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ORIGINAL RESEARCH

Biomarker Profiles in Heart Failure Patients With Preserved and Reduced Ejection Fraction

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***Background*—**Biomarkers may help us to unravel differences in the underlying pathophysiology between heart failure (HF) patients with a reduced ejection fraction (HFrEF) and a preserved ejection fraction (HFpEF). Therefore, we compared biomarker profiles to characterize pathophysiological differences between patients with HFrEF and HFpEF.

***Methods and Results*—**We retrospectively analyzed 33 biomarkers from different pathophysiological domains (inflammation, oxidative stress, remodeling, cardiac stretch, angiogenesis, arteriosclerosis, and renal function) in 460 HF patients (21% HFpEF, left ventricular ejection fraction ≥45%) measured at discharge after hospitalization for acute HF. The association between these markers and the occurrence of all-cause mortality and/or HF-related rehospitalizations at 18 months was compared between patients with HFrEF and HFpEF. Patients were 70.6±11.4 years old and 37.4% were female. Patients with HFpEF were older, more often female, and had a higher systolic blood pressure. Levels of high-sensitive C-reactive protein were significantly higher in HFpEF, while levels of pro-atrial-type natriuretic peptide and N-terminal pro-brain natriuretic peptide were higher in HFrEF. Linear regression followed by network analyses revealed prominent inflammation and angiogenesis-associated interactions in HFpEF and mainly cardiac stretch–associated interactions in HFrEF. The angiogenesis-specific marker, neuropilin and the remodeling-specific marker, osteopontin were predictive for all-cause mortality and/or HF-related rehospitalizations at 18 months in HFpEF, but not in HFrEF (P for interaction <0.05).

***Conclusions*—**In HFpEF, inflammation and angiogenesis-mediated interactions are predominantly observed, while stretch-mediated interactions are found in HFrEF. The remodeling marker osteopontin and the angiogenesis marker neuropilin predicted outcome in HFpEF, but not in HFrEF. **(*J Am Heart Assoc*. 2017;6:e003989. DOI: 10.1161/JAHA.116.003989.)**

Key Words: biomarker • heart failure • multimarker • pathophysiology

The difference in pathophysiology between heart failure with a reduced ejection fraction (HFrEF) and heart failure with a preserved ejection fraction (HFpEF) remains poorly understood, and effective treatment options are currently not available for HFpEF.[1–4] Therefore, a better understanding of the pathophysiology of HFpEF is required, which eventually may help to improve outcome.

Patient-specific biomarker profiles are useful for the purpose of monitoring disease severity and progression, to guide therapy, but also for characterizing the pathophysiology of HF.[5–9] We hypothesize that differences in biomarker levels and correlative associations between HFrEF and HFpEF may provide important insights into specific activities of pathophysiological processes.[5–9]

The aim of this study was to characterize HFpEF and HFrEF using a network analysis on an extensive set of 33 biomarkers of various pathophysiological pathways. Therefore, we investigated differences in biomarker levels, patterns of

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Accompanying Tables S1 through S5 and Figures S1 through S6 are available at http://jaha.ahajournals.org/content/6/4/e003989/DC1/embed/inline-supplementary-material-1.pdf

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correlations, and predictive value of biomarkers in patients with HFpEF and HFrEF.

Methods

Study Design and Population

Measurements of biomarkers were performed in a subcohort of the Coordinating study evaluating Outcomes of Advising and Counseling in Heart failure (COACH) trial of which rationale, design, and results have been previously described.[10,11] In short, the COACH trial studied the effects of additional intensive nurse-led support on the prognosis of 1023 chronic HF patients. A hospital admission for HF (NYHA II-IV) inclusion criteria for the COACH trial included and patients had to be at least 18 years of age. Patients were excluded if they underwent an intervention (percutaneous transluminal coronary angioplasty, coronary artery bypass graft, heart transplantation, valve replacement) in the previous 6 months or if they had a planned intervention in the following 3 months. Additionally, patients were excluded if they had an ongoing evaluation for heart transplantation.[10] Left ventricular ejection fraction (LVEF) measurements were available in 832 patients. Biomarkers were measured in blood collected from 460 patients shortly before discharge between 8:00 AM and 4:00 PM, after patients had been clinically stabilized and were considered well enough to go home. Baseline characteristics of the current substudy were comparable to the entire COACH study (Table S1). The study complies with the Declaration of Helsinki, local medical ethics committees approved the study, and all patients provided written informed consent.

