

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

Gianluigi Savarese^{1*†}, Alicia Ujjl^{1,2†}, Wouter Ouwerkerk^{3,4}, Jasper Tromp^{3,5,6,7}, Stefan D. Anker⁸, Kenneth Dickstein^{9,10}, Camilla Hage¹, Carolyn S.P. Lam^{3,6}, Chim C. Lang¹¹, Marco Metra¹², Leong L. Ng¹³, Nicola Orsini¹⁴, Nilesh J. Samani¹³, Dirk J. van Veldhuisen⁷, John G.F. Cleland¹⁵, Adriaan A. Voors⁷ and Lars H. Lund¹

¹Division of Cardiology, Department of Medicine, Karolinska Institutet, Stockholm, Sweden; ²Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands; ³National Heart Centre Singapore, Singapore; ⁴Department of Dermatology, Amsterdam UMC, University of Amsterdam, Amsterdam Infection & Immunity Institute, Amsterdam, The Netherlands; ⁵Saw Swee Hock School of Public Health, National University of Singapore, Singapore; ⁶Duke-NUS Medical School, Singapore; ⁷Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; ⁸Department of Cardiology (CVK); and Berlin Institute of Health Center for Regenerative Therapies (BCRT); German Centre for Cardiovascular Research (DZHK) partner site Berlin, Charité Universitätsmedizin Berlin, Berlin, Germany; ⁹Stavanger University Hospital, Stavanger, Norway; ¹⁰University of Bergen, Bergen, Norway; ¹¹Division of Molecular and Clinical Medicine, University of Dundee, Dundee, UK; ¹²Cardiology Unit, ASST Spedali Civili and Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy; ¹³Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, and NIHR Leicester Biomedical Research Centre, Leicester, UK; ¹⁴Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden; ¹⁵Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow, Glasgow and National Heart & Lung Institute, Imperial College, London, UK

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

Received: 20 December 2021; Revised: 4 March 2022; Accepted: 14 March 2022

*Correspondence to: Gianluigi Savarese, Department of Medicine, Cardiology Unit, Karolinska Institutet, Karolinska University Hospital, S1:02 171 77 Stockholm, Sweden. Email: gianluigi.savarese@ki.se

†These authors contributed equally as first authors.

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 $EF \leq 40\%$ 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 $\leq 50\%$ 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 ($EF \leq 40\%$) 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-adrenomedullin (proADM), Receptor for advanced glycation end product (RAGE), Soluble ST2 (sST2), Syndecan-1, Troy, Tumour necrosis factor-receptor 1 α (TNF-R1 α), 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 (LT β R), 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, TNFR1 α , NT-proBNP, and WAP-4C, together with their baseline concentration and the BIOSTAT risk score. We excluded TNFR1 α 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; LT β R, 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-adrenomedullin; PSAP- β , prosaposin- β ; RAGE, receptor for advanced glycation end product; sST2, soluble ST2; TNF-R1 α , tumour necrosis factor-receptor 1 α ; VEGFR-1, vascular endothelial growth receptor; WAP-4C, WAP 4-disulphide core domain protein HE.

