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ORIGINAL ARTICLE

Biomarker changes as surrogate endpoints in early-phase trials in heart failure with reduced ejection fraction

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Abstract

Aims No biomarker has achieved widespread acceptance as a surrogate endpoint for early-phase heart failure (HF) trials. We assessed whether changes over time in a panel of plasma biomarkers were associated with subsequent morbidity/mortality in HF with reduced ejection fraction (HFrEF).

Methods and results In 1040 patients with HFrEF from the BIOSTAT-CHF cohort, we investigated the associations between changes in the plasma concentrations of 30 biomarkers, before (baseline) and after (9 months) attempted optimization of guideline-recommended therapy, on top of the BIOSTAT risk score and the subsequent risk of HF hospitalization/all-cause mortality using Cox regression models. C-statistics were calculated to assess discriminatory power of biomarker changes/month-nine assessment. Changes in N-terminal pro-B-type natriuretic peptide (NT-proBNP) and WAP four-disulphide core domain protein HE4 (WAP-4C) were the only independent predictors of the outcome after adjusting for their baseline plasma concentration, 28 other biomarkers (both baseline and changes), and BIOSTAT risk score at baseline. When adjusting for month-nine rather than baseline biomarkers concentrations, only changes in NT-proBNP were independently associated with the outcome. The C-statistic of the model including the BIOSTAT risk score and NT-proBNP increased by 4% when changes were considered on top of baseline concentrations and by 1% when changes in NT-proBNP were considered on top of its month-nine concentrations and the BIOSTAT risk score.

Conclusions Among 30 relevant biomarkers, a change over time was significantly and independently associated with HF hospitalization/all-cause death only for NT-proBNP. Changes over time were modestly more prognostic than baseline or end-values alone. Changes in biomarkers should be further explored as potential surrogate endpoints in early phase HF trials.

Keywords Biomarkers; Surrogate endpoint; Surrogate outcome; Heart failure with reduced ejection fraction; Phase 2; Randomized trial

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Introduction

Development of therapeutic interventions for heart failure (HF) is a long and expensive process. In order to justify investment in large randomized controlled trials (RCT) for regulatory approval, and to inform guidelines and clinical practice, novel interventions need to show sufficient effect on surrogate endpoints in phase II RCTs. Indeed, a surrogate endpoint will often lie on the pathway between the disease and the outcome, and thus, changes induced by a therapy, for example, changes in a biomarker concentration over time, might predict an effect on clinically relevant endpoints in subsequent larger trials.

Currently, there are no accepted surrogate endpoints for HF trials, although N-terminal pro-B-type natriuretic peptide (NT-proBNP) is often used.[1,2] Although several phase II RCTs interventions were effective on chosen surrogate endpoints, subsequent phase III RCTs have been neutral.[3] This is for example the case of pulmonary capillary wedge whose decreased values following the treatment with nesiritide did not translate into an effect of the drug in phase III trials, or exercise duration and peak oxygen consumption that were improved by exercise training which did not reduce the risk of death or hospitalizations later in phase III.[3] Many studies have identified biomarkers that as snap-shot values predict subsequent outcomes, but there is a lack of biomarkers that are known to both change during therapy and where this change is associated with outcomes, although the totality of the evidence suggests that this may be the case for changes in NT-proBNP in PARAMOUNT [sacubitril-valsartan in HF with preserved ejection fraction (HFpEF)] and PIONEER [sacubitril-valsartan in hospitalized HF with reduced EF (HFrEF)].[4,5] The identification of easily measured, reproducible, and broadly available biomarkers as surrogate endpoints, where a favourable change in the biomarker reflects a treatment effect and thus a better outcome, should improve and expedite the design and development of phase II trials, improve confidence among funders of both phase II and III trials, and ultimately provide more therapeutic options and benefits to patients.

Therefore, the aim of this analysis was, for multiple circulating biomarkers, to assess whether and to what extent changes over time predict subsequent HF hospitalization and all-cause mortality in HFrEF and thus whether these biomarkers may serve as feasible surrogate endpoints in HFrEF phase II trials.

Methods

Study protocol and setting

We studied patients from the prospective BIOSTAT-CHF study that enrolled 2516 patients in 11 European countries.[6] Inclusion criteria were (i) age ≥18 years; (ii) symptoms of new-onset or worsening HF; (iii) objective evidence of cardiac dysfunction documented either by EF ≤ 40% or plasma concentrations of BNP and/or NT-proBNP >400 or >2000 ng/L, respectively; (iv) treatment with either oral or intravenous furosemide ≥40 mg/day or equivalent at the time of inclusion; (v) not previously treated with angiotensin-converting enzyme inhibitors/angiotensin receptor antagonists and beta-blockers or receiving ≤50% of target doses of these drugs at the time of inclusion; and (vi) be anticipated to be initiated or uptitrated with angiotensin-converting enzyme inhibitors/angiotensin receptor antagonists and/or beta-blockers by the treating physician.

Patients were enrolled between December 2010 and December 2012. At baseline, medical history, current use of medication, physical examination, and data on quality of life were recorded, and plasma, serum, and urine were sampled. Echocardiographic exam was recommended but not compulsory. Investigators and participants were asked to optimize HF treatments according to the 2012 European Society of Cardiology guidelines.[7] At 9 months, all clinical and laboratory assessments were repeated. Patients were then followed-up till 1 April 2015. The primary outcome was time to first of HF hospitalization or all-cause death. HF hospitalizations were reported by sites but not adjudicated.

The study complied with the Declaration of Helsinki. The local ethics committee approved the research protocol, and all patients provided written informed consent.

Patients and biomarkers

In the current analysis, only patients with HFrEF (EF ≤ 40%) and biomarker measurements at both baseline and month-nine were included.

