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Urinary Kidney Injury Molecule-1 and the Risk of Cardiovascular Mortality in Elderly Men

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Background and objectives KIM-1 has been suggested as a clinically relevant highly specific biomarker of acute kidney tubular damage. Yet, community-based data on the association between urinary levels of KIM-1 and the risk for cardiovascular mortality is lacking. Our aim was to investigate the association between urinary kidney injury molecule (KIM)-1 and cardiovascular mortality.

Design, setting, participants, & measurements Prospective study, using the community-based Uppsala Longitudinal Study of Adult Men (ULSAM; n=590; mean age: 77 years; baseline period: 1997-2001; median follow-up: 8.1 years; end of follow-up: 2008).

Results During follow-up, 89 participants died of cardiovascular causes (incidence rate 2.07 /100 person-years at risk). In models adjusted for cardiovascular risk factors (age, systolic blood pressure, diabetes, smoking, body mass index, total cholesterol, high-density lipoprotein cholesterol, antihypertensive treatment, lipid-lowering treatment, aspirin treatment, and history of cardiovascular disease) and markers of kidney dysfunction and damage (cystatin C-based glomerular filtration rate (GFR) and urinary albumin/creatinine-ratio), higher urinary KIM-1/creatinine (from 24-hour urine collections) was associated with higher risk for cardiovascular mortality (HR per SD-increase, 1.27; 95% CI 1.05 to 1.54; P=0.01). Participants with a combination of high KIM-1/creatinine (upper quintile, $\geq$175 ng/mmol), low GFR ($\leq$60 ml/min/1.73 m^2) and micro-/macro-albuminuria (albumin/creatinine-ratio$\geq$3g/mol) had a more then 8-fold increased risk compared to participants with low KIM-1/creatinine (<175 ng/mmol), normal GFR (>60 ml/min/1.73 m^2), and normoalbuminuria (albumin/creatinine-ratio <3g/mol), [HR 8.56, 95%CI 4.17-17.56, p<0.001].

Conclusions These findings may suggest that higher urinary KIM-1 may predispose a higher risk of cardiovascular mortality independently of established cardiovascular risk factors, GFR

and albuminuria. Additional studies are needed to further assess the utility of measuring KIM-1 in the clinical setting.

Abbreviations and Acronyms:

KIM = Kidney injury molecule

GFR = Glomerular Filtration Rate

ACR = Urinary albumin/creatinine-ratio

NGAL = neutrophil gelatinase-associated lipocalin

ULSAM = Uppsala Longitudinal Study of Adult Men

HR = Hazard Ratio

CRP = C-reactive protein

IL = interleukin

CVD = Cardiovascular Disease

BMI = Body Mass Index

SD = Standard deviation

There is growing recognition of the clinical importance of the interplay between renal damage and the development of cardiovascular disease (CVD).[1] It was recently shown that two different aspects of kidney pathology, low glomerular filtration rate (GFR) and albuminuria, provide additive and independent predictive information for the development of CVD beyond the established CVD risk factors.[2, 3] However, the role of kidney tubular damage in the development of cardiovascular disease is less studied.

Kidney injury molecule 1 (KIM-1) is a trans-membrane protein that is primarily expressed in epithelial cells in damaged regions of the proximal tubuli.[4, 5] KIM-1 has been suggested to be involved in the modulation of tubular damage and repair in response to acute kidney injury,[6, 7] but KIM-1 may also play a role in the progression of chronic kidney disease.[8-12]

Urinary levels of KIM-1 has been suggested as a clinically relevant biomarker of acute tubular injury[5] as it rises more rapidly and is more specific to tubular damage than urinary albumin/creatinine ratio (ACR) or low GFR. Urinary levels of KIM-1 are also elevated in chronic renal diseases.[13]

Previous studies have shown that higher urinary KIM-1 levels are associated higher mortality risk in patients with overt kidney disease,[14] and heart failure,[15] but the association between KIM-1 and cardiovascular mortality in the community has not been reported previously.

In the present study, we hypothesized that individuals with kidney tubular damage are at higher risk for cardiovascular mortality. Accordingly, the aim of this study was to investigate the association between a specific marker of kidney tubular damage (urinary KIM-1/creatinine) and the risk of cardiovascular and total mortality in a community-based sample

of elderly men and whether this association was independent of established cardiovascular risk factors, GFR and ACR.

