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Plasma Kidney Injury Molecule 1 in CKD: Findings From the Boston Kidney Biopsy Cohort and CRIC Studies

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Authors' Contributions: Study concept and design: IMS, AS, SSW; adjudication of histopathology: IES, HGR; measured plasma KIM-1 in CRIC and BKBC samples: VS, JVB; statistical analysis: IMS, AS, SSW; data interpretation: IMS, AS, VS, GMM, JH, JC, JK, JT, ACR, C-yH, PLK, KDL, TEM, RGN, RSV, DX, XZ, RP, IES, HGR, HIF, JVB, SSW. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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Abstract

Rationale & Objective: Plasma kidney injury molecule-1 (KIM-1) is a sensitive marker of proximal tubule injury, but its association with risks of adverse clinical outcomes across a spectrum of kidney diseases is unknown.

Study Design: Prospective, observational cohort study.

Setting & Participants: 524 individuals undergoing clinically indicated native kidney biopsy with biopsy specimens adjudicated for semiquantitative scores of histopathology by two kidney pathologists enrolled into the Boston Kidney Biopsy Cohort (BKBC) Study and 3,800 individuals with common forms of chronic kidney disease (CKD) enrolled into the Chronic Renal Insufficiency Cohort (CRIC) Study.

Exposure: Histopathologic lesions and clinicopathologic diagnosis in cross-sectional analyses, baseline plasma KIM-1 in prospective analyses.

Outcomes: Baseline plasma KIM-1 in cross-sectional analyses, kidney failure (defined as initiation of kidney replacement therapy) and death in prospective analyses.

Analytical Approach: Multivariable-adjusted linear regression models tested associations of plasma KIM-1 with histopathologic lesions and clinicopathologic diagnoses. Cox proportional hazards models tested associations of plasma KIM-1 with future kidney failure and death.

Results: In the BKBC Study, higher plasma KIM-1 levels were associated with more severe acute tubular injury, tubulointerstitial inflammation, and more severe mesangial expansion after multivariable adjustment. Participants with diabetic nephropathy, glomerulopathies, and tubulointerstitial disease had significantly higher plasma KIM-1 levels after multivariable adjustment. In the BKBC Study, 124 participants progressed to kidney failure and 85 participants died during a median follow-up time of 5 years. In the CRIC Study, 1153 participants progressed to kidney failure and 1356 participants died during a median follow-up time of 11.5 years. In both cohorts, each doubling of plasma KIM-1 was associated with an increased risk of kidney failure after multivariable adjustment (BKBC: HR 1.19, 95% CI 1.03 to 1.38 and CRIC: HR 1.10, 95% CI 1.06 to 1.15). There was no statistically significant association of plasma KIM-1 with death in either cohort.

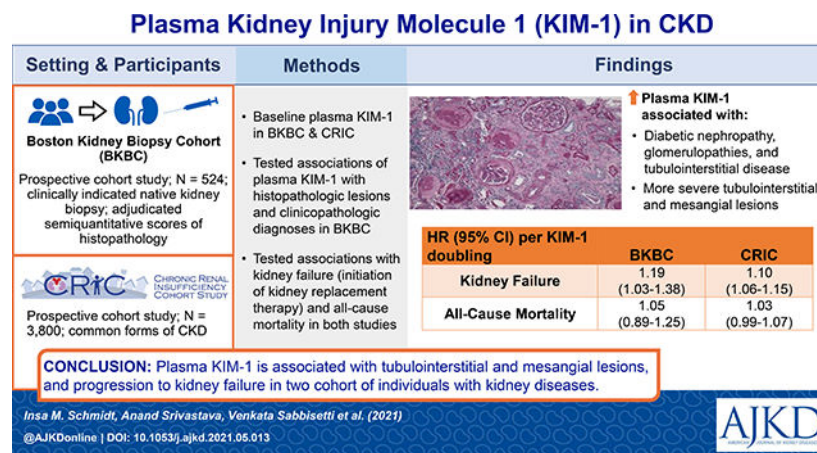
Limitations: Generalizability and unmeasured confounding.

Conclusions: Plasma KIM-1 is associated with underlying tubulointerstitial and mesangial lesions and progression to kidney failure in two cohort studies of individuals with kidney diseases.

Plain-language Summary:

Kidney tubular injury may lead to the development or progression of chronic kidney disease (CKD). Plasma KIM-1 is a sensitive marker of tubular injury, but its association with adverse clinical outcomes across a spectrum of kidney diseases is not known. In two prospective cohort studies of individuals with common and diverse forms of CKD, higher plasma KIM-1 levels were independently associated with progression to kidney failure. In individuals who underwent a native kidney biopsy with adjudicated histopathology, higher plasma KIM-1 levels were associated with more severe acute tubular injury, tubulointerstitial inflammation, and mesangial expansion. Collectively, the findings suggest that plasma KIM-1 may serve as a non-invasive tool to assess histopathologic lesions and has prognostic value across a variety of kidney diseases.

Graphical Abstract



Introduction

Kidney injury molecule-1 (KIM-1), originally identified as hepatitis A virus receptor (HAVCR1, also known as TIM-1), is a type 1 transmembrane glycoprotein strongly induced by ischemic and toxic insults to the kidney.¹⁻³ In healthy kidneys, KIM-1 is virtually undetectable. In both acute kidney injury (AKI) and chronic kidney disease (CKD), KIM-1 expression is markedly upregulated in proximal tubules.⁴⁻⁷ Animal models suggest that acute upregulation of KIM-1 appears to be protective after kidney insults, whereas chronic upregulation of KIM-1 tubular expression may lead to progressive kidney fibrosis.^{8,9}

KIM-1 expression has been demonstrated in urine samples and kidney biopsy specimens of patients with various etiologies of kidney disease.¹⁰ In animal and human studies, kidney tissue expression of KIM-1 was present in areas of inflammation and fibrosis and associated with the presence of interstitial macrophages.^{1, 10, 11} Since the ectodomain of KIM-1 is cleaved by metalloproteinases and detectable in the urine after kidney damage,⁶ the majority of the literature has focused on the investigation of urinary KIM-1.^{2, 12-24} While more

recent studies suggest that plasma KIM-1 is a risk factor for CKD progression,^{25–29} less is known about the associations of plasma KIM-1 with histopathologic lesions and the risks of adverse clinical outcomes across a spectrum of kidney diseases. To address this, we measured plasma KIM-1 levels in participants from two independent CKD cohort studies, the Boston Kidney Biopsy Cohort (BKBC) and the Chronic Renal Insufficiency Cohort (CRIC). We hypothesized that higher levels of plasma KIM-1 are associated with underlying histopathologic lesions, clinicopathologic diagnoses, and increased risks of subsequent progression to kidney failure and death.

