Patient-specific evolution of renal function in chronic heart failure patients dynamically predicts clinical outcome in the Bio-SHiFT study

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ABSTRACT

Renal dysfunction is an important component of chronic heart failure (CHF), but its single assessment does not sufficiently reflect clinically silent progression of CHF prior to adverse clinical outcome. Therefore, we aimed to investigate temporal evolutions of glomerular and tubular markers in 263 stable patients with CHF, and to det16ermine if their patient-specific evolutions during this clinically silent period can dynamically predict clinical outcome. We determined the risk of clinical outcome (composite endpoint of Heart Failure hospitalization, cardiac death, Left Ventricular Assist Device placement, and heart transplantation) in relation to marker levels, slopes and areas under their trajectories. In each patient, the trajectories were estimated using repeatedly measured glomerular markers: creatinine/estimated glomerular filtration rate (eGFR), cystatin C (CysC), and tubular markers: urinary N-acetyl-beta-D-glucosaminidase (NAG) and kidney injury molecule (KIM)-1, plasma and urinary neutrophil gelatinase-associated lipocalin (NGAL). During 2.2 years of follow-up, we collected on average 8 urine and 9 plasma samples per patient. All glomerular markers predicted the endpoint (univariable hazard ratio [95% confidence interval] per 20% increase: creatinine: 1.18 [1.07–1.31], CysC: 2.41[1.81–3.41], and per 20% eGFR decrease: 1.13[1.05–1.23]). Tubular markers, NAG, and KIM-1 also predicted the endpoint (NAG: 1.06[1.01–1.11] and KIM-1: 1.08[1.04–1.11]). Larger slopes were the strongest predictors (creatinine: 1.57[1.39–1.84], CysC: 1.76 [1.52–2.09], eGFR: 1.59[1.37–1.90], NAG: 1.26[1.11–1.44], and KIM-1: 1.64[1.38–2.05]). Associations persisted after multivariable adjustment for clinical characteristics. Thus, during clinically silent progression of CHF,

glomerular and tubular functions deteriorate, but not simultaneously. Hence, patient-specific evolutions of these renal markers dynamically predict clinical outcome in patients with CHF.

INTRODUCTION

Heart failure (HF) is the leading cause of hospitalization worldwide.[1] Despite declines in HF-related mortality as a result of current therapies, re-hospitalization rates for decompensation of chronic heart failure (CHF) remain high.[1,2] Several blood biomarkers that predict re-hospitalization and mortality have been identified in patients with CHF.[3] Still their predictive capabilities in practice are limited, and adequate risk assessment remains a challenge.[3] Estimation of renal dysfunction, which coexists and interacts with HF,[3] may improve risk stratification. Baseline glomerular dysfunction, as assessed by estimated glomerular filtration rate (eGFR), entails an unfavorable prognosis in CHF.[4–6] Besides glomerular impairment, such patients often have tubular damage due to tubulo-interstitial injury by renal tissue hypoperfusion or due to damaged glomerular barrier.[7,8] Notably, a single assessment of damaged tubules predicts adverse outcome in CHF independently of eGFR.[9–11]

It is clear that both glomerular and tubular function are important in patients with CHF, but their single assessment does not sufficiently reflect deterioration along the cardiorenal axis that occurs over time preceding adverse events. Yet the temporal evolution of renal function preceding the event may dynamically ascertain the clinically silent progression of the disease. Specifically, it would enable accurate investigation of whether, and to which degree, increasing (or decreasing) levels of renal biomarkers contribute to the patient's risk, regardless of whether these levels exceed established cut points at study baseline (i.e., a random point in time prior to event).

In the context of cardio-renal interplay, patients with CHF also display large biological heterogeneity. Renal function not only changes dynamically within a patient over time, but also differs from patient to patient. Hence, the true potential of renal markers in ascertaining individual disease progression and their accurate relation with clinical outcome can only be revealed if their patient-specific evolutions are considered. However, detailed individual temporal evolutions of renal function in CHF have never been described.

To overcome these issues, our aim was 2-fold: (i) to investigate the average (population) temporal evolutions of glomerular function (measured with plasma creatinine

[Cr], eGFR, and cystatin C [CysC]) and tubular status (measured with urinary kidney injury molecule [KIM]-1, N-acetyl-beta- D-glucosaminidase [NAG], and urinary and plasma neutrophil gelatinase-associated lipocalin [NGAL]) in stable patients with CHF; and (ii) to determine whether patient-specific (individual) evolutions of these renal biomarkers during a clinically silent period can dynamically predict clinical outcome. For this purpose we examined several aspects of the temporal evolution of each renal biomarker that may be relevant for clinical prediction.

RESULTS

Baseline characteristics

Table 1 displays the baseline characteristics. At baseline, patients who later experienced the endpoint were older; more frequently had diabetes and atrial fibrillation; had lower systolic blood pressure, higher New York Heart Association (NYHA) class, higher levels of N-terminal prohormone of brain natriuretic peptide (NT-proBNP), cardiac troponin T, CysC, urinary N-acetyl-beta-D-glucosaminidase (NAG), and plasma urinary neutrophil-gelatinase-associated-lipocalin (NGAL); and were more frequently on diuretics than the patients who remained endpoint-free.