N-terminal pro-B-type natriuretic peptide was measured by sandwich electrochemiluminescence immunoassay (Cobas, Roche). Galectin-3, myeloperoxidase (MPO), and neutrophil gelatinase-associated lipocalin (NGAL) were measured using sandwich enzyme-linked immunosorbent assays (ELISA) on a microtitre plate; angiogenin and C-reactive protein (CRP) were measured using competitive ELISAs on a Luminex® platform (Alere Inc., San Diego, CA, USA); D-dimer, endothelial cell-selective adhesion molecule (ESAM), growth differentiation factor 15 (GDF-15), lymphotoxin beta receptor (LTBR), mesothelin, neuropilin, N-terminal pro C-type natriuretic peptide (NT-proCNP), osteopontin, procalcitonin, pentraxin-3, periostin, polymeric immunoglobulin receptor (PIGR), pro-adrenomedullin (proADM), prosaposin B, receptor for advanced glycation end-products (RAGE), soluble ST-2 (sST-2), syndecan-1, tumour necrosis factor alpha receptor 1 (TNFR-1), Troy, vascular endothelial growth receptor 1 (VEGFR-1), and WAP four-disulphide core domain protein HE4 (WAP-4C) were measured using sandwich

ELISAs on a Luminex® platform (Alere Inc., San Diego, CA, USA). Atrial natriuretic peptide and BNP were also measured using Luminex multiplexed bead-based immunoassays at Alere (San Diego, California). These research assays have not been standardized to the commercialized assays used in research or in clinical use. Further, the extent to which each Alere™ assay correlates with the commercial assay is not fully characterized.

Statistical analyses

Baseline characteristics were reported as frequencies (percentages) if categorical and as median (interquartile range) if continuous. Median biomarker concentrations at baseline versus month-nine were compared by the Wilcoxon-Mann–Whitney test. A *P*-value <0.05 was considered statistically significant. As index date (start of the follow-up) for the outcome analysis we considered the date of the second biomarker measurements (at 9 months from baseline), that is, potential HF hospitalization occurred between the baseline and the month-nine biomarker measurements were not considered.

Changes in plasma concentrations of biomarkers were included in the analyses as the per cent change between the two consecutive measurements (% Δ biomarker concentrations = [month-nine biomarker concentration − baseline biomarker concentration]/baseline biomarker concentration*100). Changes in biomarkers concentrations were modelled as a quantitative predictor of outcome, accounting for both negative and positive changes. Specifically, we used restricted cubic splines to flexibly model potential nonlinearity (three knots at fixed percentiles). The associations between changes in biomarkers and the outcome from the month-nine measurement was assessed by Cox proportional regressions according to two different sequential models.

- In *Model 1*, which was performed separately for each biomarker, we included the change in biomarker concentrations from baseline to month-nine, the log baseline concentrations of the biomarker and the compact BIOSTAT risk score for 2 year HF hospitalization and all-cause mortality.[8] This risk score included age, previous HF hospitalization in the last year, peripheral oedema, systolic blood pressure, estimated glomerular filtration rate, log blood urea nitrogen, log NT-proBNP, haemoglobin, sodium, high density lipoprotein cholesterol and beta-blocker use at baseline.[8] Adjustment for multiple testing with the Holm method was used.[9]
- In *Model 2*, we assessed which changes in biomarkers were independently associated with prognosis by using a stepwise backward model (Wald test $P < 0.05$). In this model, we included those biomarkers whose changes in concentrations were associated with the risk of outcome with *P*-value <0.10 after Holm correction in Model 1. Variance inflation factor (VIF) was used to test for multi-collinearity among biomarkers and whether a pair of biomarkers was highly correlated (VIF > 10), we included only one of the biomarkers in the multivariable model.

The discriminatory power for biomarkers was assessed by C-statistic.

To assess whether changes in biomarkers' concentrations predicted prognosis on top of the month-nine concentrations, we repeated all the analyses adjusting for the BIOSTAT risk score and for month-nine rather than baseline biomarkers' concentrations. More specifically:

- Model 3 included changes in biomarkers from baseline to month-nine, the log month-nine concentrations of the biomarker and the compact BIOSTAT risk score;
- Model 4 included those biomarkers whose changes in concentrations were associated with the risk of outcome with *P*-value <0.10 after Holm correction in Model 3.

We excluded 240 patients who died before the month-nine follow-up and 494 patients who were followed for 9 months but did not have biomarkers measured at this time.

In order to evaluate the presence of a potential mortality bias, in a sensitivity analysis, we compared characteristics of patients who did have repeated measurements versus those who did not.

All statistical analyses were run by R version 3.5.1.

Results

Of 2516 patients enrolled in BIOSTAT-CHF, 1040 had HFrEF and repeated biomarker measurements (i.e. at baseline and month-nine) considered for the current analysis (Supporting Information, *Figure S1*).

Table 1 reports patient characteristics. Median age was 67 [IQR: 59–76] years and 23% were female.

Table 2 reports median [interquartile range (IQR)] baseline and month-nine biomarkers concentrations, together with the absolute and per cent variations in concentrations between the two assessments. In particular, median concentrations of 1 of 30 biomarkers did not significantly change over the time, 8 of 30 showed a significant decrease and 21 of 30 an increase in median concentrations.

Figure 1 shows the Spearman correlation matrix of the per cent changes in each biomarker versus all others. NT-proBNP and atrial natriuretic peptide (ANP) were highly correlated with BNP.

