.e., the majority of trajectories were linear and four patients rapidly progressed to ESRD within the duration of follow-up (data not shown due to large number of decliners).

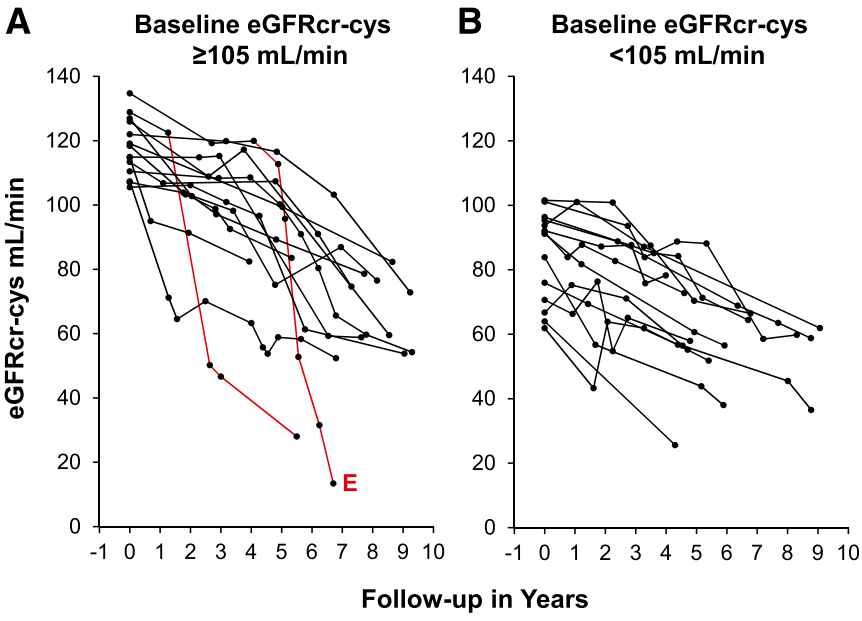

Figure 1—eGFRcr-cys trajectories in T1D patients with NA and progressive renal decline (loss ≥3.3% per year) during 4–10 years of follow-up. The trajectories are plotted in patients with baseline eGFRcr-cyst ≥105 mL/min (*A*) and in patients with baseline eGFRcr-cys <105 mL/min (*B*). Lines in red indicate presence of macroalbuminuria. E, ESRD.

Univariate Analysis of Determinants of Progressive Renal Decline

To find determinants of renal decline, we compared nondecliners with decliners with regard to baseline clinical covariates and serum concentrations of 12 markers separately in patients with NA and MA (Table 2). The values of eGFRcr-cys at last follow-up were similar to baseline for nondecliners in both groups. For decliners, however, the last values were lower (by definition) than baseline, down to 61 from 104 mL/min in NA and to 59 from 99 mL/min in MA. The median rate of eGFRcr-cys loss per year in decliners was 5.0% per year in both groups. Renal function at baseline in patients with NA was normal in nondecliners but was already lower in decliners, most likely because the onset of renal decline preceded the enrollment in the study. Similarly in patients with MA, renal function at baseline was normal in nondecliners (despite the presence of MA) but was already lower in decliners for the same reason.

During the baseline interval, AER was identical in decliners and nondecliners in the NA group, but MA developed in three times as many decliners (54%) as nondecliners (17%) during the 4 years of follow-up. In patients with MA already present during the baseline period, progression to macroalbuminuria was much more common in decliners (27%) than nondecliners (4%) during the 4 years of follow-up. By contrast, AER