Follow-up and study endpoints

From 263 patients with CHF, a total of 1912 urine and 1984 blood samples were collected with a median (interquartile range, IQR) of 8 (5–10) urine and 9 (5–10) plasma samples per patient. During a median (IQR) follow-up of 2.2 (1.4–2.5) years, 70 (27%) patients reached the primary endpoint: 56 patients were re-hospitalized for acute or worsened HF, 3 patients underwent heart transplantation, 2 patients underwent left ventricle assist device (LVAD) placement, and 9 patients died of cardiovascular causes.

Temporal evolution of glomerular function

Creatinine and eGFR. In patients who reached the composite endpoint, Cr levels on average showed an increasing pattern over time preceding the endpoint. In endpoint-free patients Cr levels were lower and remained stable during follow-up (Figure 1a). eGFR displayed similar dynamics (Figure 1b). Independently of baseline levels, repeatedly measured Cr and eGFR predicted the endpoint (per 20% increase of Cr levels: hazard ratio [95% confidence interval] 1.18 [1.07–1.31], $P = 0.004$, and

Table 1 | Patient characteristics in relation to the occurrence of the composite endpoint

Variable	Total	Composite endpoint reached		*P* value
		Yes	No	
N (%)	263 (100)	70 (27)	193 (73)	
Demographics				
Age, yr (mean ± SD)	67 ± 13	69 ± 13	66 ± 12	0.05
Men, *n* (%)	189 (72)	53 (76)	136 (70)	0.41
Clinical characteristics				
BMI, kg/m^2 (mean ± SD)	27.5 ± 4.7	27.6 ± 4.8	27.4 ± 4.7	0.80
Heart rate, bpm (mean ± SD)	67 ± 12	69 ± 13	67 ± 11	0.31
SBP, mm Hg (mean ± SD)	122 ± 20	117 ± 17	124 ± 21	0.02
DBP, mm Hg (mean ± SD)	72 ± 11	70 ± 10	73 ± 11	0.06
Features of heart failure				
NYHA class III or IV, n (%)	69 (26)	31 (44)	38 (20)	<0.001
HF-rEF n (%)	250 (95)	66 (94)	184 (95)	0.75
HF-pEF n (%)	13 (5)	4 (6)	9 (5)	
LVEF, % (mean ± SD)	32 ± 11	30 ± 11	33 ± 10	0.18
NT pro-BNP (pmol/l)*	137.3 (51.7–272.6)	282.4 (176.4–517.4)	95.3 (31.72–207.7)	<0.001
Hs-TnT (ng/l)*	18.0 (9.5–33.2)	31.9 (20.6–49.7)	13.9 (8.4–26.7)	<0.001
Etiology of heart failure, n (%)				
Ischemic	117 (44)	36 (51)	81 (42)	0.17
Hypertension	34 (13)	10 (14)	24 (12)	0.70
Secondary to valvular disease	12 (5)	5 (7)	7 (4)	0.23
Cardiomyopathy	68 (26)	15 (21)	53 (28)	0.32
Unknown or Others	32 (12)	4 (6)	28 (15)	
Medical history, n (%)				
Prior MI	96 (36)	32 (46)	64 (33)	0.06
Prior PCI	82 (31)	27 (39)	55 (28)	0.12
Prior CABG	43 (16)	13 (19)	30 (15)	0.57
Atrial fibrillation	106 (40)	36 (51)	70 (36)	0.03
Diabetes	81 (31)	32 (46)	49 (25)	0.002
Hypercholesterolemia	96 (36)	30 (43)	66 (34)	0.20
Hypertension	120 (46)	38 (54)	82 (42)	0.09
COPD	31 (12)	12 (17)	19 (10)	0.10
Medication use, n (%)				
Beta-blocker	236 (90)	61 (87)	175 (91)	0.40
ACE-I or ARB	245 (93)	63 (90)	182 (94)	0.22
Diuretics	237 (90)	68 (97)	169 (88)	0.02
Loop diuretics	236 (90)	68 (97)	168 (87)	0.02
Thiazides	7 (3)	3 (4)	4 (2)	0.28
Aldosterone antagonist	179 (68)	53 (76)	126 (65)	0.11

Table 1 | Patient characteristics in relation to the occurrence of the composite endpoint (*continued*)

