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Circulating kidney injury molecule-1 as a biomarker of renal parameters in diabetic kidney disease

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ABSTRACT

Aims/Introduction: Urinary kidney injury molecule-1 (KIM-1) has been associated with proximal tubular damage in human and animal studies. Although it has been recognized as a biomarker of acute kidney injury and chronic kidney disease, its significance in the serum remains unclear. Therefore, we examined the relationship of serum and urinary KIM-1 levels with renal parameters in patients with type 2 diabetes.

Materials and Methods: Serum and urinary KIM-1 levels, together with urinary liver-type fatty acid-binding protein, were measured in 602 patients with type 2 diabetes and an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2. These were then compared with the urinary albumin-to-creatinine ratio and eGFR.

Results: The serum and urinary KIM-1 levels were significantly different among the three (eGFR ≥60, 45–59, <45 mL/min/1.73 m^2) groups. These levels were positively associated with the albumin-to-creatinine ratio and negatively associated with eGFR. In a multivariate logistic model, both serum and urinary KIM-1 were associated with an increased albumin-to-creatinine ratio (>30 mg/g Cr), but only the serum KIM-1 was associated with a lower eGFR (<60 mL/min/1.73 m^2), after adjustment for covariates.

Conclusions: Renal parameters appear to be strongly associated with serum KIM-1, and not urinary KIM-1, in patients with type 2 diabetes and an eGFR ≥30 mL/min/1.73 m^2.

INTRODUCTION

Kidney injury molecule-1 (KIM-1) is a type I transmembrane glycoprotein that is extensively expressed at the apical membranes of proximal tubular epithelial cells during tissue regeneration after ischemic or toxic acute kidney injury, as well as when tubular epithelial cells are dedifferentiated[1,2]. Cleavage of KIM-1 by matrix metalloproteinases from the cell surface results in the release of soluble KIM-1 ectodomain into the urine[3]. Whereas KIM-1 induced by acute tubular damage has anti-inflammatory effects through phagocytosis, chronic overexpression in tubular cells leads to inflammation and interstitial fibrosis[4,5]. Indeed, there is now mounting evidence that urinary KIM-1 is a useful biomarker of tubular damage[6,7].

It was also recently reported that KIM-1 is detected in the serum, where it might serve as a biomarker of kidney damage[8]. It was primarily assumed that KIM-1 appears in the circulation due to a lack of tubular cell polarity and increased transepithelial permeability after tubular damage[8]. However, there have been conflicting findings regarding the usefulness of urinary KIM-1 compared with serum KIM-1 as a biomarker of progression in diabetic kidney disease (DKD)[8–15]. The relationship between these potential biomarkers and the urinary albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) are also unclear in patients with type 2 diabetes mellitus, because most studies have not measured both the serum and urinary KIM-1 levels at the same time.

In the present study, we therefore measured serum and urinary KIM-1 levels in addition to urinary liver-type fatty acid-binding protein (L-FABP), another marker of renal proximal

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tubular damage, in patients with type 2 diabetes mellitus and an eGFR ≥30 mL/min/1.73 m^2 to clarify the relationship between these parameters.

METHODS

Study design

The ethics committee of Kure Medical Center and Chugoku Cancer Center in Hiroshima, Japan approved this cross-sectional study. Japanese patients with type 2 diabetes mellitus were recruited for observation of the natural course of DKD at Kure Medical Center and Chugoku Cancer Center between 1 July 2014 and 31 March 2016. We excluded patients with type 1 diabetes mellitus, secondary diabetes and an eGFR <30 mL/min/1.73 m^2 (stage 4–5 chronic kidney disease). Informed consent was obtained from all patients, and the study complied with the guidelines of the Declaration of Helsinki.

Each patient's baseline anthropometric and clinical characteristics were recorded, including serum and urinary parameters. The eGFR was calculated using the following equation, specific to the Japanese population: eGFR (mL/min/1.73 m^2) = 194 × (age [years])$^{-0.287}$ × (serum creatinine [Cr; mg/dL])$^{-1.904}$ (×0.739 for women). Baseline serum and urinary samples were obtained and stored at −80°C until use.

