The incremental prognostic and clinical value of multiple novel biomarkers in heart failure

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Abstract

Aims: In recent years there has been an increase in the number of biomarkers in heart failure(HF). The clinical role for these novel biomarkers in combination is not clear.

Methods: The following novel biomarkers were measured from 628 patients recently hospitalised with decompensated HF; mid regional pro-adrenomedullin(MR-proADM), mid regional pro-atrial natriuretic peptide(MR-proANP), copeptin, high sensitivity cardiac troponin T(hs-cTnT), ST2, galectin-3, cystatin C, combined free light chains(cFLC) and high sensitivity C-reactive protein(hsCRP). The incremental prognostic value of these novel biomarkers was evaluated within an extensive model containing established predictors of mortality.

Results: During a mean(SD) follow-up of 3.2(1.5) years, 290(46%) patients died. Elevated concentrations of all of the novel biomarkers were associated with an increased unadjusted risk of mortality but only two-thirds were independent predictors following multivariable analysis. Using dichotomized cut-points from ROC analysis, MR-proADM, hs-cTnT, cFLC, hsCRP and ST2 remained independent predictors of mortality. Further dichotomization into low(0-2 elevated biomarkers) or high(at least 3 of the 5 biomarkers elevated) risk groups provided greatest incremental prognostic value(HR 2.20; 95%CI, 1.37 to 3.54; p=0.001) and improved the predictive power of the model(C-statistic 0.730 from 0.721, NRI 32.5%).

Conclusion: The novel biomarkers included in this study added little, if any, incremental prognostic value on their own to an extensive model containing established predictors of

mortality. However, following dichotomization, 5 of the novel biomarkers provided incremental prognostic value. There was a clear gradient in the risk of death with increasing numbers of elevated novel biomarkers; the presence of at least 3 identifying patients at greatest mortality risk.

Key words: Heart failure, prognosis, novel biomarkers, risk stratification

Introduction

An accurate means of predicting mortality risk in heart failure (HF) would allow clinicians to have an honest and informed discussion with patients regarding their prognosis (1). Those at low risk could be reassured. Those at high risk of dying could be considered for complex devices (including an implantable cardioverter-defibrillator - ICD), cardiac transplantation, or optimal end of life care (2).

In recent years there has been a remarkable increase in the number of biomarkers available in HF (3), many of which have been thought to hold prognostic potential (4, 5). Yet understanding of their relevance and clinical value for patients with HF in real world populations remains limited. Different biomarkers represent different pathophysiological pathways. The use of multiple biomarkers (that reflect different pathophysiological processes) in combination may be of greater prognostic value than using them in isolation. Many of these putative prognostic markers have been tested in small, selected populations with limited multivariable analyses. To date, no study has evaluated the prognostic value of multiple novel biomarkers, representing all the known pathophysiological pathways, in combination in a real-life, unselected chronic HF population.

We studied the prognostic value of several contemporary biomarkers, spanning several pathophysiological pathways in HF, in a prospective cohort of patients recently hospitalised with decompensated HF. Candidate biomarkers from several classes were studied: **(i) neurohormonal** (mid regional pro-adrenomedullin [MR-proADM], mid regional pro-atrial natriuretic peptide [MR-proANP] and copeptin), **(ii) myocyte injury**

and apoptosis (high sensitivity cardiac troponin T [hs-cTnT]), **(iii) myocyte stress and remodelling** (ST2), **(iv) extracellular-matrix remodelling** (galectin-3), **(v) extra-cardiac involvement** (cystatin C and combined free light chains [cFLC]) and **(vi) inflammation** (cFLC and high sensitivity C-reactive protein [hsCRP]).

Methods

Our study was approved by the Local Ethics Committee and complied with the Declaration of Helsinki. All patients provided written informed consent.

Study design

The study design has previously been reported (6). Briefly, we enrolled 1003 near-consecutive patients with decompensated HF from three hospitals and defined HF according to the criteria of the European Society of Cardiology (7). Eligible patients were required to be 18 years or older and an elevated B-type natriuretic peptide (BNP >100pg/ml) was mandatory. The main exclusion criteria were: primary presentation with myocardial infarction (MI), concurrent systemic disease likely to result in reduced life expectancy or cognitive impairment. Attendance for the study visit occurred 1 month post-discharge. Of 1003 patients originally enrolled, 648 patients (65%) attended the study visit. Failure to attend was due to death (n=115, 11%), deterioration in health (n=73, 7%) or withdrawal of consent (n=167, 17%).

Laboratory measurements – biomarkers studied

Whole blood was drawn from venepuncture into serum and plasma vacutainers. Samples were processed immediately by centrifugation at 3000g for 15 minutes and serum and plasma fractions were aliquoted for storage at -80°C until assay. Copeptin, MR-proADM and MR-proANP were measured on an automated BRAHMS Kryptor analyzer (BRAHMS, Hemel Hempstead, UK) with limits of detection 4.8pmol/l, 0.05nmol/l and 6pmol/l, respectively. Assay precision (coefficient of variation [CV]) ranged between 2.2-3.2%, 3.5-6.1% and 2.1-13.5% for MR-proANP, MR-proADM and copeptin, respectively. Hs-cTnT was measured on an automated e411 (Roche, Burgess Hill, UK), with a limit of detection of 3ng/ml and CV range of 1.2-5.5%. ST2 was measured using an enzyme-linked immunosorbent assay (ELISA) (R&D systems, Oxon, UK), with a limit of sensitivity of 0.1ng/ml and CV range of 4.4-10.6%. Galectin-3 was measured in our laboratory using an ELISA (provided by BG Medicine, MA, USA) according to the manufacturer's instructions with a limit of sensitivity of 1.3ng/ml and CV range of 3.2-6.2%. cFLC were measured by turbidimetry using the Combylite™ immunoassay on a SPAPLUS® automated analyzer (The Binding Site Group, Ltd, Birmingham, UK), with a limit of quantification of 0.63mg/l and CV range of 5.5-14.4%. Cystatin C was measured on a SPAPLUS® automated analyzer (The Binding Site Group, Ltd, Birmingham, UK), with a limit of detection of 0.4mg/l and CV range of 5.4-9.4%. hsCRP was measured using a Siemens immunoassay on a Siemens BN II™ nephelometer, with a limit of detection of 0.03mg/ml and CV range of 2.5-5.7.

Plasma BNP was measured using an Abbott Architect assay (Abbott Diagnostics, Maidenhead, UK). Along with BNP, all other biochemical and hematological assays were

performed in local National Health Service laboratories in Glasgow, UK, and these assays all performed adequately in the relevant national external quality assurance schemes.

Left ventricular ejection fraction

Left ventricular ejection fraction (LVEF) was measured by two-dimensional echocardiography and reduced systolic function was defined as LVEF <50% (8). Analysis was performed offline, using the biplane method of discs (modified Simpson's rule) by a single operator blinded to patient information.

Follow-up

All patients consented to be "flagged" with the Information Services Division (ISD) of the Scottish Health Service for data on in-hospital and out-of hospital deaths, held by the General Register Office for Scotland. The primary outcome measure was all-cause mortality.

Statistical Analysis

Survival time was calculated from the date of the study visit (between 16th January 2007 and 6th March 2009) until death or censoring at 31st August 2012. Univariate Cox regression analyses were performed for all novel biomarkers. All continuous variables were transformed as appropriate to normalize their distributions. The incremental prognostic value of each novel biomarker was evaluated using Cox proportional hazard models including established predictors of outcome in heart failure. The predictors

included in the baseline model were selected from the clinical model derived by the Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) investigators (9) and routine hematological and biochemical variables predictive of outcome in the subsequent CHARM analyses (10,11). These established predictors of outcome included: age, sex (men *vs* women), smoking habit (current *vs* none or past), history of HF for more than 2 years, NYHA class (III/IV vs I/II), LVEF, medical history (MI, diabetes mellitus, chronic obstructive pulmonary disease, peripheral arterial disease), left bundle branch block (LBBB) on electrocardiogram, heart rate, systolic blood pressure, body mass index, peripheral edema, bilirubin, urate, creatinine, hemoglobin, glycosylated hemoglobin, lymphocytes and red cell distribution width (RDW). B-type natriuretic peptide (BNP) was also included in the baseline model. Multivariable Cox regression analyses were performed for each novel biomarker in turn. Model performance was assessed using Harrel's C-statistic (12). The proportional hazards assumption was assessed using Shoenfield residuals. Each biomarker was dichotomized using receiver operating characteristic (ROC) curves with cut-points determined using the Kaplan Meier method (13). Univariate and multivariable analyses were performed as before using the dichotomized results. A p-value d 0.1 was considered significant. The effect of multiple elevated biomarkers was then evaluated for 1, 2 and 3 or more elevated biomarkers using univariate and multivariable analysis. Kaplan-Meier survival curves were constructed to illustrate survival of patients according to number of elevated novel biomarkers, dichotomized from ROC analysis. Curves were compared using the log rank test. All statistical analyses were performed using R version 2.15.0 or above. The net reclassification index (NRI) (14), adapted for use in survival models (15),

was used to assess if elevated levels of 3 or more novel biomarkers improved the prediction of outcome, in addition to the multivariable model. Internal validation was performed by bootstrapping, using Somers' Dxy rank correlation as the marker of validation, to evaluate the predictive performance of the data.

