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Increased plasma Kidney Injury Molecule-1 suggests early progressive renal decline in non-proteinuric patients with Type 1 diabetes

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Abstract

Progressively decreasing glomerular filtration rate (GFR), or renal decline, is seen in patients with type 1 diabetes (T1D) and normoalbuminuria or microalbuminuria. Here we examined the associations of kidney injury molecule-1 (KIM-1) in plasma and urine with the risk of renal decline and determine whether those associations are independent of markers of glomerular damage. The study group comprised patients with T1D from the 2nd Joslin Kidney Study of which 259 had normoalbuminuria and 203 had microalbuminuria. Serial measurements over 4 to 10 years of follow-up (median 8 years) of serum creatinine and cystatin C were used jointly to estimate eGFRcr-cys slopes and time of onset of CKD stage 3 or higher. Baseline urinary excretion of IgG_2 and albumin were used as markers of glomerular damage, and urinary excretion of KIM-1 and its plasma concentration were used as markers of proximal tubular damage. All patients had normal renal function at baseline. During follow-up, renal decline (eGFRcr-cys loss 3.3% or more per year) developed in 96 patients and 62 progressed to CKD stage 3. For both outcomes, the risk rose with increasing baseline levels of plasma KIM-1. In multivariable models, elevated baseline plasma KIM-1 was strongly associated with risk of early progressive renal decline, regardless of baseline clinical characteristics, serum TNFR1 or markers of glomerular damage. Thus, damage to

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proximal tubules may play an independent role in the development of early progressive renal decline in non-proteinuric patients with T1D.

Keywords

type 1 diabetes; early progressive renal decline; markers of glomerular and tubular damage

INTRODUCTION

End stage renal disease (ESRD) is a major health problem responsible for high morbidity and premature mortality in patients with Type 1 diabetes (T1D) [1,2]. Progressive renal decline leading to ESRD begins while renal function is normal and usually proceeds inexorably along a linear trajectory [3]. It develops in about 10% of patients while urinary albumin excretion is normal (NA), 30% of those with microalbuminuria (MA) and 50% of those with proteinuria [3-6]. We refer to this decline as *early progressive renal decline*: early, because it starts when renal function is normal and progressive because, once initiated, it continues until ESRD is reached [3-6].

The disease process underlying early progressive renal decline is unknown. Several systemic factors have been implicated: poor glycemic control, elevated blood pressure, and elevated serum levels of uric acid, TNFR1 and TNFR2 [5-10]. Morphological kidney studies of early progressive renal decline are nonexistent with the exception of the RASS clinical trial reported by Mauer et al. During the 5 year trial involving healthy T1D participants, significant decline in eGFR occurred in 25%. This decline was not associated with any morphological lesion in glomeruli assessed in baseline biopsies [11]. Whether it was associated with morphological lesions in tubular and interstitial compartments is unknown as this was not assessed.

To gain a view of processes taking place in kidneys, an alternative to an examination of morphology in kidney biopsies is an examination of biomarkers in plasma and urine that are specific for glomerular or tubular damage. For example, urinary albumin excretion has been viewed as a marker of glomerular damage although, in truth, it is also a marker of tubular injury that impairs albumin reabsorption. Similarly, urinary excretion of various IgG classes reflects abnormalities in the glomerular filtration barrier, and we have developed sensitive assays to measure their concentrations in urine [12]. An example of a biomarker specific to proximal tubular cell injury is the urinary concentration of Kidney Injury Molecule-1 (KIM-1). This protein was originally discovered using representational difference analysis in an effort to identify mRNAs and their encoded proteins that are up-regulated after acute ischemic kidney injury [13].

KIM-1, also known as Hepatitis A Virus Cellular Receptor 1 (HAVCR1) and T cell Ig mucin 1 (TIM1), is a transmembrane glycoprotein specifically over-expressed in damaged proximal tubules. The ectodomain of KIM-1 (approximately 90 kDa) is cleaved by matrix metalloproteinases and released into the urine [13-16]. Since its discovery, KIM-1 has emerged as a sensitive and specific urinary biomarker of kidney injury in both rodent models and humans [17-21]. After injury to proximal tubules, excess KIM-1 protein may be released not

only into the urine but also into the circulation [18]. An elevated circulating concentration of KIM-1, independent of albuminuria, predicts the risk of ESRD in patients with proteinuria and T1D. [18]

In this study of non-proteinuric patients with T1D and normal renal function, we sought to test the hypothesis that plasma and urinary KIM-1, markers of proximal tubule damage, are elevated prior to any detectable change in glomerular permeability or albuminuria. Thus proximal tubule injury may represent an early feature and potential causative factor in the development of early progressive renal decline in T1D

RESULTS

Distribution of markers of tubular and glomerular damage in the study group

The study group comprised non-proteinuric T1D patients whose renal function was normal (eGFRcr-cys > 60 ml/min) when enrolled into the 2nd Joslin Kidney Study (as described previously [5, 7-10, 19]) and who were followed for 4-10 years. The present study includes 259 patients with normoalbuminuria (NA), 203 with microalbuminuria (MA), and a comparison group of 77 healthy individuals without diabetes (NDM). Four markers were examined at baseline: two markers of tubular damage (urinary and plasma concentrations of KIM-1) and two markers of glomerular damage (urinary concentrations of albumin and IgG2).