Methods

Study Sample. The Uppsala Longitudinal Study of Adult Men (ULSAM) was started in 1970. The design and selection criteria of ULSAM have been described previously.[3, 16, 17] Further details can be found on the Internet (http://www.pubcare.uu.se/ULSAM), and in recent publications on KIM-1 in ULSAM.[18, 19] The present analyses are based on the fourth examination cycle (1997-2001)**,** when 838 men (mean age 77.5 years) were investigated. Of these, urine samples and KIM-1 measurements were available in 627 individuals. Thirty-seven participants had missing data on covariates leaving 590 individuals with data on all covariates in the present study sample. All participants gave written informed consent, and the ethics committee of Uppsala University approved the study protocol.

Outcome. The Swedish Cause-of-Death register was used to obtain the outcomes total and cardiovascular mortality. Cardiovascular mortality was defined as ICD-10 codes I00-I99 (Supplementary table 1).

Statistical Analyses. The associations of urinary KIM-1/creatinine with cardiovascular and total mortality were investigated using Cox´s proportional hazard regression using the following multivariable models:

Model A: age-adjusted (age was adjusted for in all models by using age as the timeline).

Model B: adjusted for age and established cardiovascular risk factors (known CVD at baseline, antihypertensive treatment (yes/no), lipid lowering treatment (yes/no), low-dose

aspirin treatment (yes/no), current smoking, diabetes, systolic blood pressure, BMI, total cholesterol and HDL cholesterol).

Model C: adjusted for age, established cardiovascular risk factors (as in model B), and markers of kidney damage and dysfunction (GFR and ACR).

We also performed analyses adjusting for urinary NGAL/creatinine and serum NGAL. In our primary analyses, we modelled KIM-1/creatinine as a continuous variable (expressed as 1 standard deviation (SD) increase). We also performed secondary threshold models (upper quintile vs. below the upper quintile of KIM-1/creatinine). Proportional hazards assumptions were confirmed by Schoenfeld's tests and linearity assumptions were confirmed by inspecting Martingale residuals. To gain additional insights into potential nonlinearity of the associations, we examined the Cox regression models using cubic splines with four degrees of freedom.

In order to evaluate the interplay between KIM-1/creatinine and other aspects of kidney pathology in the risk of cardiovascular mortality, we divided the participants in the following 5 groups:

1. Participants with normal GFR (>60 ml/min), normal ACR (<3g/mol) and normal KIM-1/creatinine (quintile 1-4, <175 ng/mmol [referent])

2. Participants with normal GFR (>60 ml/min), normal ACR (<3g/mol) but with high KIM-1/creatinine (quintile 5, ≥175 ng/mmol)

3. Participants with either low GFR (≤60ml/min), high ACR (≥3g/mol) or both, but with normal KIM-1/creatinine (quintile 1-4, <175 ng/mmol)

4. Participants with either low GFR (≤60ml/min) or high ACR (≥3g/mol), but with high KIM-1/creatinine (quintile 5, ≥175 ng/mmol)

5. Participants with low GFR (≤60 ml/min), high ACR (≥3g/mol), and high KIM-1/creatinine (quintile 5, ≥175 ng/mmol)

Additionally, we performed tests for effect modification by hypertension, diabetes, prevalent cardiovascular disease, micro-albuminuria and GFR by including multiplicative interaction terms of these variables and urinary KIM-1/creatinine. We also performed analyses after exclusion of participants with previous diagnosis of heart failure at baseline or during follow-up (n=100) to limit the possibility of reverse causation due to heart failure as an explanation of our findings. In secondary analyses, multiple imputation methods were used to account for the potential influence of missing data. Cumulative incidence curves were compared with Kaplan-Meier failure curves and Fine and Gray analyses were performed in order to address the issue of competing risk from non-CVD mortality.[20, 21] In secondary analyses, covariate factors were replaced by a propensity score to ensure that our adjusted models were not influenced by over-fitting.[22]

Laplace regression was used to calculate the difference in years until 10% of the participants died of cardiovascular mortality in participants with low GFR (≤60 ml/min), micro-/macro-albuminuria (≥3g/mol), and high KIM-1/creatinine (≥175 ng/mmol), respectively vs. those without these traits.[23]

We also investigated the association between KIM-1 excretion per 24 hours (KIM-1/24-hours) and the risk for cardiovascular mortality.

A two-sided p-value <0.05 was regarded as significant in all analyses. The statistical software package STATA 11.2 (Stata corp, College Station, Texas, USA) was used for all analyses.