Study and Laboratory Measurements

HFpEF was defined as having a LVEF ≥45%, measurements of high-sensitive C-reactive protein (hs-CRP), pentraxin-3, growth differentiation factor, soluble receptor of advanced glycation end-products, interleukin-6, tumor necrosis factor α, tumor necrosis factor–associated receptor 1 α, myeloperoxidase, syndecan-1, periostin, ST-2, osteopontin, pro-atrial-type natriuretic peptide (proANP), vascular endothelial growth factor receptor (VEGFR), angiogenin, end-terminal pro c-type natriuretic peptide, neuropilin-1, endothelial cell-selective adhesion molecule, neutrophil gelatinase-associated lipocalin, d-dimer, WAP 4-disulfide core domain protein HE4, mesothelin, polymeric immunoglobulin receptor, prosaposin, and TROY were measured by Alere San Diego, Inc, (San Diego, CA), using enzyme-linked immunosorbent assays. Immunoassays to ST2 were developed by Alere. This research assay by Alere has not been standardized to the commercialized assays used in research or in clinical use. Furthermore, the extent to which this Alere assay correlates with the commercial assay is not fully characterized. Galectin-3 was measured using ELISA by BG Medicine, Inc. (Waltham, MA). Transforming growth factor-β and VEGF were analyzed using a quantitative multiplexed sandwich ELISA system, SearchLightw proteome arrays (Aushon BioSystems, Billerica, MA). N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured using the Elecsys proBNP ELISA by Roche Diagnostics (Mannheim, Germany). Erythropoietin α was measured using the IMMULITEw erythropoietin ELISA by Diagnostic Products Corporation (Los Angeles, CA). Inter- and intra-assay coefficients of the assays used can be found in Table S2. Endothelin-1, interleukin-6, and cardiac-specific troponin I were measured in frozen plasma samples collected at baseline using high-sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System; Singulex Inc, Alameda, CA). Estimated glomerular filtration rate was based on the simplified Modification of Diet in Renal Disease.[12]

Study End Points

For studying the relationship between biomarker levels and outcome, the primary end point of the COACH trial was used. This end point is a combined end point consisting of all-cause mortality and/or HF-related rehospitalizations at 18 months. An independent end point committee adjudicated the end point.

Statistical Analysis

Continuous variables are presented as medians with interquartile range or means±SD where appropriate. Categorical variables are presented as numbers with percentages. Baseline characteristics and biomarker concentrations at baseline were stratified according to HFrEF and HFpEF. Intergroup differences were tested using Student *t* test or Mann–Whitney *U* test for continuous variables or χ^2 test for categorical variables. Principal component (PC) analysis was performed to correct for multiple comparisons with HFrEF and HFpEF as categorical variables, using an established statistical method described elsewhere.[13] This method is often used in -omics based studies, where there is a natural correlation between markers because of the fact that these often belong to similar pathophysiological processes.[14] Indeed, also for the 33 biomarkers employed in this study, biomarkers are clearly interrelated, belonging to several similar pathophysiological processes (Figure 1). In this situation the Bonferroni correction can be considered too conservative.[15] Here, the PC-based correction has been suggested to be more effective.[14,15] Additionally, this method has been previously successfully used in correcting for multiple comparisons in pairwise correlations.[13] A total of 21 PCs, of which the eigenvalues cumulatively explained