Laboratory measurements

We used enzyme-linked immunosorbent assays to measure serum and urinary KIM-1, respectively (cat. nos. DKM 100, DSKM 100; R&D Systems, Minneapolis, MN, USA) and urinary L-FABP (Renischem® L-FABP ELISA high sensitivity kit; CIMIC Holdings Co., Ltd., Tokyo, Japan). Two internal serum or urinary controls were included in each assay to estimate the interassay coefficient of variation. The interassay coefficients of variation for the serum and urinary KIM-1 levels were <10%, with values of 7.3% for serum KIM-1 and 8.7% for urinary KIM-1.

Uric acid (UA), lipids and hemoglobin A1c (HbA1c) were measured at Kure Medical Center and Chugoku Cancer Center using routine laboratory methods. Non-high-density lipoprotein cholesterol (non-HDL-C) levels were defined as the difference between total cholesterol and HDL-C levels. Urinary albumin was quantified by a nephelometry assay (N-assay TIA Micro Alb; Nittobo Medical Co., Ltd., Fukushima, Japan) and Cr by an enzymatic method. The ACR was then expressed in milligrams per gram of Cr (mg/g·Cr).

Statistical analysis

Statistical analysis was carried out using the SAS 9.4 software (SAS Institute, Cary, NC, USA). A two-sided *P*-value <0.05 was considered statistically significant. All categorical variables are expressed as percentages, and all continuous variables are expressed as the mean ± standard deviation or median (interquartile range). For analysis, patients were stratified based on their eGFR (<60 or ≥60 mL/min/1.73 m^2) or ACR [<30, 30–299 or ≥300 mg/g·Cr, representing normoalbuminuria, microalbuminuria and macroalbuminuria, respectively). Differences between groups were analyzed by *t*-tests for continuous variables, and χ^2-tests for dichotomous variables or by one-way analysis of variance for more than two groups. Pearson's correlation was used to assess associations among the two renal parameters (ACR and eGFR) and the three tubular damage biomarkers (serum KIM-1, urinary KIM-1 and L-FABP).

Univariate logistic regression analyses were used to examine the factors associated with lower eGFR, before multivariate logistic regression to evaluate the association of tubular damage biomarkers with lower eGFR. Candidate covariates were selected for the adjusted models as follows: age and sex were included based on biological plausibility; traditional confounders of DKD, such as HbA1c and ACR, were included based on prior study results; and covariates (type 2 diabetes mellitus duration odds ratio [OR] 1.05, 95% confidence interval [CI] 1.04–1.07, $P < 0.001$; hemoglobin OR 0.70, 95% CI 0.62–0.78, $P < 0.001$; UA OR 1.81, 95% CI 1.55–2.11, $P < 0.001$; and diastolic blood pressure (DBP) OR 0.97, 95% CI 0.95–0.98, $P < 0.001$) that were significant in the univariate logistic regression analyses. Bonferroni correction was applied for covariate selection (13 covariates; *P*-value <0.0038). After Bonferroni correction was applied, the basic model eventually comprised eight covariates (i.e., age, sex, type 2 diabetes mellitus duration, DBP, hemoglobin, UA, HbA1c and ACR). We finally selected candidate covariates for the risk of increased ACR, similar to that in lower eGFR. We added eGFR and systolic blood pressure, and removed ACR, DBP and hemoglobin from the basic model.

To examine the additive benefit of tubular damage biomarkers as predictors of lower eGFR or increased ACR, compared with the basic model alone, each biomarker was then added to the model individually and tested pairwise.

