
SYMBIOSIS

Part II

THE ROLE OF THE KIDNEYS IN HEART FAILURE AND BEYOND

CHAPTER 4

Patient-specific Evolution of Renal function in Chronic Heart Failure Patients Dynamically Predicts Clinical Outcome in the Bio-SHiFT Study

Milos Brankovic, K. Martijn Akkerhuis, Nick van Boven, Sharda Anroedh, Alina Constantinescu, Kadir Caliskan, Olivier Manintveld, Jan Hein Cornel, Sara Baart, Dimitris Rizopoulos, Hans Hillege, Eric Boersma, Victor Umans, Isabella Kardys

Kidney international. 2018; 93:952-960.

ABSTRACT

Background

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 CHF patients, and to determine if their patient-specific evolutions can dynamically predict clinical outcome.

Methods

We determined the risk of clinical outcome (composite endpoint of HF-hospitalization, cardiac death, LVAD-placement and heart transplantation) in relation to marker levels, slopes of their trajectories (increasing/decreasing patterns), and areas under their trajectories (AUCm). 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).

Results

During 2.2 years of follow-up, we collected 8 (5–10) urine and 9 (5–10) 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], eGFR: 1.59 [1.37–1.90], CysC: 1.76 [1.52–2.09]; NAG: 1.26 [1.11–1.44], and KIM-1: 1.64 [1.38–2.05]). Associations persisted after multivariable adjustment for clinical characteristics.

Conclusions

Our findings suggest that glomerular and tubular function deteriorate, but not simultaneously, during clinically silent progression of CHF. Patient-specific evolutions of these renal markers dynamically predict clinical outcome in CHF patients.

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 interact with HF[3] may improve risk stratification. Baseline glomerular dysfunction, as assessed by estimated glomerular filtration rate (eGFR), entails an unfavourable 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 cardio-renal 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 two-fold: (1) 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 (2) to determine if 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.

MATERIALS AND METHODS

The *Serial Biomarker Measurements and New Echocardiographic Techniques in Chronic Heart Failure Patients Result in Tailored Prediction of Prognosis (Bio-SHiFT)* is a prospective, observational cohort of stable patients with CHF, conducted in Erasmus MC, Rotterdam, and Noordwest Ziekenhuisgroep, Alkmaar, The 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 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.[12,13] 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 that 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 for biomarkers 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, hospitaliza-

tions for HF, MI, PCI, CABG, arrhythmias, and CVA, cardiac transplantation, left ventricular assist device (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 MI, PCI, CABG, CVA, and all-cause mortality. 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 ULN, 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 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 two 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, The Netherlands. Laboratory personnel was blinded for clinical data.

Creatinine was determined by a colorometric test by the Jaffe's reaction. Plasma was used undiluted, urine was diluted ten times in water (LLD: plasma 0,14 mg/dl, urine: 1.56 mg/ml). CysC was determined in plasma, diluted 2000 times in 0,1%BSA/PBS buffer, by ELISA (R&D systems, Minneapolis, MN) (LLD: 0.1066 µg/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). NGAL was determined in urine diluted 20 times, and plasma diluted 100 times in 0,1% BSA-PBS buffer by ELISA (R&D systems, Minneapolis, MN) (LLD: urine 5.19 ng/mL, plasma 50.3 ng/mL). All urinary biomarkers were normalized to

urinary creatinine concentrations to correct for concentration or dilution of urine.

Glomerular filtration rate (GFR) was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that has been validated in HF patients.[14] Patients were categorized using National Kidney Foundation–Kidney Disease Outcome Quality Initiative (K/DOQI) clinical practice guidelines.[15]

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 two models: (1) model with biomarker of interest plus clinical variables age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, and eGFR (for tubular markers); (2) 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, non-linear 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: (1) the repeatedly measured marker was adjusted for its baseline level (Cox model) to examine incremental value of repeated over baseline measurements (2) 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; (3) 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 (95%CI) 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:[16,17] (1) 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 (2) the area under the curve of the marker's trajectory (AUCm), indicating the cumulative effect of all the values the marker has taken in the past (Figure 1). 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.

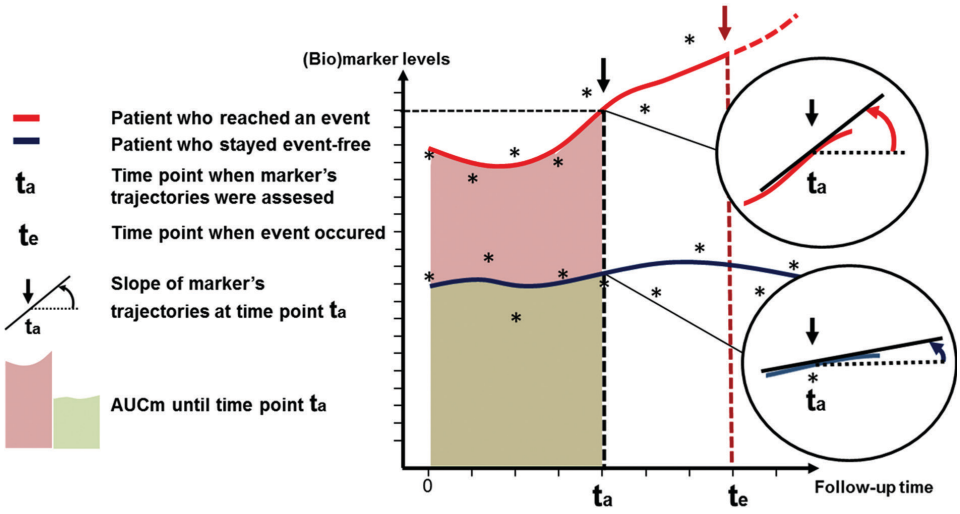


FIGURE 1 Dynamic risk prediction model using repeated marker measurements. An illustration of the underlying trajectory of a repeatedly assessed biomarker in a patient who ultimately experiences the event (solid red line) and in an event-free patient (solid blue line). Marker's levels are displayed on the y-axis and follow-up time on the x-axis. 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; *, measured marker's levels.

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 (area under the receiver operating curve) methodology.[18] For this purpose, we chose the first year as the collection time period, and we assessed two risk time-windows: 6 and 12 months after the collection time.