Results

Baseline characteristics of included participants are shown in Table 1 (excluded participants due to missing data on KIM-1/creatinine had similar values as those included, p>0.14 for all, data not shown). The Spearman correlation coefficient between KIM-1/creatinine and GFR was -0.14 (p<0.001), and between KIM-1/creatinine and albumin/creatinine 0.41 (p<0.001). During follow-up (median of 8.1 years, range 0.3-10.8 years), 198 individuals died, of which 89 deaths were due to cardiovascular causes (Table 2).

In Cox proportional hazard models, higher urinary levels of KIM-1/creatinine were significantly associated with higher risk for CVD mortality. The associations were only mildly effected by adjustments for age, cardiovascular risk factors, GFR, and urinary albumin/creatinine ratio (Model A-C, Table 2). Examination of regression splines suggests a linear increase in hazard for CVD mortality with increasing KIM-1/creatinine (Figure 1).. In secondary analysis, participants with KIM-1/creatinine levels in the highest quintile (>175 ng/mmol) were at almost a doubled risk for CVD mortality (Table 2, Figure 2). The association between urinary KIM-1/creatinine and total mortality was generally weaker as compared to the association between KIM-1/creatinine and CVD mortality (Table 2).

All aspects of kidney pathology: KIM-1/creatinine, GFR and ACR, predicted cardiovascular mortality independently of each other when included in the same model, even after further addition of cardiovascular risk factors, and serum and urinary NGAL(Table 3).

The association between urinary KIM-1/creatinine and cardiovascular mortality was essentially unaltered when missing data on covariates were imputed, and based on all 627 individuals with KIM-1 measurements (data not shown).

The first 10% died of cardiovascular mortality 4.3 years earlier (95% CI 6.2-2.4, p<0.001) among participants with KIM-1/creatinine levels in the upper quintile (>175 ng/mmol) compared with those with lower KIM-1/creatinine levels (<175 ng/mmol). The corresponding numbers for GFR<60 mL/min/1.73 m^{2}vs. for GFR> 60 mL/min/1.73 m^2 was 3.2 years earlier (95% CI 4.9-1.5, p<0.001), and for micro/macro-albuminuria (ACR>3 g/mol) vs. no micro/macro-albuminuria, 3.5 years earlier (95% CI 4.7-2.3, p<0.001).

As seen in Table 4, participants with low GFR (<60 mL/min/1.73 m^2), micro/macro-albuminuria (ACR >3g/mol), and high KIM-1/creatinine (>175 ng/mmol) had a more than 8-fold increase in risk for cardiovascular mortality, and participants with either low GFR or micro-/macro-albuminuria and high KIM-1/creatinine had a 3-fold increase in risk compared to participants with normal GFR, normo-albuminuria and low KIM-1/creatinine. A similar risk (~3-fold increase), was seen in participants with low GFR and/or micro-/macro-albuminuria but with low KIM-1/creatinine. Participants with normal GFR and normo-albuminuria but with high KIM-1/creatinine had a more than 2-fold increased risk. All these findings remained significant and were mildly attenuated after adjustments for established cardiovascular risk factors. The associations with total mortality were not as high.

The results did not substantially change after excluding participants with heart failure at baseline or during follow-up (Model B: HR for 1 SD increase in KIM-1 1.36, 95% CI 1.08-1.72, p=0.008).

The multiplicative interaction terms for prevalent cardiovascular disease, hypertension, diabetes, low GFR (<60 mL/min/1.73 m^2) or micro-albuminuria were non-significant (p>0.27 for all).

A comparison between cumulative incidence curves and Kaplan-Meier failure curves, as well as Fine and Gray analyses indicated that our results would not be compromised by competing risk from non-CVD mortality (data not shown). Neither are they likely to be a result of over-

fitting, as the findings remained unaltered in models where the covariate factors were replaced by a propensity score (data not shown).

In the 24-hour urine collections, increments of KIM-1 concentration without creatinine standardization, was also significantly associated with cardiovascular mortality in multivariable model A-C (Supplementary table 2).

The risk estimates for cardiovascular mortality for the total amount of excreted KIM-1/24 hours varied substantially in the whole cohort as compared to participants with >1000 ml urine/24 hours or participants with >1500 ml urine/24 hours (HRs ranged from 1.08 per SD increase of KIM-1/24 hours (whole sample) to 2.97 (participants with >1.500ml urine. Conversely, the KIM-1/creatinine estimate was similar in all different urine volume subgroups (Supplementary table 3).