Results

Of 648 patients completing the study visit, 628 (97%) had analysis of the entire set of novel biomarkers performed. The mean (SD) age of the overall cohort was 71 (11) years and 367 (58%) were male. The baseline clinical characteristics, including novel biomarker results, are presented in Table 1 for the overall cohort.

Overall survival following hospital admission with decompensated heart failure

The mean (SD) follow-up was 3.2 (1.5) years. Of the 628 patients with novel biomarker measurements, 290 (46%) died during the follow-up period. The multivariable analysis for the base model is shown in Table 2.

Unadjusted outcomes according to novel biomarker concentration

Elevated concentrations of all of the novel biomarkers were associated with a higher unadjusted risk of mortality (Table 3). This heightened risk was particularly evident in patients with elevated log [MR-proADM] and log [cystatin C] concentrations, with more than double an increased unadjusted risk (hazard ratios [HR] 2.83 and 2.72, respectively).

Multivariable analysis with the novel biomarkers as continuous variables

The multivariable analyses with each biomarker added individually to the baseline model are displayed in Table 4 (the full models are shown in Table 1 of the Appendix). In the base model, almost half of the predefined variables were independent predictors of an increased risk of death with a C-statistic for the overall model of 0.721 (Table 2). In the multivariable analyses, MR-proADM, hs-cTnT, ST2, cFLC, hsCRP and cystatin C remained independent predictors of mortality (defined as p d 0.1). Copeptin, MR-proANP and galectin-3 failed to add incremental prognostic value to the base model. Elevated MR-proADM and cystatin C concentrations were associated with the highest HRs, with almost double the mortality risk per log[unit] increase (HR 1.81 and p<0.01; HR 1.99 and p=0.05, respectively). In terms of adding to the prognostic strength of the overall model, hsCRP had the greatest effect (HR 1.21, p<0.001) increasing the C-statistic from 0.721 to 0.727.

Multivariable analyses using dichotomized biomarkers

Each biomarker was dichotomized using receiver operating characteristic (ROC) curves; the cut-points are displayed in Table 3. Univariate and multivariable analyses were performed as before using the dichotomized results (Tables 3 and 4). The unadjusted outcomes were similar to the continuous values, with higher levels of the entire set of dichotomized novel biomarkers associated with a higher risk of mortality (all p values <0.001). Most biomarkers were associated with an even greater risk of mortality as a dichotomized variable, than as a continuous variable, with MR-proADM and hs-cTnT associated with more than double the risk of death. Following multivariable analysis, MR-proADM, hs-cTnT, ST2, cFLC and hsCRP remained independent predictors of

mortality (defined as p d 0.1), with increased adjusted mortality risk ranging from 29-64% for these 5 novel biomarkers. In terms of adding to the prognostic strength of the overall model, cFLC had the greatest effect (HR 1.64, p<0.001) increasing the C-statistic from 0.721 to 0.729.

Number of elevated novel biomarkers - prognostic significance

Of 628 patients, 130 (20.7%) had none of the 5 significant novel biomarkers (MR-proADM, hs-cTnT, ST2, cFLC and hsCRP) elevated above the ROC cut-points and 251 (40%) had one or two elevated biomarkers. A similar proportion (n=247; 39%) of patients had ≥3 elevated biomarkers. Of these 247 patients, a similar number of patients had 3 (n=113, 45.7%) or 4 (n=103, 41.7%) elevated biomarkers, whilst few patients had an elevation of all 5 biomarkers (n=31, 12.6%). Of those with ≥3 elevated biomarkers, MR-proADM was elevated in nearly all cases (n=230, 93.1%) with hs-cTnT being the next frequently elevated biomarker (n=208, 84.2%). Similar proportions of patients had elevated hsCRP (n=180, 72.9%) and cFLC (n=172 (69.6%) whilst less than half of all patients had an elevated ST2 (n=116, 47.0%). The breakdown of all combinations of ≥3 elevated biomarkers are displayed in the appendix (Appendix Table 3). Survival curves demonstrated an increasing unadjusted mortality risk with increasing numbers of elevated biomarkers (Figure 1a), with a clear step up in mortality risk for patients with three or more elevated biomarkers (Figure 1b) who had almost four times the risk of death compared to patients with no elevated biomarkers - 3 year mortality rate for number of biomarkers elevated: 0 = 18.5%, 1 = 25.7%, 2 = 33.0% and e3 = 51.8% (Table 5). The presence of 3 or more elevated biomarkers remained an independent predictor of higher

mortality risk following multivariable analysis, with over double the mortality risk compared to patients without elevation of any of the 5 biomarkers (HR 2.10; p=0.002) (full multivariable model in Appendix Table 4).

Net reclassification index (NRI) for the addition of 3 or more elevated biomarkers to the multivariable model, at 3 years follow up, was high with an overall NRI of 0.33 (95% CI 0.16, 0.49; p<0.001). Validation by bootstrap (300 replicates) resulted in an optimism of 2%.

There were many differences in clinical characteristics according to number of biomarkers elevated (Appendix Table 5). Compared with patients with no elevated or 1-2 elevated biomarkers, patients with 3 or more elevated biomarkers were older, more often male and to have a previous diagnosis of HF. They were more symptomatic, with a greater proportion of patients in NYHA class III/IV and more had peripheral oedema. Patients with 3 or more elevated biomarkers also had more comorbidities such as myocardial infarction, hypertension, diabetes mellitus and atrial fibrillation.

Discussion

We compared the prevalence and prognostic significance of a range of novel biomarkers, individually and collectively, in a group of patients recently hospitalized with decompensated HF. Our patients had a high mortality rate, with 46% of the cohort deceased by the end of the follow-up period. The first notable, but perhaps unsurprising, finding was that all of these biomarkers individually were predictors of unadjusted mortality risk in this relatively unselected cohort. However, only half of the novel

biomarkers were independent predictors of mortality when added to a multivariable prognostic model containing established predictors of mortality risk, including BNP. Copeptin, MR-proANP and galectin-3 failed to add any incremental prognostic value to this base model. The second important finding was that, following dichotomization of the novel biomarkers, the presence of at least 3 elevated biomarkers identified patients at greatest risk of death. The predictive value of 3 or more elevated biomarkers remained after adjustment for other prognostic factors in the multivariable model - such patients had more than double the adjusted risk of death compared to patients with no elevation of any of these novel biomarkers. Furthermore, elevation of at least 3 novel biomarkers improved net reclassification in addition to an extensive multivariable model.

Novel biomarkers individually have limited incremental prognostic value

Heart failure is a complex syndrome involving many pathophysiological pathways, the components of which can be represented by various biomarkers. The main recognized pathways are the neurohormonal, cardiomyocyte injury, cardiomyocyte stress, remodelling and inflammatory processes, as well as extra-cardiac influences. To our knowledge this is the first study to study the prognostic value of multiple novel candidate biomarkers together, with representation from all of the main pathophysiological pathways.

The baseline model had reasonably good predictive power (C-statistic higher than 0.7) prior to the addition of any of the novel biomarkers as continuous variables. Although MR-proADM and ST2 provided some incremental prognostic information to the baseline

model, the importance of inflammation and extra-cardiac processes was also apparent with hsCRP and cFLC demonstrating independent predictive value. However, *individually*, these biomarkers only modestly improved the predictive power of the model. This may suggest that evaluation of one pathophysiological pathway may not give sufficient prognostic information to be clinically valuable (or that particular pathways may be more or less important in particular individuals).