Table 1 Patient characteristics at baseline and at month nine

	Baseline	Month 9	*P*-value	*N* missing baseline	*N* missing 9 months
Demographics					
Age (years)	67 [59–76]	68 [60–76]	<0.0001	0	0
Women (%)	23	-	-	0	0
Previous hospitalization (%)	31	-	-	0	0
Clinical measurements					
BMI (kg/m^2)	27 [24–31]	27 [24–31]	0.45	5	17
eGFR (mL/min/1.73 m^2)	63 [47–80]	58 [43–78]	<0.0001	64	264
Hb (g/dL)	14 [12–15]	13 [12–14]	0.0009	93	342
Heart rate (b.p.m.)	75 [66–88]	70 [61–80]	<0.0001	1	8
SBP (mmHg)	120 [110–139]	120 [110–139]	0.56	2	8
DBP (mmHg)	75 [70–84]	75 [68–80]	0.002	2	9
MAP (mmHg)	91 [83–100]	90 [83–100]	0.03	2	9
NYHA [Class III/IV (%)]	57	24	<0.0001	19	15
Co-morbidities (%)					
Smoking	15	-	-	1	-
Hypertension	59	-	-	0	-
Atrial fibrillation	41	-	-	0	-
COPD	16	-	-	0	-
Diabetes	30	-	-	0	-
Myocardial infarction	36	-	-	0	-
Stroke	9	-	-	0	-
PAD	9	-	-	0	-
Renal disease	21	-	-	0	-
Medication use					
Beta-blocker (%)	85	94	<0.0001	0	0
Beta-blocker % target dose [median (IQR)]	25 [6–50]	27 [13–50]	<0.0001	0	0
RASi (%)	77	92	<0.0001	0	0
RASi % target dose [median (IQR)]	25 [13–50]	50 [25–100]	<0.0001	0	0
Digoxin (%)	18	18	0.99	0	2
MRA (%)	54	60	0.0003	0	2
Loop diuretics (%)	100	91	<0.0001	0	2
Device therapy [ICD or CRT (%)]	17	-	-	0	-

Categorical variables are reported as percentages; continuous variables as median [interquartile range].
BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ICD, implantable cardioverter defibrillator; MAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; RASi, renin-angiotensin-system; SBP, systolic blood pressure.

Prognostic impact of changes in biomarkers concentrations (central figure)

Over a median follow-up of 1.17 [IQR: 0.74–1.57] years after the month-nine assessment, 214 of 1040 (20.6%; 17.5 per 100 patient-years) patients had a hospital admission for HF (110 patients) or died for any cause (141 patients).

In *Model 1*, after adjusting for baseline biomarker concentrations and the BIOSTAT risk score, decreases in concentrations of 22 of 30 biomarkers were individually associated with a lower risk of an adverse outcome [ANP, BNP, C-reactive protein (CRP), D-dimer, Galectin-3 (GAL-3), Growth differentiation factor (GDF-15), Mesothelin, NT-proBNP, N-terminal pro-C-type natriuretic peptide (NT-proCNP), Neuropilin, Osteopontin, Procalcitonin (PCT), Pentraxin-3, Polymeric immunoglobulin receptor (PIGR), Pro-adrenomedulin (proADM), Receptor for advanced glycation end product (RAGE), Soluble ST2 (sST2), Syndecan-1, Troy, Tumour necrosis factor-receptor 1α (TNF-R1α), Vascular endothelial growth receptor-1 (VEGFR-1) and WAP 4-disulphide core domain protein HE (WAP-4C)] (Supporting Information, *Figure S2*). In contrast, changes in Angiogenin, Cystatin-c, Endothelial cell selective adhesion molecule (ESAM), Lymphotoxin β receptor (LTβR), myeloperoxidase (MPO), neutrophil gelatinase associated lipocalin (NGAL), Periostin and Prosaposin-β (PSAP-β) did not predict subsequent outcomes (Supporting Information, *Figure S3*).

Model 2 included changes in all the biomarkers that were associated with prognosis in Model 1 with a *P*-value <0.1, together with their baseline concentrations and the BIOSTAT risk score. The full model was reduced to the final model based on *P*-value <0.05 to include changes in ANP, TNFR1α, NT-proBNP, and WAP-4C, together with their baseline concentration and the BIOSTAT risk score. We excluded TNFR1α from the final model as it showed multi-collinearity based on the VIF. Subsequently, ANP was not significantly associated with HF hospitalization/all-cause death. Therefore, only decreases in concentrations of NT-proBNP and WAP-4C were independently associated with lower risk of outcome (*Figure 2*).

Analyses were repeated adjusting for month-nine rather than baseline biomarker concentrations. Biomarkers, which entered Model 4 since their changes were associated with prognosis on top of month-nine concentration and BIOSTAT

Table 2 Biomarker measurements at baseline and at month nine, absolute and per cent changes over time