Variable	Total	Composite endpoint reached		*P* value
		Yes	No	
Glomerular function markers*				
Creatinine, mg/dl	1.18 (0.99–1.49)	1.30 (1.02–1.52)	1.17 (0.98–1.45)	0.18
eGFR, ml/min per 1.73m^2	58 (43–76)	53 (40–73)	59 (44–77)	0.16
Cystatin C, mg/l	0.73 (0.57–0.97)	0.87 (0.71–1.03)	0.70 (0.53–0.90)	<0.001
KDOQI classification, n (%)				
eGFR ≥ 90 ml/min per 1.73m^2	28 (11)	7 (10)	21 (11)	0.18
eGFR 60–89 ml/min per 1.73m^2	95 (36)	20 (28)	75 (39)	
eGFR 30–59 ml/min per 1.73m^2	119 (45)	37 (53)	82 (42)	
eGFR < 30 ml/min per 1.73m^2	21 (8)	6 (9)	15 (8)	
Tubular markers*				
NAG, U/gCr (urine)	5.9 (3.8–9.3)	8.0 (6.0–11.0)	5.1 (3.3–8.0)	<0.001
KIM-1, ng/gCr (urine)	477.2 (247.0–938.6)	589.0 (255.0–957.2)	465.1 (237.6–911.5)	0.10
NGAL, µg/gCr (urine)	17.4 (9.2–32.6)	18.2 (10.0–50.5)	17.4 (9.0–31.4)	0.20
NGAL, ng/ml (plasma)	190.1 (133.5–280.0)	260.8 (169.5–355.4)	179.2 (127.9–244.5)	<0.001

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; bpm, beats per minute; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HF-pEF, heart failure with preserved ejection fraction; HF-rEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; TIA, transitory ischemic attack.

Normally distributed continuous variables are presented as mean ± SD, and non-normally distributed variables as median and interquartile range. Categorical variables are presented as numbers and percentages.

*All biomarkers levels were presented as median (interquartile range).

per 20% eGFR decrease: 1.13 [1.05–1.1.23], $P = 0.002$) (Table 2). Similarly, their larger slopes and larger area under the curve of the marker's trajectory (AUCm) predicted the endpoint (per 20% increase of Cr slope: 1.57 [1.39–1.84], $P < 0.001$, per 20% decrease of eGFR slope: 1.59 [1.37–1.90], $P < 0.001$) (per 20% increase of Cr's AUCm: 1.10 [1.03–1.18], $P = 0.010$, and eGFR's AUCm: 1.07 [1.02–1.11], $P < 0.001$). These risk estimates remained significant even after adjustment for clinical characteristics and dose changes of HF medications during followup. After adjustment for cardiac markers, Cr's levels and AUCm lost precision, whereas eGFR remained significant (Table 2). Table S1 shows similar results for HF hospitalizations (secondary endpoint).

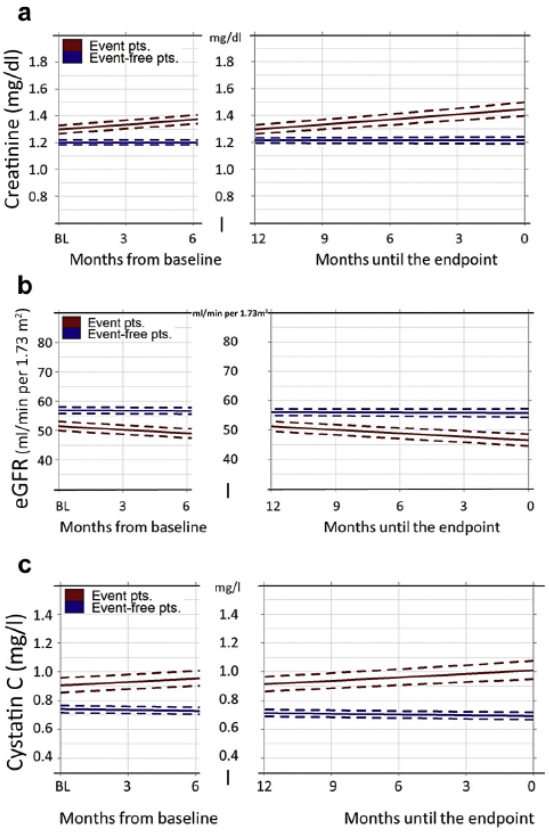

Figure 1 | Average evolution of glomerular function markers during follow-up. Average evolution in patients who reached the study endpoint (solid red line), and in endpoint-free patients (solid blue line). Dashed lines represent the 95% confidence interval. X-axis depicts the time from baseline (left part of the x-axis), and time remaining to the event (patients who experienced incident events) or last sample moment (patients who remained event-free; right part of the x-axis). Biomarker levels are presented on the y-axis. (a) Creatinine (mg/dl). (b) eGFR (ml/min per 1.73 m^2). (c) Cystatin C (µg/ml). BL, baseline; pts., patients.

Cystatin C. In patients who reached the composite endpoint, CysC showed on average higher baseline levels that increased further as the endpoint approached. In endpoint-free patients, CysC levels were lower and slightly decreased during follow-up (Figure 1c). Independently of baseline levels, CysC levels at any time during follow-up were associated with the endpoint (per 20% increase of CysC levels: 2.41 [1.81–3.41], $P < 0.001$) (Table 2). Similarly, larger slope and larger AUCm predicted the endpoint (1.76 [1.52–2.09], $P < 0.001$ and 1.32 [1.17–1.54], $P < 0.001$). These risk estimates remained significant after multivariable adjustments (Table 2). Supplementary Table S1 shows similar results for HF hospitalizations.