All analyses were performed with R Statistical Software using package JMbayes.[17,19] All tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Table 1 displays the baseline characteristics. Patients who later experienced the endpoint, at baseline were older, more frequently had diabetes and atrial fibrillation, had lower systolic blood pressure, higher NYHA class, higher levels of NT-proBNP, cardiac troponin T, CysC, urinary NAG, and plasma NGAL, and were more frequently on diuretics than the patients who remained endpoint-free.

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, years (mean ± SD)	67 ± 13	69 ± 13	66 ± 12	0.05
Men, n (%)	189 (72)	53 (76)	136 (70)	0.41
Clinical characteristics				
BMI, kg/m2 (mean ± SD)	27.5±4.7	27.6±4.8	27.4±4.7	0.80
Heart rate, b.p.m. (mean ± SD)	67±12	69±13	67±11	0.31
SBP, mmHg (mean ± SD)	122±20	117±17	124±21	0.02
DBP, mmHg (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

continued

Variable	Total	Composite endpoint reached		p-value
		Yes	No	
NT pro-BNP (pmol/L) †	137.3 (51.7–272.6)	282.4 (176.4–517.4)	95.3 (31.7–207.7)	< 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
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
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/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	28 (11)	7 (10)	21 (11)	0.18
eGFR 60-89	95 (36)	20 (28)	75 (39)	
eGFR 30-59	119 (45)	37 (53)	82 (42)	
eGFR <30	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

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; HF-pEF, heart failure with preserved ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery

bypass grafting; CVA, cerebrovascular accident; TIA, transitory ischemic attack; COPD, chronic obstructive pulmonary disease; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate. Normally distributed continuous variables are presented as mean±standard deviation (SD), and non-normally distributed variables as median and interquartile range (IQR). Categorical variables are presented as numbers and percentages.
†All biomarkers levels were presented as median (IQR).

Follow-up and study endpoints

From 263 patients with CHF, a total of 1912 urine and 1984 blood samples were collected with median (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 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 2A). eGFR displayed similar dynamics (Figure 2B). Independently of baseline levels, repeatedly measured Cr and eGFR predicted the endpoint (per 20% increase of Cr levels: HR [95%CI] 1.18 [1.07–1.31], p=0.004, and per 20% eGFR decrease: 1.13 [1.05–1.23], p=0.002) (Table 2). Similarly, their larger slopes and larger 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 follow-up. 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).

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 2C).

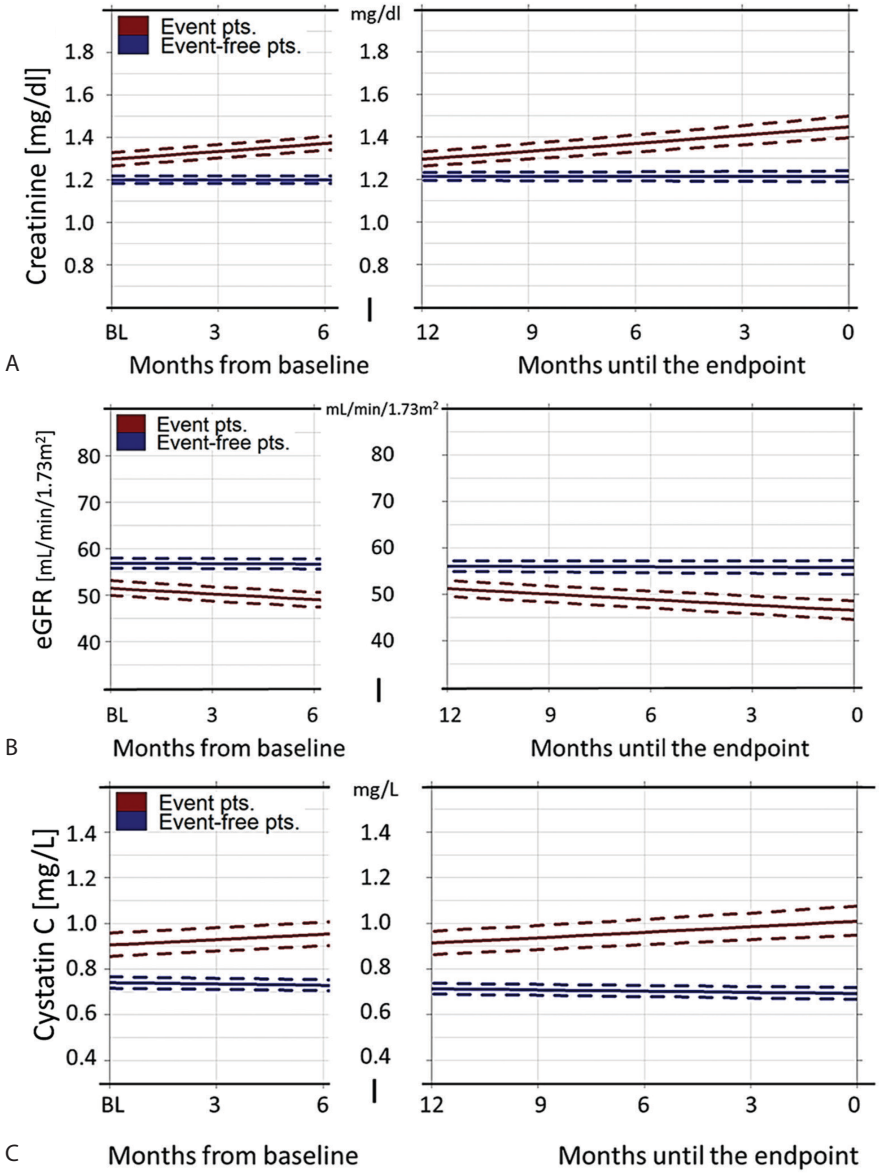


FIGURE 2 Average evolution of glomerular function markers during follow-up. Average evolution in patients who reached the study endpoint (solid red line), and in endpoint-free patients (solid blue line). Dashed lines represent the 95% confidence interval. X-axis depicts the time from baseline (left part 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. BL, baseline; pts., patients. **A.** creatinine (mg/dL); **B.** eGFR (ml/min/1.73m^2); **C.** cystatin C (µg/ml).

