
SYMBIOSIS

Part II

THE ROLE OF THE KIDNEYS IN HEART FAILURE AND BEYOND

CHAPTER 5

Glomerular Decline and Progressive Tubular Damage in Chronic Heart Failure: Clinical Determinants and Combined Value for Prognosis The Bio-SHiFT Study

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ABSTRACT

Background

Progressive tubular damage (PTD) and glomerular decline (GD) affect prognosis in chronic heart failure (CHF). We investigated clinical determinants of PTD and GD and their combined prognostic value for CHF patients.

Methods

In 263 patients, during 2.2-years, we prospectively collected 9-blood and 8-urine samples per patient. We determined slopes (biomarker change/year) of urinary tubular damage markers (N-acetyl-beta-D-glucosaminidase [uNAG], kidney injury molecule [uKIM]-1) and plasma creatinine (Cr). PTD was categorized according to uNAG or uKIM-1 (increase in neither, increase in either, and increase in both). GD was defined as increasing Cr slope. The endpoint comprised HF-hospitalization, cardiac death, LVAD-placement, and heart transplantation.

Results

Higher baseline NT-proBNP and lower eGFR independently predicted PTD (per doubling NT-proBNP: OR 1.26 [95%CI:1.07-1.49]; per 10mL/min/1.73m^2 eGFR decrease 1.16 [1.03-1.31]). Higher loop diuretic doses, lower MRA doses, and higher eGFR independently predicted GD (furosemide: per 40mg increase: 1.32 [1.08-1.62]; spironolactone: per 25mg decrease: 1.76 [1.07-2.89]; eGFR: per 10mL/min/1.73m^2 increase: 1.40 [1.20-1.63]). Lack of PTD inferred highest survival regardless of GD, but PTD and GD combined entailed poorest survival.

Conclusions

PTD and GD are associated with different clinical determinants of CHF patients. They carry the poorest prognosis when they deteriorate concurrently. PTD may be prognostically important even when glomerular function appears intact.

INTRODUCTION

Renal dysfunction is the most prevalent comorbidity among patients with chronic heart failure (CHF), and is strongly associated with clinical outcomes such as HF-related hospitalization and mortality.[1-3] Underlying hemodynamic dependence between the heart and the kidneys is widely considered as the main driver of the cardiorenal interaction leading to adverse outcomes.[4] However, other biochemical, neurohumoral, and immunological derangements also occur during the organs' interplay, which has led to the definition of cardiorenal syndrome (CRS).[5]

Because renal dysfunction entails poor prognosis in CHF, attention has focused on identifying the signals along the cardio-renal axis that precede adverse outcomes.[6] Yet, the mechanisms and the chronology according to which the failing heart damages specific renal structures that lead to CRS are poorly understood.[7] Decreased baseline glomerular function is clearly important, but glomerular decline (GD) quantified as creatinine increase over time has been shown to be an even more prominent predictor.[1] We have recently confirmed and extended these findings by using frequent, repeated GD assessment in CHF patients.[8]

Besides glomerular dysfunction, tubular damage is often present in CHF due to tubulo-interstitial injury by renal tissue hypoperfusion or due to a damaged glomerular filtration barrier.[9-11] Higher levels of tubular damage markers such as urinary N-acetyl-beta-D-glucosaminidase (uNAG) and kidney injury molecule (uKIM)-1 also entail poor prognosis in CHF.[8,11] Moreover, we have recently shown that when their levels are increasing over time (i.e., when progressive tubular damage [PTD] is present) the association with adverse outcome is even stronger.[8] Importantly, these tubular damage markers predict poor prognosis independently of patients' glomerular function.[8,11]

Taken together it appears that simultaneous biomarker-based monitoring of glomerular and tubular renal compartments carries potential for improvement of renal management of CHF patients during their outpatient follow-up. However, it has not yet been investigated which CHF patients are susceptible to PTD and which to GD. It also remains unclear how these renal biomarkers relate to prognosis when jointly assessed. These considerations are particularly interesting since in current clinical practice tubular damage markers are not routinely assessed, leaving the degree of tubular injury undetermined. Therefore, our aim was to investigate clinical determinants of PTD and GD, and their combined prognostic value for CHF patients.

METHODS

Bio-SHiFT cohort

The Serial Biomarker Measurements and New Echocardiographic Techniques in Chronic Heart Failure Patients Result in Tailored Prediction of Prognosis (Bio-SHiFT) is a prospective cohort of stable patients with CHF, conducted in Erasmus MC, Rotterdam, and Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands. Patients were included if aged ≥18 years and if CHF had been diagnosed ≥3 months ago according to European Society of Cardiology guidelines.[12] Patients were ambulatory and stable, i.e., they had not been hospitalized for HF in the past three months. The study was approved by the medical ethics committees, conducted in accordance with the Declaration of Helsinki, and registered in ClinicalTrials.gov (NCT01851538). Written informed consent was obtained from all patients. This investigation comprised 263 stable patients with CHF, who were enrolled during the first inclusion period (October 2011 until June 2013) and completed their follow-up in 2015.

Study visits

All patients were evaluated by research physicians, who collected information on HF-related symptoms, NYHA class, and performed a physical examination and collected samples. Information on HF etiology, ejection fraction, cardiovascular risk factors, comorbidities, and treatment was retrieved from hospital records. Study follow-up visits were predefined and scheduled tri-monthly (±1 month), with a maximum of 10 study follow-up visits. All patients were also routinely followed at the outpatient clinic by treating physicians who were blinded for biomarker data. Occurrence of re-hospitalizations for HF, MI, PCI, CABG, arrhythmias, CVA, cardiac transplantation, left ventricular assist device (LVAD)-placement and mortality was recorded in electronic case-report forms, and associated hospital records and discharge letters were collected. A clinical event committee, blinded for biomarker data, reviewed hospital records and discharge letters and adjudicated the study endpoints.