Distributions of baseline plasma and urinary concentrations of KIM-1 in the three study sub-groups (NDM, NA and MA) are compared in Table 1. Plasma concentrations of KIM-1 below the detection limit (0.2 pg/ml) were designated not detectable (ND). Detectable plasma concentrations of KIM-1 in T1D patients with NA or MA were combined into one distribution and divided into tertiles. This created four strata of baseline plasma concentrations of KIM-1 (ND, T1-T3). Plasma KIM-1 was not detectable in 61.5% of NDM, 35.1% of NA and 20.7% of MA (Table 1, Panel A). The frequency of plasma KIM-1 concentrations in the lowest tertile was similar in all three study sub-groups, while the frequency of elevated concentrations (upper two tertiles) rose from 17.1% of NDM to 36.3% of NA and to 62.1% of MA. The distributions of corresponding strata of urinary KIM-1 in the three study sub groups (Table 1, Panel B) is similar to the pattern in Panel A with the notable exception of a higher frequency of elevated (upper two tertiles) concentrations of urinary KIM-1 (36.3%) in NDM as compared to plasma KIM-1 (17.1%).

Characteristics of the study group according to strata of plasma KIM-1

To identify variables associated with higher baseline levels of plasma KIM-1, we examined other measured markers and relevant clinical variables in the NA and MA groups according to the four plasma KIM-1 strata. Progressively higher plasma KIM-1 concentrations were accompanied by progressively higher concentrations of KIM-1, albumin and IgG2 (Table 2). Data regarding NAG in urine and TNFR1 in serum, as previously reported by us [7, 8, 19], were used in the present study. Their levels increased with plasma levels of KIM-1 (Table 2). Regarding clinical characteristics, while age, HbA1c, and percent treated with ACE-I or ARBs increased, duration of diabetes, systolic and diastolic blood pressures and eGFRcr-cys did not differ across the four strata.

Both the duration of follow-up and the number of serum creatinine and cystatin C measurements available to estimate eGFRcr-cys and determine trajectories of change during follow-up were similar across strata. Three aspects of the trajectories of eGFRcr-cys were used to represent the association between plasma KIM-1 and early progressive renal decline. The slope of eGFRcr-cys decline became steeper, from 1.4 to 3.2 %/year (medians) between the lowest and highest stratum of plasma KIM-1. Consequently, the proportion of "decliners" (patients with eGFRcr-cys loss 3.3% per year, as defined previously [5]) increased from 5% to 47% between the same strata, and the incidence rate of progression to impaired renal function (CKD stage 3) increased from 2 to 54/1000 person-years. Each of these associations was highly significant (test for trend p<0.001).

The associations between concentrations of assayed markers and patient characteristics at baseline were examined with Spearman rank correlation (r_s) (Table 3). Tubular and glomerular markers were significantly correlated with each other but the coefficients were weak to moderate (r_s varied from 0.16 to 0.48). It should be emphasized that the correlation between concentrations of plasma and urinary KIM-1 was weak, r_s= 0.25. Serum TNFR1 was weakly correlated with markers of glomerular damage and uncorrelated with markers of tubular damage. Correlations of the markers with other patient characteristics were weak or absent. However, each of the markers was correlated with eGFRcr-cys slope. The strongest were serum TNFR1 (r_s=−0.40; p<0.001) and plasma KIM-1 (r_s=−035; p<0.001); the weakest were IgG2 (r_s=−0.16; p<0.001) and urinary KIM-1 (r_s=−0.16; p<0.01), while urinary albumin (r_s=−0.25; p<0.001) and NAG (r_s=−0.21; p<0.001) were intermediate.

Tubular and glomerular damage and early progressive renal decline: univariate analysis

Among the 482 patients in the combined NA and MA subgroups, 96 (19.9%) became decliners (eGFRcr-cys loss 3.3%/year). The frequency of decliners according to strata of markers of glomerular damage and strata of plasma KIM-1 is shown in Table 4, and the same is shown for strata of urinary KIM-1. For plasma KIM-1, the frequency increased monotonically across the four strata in both NA and MA (p<0.001 for each). Although the frequencies were consistently lower in NA than MA, the patterns of increase were not significantly different. Similarly in strata of urinary IgG2, the frequency rose monotonically with increasing plasma KIM-1 (p<0.001 for each). For urinary KIM-1, the increasing frequency of decliners across the four strata differed according to strata of markers of glomerular damage. The frequency of decliners increased across the strata in MA (p<0.001) but not in NA (p=0.45). Similarly, the frequency of decliners increased if IgG2 was above the median (p<0.001) but not if it was below (p=0.69).