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). 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 *						
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†						
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.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase of creatinine and cystatin C, and 20% eGFR decrease. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, and systolic blood pressure; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† 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**: Cox model adjusted for marker's baseline levels, LME model adjusted for sampling time; **Model 2**: Cox and LME models adjusted for the clinical variables: age, sex, diabetes, atrial fibrillation,

baseline NYHA class, diuretics, systolic blood pressure, and sampling time (LME); **Model 3**: Cox and LME models adjusted for baseline NT-proBNP and hs-cTnT, and sampling time (LME); **Model 4:** 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).

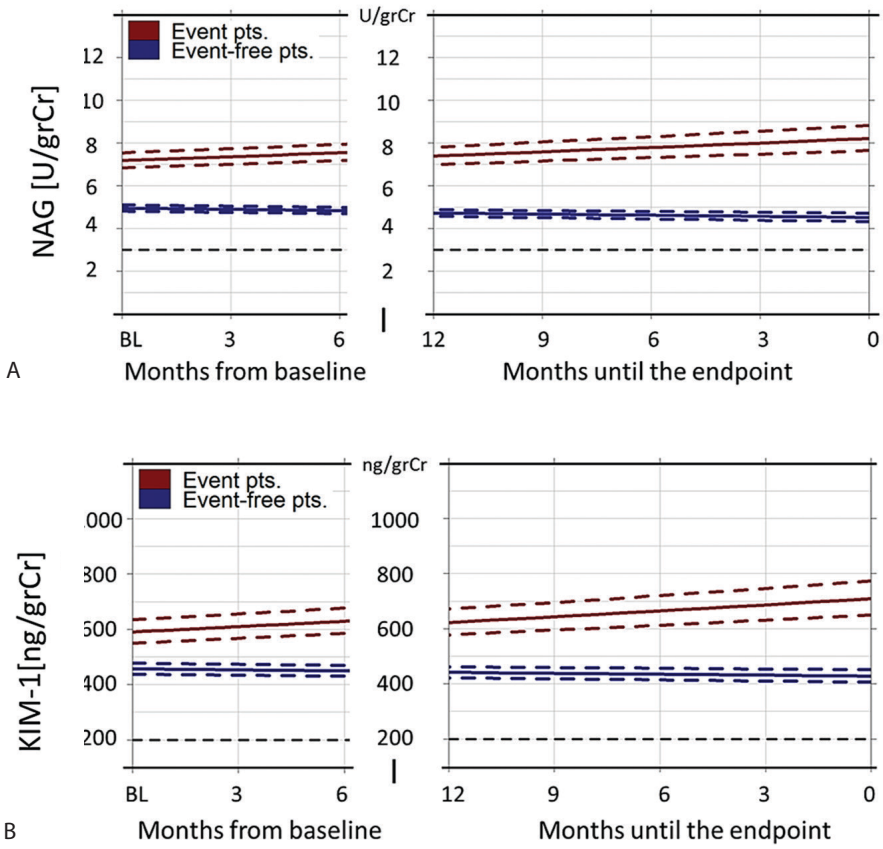


FIGURE 3 Average evolution of tubular markers, urinary NAG and KIM-1, during follow-up. For description see Figure 2. Dashed black lines represent the biomarkers' reference values. BL, baseline; pts., patients. **A.** urinary NAG (U/gCr) **B.** urinary KIM-1 (ng/gCr).

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 3A). 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 follow-up (Figure 3B). 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 multivariable adjustments (Table 3). Table S3 shows similar results for HF-hospitalizations, except for KIM-1 levels that lost significance after adjusting for cardiac markers.

Plasma and urinary NGAL

Although baseline plasma NGAL levels were higher in patients who reached the endpoint, this difference declined during follow-up (Figure S2A). The evolution of urinary NGAL levels of patients who reached the endpoint and those who did not substantially overlapped during follow-up (Figure S2B). No clear associations were found between NGAL and primary and secondary endpoints during follow-up (Tables S4 and S5).

Prospective accuracy

Table S6 shows the time-dependent AUCs 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).

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*				
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†				
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.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase of urinary NAG and KIM-1. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and eGFR; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† HRs and 95% CIs are given per 20% increase of the level, slope, and AUCm of urinary NAG and KIM-1. **Model 1**: Cox model adjusted for marker's baseline levels, LME model adjusted for sampling time; **Model 2**: Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, eGFR, and sampling time (LME); **Model 3**: Cox and LME models adjusted for baseline NT-proBNP and hs-cTnT, and sampling time (LME). **Model 4**: Time-dependent Cox adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.

Patient-specific dynamic prediction

Figure S3 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,[20] 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 two 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 ob-

servations were made using two 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[21], 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. Therefore, 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.[22] On the other hand, KIM-1 gene expression has been found to be up-regulated in a dose-dependent manner in response to direct tubular injury.[23] KIM-1 also correlated strongest with tubular damage as determined by kidney biopsies. It outperformed serum creatinine, blood urea nitrogen (BUN) and urinary NAG.[24,25] 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.[19] 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.[18] Such dynamic risk profiling can enable physicians to better detect disease progression and to make well-informed 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 S4).[26]

Study limitations

Firstly, our cohort consisted mainly of HFrEF patients. The low number of patients with HFpEF can most likely be attributed to the fact that in the Netherlands, most HFpEF patients are treated by the general practitioner or in secondary referral centres, while the current study was performed in two centres which were both tertiary referral centres. Potential inclusion bias is not a likely reason for the low HpEF rate, because all consecutive patients were screened in both participating centres. 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.

CONCLUSION

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.