Study endpoints

The composite endpoint comprised cardiac death, cardiac transplantation, LVAD implantation, and hospitalization for the management of acute or worsened HF, whichever occurred first. Cardiac death was defined as death from MI or other ischemic heart disease (ICD-10: I20-I25), death from other heart disease including HF (I30-I45 and I47-I52), sudden cardiac death (I46), sudden death undefined (R96) or unwitnessed or ill-described death (R98, R99). Hospitalization for acute

or worsened HF was defined as a hospitalization for an exacerbation of HF symptoms, in combination with two of the following: BNP or NT-proBNP >3x upper limit of normal, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral edema, increased dose or intravenous administration of diuretics, or administration of positive inotropic agents.[12]

Blood and urine analyses

Samples were collected at baseline and during study visits, and were processed and stored at -80°C. Laboratory personnel was blinded for clinical data. Batch analysis of serum was performed at Erasmus MC: NT–proBNP was analysed using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA), cardiac troponin T was also measured using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010 immunoassay analyser, Indianapolis, Indiana, USA). Plasma and urine samples were transported at -80°C to HaemoScan BV, Groningen, the Netherlands for batch analysis. Creatinine was determined by a colorometric test by the Jaffé reaction. Plasma was used undiluted, urine was diluted ten times in water (LLD: plasma 0,14 mg/dl, urine: 1.56 mg/ml). KIM-1 was determined in urine diluted 50% in 0,1% BSA/PBS buffer, by ELISA (R&D systems, Minneapolis, MN, USA) (LLD: 0.146 ng/mL). NAG was determined using a substrate p-nitrophenyl N-acetyl-β-D-glucosaminidase at pH 4.5 (Sigma, St Louis, MO, USA) (LLD: 0.485 U/L). All urinary biomarkers were normalized to urinary Cr concentrations to correct for concentration or dilution of urine.The GFR was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that has been validated in HF patients[13] and categorized using K/DOQI guidelines.[14]

Statistical analysis

To assess patient-specific slopes of renal biomarkers we performed joint modeling (JM) analysis which combines linear mixed-effects (LME) and Cox regression models.[15] The LME models apply a two component equation to construct a biomarker trajectory using its repeated measurements. The first component is a 'fixed-effect' that estimates a biomarker's average trajectory over all patients within the cohort. The second component is a 'random-effect' that estimates by how much an individual patient deviates from this average trajectory at each of study visits during follow-up. By using these two components a patient-specific biomarker trajectory is constructed. Through the random-effects component the LME models allow repeated measurements taken on the same patient to be correlated and incorporates information on the marker's biological variation in each patient (i.e., "noise" around the biomarker

regression trajectory).[16] Finally, JM combines LME and Cox models to adjust biomarker trajectories for different follow-up durations between patients.

From these biomarker trajectories, regression slopes (i.e., rates of biomarker change per year) were calculated which mathematically correspond to the first derivative of a biomarker trajectory.[16] Subsequently, patients were stratified into those in whom no tubular damage marker showed an increased slope, either uNAG or uKIM1 increased, and both markers increased during follow-up. Patients were also stratified into those with increasing Cr levels and those with stable/decreasing Cr levels.

For continuous variables, presence of a linear trend across PTD- and GD-categories was assessed by analysis of variance (ANOVA) or the Kruskal–Wallis test, when appropriate; categorical variables were tested by the $\chi 2$ trend test. Covariates that were univariably associated with PTD or GD (exploratory $p<0.10$) were entered into a multivariable logistic regression model applying proportional odds ordinal regression (for PTD) or binary logistic regression (for GD).

For associations between baseline eGFR and renal biomarkers' slopes, a linear regression analysis was performed using eGFR (per 10 mL/min/1.73m^2) as the independent variable and each of the slopes as the dependent variable on the continuous scale. The models were corrected for the study endpoints; effect heterogeneity of eGFR on study endpoints was tested by adding an interaction term.

To investigate survival rates, we used the two-sided Breslow test and the Breslow method to estimate event-time distributions. Cox regression was performed to assess hazard ratios (HR) with 95% confidence intervals (95%CI) for study endpoints. Statistical adjustments were performed by using biomarker of interest plus age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, eGFR (only for tubular damage markers) and biomarkers of myocardial stretch and damage NT-proBNP and hs-cTnT. Data on all variables were complete, except for systolic blood pressure, which was missing in <5% of patients and for which imputations were applied using patients' clinical and outcome data.

All tests were two-tailed and p-values <0.05 were considered statistically significant. All analyses were performed with SPSS (SPSS 25.0; IBM Corp., Armonk, NY),[17] and R[18] using package JMbayes.[19]

RESULTS

CHF cohort, sample collection and study endpoints

In 263 CHF patients, median age was 67±13 years, 72% were men, 26% were in New York Heart Association (NYHA) functional class III/IV, and 53% had eGFR<60

mL/min/1.73m^2.

During a median of 2.2 (IQR: 1.4–2.5) years, a total of 1984 blood and 1912 urine samples were collected (per patient: 9 [5–10] blood and 8 [5–10] urine samples). Seventy patients (27%) reached the endpoint: 56 patients were re-hospitalized for acute or worsened HF, 9 died of cardiovascular causes, 2 underwent LVAD-placement, and 3 underwent heart transplantation.

Distributions of renal biomarker slopes and their relation to baseline eGFR and study endpoints

Patients who experienced the endpoint had significantly higher slopes of uNAG (mean±SD 0.25±0.30 vs. -0.02±0.27 ln[U/gCr]/year, p<0.001), uKIM1 (0.21±0.36 vs. -0.04±0.24 ln[ng/gCr]/year, p<0.001), and plasma Cr (0.21±0.35 vs. 0.01±0.17 ln[mg/dL]/year, p<0.001) than endpoint-free patients (Figure 1).