Biomarkers (median [IQR])	Visit 1	Visit 2	Absolute difference visit 1 - visit 2	% difference visit 1 - visit 2	p-value
NT-proBNP (ng/L)	2217.50 [987.28, 4524.50]	1172.50 [407.25, 2567.25]	-618.40 [-2430.49, 146.93]	**-40.30 [-73.64, 18.06]**	<0.0001
ANP (ng/mL)	18.88 [11.92, 29.37]	23.29 [13.27, 36.59]	4.29 [-3.56, 13.92]	**25.75 [-18.72, 86.18]**	<0.0001
BNP (pg/mL)	200.62 [80.26, 404.18]	124.94 [45.51, 324.43]	-15.23 [-186.05, 71.49]	**-15.20 [-70.96, 75.64]**	<0.0001
ESAM (ng/mL)	62.53 [56.64, 69.44]	67.26 [61.29, 74.76]	5.61 [-0.89, 12.20]	**8.83 [-1.30, 19.75]**	<0.0001
LTBR (ng/mL)	0.14 [0.09, 0.21]	0.16 [0.12, 0.23]	0.02 [-0.02, 0.06]	**17.30 [-9.22, 57.74]**	<0.0001
Mesothelin (ng/mL)	52.51 [46.77, 59.54]	56.55 [48.39, 65.25]	3.89 [-2.62, 11.64]	**7.22 [-4.84, 23.16]**	<0.0001
MPO (ng/mL)	27.71 [23.13, 34.70]	28.51 [23.68, 35.27]	0.83 [-5.68, 6.71]	**2.75 [-18.27, 28.83]**	<0.0001
Neuropilin (ng/mL)	20.47 [15.97, 25.69]	22.23 [17.60, 27.51]	1.71 [-2.33, 6.29]	**9.41 [-11.06, 32.86]**	<0.0001
NT-proCNP (pg/mL)	5.20 [5.20, 12.55]	8.80 [3.23, 16.25]	-0.26 [-1.98, 6.40]	**-2.45 [-38.00, 85.90]**	<0.0001
Osteopontin (ng/mL)	209.50 [171.33, 248.57]	220.06 [183.62, 257.07]	13.02 [-22.11, 47.33]	**6.09 [-10.08, 26.99]**	<0.0001
PCT (pg/mL)	14.00 [4.75, 31.28]	19.19 [10.32, 34.49]	3.52 [-4.76, 14.59]	**30.52 [-23.62, 158.81]**	<0.0001
PSAP-B (ng/mL)	30.91 [22.40, 37.28]	28.68 [19.83, 37.63]	-1.00 [-9.45, 8.27]	**-3.04 [-30.60, 31.32]**	<0.0001
VEGFR-1 (ng/mL)	0.14 [0.14, 0.22]	0.16 [0.16, 0.23]	0.02 [-0.00, 0.04]	**12.51 [-1.80, 20.52]**	<0.0001
D-dimer (ng/mL)	101.92 [101.92, 138.45]	98.91 [98.91, 98.91]	-3.01 [-3.01, -3.01]	**-2.95 [-2.95, -2.95]**	<0.0001
Pentraxin-3 (ng/mL)	1.86 [1.13, 3.12]	1.77 [1.21, 2.68]	0.00 [-0.93, 0.72]	**0.24 [-40.35, 62.60]**	0.04
PIGR (ng/mL)	110.04 [67.65, 176.00]	185.27 [110.82, 322.65]	69.23 [12.37, 167.63]	**73.91 [12.16, 171.10]**	<0.0001
RAGE (ng/mL)	2.69 [1.88, 3.95]	2.95 [2.22, 4.12]	0.38 [-0.56, 1.19]	**14.89 [-17.68, 56.00]**	<0.0001
Syndecan-1 (ng/mL)	2.01 [0.99, 3.59]	2.42 [1.43, 3.78]	0.36 [-0.78, 1.62]	**26.71 [-29.56, 128.92]**	<0.0001
TNFR1a (ng/mL)	0.93 [0.54, 1.49]	1.11 [0.68, 1.77]	0.19 [-0.19, 0.64]	**24.64 [-20.55, 95.30]**	<0.0001
Troy (ng/mL)	0.21 [0.12, 0.37]	0.29 [0.17, 0.49]	0.07 [-0.03, 0.20]	**39.70 [-10.83, 123.57]**	<0.0001
GDF-15 (ng/mL)	3.30 [2.61, 4.11]	3.66 [2.77, 4.79]	0.34 [-0.36, 1.23]	**11.13 [-11.46, 41.61]**	<0.0001
proADM (ng/mL)	0.44 [0.29, 0.69]	0.61 [0.39, 0.98]	0.15 [-0.02, 0.38]	**41.10 [-2.82, 104.86]**	<0.0001
sST2 (ng/mL)	7.06 [3.26, 15.93]	7.85 [4.34, 13.79]	0.96 [-4.30, 4.88]	**21.78 [-41.42, 120.35]**	0.02
WAP-4C (ng/mL)	1.19 [0.68, 2.30]	1.21 [0.63, 2.64]	-0.04 [-0.49, 0.68]	**-4.43 [-37.33, 63.91]**	0.13
Periostin (ng/mL)	5.75 [3.50, 9.44]	7.63 [4.98, 12.24]	1.73 [-0.90, 4.41]	**35.81 [-10.80, 107.31]**	<0.0001
Angiogenin (ng/mL)	4692.45 [3203.51, 7303.29]	2676.41 [1801.36, 4166.88]	-1835.48 [-4168.05, -398.34]	**-43.62 [-65.77, -12.08]**	<0.0001
Cystatin-c (ng/mL)	14662.42 [10030.08, 21300.90]	11688.31 [6574.26, 19649.23]	-2645.80 [-8967.01, 5009.09]	**-18.32 [-57.12, 43.82]**	<0.0001
CRP (ng/mL)	11644.99 [4967.08, 23333.37]	4504.80 [2170.78, 10125.32]	-4730.32 [-15957.11, -18.09]	**-50.96 [-80.62, -1.46]**	<0.0001
GAL-3 (ng/mL)	19.58 [14.40, 26.64]	22.87 [18.06, 30.02]	3.39 [-2.01, 8.03]	**20.45 [-8.93, 52.14]**	<0.0001
NGAL (ng/mL)	53.29 [35.80, 85.44]	83.91 [54.80, 133.40]	29.09 [3.85, 62.97]	**60.33 [7.11, 132.04]**	<0.0001