Dichotomized cut-points – similar values to published thresholds of risk

The potential clinical value of the novel biomarkers was more apparent using dichotomized cut-points from the ROC analysis, especially when more than one biomarker was evaluated simultaneously (see below). Dichotomization into low- (0-2 elevated biomarkers) or high- (at least 3 elevated biomarkers) risk groups provided greatest incremental prognostic value statistically, and possibly a more clinically meaningful result for the practicing clinician. The cut-points yielded from ROC analysis were similar to the thresholds of risk that have previously been published for the individual biomarkers. A previous study of copeptin, MR-proADM and MR-proANP in a small group of patients with acute decompensated HF, (n=137), found similar concentrations to the present study for identifying low and high risk of 1 year mortality (16). Our dichotomized cut-point for hs-cTnT (21.9pg/ml) is extremely close to the level (20pg/ml) that has previously been shown to identify patients with acute decompensated HF at greatest risk of all-cause mortality (17). In that study patients hospitalized with HF secondary to acute myocardial infarction were also excluded, akin to our study, and follow-up exceeded one year. A large, multicenter study of patients with chronic systolic

HF (18) found median values of ST2 similar to our dichotomized cut-point (27.5ng/ml *vs* 28.4ng/ml). Patients in the highest tertile (ST2>36.3ng/ml) were at greatest risk of mortality or cardiac transplantation, over a similar median follow-up period to our study. hs-CRP has recently been studied in a large, randomized controlled clinical trial in acute decompensated HF (19). Interestingly, hs-CRP levels that had increased from baseline during hospitalization to 30 days post-discharge were associated with a greater 180-day mortality but elevated levels during admission were not. Although a threshold of risk for hs-CRP at 30-days post hospitalization is not reported, the median value (4.57mg/l) is close to our dichotomized cut-point (6.0mg/l). We have recently reported the incremental prognostic value of cFLC in HF (20), although little else is known about the potential role of this biomarker in patients with HF.

'The old and the new' – a simple multi-marker approach

An elevation of at least three out of five of the novel biomarkers significant in the dichotomized analysis (MR-proADM, hs-cTnT, ST2, cFLC and hsCRP) provided incremental prognostic value when added to a multivariable model containing established predictors of mortality in patients with HF, including BNP. Few novel markers have previously added incremental predictive information to comprehensive multivariable models containing BNP. Of particular importance in our study was MR-proADM, which was elevated in nearly all (93%) patients at greatest mortality risk (3 or more elevated biomarkers), and hs-cTnT that was elevated in the vast majority of cases (84%); hsCRP was also commonly (in 73%) elevated in these patients, as were cFLCs (70%). As mentioned earlier, individual biomarkers, or clusters of biomarkers, may potentially

identify the pathophysiological pathways which are most important in determining outcome in HF. However, hs-cTnT is arguably a non-specific marker of myocyte necrosis that is not specific to any injurious mechanism. The precise source of MR-proADM and stimuli for its production are uncertain, although the major source of this peptide may be the blood vessels.

Potential future role for multiple biomarkers in the management of HF?

All of the biomarkers in this study were measured using simple, readily available assays. In combination five of the novel biomarkers, as dichotomized variables, were powerful predictors of mortality risk, with an elevation of three or more biomarkers stratifying those at greatest risk. Incorporation of these five biomarkers into the routine blood tests that are regularly measured in patients with HF would help identify those at greatest risk. This may aid clinicians with the difficult decisions surrounding the management of such high-risk patients. On the other hand, identifying patients with no, or few, elevated biomarkers who are at lower risk may help reassure both clinicians and patients. From a practical perspective, future creation of a single panel assay to measure all five of these novel biomarkers together would be more efficient for the practicing clinician. Finally, the use of these novel biomarkers in any clinical decision making process would first require prospective testing in a randomized controlled clinical trial in a well-defined, representative population of patients with HF.

Study limitations

Only single baseline measurements of the novel biomarkers were available for the patients in this study. Longitudinal monitoring of these biomarkers may be even more useful for risk stratification and identifying patients who remain at particularly high risk. Our patients were studied one month post-hospitalization for decompensated HF and many of these biomarkers may differ between acutely decompensated and chronically stable patients (and the patients in this study were a survivor cohort). We did not have a validation cohort. Non-fatal outcomes and cause of death were not available.

Conclusions

The novel biomarkers included in this study added little, if any, incremental prognostic value individually, when added to a prognostic model containing established clinical predictors and BNP. However, following dichotomization, five of the novel biomarkers provided incremental prognostic value. There was a clear gradient in the risk of death with increasing numbers of elevated novel biomarkers; the presence of at least three elevated novel biomarkers identifying patients at greatest mortality risk. Of potential clinical value to physicians is the creation of a single panel assay to measure these five biomarkers and incorporate this information into a risk stratification tool containing the other clinical variables identified in this study to improve prognostication for patients with HF.

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immunoassay kits and measured cFLC, cystatin C and hsCRP free of charge. BG Medicine (BG Medicine, MA, USA) provided the immunoassay kits for galectin-3 free of charge.

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BG Medicine (BG Medicine, MA, USA) provided the immunoassay kits for galectin-3 free of charge. The Binding Site Group Ltd. (Birmingham, England) provided the immunoassay kits for cFLC, cystatin C and hsCRP and measured all three free of charge. We would like to thank all the patients who participated in this study.

Conflict of Interest: none declared

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Figure legends

Figure 1a: Survival curves for all-cause mortality stratified by number of biomarkers elevated (0-5) using dichotomized cut-points from the ROC analysis. Kaplan-Meier analyses show an increasing gradient of risk with increasing number of elevated biomarkers ($p<0.0001$).

Figure 1b: Survival curves for all-cause mortality stratified by numbers of biomarkers using dichotomized cut-points from the ROC analysis in combination. Kaplan-Meier analyses show patients with 3 or more elevated biomarkers are at greatest risk compared to those with none, 1 or 2 elevated biomarkers ($p<0.0001$)

