Plasma kidney injury molecule-1 in heart failure: renal mechanisms and clinical outcome

Johanna E. Emmens, BSc[a], Jozine M. ter Maaten, MD[a], Yuya Matsue, MD[a], Marco Metra, MD[b], Christopher M. O'Connor, MD[c], Piotr Ponikowski, MD[d], John R. Teerlink, MD[e], Gad Cotter, MD[f], Beth Davison, PhD[f], John G. Cleland, MD[g], Michael M. Givertz, MD[h], Daniel M. Bloomfield, MD[i], Howard C. Dittrich, MD[j], John Todd[k], Dirk J. van Veldhuisen, MD, PhD[a], Hans L. Hillege, MD, PhD[a,l], Kevin Damman, MD, PhD[a], Peter van der Meer, MD, PhD[a], Adriaan A. Voors, MD, PhD[a]

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Affiliations:

[a] University of Groningen, University Medical Center Groningen, Department of cardiology, Groningen, The Netherlands

[b] University of Brescia, Brescia, Italy

[c] Duke University Medical Center, Durham, NC, USA

[d] Medical University, Clinical Military Hospital, Wroclaw, Poland

[e] University of California at San Francisco and San Francisco Veterans Affairs Medical Center, San Francisco, CA, USA

[f] Momentum Research, Durham, NC, USA

[g] University of Hull, Kingston upon Hull, United Kingdom

[h] Brigham and Women's Hospital, Boston, MA, USA

[i] Merck Research Laboratories, Rahway, NJ, USA

[j] University of Iowa Carver College of Medicine Cardiovascular Research Center, Iowa City, IA, USA

[k] Singulex inc, Alameda, USA

[l] University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands

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Corresponding author

Prof. dr. A.A. Voors

Department of Cardiology

University Medical Center Groningen

Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

Tel: +31 (0)50 3616161

Fax : +31 (0)50 3618062

a.a.voors@umcg.nl

Abstract

Aims

Urinary Kidney Injury Molecule-1 (KIM-1) is a marker of tubular damage and associated with worse outcome in heart failure (HF). *Plasma* KIM-1 has not been described in HF.

Methods and results

In a renal mechanistic cohort of 120 chronic HF patients, we established the association between plasma KIM-1, renal invasive hemodynamic parameters (renal blood flow (^{131}I-Hippuran clearance) and measured glomerular filtration rate (GFR; ^{125}I-Iothalamate)), and urinary tubular damage markers. The association between plasma KIM-1, plasma creatinine, and clinical outcome was further explored in a cohort of 2033 acute HF patients.

Median plasma KIM-1 was 171.5 pg/mL (122.8 – 325.7) in chronic (N=99) and 295.1 pg/mL (182.2-484.2) in acute HF (N=1588). In chronic HF, plasma KIM-1 was associated with GFR ($P < 0.001$), creatinine and cystatin C. Plasma KIM-1 was associated with urinary NAG, but not with other urinary tubular damage markers. Log plasma KIM-1 predicted adverse clinical outcome after adjustment for age, gender, and GFR (Hazard Ratio (HR)=1.94 [1.07 – 3.53], P=0.030). Statistical significance was lost after correction for NT-proBNP (HR=1.61 [0.81 – 3.20], P=0.175). In acute HF, higher plasma KIM-1 levels were associated with higher creatinine, lower albumin, and presence of diabetes. Log plasma KIM-1 predicted 60-day HF rehospitalization (HR=1.27 [1.03-1.55], P=0.024), but not 180-day mortality or 60-day death or renal or cardiovascular rehospitalization.

Conclusions

Plasma KIM-1 is associated with glomerular filtration and urinary NAG, but not with other urinary tubular damage markers. Plasma KIM-1 does not predict outcome in chronic HF after correction for NT-proBNP. In acute HF, plasma KIM-1 predicts HF rehospitalization in multivariable analysis.