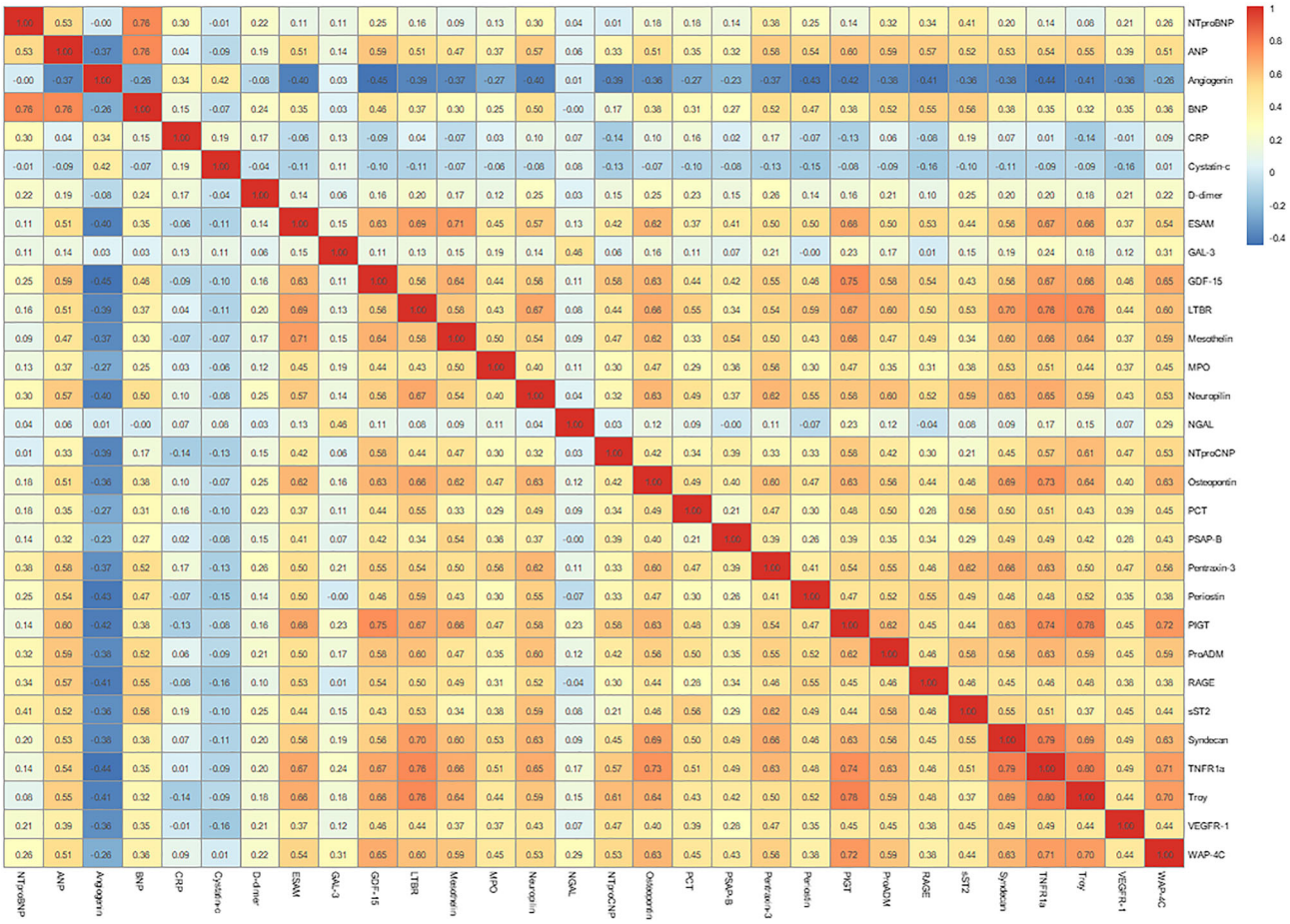
In red: changes in biomarkers associated with worse outcome in our analysis. In green: changes in biomarkers associated with better outcome in our analysis.
ANP, atrial natriuretic peptide; CRP, C-reactive protein; ESAM, endothelial cell-selective adhesion molecule; GAL-3, galectin-3; GDF-15, growth differentiation factor; LTβR, lymphotoxin β receptor; MPO, myeloperoxidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NT-proCNP, N-terminal pro-C-type natriuretic peptide; PCT, procalcitonin; PIGR, polymeric immunoglobulin receptor; proADM, pro-adrenomedulin; PSAP-β, prosaposin-β; RAGE, receptor for advanced glycation end product; sST2, soluble ST2; TNF-R1α, tumour necrosis factor-receptor 1α; VEGFR-1, vascular endothelial growth receptor; WAP-4C, WAP 4-disulphide core domain protein HE.

risk score in Model 3 (with a *P*-value <0.10), were ANP, BNP, and NT-proBNP (Supporting Information, *Figure S4*), whereas changes in other biomarkers did not predict the outcome after adjustment for their respective month-9 concentrations (Supporting Information, *Figure S5*). In Model 4, only decreasing concentrations in NT-proBNP were independently associated with lower risk of outcome (BNP was collinear with both NT-proBNP and ANP and therefore did not enter the final model) (*Figure 2*).

Discriminative power

A model fitted with only the BIOSTAT risk score at baseline, that is, testing the association between the risk score (which includes NT-proBNP) at baseline and the outcome from month-nine, not adjusting for baseline biomarkers or changes in biomarkers, resulted in a C-statistic of 0.66 for time to HF hospitalization or any death. *Table 3* shows the C-statistics of models assessing the associations between single and all biomarker concentrations at baseline only, and the outcome, adjusting for the BIOSTAT risk score (left column), and of models assessing associations between changes in single and all biomarkers and the outcome, adjusting for the BIOSTAT risk score and baseline biomarker concentrations (right column, Model 1). Adding only the baseline biomarkers concentrations to the BIOSTAT risk score did not change the C-statistic for prediction of HF hospitalization or any death. However, adding changes in biomarker concentrations over time did slightly increase the predictive ability of the model for most of the investigated biomarkers. The C-statistic for Model 2 including all significant biomarkers from Model 1

Figure 1 Spearman correlation matrix for the per cent change in biomarker levels from baseline to month nine. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ICD, implantable cardioverter defibrillator; MAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; RASi, renin-angiotensin-system; SBP, systolic blood pressure.



was 0.71, while the best C-statistic was obtained in the model including only the significant biomarkers from Model 2, that is, WAP-4C and NT-proBNP, with a C-statistic of 0.72.

Table 4 shows the C-statistics of models including only the month-nine biomarker concentrations and of the models including the change in biomarker adjusted for month-nine biomarker concentrations (Model 3) and the BIOSTAT risk score. When month-nine concentrations were added to the BIOSTAT risk score, the C-statistic for prediction of HF hospitalization or any death increased. Adding changes in biomarkers concentrations limitedly affected C-statistic (Model 3). The C-statistic for Model 4, including all the significant biomarkers from Model 3 with a *P*-value <0.10, that is, NT-proBNP, ANP, and BNP, was slightly higher compared with the individual C-statistics from Models 3 and was 0.71, while it was 0.72 whether only ANP and NT-proBNP were considered.

Sensitivity analysis

Supporting Information, *Table S1* shows the comparison of baseline characteristics between patients with versus without repeated biomarker measurements. Those who had only one measurement were generally older, more often had NYHA class III/IV, device therapy, previous hospitalization in the last year, higher NT-proBNP concentrations, and more often had comorbidities and lower use of beta-blockers and renin-angiotensin-system inhibitors.