Figure 1a: Kaplan-Meier plots for number of elevated biomarkers individually

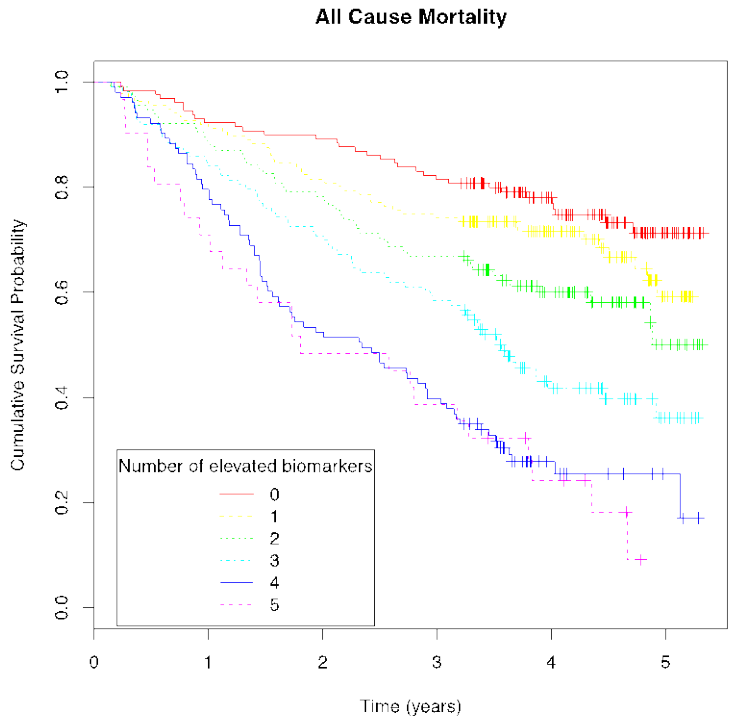

Figure 1b: Kaplan-Meier plot for number of elevated biomarkers in combination

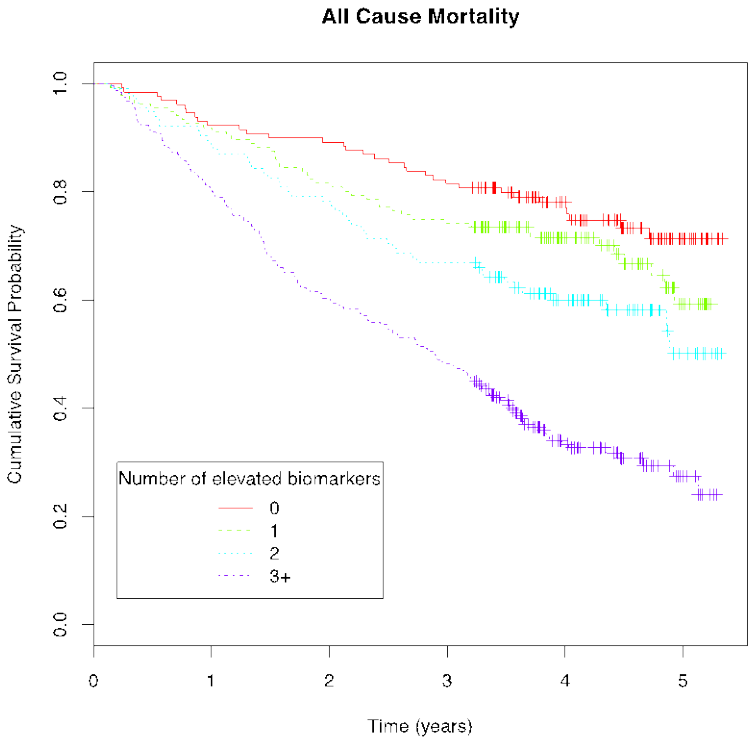

Table 1: Baseline characteristics overall

	Overall (n=628)
Demographic characteristics	
Age (years)	70.8 (10.6)
Male sex	367 (58.4)
Current smoker	137 (21.8)
Heart failure status	
Previous diagnosis HF	273 (43.5)
HF > 2years	194 (30.9)
NYHA III / IV (v I/II)	208 (33.1)
LVEF (%)	40.1 (12.1)
LVEF < 50%	466 (77.4)
Peripheral oedema	421 (67.0)
Medical history	
MI	271 (43.2)
Hypertension	404 (64.3)
Diabetes mellitus	195 (31.1)
AF	334 (53.2)
COPD	177 (28.2)
PAD	102 (16.2)
Rheumatoid arthritis	17 (2.7)
Connective tissue disease	12 (1.9)
Physiological measurements	
HR (beats per min)	77.1 (15.6)
SBP (mmHg)	130.9 (23.4)
DBP (mmHg)	67.8 (13.2)
BMI (kg/m^2)	28.6 (6.7)
LBBB	120 (19.1)
Medical therapies	
Diuretic	603 (96.0)

	Overall (n=628)
ACEI or ARB	501 (79.8)
Beta blocker	416 (66.2)
MRA	84 (13.4)
Laboratory measurements	
BNP (pg/ml)	393 [201-796]
Urea (mmol/l)	9.7 (5.1)
Creatinine (µmol/l)	117 (44)
eGFR (ml/min/1.73m^2)	50 (18)
eGFR <60ml/min/1.73m^2	337 (54)
eGFR <30ml/min/1.73m^2	50 (8.0)
Bilirubin (µmol/l)	11.6 (8.0)
Urate (mmol/l)	0.47 (0.14)
HbA1c (%)	6.3 (1.3)
Haemoglobin (g/dL)	12.5 (2.0)
White cell count (x10^9/L)	7.9 (2.4)
RDW (%)	15.6 (2.5)
Lymphocytes (x10^9/L)	1.9 (1.3)
Novel biomarkers	
Copeptin (pmol/l)	14.4 [4.7-33.1]
MR-proADM (nmol/l)	1.1 [0.9-1.5]
MR-proANP (pmol/l)	204.5 [125.8-320.2]
hs-cTnT (pg/ml)	21.1 [13.3-33.6]
ST2 (ng/ml)	19.9 [15.3-25.7]
Galectin-3 (ng/ml)	19.7 [15.3-25.7]
cFLC (mg/l)	41.9 [29.5-62.2]
hsCRP (mg/l)	4.6 [2.0-10.1]
Cystatin C (mg/l)	1.6 [1.3-2.1]

Values in parentheses are standard deviations for continuous variables or percentages for discrete variables. Values in square brackets are interquartile range for median results.

ACE indicates angiotensin-converting enzyme; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; BNP = B-type natriuretic peptide; cFLC = combined free light chains; COPD = chronic obstructive pulmonary disease; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HBA1c = glycosylated haemoglobin; HR = heart rate; hsCRP = high-sensitivity C-reactive protein; hs-cTnT = high-sensitivity cardiac troponin T; LVEF = left ventricular ejection fraction; MI = myocardial infarction; MRA = mineralocorticoid receptor antagonist; MR-proADM = mid regional pro-adrenomedullin; MR-proANP = mid regional pro-atrial natriuretic peptide; NYHA = New York Heart Association; PAD = peripheral arterial disease; RDW = red cell distribution width; SBP = systolic blood pressure

Table 2: Multivariable analysis – base model

Variable	HR (95% CI) p value
LVEF *per* 5% decrease < 50	1.00 (0.93, 1.08), 0.911
BMI *per* unit decrease < 30	1.06 (1.02, 1.10), 0.002
Age *per* 10 year	1.42 (1.21, 1.67), < 0.001
Sex (Female)	0.94 (0.71, 1.24), 0.657
Diabetes	1.01 (0.71, 1.44), 0.954
HF > 2 years	1.11 (0.85, 1.44), 0.458
NYHA III/IV	1.30 (1.00, 1.70), 0.047
SBP *per* 10 mmHg	0.99 (0.93, 1.05), 0.635
LBBB	1.09 (0.80, 1.49), 0.582
HR *per* 10bpm	1.01 (0.94, 1.10), 0.730
Peripheral edema	1.01 (0.76, 1.34), 0.960
Previous MI	1.46 (1.12, 1.89), 0.005
Current smoker	1.67 (1.20, 2.34), 0.003
PAD	0.80 (0.57, 1.12), 0.189
log lymphocytes (*per* log unit change)	0.66 (0.48, 0.91), 0.012
HbA1c (*per* %)	1.19 (1.05, 1.35), 0.005
COPD	1.27 (0.97, 1.66), 0.088
Creatinine (*per* μmol/l)	1.00 (1.00, 1.01), 0.287
log RDW (*per* log unit change)	1.49 (0.65, 3.39), 0.343
Hemoglobin (*per* g/dl)	0.94 (0.87, 1.01), 0.087
Urate (*per* mmol/l)	1.76 (0.68, 4.56), 0.244
log Bilirubin (*per* log unit change)	1.34 (1.03, 1.73), 0.029
log BNP (*per* log unit change)	1.27 (1.09, 1.47), 0.002
C-statistic	0.721

Table 3: Univariate Cox regression analysis for the novel biomarkers as continuous and dichotomized variables

	Continuous variable	Dichotomized variable
Biomarker (**)	**HR† (95% CI) p value**	**HR (95% CI) p value**
Copeptin (>16.4 pmol/l, 46.7%)	1.40 (1.25, 1.58) < 0.001	1.90 (1.50, 2.40), p = < 0.001
MR-proADM (>1.1 nmol/l, 53.3%)	2.83 (2.19, 3.66) < 0.001	2.41 (1.88, 3.10), p = < 0.001
MR-proANP (>283.0 pmol/l, 32.3%)	1.61 (1.35, 1.92) < 0.001	1.87 (1.48, 2.36), p = < 0.001
hs-cTnT (>21.9 ng/ml, 48.2%)	1.81 (1.57, 2.08) < 0.001	2.66 (2.08, 3.39), p = < 0.001
ST2 (>28.4 ng/ml, 26.1%)	1.53 (1.23, 1.90) < 0.001	1.74 (1.36, 2.22), p = < 0.001
Galectin-3 (>17.3 ng/ml, 61.5%)	1.78 (1.35, 2.35) < 0.001	1.69 (1.31, 2.17), p = < 0.001
cFLC (>51.8 mg/l, 34.4%)	1.69 (1.42, 2.00) < 0.001	1.98 (1.57, 2.50), p = < 0.001
hsCRP (>6.0 mg/l, 40.4%)	1.28 (1.16, 1.41) < 0.001	1.67 (1.32, 2.10), p = < 0.001
CystatinC (>1.6 mg/l, 52.1%)	2.72 (1.98, 3.74) < 0.001)	2.00 (1.57, 2.54), p = < 0.001

† Per log unit change ** ROC cut-point for dichotomized value, % of cohort with elevated value

Table 4: Multivariable Cox regression analysis and C-statistics for the novel biomarkers as continuous and dichotomized variables

	Continuous variable	Dichotomized variable
Biomarker	**HR[†](95%CI) p value (C-statistic)**	**HR(95%CI) p value (C-statistic)**
Copeptin	0.99 (0.85, 1.15) 0.849 (0.721)	1.06 (0.80, 1.40) 0.711 (0.722)
MR-proADM	1.81 (1.16, 2.84) 0.009 (0.724)	1.40 (1.00, 1.95) 0.052 (0.722)
MR-proANP	0.84 (0.65, 1.08) 0.169 (0.722)	0.98 (0.72, 1.33) 0.879 (0.722)
hs-cTnT	1.20 (0.98, 1.48) 0.075 (0.723)	1.48 (1.11, 1.99) 0.008 (0.723)
ST2	1.31 (1.04, 1.65) 0.024 (0.721)	1.29 (0.99, 1.68) 0.060 (0.722)
Galectin-3	1.23 (0.86, 1.78) 0.257 (0.721)	1.21 (0.90, 1.62) 0.208 (0.722)
cFLC	1.37 (1.08, 1.74) 0.011 (0.725)	1.64 (1.22, 2.19) <0.001 (0.729)
hsCRP	1.21 (1.09, 1.35) <0.001 (0.727)	1.43 (1.10, 1.84) 0.007 (0.724)
CystatinC	1.99 (1.00, 3.98) 0.051 (0.724)	1.13 (0.81, 1.57) 0.484 (0.721)