Table 2 | Associations between glomerular function markers and the composite endpoint

	Creatinine		eGFR		Cystatin C	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Baseline level[a]						
Model A	1.04 (0.99–1.09)	0.14	1.03 (0.99–1.07)	0.13	1.09 (1.05–1.14)	<0.001
Model B	1.02 (0.97–1.07)	0.49	1.02 (0.97–1.06)	0.48	1.07 (1.02–1.12)	0.007
Model C	0.98 (0.93–1.03)	0.46	0.98 (0.94–1.02)	0.28	1.00 (0.95–1.06)	0.89
Temporal evolution[b]						
Repeatedly measured levels						
Model 1	1.18 (1.07–1.31)	0.004	1.13 (1.05–1.23)	0.002	2.41 (1.81–3.41)	<0.001
Model 2	1.12 (1.02–1.23)	0.022	1.12 (1.06–1.20)	<0.001	2.16 (1.44–3.72)	<0.001
Model 3	1.05 (0.96–1.15)	0.28	1.09 (1.04–1.14)	<0.001	1.63 (1.35–2.30)	<0.001
Model 4	1.15 (1.08–1.24)	<0.001	1.10 (1.04–1.16)	<0.001	2.27 (1.99–2.59)	<0.001
Annual slope						
Model 1	1.57 (1.39–1.84)	<0.001	1.59 (1.37–1.90)	<0.001	1.76 (1.52–2.09)	<0.001
Model 2	1.65 (1.40–1.98)	<0.001	1.64 (1.38–2.02)	<0.001	2.00 (1.66–2.51)	<0.001
Model 3	1.37 (1.22–1.57)	<0.001	1.30 (1.16–1.46)	0.002	1.47 (1.32–1.66)	<0.001
Model 4	1.28 (1.16–1.43)	<0.001	1.18 (1.07–1.31)	0.001	1.63 (1.50–1.77)	<0.001
AUCm						
Model 1	1.10 (1.03–1.18)	0.010	1.07 (1.02–1.11)	<0.001	1.32 (1.17–1.54)	<0.001
Model 2	1.08 (1.01–1.15)	0.020	1.07 (1.02–1.12)	<0.001	1.23 (1.13–1.36)	<0.001
Model 3	1.04 (0.98–1.10)	0.17	1.06 (1.02–1.10)	<0.001	1.17 (1.08–1.28)	<0.001

AUCm, area under the curve of marker's trajectory; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; LME, linear mixed effects.

[a]HRs and 95% CIs are given per 20% increase of creatinine and cystatin C, and 20% eGFR decrease. Model A is unadjusted. Model B is adjusted for age, sex, diabetes, atrial fibrillation, baseline New York Heart Association class, diuretics, and systolic blood pressure. Model C is adjusted for baseline NT-proBNP and hs-cTnT.

[b]HRs and 95% CIs are given per 20% increase of the level, slope, and AUCm of creatinine and cystatin C, and 20% decrease of the level, slope, and AUCm of eGFR. Model 1 is Cox model–adjusted for marker's baseline levels and LME model–adjusted for sampling time. Model 2 is Cox and LME model–adjusted for the clinical variables age, sex, diabetes, atrial fibrillation, baseline New York Heart Association class, diuretics, systolic blood pressure, and sampling time (LME). Model 3 is Cox and LME model–adjusted for baseline NT-proBNP and hs-cTnT and sampling time (LME). Model 4 is time-dependent Cox–adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.

Temporal evolution of tubular function

Overall, we found substantial associations between NAG, KIM-1, and NGAL, but only mild associations between these tubular markers and glomerular function markers (namely CysC), when assessed during follow-up (Table S2).

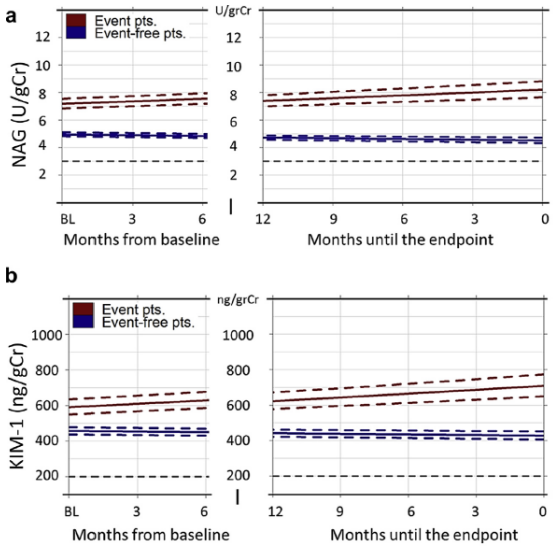

Figure 2 | Average evolution of tubular markers urinary NAG and KIM-1 during follow-up. For description see Figure 1. Dashed black lines represent the biomarkers' reference values. (a) Urinary N-acetyl-beta-D-glucosaminidase (NAG) (U/gCr). (b) Urinary kidney injury molecule-1 (KIM-1) (ng/gCr). BL, baseline; pts., patients.