Also in the combined NA and MA subgroups, 62 patients reached CKD stage 3 during 3416 person-years of follow-up (incidence rate 19/1000 person-years). The stratified analysis of the frequency of decliners as summarized in Table 4 was also performed for the incidence rate of CKD stage 3 (Figure 1). For plasma KIM-1, the incidence rate increased across the four strata in both NA and MA (p<0.0001 for each stratum) (Panel A). The incidence rate was higher in MA than NA, but the pattern of increases at higher concentrations of plasma KIM-1 did not differ in the two strata. For urinary KIM-1, the increasing incidence rate across the four strata differed according to strata of markers of

glomerular damage. The incidence rate increased across strata in MA (p=0.001) but not in NA (p=0.44) (Panel B). The stratified analysis according to urinary IgG2 gave results similar to Figure 1 (data presented in supplemental Table 1 & 2).

Markers of tubular and glomerular damage and early progressive renal decline: multivariate analysis

The association of early progressive renal decline with markers of proximal tubular damage and markers of glomerular damage, considered jointly, was evaluated with logistic regression (Table 5). The serum concentration of TNFR1 at baseline examination was examined because of its previously demonstrated importance[5].

In univariate models, the odds ratio for association of each marker with being a decliner was significant. In multivariate Model #1, these univariate associations were adjusted for clinical covariates: A1c level, baseline eGFRcr-cys, antihypertensive treatment and systolic blood pressure. The odds ratios for each marker were markedly reduced, and that for urinary KIM-1 was not significant. In Model #2, plasma KIM-1 and urinary markers (NAG, albumin and IgG2) were examined jointly (together with clinical covariates), the odds ratio for plasma KIM-1 was unaltered while that for NAG was not significant (OR for a doubling of the concentration=1.01; 95% CI: 0.82; 1.24). Regarding markers of glomerular injury, the odds ratio for albumin remained significant but diminished, and that for IgG2 was not significant. In multivariate Model #3, we included significant markers from multivariate model #2 plus serum TNFR1 (together with clinical covariates). Only the effects of plasma KIM-1 and serum TNFR1 remained significant.

In summary, the odds ratio for plasma KIM-1 was very similar in all models (unaffected by inclusions of other covariates) whereas the odds ratio for urinary albumin declined with inclusion of other covariates and was not significant after inclusion of serum TNFR1. Multivariate Model #3 was repeated in the NA and MA groups separately. The odds ratios for early renal decline for a doubling of plasma of KIM-1 were 1.28 (95%CI: 1.07; 1.54) in NA and 1.35 (95%CI: 1.15; 1.58) in MA. Similar analyses were performed to examine the association of the incidence rate of progression to CKD 3 with the examined markers using Cox analysis. The results for plasma KIM-1 were very similar to those in Table 5 although the effect of other markers was less significant (Data shown in Supplemental Table 3).

For the purpose of evaluating the relevance of plasma KIM-1 for clinical practice, a multivariate logistic model that included clinical predictors (reno-protective treatment, baseline eGFRcr-cys, HbA1c, systolic blood pressure, and serum TNFR1), was compared with a model including all these covariates plus plasma KIM-1. The area under the ROC curve increased from 0.86 to 0.89 (p=0.016).

Discussion

In this study we evaluated the relationship between markers of glomerular and proximal tubule damage and the associations of these markers with risk of early progressive renal decline in non-proteinuric patients with T1D followed for 4-10 years. While all four examined markers were elevated in T1D patients in comparison with non-diabetic controls, they were only weakly or moderately correlated among themselves (Spearman rank

correlation coefficients varied from 0.16 to 0.48). This result indicates that variations in the degree of damage to these two types of kidney structures or compartments are largely independent of each other. Thus we see elevated plasma and urine KIM-1 in patients with normal urinary albumin, and elevated urinary albumin and IgG2 in patients with no evidence of tubular damage. Recognition of this independence of damage to tubular and glomerular compartments in early diabetic nephropathy is novel.

The other important conclusion from our study is that, while all four measured markers are associated with the risk of early progressive renal decline, the associations of plasma KIM-1 and serum TNFR1 dominate, capturing all the information in the other markers. This conclusion held regardless of whether early progressive renal decline was measured as the proportion of patients with an annual eGFR loss of 3.3% (~3.5 ml/min/year) or as the time to CKD stage 3 (eGFRcr-cys 60 ml/min). The similar influence of plasma KIM-1 risk of early progressive renal decline regardless of albuminuria status indicates that early damage to proximal tubules is similarly associated with onset and progression of early renal decline in T1D.