Methods

Study Design and Oversight

The institutional review boards at the participating hospitals of each study approved the study protocol, which is in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent. The design and methods of both studies have been described previously.^{30, 31}

BKBC Study

Study Population—The BKBC Study is a prospective, observational cohort study of individuals undergoing native kidney biopsy at three tertiary care hospitals in Boston, MA. The study includes adults (> 18 years of age) who underwent a clinically indicated native kidney biopsy between September 2006 and June 2016. Exclusion criteria included the inability to provide written consent, severe anemia, pregnancy, and enrollment in competing studies. Measurements of KIM-1 were made in baseline plasma samples of 524 participants collected on the day of native biopsy.

Evaluation of Histopathology—Methods to evaluate and score histopathologic lesions in the BKBC Study were previously described.³¹ Briefly, kidney biopsies were adjudicated under light microscopy by two experienced kidney pathologists who provided semiquantitative scores of kidney inflammation, fibrosis, vascular sclerosis, and tubular injury (Supplemental Table 1). The kidney pathologists reviewed the light microscopy slides in joint pathology review sessions and verbally suggested their score for each individual histopathologic lesion severity in the presence of A.S. and/or S.S.W. If they did not initially agree, the case was discussed in more detail to obtain consensus on each individual histopathologic lesion severity. Of the 13 histopathologic lesions adjudicated, all were scored during study sessions except for grades of global or segmental glomerulosclerosis, which were taken from the biopsy report, because they were each calculated as a percentage of the total number of glomeruli. We dichotomized endocapillary glomerular inflammation, extracapillary cellular crescents, focal glomerular necrosis, and fibrocellular crescents into present/absent and then further created a single dichotomous variable named ‘glomerular inflammation’ due to the relatively low prevalence and limited range of severity for each lesion in this cohort. Glomerular inflammation was considered present if any of these lesions were present. All participants’ charts were reviewed alongside the histopathologic evaluations to provide the final primary clinicopathologic diagnosis.

Outcomes—The primary outcome was progression to kidney failure, defined as initiation of kidney replacement therapy (dialysis or kidney transplantation). The secondary outcome was death. Kidney replacement therapy status was confirmed by reviewing the electronic medical record (EMR) and linkage with the United States Renal Data System (USRDS).³¹ Mortality status was confirmed with the Social Security Death Index. Participants were followed up until the occurrence of death, voluntary study withdrawal, loss to follow-up, or February 1, 2020.

Ascertainment of Covariates—We collected participants' information at the biopsy visit, including demographics, medical history, medication lists, and pertinent laboratory data. All data were stored at the REDCap electronic data capture tools hosted at Partners Health Care.³² We used the creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation to calculate eGFR.³³ We obtained serum creatinine from the EMR on the day of biopsy. In participants for whom this was unavailable, we measured serum creatinine in blood samples collected on the day of biopsy. We obtained spot urine protein-to-creatinine ratio or urine albumin-creatinine ratio (UACR) from the date of kidney biopsy up to 3 months before biopsy from the EMR. If a participant did not have either of these values, we measured UACR from urine collected on the day of kidney biopsy. Serum and urine creatinine were measured using a Jaffe-based method, and urine albumin was measured by an immunoturbidometric method.

CRIC Study

Study Population—The CRIC Study is a multicenter, prospective, observational cohort study of individuals with CKD that was designed to investigate risk factors for progression of CKD, development of cardiovascular disease, and mortality. During Phase 1, the CRIC Study enrolled 3939 men and women aged 21 to 74 years between June 2003 and September 2008 across seven clinical centers in the United States. Individuals were included if they met specific age-defined criteria for estimated glomerular filtration rate (eGFR) of 20 to 70 ml/min/1.73m². Black and Hispanic participants were oversampled. Exclusion criteria included inability to provide consent, institutionalization, New York Heart Association class III or IV congestive heart failure, human immunodeficiency virus infection, cirrhosis, multiple myeloma, kidney cancer, recent chemotherapy or immunosuppressive therapy, polycystic kidney disease, organ transplantation, or prior treatment with dialysis for at least 1 month.³⁰ CRIC Study participants were followed annually by clinic visits, with interim phone contact at 6 months. Measurements of KIM-1 were made in baseline plasma samples of 3,800 study participants.

Outcomes—The primary outcome was progression to kidney failure, defined as initiation of kidney replacement therapy (dialysis or kidney transplantation). The secondary outcome was death. Ascertainment of kidney replacement therapy status within CRIC was supplemented by cross-linkage with the USRDS.³⁰ Participants were followed up until the occurrence of the event of interest, voluntary study withdrawal, loss to follow-up, or end of the follow-up period on January 30, 2018.

Ascertainment of Covariates—Data obtained at the baseline visit included sociodemographic characteristics, medical history, lifestyle behaviors, medications, standardized blood pressure measurements, anthropometric measures, and laboratory measurements. eGFR was calculated using the CRIC-derived estimating equation based on serum creatinine and cystatin C.³⁴ UACR was calculated as spot urine albumin concentration divided by spot urine creatinine concentration from urine samples. Urine creatinine concentration was measured using a Jaffe-based method and urine albumin concentration was measured using an immunoturbidometric method.

Sample Collection and Biomarker Assays

In the BKBC Study, blood samples were collected from participants on the day of kidney biopsy and stored at -80°C . In the CRIC Study, blood samples were collected from participants at baseline and stored at -80°C . Plasma KIM-1 was measured in BKBC and CRIC samples using multiplex microbead-based enzyme-linked immunosorbent assays on a Luminex platform as previously described.²⁵ We measured plasma KIM-1 levels using monoclonal antibody (ADI-905-906-0100, Enzo life sciences) and polyclonal antibody (AF1750, R&D systems) against KIM-1 in the BKBC and the CRIC Study, respectively. Plasma samples were diluted 1:5 in diluent buffer and 30 μl of sample or recombinant human KIM-1 protein (1750-TM, R&D systems) was incubated with microbeads coupled with KIM-1 capture antibody for one hour, washed three times with PBST, and incubated with detection antibody (BAF 1750, R&D Systems) for 45 min. Beads were washed again with PBST and incubated for 15 min with streptavidin-PE solution (Invitrogen). The signal from the fluorochrome, which is directly proportional to the amount of antigen bound at the micro-bead surface, was captured using the Bio-Plex 200 system (Bio-Rad). Data were generated and interpreted using parametric logistic regression analysis and the amount of KIM-1 in plasma was determined using Bio-Plex 200. Additional information about the plasma KIM-1 intra- and inter-assay coefficients of variation are in the Supplemental Methods.