Key words: Plasma KIM-1; heart failure; prognosis

Abbreviations

BMI Body Mass Index

BNP Brain Natriuretic Peptide

BSA Body Surface Area

BUN Blood Urea Nitrogen

CRP C-reactive protein

ELISA Enzyme-linked immonsorbent assays

ERPF Effective Renal Plasma Flow

FF Filtration Fraction

GFR Glomerular Filtration Rate

HR Hazard Ratio

KIM-1 Kidney Injury Molecule-1

LVEF Left Ventricular Ejection Fraction

NAG N-acetyl-β-D-glucosaminidase

NGAL Neutrophil Gelatinase-Associated Lipocalin

NT-proBNP N Terminal pro Brain Natriuretic Peptide

NYHA New York Heart Association

PROTECT Placebo-controlled Randomized study of the selective A(1) adenosine receptor antagonist rolofylline for patients hospitalized with acute heart failure and volume Overload to assess Treatment Effect on Congestion and renal funcTion

RBF Renal Blood Flow

WRF Worsening Renal Function

Introduction

In patients with heart failure, renal dysfunction and worsening renal function (WRF) are strong predictors of morbidity and mortality.[1] In these studies, renal function is routinely established by (estimated) glomerular filtration rate (GFR).[2,3] In addition, markers of tubular damage are elevated in heart failure, are associated with WRF, and provide additional and independent prognostic information.[4-7] A sensitive tubular marker is Kidney Injury Molecule-1 (KIM-1), a protein of the proximal tubule which is undetectable in healthy kidneys, but is thought to be expressed in fibrotic areas as a result of tubulo-interstitial damage, inflammation and tubular epithelium changes.[8,9] Urinary KIM-1 was found superior to other tubular markers in predicting WRF, response to diuretic withdrawal and was associated with outcome, even after adjustment for other known risk factors in heart failure.[4,6,10]

Recently, plasma measurements of KIM-1 have become available which have a higher clinical applicability in daily cardiology practice. In patients with both acute and chronic kidney injury, plasma KIM-1 predicted progression of renal disease.[11] However, no data are available on plasma KIM-1 measurements in patients with heart failure. Therefore, this study aims to evaluate plasma KIM-1 as a tubular and prognostic marker in patients with acute and chronic heart failure.

Methods

We first studied plasma KIM-1 in a renal mechanistic cohort of chronic heart failure patients to investigate its association with urinary tubular markers and with renal hemodynamic measurements, such as measured GFR by Iothalamate clearance and renal blood flow. Secondly, we further studied the association with plasma creatinine and its prognostic value in a cohort of 2033 patients with acute heart failure that were included in the PROTECT (Placebo-controlled Randomized study of the selective A(1) adenosine receptor antagonist rolofylline for patients hospitalized with acute heart failure and volume Overload to assess Treatment Effect on Congestion and renal funcTion) trial.

Renal mechanistic cohort

Patient population

The renal mechanistic, chronic heart failure cohort has been described in detail previously.[6] In brief, 120 stable outpatients with systolic heart failure were included at the University Medical Centre Groningen. The protocol was approved by the local medical ethical committee and all patients provided written informed consent. All patients used renin-angiotensin system blockers, and all medications had been stable for at least 1 month.

Study design

Measured GFR, effective renal plasma flow (ERPF) and renal blood flow (RBF) were measured on two consecutive days by Iothalamate clearance, which is considered the golden standard for measuring glomerular function. [12] By multiplication of the radiolabeled tracer ^{125}I-iothalamate with the ratio of the plasma and urinary clearance of ^{131}I-hippuran, this method corrects for incomplete bladder emptying and dead space. From the ratio of the GFR and ERPF the filtration fraction (FF) was calculated and presented as a percentage. GFR, ERPF and RBF are also presented with correction for 1.73 m² body surface area.

Serum laboratory assessments such as creatinine, serum blood urea nitrogen (BUN), serum albumin and serum cystatin C were measured in venous blood at baseline. Urinary measurements were performed using 24 h urine collection. Levels of urinary tubular markers were determined with ELISA for neutrophil gelatinase-associated lipocalin (NGAL), with the substrate p-nitrophenyl N-acetyl-beta-D-glucosaminide for N-acetyl-β-D-glucosaminidase (NAG) and with antibodies for urinary KIM-1. Urinary tubular activity was expressed per gram urinary creatinine. In 99 patients plasma KIM-1 was measured (lower limit of detection:2 pg/ml, upper limit:1000 pg/ml) by single molecule counting technology with the Erenna® Immunoassay System on a microtiter plate assay format (Singulex Inc, Alameda, USA).

Follow-up

We used a composite endpoint of first occurrence of all-cause death, heart transplantation, myocardial infarction or hospitalization for heart failure at three years follow-up.[13]

Acute heart failure cohort

Patient population and study design

The PROTECT study was a multicenter, double-blind, placebo-controlled study on the effects of rolofylline on symptomatic improvement of acute heart failure patients with mild to moderate renal function impairment. The details of the design, results, and conclusions have already been published.[14,15] A total of 2,033 acute heart failure patients with mild to moderate impaired renal function (creatinine clearance between 20 to 80 mL/min with Cockcroft–Gault formula) were included and randomized to rolofylline or placebo. The overall results were neutral. The study was approved by the ethics committee at each participating center, and written informed consent was obtained from all participants.