Table 2—Characteristics of nondecliners and decliners according to study group

	NA			MA		
	GFR change during follow-up			GFR change during follow-up		
Characteristics	Nondecliner (n = 249)	Decliner (n = 28)	P	Nondecliner (n = 162)	Decliner (n = 86)	P
Renal function						
eGFRcr-cys baseline	113 (103–124)	104 (91–117)	<0.005	116 (105–124)	99 (80–111)	<0.001
eGFRcr-cys last	103 (93–115)	61 (54–76)		103 (89–116)	59 (48–76)	N/A
eGFRcr-cys slope (% per year)	−1.3 (−2.1 to −0.7)	−5.0 (−7.3 to −3.8)		−1.5 (−2.1 to −0.7)	−5.1 (−7.4 to −4.0)	N/A
AER abnormality						
AER baseline (μg/min)	16 (12–22)	16 (12–22)	NS	57 (42–92)	94 (51–166)	<0.001
Onset of MA	17%	54%	<0.001	–	–	
Progression to macroalbuminuria	—	—		4%	27%	<0.001
Persistent MA	—	—		54%	50%	
Regression of MA	—	—		43%	23%	
Baseline clinical characteristics and serum markers*						
Duration of T1D (years)	19 (13–28)	28 (22–32)	<0.005	23 (15–30)	27 (16–33)	NS
Age (years) at examination	40 (28–47)	47 (44–58)	<0.001	39 (31–47)	45 (39–51)	<0.001
HbA_{1c} (%)	8.0 (7.4–8.8)	8.4 (7.5–9.3)	NS	8.1 (7.4–9.0)	8.7 (7.8–9.9)	<0.005
HbA_{1c} (mmol/mol)	64 (57–73)	68 (58–78)	NS	65 (57–75)	72 (62–85)	<0.005
Systolic blood pressure	118 (110–126)	123 (112–135)	<0.05	121 (112–130)	124 (118–133)	<0.005
Renoprotective drugs (%)	28%	69%	<0.001	69%	81%	<0.05
Serum uric acid (mg/dL)	4.1 (3.6–4.9)	4.8 (4.0–5.4)	<0.05	4.8 (4.2–5.7)	5.4 (4.2–6.4)	<0.05
Serum TNFR-1 (pg/mL)	1,256 (1,087–1,490)	1,509 (1,281–1,870)	<0.001	1,442 (1,229–1,700)	1,788 (1,454–2,220)	<0.001
Serum TNFR-2 (pg/mL)	1,986 (1,686–2,411)	2,601 (2,212–3,380)	<0.001	2,205 (1,892–2,652)	2,707 (2,180–3,385)	<0.001

Data are percents or median (25th–75th percentiles). *Concentrations of serum markers TNFs, IL-8, IP-10, MCP-1, ICAM, VCAM, Fas, and FasL in decliners and nondecliners did not differ (data not shown).

regression was twice as common in nondecliners (43%) as decliners (23%). Thus, AER was more likely to progress in patients with renal decline and more likely to be transient in nondecliners.

With regard to other baseline characteristics, decliners and nondecliners did not differ in either group with regard to sex, insulin dose, BMI, serum cholesterol (total or HDL), as well as cigarette smoking (data not shown). Significant differences between decliners and nondecliners are indicated in the bottom part of Table 2. In both study groups, age at entry into the study was older and duration of diabetes was longer for decliners than nondecliners. In addition, the decliners had poorer glycemic control and higher systolic blood pressure and were treated more frequently with renoprotective drugs than nondecliners. In both study groups, decliners had significantly higher serum concentrations of uric acid, TNFR-1, and TNFR-2 than nondecliners. Serum markers such as free TNF-α, IL-6, IL-8, IP-10, MCP-1, ICAM, VCAM, Fas, and FasL were not different between decliners and nondecliners (data not shown).

To visualize these associations, in Table 2 we graphed the risk of renal decline separately by study group according to categories of baseline characteristics that were associated with renal decline in the case-control analysis (Fig. 2). The risk of renal decline according to quartiles of baseline AER (Fig. 2*A*, *top*) was flat (~10%) across quartiles in the NA group. In MA, the risk of renal function decline was significantly higher than in NA and increased from 23% in the lowest quartile to 55% in the highest. For HbA_{1c}, the increase in risk was moderate in the NA group but strong in the MA group (Fig. 2*A*). The risk of decline increased with age in both groups (Fig. 2*A*) and increased similarly with duration of T1D because of its correlation with age (data not shown). Risk of decline also increased with systolic blood pressure in both groups (Fig. 2*A*) and increased as the number of prescribed renoprotecting treatments increased (Fig. 2*B*). Plots of the risk of renal decline according to three serum markers are shown in Fig. 2*B*. In both study groups, elevated serum uric acid increased the risk of renal decline, and the effect of an increasing serum TNFR-1 or TNFR-2 was even more striking. The effects of both TNFRs were very similar and the information in them redundant.

Multivariate Analysis of Determinants of Progressive Renal Decline

Determinants of renal decline that were significant in Table 2 and Fig. 2 were examined with multiple logistic regression analyses. In separate analyses of the NA and MA groups, the coefficients for these determinants were similar so we combined the groups and included an indicator variable for MA as one of the determinants (Table 3). The dose-response relationship appeared stronger with age (Fig. 2*A*) than with duration, so only age was included in the model. Similarly, systolic blood pressure (Fig. 2*A*) and number of prescribed renoprotective drugs (Fig. 2*B*) were correlated. We only included blood pressure in the model because the intensity of treatment was most likely secondary to the blood pressure,