Urinary KIM-1 is an established marker of proximal tubular cell injury. This protein was originally discovered as a highly up-regulated mRNA after acute ischemic kidney injury[13]. Urinary KIM-1 has been implicated as a sensitive and specific urinary marker of kidney injury in both rodent models and humans with acute impaired renal injury[13, 20, 21]. Studies evaluating urinary KIM-1 as a marker of diabetic nephropathy have been inconclusive[19, 22-26]. Due to limitations regarding study designs, statistical power and different definitions of diabetic nephropathy, the findings from these studies are difficult to interpret.

After kidney proximal tubule injury, KIM-1 is released not only into urine but also into the circulation. An elevated circulating level of KIM-1 in patients with T1D and proteinuria is a significant predictor of risk of ESRD[18]. The present study extends the domain of that association to include not only patients with less advanced nephropathy but also to the onset of early renal decline in T1D patients with normoalbuminuria.

It is important to note that the association of plasma KIM-1 with the risk of early progressive renal decline is strong and encompasses the relatively weak effects of the other urinary markers of proximal tubular damage such as urinary KIM-1 and NAG. There are no other published data that examined the role of plasma KIM-1 in the development of early progressive renal decline in T1D. Several studies have examined the effect of urinary L-FABP, another marker of proximal tubular damage, on the development and progression of abnormalities in urinary albumin excretion and on renal decline in T1D and T2D[26-30]. Similar to studies regarding the association of urinary KIM with diabetic nephropathy, the findings from these studies are also difficult to interpret. However, findings derived from the Japanese T2D cohort study showed that the concentration of urinary L-FABP was predictive for renal decline in patients with normoalbuminuria[27]. At this time we do not know whether urinary concentration of L-FABP in our cohort would be as good a predictor of renal decline as in the T2D cohort and be comparable to plasma KIM-1.

The urinary concentration of KIM-1 is less strongly associated with risk of early progressive renal decline. This association disappeared once plasma KIM-1 concentration was included in the analysis. This finding may be interpreted in two different ways. One is that both markers reflect the extent of proximal tubule damage, but the urinary concentration is representative of acute production which may vary over time while the plasma concentration represents an integration of production over time and is perhaps less susceptible to variability in time of collection. The other possibility is that these two markers may reflect different aspects of proximal tubular damage. Whereas the urinary concentration of KIM-1 reflects the extent of proximal tubule damage, the plasma concentration of KIM-1 may also reflect the extent of loss of polarity of KIM-1 expression and, perhaps, other aspects of interstitial abnormalities that may affect leakage of this marker into circulation[18]. It is worth noting that in experimental animals, prolonged KIM-1 expression led to severe tubulointerstitial damage and renal failure[31].

Finally, some strengths and limitations of our study should be acknowledged. First, our study's major strength is the large study group and long follow-up with multiple determinations of eGFRcr-cys which allowed reliable determination of early progressive renal decline. This distinguishes it from reports with follow-up too short for reliable determination of renal decline. However, the eGFRcr-cys equation that we used has not been validated in T1D and may underestimate "hyperfiltration" in T1D. This would reduce the steepness of eGFR slopes and underestimate the true frequency of early progressive renal decline. However, it would not affect the major conclusion that plasma KIM-1, as a marker of proximal tubular damage, is the major predictor of risk of early progressive renal decline. Second, our study is the first comprehensive comparison of the role of markers of glomerular and proximal tubular damage in the development of early progressive renal decline. All the same, however, it is descriptive and hypothesis generating and cannot directly shed any light on the mechanisms of early progressive renal decline in T1D beyond the focus placed on proximal tubule injury. Third, measurements of KIM-1 in this study are different from the measurements in our previous reports[18, 19]. Our current assay utilizes a monoclonal antibody for KIM-1 detection while the previous study's assay used polyclonal detection and polyclonal capture antibodies. These different assays may be responsible for significant differences in KIM-1 values in serum and urine from non-diabetic controls. In this study KIM-1 was undetectable in most controls, whereas in the previous report, KIM-1 was detectable in the plasma and urine of most controls[17]. However, both studies found KIM-1 in plasma to be a significant predictor of renal decline in early and advanced diabetic nephropathy[18].

In conclusion our findings support the view that damage of proximal tubules plays an independent role in the development of early progressive renal decline in non-proteinuric patients with T1D. It is important to further investigate the determinants/mechanisms of elevated KIM-1 in early diabetic nephropathy to develop new preventive and therapeutic programs.

STUDY DESIGN AND METHODS

The Committee on Human Subjects of the Joslin Diabetes Center approved the protocol and informed consent procedures for the 2nd Joslin Kidney Study on the Natural History of Microalbuminuria, referred to here as the 2nd JKS. A description of the Joslin Clinic T1D population, which is 95% Caucasian, and the design of the study were published previously [5, 7, 8]. In the current study we included all patients with baseline NA or MA who were followed for 4-10 years (median 8 years). The results regarding the occurrence of early progressive renal decline in these patients and the impact of systemic factors on the risk of early progressive renal decline were published recently [5].