RESULTS

Baseline characteristics

A total of 602 patients with type 2 diabetes mellitus (age 65 ± 13 years; 335 men) with available baseline serum and urine data are included in the present study. The median (25th–75th percentile) eGFR and ACR were 69 mL/min/1.73 m^2 (55–84 mL/min/1.73 m^2) and 23 mg/g·Cr (9–118 mg/g·Cr), respectively. Table 1 and Supplementary Table S1 show their clinical and demographic characteristics stratified by eGFR and ACR. There were no differences in body mass index, systolic blood pressure and non-HDL-C among the three (eGFR ≥60, 45–59, <45 mL/min/1.73 m^2) groups. Patients with decreased renal function tended to be older and male, and to have higher ACR and UA levels, longer type 2 diabetes mellitus duration, and lower DBP, HDL-C, hemoglobin and HbA1c levels. The levels of serum KIM-1, urinary KIM-1 and L-FABP increased as eGFR declined. The serum KIM-1 and L-FABP levels in the eGFR <45 mL/min/1.73 m^2 group were also significantly higher than those in the eGFR ≥60 mL/min/1.73 m^2 group or the eGFR 45–59 mL/min/1.73 m^2 group, although there was no statistical difference in the levels of urinary KIM-1 between the groups after Bonferroni correction (eGFR ≥60 vs

Table 1 | Characteristics of the study group by estimated glomerular filtration rate level

Characteristic	eGFR ≥60 mL/min/1.73 m^2 (n = 406)	eGFR 45–59 mL/min/1.73 m^2 (n = 131)	eGFR <45 mL/min/1.73 m^2 (n = 65)	P
ACR (mg/g·Cr)	19 (8–55)	33 (12–217)	179 (21–781)	<0.001
eGFR (mL/min/1.73 m^2)	80 (69–91)	53 (48–57)	40 (36–43)	<0.001
Age (years)	62 ± 13	71 ± 10	72 ± 10	<0.001
Male sex (%)	52.2	65.7	56.9	0.03
Duration (years)	14 ± 10	19 ± 11	22 ± 11	<0.001
BMI (kg/m^2)	25.3 ± 4.9	24.6 ± 3.8	25.1 ± 4.0	0.46
SBP (mmHg)	138 ± 16	140 ± 19	142 ± 22	0.20
DBP (mmHg)	79 ± 11	75 ± 12	77 ± 13	<0.001
UA (mg/dL)	5.0 ± 1.2	5.8 ± 1.2	6.3 ± 1.3	<0.001
HDL-C (mg/dL)	53 ± 13	51 ± 14	48 ± 12	<0.001
Non-HDL-C (mg/dL)	131 ± 33	126 ± 32	127 ± 32	0.19
Hemoglobin (g/dL)	13.9 ± 1.6	13.2 ± 1.7	12.4 ± 1.7	<0.001
HbA1c (%)	7.4 ± 1.1	7.2 ± 1.1	7.3 ± 1.2	0.10
L-FABP (mg/g·Cr)	3.5 (2.2–5.6)	4.4 (2.7–12.1)	11.1 (5.0–26.9)	<0.001
KIM-1 in urine (ng/g·Cr)	1.28 (0.77–1.96)	1.26 (0.81–2.10)	1.56 (1.01–2.14)	0.047
KIM-1 in sera (pg/mL)	96 (62–151)	145 (92–216)	207 (124–341)	<0.001

Data are the mean ± standard deviation, median (quartiles), or %. ACR, urinary albumin-to-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; SBP, systolic blood pressure; UA, uric acid.

eGFR 45–59 mL/min/1.73 m^2 group or eGFR 45–59 vs eGFR <45 mL/min/1.73 m^2).

Correlation among KIM-1, L-FABP, eGFR and ACR

Table 2 shows the association between the tubular damage biomarkers (serum KIM-1, urinary KIM-1 and L-FABP) and main renal parameters (eGFR and ACR). All tubular damage biomarkers were significantly associated both with eGFR and ACR. The correlations between serum KIM-1 and ACR (r = 0.51) or eGFR (r = –0.37) were stronger than that between urinary KIM-1 and ACR (r = 0.28) or eGFR (r = –0.10). All biomarkers were also associated with each other (L-FABP vs urinary KIM-1, r = 0.16; L-FABP vs serum KIM-1, r = 0.41; and urinary KIM-1 vs serum KIM-1, r = 0.43).