The following biomarkers were evaluated at baseline: albumin, BUN, creatinine, glucose, hemoglobin, potassium, sodium, total cholesterol, triglycerides, uric acid and white blood cell count were measured by ICON Laboratories, Farmingdale, New York. N Terminal pro Brain Natriuretic Peptide **(**NT-proBNP) was determined at screening using commercial assays available at study sites. NGAL and C-reactive protein (CRP) were measured in available frozen plasma samples by Alere Inc., San Diego, CA, USA. NGAL was measured using sandwich enzyme-linked immonsorbent assays (ELISA) on a microtiter plate; CRP was measured using competitive ELISAs on a Luminex platform. Plasma KIM-1, and brain natriuretic peptide (BNP) were measured from frozen plasma samples collected only at baseline by Singulex Inc, Alameda, USA. Plasma KIM-1 was determined in 1,588 baseline samples using Single Molecule Counting as described above.

Follow-up

Three endpoints that relate to heart failure and renal function were evaluated: all-cause mortality within 180 days, heart failure rehospitalization within 60 days, and death or cardiovascular or renal rehospitalization within 60 days, as described previously.[15].[16]

The endpoints were adjusted for the clinical model created for this cohort, with addition of brain natriuretic peptide (BNP). This prognostic model consisting of 8 readily available variables has shown to perform similarly compared to a more complex model.[17]

Statistical analysis

Descriptive statistics were used to examine the relation of quantiles of plasma KIM-1 to covariates. Due to different population sizes and differences in plasma KIM-1 range, plasma KIM-1 was divided into tertiles for the chronic HF cohort and into quartiles for the acute HF cohort. Prior to analyses the distribution of all variables was checked. Data are presented as mean ± SD when normally distributed, as median (interquartile range) for skewed variables and as frequencies (percentage) for categorical variables. Baseline characteristics were analyzed using ANOVA for normally distributed variables and Kruskal-Wallis for skewed variables. For categorical variables Chi-square was used. A linear trend was statistically tested with Cochran-Armitage trend test, Jonckheere-Terpstra test, and linear regression model for categorical variable, non-normally distributing continuous variable, and normally distributing continuous variable over quartiles of plasma KIM-1 in the acute heart failure cohort, after checking for non-linear trends. If necessary, variables were transformed for further analyses. Correlations of log plasma KIM-1 with established markers of tubular and glomerular function were investigated. Predictors of log plasma KIM-1 were analyzed using univariable and multivariable regression with the backward elimination method after including all variables with $p<0.10$ in univariable analyses. Cox proportional hazard models were constructed for all selected endpoints to evaluate the prognostic predictability of plasma KIM-1. The proportional hazard assumption was checked by inspection of "log-log" plots for quantiles of plasma KIM-1, and Schoenfeld residual plots. In multivariable models of the chronic heart failure cohort, log plasma KIM-1 was subsequently adjusted for age, sex, serum creatinine and BUN. In PROTECT, log plasma KIM-1 was adjusted for the clinical model created for this cohort and BNP with respect to the three outcomes.[17] A two-tailed p-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS and R: A Language and Environment for

Statistical Computing, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). ISBN 3-900051-07-0, www.r-project.org.

Results

Renal mechanistic cohort (chronic heart failure)

Patient characteristics

Baseline characteristics of the renal mechanistic cohort are summarised in table 1. In brief, mean age was 60 years (± 11.9), 81% of the patients were male, 36% had a New York Heart Association (NYHA) classification of III or IV and mean Left Ventricular Ejection Fraction (LVEF) was 28.6% (± 9.6). The median value of plasma KIM-1 was 171.5 (122.8 – 325.7) pg/mL. Higher levels of plasma KIM-1 were associated with older age ($P = 0.001$), higher N Terminal pro Brain Natriuretic Peptide (NT-proBNP) levels ($P = 0.019$), higher urinary NAG levels ($P = 0.033$) and all indices of renal function (all $P < 0.001$). No association was found between levels of plasma KIM-1 and urinary KIM-1 and urinary NGAL. The associations between plasma KIM-1 and tubular markers and GFR are presented in figure 1.