Urinary NAG. In patients who reached the composite endpoint, NAG showed on average higher baseline levels that increased further as the endpoint approached. In endpoint-free patients, NAG levels were lower and decreased during follow-up (Figure 2a). Independently of baseline levels, higher NAG levels at any time during follow-up were associated with the endpoint (per 20% increase of NAG levels: 1.06 [1.01–1.11], P = 0.018). Similarly, larger NAG slope predicted the endpoint (1.26 [1.11–1.44], P = 0.004).These risk estimates remained significant after multivariable adjustments, except for NAG slope that became insignificant after controlling for cardiac markers (Table 3). Table S3 shows similar results for HF hospitalizations, except for NAG levels that lost significance after adjusting for cardiac markers.

Urinary KIM-1. In patients who reached the composite endpoint, KIM-1 levels showed an average increasing pattern over time preceding the endpoint. In endpoint-free patients, KIM-1 levels were lower and slightly decreased during followup (Figure 2b). Independently of baseline levels, higher KIM-1 levels at any time during follow-up were associated with the endpoint (per 20% increase of KIM-1 levels: 1.08 [1.04–1.11], P < 0.001). Similarly, larger KIM-1 slope predicted the endpoint (1.64 [1.38–2.05], P < 0.001). These risk estimates remained significant after multivari-

able adjustments (Table 3). Table S3 shows similar results for HF hospitalizations, except for KIM-1 levels that lost significance after adjusting for cardiac markers.

Table 3 | Associations between tubular markers, urinary NAG and KIM-1, and the composite endpoint

	Urinary NAG		Urinary KIM-1	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Baseline levels[a]				
Model A	1.07 (1.05–1.09)	<0.001	1.02 (1.00–1.04)	0.06
Model B	1.06 (1.03–1.09)	<0.001	1.01 (0.99–1.03)	0.26
Model C	1.03 (1.00–1.06)	0.050	0.99 (0.97–1.01)	0.44
Temporal evolution[b]				
Repeatedly measured levels				
Model 1	1.06 (1.01–1.11)	0.018	1.08 (1.04–1.11)	<0.001
Model 2	1.07 (1.03–1.12)	<0.001	1.06 (1.03–1.10)	<0.001
Model 3	1.05 (1.00–1.10)	0.048	1.04 (1.01–1.07)	0.016
Model 4	1.13 (1.09–1.17)	<0.001	1.06 (1.03–1.09)	<0.001
Annual slope				
Model 1	1.26 (1.11–1.44)	0.004	1.64 (1.38–2.05)	<0.001
Model 2	1.50 (1.18–2.00)	0.002	1.78 (1.41–2.39)	<0.001
Model 3	0.81 (0.65–1.41)	0.16	1.52 (1.25–1.98)	<0.001
Model 4	1.10 (1.02–1.20)	0.009	1.12 (1.04–1.20)	0.002
AUCm				
Model 1	1.02 (0.99–1.05)	0.11	1.01 (0.99–1.02)	0.23
Model 2	1.04 (1.01–1.07)	0.01	1.01 (0.99–1.03)	0.10
Model 3	1.01 (0.98–1.05)	0.33	1.01 (0.99–1.02)	0.38

AUCm, area under the curve of marker's trajectory; CI, confidence interval; HR, hazard ratio; KIM-1, kidney injury molecule-1; LME, linear mixed effects; NAG, N-acetyl-beta-D-glucosaminidase.

[a]HRs and 95% CIs are given per 20% increase of urinary NAG and KIM-1. Model A is unadjusted. Model B is adjusted for age, sex, diabetes, atrial fibrillation, baseline New York Heart Association class, diuretics, systolic blood pressure, and estimated glomerular filtration rate. Model C is adjusted for baseline NT-proBNP and hs-cTnT.

[b]HRs and 95% CIs are given per 20% increase of the level, slope, and AUCm of urinary NAG and KIM-1. Model 1 is Cox model–adjusted for marker's baseline levels and LME model–adjusted for sampling time. Model 2 is Cox and LME model–adjusted for age, sex, diabetes, atrial fibrillation, baseline New York Heart Association class, diuretics, systolic blood pressure, estimated glomerular filtration rate, and sampling time (LME). Model 3 is Cox and LME model–adjusted for baseline NT-proBNP and hs-cTnT and sampling time (LME). Model 4 is time-dependent Cox–adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.

Plasma and urinary NGAL. Although baseline plasma NGAL levels were higher in patients who reached the endpoint, this difference declined during follow-up (Supplementary Figure S1A). The evolution of urinary NGAL levels of patients who reached the endpoint and those who did not substantially overlapped during follow-up (Supplementary Figure S1B). No clear associations were found between NGAL and primary and secondary endpoints during follow-up (Supplementary Tables S4 and S5).

Prospective accuracy

Supplementary Table S6 shows the time-dependent area under the receiver operating curve (AUC) for the different renal markers for the composite endpoint. After the 1-year collection time period, markers showed reasonably good discriminatory power both for the 6- and 12-month risk window, with slightly better accuracy for the 6-month window. The highest accuracy was found for clinical models using levels of CysC, NAG, and KIM-1 (6-month AUCs: 0.80, 0.81, and 0.80, respectively).