Table 2 | Pearson's correlation coefficients among the tubular damage biomarkers and main renal parameters

Characteristic	eGFR	L-FABP	uKIM-1	sKIM-1
ACR	–0.26*	0.67*	0.28*	0.51*
eGFR	–	–0.31*	–0.10**	–0.37*
L-FABP	–	–	0.16*	0.41*
uKIM-1	–	–	–	0.43*

*P < 0.0001, **P < 0.05. ACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein. The s and u prefixes denote serum and urinary sources, respectively.

Tubular damage biomarkers as predictors of eGFR or ACR severity

Age, hemoglobin, UA and ACR remained significant clinical predictors of lower eGFR (<60 mL/min/1.73 m^2) in the multivariate analysis (Table 3, model 1). Next, we assessed the independent effect of each biomarker on the risk of lower eGFR by adding them to model 1 with the influential clinical predictors (models 2–4) (Table 4). In these models, L-FABP (model 2) and serum KIM-1 (model 4) remained significant. We then assessed the independent effect of L-FABP and serum KIM-1 by adding them to model 1 to assess the risk of lower eGFR

Table 3 | Multivariate analysis of estimated glomerular filtration rate predictors in type 2 diabetes mellitus by clinical factors

Units of increase	Multivariate model 1	
	OR (95% CI)	P
Age (1 year)	1.08 (1.05–1.11)	<0.0001
Sex	1.39 (0.85–2.27)	0.19
Hemoglobin (g/dL)	0.71 (0.61–0.82)	<0.0001
Duration of diabetes (1 year)	1.02 (0.999–1.04)	0.07
HbA1c (1%)	0.80 (0.63–1.001)	0.051
UA (1 mg/dL)	1.99 (1.64–2.97)	<0.0001
DBP (1 mmHg)	0.99 (0.97–1.01)	0.50
ACR (1 SD = 0.76)	1.82 (1.45–2.29)	<0.0001

ACR, urinary albumin-to-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; OR, odds ratio; SD, standard deviation; T2DM, type 2 diabetes mellitus; UA, uric acid.

Table 4 | Multivariate analysis of estimated glomerular filtration rate predictors in type 2 diabetes mellitus by clinical factors and biomarkers

Units of increase	Multivariate models 2–6†	
	OR (95% CI)	*P*
Model 2: model 1 + L-FABP (1 SD = 0.45)	1.37 (1.02–1.84)	0.03
Model 3: model 1 + uKIM-1 (1 SD = 0.38)	0.86 (0.68–1.10)	0.24
Model 4: model 1 + sKIM-1 (1 SD = 0.33)	1.50 (1.14–1.99)	0.004
Model 5: model 1 + L-FABP (1 SD = 0.45)	1.35 (1.01–1.82)	0.045
+ sKIM-1 (1 SD = 0.33)	1.47 (1.12–1.92)	0.006

†The effect of each biomarker was examined separately and with pairwise combinations while controlling for clinical predictors. CI, confidence interval; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; OR, odds ratio; SD, standard deviation. The *s* and *u* prefixes denote serum and urinary sources, respectively.

Table 5 | Multivariate analysis of microalbuminuria predictors in type 2 diabetes mellitus by clinical factors

Units of increase	Multivariate model 1	
	OR (95% CI)	*P*
Age (1 year)	0.98 (0.96–0.998)	0.03
Sex	1.16 (0.81–1.65)	0.43
Duration of diabetes (1 year)	1.03 (1.01–1.05)	0.001
HbA1c (1%)	1.30 (1.11–1.52)	0.001
UA (1 mg/dL)	1.15 (0.994–1.34)	0.06
SBP (1 mmHg)	1.02 (1.01–1.03)	<0.0001
eGFR (1 SD = 0.14)	0.66 (0.52–0.83)	<0.0001

CI, confidence interval; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; SD, standard deviation; SBP, systolic blood pressure; UA, uric acid.