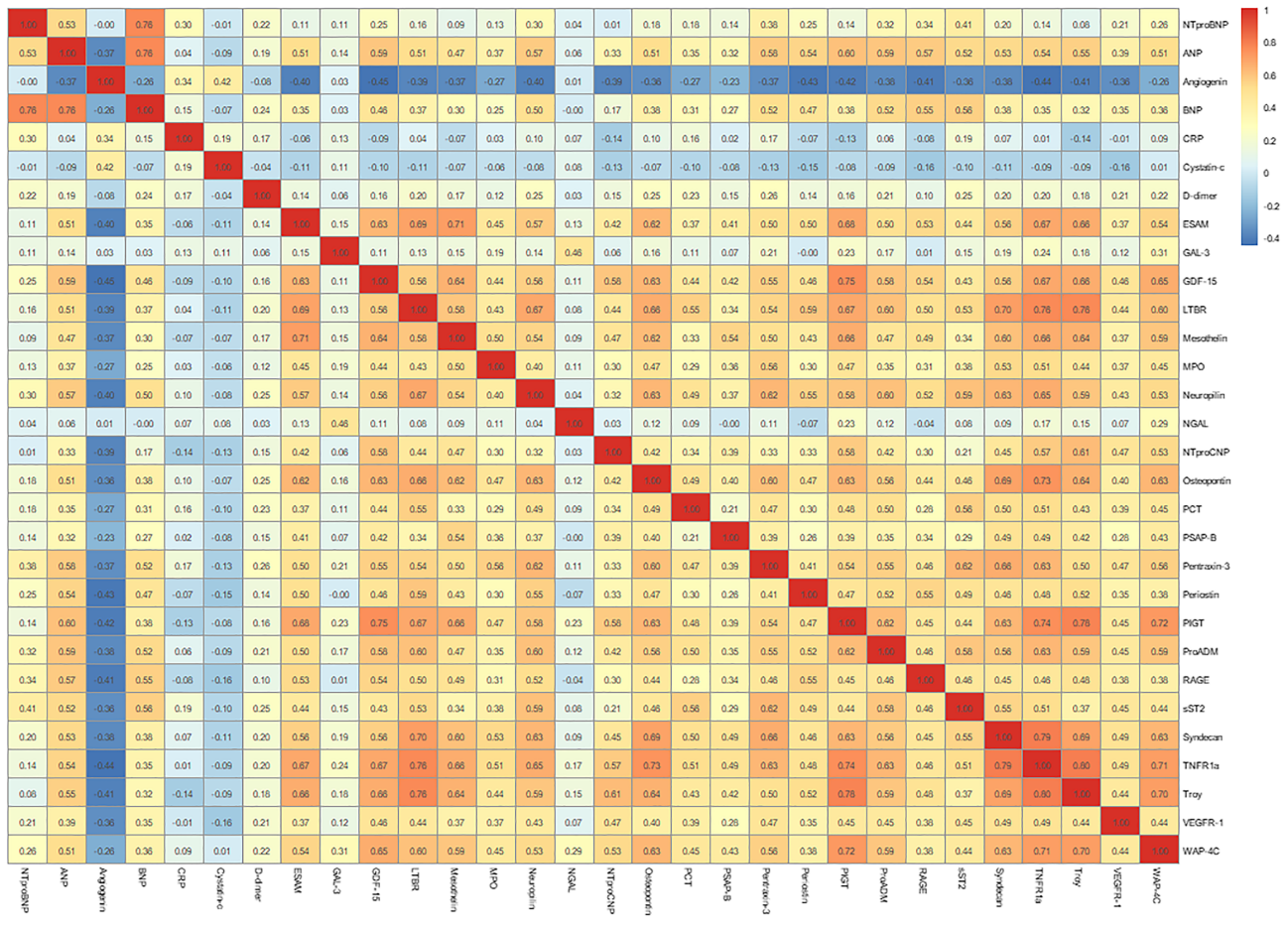
risk score in Model 3 (with a P -value <0.10), were ANP, BNP, and NT-proBNP (Supporting Information, *Figure S4*), whereas changes in other biomarkers did not predict the outcome after adjustment for their respective month-9 concentrations (Supporting Information, *Figure S5*). In Model 4, only decreasing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