Statistical Analysis

Continuous variables were expressed as means with standard deviation (SD) or medians with interquartile range (IQR) and categorical variables were presented as count with percentages. For skewed data distributions, we performed natural logarithmic transformation as appropriate. We assessed plasma KIM-1 levels in two-group comparisons using the Wilcoxon rank sum test and in multiple-group comparisons using the Kruskal-Wallis test. We used Spearman correlations to assess the association between baseline plasma KIM-1 and continuous variables. To assess differences in frequency distributions of categorical variables, normally distributed continuous variables, and non-normally distributed continuous variables by tertiles (in BKBC) or quintiles (in CRIC) of plasma KIM-1, we used chi-squared, ANOVA, and Kruskal-Wallis tests, respectively. We limited statistical analyses on histopathologic lesions to participants with adjudicated histopathology by both kidney pathologists ($n=458$, 87%) except for analyses of global or segmental glomerulosclerosis since they were taken from the biopsy report ($n=524$). Missing data on histopathologic lesions are specified in the legend of Figure 1. To calculate percent differences of plasma KIM-1 by histopathologic lesion and clinicopathologic diagnosis, we

used multivariable linear regression models. Percent differences in plasma KIM-1 were calculated by raising 2 to the power of the beta-coefficient, subtracting 1, and multiplying by 100 $[(2^{\beta} - 1) * 100]$ for each respective histopathologic lesion or clinicopathologic diagnosis from the linear regression model. Recognizing that proteinuria may be a collider variable³⁵ in analyses of histopathologic lesions and clinicopathologic diagnosis with plasma KIM-1, our primary models omitted proteinuria as an adjustment variable. Exploratory analyses were conducted with further adjustment for proteinuria.

We performed time-to-event analyses to examine the risk of the outcomes, evaluating plasma KIM-1 as a continuous variable (log base 2) in both cohorts and as tertiles (lowest tertile as the reference group) and quintiles (lowest quintile as the reference group) in the BKBC Study and the CRIC Study, respectively. We used cause-specific Cox proportional hazards regression to investigate multivariable-adjusted associations between plasma KIM-1 and outcomes. Subdistribution hazard competing risk models were not used because the goal was to understand the biologic associations of plasma KIM-1 with each outcome. Our multivariable adjustment strategy was hierarchical and based on biological and clinical plausibility of covariates as potential confounders of the association between plasma KIM-1 and outcomes. In the BKBC Study, model 1 was stratified by site and adjusted for age, sex, and race; model 2 included model 1 and further adjusted for natural log transformed proteinuria and primary clinicopathologic diagnosis, angiotensin-converting enzyme inhibitor (ACEi)/angiotensin II receptor blocker (ARB) and immunosuppression/corticosteroids use; model 3 included model 2 and further adjusted for eGFR. We tested for statistical interaction between plasma KIM-1 and primary clinicopathologic diagnosis (glomerulopathy vs. other diagnoses) for both outcomes through multiplicative interaction terms. In the CRIC Study, model 1 was stratified by site and adjusted for age, sex, race, diabetes, any cardiovascular disease (CVD), smoking status, systolic blood pressure (SBP), and body mass index (BMI); model 2 included model 1 and further adjusted for hemoglobin, natural log transformed UACR, and medications including ACEi/ARBs, statins, and antiplatelets drugs; model 3 included model 2 and further adjusted for eGFR. There were less than 5% missing data in both cohorts. Based on the Little Test,³⁶ missing data were considered as missing completely at random and complete case analysis was used for the analyses. All analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC) and STATA 15.0 (STATA Corp, College Station, TX). All statistical tests were 2-sided, and $P < 0.05$ was considered statistically significant.

Results

BKBC Study

Baseline Characteristics—Table 1 summarizes the baseline characteristics of the BKBC participants by tertiles of plasma KIM-1. The median (IQR) plasma KIM-1 concentration using the monoclonal detection antibody from Enzo Life Sciences was 210.2 (102.2 – 452.4) pg/ml. The mean age was 53 ± 17 years and the mean eGFR was 56 ± 36 ml/min/1.73m². The most common primary clinicopathologic diagnoses were proliferative glomerulonephritis (29.1%), non-proliferative glomerulopathy (18.3%), advanced glomerulosclerosis (11.3%), and diabetic nephropathy (11.1%). Plasma KIM-1

correlated negatively with eGFR ($r_s = -0.6$, $p < 0.001$) and positively with proteinuria ($r_s = 0.3$, $p < 0.001$) (Supplemental Table 2).

Associations of Plasma KIM-1 with Histopathologic Lesions and Clinicopathologic Diagnoses

—Figure 1 shows the differences in plasma KIM-1 concentrations across severity scores of histopathologic lesions. Compared to participants with less severe lesions, plasma KIM-1 levels were significantly higher in participants with more severe interstitial fibrosis/tubular atrophy, acute tubular injury, inflammation in the non-fibrosed and fibrosed interstitium, global glomerulosclerosis, mesangial expansion, and arterial and arteriolar sclerosis. Figure 2 shows adjusted differences in plasma KIM-1 concentration across histopathologic lesions and clinicopathologic diagnoses using multivariable linear regression models adjusted for age, sex, race, and eGFR. Plasma KIM-1 levels were significantly higher in participants with more severe acute tubular injury, more severe mesangial expansion, and the presence of inflammation in the non-fibrosed interstitium compared to participants with less severe lesions (Figure 2A). Plasma KIM-1 levels remained significantly higher in participants with more severe acute tubular injury and the presence of inflammation in the non-fibrosed interstitium even after adjustment for proteinuria (Supplemental Table 3). Compared to participants with minor abnormalities or relatively preserved kidney parenchyma, plasma KIM-1 levels were significantly higher in participants with diabetic nephropathy, tubulointerstitial disease, non-proliferative glomerulopathy, and proliferative glomerulonephritis (Figure 2B). Plasma KIM-1 levels remained significantly higher in participants with diabetic nephropathy and tubulointerstitial disease compared to participants with minor abnormalities or relatively preserved kidney parenchyma even after adjustment for proteinuria (Supplemental Table 4).

Associations between Plasma KIM-1 and Risks of Adverse Clinical Outcomes

—During a median follow-up time of 5 years, 124 individuals progressed to kidney failure and 85 participants died. Table 2 shows the multivariable-adjusted associations of plasma KIM-1 with subsequent kidney failure and death in the BKBC Study. In the fully adjusted model, each doubling of plasma KIM-1 was associated with a 1.19-fold increased risk of progression to kidney failure. Participants in the highest tertile of plasma KIM-1 had a higher risk of kidney failure, but the confidence interval crossed 1.0 in the fully adjusted model. There was no evidence of statistical interaction between plasma KIM-1 and primary clinicopathologic diagnosis for progression to kidney failure (P for interaction: 0.3) or death (P for interaction: 0.7) (Supplemental Table 5). Higher levels of plasma KIM-1 were associated with a higher risk of death, but these associations were not statistically significant after multivariable adjustment including eGFR (Table 2).