[†] Per log unit change

Table 5: Cox regression analysis for the number of elevated dichotomized biomarkers (0 = reference)

	Univariate analysis	Multivariable analysis
Biomarker	**HR (95% CI) p value**	**HR (95% CI) p value**
1	1.38 (0.88, 2.17) 0.162	1.02 (0.63, 1.65) 0.938
2	1.92 (1.23, 2.99) 0.004	1.35 (0.82, 2.20) 0.235)
e 3	3.88 (2.67, 5.65) < 0.001)	2.20 (1.37, 3.54) 0.001

Appendix Tables

Table 1: Full multivariable analyses with the addition of each novel biomarker individually

	Base	Copeptin	MR-proADM	MR-proANP	hs-cTnT	ST2
	HR (p)	HR (95%CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
Variable	-	0.99 (0.85, 1.15) 0.849	1.81 (1.16, 2.84), 0.009	0.84 (0.65, 1.08), 0.169	1.20 (0.98, 1.48), 0.075	1.31 (1.04, 1.65), 0.024
LVEF per 5% <50	1.00 (0.93, 1.08), 0.911	1.01 (0.93, 1.08), 0.906	1.02 (0.94, 1.10), 0.68	1.01 (0.94, 1.09), 0.817	1.00 (0.92, 1.08), 0.945	1.00 (0.93, 1.08), 0.936
BMI per unit <30	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.002	1.07 (1.03, 1.11), < 0.001	1.06 (1.02, 1.103), 0.002	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.002
Age per 10 year	1.42 (1.21, 1.67), < 0.001	1.43 (1.21, 1.68), < 0.001	1.37 (1.16, 1.62), < 0.001	1.43 (1.22, 1.683), < 0.001	1.38 (1.17, 1.62), < 0.001	1.44 (1.22, 1.70), < 0.001
Sex (female)	0.94 (0.71, 1.24), 0.657	0.94 (0.71, 1.24), 0.646	0.90 (0.68, 1.18), 0.431	0.94 (0.72, 1.242), 0.677	0.97 (0.73, 1.28), 0.808	0.96 (0.73, 1.27), 0.780
Diabetes	1.01 (0.71, 1.44), 0.954	1.01 (0.71, 1.44), 0.950	0.96 (0.67, 1.37), 0.820	1.01 (0.71, 1.442), 0.945	0.97 (0.68, 1.38), 0.847	0.99 (0.70, 1.42), 0.967
HF 2y	1.11 (0.85, 1.44), 0.458	1.11 (0.85, 1.44), 0.458	1.11 (0.85, 1.45), 0.448	1.10 (0.84, 1.436), 0.478	1.11 (0.85, 1.44), 0.457	1.08 (0.83, 1.41), 0.552
NYHA III/IV	1.30 (1.00, 1.70), 0.047	1.31 (1.00, 1.70), 0.046	1.23 (0.94, 1.61), 0.126	1.30 (1.00, 1.693), 0.048	1.27 (0.98, 1.65), 0.076	1.30 (1.00, 1.69), 0.053
SBP per 10 mmHg	0.99 (0.93, 1.05), 0.635	0.99 (0.93, 1.05), 0.627	0.99 (0.93, 1.046), 0.658	0.99 (0.93, 1.044), 0.620	0.98 (0.93, 1.04), 0.507	0.98 (0.93, 1.04), 0.522
LBBB	1.09 (0.80, 1.49), 0.582	1.09 (0.80, 1.49), 0.594	1.15 (0.85, 1.56), 0.376	1.08 (0.79, 1.473), 0.620	1.12 (0.82, 1.53), 0.467	1.15 (0.84, 1.57), 0.376
HR per 10bpm	1.01 (0.94, 1.10), 0.730	1.02 (0.94, 1.10), 0.721	1.02 (0.94, 1.11), 0.605	1.02 (0.94, 1.104), 0.666	1.01 (0.93, 1.10), 0.788	1.01 (0.93, 1.10), 0.774
Peripheral edema	1.01 (0.76, 1.34), 0.960	1.01 (0.76, 1.34), 0.972	1.00 (0.75, 1.33), 1.000	1.02 (0.77, 1.357), 0.894	1.00 (0.75, 1.33), 0.981	1.00 (0.75, 1.33), 0.987
Previous MI	1.46 (1.12, 1.89), 0.005	1.46 (1.12, 1.89), 0.005	1.46 (1.13, 1.90), 0.004	1.47 (1.13, 1.903), 0.004	1.46 (1.13, 1.89), 0.004	1.49 (1.15, 1.93), 0.003
Current smoker	1.67 (1.20, 2.34), 0.003	1.68 (1.20, 2.35), 0.003	1.60 (1.14, 2.23), 0.006	1.66 (1.18, 2.317), 0.003	1.64 (1.18, 2.30), 0.004	1.74 (1.24, 2.44), 0.001
PAD	0.80 (0.57, 1.12), 0.189	0.80 (0.57, 1.12), 0.188	0.81 (0.58, 1.12), 0.202	0.80 (0.58, 1.124), 0.202	0.81 (0.58, 1.13), 0.213	0.80 (0.58, 1.12), 0.200
log Lymphocytes	0.66 (0.48, 0.91), 0.012	0.66 (0.48, 0.91), 0.011	0.71 (0.52, 0.97), 0.033	0.66 (0.48, 0.907), 0.010	0.66 (0.48, 0.90), 0.010	0.67 (0.49, 0.92), 0.013
HbA1c	1.19 (1.05, 1.35), 0.005	1.19 (1.05, 1.35), 0.005	1.19 (1.05, 1.35), 0.005	1.18 (1.05, 1.338), 0.007	1.19 (1.05, 1.34), 0.006	1.19 (1.05, 1.35), 0.006
COPD	1.27 (0.97, 1.66), 0.088	1.26 (0.96, 1.66), 0.093	1.28 (0.98, 1.68), 0.073	1.27 (0.97, 1.664), 0.085	1.25 (0.95, 1.63), 0.114	1.28 (0.98, 1.69), 0.073
Creatinine	1.00 (1.00, 1.01), 0.287	1.00 (1.00, 1.01), 0.302	1.00 (1.00, 1.00), 0.701	1.00 (1.00, 1.006), 0.136	1.00 (1.00, 1.00), 0.578	1.00 (1.00, 1.00), 0.406
log RDW	1.49 (0.65, 3.39), 0.343	1.50 (0.66, 3.41), 0.336	1.32 (0.57, 3.03), 0.520	1.51 (0.67, 3.429), 0.325	1.46 (0.64, 3.33), 0.373	1.31 (0.57, 3.00), 0.527
Hemoglobin	0.94 (0.87, 1.01), 0.087	0.94 (0.87, 1.01), 0.087	0.96 (0.89, 1.03), 0.229	0.94 (0.87, 1.011), 0.093	0.94 (0.88, 1.02), 0.128	0.94 (0.87, 1.01), 0.092
Urate	1.76 (0.68, 4.56), 0.244	1.79 (0.68, 4.69), 0.237	1.10 (0.40, 3.00), 0.850	1.79 (0.69, 4.639), 0.229	1.49 (0.57, 3.91), 0.419	1.64 (0.63, 4.26), 0.309
log Bilirubin	1.34 (1.03, 1.73), 0.029	1.34 (1.03, 1.73), 0.028	1.28 (0.99, 1.66), 0.061	1.33 (1.03, 1.723), 0.030	1.32 (1.02, 1.71), 0.034	1.32 (1.02, 1.71), 0.038
log BNP	1.27 (1.09, 1.47), 0.002	1.27 (1.09, 1.47), 0.002	1.19 (1.02, 1.39), 0.026	1.36 (1.14, 1.63), < 0.001	1.24 (1.06, 1.44), 0.006	1.25 (1.07, 1.45), 0.004
C-statistic	0.721	0.721	0.724	0.722	0.723	0.721