REFERENCES:

1. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation*. 2013;128(4):388-400.
2. Eapen ZJ, Liang L, Fonarow GC, et al. Validated, Electronic Health Record Deployable Prediction Models for Assessing Patient Risk of 30-Day Rehospitalization and Mortality in Older Heart Failure Patients. *JACC: Heart Failure*. 2013;1(3):245-251.
3. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016.
4. 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.
5. Dries DL, Exner DV, Domanski MJ, Greenberg B, Stevenson LW. The prognostic implications of renal insufficiency in asymptomatic and symptomatic patients with left ventricular systolic dysfunction. *J Am Coll Cardiol*. 2000;35(3):681-689.
6. 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.
7. 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.
8. Tsuruya K, Eriguchi M. Cardiorenal syndrome in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2015;24(2):154-162.
9. Damman K, Voors AA, Navis G, van Veldhuisen DJ, Hillege HL. Current and novel renal biomarkers in heart failure. *Heart Fail Rev*. 2012;17(2):241-250.
10. 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.
11. Damman K, Masson S, Hillege HL, et al. Clinical outcome of renal tubular damage in chronic heart failure. *Eur Heart J*. 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. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *Eur Heart J*. 2007;28(20):2539-2550.
14. 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.
15. 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.
16. Rizopoulos D, Takkenberg JJ. Tools & techniques--statistics: Dealing with time-varying covariates in survival analysis--joint models versus Cox models. *EuroIntervention*. 2014;10(2):285-288.
17. Rizopoulos D. JM: An R Package for the Joint Modelling of Longitudinal and Time-to-Event Data. *2010*. 2010;35(9):33.
18. Rizopoulos D. Dynamic predictions and prospective accuracy in joint models for longitudinal and time-to-event data. *Biometrics*. 2011;67(3):819-829.
19. Rizopoulos D. *Joint Models for Longitudinal and Time-to-Event Data: With Applications in R*. Boca Raton: Chapman & Hall/CRC; 2012.
20. Levey AS, Inker LA, Matsushita K, et al. GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *Am J Kidney Dis*. 2014;64(6):821-835.
21. Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. *Journal of the American Society of Nephrology : JASN*. 2011;22(5):810-820.

22. Skalova S. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. *Acta Medica (Hradec Kralove)*. 2005;48(2):75-80.
23. Chiusolo A, Defazio R, Zanetti E, et al. Kidney injury molecule-1 expression in rat proximal tubule after treatment with segment-specific nephrotoxicants: a tool for early screening of potential kidney toxicity. *Toxicol Pathol*. 2010;38(3):338-345.
24. Vaidya VS, Ozer JS, Dieterle F, et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nat Biotechnol*. 2010;28(5):478-485.
25. Tonomura Y, Tsuchiya N, Torii M, Uehara T. Evaluation of the usefulness of urinary biomarkers for nephrotoxicity in rats. *Toxicology*. 2010;273(1-3):53-59.
26. https://github.com/drizopoulos/JMbayes.

SUPPLEMETARY INFORMATION

Inclusion criteria

1. Age ≥ 18 years?
2. Diagnosis Chronic Heart Failure ≥ 3 months*?
3. Written informed consent?

***Heart Failure, diagnosed ≥ 3 months ago according to the definition of the European Society of Cardiology (ESC), which includes the following features:**

- Symptoms typical of heart failure: breathlessness at rest or on exercise, fatigue, tiredness, ankle swelling
  AND
- Signs typical of heart failure: tachycardia, tachypnea, pulmonary rales, pleural effusion, raised jugular venous pressure, peripheral edema, hepatomegaly
  AND
- Objective evidence of a structural or functional abnormality of the heart at rest: cardiomegaly, third heart sound, cardiac murmurs, abnormality on the echocardiogram, raised natriuretic peptide concentration.

Heart Failure with preserved ejection fraction (HFpEF) diagnosed ≥ 3 months ago according to the definition of the ESC, which includes the following features:

- Presence of signs and/or symptoms of heart failure (as described above)
  AND
- Presence of normal or only mildly abnormal ejection fraction (LVEF ≥50%)
  AND
- Evidence of diastolic left ventricular dysfunction according to the criteria of the ESC:
  - E/E' >15
    OR
  - 15> E/E' >8 AND NT-proBNP >220 pg/ml / BNP >200 pg/ml
    OR
  - E/A <0.5 and DT >260 mm
    OR
  - Atrial fibrillation

Exclusion criteria

1. Heart Failure secondary to circulatory high-output conditions
2. Scheduled for surgery or intervention for both coronary and non-coronary indications within 6 months from inclusion
3. Severe renal failure for which dialysis is needed
4. Known moderate or severe liver disease
5. COPD Gold stage IV
6. Coexistent condition with life expectancy ≤ 1 year
7. Congenital heart disease

FIGURE S1 Inclusion and exclusion criteria.

TABLE S1 Associations between glomerular function markers and HF-hospitalizations.

	Creatinine		eGFR		Cystatin C	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Baseline level *						
Model A	1.04 (0.98–1.10)	0.17	1.04 (0.99–1.08)	0.11	1.10 (1.05–1.15)	<0.001
Model B	1.01 (0.95–1.08)	0.70	1.01 (0.96–1.07)	0.65	1.06 (1.01–1.12)	0.034
Model C	0.98 (0.92–1.04)	0.46	0.98 (0.93–1.03)	0.38	1.01 (0.95–1.07)	0.85
Temporal evolution†						
Repeatedly measured levels						
Model 1	1.22 (1.09–1.39)	<0.001	1.17 (1.08–1.27)	<0.001	2.40 (1.79–3.26)	<0.001
Model 2	1.13 (1.01–1.27)	0.032	1.15 (1.07–1.24)	<0.001	2.64 (1.63–4.31)	<0.001
Model 3	1.07 (0.96–1.18)	0.21	1.12 (1.06–1.18)	<0.001	2.04 (1.46–3.31)	<0.001
Model 4	1.19 (1.10–1.28)	<0.001	1.12 (1.06–1.19)	<0.001	2.96 (2.46–3.56)	<0.001
Annual slope						
Model 1	1.61 (1.42–1.86)	<0.001	1.65 (1.41–2.00)	<0.001	1.75 (1.50–2.05)	<0.001
Model 2	1.76 (1.45–2.17)	<0.001	1.68 (1.42–2.12)	<0.001	1.93 (1.61–2.3)	<0.001
Model 3	1.43 (1.27–1.62)	<0.001	1.36 (1.21–1.55)	<0.001	1.46 (1.31–1.68)	<0.001
Model 4	1.36 (1.21–1.52)	<0.001	1.27 (1.14–1.41)	<0.001	1.65 (1.51–1.81)	<0.001
AUCm						
Model 1	1.10 (1.02–1.19)	0.014	1.07 (1.02–1.12)	0.004	1.35 (1.7–1.63)	<0.001
Model 2	1.08 (1.01–1.16)	0.026	1.08 (1.03–1.12)	0.004	1.22 (1.11–1.38)	<0.001
Model 3	1.05 (0.98–1.12)	0.18	1.07 (1.03–1.11)	<0.01	1.20 (1.09–1.33)	<0.001

AUCm – area under the curve of marker's trajectory.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase of creatinine and cystatin C, and 20% eGFR decrease. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, and systolic blood pressure; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† 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**: Cox model adjusted for marker's baseline levels, LME model adjusted for sampling time; **Model 2**: Cox and LME models adjusted for the clinical variables: age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and sampling time (LME); **Model 3**: Cox and LME models adjusted for NT-proBNP and hs-cTnT, and sampling time (LME); **Model 4**: Time-dependent Cox adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.