Patient-specific dynamic prediction

Figure S2 shows the temporal patterns of eGFR and NAG in several individual patients from our cohort, together with their corresponding individual survival probabilities as estimated by the joint model. The figure shows that each time an additional measurement is performed in the patient, the individual survival probability is updated. Specifically, rising marker levels and worsening prognosis can be seen in the example patients who ultimately reached the composite endpoint versus stable or decreasing marker levels and more favorable prognosis in the example patients who stayed event-free.

DISCUSSION

We have shown that in patients with CHF both glomerular function (as assessed by repeatedly measured creatinine, eGFR, and CysC), and tubular function (as assessed by repeatedly measured urinary NAG and KIM-1) deteriorate over time preceding clinical outcome. Importantly, patient-specific trajectories of all glomerular markers dynamically predicted the event, and CysC was the strongest predictor. Similarly, patient-specific trajectories of urinary NAG and KIM-1 indicated progression of tubular damage in patients who later suffered adverse events. No clear associations were found between repeatedly measured plasma or urinary NGAL and the event.

Therefore, the current study does not justify its use for clinical prediction in patients with CHF.

Our findings confirm that renal function is an indivisible component of HF, and that it is clinically relevant for the monitoring of stable patients with CHF. Importantly, our results show that temporal changes in renal function remain predictive for clinical outcome despite controlling for NYHA class, cardiac markers and other clinical features, which suggests that renal dysfunction may drive adverse clinical outcomes independently of cardiac dysfunction. In addition, the results demonstrate the predictive value not only of GFR levels (single value or cumulative effects), but also of GFR slope. These findings are supported by other studies.[4,10] However, unlike previous studies, our study underscores that GFR evolution should be assessed as a function of time. In other words, information on early and late GFR changes,[12] as well as the time interval during which GFR was measured, should be taken into consideration. This recommendation is also supported by recent results from Damman et al., who found that when eGFR is assessed as a function of time, any decrease in eGFR will result in increased event rates. In previous studies, deltas in creatinine or eGFR between any 2 sampling moments were mostly used, which may have led to bias as a consequence of differences in the time periods (before the event) in which sampling was performed. In our study, the observations were made using 2 glomerular markers, creatinine and CysC, which were assessed at fixed time intervals; using more than twice as many repeated measurements as previous studies did. Notably, CysC showed the strongest association with adverse events. Considering that generation of creatinine changes when muscle wasting occurs with progression of cardiac disease, this can be of particular interest when renal function is repeatedly assessed in the same individual with CHF. Nonetheless, this issue requires further exploration.

In the setting of tubular injury, we found not only that patients with CHF experience tubular damage, but also that the damage progresses over time (months) preceding a clinical event. This extends previous findings by demonstrating that tubular markers, which were previously shown to capture acute kidney injury,[13] are also clinically relevant in chronic tubular damage in patients with CHF when followed during a prolonged time period.[11] To our best knowledge, our study is the first to simultaneously follow glomerular and tubular markers and to show that glomerular dysfunction and tubular injury, in most cases, do not progress over time in parallel. This implies that, although the failing heart affects both renal compartments, the degree of damage in these compartments is usually not temporally coupled. There-

fore, they should be viewed as different renal entities in CHF. In addition, when we examined NAG and KIM-1, we found that NAG levels will rise first, followed by a rise in KIM-1. This suggests that, although both markers are labeled as "tubular damage markers," they reflect different biological aspects of tubular injury, and their values depend on the moment in time prior to the event at which they are assessed. These findings are in line with their behavior as previously found. Increased urinary excretion of NAG has been found to occur with abnormal increases in protein traffic across the proximal tubules as a consequence of a damaged glomerular barrier.[14] On the other hand, KIM-1 gene expression has been found to be upregulated in a dose-dependent manner in response to direct tubular injury.[15] KIM-1 also correlated strongest with tubular damage as determined by kidney biopsies. It outperformed serum creatinine, blood urea nitrogen (BUN), and urinary NAG.[16,17] Thus, it appears that NAG is a marker of tubular dysfunction that shows an early initial rise, while KIM-1 can serve as a quantitative marker of tubular damage, if modeled in a time-dependent manner. Importantly, both tubular markers are relevant for clinical outcomes.

The unique advantages of our study include frequent repeated measurements at pre-specified time intervals (i.e., sampling was not left at the discretion of the treating physicians) during longer-term follow-up. This allowed us to provide an unbiased assessment of a patient's risk by using the complete temporal biomarker trajectory as assessed over the entire follow-up period. Based on this underlying trajectory, biomarker levels are used to estimate the risk of future adverse events.[18] Herewith, a window of opportunity may be gained to modify the treatment before a future event occurs. Joint modeling (JM) of patient-specific marker trajectories and survival analysis enables us to perform individualized risk predictions based on individual biomarker values. Subsequently, predictions are dynamically updated to provide real-time risk assessment whenever extra information is collected.[19] Such dynamic risk profiling can enable physicians to better detect disease progression and to make wellinformed individualized treatment decisions. Applicability of JM in daily practice is user-friendly, and an app is already available into which a patient's data (baseline and follow-up) can be uploaded (for details please see Figure S3).[20]