Appendix Table 1 Continued

	Base	Galectin-3	cFLC	hsCRP	CystatinC
	HR (p)	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
Biomarker	-	1.23 (0.86, 1.78), 0.257	1.37 (1.08, 1.74), 0.011	1.21 (1.09, 1.35), < 0.001	1.99 (1.00, 3.98), 0.051
LVEF per 5% <50	1.00 (0.93, 1.08), 0.911	1.00 (0.93, 1.08), 0.919	1.01 (0.93, 1.09), 0.891	1.01 (0.94, 1.09), 0.776	1.00 (0.93, 1.08), 0.923
BMI per unit <30	1.06 (1.02, 1.10), 0.002	1.06 (1.03, 1.10), 0.001	1.06 (1.02, 1.10), 0.005	1.07 (1.03, 1.11), < 0.001	1.06 (1.02, 1.10), 0.002
Age per 10 year	1.42 (1.21, 1.67), < 0.001	1.41 (1.19, 1.65), < 0.001	1.38 (1.17, 1.62), < 0.001	1.44 (1.22, 1.69), < 0.001	1.37 (1.17, 1.62), < 0.001
Sex (female)	0.94 (0.71, 1.24), 0.657	0.92 (0.69, 1.21), 0.536	1.01 (0.76, 1.33), 0.961	1.00 (0.76, 1.32), 0.990	0.89 (0.67, 1.18), 0.420
Diabetes	1.01 (0.71, 1.44), 0.954	1.01 (0.71, 1.43), 0.978	0.96 (0.67, 1.37), 0.800	0.98 (0.69, 1.39), 0.903	0.98 (0.69, 1.40), 0.912
HF 2y	1.11 (0.85, 1.44), 0.458	1.11 (0.85, 1.45), 0.429	1.12 (0.86, 1.47), 0.389	1.14 (0.87, 1.49), 0.333	1.07 (0.82, 1.40), 0.622
NYHA III/IV	1.30 (1.00, 1.70), 0.047	1.31 (1.01, 1.70), 0.045	1.30 (1.00, 1.70), 0.048	1.24 (0.96, 1.62), 0.104	1.30 (1.00, 1.69), 0.049
SBP per 10 mmHg	0.99 (0.93, 1.05), 0.635	0.99 (0.93, 1.05), 0.745	0.99 (0.93, 1.05), 0.671	0.99 (0.93, 1.05), 0.720	0.99 (0.93, 1.05), 0.743
LBBB	1.09 (0.80, 1.49), 0.582	1.10 (0.81, 1.50), 0.545	1.13 (0.83, 1.55), 0.437	1.15 (0.84, 1.57), 0.379	1.11 (0.82, 1.51), 0.506
HR per 10bpm	1.01 (0.94, 1.10), 0.730	1.01 (0.93, 1.10), 0.767	1.02 (0.94, 1.11), 0.635	1.01 (0.93, 1.09), 0.847	1.02 (0.94, 1.10), 0.724
Peripheral edema	1.01 (0.76, 1.34), 0.960	1.01 (0.76, 1.34), 0.965	0.99 (0.74, 1.31), 0.920	1.01 (0.76, 1.34), 0.939	0.97 (0.73, 1.30), 0.850
Previous MI	1.46 (1.12, 1.89), 0.005	1.46 (1.12, 1.89), 0.005	1.49 (1.15, 1.94), 0.003	1.55 (1.19, 2.01), 0.001	1.47 (1.13, 1.90), 0.004
Current smoker	1.67 (1.20, 2.34), 0.003	1.67 (1.20, 2.34), 0.003	1.62 (1.16, 2.27), 0.005	1.66 (1.18, 2.32), 0.003	1.60 (1.14, 2.24), 0.006
PVD	0.80 (0.57, 1.12), 0.189	0.79 (0.57, 1.10), 0.166	0.78 (0.56, 1.08), 0.136	0.78 (0.56, 1.08), 0.136	0.81 (0.58, 1.13), 0.206
log Lymphocytes	0.66 (0.48, 0.91), 0.012	0.66 (0.48, 0.91), 0.010	0.65 (0.47, 0.90), 0.009	0.69 (0.50, 0.95), 0.021	0.67 (0.49, 0.93), 0.015
HbA1c	1.19 (1.05, 1.35), 0.005	1.20 (1.06, 1.35), 0.005	1.19 (1.05, 1.35), 0.006	1.19 (1.05, 1.34), 0.006	1.20 (1.06, 1.36), 0.003
COPD	1.27 (0.97, 1.66), 0.088	1.26 (0.96, 1.65), 0.096	1.27 (0.97, 1.66), 0.087	1.20 (0.92, 1.58), 0.185	1.26 (0.96, 1.66), 0.092
Creatinine	1.00 (1.00, 1.01), 0.287	1.00 (1.00, 1.00), 0.672	1.00 (1.00, 1.00), 0.971	1.00 (1.00, 1.01), 0.270	1.00 (0.99, 1.00), 0.404
log RDW	1.49 (0.65, 3.39), 0.343	1.48 (0.65, 3.37), 0.349	1.43 (0.63, 3.24), 0.387	1.24 (0.53, 2.90), 0.615	1.38 (0.60, 3.15), 0.450
Hemoglobin	0.94 (0.87, 1.01), 0.087	0.94 (0.87, 1.01), 0.090	0.95 (0.88, 1.03), 0.186	0.95 (0.88, 1.02), 0.140	0.94 (0.87, 1.01), 0.089
Urate	1.76 (0.68, 4.56), 0.244	1.60 (0.61, 4.20), 0.338	1.57 (0.60, 4.11), 0.354	1.53 (0.59, 3.95), 0.383	1.27 (0.46, 3.48), 0.644
log Bilirubin	1.34 (1.03, 1.73), 0.029	1.32 (1.02, 1.71), 0.034	1.35 (1.04, 1.74), 0.023	1.41 (1.09, 1.83), 0.009	1.38 (1.07, 1.79), 0.015
log BNP	1.27 (1.09, 1.47), 0.002-	1.27 (1.10, 1.47), 0.002	1.27 (1.09, 1.47), 0.002	1.24 (1.07, 1.44), 0.005	1.27 (1.09, 1.47), 0.002
C-statistic		0.721	0.725	0.727	0.724