TABLE S2 **Associations between glomerular and tubular renal markers.**

	Dependent variable* – Tubular markers (%)							
	KIM-1		NAG		NGAL urine		NGAL plasma	
Independent variable*	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**
Tubular markers								
KIM-1			60 (50 to 68)	<0.001	44 (34 to 54)	<0.001	8 (4 to 12)	<0.001
NAG	52 (44 to 60)	<0.001			52 (42 to 62)	<0.001	8 (4 to 12)	<0.001
NGAL urine	32 (24 to 38)	<0.001	42 (36 to 50)	<0.001			6 (2 to 10)	0.004
NGAL plasma	46 (28 to 64)	<0.001	68 (50 to 86)	<0.001	46 (24 to 68)	<0.001		
Glomerular function markers								
Creatinine	-2 (-2 to 19)	0.79	22 (0 to 44)	0.05	16 (-8 to 40)	0.21	52 (42 to 62)	<0.001
Cystatin C	24 (6 to 42)	0.012	24 (6 to 44)	0.01	56 (36 to 78)	<0.001	70 (62 to 78)	<0.001
eGFR	2 (-16 to 20)	0.83	-26 (-44 to -6)	0.01	-30 (-52 to -8)	0.005	-46 (-54 to -16)	<0.001

* Beta coefficients and corresponding 95% confidence interval (CI) were calculated using linear mixed-effects models and are presented as percentage (%) change of dependent variable per doubling of independent variable at the same time point during follow-up.

TABLE S3 Associations between tubular damage markers, urinary NAG and KIM-1, and HF-hospitalizations.

	Urinary NAG		Urinary KIM-1	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Baseline levels*				
Model A	1.07 (1.05–1.10)	<0.001	1.01 (0.99–1.04)	0.16
Model B	1.07 (1.04–1.10)	<0.001	1.01 (0.98–1.03)	0.55
Model C	1.04 (1.01–1.07)	0.020	0.99 (0.95–1.01)	0.26
Temporal evolution†				
Repeatedly measured levels				
Model 1	1.09 (1.02–1.11)	0.006	1.06 (1.02–1.10)	0.006
Model 2	1.08 (1.03–1.12)	0.002	1.04 (1.01–1.08)	0.006
Model 3	1.03 (0.98–1.07)	0.29	1.03 (0.99–1.06)	0.09
Model 4	1.13 (1.09–1.17)	<0.001	1.07 (1.04–1.10)	<0.001
Annual slope				
Model 1	1.48 (1.21–1.99)	<0.001	1.65 (1.35–2.10)	<0.001
Model 2	1.80 (1.33–2.69)	<0.001	1.71 (1.35–2.25)	<0.001
Model 3	0.93 (0.80–1.18)	0.40	1.25 (1.13–1.39)	<0.001
Model 4	1.08 (1.00–1.18)	0.06	1.16 (1.08–1.25)	<0.001
AUCm				
Model 1	1.03 (0.99–1.06)	0.09	1.00 (0.99–1.02)	0.59
Model 2	1.04 (1.01–1.08)	0.020	1.01 (0.99–1.03)	0.31
Model 3	1.00 (0.97–1.03)	0.98	1.00 (0.98–1.02)	0.74

AUCm – area under the curve of marker's trajectory.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase in urinary NAG and KIM-1. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and eGFR; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† HRs and 95% CIs are given per 20% increase in the level, slope, and AUCm of urinary NAG and KIM-1. **Model 1**: Cox model adjusted for marker's baseline levels; **Model 2**: Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, eGFR, and sampling time (LME); **Model 3**: Cox and LME models adjusted for baseline NT-proBNP and hs-cTnT, and sampling time (LME); **Model 4**: Time-dependent Cox adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.

