Published in final edited form as:

Heart. 2010 August ; 96(16): 1297–1302. doi:10.1136/hrt.2010.194878.

Tubular damage in chronic systolic heart failure is associated with reduced survival independent of glomerular filtration rate

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Abstract

Background—The prognostic impact of reduced glomerular filtration rate (GFR) in chronic heart failure (CHF) is increasingly recognised, but little is known about tubular damage in these patients.

Objective—To investigate the prevalence of tubular damage, and its association with GFR, and prognosis in patients with CHF.

Methods and results—In 90 patients with CHF, GFR and effective renal plasma flow (ERPF) were measured (¹²⁵I iohalamate and ¹³¹I hippuran clearances). The tubular markers neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosaminidase (NAG) and kidney injury molecule 1 (KIM-1) as well as urinary albumin excretion were determined in 24 h urine collections. Mean GFR was 78±26 ml/min/1.73 m². Urinary NGAL (175 (70–346) mg/g creatinine (gCr)), NAG (12 (6–17) U/gCr) and KIM-1 (277 (188–537) ng/gCr) levels were increased compared with 20 healthy controls (all p<0.001). Urinary NAG, but not NGAL or KIM-1 correlated with GFR (r=–0.34, p=0.001) and ERPF (r=–0.29, p=0.006). Both NAG (r=0.21, p=0.048) and KIM-1 (r=0.23, p=0.033) correlated with plasma N-terminal pro-brain natriuretic peptide levels. Both urinary KIM-1 (HR=1.15 (95% CI 1.02 to 1.30) per 100 ng/gCr increase, p=0.025) and NAG (HR=1.42 (95% CI 1.02 to 1.94) per 5 U/gCr increase, p=0.039), were associated with an increased risk of death or heart failure hospitalisations, independent of GFR.

Conclusion—Tubular damage, as indicated by increased urinary concentrations of NGAL, NAG and KIM-1 is common in patients with CHF and mildly reduced GFR. Both urinary KIM-1 and NAG showed prognostic information additional to GFR. These findings suggest an important role for tubular damage and tubular markers in cardiorenal interaction in heart failure.

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Competing interests JVB is co-inventor on KIM-1 patents.

Ethics approval This study was conducted with the approval of the local medical ethics committee of the University Medical Center Groningen.

Provenance and peer review Not commissioned; externally peer reviewed.

INTRODUCTION

Reduced glomerular filtration rate (GFR) is an important independent risk factor for all-cause mortality and morbidity in patients with chronic heart failure (CHF).¹ The pathogenesis of renal impairment in CHF is multifactorial, but a main determinant is a disproportionate decrease in renal perfusion as a consequence of decreased cardiac output.² Yet, there is evidence that in many patients, CHF is associated with structural renal damage as well, including tubular abnormalities.²³ In a recent proof-of-concept study in patients with CHF, we found that increased urinary neutrophil gelatinase-associated lipocalin (NGAL) levels were highly prevalent, and associated with different indices of renal dysfunction (estimated glomerular filtration rate (eGFR) and urinary albumin excretion (UAE)), and severity of CHF—that is, levels of N-terminal pro-brain natriuretic peptide (NT-proBNP).⁴

In patients with primary renal disorders, tubulointerstitial damage is well established as the main predictor of renal outcome.⁵ Moreover, an increasing body of evidence supports the prognostic impact of urinary markers of tubular damage, in various renal disorders, such as IgA nephropathy and membranous glomerulopathy.^{6–9}

For CHF, on the other hand, the prognostic impact of urinary markers of tubular damage has not been established. In this study, therefore, we analysed the prevalence of tubular damage using a panel of different tubular marker proteins, their relationship with GFR and the association with prognosis in a well-characterised cohort of patients with CHF.

PATIENTS AND METHODS

Patient population and study design

Ninety outpatients with CHF, aged 18 years, left ventricular ejection fraction (LVEF) <45% and clinically stable, were asked to participate. All patients were receiving ACE inhibitors and/or angiotensin II receptor blockers, and all medication had to be stable for at least 1 month. In addition, 20 age- and gender-matched healthy controls were studied. All subjects gave informed consent to participate in the study, which was approved by the ethics review committees of the study centre. The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Baseline measurements included standard weight, height, systolic and diastolic blood pressure and assessment of New York Heart Association (NYHA) class heart failure. Patients underwent clearance measurements of renal function. GFR and effective renal plasma flow (ERPF) were measured by the clearances of [¹²⁵I]iothalamate and [¹³¹I]hippuran. The filtration fraction was calculated as the ratio of GFR and ERPF and expressed as percentage. eGFR was additionally determined using the simplified modification of diet in renal disease formula ($186.3 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} (\times 0.742 \text{ if female}) (\times 1.212 \text{ if black})$), for comparison between patients with CHF and controls, since the latter did not undergo radiolabelled tracer renal function measurements. Laboratory measurements comprised serum creatinine, haemoglobin levels and NT-proBNP.