Appendix Table 2: Multivariable analysis with dichotomized values

	Base	Copeptin	MR-proADM	MR-proANP	hsTnT	ST2
	HR (p)	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
Biomarker	-	1.06 (0.80, 1.40), 0.711	1.40 (1.00, 1.95), 0.052	0.98 (0.72 1.33), 0.879	1.48 (1.11, 1.99), 0.008	1.29 (0.99, 1.68), 0.060
LVEF per 5% <50	1.00 (0.93, 1.08), 0.911	1.00 (0.93, 1.08), 0.918	1.01 (0.93, 1.08), 0.895	1.01 (0.93, 1.09), 0.897	1.01 (0.93, 1.09), 0.873	1.01 (0.93, 1.09), 0.875
BMI per unit <30	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.003	1.06 (1.02, 1.10), 0.003
Age per 10 year	1.42 (1.21, 1.67), < 0.001	1.42 (1.20, 1.67), < 0.001	1.38 (1.17, 1.62), < 0.001	1.42 (1.21, 1.68), < 0.001	1.38 (1.18, 1.63), < 0.001	1.43 (1.21, 1.68), < 0.001
Sex (female)	0.94 (0.71, 1.24), 0.657	0.94 (0.72, 1.24), 0.674	0.92 (0.70, 1.21), 0.556	0.94 (0.71, 1.24), 0.654	0.97 (0.74, 1.28), 0.837	0.93 (0.71, 1.23), 0.630
Diabetes	1.01 (0.71, 1.44), 0.954	1.01 (0.71, 1.44), 0.954	1.00 (0.70, 1.42), 0.998	1.01 (0.71, 1.44), 0.948	1.01 (0.71, 1.44), 0.952	1.00 (0.70, 1.42), 0.988
HF 2y	1.11 (0.85, 1.44), 0.458	1.11 (0.85, 1.44), 0.461	1.11 (0.85, 1.45), 0.442	1.11 (0.85, 1.44), 0.462	1.09 (0.83, 1.42), 0.541	1.08 (0.83, 1.41), 0.556
NYHA III/IV	1.30 (1.00, 1.70), 0.047	1.30 (1.00, 1.69), 0.049	1.27 (0.98, 1.65), 0.075	1.31 (1.00, 1.70), 0.047	1.26 (0.97, 1.64), 0.086	1.31 (1.00, 1.70), 0.047
SBP per 10 mmHg	0.99 (0.93, 1.05), 0.635	0.99 (0.93, 1.05), 0.646	0.99 (0.93, 1.05), 0.705	0.99 (0.93, 1.05), 0.633	0.98 (0.93, 1.04), 0.493	0.98 (0.93, 1.04), 0.584
LBBB	1.09 (0.80, 1.49), 0.582	1.09 (0.80, 1.49), 0.568	1.12 (0.82, 1.52), 0.489	1.09 (0.80, 1.49), 0.587	1.09 (0.80, 1.48), 0.593	1.12 (0.82, 1.53), 0.475
HR per 10bpm	1.01 (0.94, 1.10), 0.730	1.01 (0.94, 1.10), 0.735	1.02 (0.94, 1.10), 0.673	1.02 (0.94, 1.10), 0.720	1.01 (0.93, 1.10), 0.783	1.01 (0.93, 1.10), 0.794
Peripheral edema	1.01 (0.76, 1.34), 0.960	1.01 (0.76, 1.34), 0.943	1.01 (0.76, 1.35), 0.925	1.01 (0.76, 1.34), 0.953	0.96 (0.72, 1.28), 0.775	1.00 (0.75, 1.33), 0.996
Previous MI	1.46 (1.12, 1.89), 0.005	1.45 (1.12, 1.88), 0.005	1.47 (1.13, 1.91), 0.004	1.46 (1.12, 1.89), 0.005	1.42 (1.09, 1.84), 0.009	1.46 (1.13, 1.89), 0.004
Current smoker	1.67 (1.20, 2.34), 0.003	1.67 (1.19, 2.33), 0.003	1.63 (1.16, 2.27), 0.004	1.67 (1.19, 2.34), 0.003	1.67 (1.19, 2.33), 0.003	1.69 (1.21, 2.36), 0.002
PAD	0.80 (0.57, 1.12), 0.189	0.80 (0.57, 1.12), 0.194	0.79 (0.56, 1.10), 0.159	0.80 (0.57, 1.12), 0.193	0.80 (0.57, 1.12), 0.180	0.82 (0.58, 1.14), 0.236
log Lymphocytes	0.66 (0.48, 0.91), 0.012	0.66 (0.48, 0.91), 0.012	0.68 (0.49, 0.93), 0.016	0.66 (0.48, 0.91), 0.012	0.66 (0.48, 0.91), 0.010	0.67 (0.49, 0.92), 0.014
HbA1c	1.19 (1.05, 1.35), 0.005	1.19 (1.05, 1.35), 0.006	1.18 (1.04, 1.33), 0.010	1.19 (1.05, 1.35), 0.006	1.16 (1.02, 1.31), 0.019	1.19 (1.05, 1.35), 0.006
COPD	1.27 (0.97, 1.66), 0.088	1.27 (0.97, 1.66), 0.088	1.29 (0.99, 1.70), 0.064	1.27 (0.97, 1.66), 0.087	1.25 (0.96, 1.65), 0.103	1.26 (0.96, 1.66), 0.092
Creatinine	1.00 (1.00, 1.01), 0.287	1.00 (1.00, 1.01), 0.373	1.00 (1.00, 1.00), 0.638	1.00 (1.00, 1.01), 0.286	1.00 (1.00, 1.00), 0.544	1.00 (1.00, 1.00), 0.420
log RDW	1.49 (0.65, 3.39), 0.343	1.46 (0.64, 3.35), 0.368	1.33 (0.58, 3.07), 0.502	1.49 (0.65, 3.38), 0.344	1.39 (0.61, 3.18), 0.434	1.34 (0.59, 3.08), 0.485
Hemoglobin	0.94 (0.87, 1.01), 0.087	0.94 (0.87, 1.01), 0.087	0.95 (0.88, 1.02), 0.135	0.94 (0.87, 1.01), 0.089	0.94 (0.88, 1.02), 0.125	0.94 (0.87, 1.01), 0.088
Urate	1.76 (0.68, 4.56), 0.244	1.71 (0.65, 4.49), 0.280	1.24 (0.45, 3.45), 0.677	1.77 (0.68, 4.58), 0.242	1.41 (0.54, 3.69), 0.487	1.73 (0.67, 4.49), 0.261
log Bilirubin	1.34 (1.03, 1.73), 0.029	1.33 (1.03, 1.73), 0.030	1.32 (1.02, 1.71), 0.035	1.34 (1.03, 1.73), 0.029	1.30 (1.00, 1.68), 0.049	1.32 (1.02, 1.71), 0.036
log BNP	1.27 (1.09, 1.47), 0.002	1.26 (1.09, 1.47), 0.002	1.24 (1.07, 1.44), 0.004	1.27 (1.08, 1.50), 0.004	1.24 (1.07, 1.44), 0.004	1.25 (1.08, 1.45), 0.003
C-statistic	0.721	0.722	0.722	0.722	0.723	0.722

Appendix Table 2 Continued

	Base	Galectin-3	cFLC	HS CRP	Cystatin C
	HR (p)	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p	HR (95% CI) p
Biomarker	-	1.21 (0.90, 1.62), 0.208	1.64 (1.22, 2.19), < 0.001	1.43 (1.10, 1.84), 0.007	1.13 (0.81, 1.57), 0.484
LVEF per 5% < 50	1.00 (0.93, 1.08), 0.911	1.01 (0.93, 1.09), 0.881	1.01 (0.93, 1.09), 0.871	1.01 (0.93, 1.09), 0.855	1.01 (0.93, 1.09), 0.865
BMI per unit < 30	1.06 (1.02, 1.10), 0.002	1.06 (1.03, 1.10), 0.001	1.06 (1.02, 1.10), 0.002	1.07 (1.03, 1.11), < 0.001	1.06 (1.02, 1.10), 0.002
Age per 10 year	1.42 (1.21, 1.67), < 0.001	1.40 (1.19, 1.65), < 0.001	1.35 (1.15, 1.59), < 0.001	1.43 (1.21, 1.68), < 0.001	1.40 (1.19, 1.66), < 0.001
Sex (female)	0.94 (0.71, 1.24), 0.657	0.92 (0.70, 1.21), 0.543	1.00 (0.76, 1.32), 1.000	0.99 (0.75, 1.31), 0.966	0.93 (0.71, 1.23), 0.603
Diabetes	1.01 (0.71, 1.44), 0.954	1.01 (0.71, 1.43), 0.977	0.95 (0.67, 1.36), 0.794	0.99 (0.69, 1.41), 0.947	1.00 (0.70, 1.42), 0.988
HF 2y	1.11 (0.85, 1.44), 0.458	1.11 (0.85, 1.45), 0.427	1.14 (0.87, 1.49), 0.333	1.13 (0.87, 1.48), 0.361	1.09 (0.84, 1.43), 0.516
NYHA III/IV	1.30 (1.00, 1.70), 0.047	1.30 (1.00, 1.69), 0.049	1.31 (1.01, 1.70), 0.044	1.25 (0.96, 1.62), 0.102	1.30 (1.00, 1.69), 0.050
SBP per 10 mmHg	0.99 (0.93, 1.05), 0.635	0.99 (0.93, 1.05), 0.746	0.99 (0.94, 1.05), 0.802	0.99 (0.94, 1.05), 0.747	0.99 (0.93, 1.05), 0.686
LBBB	1.09 (0.80, 1.49), 0.582	1.10 (0.81, 1.50), 0.557	1.15 (0.84, 1.57), 0.386	1.14 (0.83, 1.55), 0.425	1.09 (0.80, 1.49), 0.568
HR per 10bpm	1.01 (0.94, 1.10), 0.730	1.01 (0.93, 1.10), 0.805	1.01 (0.93, 1.10), 0.748	1.01 (0.93, 1.09), 0.840	1.01 (0.94, 1.10), 0.737
Peripheral edema	1.01 (0.76, 1.34), 0.960	1.01 (0.76, 1.34), 0.968	1.01 (0.76, 1.35), 0.924	1.02 (0.76, 1.35), 0.916	1.01 (0.76, 1.34), 0.945
Previous MI	1.46 (1.12, 1.89), 0.005	1.44 (1.11, 1.87), 0.006	1.55 (1.19, 2.01), 0.001	1.50 (1.16, 1.95), 0.002	1.46 (1.13, 1.90), 0.004
Current smoker	1.67 (1.20, 2.34), 0.003	1.67 (1.20, 2.34), 0.003	1.60 (1.15, 2.24), 0.006	1.63 (1.17, 2.29), 0.004	1.65 (1.18, 2.31), 0.004
PAD	0.80 (0.57, 1.12), 0.189	0.79 (0.56, 1.10), 0.161	0.79 (0.57, 1.10), 0.166	0.78 (0.56, 1.09), 0.150	0.80 (0.57, 1.12), 0.192
log Lymphocytes	0.66 (0.48, 0.91), 0.012	0.67 (0.49, 0.92), 0.013	0.66 (0.48, 0.90), 0.010	0.68 (0.50, 0.93), 0.016	0.67 (0.48, 0.92), 0.012
HbA1c	1.19 (1.05, 1.35), 0.005	1.19 (1.06, 1.35), 0.005	1.18 (1.05, 1.34), 0.007	1.19 (1.05, 1.34), 0.006	1.19 (1.05, 1.35), 0.006
COPD	1.27 (0.97, 1.66), 0.088	1.27 (0.96, 1.66), 0.090	1.26 (0.96, 1.65), 0.094	1.23 (0.93, 1.61), 0.144	1.27 (0.97, 1.67), 0.084
Creatinine	1.00 (1.00, 1.01), 0.287	1.00 (1.00, 1.00), 0.508	1.00 (1.00, 1.00), 0.776	1.00 (1.00, 1.01), 0.290	1.00 (1.00, 1.01), 0.502
log RDW	1.49 (0.65, 3.39), 0.343	1.50 (0.66, 3.43), 0.333	1.39 (0.61, 3.14), 0.435	1.36 (0.59, 3.13), 0.468	1.46 (0.64, 3.34), 0.366
Hemoglobin	0.94 (0.87, 1.01), 0.087	0.94 (0.87, 1.01), 0.090	0.95 (0.88, 1.02), 0.163	0.95 (0.88, 1.02), 0.150	0.94 (0.87, 1.01), 0.100
Urate	1.76 (0.68, 4.56), 0.244	1.53 (0.58, 4.06), 0.390	1.47 (0.56, 3.83), 0.434	1.70 (0.66, 4.42), 0.273	1.59 (0.59, 4.29), 0.363
log Bilirubin	1.34 (1.03, 1.73), 0.029	1.32 (1.02, 1.71), 0.034	1.33 (1.03, 1.72), 0.028	1.39 (1.08, 1.81), 0.012	1.35 (1.04, 1.75), 0.025
log BNP	1.27 (1.09, 1.47), 0.002	1.27 (1.10, 1.48), 0.001	1.27 (1.09, 1.47), 0.002	1.24 (1.07, 1.44), 0.004	1.26 (1.09, 1.46), 0.002
C-statistic	0.721	0.722	0.729	0.724	0.721