Discussion

In patients with HFrEF in BIOSTAT-CHF, which included patients with worsening symptoms and indications for initiation

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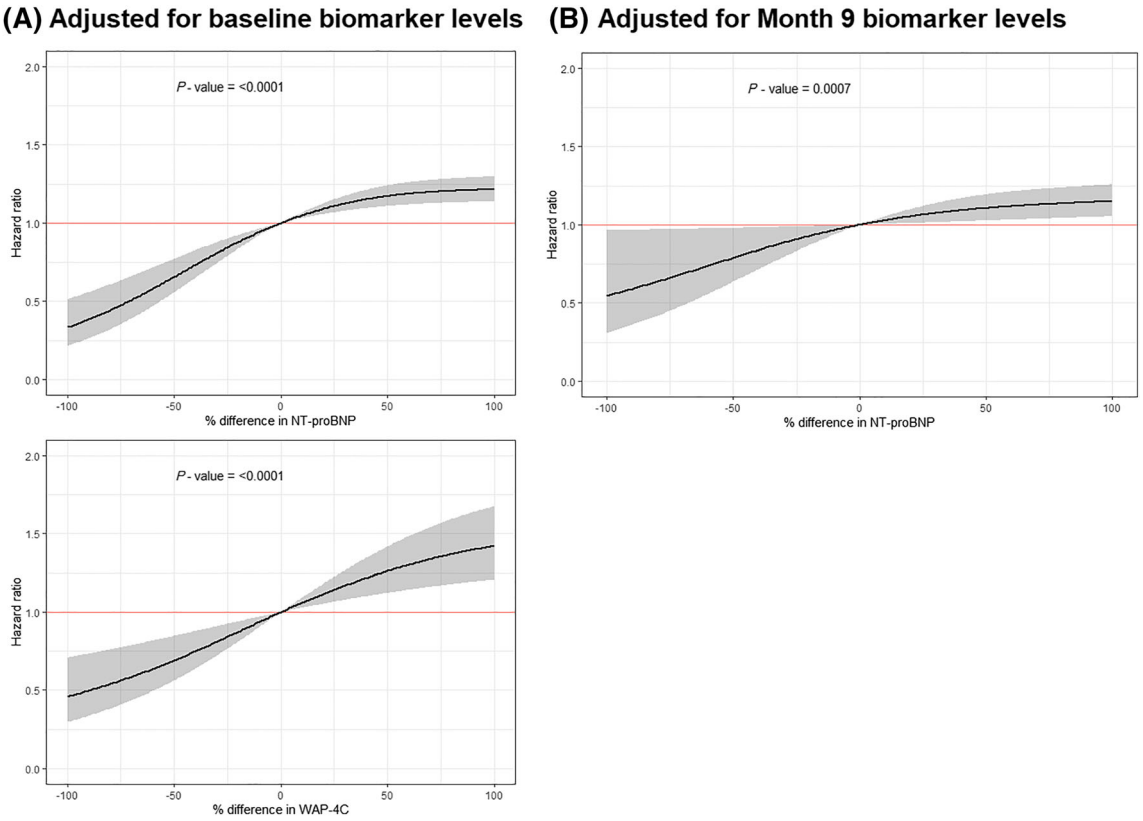
Figure 2 Per cent changes in concentrations of biomarkers from baseline to month nine independently and significantly associated with heart failure (HF) hospitalization/all-cause death (Model 2). All the changes in biomarkers concentrations significantly associated with all-cause death/HF hospitalization at Model 1 entered Model 2 together with their (A) baseline biomarker concentrations, (B) Month 9 biomarker concentrations, and BIOSTAT risk score. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ICD, implantable cardioverter defibrillator; MAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; RASi, renin-angiotensin-system; SBP, systolic blood pressure.



and/or uptitration of guidelines recommended medical therapy, changes in concentrations of NT-proBNP and WAP-4C over the subsequent 9 months were independently associated with a reduced risk of subsequent hospitalization for HF or all-cause mortality, after adjusting for their baseline concentrations and other patient characteristics (in the form of the BIOSTAT risk score). Including changes in these biomarkers on top of baseline biomarker concentrations and the BIOSTAT risk score improved the discriminatory power from a C-statistic of 0.66 to 0.72. This may at a first glance not appear any better than that of many single measurement or composite biomarkers or risk scores (C-statistics generally in the low 0.70's in HF). However, it is notable that changes in biomarkers could achieve C-statistics above 0.70 *on top of* baseline biomarkers and risk score. Furthermore, a change over time of a biomarker may be the best surrogate to reflect a treatment effect over time. Arguably, a follow-up value alone (rather than change) may to some extent reflect a treatment effect up to that follow-up time point. However, changes in NT-proBNP (but not WAP-4C) still independently predicted the outcome on top of month-nine values and risk scores, although it added only marginally to discriminatory power.

Need for surrogate end-points in heart failure with reduced ejection fraction

The use of surrogate endpoints in RCTs is convenient and necessary in early-phase non-outcomes driven clinical trials since it reduces the sample size and thus the number of subjects exposed to interventions that may not be beneficial or may be even harmful, and reduces the trial duration from years to months and thus the overall costs. Furthermore, use of surrogate endpoints in trials provides important mechanistic insights about the intervention. However, treatments usually target multiple pathways and thus may have multiple effects, and there is continued misunderstanding of the difference between risk markers (associations) and risk factors (causality).[10,11] Consequently, assessing the efficacy of a drug

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Table 3 C-statistics for the change in biomarker + baseline biomarker + BIOSTAT risk score compared with the BIOSTAT risk score alone