When examining baseline eGFR as a continuous variable, eGFR was inversely associated with uNAG and uKIM-1 slopes (i.e., greater PTD was present in patients with lower baseline eGFR), but positively associated with Cr slope (i.e., greater GD was present in patients with higher baseline eGFR). No interactions were found between baseline eGFR and study endpoints (Table 1).

TABLE 1 Slopes of renal biomarkers according to baseline renal function and study endpoints.

Biomarker slopes	β (95% confidence interval)	p-value
uNAG		
Baseline eGFR (per 10 mL/min/1.73m^2 increase)	-0.02(-0.03 to -0.01)	0.030
Study endpoint (yes)	0.26 (0.19 to 0.34)	<0.001
Interaction (eGFR x study endpoint)	**	0.99
uKIM1		
Baseline eGFR (per 10 mL/min/1.73m^2 increase)	-0.02 (-0.03 to -0.01)	0.017
Study endpoint (yes)	0.24 (0.16 to 0.31)	<0.001
Interaction (eGFR x study endpoint)	**	0.69
Creatinine		
Baseline eGFR (per 10 mL/min/1.73m^2 increase)	0.02 (0.01 to 0.04)	<0.001
Study endpoint (yes)	0.21 (0.14 to 0.27)	<0.001
Interaction (eGFR x study endpoint)	**	0.37

** Coefficient not presented since interaction was not significant. Abbreviations: eGFR, estimated glomerular filtration rate; uNAG, urinary N-acetyl-β-D-glucosaminidase; uKIM1, urinary kidney injury molecule 1.

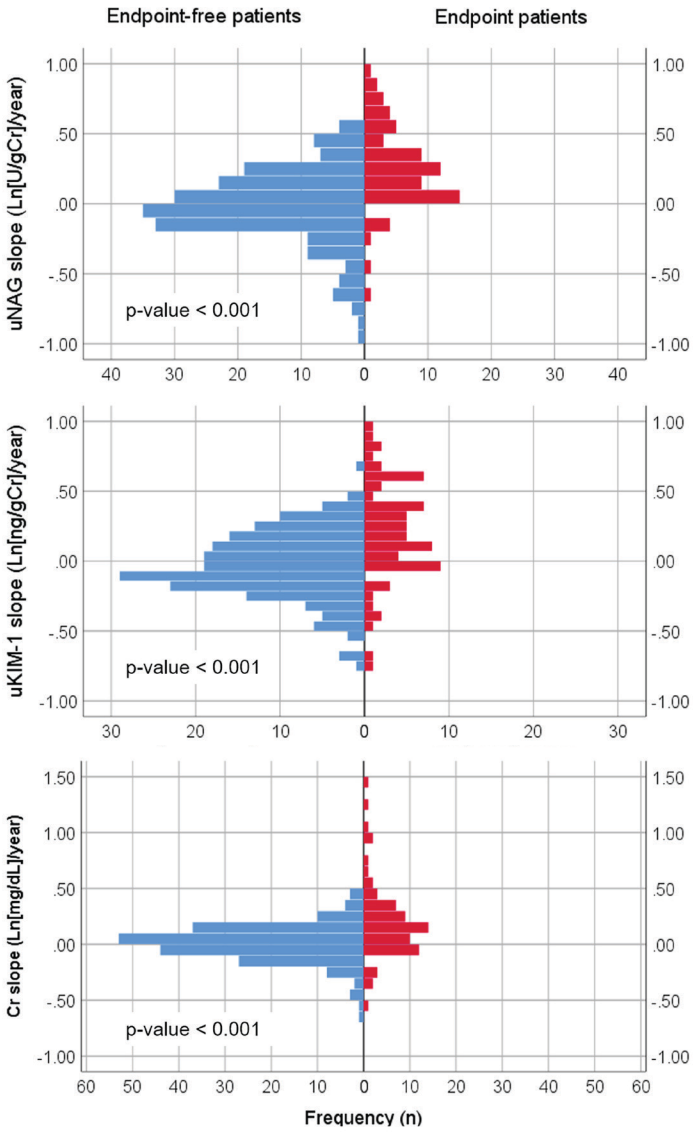


FIGURE 1 Distributions of slopes of renal biomarkers prior to study endpoints. X-axis displays percentage of patients who experienced the event (red) and those who did not (blue), Y-axis displays the estimated slopes on the continuous scale, where positive numbers correspond to increasing slopes and negative numbers correspond to decreasing slopes. T-test was used test the average difference between patient with and without event.

When categorizing patients according to baseline eGFR, we found that patients who experienced the endpoint had higher slopes of all three renal biomarkers than those who did not across all eGFR categories. We also found a tendency towards

more frequent occurrence of PTD and less frequent occurrence of GD in lower eGFR categories (Figure 2).