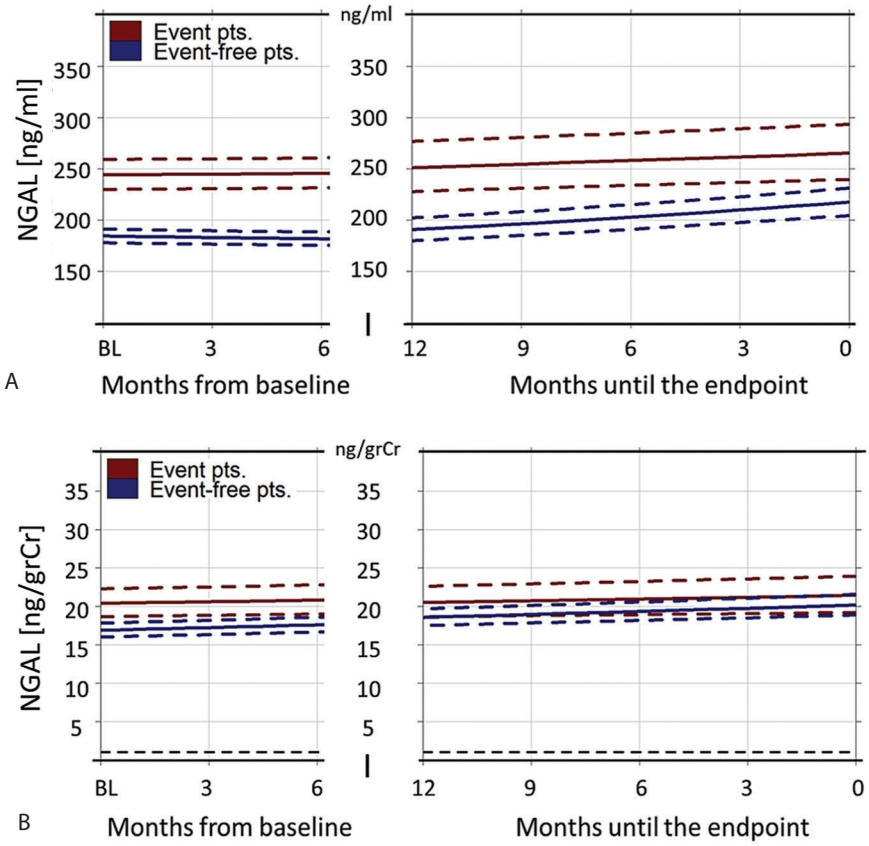


FIGURE S2 Average evolution of tubular markers, urinary and plasma NGAL, during follow-up. Average evolution in patients who reached the study endpoint (solid red line), and in event-free patients (solid blue line). Dashed lines represent the 95% confidence interval. X-axis depicts the time from baseline (left part of the x-axis), and the time remaining to the event (patients who experienced incident events) or last sampling moment (patients who remained event-free) (right part of the x-axis). Biomarker levels are presented on the y-axis. Dashed black lines represent the biomarkers' reference values (<1 µg/gCr). **A.** plasma NGAL(ng/ml); **B.** urinary NGAL(µg/gCr). BL, baseline; pts., patients.

TABLE S4 Associations between tubular markers, urinary and plasma NGAL, and the composite endpoint.

	urinary NGAL		plasma NGAL	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Baseline levels*				
Model A	1.01 (1.00–1.02)	0.08	1.08 (1.04–1.11)	<0.001
Model B	1.01 (0.99–1.03)	0.21	1.06 (1.02–1.10)	0.004
Model C	0.99 (0.97–1.01)	0.95	1.01 (0.97–1.04)	0.74
Temporal evolution†				
Repeatedly measured levels				
Model 1	0.94 (0.65–1.35)	0.78	1.01 (0.94–1.09)	0.75
Model 2	X		x	
Model 3	x		x	
Annual slope				
Model 1	x		x	
Model 2	x		x	
Model 3	x		x	
Model 4	x		x	
AUCm				
Model 1	x		x	
Model 2	x		x	
Model 3	x		x	

AUCm – area under the curve of marker's trajectory.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase of urinary and plasma NGAL. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and eGFR; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† HRs and 95% CIs are given per 20% increase of the level, slope, and AUCm of urinary and plasma NGAL. **Model 1**: Cox model adjusted for marker's baseline levels, LME model adjusted for sampling time; **Model 2**: Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, eGFR, and sampling time (LME); **Model 3**: Cox and LME models adjusted for baseline NT-proBNP and hs-cTnT, and sampling time (LME). **Model 4**: Time-dependent Cox adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.
x The models were not performed because repeatedly measured level was not significant.

TABLE S5 Associations between tubular markers, urinary and plasma NGAL, and HF-hospitalizations.

	urinary NGAL		plasma NGAL	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**
Baseline levels*				
Model A	1.02 (1.00–1.03)	0.06	1.09 (1.05–1.13)	<0.001
Model B	1.01 (0.99–1.03)	0.26	1.06 (1.02–1.10)	0.007
Model C	1.00 (0.99–1.02)	0.90	1.01 (0.97–1.06)	0.49
Temporal evolution†				
Repeatedly measured levels				
Model 1	0.99 (0.95–1.03)	0.68	0.99 (0.91–1.09)	0.84
Model 2	x		x	
Model 3	x		x	
Model 4	x		x	
Annual slope				
Model 1	x		x	
Model 2	x		x	
Model 3	x		x	
Model 4	x		x	
AUCm				
Model 1	x		x	
Model 2	x		x	
Model 3	X		x	

AUCm – area under the curve of marker's trajectory.
* Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 20% increase of urinary and plasma NGAL. **Model A**: unadjusted; **Model B**: adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and eGFR; **Model C**: adjusted for baseline NT-proBNP and hs-cTnT.
† HRs and 95% CIs are given per 20% increase of the level, slope, and AUCm of urinary and plasma NGAL. **Model 1**: Cox model adjusted for marker's baseline levels, LME model adjusted for sampling time; **Model 2**: Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, eGFR, and sampling time (LME); **Model 3**: Cox and LME models adjusted for baseline NT-proBNP and hs-cTnT, and sampling time (LME). **Model 4**: Time-dependent Cox adjusted for total daily equivalent doses of carvedilol, enalapril, furosemide, and spironolactone during follow-up.
x The models were not performed because repeatedly measured level was not significant.