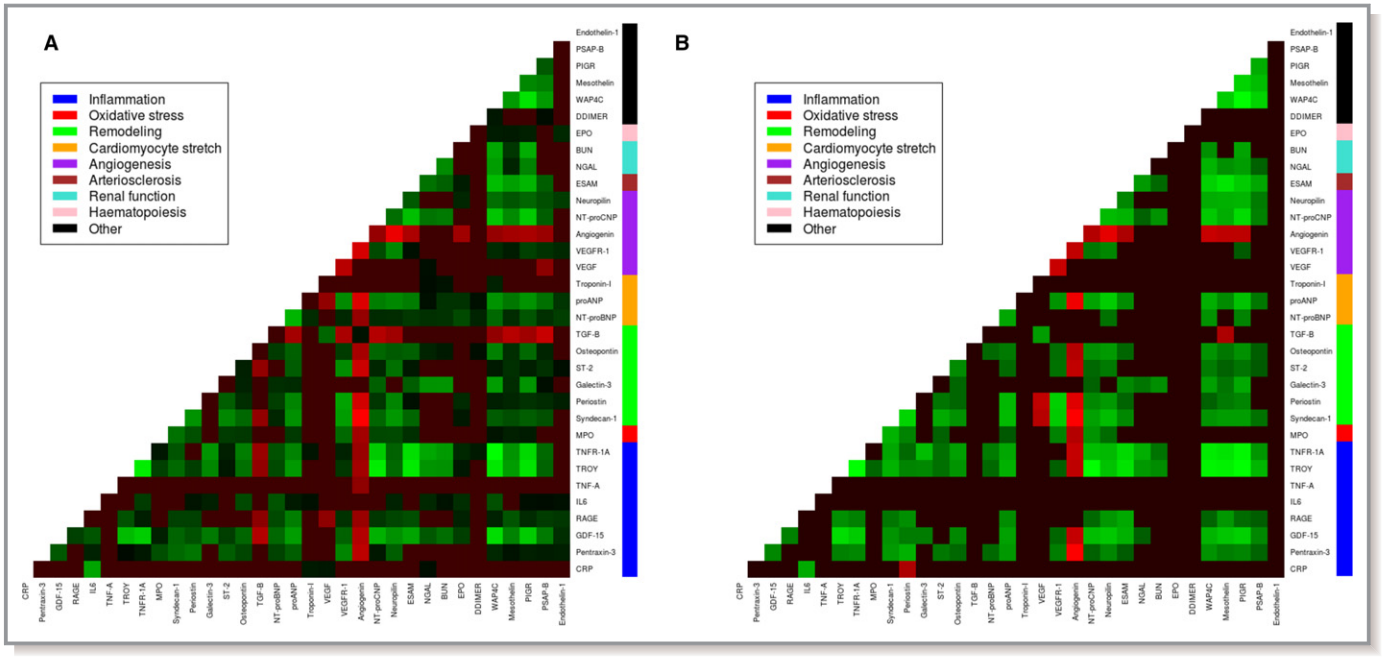

Figure 1. Heatmaps depicting correlation between biomarkers in HFrEF (A) and HFpEF (B). Biomarker correlations that did not pass the corrected *P*-value (0.05/21) are black. Red entails a negative correlation, green entails a positive correlation. BUN indicates blood urea nitrogen; CRP, C-reactive protein; EPO, erythropoietin; ESAM, endothelial cell-selective adhesion molecule; GDF-15, growth differentiation factor 15; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IL-6, interleukin 6; MPO, myeloperoxidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NT-proCNP, amino terminal pro-C-type natriuretic peptide; PIGR, polymeric immunoglobulin receptor; proANP, pro-atrial-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; RAGE, receptor of advanced glycation end-products; ST-2, suppression of tumorigenicity 2; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; TNF-α-R1a, tumor necrosis factor α receptor 1a; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WAP4C, WAP 4 disulfide core domain protein.

>95% of the variation observed in the data set when comparing HFrEF with HFpEF, were found. The corrected significance level for multiple testing was thus set at $P<0.05/21$, equating to an adjusted *P*-value cut-off of 0.00238. To correct for multiple comparison for interbiomarker correlations, $0.05/[PC\times(PC-1)/2]$ was used for the adjusted *P* cut-off value, where PC is the number of principal components found. To study the influence of clinical confounders on biomarker-level differences between HFrEF and HFpEF, logistic regression was performed. Here, HFpEF is coded as 1 and HFrEF as 0. An odds ratio above 1 signifies that higher levels are associated with HFpEF. Associations were corrected for age, sex, estimated glomerular filtration rate, a history of diabetes mellitus, and other clinical covariates that significantly differed between HFrEF and HFpEF. Next, a Spearman's rank correlation coefficient was calculated for each possible biomarker pair in the HFrEF cohort of patients and the procedure was repeated for HFpEF. This resulted in 2 sets of *R*-values with associated *P*-values for both HFrEF and HFpEF. To adjust for multiple testing, only those correlations passing the adjusted *P*-value cut-off calculated from the PC analysis were deemed statistically significant and subsequently retained. These significant correlation coefficients for HFrEF and HFpEF were then graphically displayed as heatmaps with associated disease domains for all biomarkers. Network analysis was performed to analyze associations between biomarkers in HFrEF and HFpEF. First, all significant associations found within HFrEF and HFpEF were separately depicted as circular networks. Next, significant associations between biomarkers exclusive to HFrEF and HFpEF were identified. To ascertain whether these associations were significantly different, the Fishers z-transformation test was used to compare *R*-values between HFrEF and HFpEF. The *P*-values from these associations were corrected using the PC analysis method described above.

For outcome analysis, a univariable interaction test was performed between the (log2-transformed) biomarker and HF status (HFrEF versus HFpEF). The interaction test was then bootstrapped with 1000 iterations to validate the results. Following this, a multivariable interaction test was performed correcting for the COACH risk engine. The COACH risk engine includes sex, age, pulse pressure, diastolic blood pressure, history of stroke, history of diabetes mellitus, estimated glomerular filtration rate, atrial fibrillation, myocardial infarction, peripheral arterial disease, and levels of NT-proBNP and sodium and is powered for the primary end point used in this study, as published elsewhere.[16] The relationship of the primary end point with biomarkers, showing a significant interaction

with HF status and outcome, was then graphically depicted using Kaplan–Meier curves. To correct for potential optimism and given the limited sample size, we bootstrapped the estimates with 1000 iterations.[17] The significance of a difference between tertiles of biomarker levels and association with outcome was tested using the Log-rank test. Univariable and multivariable associations of biomarkers with outcome were tested using the Cox regression. Tests performed were 2-tailed and a *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using STATA version 13.0 (StataCorp LP, College Station, TX) and R, version 3.2.3.