Study limitations

Firstly, our cohort consisted mainly of patients with HF and reduced ejection fraction. The low number of patients with HF and a preserved ejection fraction can most likely be attributed to the fact that in the Netherlands, most such patients

are treated by the general practitioner or in secondary referral centers, while the current study was performed in 2 centers that were both tertiary referral centers. Potential inclusion bias is not a likely reason for the low HF and preserved ejection fraction rate, because all consecutive patients were screened in both participating centers. Secondly, enrolled CHF patients were in a better health condition than previously reported CHF populations. Yet, we were able to demonstrate, even in this "less sick" CHF population that evolutions of glomerular and tubular dysfunction predict clinical outcome. Thus, it is possible that these markers could perform even better in more sick CHF patients. Thirdly, although we adjusted for several confounders, residual confounding may be present. However, we corrected all urinary markers for concentration or dilution of urine caused by diuretics during follow-up. Furthermore, treating physicians were blinded to biomarker data to exclude bias by treatment effect. Finally, although our findings underscore the importance of regular monitoring of both glomerular and tubular function in CHF, routine evaluation of kidneys should always be seen in the light of the patient's clinical status.

Altogether, our findings demonstrate that glomerular function (as assessed by creatinine, eGFR, and CysC), and tubular function (as assessed by urinary NAG and KIM-1) deteriorate, but not simultaneously, during clinically silent progression of CHF over time preceding adverse events. Patient-specific temporal evolutions of these repeatedly measured renal markers dynamically predict clinical outcome in CHF patients, and are useful for individual risk profiling.

MATERIALS AND METHODS

The Bio-SHiFT study is a prospective, observational cohort study of stable patients with CHF, conducted at Erasmus Medical Center (Rotterdam, Netherlands) and Noordwest Ziekenhuisgroep (Alkmaar, Netherlands). Patients were recruited during their regular visits to the cardiology outpatient clinics of these hospitals. For this purpose, consecutive patients were screened according to the inclusion and exclusion criteria specified in Supplementary Figure S1, and eligible patients were asked for informed consent. The main inclusion criteria were age ≥18 years, capability of understanding and signing informed consent, and diagnosis of CHF ≥3 months ago according to European Society of Cardiology guidelines (Supplementary Figure S4).[21,22] Patients were ambulatory and stable (i.e., they had not been hospitalized for HF in the past 3 months). The study was approved by the medical ethics committees, conducted in accordance with the Declaration of Helsinki, and registered

with ClinicalTrials.gov (NCT01851538). Written informed consent was obtained from all patients who participated in the study. This investigation comprised 263 stable patients with CHF enrolled during the first inclusion period (October 2011 until June 2013).

Baseline assessment

All patients were evaluated by research physicians, who collected information on HF-related symptoms, NYHA class, and performed a physical examination, including blood pressure, heart rate, and body mass index. Information on HF etiology, left ventricular ejection fraction, cardiovascular risk factors, medical history, and medical treatment was retrieved primarily from hospital records and was checked if ambiguities were present. History of cardiovascular and other comorbidities was defined as a clinical diagnosis of these conditions. Non-fasting blood and urine samples were collected, as described below.

Follow-up and study endpoints

During the study, all patients were routinely followed at the outpatient clinic by treating physicians who were blinded to biomarker sampling and results. Study follow-up visits were predefined and scheduled every 3 months (±1 month was allowed), with a maximum of 10 study follow-up visits. At each study follow-up visit, a short medical evaluation was performed and samples were collected. All medication changes and occurrence of adverse cardiovascular events since the previous visit were recorded in electronic case report forms. During follow-up, hospitalizations for HF, myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, arrhythmias, cerebrovascular accident, cardiac transplantation, LVAD implantation, and mortality were recorded in the electronic case report forms, and associated hospital records and discharge letters were collected. Subsequently, a clinical event committee, blinded to the biomarker sampling and results, reviewed hospital records and discharge letters and adjudicated the study endpoints.

The primary endpoint comprised the composite of cardiac death, cardiac transplantation, LVAD implantation, and hospitalization for the management of acute or worsened HF, whichever occurred first. Secondary endpoints included individual components of the primary endpoint, and also myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, cerebrovascular accident, and all-cause mortality. Cardiac death was defined as death from myocardial in-

farction 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 2 of the following: BNP or NT-proBNP > 3x ULN, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral edema, increased dose or i.v. administration of diuretics, or administration of positive inotropic agents.[21]

Blood and urine analysis

Blood and urine samples were collected at baseline and at each study follow-up visit and were processed and stored at a temperature of –80°C within 2 hours after collection. The biomarker measurements performed for this study did not lead to drug adjustments and all patients received usual care. Batch analysis of plasma and urine samples was performed at HaemoScan BV (Groningen, Netherlands) using methods described in the supplementary text. All urinary biomarkers were normalized to urinary creatinine concentrations to correct for concentration or dilution of urine.