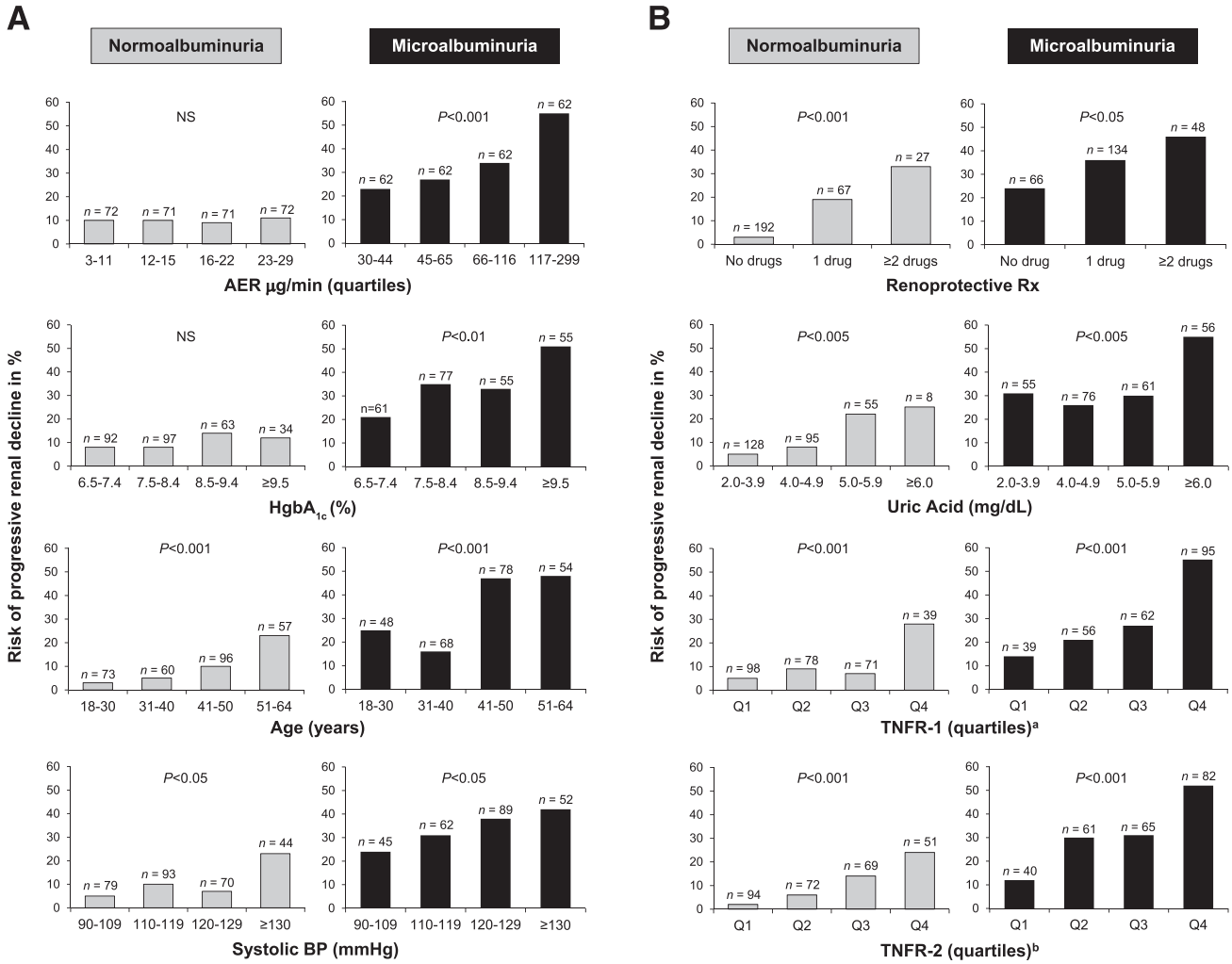

Figure 2—Risk of progressive renal decline according to categories of baseline clinical characteristics (*A*) and serum markers (*B*) and according to study groups. [a]Cut points for 25th, 50th, and 75th percentiles were 1,173, 1,394, and 1,685 pg/mL; [b]cut points for 25th, 50th, and 75th percentiles were 1,810, 2,186, and 2,690 pg/mL.

not the reverse. In a cross-tabulation of patients according to both serum uric acid and TNFR-1, the risk of renal decline increased with each one alone, but their effects were not additive. This is reflected in the significant odds ratio (OR) but smaller than 1.00 for their interaction term in the multiple logistic model (Table 3). An increase in one diminishes the effect of the increase in the other. For example, at the 25th percentile of TNFR-1 (1,170 pg/mL), the OR for the 75th vs. 25th percentiles of uric acid is 3.72, whereas at the 75th percentile of TNFR-1 (1690 pg/mL), it is 1.58. The relationship is symmetric. At the 25th percentile of uric acid (3.8 mg/dL), the OR for the 75th vs. 25th percentiles of TNFR-1 is 4.68, whereas at the 75th percentile of uric acid (6.4 mg/dL), it is 1.99.