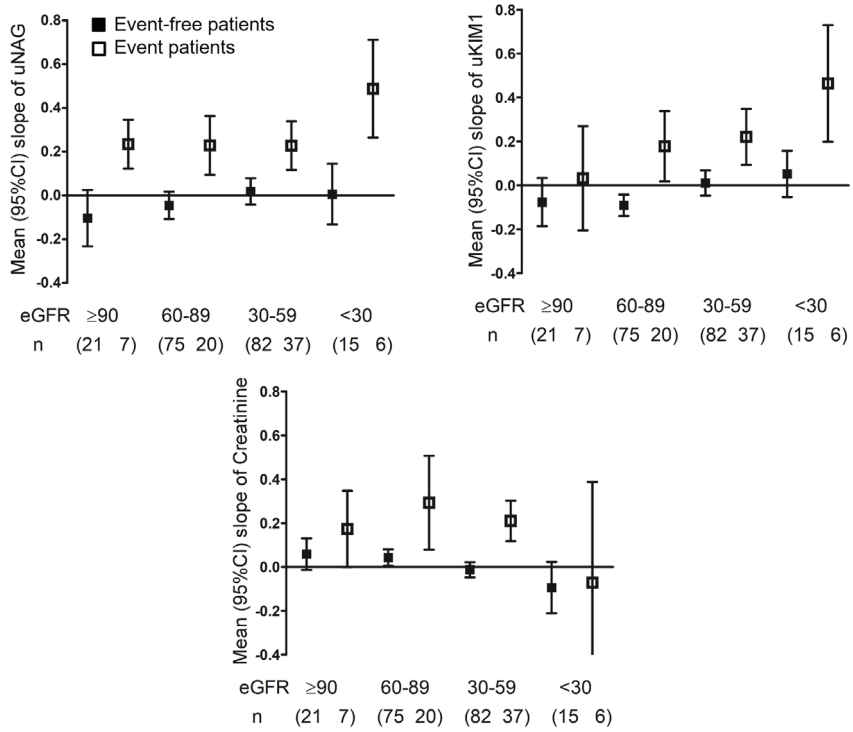


FIGURE 2 Average slopes of renal biomarkers stratified by baseline eGFR category. X-axis displays eGFR categories with absolute number of patients (n), and Y-axis displays the average slopes with 95% confidence intervals, where positive numbers correspond to increasing slopes and negative numbers correspond to decreasing slopes. Black horizontal line depicts stable (zero) slope.

Associations of clinical characteristics with PTD and GD

Seventy five percent of patients (196 of 263) had increasing slope of either uNAG or uKIM1. Of those, both markers were increasing in 43% (85 of 196). Table 2 shows that patients in higher PTD-categories, had higher baseline levels of NT-proBNP, cardiac troponin-T and Cr (eGFR was lower); more frequently diabetes, NYHA class III/IV, and cardiac resynchronization therapy (CRT), and were older. After multivariable adjustments, higher NT-proBNP and lower eGFR levels remained independent clinical predictors of PTD severity (per doubling of NT-proBNP adj. OR 1.26 [95%CI 1.07-1.49], p=0.006; and per 10 mL/min/1.73m^2 eGFR decrease 1.16 [1.03-1.31], p=0.016) (Table 3).

TABLE 2 **Patient characteristics stratified by uNAG and uKIM1 slopes.**

	uNAG&uKIM1 stable/decreased slope (n=67)	uNAG or uKIM1 increased slope (n=111)	uNAG&uKIM1 increased slope (n=85)	p-value
Clinical features				
Age years	65 (57 to 72)	69 (60 to 77)	70 (62 to 79)	0.017*
Men	49 (73)	80 (72)	60 (71)	0.73
HF-rEF	66 (98)	104 (94)	80 (94)	0.24
Ischemic etiology	28 (42)	48 (43)	41 (48)	0.41
BMI kg/m^2	27.1 (25.0 to 30.9)	26.2 (24.1 to 29.0)	26.3 (24.2 to 30.3)	0.55
Heart rate b.p.m.	65 (60 to 74)	66 (60 to 72)	68 (60 to 76)	0.18
SBP mmHg	122 (110 to 135)	120 (106 to 140)	120 (108 to 132)	0.70
DBP mmHg	74 (61 to 82)	73 (65 to 80)	70 (60 to 79)	0.08
Congestion [b]	38 (57)	75 (68)	56 (66)	0.27
NYHA III/IV	9 (13)	28 (25)	32 (38)	0.001*
CRT	27 (41)	35 (32)	18 (21)	0.009*
Medical history				
Prior MI	23 (36)	39 (36)	32 (39)	0.69
Atrial fibrillation	23 (36)	48 (45)	34 (40)	0.65
Diabetes	14 (21)	34 (31)	33 (39)	0.018*
Hypertension	27 (41)	50 (46)	43 (52)	0.18
COPD	8 (12)	10 (9)	13 (16)	0.42
Medication prevalence (%) /average total daily dose (mg)				
Beta-blocker	95/45	91/43	83/47	0.50 [a]
ACE-I/ARBs	96/25	92/25	93/23	0.92 [a]
Loop diuretics	85/77	88/78	96/93	0.35 [a]
MRAs	73/23	68/23	63/23	0.88 [a]
Biomarkers				
NT-proBNP ng/L	592 (158 to 1690)	1196 (448 to 2105)	1650 (857 to 3525)	<0.001*
cTnT ng/L	12.6 (7.5 to 27.2)	17.1 (9.6 to 32.7)	22.4 (13.7 to 43.2)	<0.001*
Glomerular indices				
Creatinine mg/dl	1.10 (0.92 to 1.26)	1.18 (0.97 to 1.43)	1.31 (1.05 to 1.72)	<0.001*
eGFR	70 (48 to 79)	57 (44 to 76)	50 (37 to 71)	<0.001*
eGFR<60	22 (33)	63 (57)	55 (65)	<0.001*
Tubular damage markers				
uNAG, U/gCr	5.2 (2.7 to 10.1)	5.8 (4.0 to 9.1)	6.8 (4.6 to 9.1)	0.22
uKIM1, ng/gCr	447 (235 to 926)	500 (247 to 904)	540 (249 to 994)	0.44

BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; NYHA class, New York Heart Association class; HF-REF, Heart failure with reduced ejection fraction;

eGFR, estimated glomerular filtration rate; MI, myocardial infarction; CVA, cerebrovascular accident; TIA, transitory ischemic attack; COPD, chronic obstructive pulmonary disease; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers, MRA, mineralocorticoid receptor antagonist; cTnT, cardiac troponin T; CRP, C-reactive protein. eGFR, estimated glomerular filtration rate; uNAG, urinary N-acetyl-β-D-glucosaminidase; uKIM1, urinary kidney injury molecule 1. For reasons of uniformity continuous variables are presented as medians (25th to 75th percentiles) and categorical variables are presented as n (%). p-values signify trend across groups and the asterisk indicates p<0.05.
[a] p-value for the difference in average total daily dose.
[b] Congestion was considered present if ≥2 symptoms or signs were present at baseline (dyspnea, orthopnea, fatigue, elevated jugular venous pressure, presence of rales/crackles and pedal oedema).