Urine analysis and markers of tubular damage

For patients and controls 24 h urine was collected and UAE determined. Urinary creatinine was determined to correct for concentration of the urine. NGAL was determined by a commercially available ELISA test kit from Antibody Shop (Gentofte, Denmark). Samples were diluted 500 times in dilution buffer supplied with the test kit. NGAL was expressed in ng/ml and values were also corrected for urinary creatinine concentration. The enzyme N-acetyl-β-D-glucosaminidase (NAG) was evaluated using the substrate p-nitrophenyl N-

acetyl- β -D-glucosaminide (Sigma, St Louis, Missouri, USA) in citrate buffer at pH 4.5. After 60 min at 37°C, 1 M Na₂CO₃ was added to the mixture to terminate the reaction and to develop a yellow colour released from the converted substrate. Controls were obtained from each sample by addition of Na₂CO₃ at time=0. The colour was measured at 405 nm by a microtitre plate reader and values for controls were subtracted. A standard curve was prepared with NAG. Urinary NAG activity was expressed as U/g urinary creatinine (U/gCr). Urinary kidney injury molecule 1 (KIM-1) measurements were performed using microsphere-based Luminex xMAP technology with polyclonal antibodies raised against the human KIM-1 ectodomain. For measurements, 30 μ l of urine samples was analysed in duplicate. The lowest limit of detection for this assay is 0.125 ng/ml. The interassay and intra-assay variability was <20%. The urinary KIM-1 level was normalised to the urinary creatinine concentration.

Follow-up and prognosis

Patients' follow-up started after [¹²⁵I]iothalamate and [¹³¹I] hippuran clearances were determined. Follow-up data were based upon the patients records available at our outpatient clinic. All patients routinely visited our clinic for heart failure treatment. Information was gathered from local general practitioners when necessary. None of the patients were lost to follow-up. A combined clinical outcome parameter was defined consisting of the first occurrence of either death, heart transplantation, cardiovascular event (myocardial infarction or primary percutaneous transluminal coronary angioplasty) or first hospitalisation for heart failure with a maximum 30 months' follow-up.

Statistics

Data are given as mean $\pm$ SD when normally distributed, as median and IQR when distribution was skewed, and as frequencies and percentages for categorical variables. Differences between patients and controls were tested using Mann–Whitney U or Student t testing, where appropriate. One-sided analysis of variance or Kruskal–Wallis were used for multiple comparisons, where appropriate. Spearman's correlation coefficients were calculated between NAG, NGAL and other variables. Testing of equality of correlations was carried out using Fisher's Z transformation for the correlation coefficients. Survival analysis was carried out using Cox proportional hazard analysis. In the multivariate analyses, we first constructed a model with the individual urinary markers and GFR. In a second model, GFR was replaced by serum creatinine. In the final model, we adjusted for a number of predefined confounding factors (age, gender, LVEF, blood pressure, haemoglobin levels, GFR, NT-proBNP and UAE). Testing for interaction showed no significant ($p < 0.1$) interactions between the investigated variables. All reported probability values are two-tailed, and a p value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS, Chicago version 12.0 and STATA, College Station, Texas, version 10.0.

RESULTS

Clinical characteristics of patients and controls are summarised in table 1. The majority of patients were in NYHA functional class heart failure II and III, with a mean LVEF of $28 \pm 9\%$. Mean GFR was mildly impaired (78 ± 26 ml/min/1.73m²), with a correspondingly decreased ERPF (282 ± 83 ml/min/1.73m²). The urinary concentration of markers of tubular damage, including NAG (12 (6.2–17) U/gCr), NGAL (175 (70–346) μ g/gCr) and KIM-1 (277 (188–537) ng/gCr) were significantly elevated in patients with CHF compared with healthy controls (NAG 1.6 (0.7–2.2) U/gCr, NGAL 37 (6–58) μ g/gCr and KIM-1 (136 (63–195) ng/gCr), respectively (all $p < 0.001$) (figure 1). Even after adjustment for the difference

in eGFR, urinary NGAL, NAG and KIM-1 levels were significantly higher in patients with CHF (all $p < 0.001$) compared with controls.