Table 6 | Multivariate analysis of microalbuminuria predictors in type 2 diabetes mellitus by clinical factors and biomarkers

Units of increase	Multivariate models 2–6†	
	OR (95% CI)	*P*
Model 2: model 1 + L-FABP (1 SD = 0.45)	3.88 (2.93–5.14)	<0.0001
Model 3: model 1 + uKIM-1 (1 SD = 0.38)	1.84 (1.47–2.31)	<0.0001
Model 4: model 1 + sKIM-1 (1 SD = 0.33)	2.26 (1.81–2.83)	<0.0001
Model 5: model 1 + L-FABP (1 SD = 0.45)	3.63 (2.71–4.86)	<0.0001
+ sKIM-1 (1 SD = 0.33)	1.98 (1.54–2.55)	<0.0001
Model 6: model 1 + L-FABP (1 SD = 0.45)	3.78 (2.84–5.03)	<0.0001
+ uKIM-1 (1 SD = 0.38)	1.63 (1.29–2.05)	<0.0001
Model 7: model 1 + L-FABP (1 SD = 0.45)	3.63 (2.70–4.87)	<0.0001
+ sKIM-1 (1 SD = 0.33)	1.75 (1.33–2.29)	<0.0001
+ uKIM-1 (1 SD = 0.38)	1.35 (1.06–1.72)	0.02

†The effect of each biomarker was examined separately and with pairwise combinations while controlling for clinical predictors. CI, confidence interval; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid-binding protein; OR, odds ratio; SD, standard deviation. The *s* and *u* prefixes denote serum and urinary sources, respectively.

(Table 4, model 5). Elevated levels of both L-FABP and serum KIM-1 were significantly associated with the risk of lower eGFR, independent of all relevant covariates. We further carried out analysis to identify predictors of eGFR <45 mL/min/1.73 m^2. L-FABP (OR 1.45, 95% CI 0.99–2.15, $P = 0.05$) and serum KIM-1 (OR 1.43, 95% CI 0.98–2.10, $P = 0.06$)] were almost, but not quite, significant after adjustment for relevant clinical covariates (data not shown).

As with the eGFR, many clinical covariates were associated with an ACR ≥30 mg/g·Cr in the univariate logistic regression analysis. Notably, age, type 2 diabetes mellitus duration, HbA1c, systolic blood pressure and eGFR remained significant in the multivariate analysis of clinical predictors (Table 5, model 1). When we then assessed the independent effect of each tubular biomarker on the risk of worsened albuminuria by adding them to model 1 (Table 6), each biomarker remained significant in the presence of the clinical predictors (models 2–4). To assess the independent effects of serum and urinary KIM-1 for predicting increased albuminuria, we then added each of these to model 2 to assess risk (models 5 and 6, respectively). We noted that elevated levels of both serum and urinary KIM-1 were significantly associated an increased risk of albuminuria despite the other clinical predictors and L-FABP.

In the final model, we assessed the ability of all tubular damage biomarkers to predict increased albuminuria by adding them simultaneously to model 1 (Table 6, model 7). Elevated levels of each tubular damage biomarker were significantly associated with a greater risk of increased albuminuria. In particular, serum KIM-1 appeared to be a more useful predictor of increased albuminuria than urinary KIM-1.

DISCUSSION

In the present large cross-sectional study, we showed that two renal parameters (eGFR and ACR) were more strongly associated with serum KIM-1 than with urinary KIM-1 in Japanese patients with type 2 diabetes mellitus and an eGFR ≥30 mL/min/1.73 m^2 (i.e., chronic inflammatory state). Furthermore, after adjustment for relevant clinical covariates, serum KIM-1 remained independently associated with both renal parameters, whereas urinary KIM-1 was only associated with the ACR.

Urinary KIM-1 has reportedly been independently associated with ACR and eGFR in a large cross-sectional study of five combined cohorts (i.e., chronic renal insufficiency cohort [CRIC], atherosclerosis risk in communities, prospective investigation of the vasculature in Uppsala seniors, Pima Indian cohort and Uppsala longitudinal study of adult men), covering patients with a wide range of kidney function[16]. Consistent with the present study, albuminuria was strongly associated with an elevated urinary KIM-1 in all five cohorts. However, a significant association between urinary KIM-1 and eGFR was only observed in the CRIC cohort, which uniquely comprised patients with chronic kidney disease (typically advanced stage).