Linear regression analysis for plasma KIM-1

Variables associated with log plasma KIM-1 in univariable linear regression analysis are presented in supplementary table 1. In multivariable linear regression higher levels of log plasma KIM-1 were associated with older age (Beta = 0.016, $P = 0.023$), more frequent use of an aldosterone antagonist (β = 0.439, $P = 0.009$), higher log serum BUN levels (β = 0.520, $P = 0.040$) and higher log urinary NAG levels (β = 0.213, $P = 0.033$), r^2 for the model = 0.604 (table 2). Log urinary KIM-1 was not significantly associated with log plasma KIM-1 levels.

Relationship with clinical outcome

In total, 38 patients experienced an event, among which were 14 all-cause deaths, 18 hospitalizations for heart failure, 5 heart transplantations and 1 myocardial infarction. In figure 2, Kaplan-Meier event-

free survival curves for tertiles of plasma KIM-1 showed that higher levels of plasma KIM-1 (tertile 3) are associated with reduced survival (*P* = 0.019). Cox regression analysis for the combined endpoint is presented in table 4a. Higher log plasma KIM-1 levels were associated with adverse clinical outcome outcome (Hazard Ratio (HR) = 2.69 (1.52 – 4.76), *P* = 0.001), even after adjustment for age, gender, and GFR per BSA (HR = 1.94 (1.07 – 3.53), *P* = 0.030). The statistical significance was however lost after correction for NT-proBNP (HR = 1.61 (0.81 – 3.20), *P* = 0.175).

Acute heart failure cohort

Patient characteristics

Baseline characteristics of the study population and associations of plasma KIM-1 with the other PROTECT variables are presented in table 3. In brief, mean age was 70.9 years (± 11.2), 66% of the patients were male, and mean LVEF was 32.5% (± 13.2). The median value of plasma KIM-1 was 295.1 (182.2-484.2) pg/mL. Higher plasma KIM-1 levels were, among others, associated with older age (*P* < 0.001), higher systolic blood pressure (*P* < 0.001), higher Body Mass Index (BMI) (*P* = 0.001), higher LVEF (*P* = 0.004), higher frequency of Heart Failure with preserved Ejection Fraction (*P* = 0.011), higher NT-proBNP levels (*P* < 0.001), higher CRP levels (*P* < 0.001), higher plasma NGAL levels (*P* < 0.001) and a history of hypertension (*P* = 0.001), diabetes (*P* < 0.001), and dyslipidemia (*P* = 0.001). The incidence of worsening renal function, defined as 0.3 mg/dl increase in creatinine at day 4, was not different over baseline quartiles of plasma KIM-1 (*P* = 0.742).

Linear regression analysis for plasma KIM-1

The univariable regression analysis for plasma KIM-1 in acute heart failure is presented in supplementary table 1. In multivariable regression plasma KIM-1 was associated with presence of diabetes (β = 0.188, *P* < 0.001), lower albumin levels (β = -0.164, *P* = <0.001), higher BUN levels (β = 0.104, *P* = 0.004), higher creatinine levels (β = 0.088, *P* = 0.013), higher total cholesterol levels (β = 0.079, *P* = 0,004), higher systolic blood pressure (β = 0,07, *P* = 0.008), higher triglyceride levels (β = 0.058, *P* = 0.028), older age (β = 0.057, *P* = 0.021), and higher CRP levels (β = 0.052, *P* = 0.034), r^2 of the model = 0.137 (table 2).

Relationship with clinical outcome

Cox regression analysis for the endpoints is presented in table 4b. Log plasma KIM-1 was associated with all-cause mortality within 180 days (HR = 1.21 (1.03-1.43), *P* = 0.020) and heart failure rehospitalization within 60 days (HR = 1.29 (1.08-1.55), *P* = 0.006). Log plasma KIM-1 was an independent predictor for heart failure rehospitalization even after adjustment for a previous selected model that includes age, history of heart failure hospitalization, peripheral edema, systolic blood pressure, sodium, BUN, creatinine, and albumine (HR = 1.26 (1.03-1.54), *P* = 0.028) and these 8 variables plus BNP (HR 1.27 (1.03-1.55), *P* = 0.024). Supplementary figure 2, Kaplan-Meier event-free survival curves for quartiles of plasma KIM-1 showed that higher levels of plasma KIM-1 (quartile 4) are associated with a slightly higher probability of heart failure rehospitalization (*P* = 0.066). This is confirmed in supplementary table 2, in which the association of plasma KIM-1 with outcome is analyzed for quartiles of plasma KIM-1.