Correlations with markers of tubular damage

Urinary NAG, NGAL and KIM-1 levels only showed weak, nonsignificant associations with each other (table 2). Urinary NAG levels were significantly lower in men than in women, while both urinary NGAL and KIM-1 levels increased with advancing age. Table 2 shows relationships between urinary NAG, NGAL and KIM-1 and different cardiorenal functional parameters including GFR, plasma NT-proBNP and UAE. The relationship between urinary NAG and GFR ($r = -0.34$, $p = 0.001$) and ERPF ($r = -0.29$, $p = 0.006$) was stronger than the relationship of urinary KIM-1 and NGAL with these renal indices. Both urinary NAG ($r = 0.21$, $p = 0.048$) and urinary KIM-1 levels ($r = 0.23$, $p = 0.033$) showed a significant association with NT-proBNP levels. Urinary NAG, NGAL or KIM-1 did not show any significant correlation with blood pressure, haemoglobin levels or LVEF.

Data on tubular markers, stratified for eGFR according to the definition of chronic kidney disease (CKD) (< 60 ml/min/1.73m²) are given in figure 2. These show that tubular markers are elevated in CHF, even when renal function is preserved. Yet, with a decrease in renal function a further elevation of NAG is observed, while urinary NGAL and KIM-1 levels did not differ between patients with CHF with or without CKD. Urinary levels of NAG, KIM-1 and NGAL did not significantly differ between patients with or without diuretics, β blockers, digoxin or aldosterone receptor antagonists.

Relationship with prognosis

In 30 months' follow-up, in total 37 events were recorded, of which 17 were the first event of an individual patient. These consisted of seven deaths and 10 first admissions to hospital for worsening CHF, combining to a total of 19% of patients with an event. Both urinary NGAL (HR=1.02 per 100 μ g/gCr (95% CI 0.87–1.20), $p = 0.796$) and UAE (HR=1.17 per 10 mg/24 h increase (95% CI 0.90 to 1.52), $p = 0.251$) showed no significant relationship with prognosis. Urinary NAG was a significant predictor of the combined end point (HR=1.43 per 5 U/gCr increase (95% CI 1.10 to 1.84), $p = 0.007$). Urinary KIM-1 concentrations were also related to prognosis (HR=1.13 per 100 ng/gCr increase (95% CI 1.00 to 1.28), $p = 0.047$). Both NAG and KIM-1 showed additional prognostic information next to GFR (table 3). Even after adjustment for other known risk factors in CHF, both urinary NAG (HR=1.46 (95% CI 0.98 to 2.17), $p = 0.066$) and urinary KIM-1 (HR=1.16 (95% CI 1.00 to 1.35), $p = 0.046$) remained borderline significantly associated with outcome. Finally, figure 3 shows the relationship of urinary NGAL, NAG and KIM-1 with prognosis after adjustment for GFR.

DISCUSSION

Our study demonstrates, first, considerably increased urinary markers for tubular damage in patients with CHF and a relatively mild impairment of GFR. Both urinary KIM-1 and NAG levels showed a relationship with plasma NT-proBNP concentrations, while urinary NAG levels were associated with impaired renal function and perfusion. Next, remarkably, increased urinary KIM-1 and NAG levels were associated with an increased risk of death or heart failure hospitalisations, independently of GFR and UAE. This is the first report on the independent prognostic impact of urinary tubular markers for hard cardiac end points in CHF.

Tubular marker proteins in patients with CHF

In this analysis, urinary NGAL, NAG and KIM-1 levels were substantially increased in patients with CHF compared with controls, even after adjustment for the lower (e)GFR levels. Online supplementary tables 1A–C show the way in which these values compare with other patient populations in, mostly, renal disease. The sensitivity and specificity for (histological) tubulointerstitial damage of NAG, NGAL and KIM-1 has mainly been studied in patients with renal disease, showing strong sensitivity and reasonable specificity for the presence of tubular damage.⁶⁷¹⁰ In addition, urinary concentrations of NAG, NGAL and KIM-1 showed strong correlations with the extent of morphological tubulointerstitial damage in different studies.⁶⁸¹¹