Discussion

In a cohort of free-living elderly men, higher urinary KIM-1/creatinine was associated with a higher risk for cardiovascular mortality. These associations remained robust after adjustments for established cardiovascular risk factors and established markers for kidney damage and dysfunction (ACR, and GFR). Participants who had a combination of high KIM-1, low GFR, and micro-/macro-albuminuria had the highest cardiovascular risk. Interestingly, our data indicates that urinary KIM-1 also identifies a subgroup of individuals with higher cardiovascular risk in those with no other signs of kidney damage or dysfunction. Our data confirm and extend previous studies suggesting a link between kidney tubular damage and the development of CVD.[24]

Comparison with the literature

Higher urinary levels of KIM-1 have been suggested to provide prognostic information regarding mortality risk in patients with renal disease,[13, 14] or heart failure.[15] For instance, in a study of hospitalized patients with acute renal failure, higher urinary KIM-1 was associated with higher risk of dialysis requirement or death,[14] and in kidney transplant patients, higher urinary KIM-1 was associated with an increased risk of graft loss.[13, 25] In patients with overt heart failure, higher urinary KIM-1 was associated with higher risk for mortality and heart failure morbidity.[15] Moreover, we recently reported that higher urinary KIM-1 is associated with and increased risk for incident heart failure hospitalizations in the present community-based study cohort.[26] Yet, we are aware of no previous studies that have reported the association between urinary KIM-1 and cardiovascular mortality in the community. However, both circulating and urinary levels of NGAL, another tubular damage biomarker, has also been shown to be an independent predictor of cardiovascular mortality.[27] In the present study, the independent association between KIM-1/creatinine and cardiovascular mortality was not influenced after adding both urinary and circulating NGAL to the multivariate model. Interestingly, in this model neither urinary nor circulating NGAL were significant predictors of cardiovascular mortality (Table 3), perhaps indicating that KIM-1/creatinine is superior to NGAL as a prognostic marker.

Potential Mechanisms

Previous experimental studies have illustrated different functions of KIM-1 in various renal diseases including protective functions in acute kidney injury and damaging functions in chronic kidney disease.[5-7] Even though it is not possible to establish causality in our observational study, there are some potential pathways that could explain the present associations:

First, higher urinary levels of KIM-1 have been reported in patients with impaired GFR and micro- or macro-albuminuria,[25, 28] two aspects of kidney disease that has been shown to be

closely associated with an increased cardiovascular risk.[2, 3] The fact that KIM-1 predicted cardiovascular death beyond these established markers of kidney damage and dysfunction indicates that KIM-1 portrays an aspect of kidney pathology that is not fully reflected by levels of GFR or albuminuria. It is also possible that KIM-1 predicts the deterioration of kidney function which in turn leads to an increased cardiovascular risk.[8, 29]

Second, CVD and CKD share several common risk factors including hypertension, hypercholesterolemia, diabetes, inflammation, oxidative stress, and RAAS activation[30].[31] Yet, the multivariable models in the present study indicate that these are not major pathways that explain our findings.

Finally, it is possible that the results of the present study are due to reverse causation, i.e. that higher urinary KIM-1 levels are a consequence of prevalent cardiovascular disease, perhaps due to atherosclerosis in the small vessels of the kidney or by prevalent heart failure. For instance, heart failure patients have higher urinary KIM-1 compared to healthy controls,[32] and higher KIM-1 predicts mortality and hospitalizations in this patient group.[15, 33] However, in the present study, the association between urinary KIM-1 and cardiovascular mortality was similar in participants free from CVD at baseline, after further exclusion of participants that developed heart failure during follow-up, which would argue against reverse causation due to prevalent cardiovascular disease or heart failure as an explanation of our findings.