Appendix Table 3: Combinations of elevated biomarkers when 3 or more elevated

MR-proADM	hsTnT	hsCRP	cFLC	ST2	Number of patients (%)
	X		X	X	1 (0.4%)
X			X	X	1 (0.4%)
		X	X	X	3 (1.2%)
	X	X	X	X	4 (1.6%)
	X	X		X	4 (1.6%)
	X	X	X		5 (2.0%)
X		X		X	7 (2.8%)
X		X	X	X	8 (3.2%)
X	X			X	15 (6.1%)
X	X		X	X	19 (7.7%)
X		X	X		20 (8.1%)
X	X	X		X	23 (9.3%)
X	X	X			26 (10.5%)
X	X		X		31 (12.6%)
X	X	X	X	X	31 (12.6%)
X	X	X	X		49 (19.8%)
230 (93.1%)	208 (84.2%)	180 (72.9%)	172 (69.7%)	116 (47.0%)	

Appendix Table 4: Full multivariable analysis with number of elevated biomarkers

	HR (95% CI) p	HR (95% CI) p
1 elevated		1.02 (0.63, 1.65), 0.938
2 elevated		1.35 (0.82, 2.20), 0.235
e 3 elevated		2.20 (1.37, 3.54), 0.001
LVEF per 5% decrease < 50	1.00 (0.93, 1.08), 0.911	1.02 (0.94, 1.10), 0.686
BMI per unit decrease < 30	1.06 (1.02, 1.10), 0.002	1.06 (1.02, 1.10), 0.005
Age per 10 year	1.42 (1.21, 1.67), < 0.001	1.35 (1.15, 1.60), < 0.001
Sex (female)	0.94 (0.71, 1.24), 0.657	0.98 (0.75, 1.30), 0.903
Diabetes	1.01 (0.71, 1.44), 0.954	0.99 (0.69, 1.42), 0.963
HF 2y	1.11 (0.85, 1.44), 0.458	1.06 (0.81, 1.39), 0.650
NYHA III/IV	1.30 (1.00, 1.70), 0.047	1.21 (0.92, 1.57), 0.168
SBP per 10 mmHg	0.99 (0.93, 1.05), 0.635	0.99 (0.93, 1.05), 0.667
LBBB	1.09 (0.80, 1.49), 0.582	1.22 (0.89, 1.66), 0.225
HR per 10bpm	1.01 (0.94, 1.10), 0.730	1.01 (0.93, 1.10), 0.746
Peripheral edema	1.01 (0.76, 1.34), 0.960	0.97 (0.73, 1.29), 0.840
Previous MI	1.46 (1.12, 1.89), 0.005	1.49 (1.15, 1.93), 0.003
Current smoker	1.67 (1.20, 2.34), 0.003	1.59 (1.14, 2.23), 0.007
PAD	0.80 (0.57, 1.12), 0.189	0.79 (0.57, 1.11), 0.172
log Lymphocytes	0.66 (0.48, 0.91), 0.012	0.71 (0.52, 0.97), 0.032
HbA1c	1.19 (1.05, 1.35), 0.005	1.14 (1.01, 1.30), 0.036
COPD	1.27 (0.97, 1.66), 0.088	1.26 (0.96, 1.66), 0.091
Creatinine	1.00 (1.00, 1.01), 0.287	1.00 (1.00, 1.00), 0.828
log RDW	1.49 (0.65, 3.39), 0.343	1.03 (0.44, 2.42), 0.939
Hemoglobin	0.94 (0.87, 1.01), 0.087	0.95 (0.88, 1.02), 0.161
Urate	1.76 (0.68, 4.56), 0.244	1.01 (0.37, 2.73), 0.992
log Bilirubin	1.34 (1.03, 1.73), 0.029	1.34 (1.03, 1.73), 0.686
log BNP	1.27 (1.09, 1.47), 0.002	1.21 (1.04, 1.40), 0.005
	0.721	0.730

Appendix Table 5: Clinical characteristics according to number of biomarkers elevated

Variable	All subjects (n=628)	0 elevated (n=130)	1 - 2 elevated (n=251)	3+ elevated (n=247)	P-value
Demographic Characteristics					
Age (years)	70.82 (10.62)	65.8 (11.9)	70.2 (10.5)	74.1 (8.7)	< 0.001
Sex (Male)	367 (58.4)	67 (51.5)	134 (53.4)	166 (67.2)	0.002
Current smoker	137 (21.8%)	29 (22.3%)	58 (23.1%)	50 (20.2%)	0.733
Heart Failure Status					
Previous diagnosis HF	273 (43.5)	39 (30.0)	100 (39.8)	134 (54.3)	<0.001
HF >2 years	194 (30.9%)	26 (20.0%)	69 (27.5%)	99 (40.1%)	< 0.001
NYHA III / IV (v I/II)	208 (33.1%)	25 (19.2%)	73 (29.1%)	110 (44.5%)	< 0.001
LVEF	40.1 (12.1)]	42.3 (11.3)	39.5 (12.8)	39.5 (11.7)	0.068
LVEF (<50)	466 (77.4%)	87 (70.2%)	190 (79.2%)	189 (79.4%)	0.096
Peripheral oedema	421 (67.0%)	59 (45.4%)	164 (65.3%)	198 (80.2%)	< 0.001
Medical History					
MI	285 (45.4%)	47 (36.2%)	110 (43.8%)	128 (51.8%)	0.012
Hypertension	404 (64.3%)	67 (51.5%)	159 (63.3%)	178 (72.1%)	< 0.001
Diabetes mellitus	195 (31.1%)	19 (14.6%)	78 (31.1%)	98 (39.7%)	< 0.001
AF	334 (53.2%)	57 (43.8%)	124 (49.4%)	153 (61.9%)	0.001
COPD	177 (28.2%)	34 (26.2%)	68 (27.1%)	75 (30.4%)	0.609
PAD	102 (16.2%)	13 (10.0%)	41 (16.3%)	48 (19.4%)	0.056
Physiological measurements					
HR	77.1 (15.6)	78.6 (15.6)	77.7 (15.3)	75.7 (15.8)	0.171
SBP	130.9 (23.4)	130.8 (21.4)	131.0 (23.1)	131.0 (24.8)	0.995
DBP	67.8 (13.2)	69.6 (12.9)	68.8 (13.3)	65.8 (13.2)	0.009
BMI	28.6 (6.7)	27.8 (6.1)	28.8 (6.7)	28.9 (7.0)	0.253
LBBB	120 (19.1%)	18 (13.8%)	53 (21.1%)	49 (19.8%)	0.214

Values are mean (SD) or N (%). P-values from one-way ANOVA or Fisher test