NGAL is a protein of the lipocalin family and is normally secreted in small amounts in the lung, kidney, trachea, stomach and colon tissue.¹² In patients with acute kidney injury, both serum and urinary concentrations rise massively in response to tubular ischaemia.¹² Furthermore, acute tubular necrosis caused by heart failure-induced hypotension was shown to be associated with higher expression of renal NGAL.⁹ NAG is a lysosomal brush border enzyme found in proximal tubular cells.⁵⁷ It is found at elevated urinary concentrations in acute and chronic kidney disease, as well as in diabetic patients and in patients with essential hypertension.^{13–15} The elevated NAG excretion in renal disease is correlated with proteinuria, and is reduced by antiproteinuric treatment by renin–angiotensin–aldosterone system blockade.¹⁴ In patients with CHF without overt renal impairment, urinary NAG levels were already higher than in the normal population, but not as high as in this study.¹⁵ Finally, KIM-1 is a transmembrane protein with levels that are elevated in acute renal disease of various origin, in diabetes, and various other causes of chronic renal failure in humans as well as rats with proteinuria and preserved renal function.^{1116–18} Furthermore, the extent of tubulointerstitial damage and fibrosis has been associated with urinary KIM-1 concentrations, and KIM-1 mRNA levels correlate strongly with urinary KIM-1 concentration in rats exposed to bilateral renal ischaemia.¹⁰

In our present study, the inter-relationships between urinary NAG, NGAL and KIM-1 were modest, and non-significant. This is in disagreement with findings in CKD. Reasons for this discrepancy may be the small sample size, but may also be differences in the pathophysiology of renal damage in CHF as compared with primary renal disease, as well as the extent of tubular injury in patients with CHF. In addition, we found differences between associations of NAG, NGAL and KIM-1, respectively, with clinical and cardiorenal parameters. Only urinary NAG showed a relationship with estimates of glomerular function, which might suggest partial dependency on glomerular filtration, limiting sensitivity for actual tubular damage. In contrast, neither KIM-1, nor NGAL showed a relationship with glomerular indices, suggesting a better specificity for tubulointerstitial damage.

Pathophysiology of tubular damage in CHF

The impairment of GFR in CHF is mainly driven by renal perfusion impairment and thought to be reversible upon restoration of renal perfusion.¹⁹ However, reports in experimental animal settings suggest that hypoperfused kidneys may develop structural damage as well.²⁰ Furthermore, chronic renal hypoxia is considered as a common final pathway in end-stage renal disease, and is also related to tubulointerstitial damage.²¹ Additionally, tubulointerstitial damage itself may predispose to a vicious circle of kidney injury and hypoxia, leading to chronic renal insufficiency.²² Therefore, patients with CHF, may be at risk for development of hypoxic tubulo(interstitial) damage. Indeed, we were able to demonstrate that urinary NAG showed the strongest relationship with ERPF and GFR, supporting this hypothesis. However, the associations of NGAL and KIM-1 with either the degree of renal impairment or reduced ERPF were weak and non-significant. One reason for

this contrast may be that urinary concentrations of NGAL rises massively in response to acute (tubular) renal injury, and also very quickly decreases when the initiating trigger has vanished.²³²⁴ The discrepancy with urinary KIM-1 levels is much less clear and should be the focus of further studies.

Interestingly, we recently showed that venous congestion is also an important determinant of GFR in CHF, with lower GFR in subjects with the highest venous pressure. This might be due to chronically increased renal interstitial pressure and consequent renal damage.²⁵²⁶ In agreement with this hypothesis, we observed a significant relationship between both urinary NAG and KIM-1 levels with NT-proBNP in our present analysis.

Next to reduced GFR and now also tubular damage, albuminuria is often seen in CHF.³ Albuminuria is assumed to be caused by glomerular leakage or damage, which often precedes reduced GFR, but these two do not always coexist. In addition, albuminuria may reflect impairment of tubular reabsorption of proteins. In our present cohort, we were unable to establish a relationship between markers of tubular damage and UAE. This is in disagreement with findings in primary renal failure, in which both NGAL and NAG correlated well with UAE.⁵⁶ Of note, all patients were receiving renin-angiotensin system blocking treatment, which will have reduced albuminuria and might have influenced the associations between tubular damage and UAE.