Clinical Implications

As KIM-1 expression is virtually absent in normal kidneys,[34] but rise rapidly in response to tubular damage,[35] KIM-1 has been put forward as a clinically useful marker for acute tubular kidney damage.[14] In fact, recent studies have suggested the assessment of biomarkers reflecting tubular damage should be included in the diagnostic criteria for acute kidney injury.[36] However, in the present study urinary KIM-1 predicted cardiovascular mortality in a

community based sample where the prevalence of individuals with acute kidney injury likely was very low. Thus, our data indicate that urinary KIM-1 levels should not merely be considered as a marker for acute tubular damage but perhaps also a marker for chronic tubular damage that predisposes to an increased risk for cardiovascular mortality. Whether tubular damage should be considered in the diagnostic criteria for chronic kidney damage remains to be established. The clinical utility of measuring urinary KIM-1 or other markers of kidney tubular damage, such as urinary NGAL, cystatin, interleukin -18, fatty-acid binding proteins, microglobulines, or N-acetyl-β-D-glucosaminidase,[37] for risk prediction purposes in patients and in the community needs to be evaluated in further large scale studies. Previous studies have suggested that treatment with diuretics or pharmacological inhibition of the RAAS reduces urinary levels of KIM-1.[38, 39] However, it is not known whether this reduction of KIM-1 levels corresponds to a reduction of cardiovascular risk.

KIM-1 without creatinine standardization and the total excreted amount of KIM-1/24 hours also predicted cardiovascular mortality, but the strength of the association with KIM-1/24 hours varied substantially in the whole cohort as compared to participants with >1000 ml urine/24 hours or participants with >1500 ml urine/24 hours. The highest risk estimate for KIM-1/24 hours was seen in those who most likely had the highest validity of the 24-hour collection of urine. In contrast, the KIM-1/creatinine risk estimate was similar in all subgroups. This finding raises the question of what would be the best way to measure urinary KIM-1 in clinical practice. This is, of course, also an issue of feasibility; in clinical practice it is difficult to get reliable 24-hour urine collections. We would like to emphasize that given the limited size of the different strata, no firm conclusions should be drawn based on this exploratory sub-group analysis.

Strengths and Limitations. Strengths of the study include the community-based sample, the longitudinal study design with long follow-up time and the detailed characterization of the study participants. Other strengths include the high quality and completeness of ascertainment of population registers in Sweden.[40, 41] The main limitation of this study is that we only had access to data in elderly men, extrapolations of these findings to women, other ethnicities, and other age-groups have to be done with caution. Further studies are needed to evaluate our secondary aim, and age and sex specific thresholds for what should be considered a normal KIM-1/creatinine and to evaluate the clinical relevance of the suggested threshold for urinary KIM-1/creatinine in the present study (175 ng/mmol). We had only access to single eGFR, ACR and KIM-1/creatinine measurements. Also, biomarker levels measured in urine may be biased by the large variation in urine volume or concentration; however, we minimized the risk of this by standardizing the KIM-1 levels for urinary creatinine. Nor did we have access to serum creatinine levels and could thus not calculate eGFR based on the new combined equation.[42] Another limitation is the fact that some of our multivariable models were adjusted for a large number of confounders which potentially could lead to bias and uncertain risk estimates due to over-fitting. Some of the groups we studied were fairly small as well and the point estimates should thus be interpreted with caution. However, the consistency of the results across all multivariable models and in models where we replaced the covariate factors with a propensity score would argue against over-fitting as an explanation of our findings.[22]

Conclusions

Our findings show that kidney tubular damage may predispose to higher risk for cardiovascular mortality in the community-based setting. Further studies are needed in order to firmly establish a causal pathophysiological role for KIM-1 in the development of

cardiovascular disease, and to evaluate the utility of measuring urinary KIM-1 in the clinical setting.

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TABLE 1 Baseline Characteristics of all men and stratified by quintiles if KIM-1/creatinine (Q1-4 VS Q5)

Variable	All	Q1-Q4	Q5	p-value
N	590	472	118	Q1-Q4 vs Q5
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	77.5 (0.8)	77.5 (0.8)	77.5 (0.7)	0.72
BMI (kg/m^2)	26.3 (4.5)	26.2 (3.4)	26.7 (3.8)	0.15
Systolic blood pressure (mmHg)	150 (21)	150 (20)	153 (24)	0.20
Cholesterol (mmol/L)	97.3 (18)	97.3 (18)	95.5 (20)	0.17
HDL cholesterol (mmol/L)	23.4 (5.4)	23.4 (5.4)	23.4 (5.4)	0.10
GFR (mL/min/1.73 m^2)	74 (17)	75 (16)	69 (20)	
	Median (25th to 75th percentile)	Median (25th to 75th percentile)	Median (25th to 75th percentile)	
Albumin/creatinine ratio (g/mol)	0.7 (0.3-2.0)	0.6 (0.3-1.6)	1.6 (0.7-1.5)	<0.001
KIM-1/creatinine ratio (ng/mmol)	98 (55-152)	78 (49-115)	228 (199-301)	<0.001
	n (%)	n (%)	n (%)	
GFR<60mL/min/1.73 m^2	124 (21)	82 (17)	42 (36)	<0.001
Micro-/Macro albuminuria (>3g/mol)	110 (19)	67 (14)	43 (36)	<0.001
Current smoking	42 (7)	28 (6)	14 (12)	0.03
Diabetes	78 (13)	54 (11)	24 (20)	0.01
Anti-hypertensive medication	281 (48)	215 (46)	66 (66)	0.04
Lipid-lowering medication	110 (19)	80 (17)	30 (25)	0.03
NSAID	32 (5)	22 (5)	10 (9)	0.10
Low-dose aspirin	166 (28)	122 (26)	44 (37)	0.01
History of cardiovascular disease	153 (26)	116 (25)	37 (31)	0.13
History of Ischemic heart disease	109 (18)	79 (17)	30 (25)	0.03
History of Stroke	54 (9)	43 (9)	11 (9)	0.94
History of Heart failure	25 (4)	18 (4)	7 (6)	0.31