Model	C-statistic	
BIOSTAT risk score[a]	0.66	Change in risk model not available
	Baseline biomarker C-statistic	**Baseline biomarker + change in biomarker (Model 1) C-statistic**
+ NT-proBNP (ng/mL)[b]	Included in risk score	0.70
+ ANP (ng/mL)	0.66	0.70
+ BNP (pg/mL)	0.66	0.70
+ CRP (ng/mL)	0.66	0.67
+ D-dimer (ng/mL)	0.66	0.66
+ GAL-3 (ng/mL)	0.66	0.66
+ GDF-15 (ng/mL)	0.66	0.69
+ Mesothelin (ng/mL)	0.66	0.66
+ Neuropilin (ng/mL)	0.66	0.68
+ NT-proCNP (pg/mL)	0.66	0.67
+ Osteopontin (ng/mL)	0.66	0.67
+ PCT (pg/mL)	0.66	0.68
+ Pentraxin-3 (ng/mL)	0.66	0.69
+ PIGR (ng/mL)	0.66	0.67
+ proADM (ng/mL)	0.66	0.68
+ RAGE (ng/mL)	0.66	0.68
+ sST2 (ng/mL)	0.66	0.70
+ Syndecan (ng/mL)	0.66	0.68
+ TNF-R1α (ng/mL)	0.66	0.67
+ Troy (ng/mL)	0.66	0.67
+ VEGFR-1 (ng/mL)	0.66	0.68
+ WAP-4C (ng/mL)	0.66	0.70
	Multivariable model with baseline biomarkers	**Multivariable model with baseline biomarker + change in biomarker (Model 2)**
+ All above biomarkers	0.63	0.71
+ WAP-4C and NT-proBNPb only	0.66	0.72

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ICD, implantable cardioverter defibrillator; MAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; RASi, renin-angiotensin-system; SBP, systolic blood pressure.

[a]The model included age, previous HF hospitalization in the last year, peripheral oedema, systolic blood pressure, estimated glomerular filtration rate (eGFR), log blood urea nitrogen (BUN), log N-terminal pro-B type natriuretic peptide (NT-proBNP), haemoglobin, sodium, high density lipoprotein (HDL) cholesterol and beta-blocker use at baseline.

[b]BIOSTAT risk model includes baseline NT-proBNP.

Table 4 C-statistics for the change in biomarker + month-nine biomarker + BIOSTAT risk score compared with the BIOSTAT risk score alone

Model	C-statistic	
BIOSTAT risk score[a]	0.66	Change in risk model not available
	Month-nine biomarker C-statistic	**Month-nine biomarker + change in biomarker (Model 3) C-statistic**
+ NT-proBNP (ng/mL)	0.70	0.71
+ ANP (ng/mL)	0.69	0.70
+ BNP (pg/mL)	0.70	0.70
	Multivariable model with month-nine biomarkers	**Multivariable model with month-nine biomarker + change in biomarker (Model 4)**
+ All above biomarkers	0.70	0.71
+ ANP and NT-proBNP[b] only	0.71	0.72

BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; ICD, implantable cardioverter defibrillator; MAP, mean arterial pressure; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PAD, peripheral artery disease; RASi, renin-angiotensin-system; SBP, systolic blood pressure.

[a]The model included age, previous HF hospitalization in the last year, peripheral oedema, systolic blood pressure, estimated glomerular filtration rate (eGFR), log blood urea nitrogen (BUN), log N-terminal pro-B type natriuretic peptide (NT-proBNP), haemoglobin, sodium, high density lipoprotein (HDL) cholesterol and beta-blocker use at baseline.

[b]BIOSTAT risk model includes baseline NT-proBNP. BNP did not enter Model 2 since collinear with NT-proBNP.

focusing only on one intermediate effect, that is, one surrogate endpoint targeting only one pathway, may lead to neglecting other beneficial or even harmful effects. Indeed, inappropriate surrogate endpoints may lead to positive phase II trials followed by neutral (or negative) phase III trials, and to negative phase II trials preventing subsequent successful phase III trials.

Thus, there is a critical need for feasible surrogate endpoints for HF trials.[1] Indeed, changes in hemodynamic measurements, quality of life, left ventricular performance and exercise capacity have been inconsistently shown to be associated with prognosis.[12,13] Among neurohormones, worse prognosis has been reported in patients with higher norepinephrine concentrations and with increasing norepinephrine concentrations over time.[14] However, RCTs showed that inotropes, although significantly reducing norepinephrine concentrations over the time, also increased the risk of mortality, limiting the role of norepinephrine as potential surrogate endpoint.[13,15,16] Both BNP and NT-proBNP concentrations have been associated with mortality and HF hospitalization risk in patients with HFrEF.[17,18] However, although meta-analyses of RCTs reported a link between a reduction in natriuretic peptides concentrations over the time and reduced risk of HF hospitalization, similar findings were not shown for mortality risk.[19,20] Additionally, whether NT-proBNP/BNP guided therapy may be a beneficial approach in HFrEF patients is still debated, with several RCTs and meta-analyses reporting contrasting results.[21,22] These observations raise important questions regarding the use of natriuretic peptides in phase II RCTs for decision making regarding phase III RCTs.

Potential surrogate end-points in heart failure with reduced ejection fraction

Previous studies have reported a prognostic role for natriuretic peptides plasma concentrations and improved prognosis associated with a reduction in natriuretic peptides concentrations over the time.[17–20,23] With sacubitril-valsartan in both PARAMOUNT (HFpEF) and PIONEER (hospitalized HFrEF), endpoints included changes in NT-proBNP over time, and reductions during treatment in these trials appear to be consistent with a benefit on morbidity and mortality in PARAGON-HF (at least in the lower spectrum of HFpEF) and in PARADIGM-HF (HFrEF).[4,5,24,25] These findings suggest both that NT-proBNP may be a useful surrogate marker, and that a change over time may be a useful way to assess that biomarker. We hypothesized that other biomarkers may be similarly or more useful when assessed as changes over time, but our findings suggest that among those tested, NT-proBNP is the best. Our analysis contributes to highlight a potential use for biomarkers linked with the cardiomyocyte stretch/injury pathophysiological domain as surrogates for hard outcomes in trials. Indeed, we showed an association between reductions in NT-proBNP concentrations over the time and improved risk of HF hospitalization/all-cause mortality after adjustment for patients' characteristics, its baseline concentration, and also baseline/changes in concentrations of all those biomarkers which entered Model 2. Additionally, changes in NT-proBNP improved discrimination for HF hospitalization/all-cause death on top of baseline concentrations. Notably, changes in NT-proBNP predicted prognosis also on top of month-nine NT-proBNP, patients' characteristics, month-nine ANP and changes in ANP (BNP did not enter the final model because collinear with NT-proBNP). Therefore, according to these data, NT-proBNP may be the preferred choice as surrogate endpoint among the other natriuretic peptides, with ANP having also other limitations such as shorter half-time (3–5 min) compared with BNP (23 min) and NT-proBNP (120 min).[26] Adding changes in NT-proBNP to a model already including month-nine NT-proBNP marginally improved discrimination for HF hospitalization/all-cause death. Thus, while follow-up NT-proBNP alone may be useful in assessing a treatment effect, adding the change over time provided additional, albeit modest, prognostic value and discrimination.[27]