Tubular damage and prognosis

Only limited data are available on the relationship between the presence of tubulointerstitial injury as measured by histological abnormalities or increased levels of urinary marker proteins and prognosis, even in primary kidney disease. In patients with acute kidney injury, both urinary NAG and KIM-1 predicted the occurrence of hospital death or need for dialysis, although only urinary NAG remained an independent predictor after adjustment.⁷ In primary glomerulonephritis, urinary NAG levels were significant predictors of functional outcome.⁵ Urinary KIM-1 levels were independent predictors of graft loss in renal transplant recipients.²⁷ Our present cohort of patients with CHF further emphasises the prognostic importance of both urinary NAG and KIM-1, as markers for outcome. Both markers of tubular damage were predictors of the combined end point of death, heart failure hospitalisation and heart transplantation, in spite of the limited number of events. Remarkably, for both markers their prognostic importance was independent of GFR. This might implicate that for risk profiling, the presence of tubular damage may be an additive component related to prognosis in patients with CHF, which is independent of generally impaired GFR. The absence of any relationship with prognosis of urinary NGAL warrants further investigation, especially in patients who frequently develop acute worsening or renal function. One explanation may be that NGAL is localised in the distal part of the loop of Henle, while NAG and KIM-1 are primarily found in the proximal tubule, which may suggest that damage to the latter part of the tubule is far more important for clinical outcome. Finally, future studies targeting reduction of these markers are needed to assess the potential application of these markers in clinical practice.

Limitations

This study is hampered by its small size, and a limited number of events. Therefore observed associations may not represent the general CHF population. As renal biopsies are not warranted in this population, we have no data on tubulointerstitial morphology to support the specificity for tubular damage in this population. Furthermore, all patients in this study were receiving renin-angiotensin system blocking medication, which has been shown to reduce urinary excretion of both KIM-1 and NAG, along with proteinuria.¹⁴ Our study shows cross-sectional data with follow-up, but no serial measurements are available. We did

not investigate the effect of treatment on the observed relationships, and did not assess the effect of therapeutic interventions on these markers. For these reasons, our study is hypothesis generating, and the inter-relationships between these tubular markers and their specificity and sensitivity for renal tubular damage, and for cardiac and renal outcome should be the focus of larger prospective studies in both acute and chronic heart failure.

CONCLUSION

Tubular damage, as measured by increased levels of urinary tubular damage markers is common in patients with CHF. The prognostic impact of these tubular markers, especially urinary KIM-1 and NAG, is additive to that of impaired GFR, and seems independent of the prevalence of albuminuria. These urinary proteins may be new non-invasive markers of renal dysfunction and prognosis in these patients.

Acknowledgments

Funding KD is supported by the Netherlands Heart Foundation (grant 2006B157). AAV and DJvV are clinical established investigators of the Netherlands Heart Foundation (grants 2006T37 and D97-017, respectively). JVB is supported by the National Institute of Health (grants DK39773 and DK74099). Other Funders: NIH.

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Figure 1. Urinary NGAL, NAG and KIM-1 levels in patients with CHF versus controls. Shown are box plots with median and interquartile ranges (box) and 5—95% ranges (error bars) of urinary NAG, NGAL and KIM-1 levels. * $p < 0.0001$ versus controls. CHF, chronic heart failure; KIM-1, kidney injury molecule 1; NAG, N-acetyl- β -D-glucosaminidase; NGAL, Neutrophil gelatinase-associated lipocalin.

Figure 2.

Relationship between CKD and urinary NAG, NGAL and KIM-1. Shown are box plots with median and interquartile ranges (box) and 5—95% ranges (error bars) of urinary NAG, NGAL and KIM-1 levels. * $p < 0.001$ versus controls; † $p = 0.008$ versus no CKD. CHF, chronic heart failure; CKD, chronic kidney disease; KIM-1, kidney injury molecule 1; NAG, N-acetyl- β -D-glucosaminidase; NGAL, Neutrophil gelatinase-associated lipocalin.

Figure 3.

Relationship of tubular markers, urinary albumin excretion and GFR with prognosis. GFR is shown as decreasing GFR. GFR, glomerular filtration rate; KIM-1, kidney injury molecule 1; NAG, N-acetyl- β -D-glucosaminidase; NGAL, Neutrophil gelatinase-associated lipocalin.