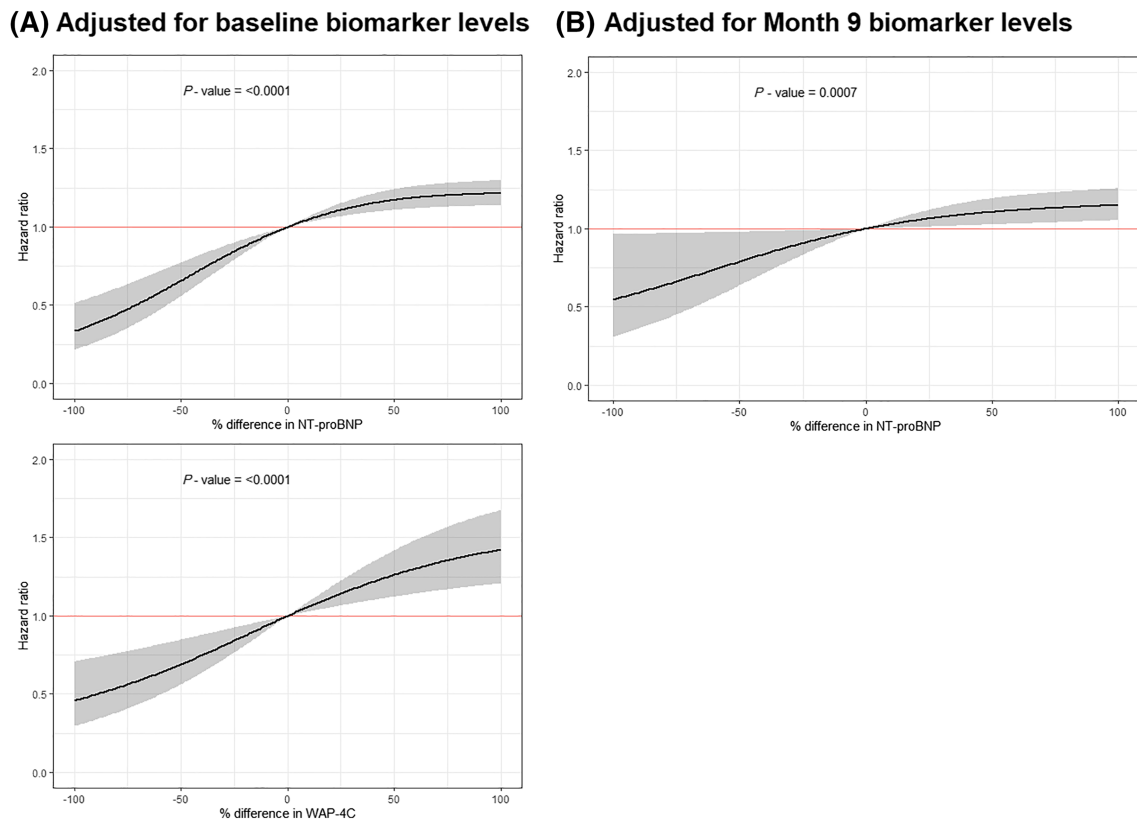
Sensitivity analysis

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

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

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	Baseline biomarker + change in biomarker (Model 1)
	C-statistic	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-R1 α (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-proBNP 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.

^aThe 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.

^bBIOSTAT 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	Month-nine biomarker + change in biomarker (Model 3)
	C-statistic	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.

^aThe 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.

^bBIOSTAT 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, Boehringer Ingelheim, MSD, Menarini Pharma, Novartis, Novo Nordisk, Vifor Pharma, outside the submitted work.

MM reports personal fees from Actelion, Amgen, AstraZeneca, Abbott vascular, Bayer, Servier, Edwards Therapeutics, Livanova, Vifor pharma, WindTree Therapeutics, as member of Trials' Committees or advisory boards or for speeches at sponsored meetings in the last 3 years. LLN has no conflict of interest related to the current work. NO has no conflict of interest related to the current work. NJS has no conflict of interest related to the current work. DJV: has no conflict of interest related to the current work. JGFC reports personal fees from Abbott, personal fees from Amgen, grants and personal fees from Bayer, grants and personal fees from Bristol Myers Squibb, personal fees from Novartis, personal fees from Medtronic, personal fees from Idorsia, grants and personal fees from Vifor, grants and personal fees from Pharmacosmos, grants and personal fees from Cytokinetics, personal fees from Servier, personal fees and non-financial support from Boehringer-Ingelheim, personal fees from Astra-Zeneca, personal fees from Innolife, personal fees from Torrent, grants and personal fees from Johnson & Johnson, grants and personal fees from Myokardia, personal fees from Respicardia, grants and personal fees from Stealth Biopharmaceuticals, grants and personal fees from Viscardia, outside the submitted work.

AAV acted as a consultant for Amgen, Bayer AG, Boehringer Ingelheim, Cytokinetics, Merck, Myokardia, Novartis, and received research support from NovoNordisk, Roche Diagnostics. LHL reports consulting fees from Lexicon, Merck, Pharmacosmos, and Myokardia, lecture fees and consulting fees from Vifor, Bayer, Medscape, grant support, lecture fees, and consulting fees AstraZeneca, and Novartis, grant support and consulting fees from Relypsa and Boehringer Ingelheim, grant support from Boston Scientific, and lecture fees from Abbot.

Funding

BIOSTAT-CHF was funded by a grant from the European Commission (FP7-242209-BIOSTAT-CHF; EudraCT 2010-020808-29). This study has been partially funded by the EU/EFPIA Innovative Medicines Initiative 2 Joint Undertaking BigData@Heart grant no. 116074. LHL was supported by Karolinska Institutet, the Swedish Research Council [grant 523-2014-2336], the Swedish Heart Lung Foundation [grants

20150557, 20190310], and the Stockholm County Council [grants 20170112, 20190525].

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).