GFR was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that has been validated in HF patients.[23] Patients were categorized using National Kidney Foundation–Kidney Disease Outcome Quality Initiative (K/DOQI) clinical practice guidelines.[24]

Statistical analysis

Biomarkers measured at baseline. The association between baseline marker levels and the study endpoint was examined by Cox regression analysis. If skewed, ^{2}log-tranformation of continuous variables was used for further analyses. Analyses were first performed univariably, then statistical adjustments were performed by using 2 models: (i) model with biomarker of interest plus clinical variables age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, and eGFR (for tubular markers); and (ii) model with biomarker of interest plus 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. The proportional hazards (PH) assumption was evaluated by plotting transformed Kaplan-Meier estimates, and by evaluating scaled Schoenfeld residuals.

Repeatedly measured biomarkers. We applied a joint modeling (JM) of linear mixed-effects (LME) models to assess the true underlying trajectory of a repeatedly measured marker, and a Cox survival analysis to analyze the association of this trajectory with the study endpoint. For both the fixed- and random-effects parts of LME, nonlinear evolutions were tested using restricted cubic splines. If the model was not significantly improved, a linear evolution was retained. All markers were adjusted for the sampling time during follow-up. Additional statistical adjustments were as follows: (i) the repeatedly measured marker was adjusted for its baseline level (Cox model) to examine incremental value of repeated over baseline measurements; (ii) Cox and LME models were adjusted for the clinical variables age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, and eGFR (for tubular markers) to examine incremental value of the renal markers over the patients' clinical characteristics; (iii) Cox and LME models were adjusted for biomarkers of myocardial stretch and damage (NT-proBNP and hs-cTnT) to examine the incremental value of the renal markers over these commonly used cardiac markers. Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs) per 20% change in biomarkers levels.

To investigate the independent predictive value of these renal markers on the study endpoints, all individual temporal biomarker patterns derived from the joint models were extracted and subsequently entered simultaneously with HF medication doses (repeatedly assessed during follow-up) into a time-dependent Cox analysis.

Parameterization of marker's trajectory. The above-described analyses estimate the instantaneous risk based on repeatedly measured marker levels. However, in the context of repeated measurements, we also estimated the following aspects:[25,26] (i) the time-dependent slope (or rate of change) of the marker's trajectory, indicating whether and by how much the levels are increasing or decreasing at any point in time, which corresponds to the first derivative of the marker's trajectory; and (ii) the AUCm, indicating the cumulative effect of all the values the marker has taken in the past (Figure 3). The results are presented as HRs [95% CI] per 20% change in the annual slope (delta of the marker's levels/year) and the AUCm.

Prospective accuracy. We determined the longitudinal marker's predictive accuracy (i.e., the ability of a marker to discriminate between a patient who experiences the event within a given time window after the last measurement and the patient who does not experience the event within that same time window) using the time-dependent AUC methodology.[19] For this purpose, we chose the first year as the

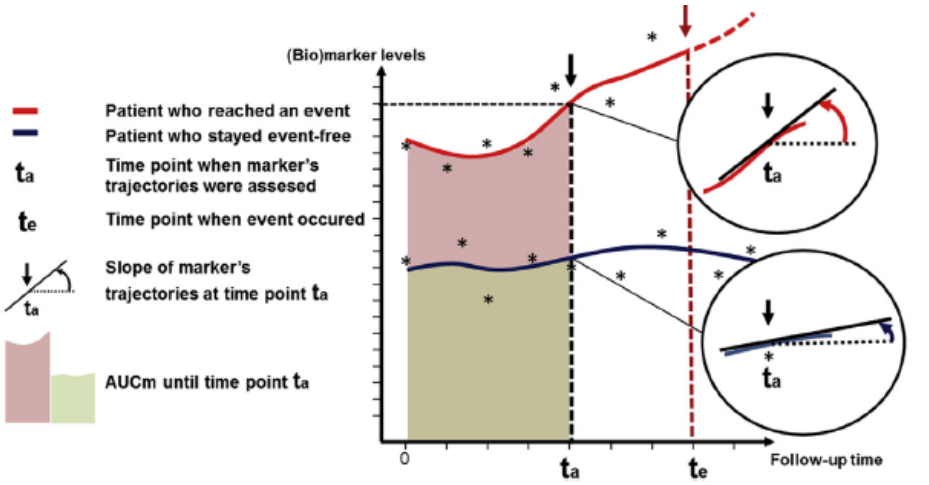

Figure 3 | Dynamic risk prediction model using repeated marker measurements. An illustration of the underlying trajectory of a repeatedly assessed biomarker in a patient who ultimately experiences the event (solid red line) and in an event-free patient (solid blue line). Marker's levels (asterisks) are displayed on the y-axis and follow-up time on the x-axis. The figure shows different types of parameterization that can be examined: marker's levels at any point in time (ta), slope of the marker's trajectory at any point in time (ta), and the area under the curve of marker's trajectory (AUCm) up to the same point in time (ta). te, time when the event occurred.

collection time period, and we assessed 2 risk time-windows: 6 and 12 months after the collection time.

All analyses were performed with R package JMbayes statistical software.[18,26] All tests were 2-tailed, and P values < 0.05 were considered statistically significant.

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