CRIC Study

Baseline Characteristics—Table 3 summarizes baseline characteristics of 3,800 CRIC Study participants by quintiles of plasma KIM-1 concentration. The median (IQR) plasma KIM-1 concentration using the polyclonal detection antibody from R&D was 1073.0 (557.5 – 2052.4) pg/ml. The mean age was 58 ± 11 years, and the mean eGFR was 45 ± 17 ml/min/1.73m². Participants who had higher levels of plasma KIM-1 were more likely to be non-white, had a greater prevalence of diabetes mellitus and CVD, had higher SBP, and

had lower hemoglobin. Plasma KIM-1 had a negative correlation with eGFR ($r_s = -0.3$, $p < 0.001$) and a positive correlation with UACR ($r_s = 0.4$, $p < 0.001$) (Supplemental Table 2).

Associations between Plasma KIM-1 and Risks of Adverse Clinical Outcomes

—During a median follow-up time of 11.5 years, 1153 participants progressed to kidney failure and 1356 participants died. Table 4 shows multivariable-adjusted associations of plasma KIM-1 with subsequent kidney failure and death in the CRIC Study. In the fully adjusted model, each doubling of plasma KIM-1 was associated with a 1.10-fold increased risk for progression to kidney failure. Participants in the highest quintile of plasma KIM-1 had a 1.58-fold increased risk of progression to kidney failure compared to participants in the lowest quintile. Higher levels of plasma KIM-1 were associated with a higher risk of death, but this association was attenuated and no longer statistically significant after further adjustment for eGFR (Table 4).

Discussion

Kidney tubular injury leads to a decrease in kidney function, most commonly associated with the clinical syndrome of AKI, but tubular injury may be interconnected to the development and progression of CKD.³⁷ In two prospective cohort studies that included common forms of CKD as well as more rare glomerular, interstitial, and vascular diseases, we found a consistent association between higher levels of plasma KIM-1, a sensitive marker of tubular injury, and an increased risk of progression to kidney failure. In individuals who underwent a native kidney biopsy with adjudicated histopathology, we confirmed that higher plasma KIM-1 levels were associated with more severe acute tubular injury, and we further identified associations between higher levels of plasma KIM-1 with tubulointerstitial inflammation and more severe mesangial expansion independent of kidney function. The results provide additional evidence for the importance of kidney tubular injury in glomerulopathies and more common forms of CKD, such as diabetic kidney disease. Our findings demonstrate that plasma KIM-1 has prognostic value across a spectrum of kidney diseases and may serve as a tool in the non-invasive assessment of kidney tubular injury.

KIM-1 is strongly expressed in proximal tubules in response to hypoxic or nephrotoxic events.^{1, 6, 38} While tubular KIM-1 expression is nearly undetectable in healthy kidney tissue, multiple human studies have demonstrated an elevated expression not only in AKI but also in the setting of chronic kidney damage.^{4, 6, 7, 39} Rodent models of CKD showed that sustained KIM-1 upregulation triggers the development of fibrosis with associated monocyte chemotactic protein 1 (MCP-1)-mediated macrophage chemotaxis.⁸ Our finding of an association between higher levels of plasma KIM-1 and more severe acute tubular injury and tubulointerstitial inflammation confirms similar observations in animal and smaller cross-sectional human studies, which identify the tubules as the primary site for KIM-1 expression.^{6, 11} Our findings of higher plasma KIM-1 levels in those with more severe mesangial expansion could reflect downstream tubular ischemia from abnormal glomerular capillaries and post-glomerular blood flow.¹⁰ The observed associations of plasma KIM-1 with tubulointerstitial inflammation and mesangial expansion provide additional evidence that tubular injury and sustained KIM-1 expression may be implicated

in the progressive cycle of interstitial inflammation and fibrosis, glomerular ischemia, and microvascular rarefaction.^{8, 40}

Urinary and plasma KIM-1 are correlated but each may reflect separate processes of injury, which could lead to differences in the prognostic value for kidney disease progression.²⁵ Urinary KIM-1 was associated with lower eGFR and greater proteinuria across 5 cohorts of individuals with CKD,² which was similar in magnitude to the associations of plasma KIM-1 with eGFR and proteinuria in the present study. Some, but not all, studies of urinary KIM-1 in kidney diseases have shown that KIM-1 was a risk factor for future kidney disease progression after adjustment for eGFR and proteinuria.^{13, 14, 16, 20–24} Studies that examined plasma KIM-1 in individuals with diabetes have demonstrated prognostic value for the development or progression of CKD.^{26–28} Two studies that measured both plasma and urinary KIM-1 showed that higher plasma, but not urinary, KIM-1 was associated with an increased risk of early kidney function decline in individuals with type 1 and type 2 diabetes without CKD.^{26, 28} In participants with early and advanced diabetic kidney disease from the ACCORD and VA NEPHRON-D clinical trials, respectively, higher levels of plasma KIM-1 were associated with worsening kidney function over time.²⁷ Similar associations have been reported between higher levels of plasma KIM-1 and the development of CKD in healthy individuals²⁹ and progression to kidney failure in individuals with moderate to severe CKD.⁴¹ One potential explanation for the discordance in prognostic value for urinary and plasma KIM-1 is that urinary KIM-1 may reflect the extent of acute tubular damage and KIM-1 production in response to injury, whereas plasma KIM-1 may better mirror the integral of injury over time and continuing production, being released into systemic circulation in response to a loss of tubular cell polarity.^{25, 29, 42, 43}

We extend the findings in the prior studies of plasma KIM-1 by demonstrating a consistent association of higher plasma KIM-1 levels with progression to kidney failure in cohorts of individuals with both common and more rare forms of kidney disease. Notably, our findings were similar in individuals with and without glomerulopathies, which is consistent with results from the Chronic Kidney Disease in Children (CKID) Study that included both glomerular diseases as well as congenital anomalies of the kidney and urinary tract.⁴⁴ While we found a nominally higher risk of death in individuals with higher levels of plasma KIM-1, these associations were attenuated by eGFR and no longer statistically significant after adjustment.