TABLE 3 Independent clinical predictors of PTD severity and GD.

	Multivariable model *	
	OR (95% CI)	**p-value**
PTD (dependent variable)[a]		
NT-proBNP (per doubling)	1.26 (1.07-1.49)	p=0.006
eGFR (per 10 mL/min/1.73m^2 decrease)	1.16 (1.03-1.31)	p=0.016
GD (dependent variable)[b]		
Loop diuretics (per 40 mg furosemide dose increase)	1.32 (1.08-1.62)	p=0.006
MRAs (per 25 mg spironolactone dose decrease)	1.76 (1.07-2.89)	p=0.025
eGFR (per 10 mL/min/1.73m^2 increase)	1.40 (1.20-1.63)	p<0.001

OR indicates odds ratio for having GD or more severe PTD; 95%CI, 95% confidence interval for the corresponding OR; GD, glomerular decline; PTD, progressive tubular damage; eGFR, estimated glomerular filtration rate, MRAs, mineralocorticoid receptor antagonists.
[a] Covariates that were found to be different across PTD categories with p<0.10 (Table 2) were entered into a multivariable ordinal regression model, and those were age, diastolic blood pressure, NT-proBNP, hs-cTnT, eGFR, NYHA class, diabetes, use of cardiac resynchronization therapy (CRT).
[b] Covariates that were found to be different between GD and non-GD subgroup with p<0.10 (Table 3) were entered into a multivariable binary regression model, and those were diastolic blood pressure, NT-proBNP, hs-cTnT, eGFR, NAG, prior myocardial infarction, hypertension, atrial fibrillation, loop diuretics and MRAs doses.
* only covariates with p-value <0.05 were presented in the table

Fifty eight percent of patients (153 of 263) had increasing Cr slope. Table 4 shows that these patients had higher baseline levels of NT-proBNP, cardiac troponin-T and uNAG, more frequently had a history of myocardial infarction, and were given higher doses of loop diuretics and lower doses of mineralocorticoid receptor blockers (MRAs). After multivariable adjustments, higher doses of loop diuretics, lower MRA doses, and higher eGFR levels remained independent clinical predictors of GD (per 40 mg increase of furosemide equivalent dose adj. OR 1.32 [1.08-1.62], p=0.006; per 25 mg decrease of spironolactone equivalent dose 1.76 [1.07-2.89], p=0.025; per 10 mL/min/1.73m^2 eGFR increase 1.40 [1.20-1.63], p<0.001) (Table 3).

TABLE 4 **Patient characteristics stratified by creatinine slope.**

	Cr stable/decreased slope (n=110)	Cr increased slope (n=153)	p-value
Clinical features			
Age years	66 (57–75)	69 (60–77)	0.17
Men	74 (67)	115 (75)	0.16
HF-rEF	104 (95)	146 (95)	0.75
Ischemic etiology	45 (41)	72 (47)	0.32
BMI kg/m^2	26.6 (24.2–30.3)	26.4 (24.4–30.1)	0.90
Heart rate b.p.m.	68 (60-77)	66 (60–73)	0.42
SBP mmHg	120 (110–136)	120 (106–132)	0.38
DBP mmHg	75 (66–80)	70 (60-80)	0.07
Congestion	73 (66)	96 (63)	0.55
NYHA III/IV	32 (29)	37 (24)	0.37
CRT	37 (34)	44 (29)	0.40
Medical history			
Prior MI	32 (29)	64 (42)	0.034*
Atrial fibrillation	38 (35)	68 (44)	0.10
Diabetes	29 (26)	52 (34)	0.19
Hypertension	43 (39)	77 (50)	0.07
COPD	9 (8)	22 (14)	0.12
Medication prevalence (%) /average total daily dose (mg)			
Beta-blocker	87 / 48	92 / 42	0.50[a]
ACE-I/ARBs	95 / 22	92 / 26	0.17[a]
Loop diuretics	87 / 62	92 / 97	0.002*[a]
MRAs	69 / 25	67 / 22	0.034*[a]
Cardiac biomarkers			
NT-proBNP ng/L	907 (293–2130)	1406 (520–2804)	0.033*
cTnT ng/L	14.3 (8.5–28.3)	20.6 (10.7–39.1)	0.012*
Glomerular indices			
Creatinine mg/dl	1.29 (1.08–1.63)	1.11 (0.92–1.38)	<0.001*
eGFR	50 (38-70)	63 (48–81)	<0.001*
eGFR<60	71 (65)	69 (45)	0.002*
Tubular damage markers			
uNAG, U/gCr	5.5 (3.4-8.6)	6.6 (4.0–9.4)	0.044
uKIM1, ng/gCr	467.4 (238.3–840.6)	507.6 (247.2–994.1)	0.20

For description please see Table 2; p-values signify a trend across groups. * p<0.05.
[a] p-value for the difference in the average total daily dose.

Study endpoint-free survival and prognosis

Figure 3A displays estimated survival distributions of CHF patients stratified by uNAG and uKIM1 slopes. Survival rates were lowest when both biomarkers were increased, followed by survival rates when either marker's slope was increased (p for trend <0.001). Hazard ratios were significantly higher as compared to the category of patient in whom both markers were stable or decreasing during follow-up (uNAG or uKIM-1 slope increased: adj. HR 4.2 [95%CI: 1.2-13.9], p=0.021; uNAG & uKIM-1 slopes increased: 8.1 [2.4-26.6], p=0.001). These estimates were independent of the patients' clinical characteristics, baseline eGFR, NT-proBNP, and cardiac troponin T.