Discussion

In this study, we investigated the reliability of plasma KIM-1 as a marker of tubular kidney dysfunction in patients with chronic and acute heart failure and its associations with adverse clinical outcome. Plasma KIM-1 is associated with glomerular function and with urinary NAG, but not with urinary tubular damage markers and has a moderate association with clinical outcome. This study is the first to study plasma KIM-1 in heart failure.

Marker position of plasma KIM-1

Previous studies showed that urinary markers of tubular damage provide additional prognostic information independent from glomerular function in patients with heart failure.[5-7] In addition, urinary KIM-1 appeared to be a suitable marker to assess tubular damage and was the best predictor of worsening renal function.[4,6] Recently, an assay to assess plasma KIM-1 has become available.

Contrary to expectations, our study shows that plasms KIM-1 is only moderately associated with other markers of tubular damage, but is strongly associated with glomerular function. We only found a moderate association with urinary NAG over tertiles of plasma KIM-1. However, urinary NAG was the only marker of tubular damage that was associated with GFR, so therefore the association between urinary NAG and plasma KIM-1 might be explained through its association with GFR. Both urinary KIM-1 and urinary NGAL had no association with either GFR or plasma KIM-1.

In a previous study in patients with kidney disease, plasma KIM-1 was strongly associated with renal injury and decreasing GFR, and they found an association between plasma KIM-1 and urinary KIM-1.[11] The absence of a correlation between plasma KIM-1 and other renal tubular markers, such as urinary NGAL and urinary KIM-1 might have several explanations.

Plasma KIM-1 is encoded by the HAVCR1 gene.[18] This gene is widely expressed in the human body, and highest levels are found in the testis and kidney. Circulating plasma KIM-1 might be originating from other organs and is possibly not specifically linked to renal (tubular) damage. The previous study in patients with kidney disease already showed that plasma KIM-1 levels are detectable in healthy volunteers and thus in the absence of renal (tubular) damage.[11] Due to the pathologic changes that are associated with heart failure, it is conceivable that the lungs or the heart itself sheds KIM-1. Furthermore, congestion of the hepatic and portal vessels or impaired perfusion may also produce pathologic changes in the liver, spleen and intestines.[19] Possible difficulties of the kidney in handling plasma KIM-1 could play a role, which could be due to the size of KIM-1 (104 kDa).[20] Small-molecularweight proteins (<40 kDa) are freely filtered by the glomeruli, whereas bigger proteins (>100 kDa) are almost completely impeded by the glomerular filtration barrier.[21] This further implies that plasma KIM-1 probably does not originate from the tubulus. Furthermore, in previous research plasma NGAL also demonstrated to be associated with decreasing glomerular function,[22] which is in line with our findings regarding plasma KIM-1. Plasma KIM-1 might be associated with the severity of heart failure and comorbidities, which is reflected by the association of higher levels of plasma KIM-1 with higher levels of NT-proBNP, BNP and a remarkable association with characteristics of the metabolic syndrome in the acute HF cohort, as expressed by the relationship of higher plasma KIM-1 with higher BMI, diabetes, dyslipidemia, and hypertension.

Plasma KIM-1 and adverse clinical outcome

Plasma KIM-1 is moderately associated with adverse clinical outcome. In chronic heart failure, the association with outcome was maintained even after correction for GFR, but statistical significance was lost after correction for NT-proBNP. In acute heart failure plasma KIM-1 was a significant predictor of heart failure rehospitalization in multivariable analysis, but not for other endpoints. When plasma KIM-1 was evaluated in quartiles, the association with heart failure rehospitalization was only significant for the highest quartile of plasma KIM-1 levels. Plasma KIM-1 showed strong associations with severity of heart failure in our study, which could explain why plasma KIM-1 predicted heart failure rehospitalization but not other endpoints.

Strengths and limitations of the study

This is the first study investigating the role of plasma KIM-1 in heart failure. We compared plasma KIM-1 with several renal indices, and in particular with measured GFR, measured by Iothalamate clearance. This increases the reliability of the data for positioning plasma KIM-1 as a marker in this cohort. Furthermore, we investigated plasma KIM-1 in both acute and chronic heart failure. However, all analyses are retrosepctive and the renal mechanistic cohort is a relatively small, single centre cohort with mainly young male patients, with reasonably preserved GFR. Additionally, different assays were used for the measurement of KIM-1 in urine and in plasma.

Conclusion and implications

Plasma KIM-1 is associated with glomerular filtration and with urinary NAG, but not with urinary tubular damage markers. Plasma KIM-1 is only moderately associated with clinical outcome. This study implicates that plasma KIM-1 is probably not clinically applicable as a tubular damage marker in heart failure patients.