Table 1

Baseline characteristics and relationship between quartiles of NAG

Variable	Total population (n=90)	Controls (n=20)	p-Value
Age (years)	59±11	58±4	0.91
Gender, n (% male)	71 (79)	16 (80)	0.91
NYHA (I/II/III or IV (%))	16/47/37	NA	NA
Ischaemic aetiology, n (%)	43 (48)	NA	NA
LVEF (%)	28±9	NA	NA
Systolic BP (mm Hg)	121±20	120±11	0.67
Diastolic BP (mm Hg)	69±11	71±8	0.30
Haemoglobin (g/dl)	14±1.3	14±1.0	0.99
NT-proBNP (pg/ml)	515 (219–1283)	84 (57–108)	<0.0001
Renal indices			
Serum creatinine (μmol/l)	112±36	79±13	<0.001
eGFR (ml/min/1.73m ²)	64±17	90±12	<0.0001
GFR (ml/min/1.73m ²)	78±26	NA	NA
ERPF (ml/min/1.73m ²)	282±83	NA	NA
Filtration fraction (%)	27±5	NA	NA
UAE (mg/day)	5.1 (3.4–10)	1.5 (1.2–1.9)	<0.0001
Tubular markers			
NGAL (μg/gCr)	175 (70–346)	37 (6–58)	<0.0001
NAG (U/gCr)	12 (6.2–17)	1.6 (0.7–2.2)	<0.0001
KIM-1 (ng/gCr)	277 (188–537)	136 (63–195)	<0.0001
Treatment (n (% use))			
RAS inhibitor	90 (100)	0	NA
β Blocker	78 (87)	0	NA
Diuretic	63 (70)	0	NA
Aldosterone receptor blocker	29 (32)	0	NA

BP, blood pressure; eGFR, estimated glomerular filtration rate; ERPF, effective renal plasma flow; 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; RAS, renin-angiotensin system; UAE, urinary albumin excretion.

Table 2

Correlation between markers of tubular damage and renal function parameters

Variable	Univariate correlation coefficient (95% CI)		
	NGAL	NAG	KIM-1
Tubular markers			
NGAL	NA	0.15 (−0.06 to 0.35)	0.16 (−0.05 to 0.35)
NAG	0.15 (−0.06 to 0.35)	NA	0.13 (−0.08 to 0.33)
KIM-1	0.16 (−0.05 to 0.35)	0.13 (−0.08 to 0.33)	NA
Renal function measurements			
GFR	−0.07 (−0.27 to 0.14)	−0.34 (−0.51 to −0.14) ^{**}	−0.01 (−0.22 to 0.20)
ERPF	−0.06 (−0.26 to 0.15)	−0.29 (−0.47 to −0.09) ^{**}	−0.11 (−0.31 to 0.10)
Filtration fraction	0.04 (−0.17 to 0.25)	−0.16 (−0.36 to 0.05)	0.17 (−0.04 to 0.36)
UAE	0.10 (−0.11 to 0.30)	0.16 (−0.05 to 0.35)	0.21 (−0.01 to 0.40)
NT-proBNP	−0.03 (−0.24 to 0.18)	0.21 (0.00 to 0.40) [*]	0.23 (0.02 to 0.42) [*]

N=90, all patients with chronic heart failure.

^{*} p<0.05;

^{**} p<0.01.

ERPF, effective renal plasma flow; GFR, glomerular filtration rate; KIM-1, kidney injury molecule 1; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain natriuretic peptide; UAE, urinary albumin excretion.

Univariate and adjusted Cox proportional hazard analysis for urinary NAG, NGAL and KIM-1 as predictors for the combined end point

Table 3

Predictor variable	Urinary NGAL (per 100 µg/gCr) (HR (95% CI))	p Value	Urinary NAG (per 5 U/gCr) (HR (95% CI))	p Value	Urinary KIM-1 (per 100 ng/gCr) (HR (95% CI))	p Value
Unadjusted	1.02 (0.87 to 0.20)	NS	1.43 (1.10 to 1.84)	0.007	1.13 (1.00 to 1.28)	0.047
Adjusted for GFR	1.01 (0.88 to 1.16)	NS	1.42 (1.02 to 1.94)	0.039	1.15 (1.02 to 1.30)	0.025
Adjusted for serum creatinine	1.03 (0.89 to 1.19)	NS	1.49 (1.11 to 2.00)	0.008	1.18 (1.04 to 1.33)	0.009
Adjusted for Age, gender, LVEF, SBP, DBP, Hb, GFR, NT-proBNP and UAE	1.01 (0.85 to 1.19)	NS	1.46 (0.98 to 2.17)	0.066	1.16 (1.00 to 1.35)	0.046

N=90, all patients with chronic heart failure.

CI, confidence interval; DBP, diastolic blood pressure; gCr, gram urinary creatinine; GFR, glomerular filtration rate; Hb, haemoglobin levels; HR, hazards 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; SBP, systolic blood pressure; UAE, urinary albumin excretion.