Our prospective findings confirm that plasma KIM-1 may serve as a tool to enrich selection of high-risk individuals with kidney disease for enrollment in clinical trials.^{27, 45, 46} Although there was overlap of plasma KIM-1 levels across histopathologic severity grades, our findings suggest that plasma KIM-1 may be able to provide a non-invasive estimate of more severe acute tubular injury independent of kidney function, which warrants further investigation. While we are unable to demonstrate a causal association of plasma KIM-1 with histopathology or CKD progression, confirmation of our findings may show that plasma KIM-1 is able to serve as a tool to assess for kidney tubular injury^{6, 47} or to monitor response to treatment in the setting of a clinical trial⁴⁸ for the development of therapies across a variety of chronic kidney diseases.^{49–51}

Strengths of our study are the inclusion of two large cohorts of individuals with established kidney disease. Both studies have a long duration of follow-up and low rates of missing outcome data. Specific strengths of the BKBC Study include biopsy-confirmation of the underlying cause of kidney disease, adjudicated histopathologic scores on lesion severity, and the inclusion of individuals with a diverse range of kidney diseases. Specific strengths of the CRIC Study include the adjustment for a large number of potential confounders. Our study has limitations that warrant consideration. The ascertainment of variables differed across the two cohorts, but we were able to adjust for major confounders, including eGFR and proteinuria in both cohorts. Plasma KIM-1 was measured using different platforms, so direct comparisons of absolute levels cannot be done across the two cohorts, which may limit generalizability of the findings. Although we did not find a statistically significant association between the amount of time in storage and plasma KIM-1 concentration using multivariable regression analyses, we cannot rule out that the duration of storage time may have impacted the stability of plasma KIM-1 which could have altered plasma KIM-1 levels. Given the heterogeneity of the BKBC Study, the number of individuals within each clinicopathologic diagnostic category was small, particularly for rare diseases. We did not measure urinary KIM-1 in the BKBC Study and therefore we are unable to compare plasma KIM-1 with urinary KIM-1 as a biomarker of histopathologic lesions. While the effect sizes between plasma KIM-1 and adverse clinical outcomes were relatively modest, additional research is warranted to determine if multi-marker panels that include plasma KIM-1 are able to improve risk estimation for CKD progression. Although we adjusted for a number of clinical predictors, there may be residual confounding from unmeasured confounders.

In conclusion, we found that higher levels of plasma KIM-1 are associated with more severe tubulointerstitial and mesangial lesions and progression to kidney failure in two cohort studies of individuals with kidney diseases. The strong associations with histopathologic lesions and progression to kidney failure suggest that plasma KIM-1 may potentially serve as a kidney-specific marker to enhance the estimation of the risk of progression to kidney failure across a diverse spectrum of kidney diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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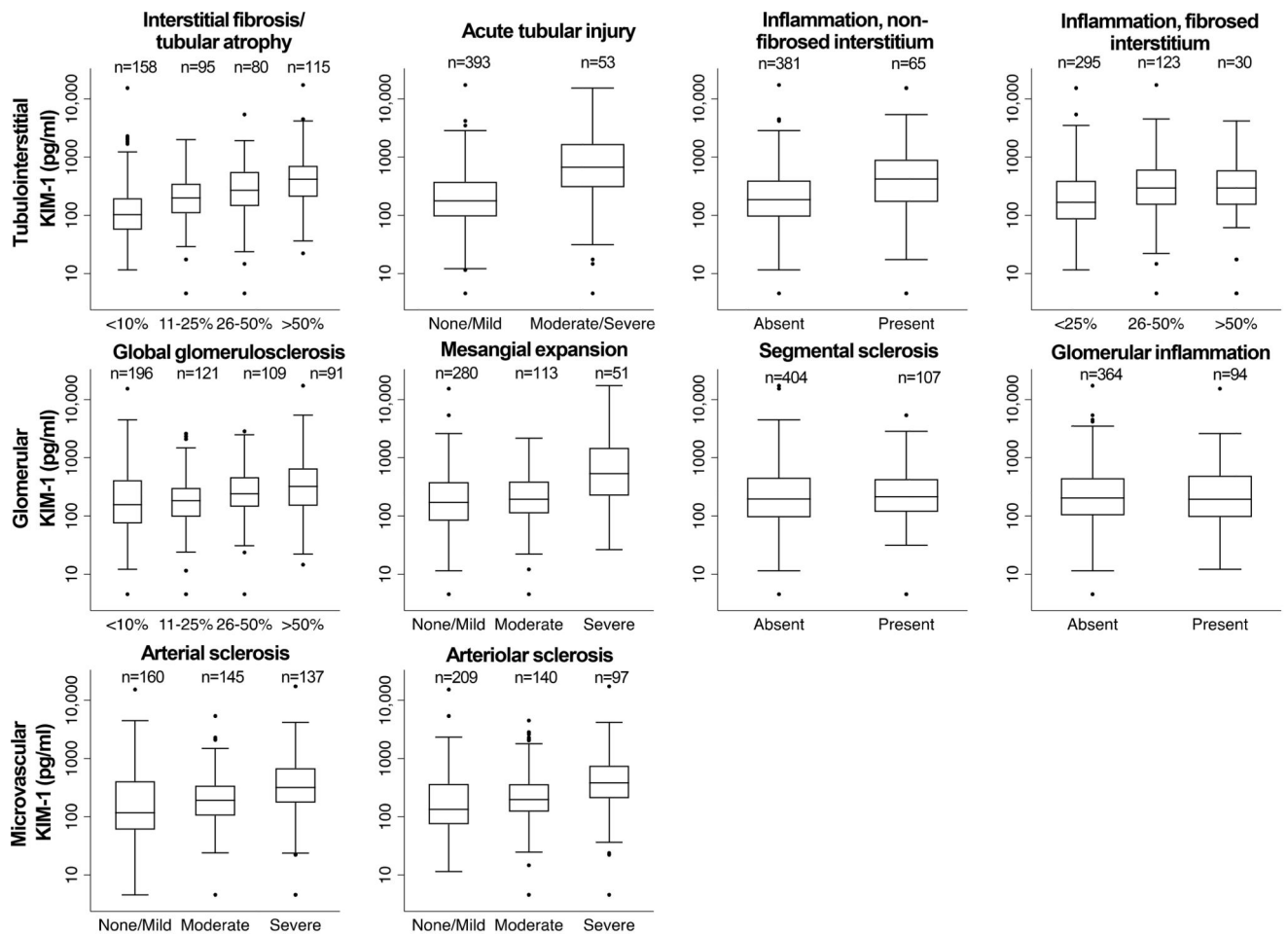


Figure 1. Differences in plasma KIM-1 by histopathologic lesion.