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Figure Legends

Figure 1. Relationship between tubular markers and GFR per BSA and tertiles of plasma KIM-1 in the renal mechanistic cohort.

Relationship between tertiles of plasma KIM-1 and a) urinary KIM-1, b) urinary NGAL, c) urinary NAG, and d) GFR per BSA. Abbreviations: KIM-1: Kidney Injury Molecule-1, NAG: N-acetyl-β-D-glucosaminidase, NGAL: Neutrophil Gelatinase-Associated Lipocalin, GFR: glomerular filtration rate, BSA: body surface area

Figure 2. Kaplan-Meier survival curve for tertiles of plasma KIM-1 in the renal mechanistic cohort.

Log-rank P = 0.019. Abbreviations: KIM-1: Kidney Injury Molecule-1

Table 1. Baseline characteristics and relationship between tertiles of plasma KIM-1 in the renal mechanistic cohort

	Tertile 1 (n=33)	**Tertile 2 (n=33)**	**Tertile 3 (n=33)**	**P-value**
Plasma KIM-1 (pg/ml)	103.1 (72.1 – 122.9)	171.5 (149.3 – 207.7)	353.9 (318.6 – 421.9)	
Clinical characteristics				
Age (years)	54.4 ± 12.0	61.1 ± 10.7	64.8 ± 11.0	0.001
Gender, n (% male)	27 (82)	25 (76)	28 (85)	0.634
BMI (kg/m²)	27.1 ± 3.4	27.9 ± 3.7	27.6 ± 3.4	0.601
NYHA III/IV, n (%)	12 (36)	10 (30)	14 (42)	0.895
LVEF (%)	29.2 ± 8.8	29.1 ± 9.8	28.3 ± 9.8	0.902
Diabetes, n (%)	1 (3)	3 (9)	7 (21)	0.057
Systolic blood pressure (mm Hg)	120.7 ± 20.8	118.1 ± 18.5	119.6 ± 21.8	0.879
NT-proBNP (ng/L)	520.0 (284.9– 881.2)	510.6 (212.0– 1973.0)	1055.0 (483.1– 2755.0)	0.019
Prior medication use				
ACE-inhibitor, n (%)	30 (91)	26 (79)	26 (79)	0.321
ARB, n (%)	3 (9)	8 (24)	6 (18)	0.259
Beta-blocker, n (%)	29 (88)	30 (91)	24 (73)	0.099
Diuretics, n (%)	19 (19)	19 (19)	25 (25)	0.208
Aldosterone antagonist, n	5 (15)	10 (30)	12 (36)	0.137

(%)				
Digoxin, n (%)	0 (0)	0 (0)	2 (6)	0.130
Renal function				
GFR per BSA (mL/min/1.73m²)	86.4 ± 22.5	76.4 ± 23.9	58.2 ± 24.7	<0.001
ERPF per BSA (mL/min/1.73m²)	306.0 ± 72.1	276.8 ± 82.2	220.7 ± 81.3	<0.001
RBF per BSA (mL/min/1.73m²)	528.9 ± 133.5	482.7 ± 151.0	371.0 ± 145.4	<0.001
Creatinine (μmol/L)	94.0 (88.0-108.5)	104.0 (89.0-117.5)	125.0 (96.0-147.0)	0.003
BUN (mg/dL)	17.6 (14.4-19.9)	19.9 (16.5-28.6)	24.4 (20.4-39.6)	<0.001
Cystatin C (mg/L)	0.74 (0.68–0.79)	0.77 (0,67–0.95)	1.00 (0.86–1.26)	<0.001
Urinary markers				
Urinary KIM-1 (ng/gCr)	277.6 (188.4–546.5)	376.1 (229.5–587.7)	407.0 (219.6–740.9)	0.526
Urinary NGAL (ug/gCr)	178.4 (53.6–357.2)	152.5 (64.0–359.8)	195.9 (78.4–351.8)	0.892
Urinary NAG (U/gCr)	9.2 (5.6–13.2)	6.6 (5.1–14.2	13.6 (8.6–19.7)	0.033
Urinary creatinine (mmol/L)	6.7 (5.1–9.6)	7.2 (5.4–9.6)	6.1 (4.8 – 8.3)	0.345
Urinary albumin (mg/L)	6.7 (3.8–10.4)	5.9 (3.1–12.3)	5.7 (3.4–26.3)	0.895