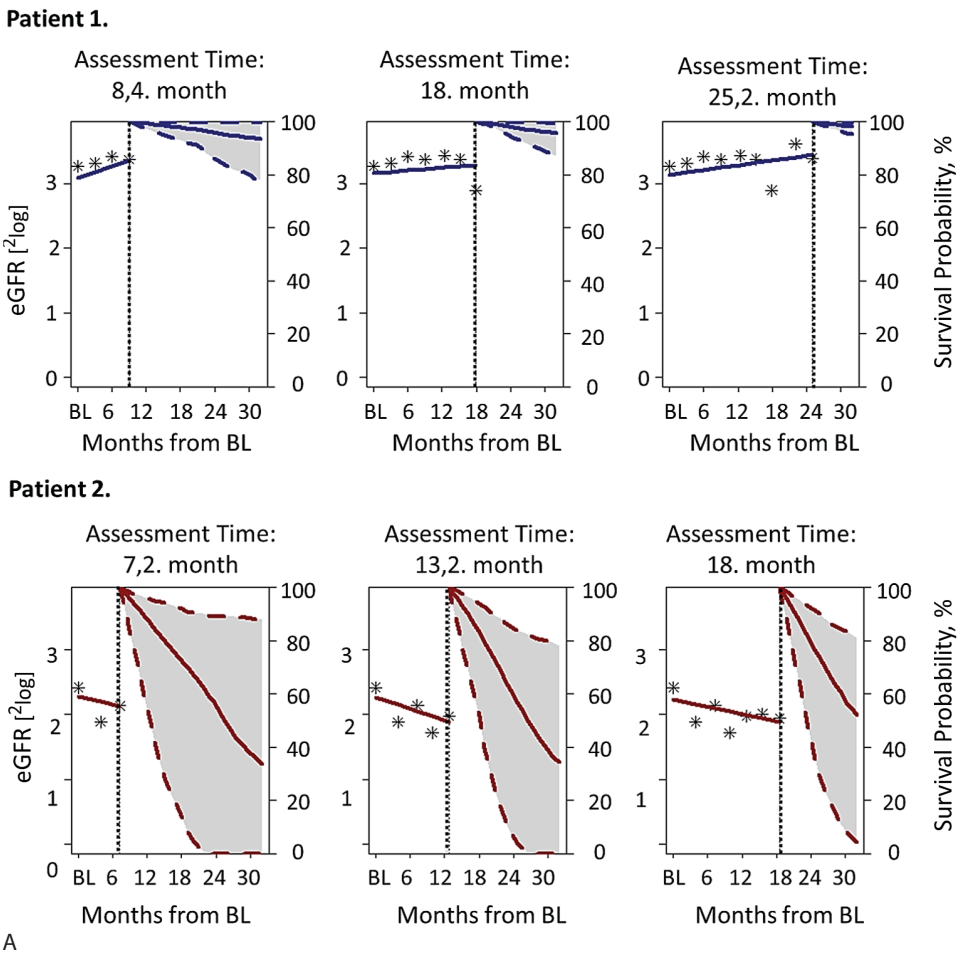
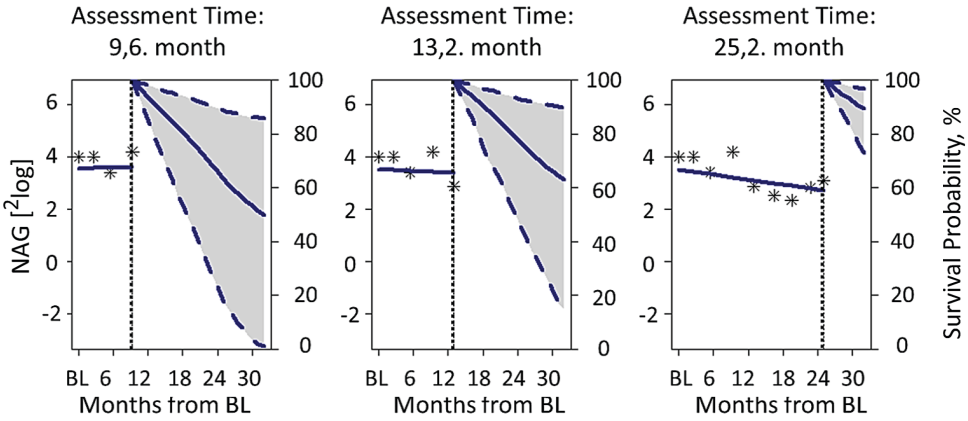
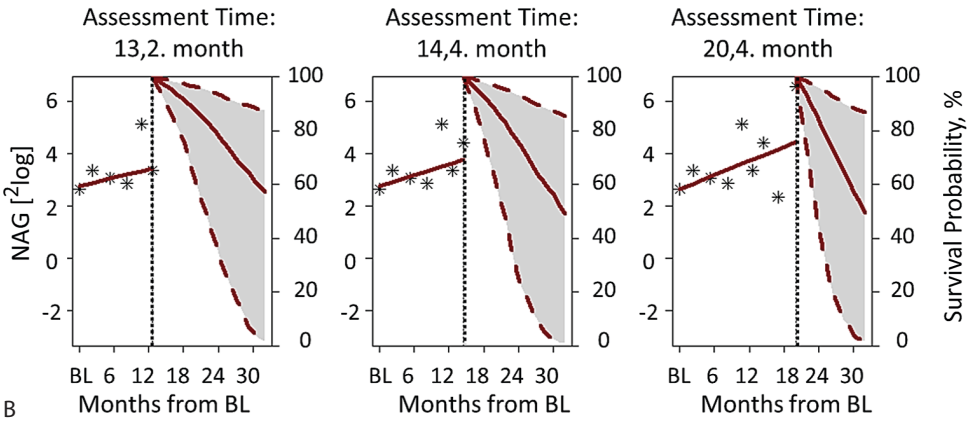


FIGURE S3 Clinical scenarios where a patient's risk is dynamically profiled using patient-specific trajectories. The solid red lines depict patients who experienced the study endpoint, and the solid blue lines depict patients who did not. X-axis depicts follow-up time in months starting from baseline (BL). Biomarker levels (on ²log scale) are displayed on the primary (left) Y-axis and survival probability (%) on the secondary (right) Y-axis. *Patient-specific marker's trajectory* with scatter points is displayed left of the vertical dotted black line. To the right of this line, the corresponding *conditional survival probability curve* is displayed with 95% confidence intervals (grey area). To show how this conditional survival probability curve is dynamically updated every time an extra measurement is recorded, we have provided three time-points at which the risk was assessed. For each of the four patients, we considered: (1) information on their measurements up to these three time-points and (2) the fact that they had survived up to each of the time-points. This information was then jointly modeled to provide the conditional survival probability curve for the remaining time period until the study ended (i.e., the patients suffered the event or were censored).
*Conditional – given that the patient survived up to the time interval during which measurements were collected.

Patient 3.



Patient 4.



B

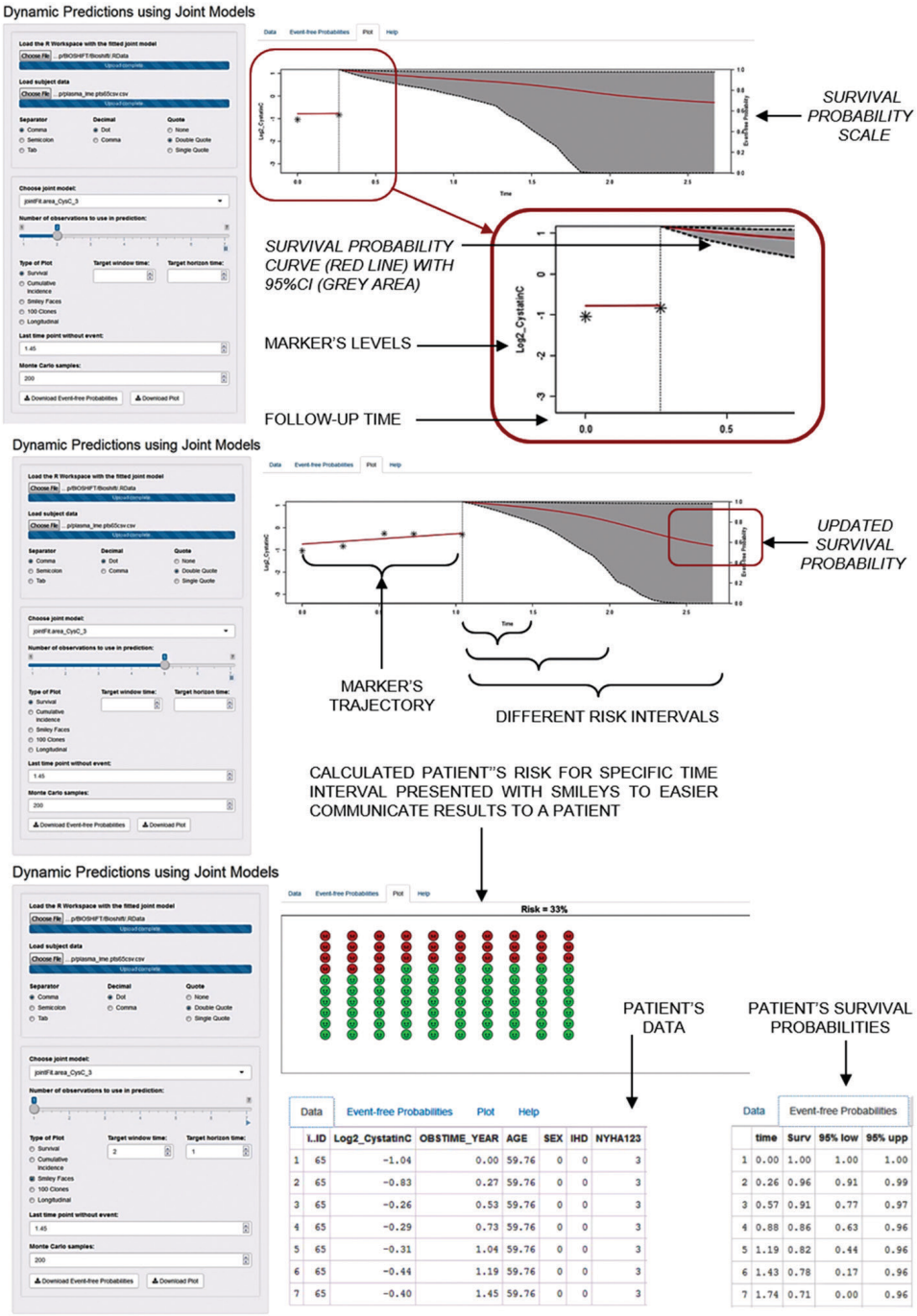
Scenario A. For the first patient (who did not experience the endpoint), we notice high baseline eGFR levels and high conditional survival probability. Conversely, the second patient (who ultimately experienced the endpoint) exhibits lower baseline eGFR levels, that continue to decline during follow-up. This eGFR decline corresponds to decline in the patient's conditional survival probability.

Scenario B. For the third patient (who did not experience the endpoint), we notice slightly higher NAG levels than for the fourth patient (who ultimately experienced the endpoint) at the moment of the first assessment. Logically, the conditional survival probability for the third patient is slightly lower than for the fourth patient. Yet the third patient exhibits a decline in NAG levels during follow-up, and the patient's conditional survival probability profile improves. Conversely, in the fourth patient NAG levels increase over time preceding the endpoint, which reduces the patient's survival probability.

TABLE S6 The longitudinal marker's accuracy.

Renal Markers	Risk Time Window	AUC (t)	
		Clinical model	Biomarkers model
Repeatedly measured levels			
Creatinine			
	6 months	0.77	0.75
	12 months	0.72	0.76
eGFR			
	6 months	0.77	0.70
	12 months	0.73	0.72
Cystatin C			
	6 months	0.80	0.77
	12 months	0.74	0.72
NAG			
	6 months	0.81	0.77
	12 months	0.76	0.79
KIM-1			
	6 months	0.80	0.75
	12 months	0.72	0.76
Annual slope			
Creatinine			
	6 months	0.64	0.62
	12 months	0.67	0.69
eGFR			
	6 months	0.64	0.62
	12 months	0.68	0.69
Cystatin C			
	6 months	0.78	0.77
	12 months	0.71	0.72
NAG			
	6 months	0.76	0.73
	12 months	0.73	0.71
KIM-1			
	6 months	0.61	0.66
	12 months	0.65	0.72

We determined the longitudinal marker's predictive accuracy (i.e., an ability of a marker to discriminate between a patient who experiences the endpoint within a given risk time window after the last measurement, and the patient who does not experience the event within the same risk time window) using the time-dependent AUC. For this purpose, we chose the first year as the collection time period, and we assessed two risk time windows: 6 and 12 months after collection time. We determined the predictive accuracy of the marker's levels and slopes in two multivariable adjusted models: a) clinical model: Cox and LME models adjusted for age, sex, diabetes, atrial fibrillation, NYHA class, diuretics, systolic blood pressure, eGFR (for NAG and KIM-1), and sampling time (LME); b) biomarker model: Cox and LME models adjusted for NT-proBNP and hs-cTnT, and sampling time (LME).



	I_ID	Log2_CystatinC	OBSTIME_YEAR	AGE	SEX	IHD	NYHA123
1	65	-1.04	0.00	59.76	0	0	3
2	65	-0.83	0.27	59.76	0	0	3
3	65	-0.26	0.53	59.76	0	0	3
4	65	-0.29	0.73	59.76	0	0	3
5	65	-0.31	1.04	59.76	0	0	3
6	65	-0.44	1.19	59.76	0	0	3
7	65	-0.40	1.45	59.76	0	0	3

	time	Surv	95% low	95% upp
1	0.00	1.00	1.00	1.00
2	0.26	0.96	0.91	0.99
3	0.57	0.91	0.77	0.97
4	0.88	0.86	0.63	0.96
5	1.19	0.82	0.44	0.96
6	1.43	0.78	0.17	0.96
7	1.74	0.71	0.00	0.96

FIGURE S4 An app interface using joint modeling approach to calculate and communicate the risk in an individual patient.