Boxplots show differences between grades of adjudicated histopathologic lesions and plasma KIM-1 on a log scale. Boxplots show median and interquartile range (IQR) of plasma KIM-1 measured using a monoclonal detection antibody. Whiskers represent the IQR of the lower and upper quartile (25th and 75th percentile, respectively). P-values obtained from Wilcoxon or Kruskal-Wallis tests: $p < 0.001$ for interstitial fibrosis/tubular atrophy, acute tubular injury, inflammation in the fibrosed and non-fibrosed interstitium, global glomerulosclerosis, mesangial expansion, arterial and arteriolar sclerosis; $p = 0.7$ for glomerular inflammation; and $p = 0.4$ for segmental sclerosis. 3 individuals had KIM-1 levels below the lower limit of quantification (LLOQ) which were imputed as LLOQ/2. Data on histopathologic lesion scores were missing for $n = 7$ (global glomerulosclerosis), $n = 13$ (segmental sclerosis), $n = 10$ (interstitial fibrosis/tubular atrophy), $n = 12$ (acute tubular injury), $n = 12$ (inflammation in the non-fibrosed interstitium), $n = 10$ (inflammation in the fibrosed interstitium), $n = 14$ (mesangial expansion), $n = 16$ (arterial sclerosis), and $n = 12$ (arteriolar sclerosis).

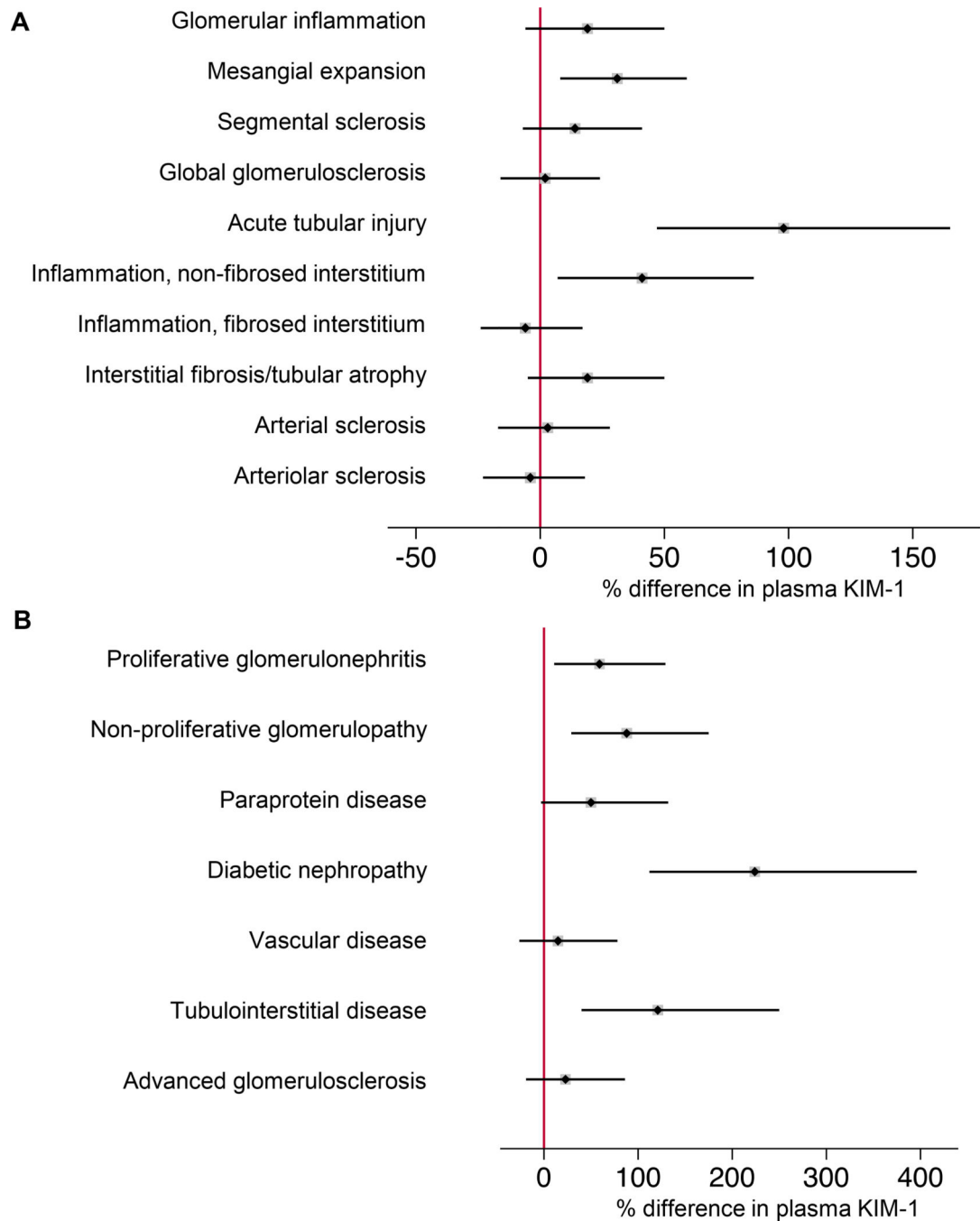


Figure 2. Adjusted differences in plasma KIM-1 by histopathologic lesion and clinicopathologic diagnosis.

Models were fit using log-transformed plasma KIM-1 as the outcome and histopathologic lesion (A) and clinicopathologic diagnosis (B) as the predictor variable. Percent differences are derived from linear regression models of log base 2 plasma KIM-1. Percent differences in plasma KIM-1 were calculated by raising 2 to the power of the beta-coefficient, subtracting 1, and multiplying by 100, $[(2^{\beta} - 1) * 100]$, for each respective histopathologic lesion or clinicopathologic diagnosis from the linear regression model. Each individual model was adjusted for age, sex, race, and eGFR.

A. Reference is absence of lesion for glomerular inflammation ($p=0.2$), segmental sclerosis ($p=0.2$), inflammation in the non-fibrosed interstitium ($p=0.01$); Reference is none/mild lesion severity for mesangial expansion ($p=0.006$), acute tubular injury ($p<0.001$), arterial sclerosis ($p=0.8$), arteriolar sclerosis ($p=0.7$); Reference is 0-25% of cortical volume affected for global glomerulosclerosis ($p=0.8$), inflammation in the fibrosed interstitium ($p=0.6$), interstitial fibrosis/tubular atrophy ($p=0.1$).

B. Reference is near-normal tissue (comprised of individuals with minor abnormalities or relatively preserved parenchyma). Proliferative glomerulonephritis, $p=0.01$; non-proliferative glomerulopathy, $p<0.001$; paraprotein disease, $p=0.07$; diabetic nephropathy, $p<0.001$; vascular disease, $p=0.5$; tubulointerstitial disease, $p<0.001$; advanced glomerulosclerosis, $p=0.3$.

Table 1. Baseline characteristics of the BKBC Study participants by tertiles of plasma KIM-1.