Abbreviations: ARB: angiotensin II receptor blocker BMI: body mass index, BSA: body surface area, BUN: blood urea nitrogen, ERPF: effective renal plasma flow, FF: filtration fraction, GFR: glomerular filtration rate, KIM-1: kidney injury molecule-1, LVEF: left ventricular ejection fraction, NAG: N-acetyl-β-D-glucosaminidase**,** NGAL: Neutrophil Gelatinase-Associated Lipocalin**,** NT-proBNP: N Terminal pro Brain Natriuretic Peptide**,** NYHA: New York Heart Association, RBF: renal blood flow

Table 2. Multivariable linear regression for plasma KIM-1 in the renal mechanistic cohort and acute heart failure cohort

Variables	Standardized Beta	T-value	P-value
Renal mechanistic cohort			
Log BUN	0.337	2.096	0.040
Aldosterone antagonist	0.302	2.696	0.009
Age	0.275	2.337	0.023
GFR per BSA	0.267	1.539	0.129
Log urinary NAG	0.245	2.175	0.033
Beta blocker	-0.131	-1.266	0.210
Gender	0.107	0.986	0.328
Log urinary KIM-1	0.092	0.846	0.401
Log NT-proBNP	0.042	0.350	0.727
Acute heart failure cohort			
Diabetes	0.188	7.529	<0.001
Albumin	-0.164	-6.431	<0.001
Log BUN	0.104	2.847	0.004
Log Creatinine	0.088	2.479	0.013
Total Cholesterol	0.079	2.862	0.004
Systolic blood pressure	0.07	2.674	0.008
Triglycerides	0.058	2.196	0.028
Age	0.057	2.314	0.021
CRP	0.052	2.116	0.034

Abbreviations: BMI: body mass index, BUN: blood urea nitrogen, CRP: C-reactive protein, GFR: glomerular filtration rate, KIM-1: kidney injury molecule-1, NAG: N-acetyl-β-D-glucosaminidase, NT-proBNP: N terminal pro brain natriuretic peptide

Table 3. Baseline characteristics and relationship between quartiles of plasma KIM-1 in acute heart failure

	Q1	**Q2**	**Q3**	**Q4**	**P-value for trend**
	(n=397)	(n=397)	(n=397)	(n=397)	
KIM-1 value (pg/ml) (median, [min-max])	133.96 [2.00, 182.18]	231.33 [182.19, 295.05]	371.73 [295.15, 483.60]	735.32 [485.86, 1000.00]	
Age (years)	68.65 ±12.72	71.69 ±11.14	71.67 ±10.09	71.46 ±10.32	<0.001
Male gender (%)	272 (68.5)	272 (68.5)	250 (63.0)	254 (64.0)	0.718
Systolic blood pressure (mmHg)	122.61 ±16.86	122.93 ±17.28	124.32 ±16.98	129.10 ±17.92	<0.001
BMI (kg/m²)	27.93 ±6.17	28.45 ±5.70	29.05 ±6.04	29.57 ±5.89	<0.001
Rolofylline administration (%)	260 (65.5)	265 (66.8)	265 (66.8)	272 (68.5)	0.391
LVEF (%)	30.2 ±12.4	31.4 ±12.5	34.4 ±13.9	34.0 ±13.6	<0.001
HFpEF (LVEF≥45) (%)	27 (14.7)	35 (17.9)	42 (22.7)	48 (24.1)	0.011
WRF (%)	69 (18.2)	71 (18.4)	69 (18)	71 (19.3)	0.742
Prior medication use					
ACEI (%)	250 (63.1)	268 (67.5)	238 (59.9)	236 (59.4)	0.087
ARB (%)	54 (13.6)	57 (14.4)	67 (16.9)	63 (15.9)	0.253
Beta blocker (%)	298 (75.3)	290 (73.0)	304 (76.6)	303 (76.3)	0.486
Calcium channel blocker (%)	51 (12.9)	46 (11.6)	48 (12.1)	80 (20.2)	0.004
Aldosterone inhibitor (%)	200 (50.5)	202 (50.9)	165 (41.6)	149 (37.5)	<0.001
Digoxin (%)	123 (31.1)	126 (31.7)	108 (27.2)	100 (25.2)	0.029
Medical history					
Hypertension (%)	293 (73.8)	322 (81.1)	319 (80.4)	337 (84.9)	<0.001