In Figure 3B, patients with increasing Cr slope had lower survival rates than their counterparts (p=0.012). The hazard in these patients was also significantly higher and independent of patients' clinical characteristics, NT-proBNP, and cardiac troponin T (Cr slope increased: HR 1.9 [1.1-3.3], p=0.025).

Figure 4 displays the Kaplan-Meier curves of patients stratified by uNAG, uKIM1, and Cr. The figure shows that when the slopes of tubular damage markers were stable or improving, glomerular decline did not affect survival rates. However, if either uNAG or uKIM1 slope increased, the survival rates decreased. Finally, the lowest survival rates were in patients who had increasing slopes of all three renal biomarkers (p for trend <0.001).

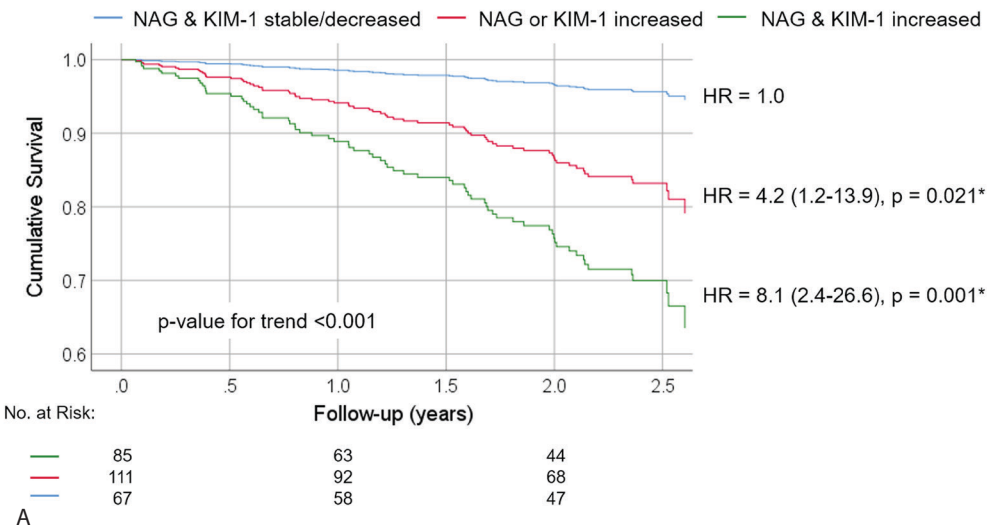
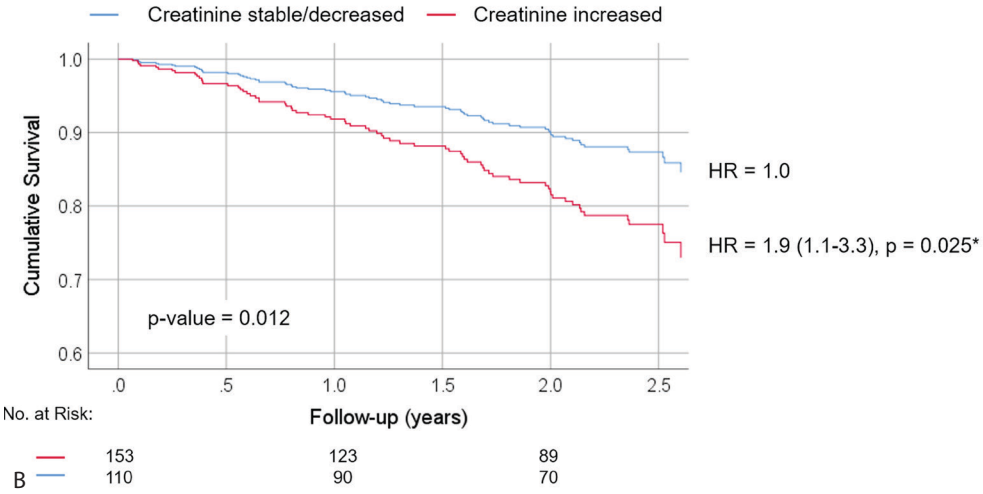


FIGURE 3 Kaplan-Meier Survival Curves stratified by slopes of renal biomarkers. Shown are Kaplan-Meier (KM) curves for the cumulative event-free survival of the composite of HF-rehospitalization, cardiac death, LVAD placement, and heart transplantation. **A**. KM curves are stratified by whether both uNAG and uKIM1 slopes were



decreasing/stable (blue); either uNAG or uKIM1 slope was increasing (red); or both uNAG and uKIM1 slopes were increasing (green); **B.** KM curves are stratified by whether creatinine slope was decreasing/stable (blue) or increasing (red). *adjusted for age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, eGFR (only for tubular damage biomarkers), NT-proBNP, and hs-cTnT.