References

- Solomon SD, Pfeffer MA. The future of clinical trials in cardiovascular medicine. *Circulation*. 2016; **133**: 2662–2670.
- Zannad F, Garcia AA, Anker SD, Armstrong PW, Calvo G, Cleland JG, Cohn JN, Dickstein K, Domanski MJ, Ekman I, Filippatos GS, Gheorghide M, Hernandez AF, Jaarsma T, Koglin J, Konstam M, Kupfer S, Maggioni AP, Mebazaa A, Metra M, Nowack C, Pieske B, Pina IL, Pocock SJ, Ponikowski P, Rosano G, Ruilope LM, Ruschitzka F, Severin T, Solomon S, Stein K, Stockbridge NL, Stough WG, Swedberg K, Tavazzi L, Voors AA, Wasserman SM, Woehrl H, Zaleski A, McMurray JJ. Clinical outcome endpoints in heart failure trials: A European Society of Cardiology Heart Failure Association consensus document. *Eur J Heart Fail*. 2013; **15**: 1082–1094.
- Bikdeli B, Punnanithinont N, Akram Y, Lee I, Desai NR, Ross JS, Krumholz HM. Two decades of cardiovascular trials with primary surrogate endpoints: 1990–2011. *J Am Heart Assoc*. 2017; **6**.
- Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, Shi V, Bransford T, Takeuchi M, Gong J, Lefkowitz M, Packer M, McMurray JJ. Prospective comparison of AwARBoMOhfwpefl. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: A phase 2 double-blind randomised controlled trial. *Lancet*. 2012; **380**: 1387–1395.
- Velazquez EJ, Morrow DA, DeVore AD, Duffy CI, Ambrosy AP, McCague K, Rocha R, Braunwald E, PIONEER-HF Investigators. Angiotensin-Nepriylsin inhibition in acute decompensated heart failure. *N Engl J Med*. 2019; **380**: 539–548.
- Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng L, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Zwiderman AH, Metra M. A systems BIOlogy study to Tailored treatment in chronic heart failure: Rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail*. 2016; **18**: 716–726.
- McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Kober L, Lip GY, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Ronnevik PK, Rutten FH, Schwitzer J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A, Guidelines ESCCfP. 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**: 1787–1847.
- Voors AA, Ouwerkerk W, Zannad F, van Veldhuisen DJ, Samani NJ, Ponikowski P, Ng LL, Metra M, Ter Maaten JM, Lang CC, Hillege HL, van der Harst P, Filippatos G, Dickstein K, Cleland JG, Anker SD, Zwiderman AH. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail*. 2017; **19**: 627–634.
- Chen SY, Feng Z, Yi X. A general introduction to adjustment for multiple comparisons. *J Thorac Dis*. 2017; **9**: 1725–1729.
- Lund LH, Stehlik J. Risk scores and biomarkers in heart failure: A journey to predictive accuracy and clinical utility. *J Heart Lung Transplant*. 2016; **35**: 711–713.
- Lund LH, Gabrielsen A. Biomarkers in advanced heart failure--pathophysiology leading to clinical use? *J Heart Lung Transplant*. 2014; **33**: 1213–1214.
- Ciani O, Piepoli M, Smart N, Uddin J, Walker S, Warren FC, Zwisler AD, Davos CH, Taylor RS. Validation of exercise capacity as a surrogate endpoint in exercise-based rehabilitation for heart failure: A meta-analysis of randomized controlled trials. *JACC Heart Fail*. 2018; **6**: 596–604.
- Anand IS, Florea VG, Fisher L. Surrogate endpoints in heart failure. *J Am Coll Cardiol*. 2002; **39**: 1414–1421.
- Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G, Cohn JN, Val-He FTI. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the valsartan heart failure trial (Val-HeFT). *Circulation*. 2003; **107**: 1278–1283.
- Hampton JR, van Veldhuisen DJ, Kleber FX, Cowley AJ, Ardia A, Block P, Cortina