Diabetes (%)	109 (27.5)	151 (38.0)	202 (50.9)	271 (68.3)	<0.001
Dyslipidemia (%)	197 (49.6)	174 (43.9)	203 (51.1)	233 (58.7)	0.002
Biomarkers					
Albumin (g/dL)	4 (3.7-4.3)	3.9 (3.6-4.1)	3.8 (3.5-4.1)	3.7 (3.5-4)	<0.001
BNP (pg/mL)	357.7 (219.4-664)	474.1 (282.3-798.9)	489.8 (274.8-850.4)	463.6 (255-861.6)	0.003
BUN (mg/dl)	24 (18-32)	30 (22-41)	32 (24-44)	34.5 (25-48)	<0.001
NT-proBNP (pg/mL)	3000 (496.0-62810.0)	3000 (1249.0-154312.0)	3000 (1388.0-63501.0)	3000 (646.2-48778.0)	<0.001
Total cholesterol (mg/dl)	144.5 (116-169.2)	139 (118-168)	136 (155-168.8)	144 (119.2-184)	0.035
Creatinine (mg/dl)	1.2 (1-1.5)	1.4 (1.1-1.7)	1.4 (1.2-1.8)	1.6 (1.2-2)	<0.001
CRP (ng/mL)	10787.5 (5751.1-23260.7)	13289 (7458.5-26374.6)	15434.9 (8064-29171.1)	16377.1 (8305.6-32659.8)	<0.001
Glucose (mg/dL)	124 (103-154)	124 (101-159)	126 (103-164)	136 (106-185)	<0.001
Hemoglobin (g/dL)	12.9 (11.6-14.2)	12.6 (11.4-13.9)	12.4 (11.2-13.7)	12 (10.9-13.4)	<0.001
NGAL (ng/mL)	63.2 (42.4-95.5)	77.7 (52-119.4)	89.8 (56.5-145.5)	108.7 (71-180.7)	<0.001
Potassium (mmol/L)	4.2 (3.8-4.6)	4.3 (3.9-4.6)	4.2 (3.8-4.6)	4.2 (3.9-4.7)	0.096
Triglycerides (mmol/L)	84 (63-112)	82 (60-110)	87 (65-119)	101 (72-142)	<0.001
Uric acid (mg/dL)	8.3 (6.8-10.2)	8.8 (7.2-10.6)	9.4 (7.6-10.8)	8.7 (7.2-10.4)	0.064

Abbreviations: ACEI: ACE-inhibitor, ARB: angiotensin II receptor blocker, BMI: body mass index, BNP: brain natriuretic peptide, BUN: blood urea nitrogen, CRP: C-reactive protein, HFpEF: heart failure with preserved ejection fraction, KIM-1: kidney injury molecule-1, LVEF: left ventricular ejection fraction, NGAL: Neutrophil Gelatinase-Associated Lipocalin, NT-proBNP: N terminal pro brain natriuretic peptide, WRF: worsening renal function

Table 4a. Cox Regression analysis for the combined endpoint in the renal mechanistic cohort

Variable	Hazard ratio (95% CI)	P-value
Per log plasma KIM-1	2.69 (1.52 – 4.76)	0.001
- **Adjusted for age and gender**	2.69 (1.51 – 4.81)	0.001
- **Adjusted for above and GFR per BSA**	1.94 (1.07 – 3.53)	0.030
- **Adjusted for above and log NT-proBNP**	1.61 (0.81 – 3.20)	0.175
- **Adjusted for above and log BUN**	1.61 (0.81 – 3.23)	0.178

Abbreviations: BSA: body surface area, BUN: blood urea nitrogen, CI: confidence interval, GFR: glomerular filtration rate, HR: Hazard Ratio, KIM-1: kidney injury molecule-1, NT-proBNP: N terminal pro brain natriuretic peptide

Table 4b. Cox regression analysis for 3 outcomes in acute heart failure

Outcomes	**Univariable Cox**		**Adjusted for age, previous heart failure hospitalization, peripheral edema, SBP, sodium, urea, creatinine and albumin**		**Adjusted for the previous model + BNP**	
	HR per log plasma KIM-1 (95% CI)	**P-value**	**HR per log plasma KIM-1 (95% CI)**	**P-value**	**HR per log plasma KIM-1 (95% CI)**	**P-value**
Death within 180 days	1.21 (1.03-1.43)	0.020	1.03 (0.89-1.21)	0.699		
Heart failure Rehospitalization within 60 days	1.29 (1.08-1.55)	0.006	1.26 (1.03-1.54)	0.028	1.27 (1.03-1.55)	0.024
Death or Cardiovascular or Renal Rehospitalization within 60 days	1.05 (0.93-1.19)	0.396				

Abbreviations: BNP: brain natriuretic peptide, CI: confidence interval, HR: Hazard Ratio, SBP: systolic blood pressure