Study Groups

Briefly, 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 1st phase of the study, January 1, 2003 through December 31, 2006, we recruited T1D patients with microalbuminuria and an equal number of T1D patients with normoalbuminuria. Eligibility criteria included residence in New England, T1D diagnosed before age 40 years and age 18–64 years at the study enrollment. Not eligible for the study were patients with macroalbuminuria (median pre-enrollment AER 300 μg/min), on dialysis, had renal transplant, or had a history of HIV or hepatitis C infection.

For eligible patients 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 to classify them to those with normoalbuminuria (2 year pre-enrollment median AER <30 μg/min) and those with microalbuminuria (2 year pre-enrollment median AER 30-299 μg/min). The methods of determining of ACR and converting it to AER were described previously [5]. The study aimed to enroll eligible patients with MA and a similar number of eligible patients taken randomly from the much larger pool of patients with NA. Patients who had been identified as eligible were invited for the study and they were examined during a routine visit to the Clinic with specimens of blood and urine taken for laboratory determinations and storage in −85 C.

As of the end of 2006, we had examined 304 patients with MA and 363 patients with NA. These patients have been followed with a goal to obtain blood and urine specimens at least every 2 years. Collection of research specimens occurs during patients' routine clinic visits. Patients with less frequent visits to the clinic or those who stopped coming to the clinic were examined at their homes. Specimens obtained during follow-up were stored at −80°C. As of the end of 2012 248 patients with MA (82%) and 286 (79%) of those with NA have been followed for 4 to 10 years (median 8 years). The results regarding the occurrence of early progressive renal decline in these patients have been published recently [5]. For the current study we aimed to use all patients from the previous report, however, the baseline urinary and plasma specimens were available only for 203 patients with MA and 259 patients with NA. Patients with specimens not available were not different from patients for whom the specimens were available with regard to any clinical characteristic.

For comparison of the distribution of KIM-1 markers in T1D with non-diabetics, we used a panel of 77 healthy volunteers. These individuals were parents of T1D patients and were recruited between 2003 and 2006 for genetic studies. At examination patients were 45-74 years of age, had normal renal function and normoalbuminuria. Their blood and urine specimens were stored in −85 C.

Laboratory Measurements

Detailed laboratory protocols to measure baseline urinary concentrations of albumin, creatinine, IgG2, NAG and serum TNFR1, and serum creatinine and cystatin C in specimens obtained at baseline and during follow-up examinations were described in previous publications [5, 7, 12]. The eGFRcr-cys was estimated using the combined creatinine-cystatin C CKD-EPI formula [32] and was used to estimate eGFRcr-cys slopes (see below).

Plasma and urinary KIM-1 concentrations were measured in specimens obtained at enrollment into the 2nd JKS and stored in −80°C until the measurements. Concentrations of KIM-1 in plasma and urine were determined by a particle-enhanced, sandwich-type immunoassay using a detection system based on flow cell fluorometry (Luminex Inc., Billerica, MA). We used Human Magnetic Kidney Biomarker Panel (R&D Systems; Minneapolis, US) with a monoclonal capture antibody specific to the fragment of the KIM-1 peptide chain. The validation parameters for this assay, such as recovery, cross-reactivity, and linearity of sample values upon dilution of samples had been determined by the manufacturer. The KIM-1 concentrations in plasma and in urine, that were not detected by the assay used, were assigned to the LOD value (0.2pg/ml) - for the purpose of the further quantitative statistical analyses, i.e. correlation analysis, logistic regression, COX hazard proportional regression. All assays included technical sample replicates that were frozen at the beginning of the study, transferred to aliquots and used for assay normalization. On this basis, our intra-assay coefficients of variation were 9.2 % for plasma (based on measurements of 16 sample replicates), and 11.5% for urine KIM-1, and inter-assay coefficients of variation were 18.0 % for plasma KIM-1 and 21.1 % for urine KIM-1. All tested samples were treated and stored under the same conditions, and did not undergo repeated freeze-thawing cycles. In the analyses we did find a weak correlation between duration of storage and levels of plasma and urine KIM-1. The Spearman correlation coefficient between time of storage and plasma KIM-1 and urinary KIM-1 was not significant in micro-albuminurics. The correlation between plasma KIM-1 and time of storage was r=−0.1 (p=0.15). Time of storage did not influence the association between plasma KIM-1 and renal function decline in multivariate analyses. In normoalbuminurics, Spearman correlation coefficient between time of storage and plasma KIM-1 was not significant (p=0.4). For urinary creatinine adjusted KIM-1 it was r=−0.22 (p=0.001), and r=−0.24 (p=0.001) without adjustment for creatinine. However, time of storage did not influence the association between urinary KIM-1 and renal function decline or with time to CKD3 in multivariate analyses. The Results presented in this report include only baseline measurements of urinary ACR, IgG2, KIM-1, NAG and plasma KIM-1, and serum TNFR1. Similarly all clinical covariates determined at baseline examination were used in the analyses.