Plasma KIM-1 (pg/mL)*	All participants 4.6 – 17305.3 (n = 524)	Tertile 14.6 – 125.2 (n = 174)	Tertile 2125.4 – 342.3 (n = 175)	Tertile 3343.3 – 17305.3 (n = 175)	p-value
Clinical Characteristics					
Age, years	52.8 ± 16.6	46.4 ± 15.3	55.7 ± 16.0	56.4 ± 16.4	<0.001
Female	267 (51.0)	104 (59.8)	83 (47.4)	80 (45.7)	0.02
Race					0.2
White	334 (63.7)	104 (59.8)	118 (67.4)	112 (64.0)	
Black	104 (19.8)	32 (18.4)	32 (18.3)	40 (22.9)	
Other	86 (16.4)	38 (21.8)	25 (14.3)	23 (13.1)	
eGFR, ml/min/1.73m ²	56.4 ± 36.0	81.8 ± 34.1	54.2 ± 31.6	33.4 ± 23.5	<0.001
Proteinuria, g/g creatinine	1.6 [0.4 – 4.0]	1.0 [0.3 – 2.6]	1.5 [0.3 – 3.7]	2.6 [0.7 – 6.7]	<0.001
Reason for Biopsy**					
Proteinuria	306 (58.4)	110 (63.2)	113 (64.6)	83 (47.4)	0.001
Hematuria	127 (24.2)	57 (32.8)	38 (21.7)	32 (18.3)	0.004
Nephrotic syndrome	70 (13.4)	24 (13.8)	18 (10.3)	28 (16.0)	0.3
Nephritic syndrome	12 (2.3)	7 (4.0)	3 (1.7)	2 (1.1)	0.2
Abnormal eGFR	271 (51.7)	56 (32.2)	93 (53.1)	122 (69.7)	<0.001
Primary Clinicopathologic Diagnosis					
Proliferative glomerulonephritis	150 (29.1)	64 (37.0)	45 (26.3)	41 (24.0)	<0.001
Non-proliferative glomerulopathy	94 (18.3)	34 (19.7)	36 (21.1)	24 (14.0)	
Advanced glomerulosclerosis	58 (11.3)	18 (10.4)	25 (14.6)	15 (8.8)	
Diabetic nephropathy	57 (11.1)	4 (2.3)	14 (8.2)	39 (22.8)	
Vascular disease	44 (8.5)	12 (6.9)	23 (13.5)	9 (5.3)	
Paraprotein-related disease	42 (8.2)	17 (9.8)	11 (6.4)	14 (8.2)	
Tubulointerstitial disease	39 (7.6)	3 (1.7)	11 (6.4)	25 (14.6)	
Other	31 (6.0)	21 (12.1)	6 (3.5)	4 (2.3)	

Plasma KIM-1 (pg/mL)*	All participants 4.6 – 17305.3 (n = 524)	Tertile 14.6 – 125.2 (n = 174)	Tertile 2125.4 – 342.3 (n = 175)	Tertile 3343.3 – 17305.3 (n = 175)	p-value
Comorbidities					
Diabetes mellitus	120 (22.9)	13 (7.5)	42 (24.0)	65 (37.1)	<0.001
Hypertension	279 (53.2)	65 (37.4)	98 (56.0)	116 (66.3)	<0.001
Systemic lupus erythematosus	77 (14.7)	49 (28.2)	16 (9.1)	12 (6.9)	<0.001
Hepatitis C	10 (1.9)	2 (1.1)	0 (0.0)	8 (4.6)	0.005
Malignancy	82 (15.6)	18 (10.3)	35 (20.0)	29 (16.6)	0.04
Medications					
ACEi/ARB	243 (46.4)	77 (44.3)	92 (52.6)	74 (42.3)	0.1
MRA	12 (2.3)	1 (0.6)	5 (2.9)	6 (3.4)	0.2
Calcium channel blockers	138 (26.3)	38 (21.8)	45 (25.7)	55 (31.4)	0.1
Beta-blockers	168 (32.1)	30 (17.2)	58 (33.1)	80 (45.7)	<0.001
Immunosuppression	94 (17.9)	34 (19.5)	31 (17.7)	29 (16.6)	0.8
Corticosteroids	97 (18.5)	42 (24.1)	32 (18.3)	23 (13.1)	0.03

Data presented as mean \pm standard deviation, median [IQR], and count with frequencies (%) for binary and categorical variables. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; MRA, mineralocorticoid receptor blocker; eGFR, estimated glomerular filtration rate

* Monoclonal detection antibody; 3 individuals had KIM-1 levels below the lower limit of quantification (LLOQ) which were imputed as LLOQ/2.

** Reasons for biopsy were based on the referring nephrologist's judgement. Percentages do not add to 100 as there may have been more than one reason for kidney biopsy. Percentages do not add to 100 as there may have been more than one reason for kidney biopsy

*** Nine individuals had insufficient tissue to make a clinicopathologic diagnosis

Table 2.

Plasma KIM-1 and the risk of adverse clinical outcomes in the BKBC Study.

Plasma KIM-1	Events	Events per 100 person-years	Model 1	p-value	Model 2	p-value	Model 3	p-value
Kidney Failure								
Tertile 1	19	2.1	Reference		Reference		Reference	
Tertile 2	37	5.4	2.39 (1.35 – 4.22)	0.003	2.35 (1.30 – 4.27)	0.005	1.40 (0.77 – 2.56)	0.3
Tertile 3	68	12.3	5.12 (3.00 – 8.73)	<0.001	4.62 (2.57 – 8.32)	<0.001	1.46 (0.80 – 2.64)	0.2
Continuous, per doubling KIM-1	124	5.8	1.66 (1.48 – 1.87)	<0.001	1.65 (1.43 – 1.90)	<0.001	1.19 (1.03 – 1.38)	0.02
Mortality								
Tertile 1	12	1.2	Reference		Reference		Reference	
Tertile 2	31	3.6	2.17 (1.09 – 4.29)	0.03	1.93 (0.96 – 3.90)	0.07	1.46 (0.72 – 2.96)	0.3
Tertile 3	42	5.0	2.92 (1.51 – 5.68)	0.002	2.63 (1.30 – 5.34)	0.007	1.48 (0.69 – 3.18)	0.3
Continuous, per doubling KIM-1	85	3.1	1.25 (1.09 – 1.43)	0.001	1.23 (1.06 – 1.44)	0.008	1.05 (0.89 – 1.25)	0.6

Results are expressed as hazard ratios (95% CI); Kidney failure, defined as initiation of kidney replacement therapy.

Model 1 is stratified by site and adjusted for age, sex, and race.

Model 2 is model 1 and further adjusts natural log transformed proteinuria, primary clinicopathologic diagnosis, ACEi/ARB use, and immunosuppression/corticosteroids.

Model 3 is model 2 and further adjusts for eGFR

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate

Table 3. Baseline characteristics of the CRIC Study participants by quintiles of plasma KIM-1.