CONCLUSIONS

In this large follow-up study, we showed that progressive renal decline in T1D began during NA and, similar to MA, it was determined by multiple factors. We conclude that this early progressive renal decline can be considered the primary clinical manifestation of disease process leading to impaired renal function and eventually to ESRD in T1D. Changes in AER observed during follow-up, such as the onset of MA or its progression to macroalbuminuria, were either caused by or developed in parallel to the early renal decline in T1D.

In our study of T1D patients with NA, progressive renal decline developed in 10% (decliners) within 4–10 years of follow-up, whereas only 2.5% was expected if these patients had been nondiabetics. The risk of renal decline did not vary within the normal range of AER in NA but increased with increasing AER in the MA range. The rate of eGFRcr-cys loss was continuous (progressive decline) in the majority of decliners and could be represented as a linear slope. These slopes varied widely in their steepness. For example, in the study, eGFRcr-cys loss was so rapid in five patients (one with NA and four with MA) that they progressed from normal renal function to ESRD within the 5–10 years of observation, whereas the eGFRcr-cys loss in other decliners was slow so that progression to ESRD might take up to 30 years. These findings are

Table 3—Multiple logistic analysis of determinants of renal decline in both study groups combined

Baseline determinants	OR	95% CI	χ^2	*P*
Age (per 10 years)	1.53	1.18–1.97	10.7	0.0011
HbA_{1c} (per 1%)	1.60	1.31–1.95	20.9	<0.0001
Systolic BP (per 10 mmHg)	1.34	1.09–1.63	8.4	0.0038
MA vs. NA	2.28	1.28–4.08	7.7	0.0054
Uric acid (per 0.5 mg/dL)	1.86	1.33–2.62	12.8	0.0003
TNFR-1 (per 200 pg/mL)	2.93	1.90–4.52	23.6	<0.0001
Interaction (TNFR-1 and uric acid)	0.94	0.91–0.97	12.2	0.0005

BP, blood pressure.

similar to the renal decline patterns we observed in T1D patients with macroalbuminuria (12).

Progressive renal decline was present in a significant proportion of T1D patients when their urinary albumin excretion was normal, and it increased the risk of onset of MA several fold during follow-up in comparison with nondecliners (51 vs. 17%). This finding provides a very strong evidence that early progressive renal decline is the primary event in the development of diabetic nephropathy. Furthermore, once early progressive renal decline and MA were both present, the risk of progression to macroalbuminuria during follow-up was several fold higher in comparison with nondecliners with MA (28 vs. 4%). Conversely, the frequency of regression of MA in nondecliners was twice that in decliners. Thus, the abnormal AER that occurred in the presence of early progressive renal decline was more likely to be progressive, whereas the abnormal excretion that occurred in nondecliners was more likely to be transient.

These findings are not consistent with the generally assumed model of diabetic nephropathy in which renal decline is a consequence of or follows macroalbuminuria (1). Instead, our findings indicate that either both are loosely correlated phenotypes of a disease process that underlies diabetic nephropathy and leads to ESRD or that early progressive renal decline is one of the causes of albuminuria. The former interpretation of our findings explains reports that showed that albuminuria and renal decline are uncoupled phenotypes in T1D (2,13,14) and T2D (15). The latter should be considered as a new hypothesis and should be studied further.

Recognition that multiple clinical factors contribute in a similar way to renal decline in NA and MA strengthens our hypothesis that progressive renal decline is the primary clinical abnormality of diabetic nephropathy. Following, we discuss what is known of these factors. As mentioned earlier, the effect of renal decline on the risk of the onset of MA and its progression to macroalbuminuria may account in large degree for a higher occurrence of renal decline in our collection of patients ascertained for the presence of MA. In other words, early renal decline leads to MA, so patients with MA should be enriched with those at risk for renal decline. The effect of HbA_{1c} on renal decline was recently recognized (16). In our study, the effect of HbA_{1c} on risk of early renal decline was strong. However, the mechanisms through which hyperglycemia contributes to early progressive renal decline independently from AER abnormalities are unknown. The effect of increasing age on renal decline is recognized in nondiabetic individuals >40 years of age. The GFR loss of >3.3% per year (about 4 mL/min/year) occurs in <3% of the healthy population (11). It is unknown why such GFR loss occurred almost 5–15 times more frequently in T1D patients 40 years of age or more. In part, this effect was due to a long duration of diabetes. Since these two covariates were closely correlated, it was impossible to separate their effects in this study. The risk of early progressive renal decline increased with increasing systolic blood pressure, a well-documented risk factor for progression to ESRD (17). Antihypertensive and renoprotective drugs were prescribed in patients with elevated blood pressure and MA. In univariate analysis, both elevated systolic blood pressure and treatment with these drugs increased the risk of early progressive renal decline. However, in multivariate analysis, the effect of the latter declined significantly, indicating that antihypertensive and renoprotective treatment had an inconsequential effect on the risk of early progressive renal decline. Similar findings were recently reported in the Renin-Angiotensin System Study (RASS) clinical trial (18).