Definition of early progressive renal decline

Early progressive renal decline was defined using two indices: significant eGFR cr-cys loss exceeding 3.3%/year (~ 3 ml/min/year) and progression to CKD Stage 3 defined by an eGFRcr-cys <60ml/min/1.73m^2. The first index was determined by analyzing the longitudinal measures of log transformed eGFRcr-cys values using linear mixed-effects regression (PROC MIXED, SAS 9.3, SAS Institute, Cary, NC). This approach takes into account the correlation between follow-up observations from a patient taken at varying intervals and it yields individual-specific slope coefficients which are re-expressed as percent change per year in eGFRcr-cys[5]. Significant eGFRcr-cys loss was defined as a negative slope equal to or steeper than −3.3%/per year, and the patient is referred to as a "decliner". Patients with less steep slopes are referred to as "non-decliners". This criterion, an eGFRcr-cys loss of 3.3% per year or more, has been used in previous reports [5, 33] and corresponds to the 2.5th percentile of the distribution of annual renal function loss in a general population[34]. The second index was determined by evaluating eGFRcr-cys trajectories over time. If at any time eGFRcr-cys reached 60 ml/min/1.73m^2 patient was classified as one developing Chronic Kidney Disease stage 3 or greater (CKD 3). Time of reaching such value of eGFRcr-cys was considered as the onset of CKD stage 3.

Statistical analysis

The statistical analyses were performed in SAS Version 9.3 (SAS Institute, Cary, NC). Differences in clinical characteristics and renal outcomes across strata of baseline plasma KIM-1 and other markers were tested with Cochran-Armitrage test for categorical variables, with linear trend regression for continuous variables and with log-rank trend test for failure-time outcome. Correlations among the values of tubular, glomerular markers and quantitative clinical characteristics were assessed with Spearman coefficients. Univariate and multiple logistic regression or Cox regression models were used to assess effects of baseline characteristics on risk of early progressive renal decline. The baseline characteristics, such as antihypertensive treatment, sex, BMI, A1c, diabetes duration, systolic blood pressure and eGFRcr-cys were considered potential confounders. We considered a single time point measurement of the markers, including ACR, for the purpose of regression analyses. Only the variables significant at p<0.05 were retained in final models. In addition, we included the baseline concentration of an inflammatory protein; serum TNFR1, a well-known predictor of renal decline, in the regression equation. Marker concentrations were transformed to their logarithms. To assess the improvement in logistic regression model performance accomplished by adding plasma KIM-1 to clinical covariates for renal decline and baseline TNFR1 concentration, we used the difference in area under the ROC curves. All statistical tests were two-tailed and p<0.05 was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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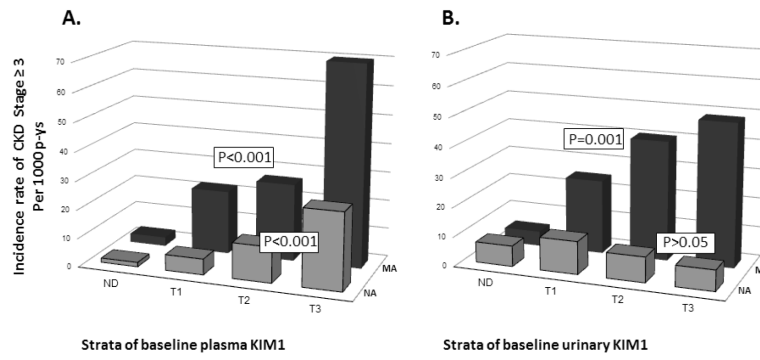
Figure 1.
Incidence rate of CKD 3 per 1000 person years according to baseline concentration of
KIM-1 in plasma (Panel A) and urine (Panel B), shown separately for patients with NA and
MA
(See Supplemental Tables 1 and 2 for numbers of events and person-years.)
P values are for a test for trend.

Table 1

Distribution of baseline plasma and urine concentrations of KIM-1 in study subgroups: non-diabetic controls (NDM), T1D patients with normo-albuminuria (NA) or micro-albuminuria (MA)

Panel A:

	Stratum of baseline plasma KIM-1 *				
	ND	T1	T2	T3	Total
NDM	61.5% [46]	22.4% [17]	15.8% [12]	1.3% [1]	100% [76]
NA	35.1% [91]	28.6% [74]	20.1% [52]	16.2% [42]	100% [259]
MA	20.7% [42]	17.2% [35]	29.6% [60]	32.5% [66]	100% [203]

Panel B:

	Stratum of baseline urine KIM-1 **				
	ND	T1	T2	T3	Total
NDM	40.3% [31]	23.4% [18]	25.9% [20]	10.4% [8]	100% [77]
NA	32.5% [82]	19.5% [49]	25.4% [64]	22.6% [57]	100% [252]
MA	11.1% [22]	32.8% [65]	26.8% [53]	29.3% [58]	100% [198]

[ ] number of individuals

*
Definition of strata of baseline concentration of KIM-1 plasma:

ND: not detectable (<0.2 pg/ml)

T1-T3: tertiles of the distribution of detectable values of plasma KIM-1 in all T1D patients

Cut points for tertiles (33rd and 67th percentiles) were 11 and 21 pg/ml.