Q= Quintiles. Data are mean ± standard deviation for continuous variables and n (%) for categorical variables. History of cardiovascular disease was defined as a history of hospitalization for ischemic heart disease, cerebrovascular disease, or heart failure prior to baseline.

TABLE 2

The Association between KIM-1/creatinine and Cardiovascular and Total Mortality: Incidence Rates and Multivariable Cox regression

	Number of events / numbers at risk	Incidence rates (per 100 person-years at risk, 95%CI)	Model A Hazard ratio (95%CI)	Model B Hazard ratio (95%CI)	Model C Hazard ratio (95%CI)
Cardiovascular mortality					
Continuous models					
1 SD increase (91 ng/mmol)			1.50 (1.29-1.75) ‡	1.45 (1.22-1.71) ‡	1.27 (1.05-1.54)†
Threshold models					
Quintile 1-4 (<175 ng/mmol)	59/472	16.6 (12.9-21.4)	referent	referent	referent
Quintile 5 (≥175 ng/mmol)	30/118	38.1 (26.7-54.5)	2.34 (1.51-3.64)‡	2.08 (1.33-3.27)†	1.72 (1.07-2.76)*
Total mortality					
Continuous models					
1 SD increase			1.30 (1.16-1.47) ‡	1.24 (1.10-1.41) ‡	1.12 (0.98-1.29)
Threshold models					
Quintile 1-4 (<175 ng/mmol)	148/472	41.6 (35.4-48.9)	referent	referent	referent
Quintile 5 (≥175 ng/mmol)	50/118	63.6 (48.2-83.9)	1.56 (1.13-2.14) †	1.42 (1.02-1.97)*	1.20 (0.85-1.69)

*p<0.05, † p<0.01, ‡ p<0.001

Model A age
Model B age and established cardiovascular risk factors (known CVD at baseline, antihypertensive treatment, lipid lowering treatment, low-dose aspirin treatment, current smoking, diabetes, systolic blood pressure, BMI, total cholesterol and HDL cholesterol)
Model C age, established cardiovascular risk factors, eGFR, and albumin/creatinine ratio

Table 3 The association between urinary KIM-1/creatinine and cardiovascular mortality, adjusted for established cardiovascular risk factors, glomerular filtration rate, urinary albumin/creatinine ratio, and serum and urinary NGAL. urinary NGAL, with hazard ratios for all covariate factors shown.

Variable	Hazard Ratio	95% CI	p-value
KIM-1/creatinine (ng/mmol)	1.27	1.05-1.54	0.01
BMI (kg/m^2)	0.88	0.70-1.11	0.28
Systolic blood pressure (mmHg)	0.96	0.77-1.19	0.71
Cholesterol (mmol/L)	0.90	0.72-1.13	0.37
HDL cholesterol (mmol/L)	1.20	0.93-1.54	0.16
GFR (mL/min/1.73 m^2)	0.98	0.97-0.99	0.01
Albumin/creatinine ratio (g/mol)	1.16	1.01-1.32	0.03
Current smoking	0.75	0.35-1.63	0.46
Diabetes	2.15	1.24-3.73	0.006
Anti-hypertensive medication	1.47	0.88-2.46	0.14
Lipid-lowering medication	0.80	0.45-1.41	0.43
Low-dose aspirin	1.06	0.64-1.75	0.82
History of cardiovascular disease	2.06	1.23-3.46	0.006
Serum NGAL (µg/L)	1.02	0.83-1.25	0.88
Urinary NGAL/creatinine (µg/mmol)	0.97	0.79-1.18	0.74

Data are multivariable hazard ratios when all variables were simultaneously included in the model. Hazard ration for continuous variables are expressed per standard deviation increase.