- A, Cserhalmi L, Follath F, Jensen G, Kayanakis J, Lie KI, Mancina G, Skene AM. Randomised study of effect of ibopamine on survival in patients with advanced severe heart failure. Second prospective randomised study of ibopamine on mortality and efficacy (PRIME II) Investigators. *Lancet*. 1997; **349**: 971–977.
16. Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M, Wiltse C, Wright TJ, Investigators M. Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). *Eur J Heart Fail*. 2003; **5**: 659–667.
 17. Savarese G, Orsini N, Hage C, Dahlstrom U, Vedin O, Rosano GMC, Lund LH. Associations with and prognostic and discriminatory role of N-terminal pro-B-type natriuretic peptide in heart failure with preserved versus mid-range versus reduced ejection fraction. *J Card Fail*. 2018; **24**: 365–374.
 18. York MK, Gupta DK, Reynolds CF, Farber-Eger E, Wells QS, Bachmann KN, Xu M, Harrell FE Jr, Wang TJ. B-type natriuretic peptide levels and mortality in patients with and without heart failure. *J Am Coll Cardiol*. 2018; **71**: 2079–2088.
 19. Savarese G, Musella F, D'Amore C, Vassallo E, Losco T, Gambardella F, Cecere M, Petraglia L, Pagano G, Fimiani L, Rengo G, Leosco D, Trimarco B, Perrone-Filardi P. Changes of natriuretic peptides predict hospital admissions in patients with chronic heart failure. A meta-analysis. *JACC: Heart Failure*. 2014; **2**: 148–158.
 20. Vaduganathan M, Claggett B, Packer M, McMurray JJV, Rouleau JL, Zile MR, Swedberg K, Solomon SD. Natriuretic peptides as biomarkers of treatment response in clinical trials of heart failure. *JACC: Heart Failure*. 2018; **6**: 564–569.
 21. Savarese G, Trimarco B, DelleGrottaglie S, Prastaro M, Gambardella F, Rengo G, Leosco D, Perrone-Filardi P. Natriuretic peptide-guided therapy in chronic heart failure: A meta-analysis of 2,686 patients in 12 randomized trials. *PLoS ONE*. 2013; **8**: e58287.
 22. Felker GM, Anstrom KJ, Adams KF, Ezekowitz JA, Fiuzat M, Houston-Miller N, Januzzi JL Jr, Mark DB, Pina IL, Passmore G, Whellan DJ, Yang H, Cooper LS, Leifer ES, Desvigne-Nickens P, O'Connor CM. Effect of natriuretic peptide-guided therapy on hospitalization or cardiovascular mortality in high-risk patients with heart failure and reduced ejection fraction: A randomized clinical trial. *JAMA*. 2017; **318**: 713–720.
 23. Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, Coats AJS, Metra M, Mebazaa A, Ruschitzka F, Lainscak M, Filippatos G, Seferovic PM, Meijers WC, Bayes-Genis A, Mueller T, Richards M, Januzzi JL Jr, Heart Failure Association of the European Society of Cardiology. Practical guidance on the use of natriuretic peptide concentrations. *Eur J Heart Fail*. 2019; **21**: 715–731.
 24. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, Martinez F, Packer M, Pfeffer MA, Pieske B, Redfield MM, Rouleau JL, van Veldhuisen DJ, Zannad F, Zile MR, Desai AS, Claggett B, Jhund PS, Boytsov SA, Comin-Colet J, Cleland J, Düngen HD, Goncalvesova E, Katova T, Kerr Saraiva JF, Lelonek M, Merkely B, Senni M, Shah SJ, Zhou J, Rizkala AR, Gong J, Shi VC, Lefkowitz MP, PARAGON-HF Investigators and Committees. Angiotensin-Nephrilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med*. 2019; **381**: 1609–1620.
 25. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR, PARADIGM-HF Investigators and Committees. Angiotensin-nephrilysin inhibition versus enalapril in heart failure. *N Engl J Med*. 2014; **371**: 993–1004.
 26. Felker GM, Petersen JW, Mark DB. Natriuretic peptides in the diagnosis and management of heart failure. *CMAJ*. 2006; **175**: 611–617.
 27. Kubanek M, Goode KM, Lanska V, Clark AL, Cleland JG. The prognostic value of repeated measurement of N-terminal pro-B-type natriuretic peptide in patients with chronic heart failure due to left ventricular systolic dysfunction. *Eur J Heart Fail*. 2009; **11**: 367–377.
 28. de Boer RA, Cao Q, Postmus D, Damman K, Voors AA, Jaarsma T, van Veldhuisen DJ, Arnold WD, Hillege HL, Silljé HHW. The WAP four-disulfide Core domain protein HE4: A novel biomarker for heart failure. *JACC: Heart Failure*. 2013; **1**: 164–169.
 29. Piek A, Meijers WC, Schrotten NF, Gansevoort RT, de Boer RA, Silljé HH. HE4 serum levels are associated with heart failure severity in patients with chronic heart failure. *J Card Fail*. 2017; **23**: 12–19.
 30. Kilci H, Altinbilek E, Cetinkal G, Sigirci S, Kocas BB, Yildiz SS, Kiliçkesmez KO. Relation of a novel fibrosis marker and post-myocardial infarction left ventricular ejection fraction in revascularized patients. *Biomark Med*. 2021; **15**: 1651–1658.
 31. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, Drescher C, Urban N, Hellstrom KE. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res*. 2003; **63**: 3695–3700.