**
Definition of strata of baseline concentration of KIM-1 in urine:

ND: not detectable (<0.2 pg/ml)

T1-T3: tertiles of the distribution of detectable values of urinary KIM-1 in all T1D patients

Cut points for tertiles (33rd and 67th percentiles) were 58 and 208 pg per mg of urinary creatinine.

Table 2

Characteristics of non-proteinuric patients with T1D according to stratum of plasma baseline concentration of KIM-1.

Characteristic	Stratum of baseline plasma KIM-1 [*]				p for trend
	ND N=133	T1 N=109	T2 N=110	T3 N=110	
Baseline Data					
Examined markers					
KIM-1 in plasma pg/ml [**]	------	2.1 (0.2; 10.9)	13.9 (11; 20.9)	32.8 (21; 858)	By design
KIM-1 in urine pg/mg creatinine[†]	70 (24; 166)	87 (29; 173)	90 (28; 211)	179(82; 393)	<0.001
NAG in urine U/g creatinine[†]	2.02 (0.67; 3.)	2.16 (1.38; 3.51)	2.69 (1.49; 4.19)	3.92 (2.18; 6.37)	<0.001
ACR in urine ug/mg creatinine[†]	10.3 (6; 23)	12.0 (7; 30)	19.8 (7; 43)	26.8 (10; 77)	<0.001
IgG2 in urine ng/mg creatinine[†]	809 (228; 2493)	755 (315; 2375)	1026 (318; 3152)	1142(286; 4737)	0.02
TNFR1 in serum ng/ml	1.31 (1.10; 1.54)	1.31 (1.13; 1.58)	1.44 (1.20; 1.74)	1.40 (1.19; 1.88)	<0.001
Other characteristics:					
Age (y)	39 (28; 46)	40 (30; 47)	42 (34; 48)	45 (34; 53)	0.002
Duration of DM (y)	20 (13; 29)	20 (14; 28)	24 (15; 31)	23 (15; 30)	n.s.
HbA1c (%)	8.1 (7.4; 8.7)	8.0 (7.3; 8.9)	8.2 (7.4; 9.2)	8.4 (7.7; 9.4)	0.001
HbA1c (mmol/mol)	65 (57; 72)	64 (56; 74)	66 (57; 77)	68 (61; 79)	NA
SysBP (mmHg)	120 (110; 127)	120 (111; 130)	120 (110; 128)	123 (116; 130)	0.035
DiaBP (mmHg)	70 (68; 78)	71 (69; 78)	70 (69; 78)	72 (69; 79)	n.s.
Rx ACE-I & ARB (%)	41	42	56	64	<0.001
eGFRcr-cys ml/min	115 (104; 125)	112 (102; 122)	113 (101; 121)	111 (92; 121)	0.002
Follow-up data					
Duration of follow-up (y)	8 (6; 9)	8 (6; 9)	8 (6; 9)	8 (5; 9)	n.s.
Number of eGFRcr-cys determinations	6 (4; 8)	5 (4; 7)	6 (4; 6)	6 (4; 6)	n.s.
eGFRcr-cys loss in %/y	−1.4 (−2.1; −0.7)	−1.6 (−2.4; −0.1)	−2.0 (−3.1; −1.0)	−3.1 (−5; −1.4)	<0.001
% decliners (eGFRcr-cys loss 3.3%/y)	4.5	13.8	21.4	47.2	<0.001
CKD 3 incidence rate/1000 p-y	2.0 (2/999)[§]	10.3 (8/773)[§]	21.1 (16/760)[§]	54.0 (36/668)[§]	<0.001

[*] For definition of baseline plasma KIM-1 strata see legend to Table 1

[**] Plasma KIM-1 data are median (minimum; maximum). Others are median (25th; 75th percentile) or percent.

†Concentrations of urinary markers were normalized for urinary creatinine concentrations.

§[number of CKD Stage 3 cases/number of person years]

Table 3

Spearman rank correlation coefficients between baseline concentrations of measured markers and relevant clinical characteristics

Baseline	Plasma KIM-1	Serum TNFR1	Urinary KIM-1	Urinary NAG	Urinary Albumin	Urinary IgG2
	Spearman rank correlation coefficients (r_s)					
Plasma KIM-1	NA					
Serum TNFR1	0.16 **	NA				
Urinary KIM-1	0.25 ***	n.s	NA			
Urinary NAG	0.30 ***	n.s.	0.40 ***	NA		
Urinary Albumin	0.30 ***	0.25 ***	0.32 ***	0.41 ***	NA	
Urinary IgG2	0.16 **	0.19 ***	0.38 ***	0.38 ***	0.48 ***	NA
Age	0.15 *	0.27 ***	n.s.	n.s.	n.s.	n.s.
Duration	ns.	n.s.	n.s.	n.s.	n.s.	n.s.
HbA1c	0.17 **	n.s.	n.s.	0.24 **	0.17 **	n.s.
SysBP	n.s.	n.s.	n.s.	0.12	0.14 **	n.s.
DiaBP	n.s.	n.s.	n.s.	n.s.	0.17 **	n.s.
eGFRcr-cys baseline	−0.14 **	−0.55 ***	n.s.	n.s.	n.s.	−0.15 **
Follow-up						
eGFRcr-cys slope	−0.34 ***	−0.40 ***	−0.16 **	−0.21 **	−0.25 ***	−0.22 ***

Concentration of urinary markers was adjusted for urinary creatinine concentration.

*** $P<0.0001$;

** $P<0.001$;

* $P<0.01$

Table 4

Frequency of Decliners (eGFRcr-cys loss ≥ 3.3%/y) according to concentrations of markers of glomerular damage and according to plasma or urinary concentration of KIM-1

Marker of	ND	T1	T2	T3	p for trend
Glomerular damage	*Stratum of baseline plasma KIM-1* *				
			% Decliners		
Urinary Albumin **					
NA	2.2% (2/91)	9.5% (7/74)	13.5% (7/52)	23.8% (10/42)	<0.001
MA	9.5% (4/42)	22.9% (8/35)	28.3% (17/60)	62.1% (41/66)	<0.001
Urinary IgG2 †					
Below median	1.4% (1/72)	9.0% (6/64)	19.2% (10/52)	33.3% (15/45)	<0.001
Above median	9.8% (5/51)	21.4% (9/42)	23.0% (12/52)	56.5% (35/62)	<0.001
	Stratum of baseline urinary KIM-1 *				
			% Decliners		
Urinary Albumin **					
NA	9.8% (8/82) §	12.2% (6/49)	6.3% (4/64)	10.5% (6/57)	n.s.
MA	9.1% (2/22)	36.9% (24/65)	26.4% (14/53)	51.7% (30/58)	< 0.001
Urinary IgG2 †					
Below median	11.3% (9/80)	18.3% (13/78)	6.5% (3/46)	9.7% (4/41)	n.s.
Above median	11.1% (4/36)	32.6% (15/46)	25.0% (18/72)	42.2% (35/83)	0.00

*
For definition of strata of baseline plasma and urinary KIM-1 see legend to Table 1

**
For definition of NA and MA see methods

†
Median IgG2 in urine was 1032 ng per 1 mg of urinary creatinine

§
(number of decliners/number of patients at risk)

Table 5

Logistic regression analysis of the risk of being a Decliner according to baseline concentrations of markers of tubular and glomerular damage in non-proteinuric patients with T1D

	Univariate Model	Multivariate Model #1	Multivariate Model #2	Multivariate Model #3
		Odds Ratio (95% CI) for a doubling of the marker concentration		
Marker of tubular damage:				
Plasma KIM-1	1.43 (1.29; 1.60)	1.35 (1.20; 1.51)	1.32 (1.17; 1.47)	1.33 (1.19; 1.49)
Urinary KIM-1 *	1.12 (1.06; 1.18)	n.s.	NA	NA
Urinary NAG *	1.43 (1.21; 1.70)	1.23 (1.02; 1.48)	n.s.	NA
Marker of glomerular damage:				
Urinary Albumin *	1.67 (1.47; 1.90)	1.42 (1.22; 1.65)	1.26 (1.05; 1.51)	n.s.
Urinary IgG2 *	1.26 (1.17; 1.37)	1.17 (1.07; 1.28)	n.s	NA
		Odds Ratio (95% CI) for an increase of one quartile of the concentration of TNFR1		
Serum TNFR1	3.83 (2.77; 5.31)	2.64 (1.73; 4.03)	NA	2.01 (1.24; 3.26)

*
Concentrations of urinary markers were adjusted for urinary creatinine concentration.

The non-detectable concentrations of markers were extrapolated to the values of limit of detection for the respective marker

Univariate Model – odds ratio for each marker without consideration of other covariates.

Multivariate Model #1 – odds ratio for each marker after adjustment for clinical covariates such as eGFRcr-cys, HbA1c, antihypertensive treatment, and systolic blood pressure.

Multivariate Model #2 – odds ratio for each marker after adjustment for significant clinical covariates (as above) and other significant markers excluding serum TNFR1.

Multivariate Model #3 – odds ratio for markers which remained significant after adjustment for clinical covariates (as above) and inclusion of serum TNFR1.