Our data support a potential role also for changes in WAP-4C as prognostic marker and surrogate endpoint in HFrEF. Indeed, a reduction of WAP-4C over 9-month follow-up was independently associated with lower HF hospitalization/all-cause mortality, and adding changes in concentrations of this biomarker to a model including patient characteristics and corresponding biomarker baseline concentrations improved discrimination. However, changes in NT-proBNP did, but changes in WAP-4C did not predict prognosis on top of month-nine concentrations. Previous studies report higher WAP-4C concentrations independently predicting increased risk of HF hospitalization/all-cause death, symptoms and disease severity in HF populations including mainly HFrEF patients,[28,29] and also decreased EF in STEMI revascularized patients.[30] WAP-4C is a protein with antimicrobial and immunomodulatory properties and an accepted biomarker for ovarian carcinoma.[31] Its role in HF has not been elucidated but may be linked to inflammation and immunomodulation. Whether changes in WAP-4C concentrations over time may predict HF treatments' effect requires future investigation.

Study limitations

We tested the association between changes in 30 biomarker concentrations and outcomes, thus there could be chance of false positive findings although we did adjust for multiple testing in our main analysis. Many biomarkers were measured by a multiplexed bead-based immunoassay (Alere, San Diego, California) which has not been correlated with

commercial assays used clinically or in research. Some more recently emerging biomarkers in HFrEF, for example, CA-125, were not available at the time of this analysis in BIOSTAT-CHF. Patient follow-up ended in 2015, therefore new advances in HF therapy have not been captured in this study. Future studies should elucidate on these aspects. Finally, our analysis could be prone to mortality bias. Indeed, in a sensitivity analysis we showed that patients with two biomarker concentrations measurements were less sick as compared with those with only the baseline assessment.

Conclusions

In patients with HFrEF, among 30 biomarkers changes in NT-proBNP best predicted risk of HF hospitalization/all-cause mortality after adjustments for baseline and month-nine concentrations of NT-proBNP, the BIOSTAT risk score, that is, patient characteristics. Thus, changes over time in NT-proBNP may serve as useful surrogate markers of therapeutic response and thus surrogate endpoints in for early-phase HFrEF trials. These findings should be validated in future RCTs and such validation can be incorporated in RCT design.

Conflict of interest

GS reports grants and personal fees from Vifor, grants and non-financial support from Boehringer Ingelheim, personal fees from Societa' Prodotti Antibiotici, grants from MSD, grants and personal fees from AstraZeneca, personal fees from Roche, personal fees from Servier, grants from Novartis, personal fees from GENESIS, personal fees from Cytokinetics, personal fees from Medtronic, grants from Merck, grants from Bayer outside the submitted work. AU has nothing to disclose. WO has nothing to disclose. JT declares personal fees from Roche diagnostic and US2.ai, outside the submitted work. SDA reports receiving fees from Abbott, Bayer, Boehringer Ingelheim, Cardiac Dimension, Cordio, Impulse Dynamics, Novartis, Occlutech, Servier, and Vifor Pharma, and grant support from Abbott and Vifor Pharma. KD declares no conflict of interest related to the current work. CH reports consulting fees from Novartis and Roche Diagnostics and speaker and honoraria from MSD, supported by the Swedish Research Council [grant 20180899]. CSL is supported by a Clinician Scientist Award from the National Medical Research Council of Singapore; has received research support from Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic, and Vifor Pharma; has served as consultant or on the Advisory Board/Steering Committee/Executive Committee for Abbott Diagnostics, Amgen, Applied Therapeutics, AstraZeneca, Bayer, Biofourmis, Boehringer Ingelheim, Boston Scientific, Corvia Medical, Cytokinetics, Darma Inc., Eko.ai Pte Ltd, JanaCare, Janssen Research & Development LLC, Medtronic, Menarini Group, Merck, MyoKardia, Novartis, Novo Nordisk, Radcliffe Group Ltd., Roche Diagnostics, Stealth BioTherapeutics, The Corpus, Vifor Pharma and WebMD Global LLC; and serves as co-founder & non-executive director of EKo.ai Pte Ltd. CCL received fees and/or research grants from AstraZeneca, Boehringher Ingelheim, MSD, Menarini Pharma, Novartis, Nono Nordisk, Vifor Pharma, outside the submitted work.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline patient characteristics of patients with and without repeat measurements (i.e. both at Baseline and Month 9).

Figure S1. Flow chart reporting patients' inclusion.

Figure S2. Statistically significant associations between continuous percent changes in biomarkers concentrations from Baseline to Month 9 and subsequent risk of heart failure (HF) hospitalization/all-cause death, adjusted for the BIOSTAT risk score and baseline biomarker concentrations but not for other biomarkers (Model 1).

Figure S3. Non-statistically significant associations between continuous percent changes in biomarkers concentrations from Baseline to Month 9 and subsequent risk of heart failure (HF) hospitalization/all-cause death, adjusted for the BIOSTAT risk score and baseline biomarker concentrations but not for other biomarkers (Model 1).

Figure S4. Statistically significant associations between continuous percent changes in biomarkers concentrations from Baseline to Month 9 and subsequent risk of heart failure (HF) hospitalization/all-cause death, adjusted for the BIOSTAT risk score and Month 9 biomarker concentrations but not for other biomarkers (Model 3).

Figure S5. Non-statistically significant associations between continuous percent changes in biomarkers levels from Baseline to Month 9 and subsequent risk of heart failure (HF) hospitalization/all-cause death, adjusted for the BIOSTAT risk score and month 9 biomarker levels but not for other biomarkers (Model 3).

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