The other cohorts included comparatively more patients without chronic kidney disease or who had diabetes and glomerular hyperfiltration. Thus, the association between urinary KIM-1 and eGFR appears to be weak when compared with that between urinary KIM-1 and ACR, especially in individuals with well-maintained eGFR.

In a longitudinal study, baseline urinary KIM-1 was reported to predict progression to end-stage renal disease or faster decline in eGFR in patients with type 1 diabetes mellitus and macroalbuminuria[10,12]. However, both studies also showed that urinary KIM-1 was no longer a predictor after adjustment for known risk factors, such as albuminuria. In Pima Indians with type 2 diabetes mellitus, urinary KIM-1 also failed to predict progression to end-stage renal disease, although L-FABP did predict progression[17]. By contrast, Vaidya *et al.*[11] showed that a lower urinary KIM-1 level was associated with regression to normoalbuminuria in patients with type 1 diabetes mellitus and microalbuminuria.

In other research, Sabbisetti *et al.*[8] showed that serum and urinary KIM-1 levels were elevated in mouse models of acute and chronic kidney injury induced by renal bilateral ischemia-reperfusion injury and unilateral ureteral obstruction, respectively. Both levels were also elevated in a rat model of toxin-induced injury to the proximal tubule. Furthermore, baseline serum KIM-1 was associated with declining renal function and predicted progression to end-stage renal disease in a relatively small number of patients with type 1 diabetes mellitus and macroalbuminuria. Thus, they argued that serum KIM-1 reflected the integrated consequence of proximal tubular injury over time. Since then, the usefulness of serum KIM-1 has been confirmed as a predictor of renal functional decline in cohorts with both early DKD (second Joslin kidney study, action to control cardiovascular risk in diabetes) and advanced DKD (veterans affairs nephropathy in diabetes)[9,15].

Another marker of tubulointerstitial damage, L-FABP[18], is also independently associated with ACR and eGFR, as we showed with the serum KIM-1 in the present study. However, in contrast to serum KIM-1, it appeared that L-FABP was more strongly associated with the ACR than with the eGFR. Although an increased ACR is generally considered a marker of glomerular damage, it is also partly considered a consequence of impaired renal tubular reabsorption. This is because albumin that passed across the glomerular basement membrane is reabsorbed in the proximal tubule. Given the strong association between L-FABP and serum KIM-1, rather than urinary KIM-1, it is plausible that the serum KIM-1 level is associated with not only glomerular damage, but also tubular damage in DKD.

One might think that urinary markers reflect tubular damage more directly; however, serum KIM-1 is more strongly associated with eGFR than urinary KIM-1 in the present study. Nowak *et al.*[15] speculated that urinary KIM-1 reflects the current damage of proximal tubular cell, whereas serum KIM-1 reflects the integration of production from proximal tubular cell over time. Therefore, serum KIM-1 might more precisely reflect both albuminuria and eGFR than urinary KIM-1.

How can both urinary and serum KIM-1 be strongly associated with albuminuria than with eGFR? In patients with classical diabetic nephropathy, persistent albuminuria affects proximal injury and is followed by GFR decline as a result of interstitial injury, which represents long-term renal damage. An increase in urinary KIM-1 might perceptively reflect proximal tubular injury, and this increase starts from an extremely early stage of DKD. Serum KIM-1 might also reflect interstitial injury as a result of accumulated proximal tubular injury. Therefore, serum KIM-1 increased linearly, although urinary KIM-1 did not increase linearly, as GFR declined in the present study.

The main limitation of the present study was its cross-sectional design, which precluded conclusions about temporal relations or mechanisms of action. Another limitation was that we estimated renal function from creatinine-based prediction equations, because direct radioisotope GFR measurements would have been difficult to accomplish in such a large cohort. However, a notable strength is that we measured and compared both serum and urinary KIM-1 with other recognized measures.

In conclusion, the present study provides clinical evidence that serum and urinary KIM-1 levels are associated with renal parameters of declining kidney function in a relatively large number of Japanese patients with type 2 diabetes mellitus, with the association being strongest for serum KIM-1.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Patients' characteristics by albuminuria level.