Plasma KIM-1 (pg/mL)*	All Participants 16.5 – 965023.4 (n = 3800)	Quintile 1 12.6 – 484.5 (n = 760)	Quintile 2 485.6 – 830.0 (n = 760)	Quintile 3 831.7 – 1374.0 (n = 760)	Quintile 4 1374.8 – 2444.4 (n = 759)	Quintile 5 2446.1 – 965023.4 (n = 761)	p-value
Clinical characteristics							
Age, years	57.7 ± 11.0	56.4 ± 11.3	58.5 ± 10.6	58.4 ± 11.0	58.1 ± 11.1	56.8 ± 10.9	<0.001
Female	1694 (44.6)	336 (44.2)	341 (44.9)	347 (45.7)	344 (45.3)	326 (42.8)	0.8
Race							
White	1587 (41.8)	360 (47.4)	371 (48.8)	339 (44.6)	283 (37.3)	234 (30.7)	<0.001
Black	1570 (41.3)	310 (40.8)	287 (37.8)	312 (41.1)	317 (41.8)	344 (45.2)	
Hispanic	489 (12.9)	55 (7.2)	82 (10.8)	78 (10.3)	129 (17.0)	145 (19.1)	
Other	154 (4.1)	35 (4.6)	20 (2.6)	31 (4.1)	30 (4.0)	38 (5.0)	
eGFR (ml/min/1.73m ²)	44.9 ± 16.9	53.0 ± 18.4	47.7 ± 16.1	43.5 ± 15.7	41.1 ± 15.0	39.1 ± 15.4	<0.001
UA CR, mg/g	51.6 [8.6 – 453.5]	12.4 [5.1 – 62.1]	25.7 [6.6 – 161.5]	53.8 [9.5 – 335.5]	153.2 [18.3 – 873.3]	433.8 [34.0 – 2227.0]	<0.001
Hemoglobin, g/dL	12.6 ± 1.8	13.2 ± 1.6	12.8 ± 1.8	12.6 ± 1.7	12.3 ± 1.7	12.1 ± 1.8	<0.001
Total cholesterol	183.6 ± 45.5	177.5 ± 38.9	179.0 ± 40.7	180.8 ± 43.5	186.0 ± 46.6	194.6 ± 54.1	<0.001
Comorbidities							
Hypertension	3271 (86.1)	600 (78.9)	652 (85.8)	667 (87.8)	654 (86.2)	698 (91.7)	<0.001
Diabetes mellitus	1842 (48.5)	236 (31.1)	320 (42.1)	352 (46.3)	444 (58.5)	490 (64.4)	<0.001
SBP, mm Hg	128.6 ± 22.2	122.3 ± 20.2	125.0 ± 19.4	126.9 ± 21.0	131.5 ± 22.2	137.1 ± 25.0	<0.001
BMI, kg/m ²	32.1 ± 7.8	31.5 ± 7.2	32.3 ± 7.7	32.3 ± 8.2	32.3 ± 7.9	32.2 ± 7.8	0.2
Any CVD	1269 (33.4)	189 (24.9)	270 (35.5)	254 (33.4)	277 (36.5)	279 (36.7)	<0.001
Current smoking	496 (13.1)	83 (10.9)	98 (12.9)	88 (11.6)	113 (14.9)	114 (15.0)	0.06
Medications**							
ACEI/ARB use	2594 (68.8)	495 (65.4)	506 (66.9)	541 (71.9)	513 (68.2)	539 (71.4)	0.02
Statins	2087 (55.3)	368 (48.6)	401 (53.0)	430 (57.2)	443 (58.9)	445 (58.9)	<0.001
Antiplatelets	1731 (45.9)	327 (43.2)	369 (48.8)	338 (44.9)	338 (44.9)	359 (47.5)	0.2

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Data presented as mean \pm standard deviation, median [IQR], and count with frequencies (%) for binary and categorical variables. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SBP, systolic blood pressure; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio

* Polyclonal detection antibody; 2 individuals had KIM-1 levels below the lower limit of quantification (LLOQ) which were imputed as LLOQ/2.

** Data on medications were missing for 28 individuals.

Table 4.

Plasma KIM-1 and the risks of adverse clinical outcomes in the CRIC Study.

Plasma KIM-1	Events	Events per 100 person-years	Model 1	p-value	Model 2	p-value	Model 3	p-value
Kidney Failure								
Quintile 1	100	1.3	Reference		Reference		Reference	
Quintile 2	140	1.9	1.35 (1.05 – 1.75)	0.02	1.03 (0.79 – 1.34)	p=0.8	1.03 (0.79 – 1.35)	0.8
Quintile 3	211	3.2	2.15 (1.69 – 2.73)	<0.001	1.29 (1.01 – 1.66)	p=0.04	1.14 (0.89 – 1.46)	0.3
Quintile 4	292	5.1	2.81 (2.23 – 3.55)	<0.001	1.40 (1.10 – 1.79)	p=0.01	1.22 (0.95 – 1.55)	0.1
Quintile 5	410	8.8	4.64 (3.70 – 5.81)	<0.001	1.86 (1.45 – 2.37)	p<0.001	1.58 (1.24 – 2.02)	<0.001
Continuous, per doubling KIM-1	1153	3.6	1.24 (1.21 – 1.28)	<0.001	1.13 (1.09 – 1.17)	p<0.001	1.10 (1.06 – 1.15)	<0.001
Mortality								
Quintile 1	182	2.2	Reference		Reference		Reference	
Quintile 2	235	3.0	1.08 (0.89 – 1.31)	0.4	1.04 (0.85 – 1.27)	0.7	1.01 (0.83 – 1.24)	0.9
Quintile 3	269	3.5	1.28 (1.06 – 1.55)	0.01	1.14 (0.93 – 1.39)	0.2	1.04 (0.85 – 1.27)	0.7
Quintile 4	317	4.5	1.45 (1.20 – 1.75)	<0.001	1.23 (1.01 – 1.50)	0.04	1.14 (0.93 – 1.39)	0.2
Quintile 5	353	5.0	1.63 (1.35 – 1.97)	<0.001	1.23 (1.00 – 1.51)	0.05	1.13 (0.92 – 1.39)	0.2
Continuous, per doubling KIM-1	1356	3.6	1.09 (1.06 – 1.13)	<0.001	1.04 (1.00 – 1.08)	0.03	1.03 (0.99 – 1.07)	0.1

Results are expressed as hazard ratios (95% CI); Kidney failure, defined as initiation of kidney replacement therapy

Model 1 is stratified by site and adjusted for age, sex, race, diabetes, any CVD, smoking status, SBP, and BMI.

Model 2 is model 1 and further adjusts for ACEi/ARB use, statins, antiplatelet drugs, hemoglobin, and natural log transformed UACR.

Model 3 is model 2 and further adjusts for eGFR

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SBP, systolic blood pressure; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio