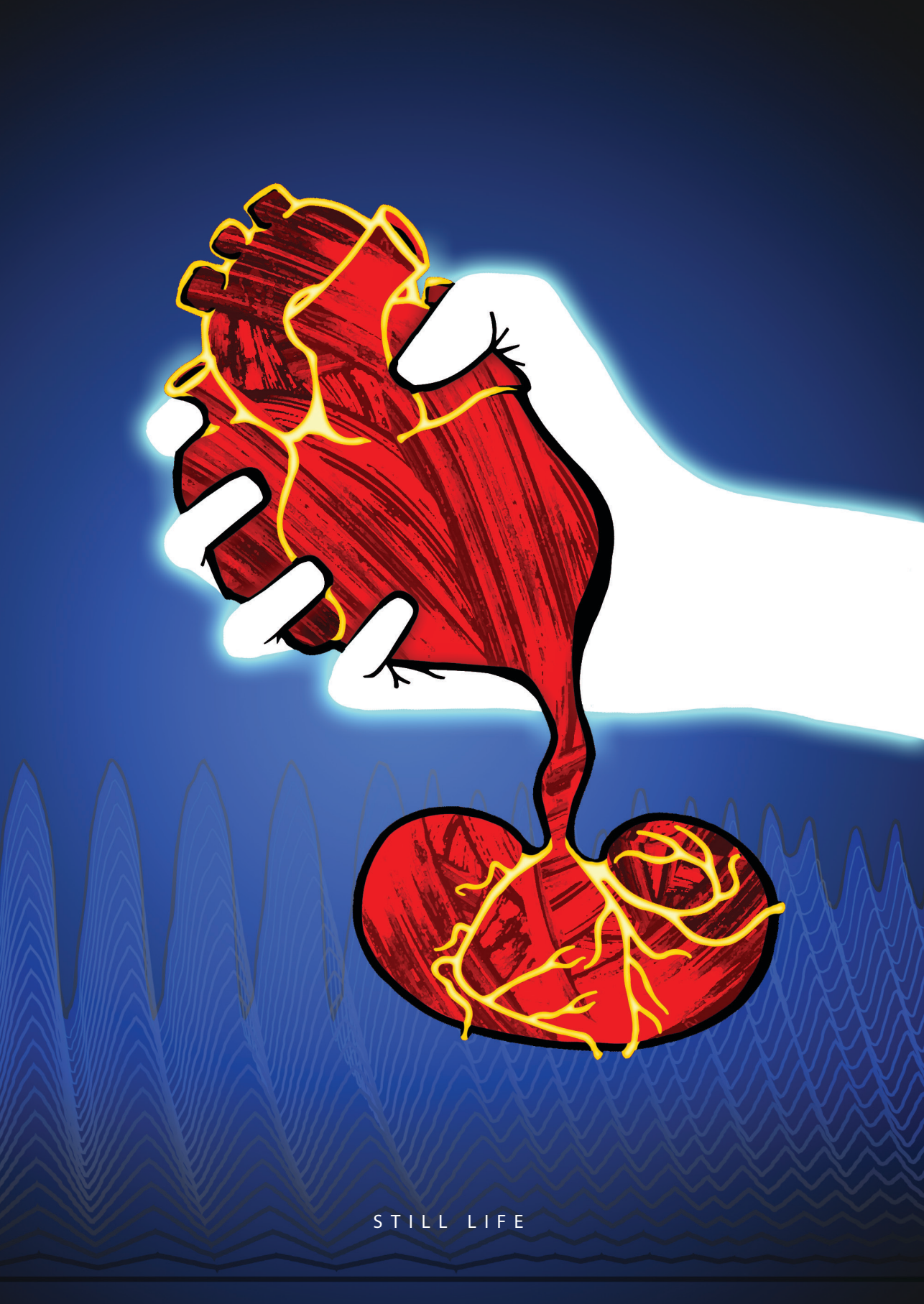
STILL LIFE

Part IV

LESSONS LEARNED FROM CLINICAL PRACTICE

CHAPTER 11

Real-life use of Neurohormonal Antagonists and Loop Diuretics in Chronic Heart Failure Analysis of Serial Biomarker Measurements and Clinical Outcome

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ABSTRACT

Background

We determined the temporal effects of neurohormonal antagonists and loop diuretics on serially assessed cardio-renal biomarkers, functional status, and clinical outcomes in patients with chronic heart failure (CHF) with reduced ejection fraction.

Methods

In 250 CHF patients, we measured 3-monthly in blood: NT-proBNP, troponin T, C-reactive protein, creatinine, cystatin C; and in urine: N-acetyl-beta-D-glucosaminidase and kidney-injury-molecule-1.

Results

ACE-inhibitors/ARB were inversely associated with cardiac impairment, inflammation and renal tubular damage, but not with glomerular dysfunction. Diuretics were associated with worse biomarker profiles and with a hazard ratio for adverse clinical outcome of 1.12 (95%CI:1.03–1.22) per 40 mg higher doses. ACE-inhibitors/ARBs were more frequently down-titrated and diuretics more frequently up-titrated in patients who experienced endpoints than in those who did not.

Conclusions

In conclusion, decrease or withholding of ACE-inhibitors/ARBs solely based on glomerular function is not justified because of the beneficial effects on the heart, inflammation, and renal tubules. Higher and increase in diuretic doses mark progression towards end-stage CHF.

INTRODUCTION

In randomized clinical trials (RCTs), neurohormonal antagonists significantly reduce mortality in chronic heart failure (CHF) with reduced ejection fraction.[1-4] In clinical practice, however, optimization of neurohormonal antagonist doses to guideline recommendations is often not reached.[5] Moreover, the temporal effects of dose adjustments of these agents during clinical follow-up of "real-life" patients with CHF are uncertain.

Although guidelines also recommend the use of loop diuretics due to their beneficial effect on symptoms and signs of congestion, no large RCTs have been conducted to prove their efficacy on survival.[6] While longitudinal data on the temporal effects of loop diuretics are absent, studies using cross-sectional data have suggested that the loop diuretics are associated with reduced survival.[7-9] Yet, it is unclear whether this association between poor survival and non-randomized use of diuretics is causal or a reflection of the progressive underlying disease with progressive congestion.[7] Hence, higher doses of loop diuretics will be given to the patients with more severe CHF. However, excessive diuresis may also lead to excessive neurohormonal activation and renal dysfunction, thereby potentially increase mortality.[10,11]

For these reasons identifying the temporal effects of neurohormonal antagonists and loop diuretics on serially assessed patients' functional status and multiple cardio-renal biomarkers, could help to better use of these agents and potentially improve outcomes. The multiple-biomarker strategy enables us to investigate simultaneously the effects of HF medication doses on the evolution of different pathophysiological processes (myocardial stretching and damage, inflammation, renal injury and dysfunction) that occur in CHF regardless of its underlying cause. Similarly, serial measures enable us to control for time-varying health status of patients, thereby providing less biased risk estimates.

In this prospective longitudinal study, our aim was (1) to determine the temporal effects of neurohormonal antagonists and loop diuretics on serially assessed New York Heart Association (NYHA) functional classification, natriuretic peptide NT-proBNP, cardiac troponin T (hs-cTnT), C-reactive protein (CRP), creatinine and cystatin C, and urinary N-acetyl-ß-D glucosaminidase (NAG) and kidney-injury-molecule (KIM)-1, at predefined 3-month intervals during ≥ 2-year outpatient follow-up; (2) to investigate the temporal associations between dose adjustments of these HF medications and clinical outcomes.

MATERIALS AND METHODS

The Serial Biomarker Measurements and New Echocardiographic Techniques in Chronic Heart Failure Patients Result in Tailored Prediction of Prognosis (BioSHiFT) is a prospective observational cohort of patients with CHF, conducted in Erasmus MC, Rotterdam, and Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands. Patients were included if aged ≥18 years, capable of understanding and signing informed consent, and if CHF had been diagnosed ≥3 months ago according to European Society of Cardiology guidelines (Figure S1).[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. This investigation comprised 263 stable CHF patients enrolled during the first inclusion period (October 2011 until June 2013). Since the effect of certain HF medications, such as RAAS inhibitors, is less firmly established in HFpEF patients than in HFrEF patients, and since 95% of the study population had HFrEF, in this paper we focused on the HFrEF patients (n=250). However, all analyses were also repeated in the full cohort (n=263).

Baseline assessment

All patients were evaluated by research physicians, who collected information on HF-related symptoms, NYHA classification, 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 in case of ambiguities. History of myocardial infarction (MI), percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), valvular heart disease, atrial fibrillation or other arrhythmias, cerebrovascular accident (CVA), diabetes mellitus, hypercholesterolemia, hypertension, and COPD were defined as a clinical diagnosis of these conditions, as reported by the treating physician in the medical chart.

Study follow-up and endpoints

Study follow-up visits were predefined and scheduled every 3 months (±1 month was allowed), with a maximum of 10 study follow-up visits (for details see Figure 1 and Table S2). At each study follow-up visit, a research physician performed a

short medical evaluation and collected samples. In parallel, all patients completed their standard outpatient clinic visits at their treating physicians' offices. Treating physicians were unaware of the biomarker results. All medication changes and occurrence of adverse cardiovascular events since the previous visit were recorded in electronic case report forms.

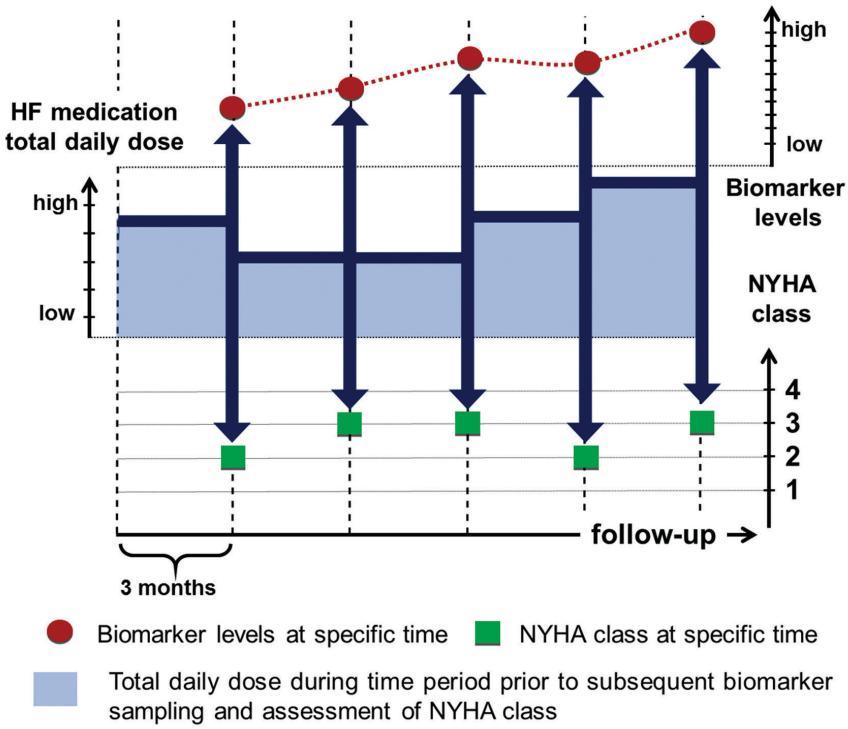


FIGURE 1 Schematic depiction of the analysis of the temporal lagged effects of HF medication doses on NYHA functional classification and biomarker profiles during follow-up. Study follow-up visits were predefined and scheduled every 3 months (X-axis). At these visits a research physician performed a medical evaluation, assessing NYHA functional class (green rectangle), and collecting blood and urine samples for biomarker measurement (red dots). All HF medication changes that had occurred after the previous visit were recorded and calculated as total daily equivalent doses (light blue area); subsequently these doses were related to NYHA class and biomarker profiles at the next outpatient visit (dark blue arrows; temporal lagged effect). All patients were followed until they reached the composite endpoint or until they were censored. To account for differences in the moments in time at which sampling was performed in individual patients and the fact that some patients reached the event and some did not, analyses were adjusted for sampling time and whether or not the patient had an event (for details see statistical analyses).

During follow-up, hospitalizations for HF, MI, PCI, CABG, arrhythmias, CVA, cardiac transplantation, left ventricular assist device (LVAD) implantation and mortality were recorded and associated hospital records and discharge letters were collected. Subsequently, a clinical event committee, blinded to the biomarker 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 and stored at -80°C. Biomarkers were measured batchwise after follow-up was completed. Laboratory personnel was blinded for clinical data and patients outcomes. Serum NT–proBNP and cardiac troponin T were analyzed by electrochemiluminesence immunoassays (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA) (LLD: 0.6 pmol/L and 3 ng/L respectively). Serum CRP was measured by immunoturbidimetric assay (Roche Hitachi 912 chemistry analyser, Basel, Switzerland) (LLD: 0.3 mg/L). Creatinine was determined by a colorometric test by the Jaffe reaction (LLD: plasma 0,14 mg/dl, urine: 1.56 mg/ml). Plasma CysC was determined by ELISA (R&D systems, Minneapolis, MN) (LLD: 0.1066 μg/mL). Urinary KIM-1 was determined by ELISA (R&D systems, Minneapolis, MN, USA) (LLD: 0.146 ng/mL), and NAG was determined using a substrate p-nitrophenyl N-acetyl-β-D-glucosaminidase at pH 4.5 (Sigma, St Louis, MO, USA) (LLD: 0.485 U/L). All urinary biomarker 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]

Statistical analyses

Categorical data are summarized by numbers and percentages; continuous data when normally distributed by mean ± standard deviation (SD) and when skewed by median and interquartile range (IQR). Differences between patients with the event and event-free patients were evaluated by the Mann-Whitney U test or Student T test.

The total daily doses (TDD) were converted to equivalents according to ESC guidelines[6] (Table S1). Furosemide equivalent dose above 500 mg (n=7) were excluded from the analysis. To calculate per patient the relative number of up-titrations and down-titrations, the number of times the dose was changed (compared with the previous visit) of a particular patient was divided by the total number of this patient's outpatient visits.

Linear mixed-effects (LME) models were applied to estimate the evolution of HF medication doses over time. Intercept and slope were included in the random-effects design matrix. To achieve normal distributions, biomarkers were ^{2}log-transformed and TDD were $\sqrt{}$-transformed for the analyses.

LME models were also applied to assess the temporal effects of HF medication doses at the current visit on NYHA class and biomarkers at the subsequent outpatient visit (i.e., temporal lagged effect) during follow-up (Figure 1). For this analysis, we used only complete data on all variables (medication, NYHA class, and biomarkers) at corresponding time points during follow-up (per patient: a median of 8 time points). The models were adjusted for sampling time (in the fixed- and random-effects part), and whether or not the patient had an event (in the fixed-effect part). To allow direct comparison of the effects of HF medication on different biomarkers, we used Z-scores (i.e., standard deviation differences from their means). Thus, the effects are depicted as per 1SD increase of HF medication.

Time-dependent Cox survival analysis was applied to investigate the associations between HF medication doses and the study endpoints. Analyses were performed univariably, and then adjusted for potential confounders: age, gender, diabetes, and repeatedly assessed NYHA class, NT-proBNP and eGFR during follow-up. Covariates were chosen based on pathophysiological considerations and were limited in number because we took into account the number of events that occurred during follow-up (and required minimum of 10 outcome events per covariate).

All analyses were performed with R Statistical Software Version 3.[15] All tests were two-tailed and p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics, Follow-up, and Clinical Outcomes

Table 1 shows baseline clinical and biomarker characteristics of the 250 HFrEF patients. Patients who later experienced the endpoint, at baseline were older, more frequently had diabetes, atrial fibrillation, and history of myocardial infraction, and had lower systolic blood pressure, higher NYHA class, and higher levels of NT-proBNP, cardiac troponin T, CRP, cystatin C, and urinary NAG than patients who remained endpoint-free.

During a median (IQR) follow-up of 2.2 (1.4–2.5) years, we drew a median of 9 blood (IQR: 5–10) and 8 urine (IQR: 5–10) samples per patient, and assessed NYHA functional classification and HF medication 9 (IQR: 5–11) times. Of the HFrEF patients, a total of 66 (26%) patients reached the composite endpoint: 53 patients were re-hospitalized for acute or worsened HF, 8 patients died of cardiovascular causes, 3 underwent heart transplantation, and 2 underwent LVAD placement.

TABLE 1 **Baseline characteristics.**

	Total	Composite endpoint reached		p-value
		Yes	No	
	n = 250	66	184	
Demographics				
Age, years*	66 ± 13	69 ± 13	65 ± 12	0.042
Men, n (%)	184 (74)	52 (79)	132 (72)	0.27
Clinical characteristics				
BMI, kg/m^2 *	27.4 ± 4.7	27.3 ± 4.7	27.5 ± 4.7	0.78
Heart rate, b.p.m.*	67 ± 11	68 ± 13	66 ± 11	0.26
SBP, mmHg*	122 ± 21	116 ± 18	123 ± 21	0.021
DBP, mmHg*	72 ± 11	70 ± 10	73 ± 11	0.052
Features of heart failure				
NYHA class III/IV, n (%)	62 (25)	29 (44)	33 (18)	<0.001
LVEF, % *	30 ± 10	29 ± 9	31 ± 10	0.52
Etiology of heart failure, n (%)				
Ischemic	116 (46)	36 (54)	80 (43)	0.12
Hypertension	31 (13)	8 (12)	23 (12)	0.94
Valvular disease	10 (4)	5 (8)	5 (3)	0.08
Cardiomyopathy	63 (25)	13 (20)	50 (27)	0.23

continued

	Total	Composite endpoint reached		p-value
		Yes	No	
n =	250	66	184	
Unknown or Others	30 (12)	4 (6)	26 (14)	
Medical history, n (%)				
Prior MI	95 (38)	32 (48)	63 (34)	0.041
Prior PCI	81 (32)	26 (39)	55 (30)	0.16
Prior CABG	42 (17)	12 (18)	30 (16)	0.73
Atrial fibrillation	97 (39)	33 (50)	64 (35)	0.030
Diabetes	77 (31)	29 (44)	48 (26)	0.007
Hypercholesterolemia	94 (38)	29 (44)	65 (35)	0.22
Hypertension	113 (45)	34 (51)	79 (43)	0.23
COPD	31 (12)	12 (18)	19 (10)	0.10
NT-proBNP (pmol/L) †	133.1(44.9–274.4)	297.4 (176.4–524.6)	93.9 (29.1–205.0)	<0.001
Hs-TnT (ng/L) †	17.7 (9.3–32.8)	30.1 (19.7–48.6)	13.8 (8.2–27)	<0.001
C-reactive protein mg/L †	2.2 (0.9–4.9)	2.9 (1.4–5.4)	1.8 (0.7–4.3)	0.016
Glomerular function markers †				
Creatinine, mg/dl	1.18 (0.99–1.49)	1.32 (1.02–1.51)	1.17 (0.97–1.48)	0.14
eGFR, mL/min/1.73m^2	58 (42–77)	53 (39–73)	60 (44–78)	0.24
Cystatin C, mg/L	0.73 (0.57–0.97)	0.86 (0.70–1.02)	0.70 (0.52–1.18)	<0.001
KDOQI classification, n (%)				
eGFR ≥90	28 (11)	7 (11)	21 (11)	0.59
eGFR 60-89	92 (37)	20 (30)	72 (39)	
eGFR 30-59	110 (44)	33 (50)	77 (42)	
eGFR <30	20 (8)	6 (9)	14 (8)	
Tubular markers †				
NAG, U/gCr [urine]	5.8 (3.7–9.1)	7.9 (5.9–10.8)	5.1 (3.2–8.0)	<0.001
KIM-1, ng/gCr [urine]	488.6 (246.6–935.2)	589.0 (259.6–1802.7)	462.8 (236.2–900.6)	0.14

BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; NYHA class, New York Heart Association class; 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 and interquartile range (IQR).

Associations of temporal changes in repeatedly assessed HF medication doses with temporal changes in NYHA classification and biomarker profiles during follow-up

Figure 2 shows average temporal lagged effects of repeatedly assessed HF medication doses on subsequent NYHA classification and biomarkers profiles during follow-up.

NYHA functional classification

Higher repeatedly assessed furosemide equivalent doses were associated with higher (i.e., worse) NYHA class values during follow-up. At any time-point during follow-up, one SD increase in equivalent dose of furosemide was related to a 0.10 (95% CI: 0.04–0.15) points higher NYHA class (p<0.001) at the next follow-up visit. Conversely, higher doses of carvedilol and enalapril equivalents were associated with lower (i.e., better) NYHA class values during follow-up: one SD increase in equivalent dose of carvedilol with a 0.06 (0.01–0.12) points lower value (p=0.036), and one SD increase in equivalent dose of enalapril with a 0.07 (0.02–0.13) points lower value (p=0.011).

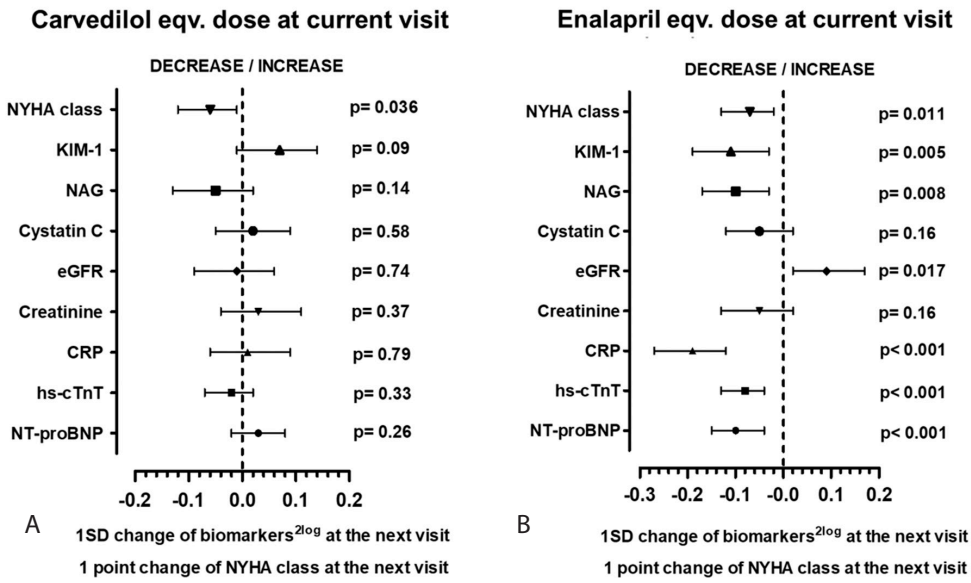
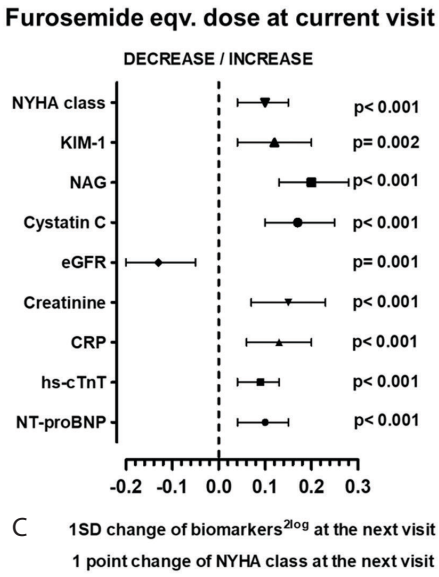
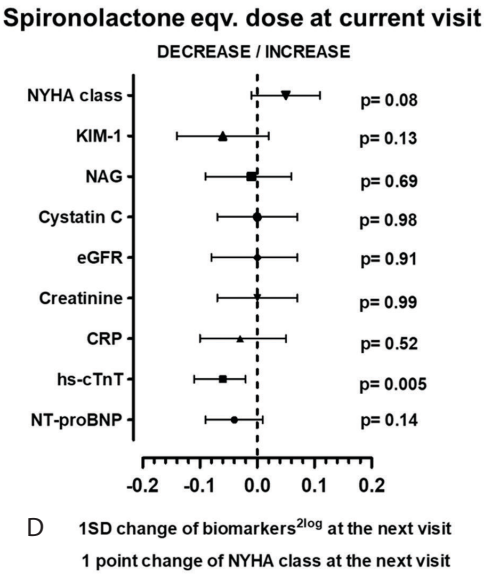


FIGURE 2 Associations of temporal changes in repeatedly assessed HF medication doses with temporal changes in NYHA classification and biomarker profiles in HFrEF patients. The HF medication effects are given as β (95% confidence interval) SD change in ^{2}log-biomarkers levels per 1SD increase in HF medication √-dose. This method of standardization (i.e., per SD) allows





Scale: HFrEF patients	Mean-1SD	Mean	Mean+1SD
HF medication - independent variable			
Carvedilol eqv., mg.	8	32	71
Enalapril eqv., mg.	4	17	39
Furosemide eqv., mg.	9	52	131
Spironolactone eqv., mg.	1	11	30
Biomarkers - dependent variable			
NT-proBNP, pmol/L	24.2	92.4	353.8
hs-cTnT, ng/L	7.4	17.0	39.3
CRP, mg/L	0.7	2.4	7.6
Creatinine, mg/L	0.87	1.21	1.69
eGFR, mL/min/1.73m^2	37	56	84
Cystatin C, µg/mL	0.50	0.74	1.10
NAG, U/gCr [urine]	2.2	4.9	11.1
KIM-1, ng/gCr [urine]	197.2	457.8	1062.7

E

a direct comparison of the effects of HF medication doses on different biomarkers. **A.** Carvedilol equivalent doses, **B.** Enalapril equivalent doses, **C.** Furosemide equivalent doses, **D.** Spironolactone equivalent doses, **E.** Table shows conversion factors for HF medication doses and biomarker levels from logarithmic to linear scale

Myocardial stretching and damage

At any time-point during follow-up, one SD increase in equivalent dose of furosemide was related to a 0.10 SD (0.04–0.15) higher NT-proBNP value ($p<0.001$) at the next follow-up visit, as measured on the ^{2}log scale. As an example on the linear scale, these findings read as follows: in HFrEF patients an increase in furosemide dose from 52 (mean value) to 131 mg (mean+1SD) at the current visit corresponds to an increase in NT-proBNP from 92.4 (mean value) to 118.5 pmol/L (mean value + 0.10 SD) at the next visit. Similarly, one SD increase in equivalent dose of furosemide was also related to a 0.09 SD (0.04–0.13) higher hs-cTnT value ($p<0.001$), as measured on the ^{2}log scale (Figure 2).

At any time-point during follow-up, an increase in equivalent dose of enalapril was associated with lower NT-proBNP and hs-cTnT at the next follow-up visit (one SD higher dose: 0.10 SD [0.04–0.15] lower NT-proBNP values, $p<0.001$; and 0.08 SD [0.04–0.13] lower hs-cTnT values, $p<0.001$) (for details on linear scale see Figure 2).

Inflammation

At any time-point during follow-up, one SD increase in equivalent dose of furosemide dose was related to a 0.13 SD (0.06–0.20) higher CRP value ($p<0.001$) at the next follow-up visit, as measured on the ^{2}log scale. Conversely, higher enalapril equivalent doses were associated with lower CRP levels (one SD higher dose: 0.19 SD [0.12–0.27] lower CRP values, $p<0.001$) (for details on the linear scale see Figure 2).

Renal function and injury

At any time-point during follow-up, one SD increase in equivalent dose of furosemide was related to a 0.13 SD (0.05–0.20) lower eGFR ($p=0.001$), and to 0.17 SD (0.10–0.25) higher cystatin C ($p<0.001$) at the next follow-up visit, as measured on the ^{2}log scale. Associations were also present with greater tubular damage (one SD higher dose: 0.20 SD [0.13–0.28] higher NAG values, $p<0.001$; and 0.12 SD [0.04–0.20] higher KIM-1 values, $p<0.001$) at the next follow-up visit.

At any time-point during follow-up, increase in equivalent dose of enalapril was associated with less tubular damage at the next follow-up visit (one SD higher dose: 0.10 SD [0.03–0.17] lower NAG values, $p=0.008$; and 0.11 SD [0.03–0.19] lower KIM-1 values, $p=0.005$) (for details on the linear scale see Figure 2). Of note, glomerular function improved numerically with higher doses of enalapril equivalents, but this was not statistically significant (Figure 2).

HF medication and clinical outcomes: prevalence of use and frequency of change

At baseline, loop diuretics were given more frequently to patients who experienced adverse events than to event-free patients (97 vs. 89%, p=0.021) (Figure 3). During follow-up, patients who experienced the event had more than twice as many up-titrations of diuretics than event-free patients (8 vs. 3%, p=0.038) (Figure 4). The frequency of unchanged dose during follow-up was numerically, but not statistically, higher in event patients (11 vs. 5%, p=0.10). Importantly, such patients also had more than twice as many down-titrations of ACE-inhibitors/ARBs (5 vs. 2%, p=0.018). In contrast, event-free patients had more up-titrations of ACE-inhibitors/ARBs (0.2 vs. 1.5%, p=0.047).

HF medication and clinical outcomes: average evolutions of total daily doses

At baseline, patients who later experienced adverse events were given significantly higher doses of loop diuretics than patients who remained event-free (94 vs. 43 mg, p<0.001). This difference in average dose remained significant during follow-up (p<0.001), and further increased in the time-period prior to event (Figure 5).

At baseline, the average dose of ACE-inhibitors/ARBs was numerically, but not statistically, lower in patients who experienced the event than in event-free patients (15 vs. 19 mg, p=0.12). However, the average dose significantly decreased in the time-period prior to the event (p=0.015 for the difference during follow-up between patients with events and without events). We also found a tendency towards a simultaneous decrease in ACE-inhibitor/ARB doses and increase in loop diuretic doses in the same patient over time preceding the event. However, this was not the case in event-free patients (Figure S2).

At baseline, patients who experienced adverse events were given, on average, numerically higher doses of mineralocorticoid receptor antagonists (MRAs) than event-free patients (13 vs. 11 mg, p=0.11). However, a decrease in average MRAs dose was observed during follow-up in event patients (Figure 5).

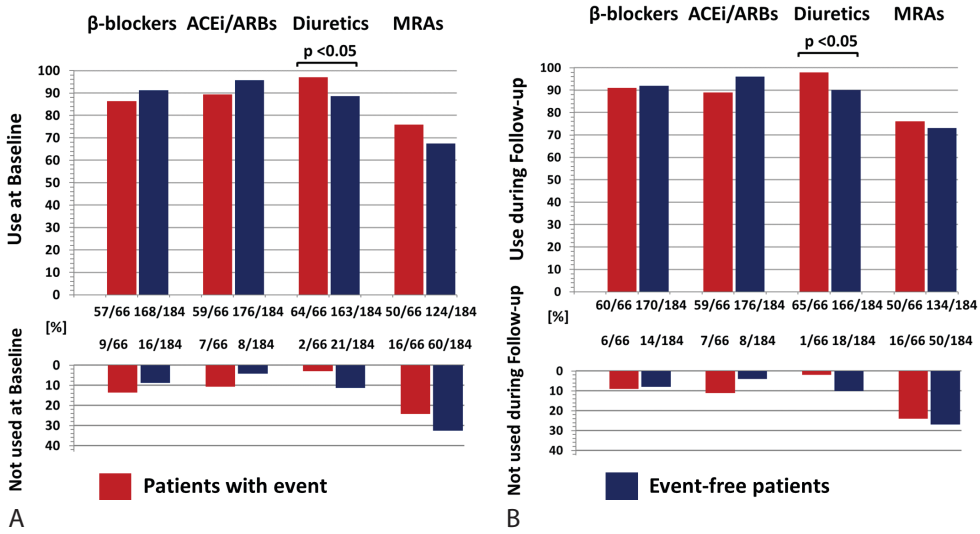


FIGURE 3 Prevalence of HF medication use. Mann-Whitney U test was applied to test the difference between patients who experienced the event and event-free patients. **A.** HF medication use at baseline **B.** HF medication use during follow-up.

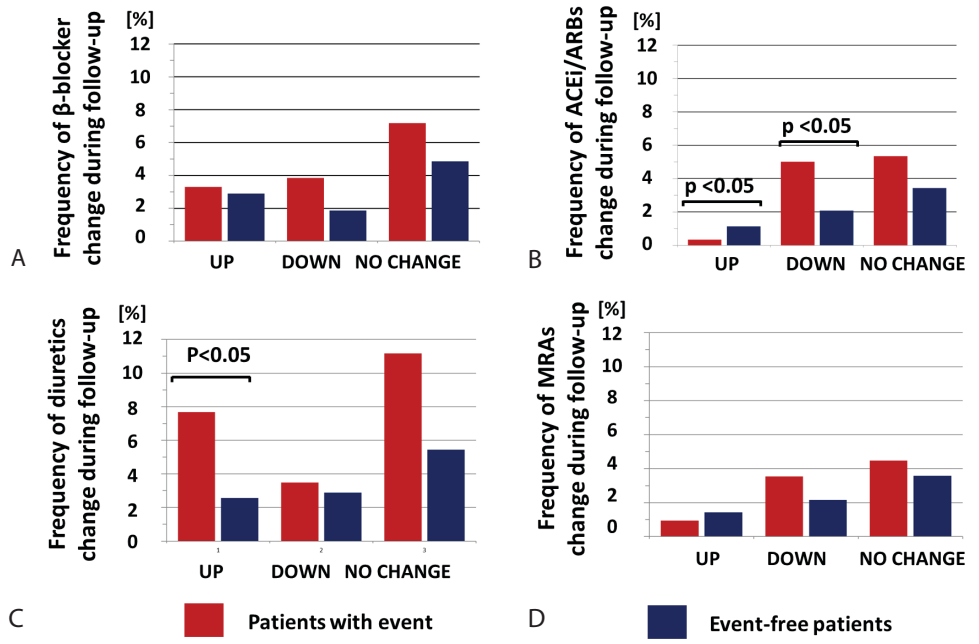


FIGURE 4 Frequency of HF medication change (up-titration/down-titration/no change) during follow-up. Mann-Whitney U test was applied to test the difference between patients who experienced the event and event-free patients. **A.** β-blockers, **B.** ACE-inhibitors/ARBs, **C.** Diuretics, **D.** Mineralocorticoid receptor antagonist (MRAs).

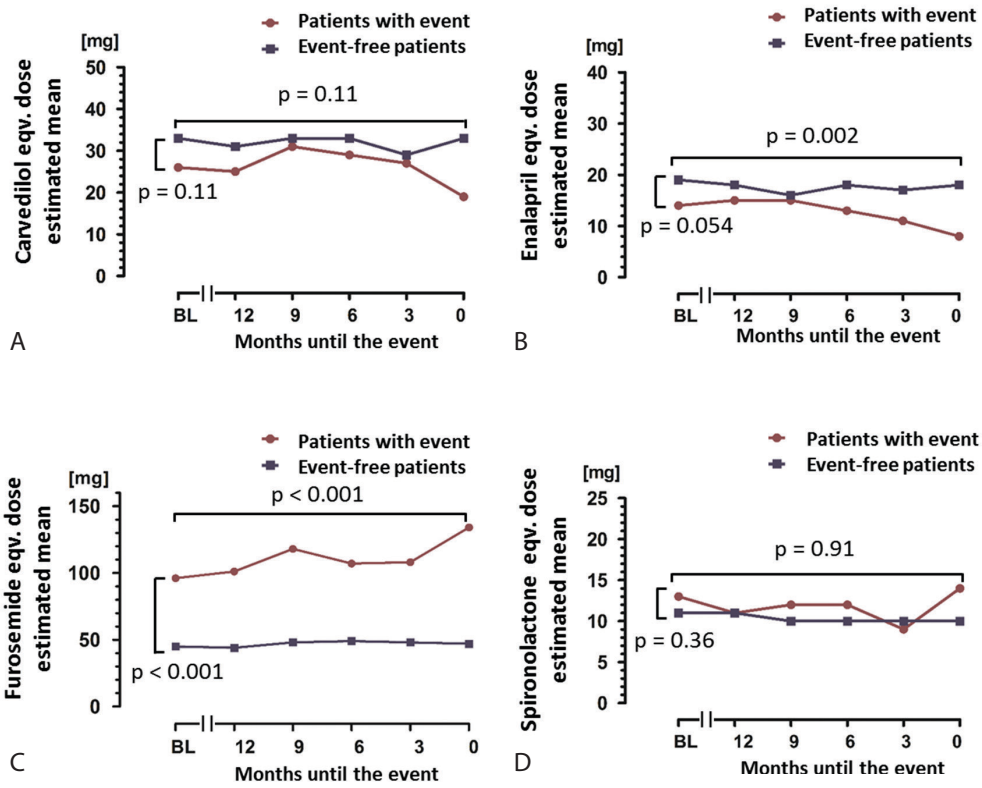


FIGURE 5 Average evolutions of HF medication total daily doses at baseline and during 1-year preceding the event (patients with incident endpoints) or last sample moment (event-free patients). X-axis displays baseline (BL) and the time (months) preceding the event or last sampling moment (time 0). Y-axis displays estimated mean of HF medication total daily dose at each time moment during follow-up. T-test was applied to test the differences at baseline, and mixed-effects models were used to test the difference during follow-up.

HF medication and clinical outcomes: time-dependent survival analysis

Table 2 displays the results of the time-dependent survival analysis. Higher doses of diuretics are independently associated with higher risk of events (per 40 mg increase: HR (95%CI) 1.12 (1.03–1.22), p=0.009). In addition, lower enalapril equivalent doses were univariably associated with increased risk (per 40 mg decrease: 2.41 [1.19–4.88], p=0.014), which did not persist after multivariable adjustment.

TABLE 2 Time-dependent survival analysis of total daily doses in HF medication and the risk of clinical events during follow-up.

HF medication	Unadjusted		Model 1		Model 2	
	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
β-blockers per 50 mg increase:	0.79 (0.53–1.19)	0.27	0.80 (0.52–1.23)	0.32	0.92 (0.62–1.36)	0.67
ACEi/ARBs per 40 mg decrease:	2.41 (1.19–4.88)	0.014	2.44 (1.20–4.97)	0.014	1.27 (0.65–2.48)	0.48
Loop diuretics per 40 mg increase:	1.24 (1.16–1.33)	<0.001	1.25 (1.17–1.34)	<0.001	1.12 (1.03–1.22)	0.009
MRAs per 25 mg increase:	0.91 (0.58–1.43)	0.68	0.97 (0.61–1.55)	0.90	0.94 (0.59–1.51)	0.81

β-blockers, β-adrenergic receptor blockers; ACE-inhibitors/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; MRAs, mineralocorticoid receptor antagonists. Hazard ratios (HR) with 95% confidence intervals (CI) are given for unadjusted model; **Model 1**: HF medications adjusted for one another; and **Model 2**: HF medications adjusted for age, sex, diabetes + repeatedly assessed: NYHA classification, NT-proBNP and eGFR.

Sensitivity analysis

All above-described analyses were also performed in the full cohort (n=263) which additionally included the HFpEF patients. Results were essentially the same (data not shown).

DISCUSSION

This study is the first to investigate the temporal relationship between medical therapy for HF and detailed biomarker profiles in patients with CHF with reduced ejection fraction. We found that higher ACE-inhibitor/ARB doses are associated with less cardiac impairment, lower inflammation, and less renal tubular damage. No association was observed between higher ACE-inhibitor/ARB doses and glomerular impairment. In contrast, higher loop diuretic doses were associated with worsening of the biomarkers profiles and poor prognosis. We also found that patients who experienced incident clinical events had significantly more down-titrations of ACE-inhibitors/ARBs, and more up-titrations of loop diuretics in the time-period prior to the event. Altogether, these findings challenge the down-titration or withholding of ACE-inhibitors/ARBs solely based on creatinine or eGFR,

and thus carry potential implications for treatment of patients with CHF. Likewise, "renoprotective" treatment targeted at the tubules may be even more effective than treatment aiming at improving renal function in terms of GFR.

CHF and renal dysfunction are highly prevalent, share many risk factors (diabetes, hypertension, hyperlipidemia), and interact to worsen the prognosis.[10,16] Yet, patients with CHF and eGFR <30 ml/min/1.73m^2 have systematically been excluded from RCTs that showed efficacy of ACE-inhibitors/ARBs in reversing cardiac remodeling and improving outcome.[6] Moreover, some reports indicated that use of ACE-inhibitors/ARBs might precipitate acute renal failure.[17,18] This may result in suboptimal dosing of ACE-inhibitors/ARBs in clinical practice as eGFR declines.[19,20] In a recent multicenter study including 11 European countries, lower eGFR remained an independent predictor for suboptimal dosing of ACE-inhibitors/ARBs.[21] In contrast, nephrology guidelines recommend the use of ACE-inhibitors/ARBs in patients with eGFR <30 ml/min/1.73m^2.[22,23] In fact, a pooled analysis of 11 randomized clinical trials has demonstrated a consistent protective effect of ACE-inhibitors on progression of kidney disease.[24] Importantly, ACE-inhibitors impede progression of proteinuria independently of their antihypertensive effect.[25,26] Our study extends these findings by exclusively showing that ACE-inhibitors/ARBs reduce renal tubular damage in patients with CHF. This was demonstrated by two tubular markers (urinary NAG and KIM-1) that were previously found to be strongly associated with tubular damage in patients with acute renal injury,[27] but were also associated with adverse clinical outcomes such as HF re-hospitalisation and mortality in patients with CHF.[28,29] In line with this, our findings indicate that these urinary biomarkers may also be clinically useful for monitoring the kidney's response to ACE-inhibitors/ARBs in patients with CHF. Furthermore, we found, although not significantly, a tendency towards improvement of glomerular function with higher ACE-inhibitor/ARB doses. This is indirectly supported by Frohlich et al., who found that down-titration of ACE-inhibitors/ARBs from higher doses does not improve renal function.[30] Our results also suggest that higher ACE-inhibitors/ARBs doses are associated with lower inflammation in CHF, as shown by repeatedly measured CRP levels. This anti-inflammatory effect of ACE-inhibitors/ARBs,[31,32] although not consistently proven, may be an additional link to improved survival in CHF. This raises the question whether a decrement in renin-angiotensin-aldosterone system (RAAS) blockage is justified solely based on creatinine or eGFR. This issue is especially important in subgroups of patients in whom we found that decrease in ACE-inhibitors/ARBs and increase in diuretics dose occurs in parallel.

As for the effects of diuretics, our time-dependent survival analysis showed that every 40 mg increase in furosemide equivalent dose independently increases the instantaneous risk for 13% (4–19%). This corresponds well with 11% (8–14%) found by Damman et al. in their propensity-matched study of 5011 CHF patients.[7] Yet a 50% reduction in the risk after correction for time-varying health status of patients indicates that substantial confounding by severity of HF is present in the crude risk estimates of loop diuretics.This study is not the first to report an association between these agents and poor prognosis in CHF. However, a unique advantage of this study is frequent repeated assessment both of NYHA functional classification and different cardio-renal biomarkers, which allowed us to thoroughly evaluate the temporal effect of HF medication dosage adjustments in CHF. To this end, we found that higher loop diuretics doses were associated with a deterioration of the complete biomarker profiles, with the largest effect being on the kidneys (glomeruli and tubules). This temporal association between loop diuretics and the levels of glomerular and tubular markers might be of particular importance for optimizing diuretic therapy in such a way that congestion is treated adequately but at the same time, renal injury is not caused. However, in-depth studies on these tubular markers, preferably interventional in nature, are needed to provide definite recommendations on the potential use of biomarker-guided loop diuretic treatment in CHF. Taken together, it is clear that higher, and increase in, loop diuretic doses during follow-up mark progression of CHF. Notably, the effects we found for potassium-sparing diuretics differed from those found for loop diuretics. Higher MRA doses were not significantly associated with adverse biomarker profiles or adverse clinical outcomes. This may in part be attributed to the differences in the mechanisms of action between loop diuretics and MRAs. While the former have been shown to up-regulate the RAAS, the latter result in (beneficial) RAAS blockage. Yet, although efficacy of MRAs has been demonstrated in trials, in our study higher doses only showed a statistically significant association with lower cardiac troponin levels over follow-up; other beneficial effects could not be demonstrated. To this end, in other CHF cohorts, under-prescription of MRAs was found to be a stumbling stone for observing beneficial effects[33-35]

Study limitations

First, although this study was not randomized, its repeated-measures design allows for stronger claims of causality than can be made in previous observational studies. Nevertheless, risk assessment may have been biased by unmeasured confounding although we adjusted for several time-varying variables. Second, our analysis could

not take into account reasons for the dose adjustments. Yet, it is likely that reasons are similar to those identified by Ouwerkerk et al., since our patients were recruited from Dutch hospitals as was the majority of patients in their study.[21] Third, we cannot comment on the anti-proteinuric effect of ACE-inhibitors/ARBs in CHF since we did not measure proteinuria. However, we showed that these agents were associated with less tubular damage which may share similar mechanisms. Importantly, a protective tubular effect was shown by NAG and also by KIM-1, which was qualified as the biomarker for kidney toxicity in preclinical settings (i.e, safety assessment in rats) by the Food and Drug Administration and European Medicines Agency.[36] While we examined a wide array of biomarkers, other biomarkers that were not assessed here may also be relevant and should be investigated in future studies. With the rise of modern –omics technologies, multiple biomarkers that carry potential for heart failure are expected to emerge in the near future. Finally, of note is that the proportion of patients with HFpEF in the current study was low. This may 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. We do not deem potential inclusion bias a likely reason for the low proportion HFpEF, because all consecutive patients were screened in both participating centres.

CONCLUSION

In conclusion, decrease or withholding of ACE-inhibitors/ARBs solely based on glomerular function is not justified because of the beneficial effects on the heart, inflammation, and renal tubules. Furthermore, higher and increase in loop diuretic doses during follow-up mark progression towards end stage CHF.

REFERENCES:

1. The CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *N Engl J Med.* 1987;316(23):1429-1435.
2. Granger CB, McMurray JJ, Yusuf S, et al. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet.* 2003;362(9386):772-776.
3. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med.* 1999;341(10):709-717.
4. Packer M, Coats AJ, Fowler MB, et al. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med.* 2001;344(22):1651-1658.
5. Maggioni AP, Anker SD, Dahlstrom U, et al. Are hospitalized or ambulatory patients with heart failure treated in accordance with European Society of Cardiology guidelines? Evidence from 12,440 patients of the ESC Heart Failure Long-Term Registry. *Eur J Heart Fail.* 2013;15(10):1173-1184.
6. 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.
7. Damman K, Kjekshus J, Wikstrand J, et al. Loop diuretics, renal function and clinical outcome in patients with heart failure and reduced ejection fraction. *Eur J Heart Fail.* 2016;18(3):328-336.
8. Ahmed A, Young JB, Love TE, Levesque R, Pitt B. A propensity-matched study of the effects of chronic diuretic therapy on mortality and hospitalization in older adults with heart failure. *Int J Cardiol.* 2008;125(2):246-253.
9. Eshaghian S, Horwich TB, Fonarow GC. Relation of loop diuretic dose to mortality in advanced heart failure. *Am J Cardiol.* 2006;97(12):1759-1764.
10. 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.
11. Francis GS, Benedict C, Johnstone DE, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation.* 1990;82(5):1724-1729.
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. R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
16. Filippatos G, Farmakis D, Parissis J. Renal dysfunction and heart failure: things are seldom what they seem. *Eur Heart J. 2014;35(7):416-418.*
17. De Vecchis R, Di Biase G, Ariano C, et al. ACE-inhibitor therapy at relatively high doses and risk of renal worsening in chronic heart failure. *Arq Bras Cardiol.* 2011;97(6):507-516.
18. Fonarow GC, Yancy CW, Heywood J, Committee ASA, Study G, and I. Adherence to heart failure quality-of-care indicators in us hospitals: Analysis of the adhere registry. *Arch*

Intern Med. 2005;165(13):1469-1477.

19. Ezekowitz J, McAlister FA, Humphries KH, et al. The association among renal insufficiency, pharmacotherapy, and outcomes in 6,427 patients with heart failure and coronary artery disease. *J Am Coll Cardiol.* 2004;44(8):1587-1592.
20. Berger AK, Duval S, Manske C, et al. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in patients with congestive heart failure and chronic kidney disease. *Am Heart J.* 2007;153(6):1064-1073.
21. Ouwerkerk W VA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL , Lang CC, ter Maaten JM, Ng LL , Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Metra M, Zwinderman AH. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: a prospective European study. *Eur Heart J.* 2017;00:1-10 doi:10.1093/eurheartj/ehx1026.
22. National Kidney F. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Di.* 2002(39):1-266.
23. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl.* 2013;3:1-150.
24. Kent DM, Jafar TH, Hayward RA, et al. Progression risk, urinary protein excretion, and treatment effects of angiotensin-converting enzyme inhibitors in nondiabetic kidney disease. *J Am Soc Nephrol.* 2007;18(6):1959-1965.
25. Viberti G, Mogensen CE, Groop LC, Pauls JF. Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. European Microalbuminuria Captopril Study Group. *Jama.* 1994;271(4):275-279.
26. Ravid M, Savin H, Jutrin I, Bental T, Katz B, Lishner M. Long-term stabilizing effect of angiotensin-converting enzyme inhibition on plasma creatinine and on proteinuria in normotensive type II diabetic patients. *Ann Intern Med.* 1993;118(8):577-581.
27. 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.
28. van Veldhuisen DJ, Ruilope LM, Maisel AS, Damman K. Biomarkers of renal injury and function: diagnostic, prognostic and therapeutic implications in heart failure. *European heart journal.* 2016;37(33):2577-2585.
29. Damman K, Masson S, Hillege HL, et al. Clinical outcome of renal tubular damage in chronic heart failure. *European heart journal.* 2011;32(21):2705-2712.
30. Frohlich H, Nelges C, Tager T, et al. Long-term changes of renal function in relation to ace inhibitor/angiotensin receptor blocker dosing in patients with heart failure and chronic kidney disease. *Am Heart J.* 2016;178:28-36.
31. Verma S, Lonn EM, Nanji A, et al. Effect of angiotensin-converting enzyme inhibition on C-reactive protein levels: the ramipril C-reactive pRotein randomized evaluation (4R) trial results. *Can J Cardiol.* 2009;25(7):e236-240.
32. Marchesi C, Paradis P, Schiffrin EL. Role of the renin-angiotensin system in vascular inflammation. *Trends Pharmacol Sci.* 2008;29(7):367-374.
33. Ferreira JP, Rossignol P, Machu JL, et al. Mineralocorticoid receptor antagonist pattern of use in heart failure with reduced ejection fraction: findings from BIOSTAT-CHF. *Eur J Heart Fail. 2017;19(10):1284-1293.*
34. Lachaine J, Beauchemin C, Ramos E. Use, tolerability and compliance of spironolactone in the treatment of heart failure. *BMC Clin Pharmacol. 2011;11:4.*
35. Margolis J, Gerber RA, Roberts C, Gheorghiade M. Adherence to aldosterone-blocking agents in patients with heart failure. *Am J Ther.* 2010;17(5):446-454.
36. Dieterle F, Sistare F, Goodsaid F, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium. *Nat Biotechnol.* 2010;28(5):455-462.

SUPPLEMENTARY INFORMATION

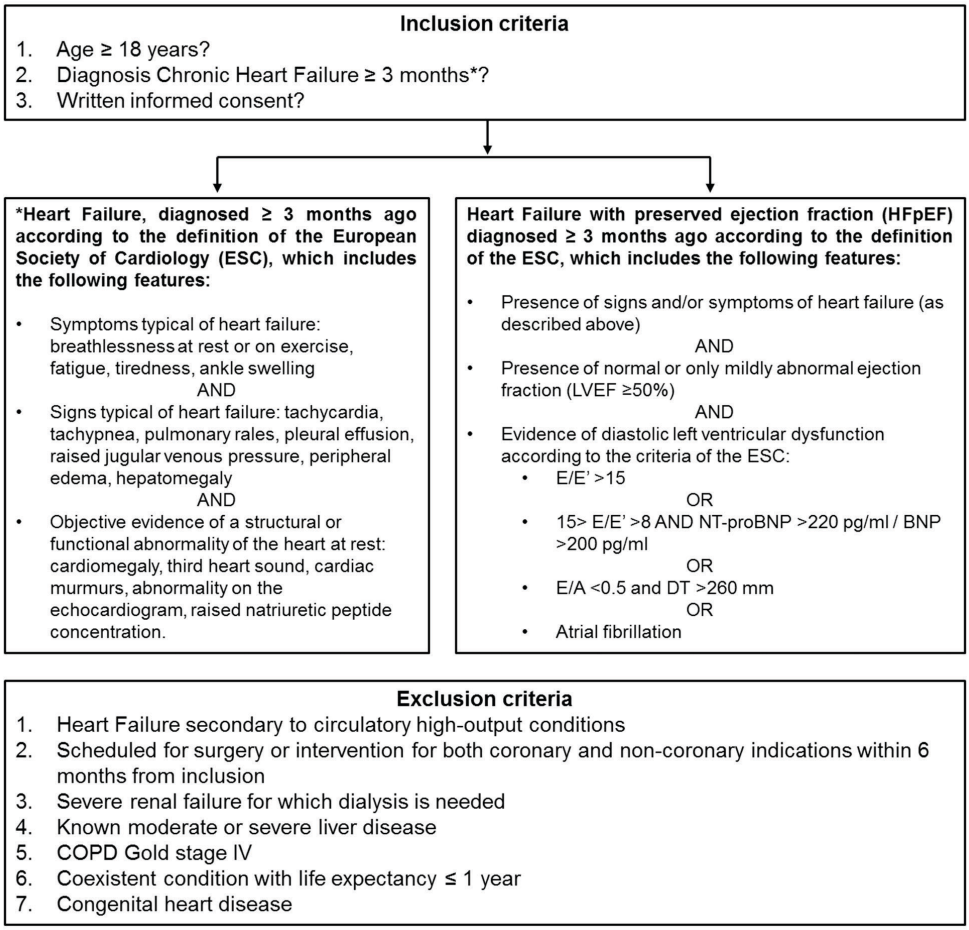


FIGURE S1 Inclusion and exclusion criteria.

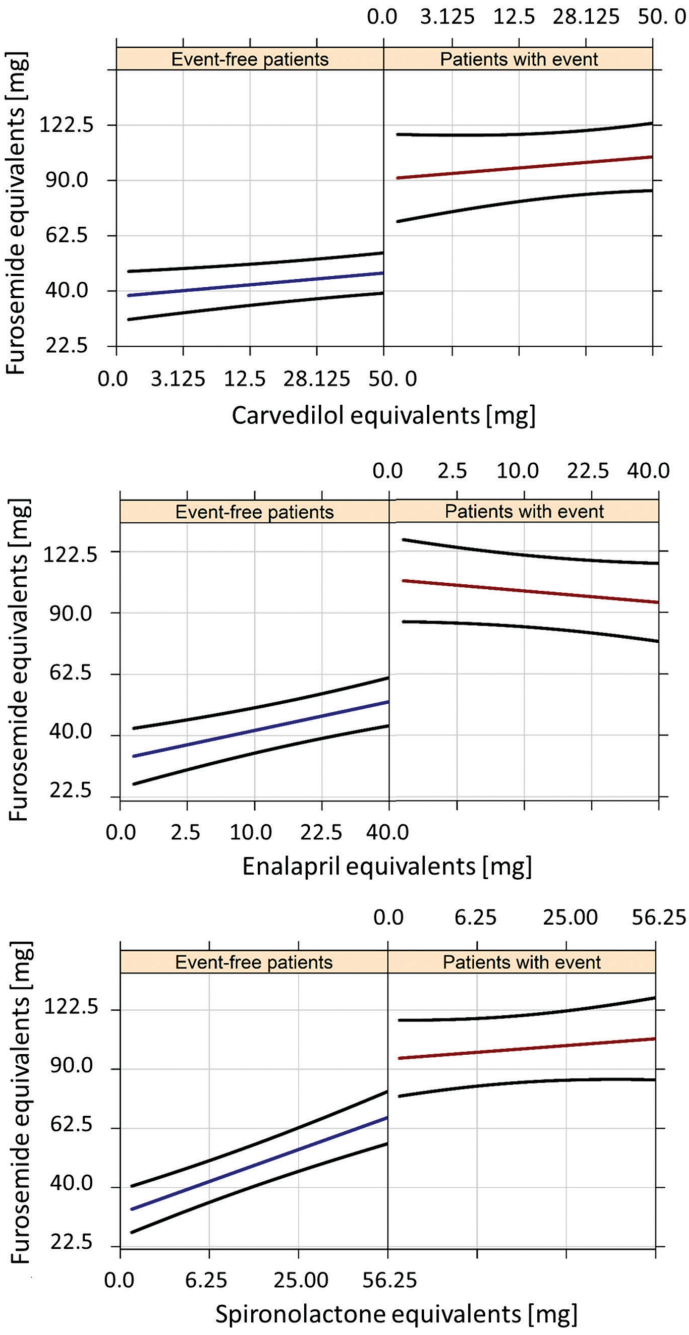


FIGURE S2 Average evolutions of furosemide equivalent doses in relation to equivalent doses of carvedilol, enalapril, and spironolactone within the same patient at the same time during follow-up, stratified by event status.

TABLE S1 **Total daily dose equivalents and conversion factors for ACE-inhibitors/ARBs, β-blockers, MRAs and loop diuretics/thiazides.**

Drug Category	Maximal Dose (Target Dose)	Equivalency Conversion
ACE-inhibitors	**Total Daily Dose (mg)**	**Enalapril Dose Conversion Factor**
Enalapril	40	x 1
Lisinopril	40	x 1
Captopril	150	/ 3.75
Quinapril	40	x 1
Ramipril	10	x 4
Fosinopril	40	x 1
Perindopril	16	x 2.5
Trandolapril	4	x 10
ARB	**Total Daily Dose (mg)**	**Enalapril Dose Conversion Factor**
Candesartan	32	x 1.25
Losartan	50	/ 1.25
Valsartan	320	/ 8
Irbesartan	150	/3.75
β-lockers	**Total Daily Dose (mg)**	**Carvedilol Dose Conversion Factor**
Carvedilol	50	x 1
Bisprolol	10	x 5
Metoprolol tartrate	100	/ 2
Atenolol	50	x 1
Celiprolol	200	/ 4
Labetalol	100	/ 2
Nebivolol	10	x 5
Aldosterone Antagonists	**Total Daily Dose (mg)**	**Spironolactone Dose Conversion Factor**
Spironolactone	25	x 1
Eplerenone	50	/ 2
Loop Diuretic/thiazides	**Total Daily Dose (mg)**	**Furosemide Dose Conversion Factor**
Furosemide	40	x 1
Bumetanide	1	x 40
Torsemide	20	x 2
Hydrochlorothiazide	12.5	x 3.2
Chlorothiazide	36	x 1.44

TABLE S2 Details on HF medication doses and biomarkers data at baseline and during each follow-up visit from the whole study population.

		BL	F-up 1	F-up 2	F-up 3	F-up 4	F-up 5	F-up 6	F-up 7	F-up 8	F-up 9	F-up 10
HF medication*	n[a]	263	251	225	201	209	188	162	140	161	108	88
Carvedilol eqv		7, 32, 73	7, 31, 72	9, 32, 70	8, 33, 74	11, 34, 71	8, 32, 70	8, 31, 70	10, 35, 76	7, 31, 70	8, 30, 67	10, 33, 70
Enalapril eqv		4, 18, 41	4, 17, 41	4, 17, 40	3, 16, 39	4, 17, 40	3, 16, 39	4, 15, 35	3, 15, 36	3, 16, 38	5, 18, 41	3, 14, 34
Furosemide eqv		11, 57, 139	9, 52, 130	10, 54, 134	7, 56, 153	6, 60, 168	6, 56, 157	5, 62, 181	4, 49, 142	4, 55, 165	5, 47, 130	3, 50, 153
Spironolactone eqv		1, 11, 31	1, 11, 31	1, 11, 30	1, 10, 29	1, 11, 30	1, 10, 30	1, 11, 31	1, 10, 28	1, 10, 27	1, 10, 28	1, 9, 27
Serum**	n[b]	263	243	224	199	205	189	168	152	159	104	74
NT-proBNP pmol/L		29.7, 112.8, 428.3	26.8, 102.1, 388.9	25.6, 100.1, 390.7	24.8, 94.3, 358.3	23.7, 90.2, 343.7	24.9, 92.2, 341.5	24.8, 89.1, 320.4	22.7, 85.4, 321.7	24.4, 88.9, 323.8	20.9, 88.9, 378.4	26.4, 88.5, 296.9
Hs-cTnT ng/L		7.8, 18.1, 42.0	7.5, 17.7, 41.6	7.6, 17.9, 41.8	7.2, 17.2, 40.9	7.3, 16.9, 39.3	8.0, 17.4, 37.7	7.5, 17.3, 39.8	6.9,16.0, 37.3	7.5, 16.6, 36.7	7.8, 18.1, 41.8	8.9, 18.1, 37.0
CRP mg/L		0.7, 2.1, 6.6	0.7, 2.3, 7.7	0.7, 2.4, 8.0	0.8, 2.4, 7.3	0.7, 2.4, 7.8	0.8, 2.6, 8.0	0.8, 2.5, 7.9	0.8, 2.3, 6.8	0.9, 2.6, 7.9	0.9, 2.3, 8.8	0.7, 2.6, 9.3
Plasma**	n[b]	263	244	223	199	207	190	167	152	160	105	74
Creatinine mg/dl		0.90, 1.23, 1.68	0.90, 1.22, 1.65	0.89, 1.24, 1.74	0.87, 1.23, 1.74	0.87, 1.23, 1.75	0.88, 1.21, 1.67	0.83, 1.13, 1.53	0.82, 1.12, 1.53	0.87, 1.22, 1.73	0.84, 1.18, 1.65	0.83, 1.25, 1.86
eGFR_mL/ in/1.73m2		37, 55, 83	38, 55, 81	36, 54, 82	36, 55, 83	36, 55, 84	38, 55, 81	42, 61, 88	42, 60, 88	36, 55, 84	38, 57, 85	34, 54, 84
Cystatin C mg/L		0.50, 0.74, 1.09	0.52, 0.79, 1.20	0.55, 0.80, 1.16	0.48, 0.72, 1.09	0.52, 0.74, 1.05	0.51, 0.76, 1.12	0.50, 0.72, 1.02	0.43, 0.68, 1.07	0.50, 0.71, 1.02	0.43, 0.67, 1.03	0.46, 0.70, 1.05
Urine**	n[b]	263	228	206	191	203	183	162	147	155	103	71
NAG U/gCr		2.8, 5.8, 11.9	2.4, 5.4, 12.1	2.3, 4.9, 10.5	2.2, 5.0, 10.9	2.0, 4.9, 11.7	2.1, 4.9, 11.1	1.9, 4.5, 10.6	2.0, 4.7, 10.9	2.1, 4.7, 10.6	1.8, 4.5, 11.1	2.3, 4.6, 9.3
KIM-1 ng/gCr		205.0, 493.8, 1189.1	208.3, 492.3, 1163.8	187.9, 451.3, 1084.2	204.2, 461.3, 1041.8	193.8, 441.3, 1004.9	208.6, 470.7, 1062.0	199.2, 464.1, 1081.2	194.3, 467.4, 1124.6	210.4, 494.4, 1161.8	198.4, 469.0, 1108.8	184.2, 432.4, 1015.1

BL, baseline; F-up, follow-up; eqv. equivalents. [a] number of times HF medication was assessed per follow-up visit [b] number of measured samples per follow-up visit * Geometric mean value ± 1 standard deviation (SD) (i.e., mean - SD, mean, mean + SD) per follow-up visit of √-transformed medication total daily doses presented on the linear (natural) scale. Results are based on the data from the total study population. ** Geometric mean ± 1 SD (mean - SD, mean, mean + SD) per follow-up visit of [2]log-transformed biomarker values presented on the linear (natural) scale. Results are based on the data from the total study population.