TABLE 4

Data Portraying the Interplay between KIM-1/creatinine, GFR and Albuminuria and the Risk for Cardiovascular Mortality: Cox regression

		All-cause Mortality			Cardiovascular Mortality	
Groups according to GFR- albuminuria-, and KIM-1/creatinine status		**Model A**	**Model B**		**Model A**	**Model B**
	Number of events / numbers at risk	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**	**Number of events / numbers at risk**	**Hazard ratio (95% CI)**	**Hazard ratio (95% CI)**
Normal GFR, normal ACR and normal KIM-1/creatinine	82/342	referent	referent	30/342	referent	referent
Normal GFR, normal ACR, and high KIM-1/creatinine	19/52	1.70 (1.03-3.80)*	1.60 (0.97-2.65)	10/52	2.45 (1.20-5.01)*	2.29 (1.11-4.70)*
Low GFR, high ACR or both, and normal KIM-1/creatinine	66/131	2.42 (1.75-3.25) ‡	2.18 (1.55-3.06) ‡	29/131	2.93 (1.76-4.90) ‡	2.35 (1.38-4.01) †
Low GFR or high ACR, and high KIM-1/creatinine	19/45	2.14 (1.30-3.52) †	1.84 (1.09-3.12)*	10/45	3.09 (1.51-6.33) †	2.44 (1.14-5.19)*
Low GFR, high ACR and high KIM-1/creatinine	12/20	3.69 (2.01-6.77)‡	3.31 (1.76-6.23)‡	10/20	8.56 (4.18-17.56)‡	6.87 (3.21-14.73)‡

Normal GFR ≥ 60 ml/min/1.73 m^2 and low GFR (≤ 60 mL/min/1.73 m^2), normal ACR (albumin/creatinine ratio) <3g/mol and micro/macro-albuminuria (ACR $\geq$3g/mol), normal KIM-1/creatinine < 175 ng/mmol and high KIM-1/creatinine ($\geq$175 ng/mmol).

Model A adjusted for age.

Model B adjusted for age and established cardiovascular risk factors (known CVD at baseline, antihypertensive treatment, lipid lowering treatment, low-dose aspirin treatment, current smoking, diabetes, systolic blood pressure, BMI, total cholesterol and HDL cholesterol)

* † p<0.01, ‡ p<0.001

Supplementary Table 1. Specific causes of cardiovascular death

Mortality cause	No of CVD-deaths
Ischemic heart disease	46
Cerebrovascular disease	14
Heart Failure	10
Other cardiovascular disease (including, generalized atherosclerosis, malignant hypertension, arrhythmias, valvular disease, aneurysms)	19
Total	89

Supplementary Table 2. The association between KIM-1 concentrations (without creatinine standarization) and cardiovascular and total mortality: multivariable Cox regression

	Model A Hazard ratio (95%CI)	Model B Hazard ratio (95%CI)	Model C Hazard ratio (95%CI)
Continuous models			
1 SD increase	1.29 (1.09-1.51) †	1.30 (1.10-1.55) †	1.22 (1.01-1.47)*

*p<0.05, † p<0.01, ‡ p<0.001
Model A age
Model B age and established cardiovascular risk factors (known CVD at baseline, antihypertensive treatment, lipid lowering treatment, low-dose aspirin treatment, current smoking, diabetes, systolic blood pressure, BMI, total cholesterol and HDL cholesterol)
Model C age, established cardiovascular risk factors, eGFR, and albumin/creatinine ratio

Supplementary Table 3. The association between KIM-1/ 24 hours, KIM-1/creatinine and cardiovascular mortality.