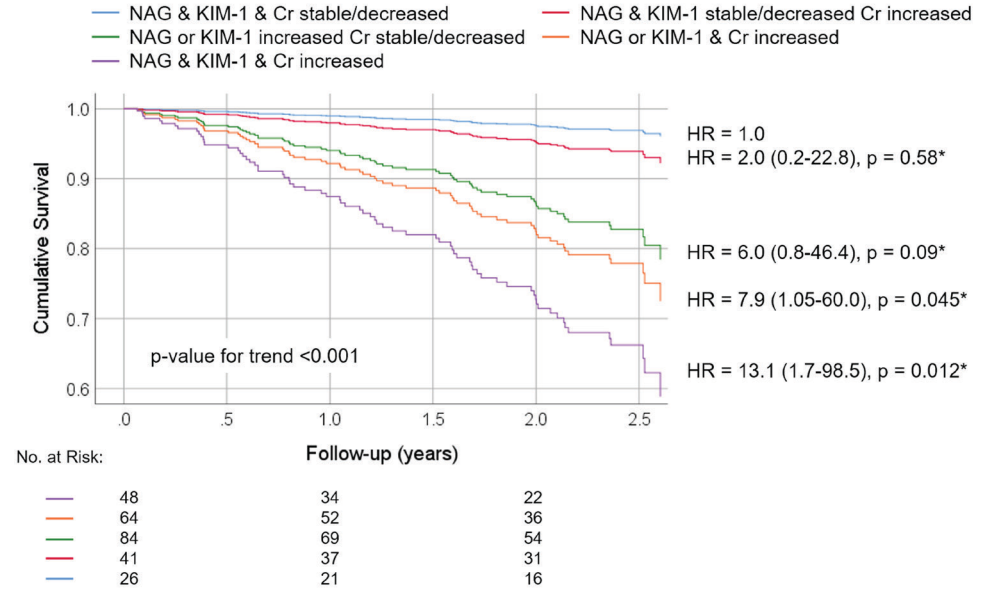


FIGURE 4 Kaplan-Meier Survival Curve stratified by combined slopes of renal biomarkers. KM curves are stratified by whether slopes of all three renal biomarkers were decreasing/stable (blue); uNAG and uKIM1 slopes were decreasing/stable, but creatinine (Cr) slope was increasing (red); either uNAG or uKIM1 slope was increasing but creatinine slope was decreasing/stable (green); either uNAG or uKIM1 slope was increasing, and Cr slope was

increasing (orange); and slopes of all three biomarkers were increasing (purple). *adjusted for age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, NT-proBNP, and hs-cTnT.

DISCUSSION

This study is the first to assess combined effects of PTD and GD on clinical endpoint-free survival during outpatient follow-up of patients with CHF. We show that patients in whom both renal compartments deteriorate over time have the lowest endpoint-free survival. Conversely, the highest endpoint-free survival was observed in patients without signs of PTD, regardless of their Cr slope pattern. To our best knowledge, this is also the first study to identify clinical predictors of PTD severity in CHF. Of note, these determinants differ from those found in GD, which strengthens the recommendation that glomerular and tubular damage markers should be jointly assessed.

Renal function may act as a barometer of cardiac function in CHF. [20] However, because of the multi-factorial nature of cardiorenal interactions, merely assessing the glomerular filtration rate of the kidney may be suboptimal for decision-making. Our study confirms this, and provides an additional evidence that the failing heart affects glomerular and tubular compartments differently over time. In this study, one of the striking findings is that the change in tubular markers may be even clinically more relevant than the change in Cr. Importantly, the rates of change in each aspect of the kidney (glomerular and tubular) provide incremental prognostic information, and together may further identify higher-risk individuals and herewith improve clinical monitoring of CHF patients. These kidney-specific signals may, therefore, help physicians to better, and timely, target medical therapy before the future event occurs. It could also be speculated that "renoprotective" treatment targeted at the tubules may be even more effective than treatment aiming at improving renal function in terms of GFR by means of afferent/efferent vasodilating agents. However, interventional studies on these tubular damage markers are needed to provide definite answers in this matter.

In patients who had PTD, we found lower baseline eGFR. This suggests that patients who had fewer functioning nephrons, were more susceptible to tubular deterioration. This may be attributed to work-overload in residual nephrons to compensate renal function.[21] Despite the loss in total GFR, compensatory hyperfiltration in these nephrons may exceed tubular capacity leading to their progressive damage. These patients more frequently had diabetes, which may also have contributed to PTD. Similarly, other clinical determinants such as aging kidneys and severity of HF (higher cardiac markers, NYHA class, and CRT) indicate that

factors that are related to more severe HF, also cause tubule-specific kidney damage. Importantly, our findings suggest that simultaneous assessment of both uNAG and uKIM-1 translates into better risk stratification of patients than assessment of either one alone. Importantly, these biomarkers predicted poor survival even in patients with apparently stable glomerular function during outpatient follow-up.

GD was found to be associated with higher baseline eGFR which is supported by several previous studies.[22-24] However, this finding is inconsistent with the general opinion that GD (defined as worsening renal function [WRF] with delta-Cr >0.03mg/dl) occurs more frequently in CHF patients that have impaired GFR already at baseline.[25] However, has also been reported that when studies defined WRF as eGFR change instead of Cr change,[23,24] paradoxically, the patients with WRF had lower baseline Cr levels. Interestingly, in the studies that reported lower baseline Cr levels in patient with GD, average baseline Cr was 1.15 mg/dl,[22-24] whereas in the studies that reported higher baseline Cr levels in patients with GD, average baseline Cr was 1.41 mg/dl (average of all reported values in CHF cohorts on WRF).[1] Thus, it seems that studies in which baseline renal impairment was associated with GD recruited patients with worse baseline renal function than those in which the opposite was found. Furthermore, the dissimilar degree of tubular damage could have affected this relationship, as higher tubular damage relates to glomerular decline. However, a definite answer cannot be given because many studies lack these data. Moreover, closer monitoring of patients who already had impaired GFR could have also increased the likelihood of finding WRF in these patients,[26] and particularly if sampling was not fixed but left at the discretion of the treating physician.[27] Finally, a "regression to the mean" could also account for observed discrepancies. As for our study, the observations were made using more than twice as many repeated measurements as in each of the previous studies, samples were collected at fixed time intervals, and the treating physicians were unaware of biomarker data. This further strengthens our suggestion that GD should not be disregarded in CHF patients with relatively intact GFR. Finally, higher doses of loop diuretics and lower MRA doses were identified in glomerular decliners and are supported by previous studies. [1,26]

Study limitations

Several limitations merit consideration. First, this study lacked direct GFR measurement. Second, we cannot comment on the effects of glomerular permeability on clinical outcome since we did not measure proteinuria. Third, although trials on this subject are lacking, and causal inference is limited by the observational nature of our study, the repeated-measures design of this study allows for stronger claims of true associations than previous studies do.