Of the 12 serum markers previously examined in cross-sectional analyses of this cohort (6–8), only the concentrations of uric acid, TNFR-1, and TNFR-2 significantly contributed to renal decline. The effects of these markers on the risk of renal decline were equally strong in patients with NA or MA. The congruence of these determinants in MA and NA is further evidence for our hypothesis that early progressive renal function is the primary disease process underlying the development of renal decline in T1D and leads to ESRD.

The mechanisms underlying the association between high normal values of serum uric acid and increased risk of renal decline are unclear at this time. Serum uric acid has proinflammatory properties and may act as either a pro-oxidant or antioxidant molecule depending on the circumstances (19). In vitro, exposure to uric acid leads to upregulation of cyclooxygenase-2, specific mitogen-activated protein kinases, various inflammatory mediators, and the renin-angiotensin system, as well as to inhibition of endothelial nitric oxide production (20–22), which may all contribute to the development of kidney damage. In vivo, rats rendered hyperuricemic by means of a uricase inhibitor develop an afferent arteriolopathy that decreases luminal diameter and produces renal ischemia, leading to glomerulosclerosis and tubulointerstitial fibrosis (23). Similar histological changes occur in humans with gouty nephropathy (24). Lowering serum uric acid concentration with allopurinol attenuates these histological and functional changes, although this effect may be due in part to reduced

oxidative stress resulting from xanthine-oxidase inhibition (19). It is intriguing to postulate that lowering serum uric acid in patients at risk for renal decline may be an effective intervention to reduce the risk of renal decline in T1D (25). Preparations for such clinical trials are under way (26).

Our previous studies described the strong effect of serum concentrations of TNFR-1 or TNFR-2 on the risk of advanced stages of renal decline such as CKD-EPI $\geq$33 or ESRD (7,27). This study extends the demonstration of their effects to the onset of the process of renal decline itself. We do not know how elevated concentrations of TNFRs initiate renal decline and lead to renal failure. However, some hypotheses were excluded by this study. For example we showed that serum TNF-α is not involved directly or indirectly, through regulation of serum TNFRs, in the development of renal decline. Furthermore, we excluded the role of several circulating adhesion molecules and chemokines (VCAM, ICAM, IL-6, IL-8, IP-10, and MCP-1) in the etiology of renal decline as potential downstream effectors of TNFRs (8).

An intriguing finding of the current study is the negative interaction between serum uric acid and TNFR-1 on the risk of renal decline in both NA and MA, meaning that the risk of renal decline for patients with elevated serum uric acid and serum TNFR-1 is less than the sum of the individual risks associated with the two predictors. This suggests that the predisposing effects of serum uric acid and TNFR-1 converge on some common pathway that cannot be further activated by one factor if it has already been turned on by the other. The nature of this putative pathway is unknown at this time.

Finally, the limitations of our study must be considered. First, our major weakness is the lack of direct measurements of GFR. The eGFRcr-cys equation has not been validated in T1D, and this type of eGFR assessment may significantly underestimate "hyperfiltration" in T1D, therefore reducing the steepness of eGFR slopes and underestimating the true frequency of early progressive renal decline. Second, our study is descriptive and hypothesis generating, and it cannot directly shed any light on the mechanisms of early progressive renal decline in T1D. However, it provides novel data that question many assumptions about the natural history of diabetic nephropathy in T1D. Third, this study was conducted in T1D and the generalizability of the findings to T2D is uncertain.

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Author Contributions. A.S.K. designed the study, supervised data collection and data analysis, and wrote the manuscript. M.A.N. and T.G. contributed to data collection and reviewed the manuscript. J.S. analyzed data and reviewed and edited the manuscript. A.S. contributed to data collection, analyzed data, and reviewed the manuscript. J.H.E. contributed to data collection and reviewed and edited the manuscript. A.D. contributed to discussion and reviewed and edited the manuscript. J.H.W. designed the study and contributed to data analysis and writing and editing 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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