	Number of events/ numbers at risk	KIM-1/ 24 hours	KIM-1/creatinine
		HR (95 % CI)	HR (95 % CI)
Whole sample	89/590	1.08 (0.94-1.24)	1.50 (1.29-1.75)
Individuals with >1 l urine/24 hours	64/402	1.86 (1.08-3.23)	1.51 (1.28-1.78)
Individuals with >1.5 l urine/24 hours	34/195	2.97 (1.41-6.27)	1.56 (1.29-1.89)

Figure 1. Cubic regression Splines of The Unadjusted Association Between Urinary KIM-1/creatinine and Cardiovascular Mortality
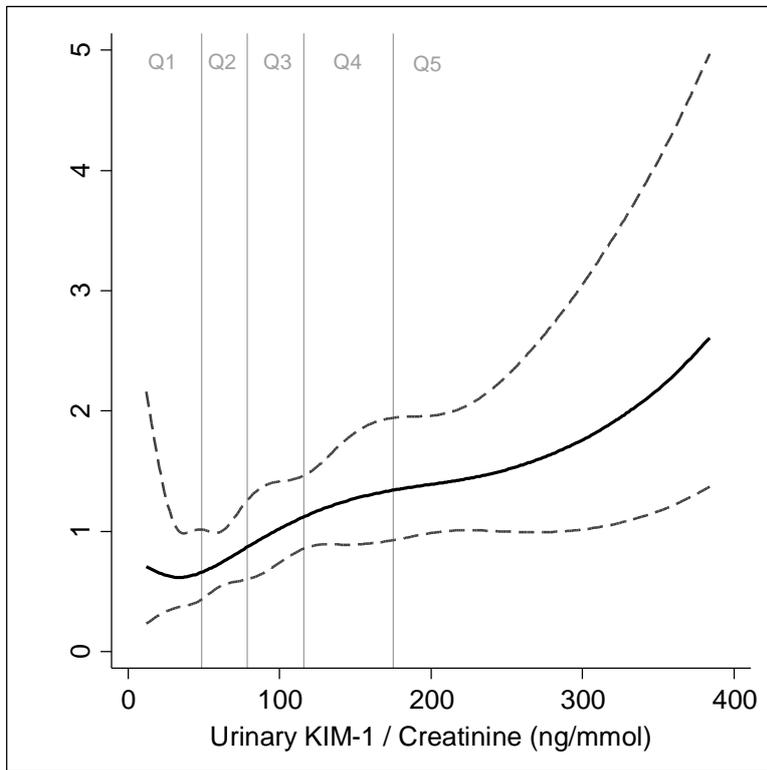
Q= Quintiles, Black line indicates estimated hazard ratios (with 95 % confidence intervals).

FIGURE 2. The Cumulative Incidence of Cardiovascular Mortality
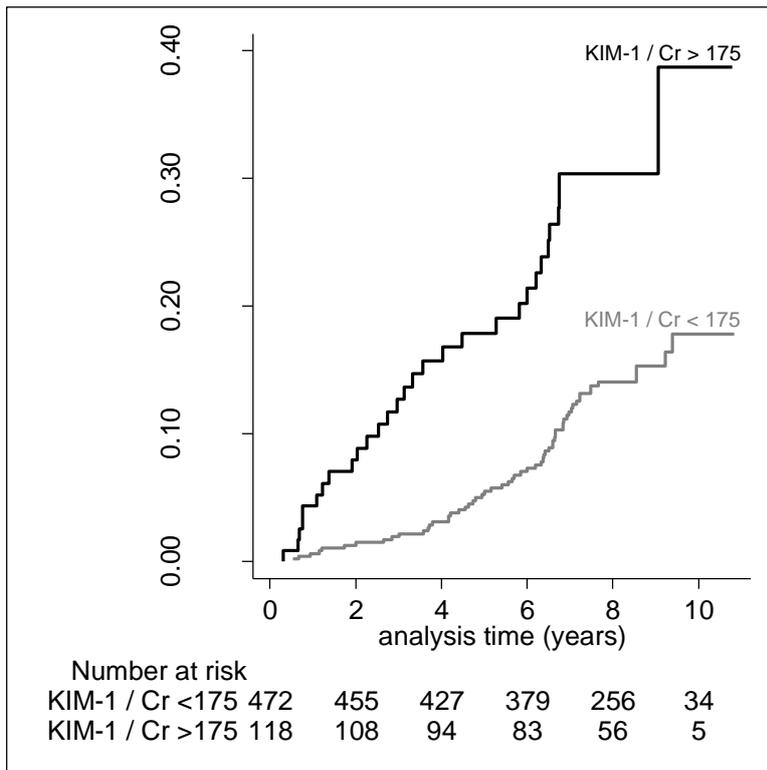
Analyzed by Above vs. Below Urinary KIM-1/creatinine 175 ng/mmol (quintile 5 vs. quintile

1-4)