CONCLUSION

Progressive tubular damage and glomerular decline are coupled with different clinical profiles of CHF patients, and those in whom both renal compartments deteriorated had the poorest prognosis. Slopes of urinary tubular damage markers uNAG and uKIM-1 appear to be clinically important even without concomitant glomerular decline, which is of particular interest since in current clinical practice these markers are not routinely assessed and the degree of tubular injury remains undetermined.

REFERENCES:

1. Damman K, Valente MA, Voors AA, O'Connor CM, van Veldhuisen DJ, Hillege HL. Renal impairment, worsening renal function, and outcome in patients with heart failure: an updated meta-analysis. Eur Heart J. 2014;35(7):455-469.
2. Smith GL, Lichtman JH, Bracken MB, et al. Renal impairment and outcomes in heart failure - Systematic review and meta-analysis. J Am Coll Cardiol. 2006;47(10):1987-1996.
3. Hillege HL, Girbes AR, de Kam PJ, et al. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. Circulation. 2000;102(2):203-210.
4. Damman K, Testani JM. The kidney in heart failure: an update. Eur Heart J. 2015;36(23):1437-1444.
5. Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. J Am Coll Cardiol. 2008;52(19):1527-1539.
6. Bock JS, Gottlieb SS. Cardiorenal syndrome: new perspectives. *Circulation*. 2010;121(23):2592-2600.
7. Di Lullo L, Bellasi A, Barbera V, et al. Pathophysiology of the cardio-renal syndromes types 1-5: An uptodate. *Indian Heart J*. 2017;69(2):255-265.
8. Brankovic M, Akkerhuis KM, van Boven N, et al. Patient-specific evolution of renal function in chronic heart failure patients dynamically predicts clinical outcome in the Bio-SHiFT study. *Kidney international. 2018;93(4):952-960*.
9. *Goldfarb M, Abassi Z, Rosen S, Shina A, Brezis M, Heyman SN. Compensated heart failure predisposes to outer medullary tubular injury: studies in rats. Kidney Int. 2001;60(2):607-613*.
10. *Tsuruya K, Eriguchi M. Cardiorenal syndrome in chronic kidney disease. Curr Opin Nephrol Hypertens*. 2015;24(2):154-162.
11. Damman K, Masson S, Hillege HL, et al. Clinical outcome of renal tubular damage in chronic heart failure. *European heart journal. 2011;32(21):2705-2712*.
12. *McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J*. 2012;33(14):1787-1847.
13. McAlister FA, Ezekowitz J, Tarantini L, et al. Renal Dysfunction in Patients With Heart Failure With Preserved Versus Reduced Ejection Fraction Impact of the New Chronic Kidney Disease-Epidemiology Collaboration Group Formula. *Circ-Heart Fail. 2012;5(3):309-314*.
14. *National Kidney F. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1-266*.
15. *Rizopoulos D. Joint Models for Longitudinal and Time-to-Event Data: With Applications in R. Boca Raton: Chapman & Hall/CRC; 2012*.
16. *Brankovic M, Kardys I, Hoorn EJ, Baart S, Boersma E, Rizopoulos D. Personalized dynamic risk assessment in nephrology is a next step in prognostic research. Kidney international. 2018*.
17. *IBM Corp. Released 2017. IBM SPSS Statistics for Windows VA, NY: IBM Corp*. .
18. *R Core Team (year and version used). R: A language and environment for statistical computing. R Foundation for Statistical Computing V, Austria. URL* https://www.R-project.org/.
19. Rizopoulos D. The R Package JMbayes for Fitting Joint Models for Longitudinal and Time-to-Event Data Using MCMC. *Journal of Statistical Software. 2016;72(7):46*.
20. *de Silva R, Nikitin NP, Witte KK, et al. Incidence of renal dysfunction over 6 months in patients with chronic heart failure due to left ventricular systolic dysfunction: contributing factors and relationship to prognosis. Eur Heart J. 2006;27(5):569-581*.
21. *Helal I, Fick-Brosnahan GM, Reed-Gitomer B, Schrier RW. Glomerular hyperfiltration: definitions, mechanisms and clinical implications. Nat Rev Nephrol. 2012;8(5):293-300*.
22. *Jose P, Skali H, Anavekar N, et al. Increase in creatinine and cardiovascular risk in patients with systolic dysfunction after myocardial infarction. J Am Soc Nephrol. 2006;17(10):2886-2891*.
23. *Khan NA, Ma I, Thompson CR, et al. Kidney function and mortality among patients*

with left ventricular systolic dysfunction. J Am Soc Nephrol. 2006;17(1):244-253.

24. *Rossignol P, Cleland JG, Bhandari S, et al. Determinants and consequences of renal function variations with aldosterone blocker therapy in heart failure patients after myocardial infarction: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study. Circulation. 2012;125(2):271-279.*
25. *Damman K, Navis G, Voors AA, et al. Worsening renal function and prognosis in heart failure: systematic review and meta-analysis. J Card Fail. 2007;13(8):599-608.*
26. *Damman K, Masson S, Hillege HL, et al. Tubular damage and worsening renal function in chronic heart failure. JACC Heart Fail.* 2013;1(5):417-424.
27. Breidthardt T, Socrates T, Noveanu M, et al. Effect and clinical prediction of worsening renal function in acute decompensated heart failure. *Am J Cardiol. 2011;107(5):730-735.*