Results

Patient Characteristics

The 460 patients in this cohort had a mean age of 70.6±11.1 years and 37.4% were female. Most patients were in NYHA class III (52%) with a mean LVEF of 32.5±14.0% (Table 1). Ninety-six patients had HFpEF (21%). Patients with HFpEF in this cohort were relatively older (74.5 years versus 69.6 years, *P*<0.001) and more often female (51.0% versus 33.8%, *P*=0.002). Additionally, patients with HFpEF were found to have a higher systolic blood pressure (126.6 mm Hg

Table 1. Baseline Characteristics

	Total Cohort (n=460)	HFrEF (LVEF <45%) (n=364)	HFpEF (LVEF ≥45%) (n=96)	*P* Value
LVEF (%)	32.5±14.0	26.7±8.5	54.4±7.5	NA
Demographics and HF characteristics				
Age, y	70.6±11.1	69.6 (11.2)	74.5 (10.0)	<0.001*
Female sex, n (%)	172 (37.4%)	123 (33.8%)	49 (51.0%)	0.002*
NYHA class (at discharge) II/III/IV, %	44/52/4	42/54/4	55/41/4	0.064
Previous HF hospitalization, n (%)	155 (33.7%)	118 (32.4%)	37 (38.5%)	0.260
Clinical signs				
BMI, kg/m^2	27.0±5.6	26.8±5.5	28.0±5.7	0.08
Systolic BP, mm Hg	117.9±21.3	115.6±20	126.6±23.1	<0.001*
Diastolic BP, mm Hg	68.9±12.3	68.9±12.4	68.9±12.1	0.980
eGFR, mL/min per 1.73 m^2	54.9±20.5	55.1±20.4	53.8±21.1	0.580
Heart rate, bpm	74.2±13.4	74.7±13.8	72.2±11.8	0.110
Medical history, n (%)				
Myocardial infarction	187 (40.7%)	161 (44.2%)	26 (27.1%)	0.002*
Hypertension	191 (41.5%)	143 (39.3%)	48 (50.0%)	0.058
Diabetes mellitus	135 (29.3%)	104 (28.6%)	31 (32.3%)	0.048*
COPD	130 (28.3%)	99 (27.2%)	31 (32.3%)	0.320
Atrial fibrillation/flutter	209 (45.4%)	159 (43.7%)	50 (52.1%)	0.140
Anemia	128 (27.8%)	92 (25.3%)	36 (37.5%)	0.017*
Medication, n (%)				
ACE-inhibitor/ARB	378 (82.2%)	311 (85.4%)	67 (69.8%)	<0.001*
β-Blocker	312 (67.8%)	255 (70.1%)	57 (59.4%)	0.005*
Diuretic	440 (95.7%)	350 (96.2%)	90 (93.8%)	0.300
Statin	183 (39.8%)	153 (42.0%)	30 (31.2%)	0.055
Digoxin	155 (33.7%)	120 (33.0%)	35 (36.5%)	0.052
Laboratory				
Hemoglobin, g/dL	8.5 (7.7, 9.2)	8.6 (7.8, 9.3)	8.1 (7.2, 8.8)	<0.001*
Sodium, mEq/L	138.6±4.3	138.6±4.4	138.6±4.2	0.973
Potassium, mEq/L	4.2 (3.9, 4.6)	4.3 (3.9, 4.6)	4.1 (3.7, 4.6)	0.214

ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; LVEF, left ventricular ejection fraction; NA, not available; NYHA, New York Heart Association.

**P*-value lower than the significance treshhold of 0.05.

versus 115.6 mm Hg, $P<0.001$) compared to patients with HFrEF. Furthermore, patients with HFpEF used fewer angiotensin-converting enzyme inhibitors (55.2% versus 76.9%, $P<0.001$) and β-blockers (59.4% versus 70.1%, $P<0.001$) at discharge.

Biomarker Levels in HF With Reduced and Preserved Ejection Fraction

PC analysis revealed 21 principal components that accounted for a cumulative proportion of variance of 95% between HFrEF and HFpEF, which were subsequently used for adjusting the *P*-value significance threshold ($P<0.05/21$; Figure S1). Table 2 shows the baseline biomarker concentrations stratified according to HFrEF and HFpEF where *P*-values shown are corrected for multiple testing. Levels of hs-CRP were higher in HFpEF (3.6 mg/L versus 2.1 mg/L, $P=0.001$) and levels of pentraxin-3 were higher in HFrEF (3.9 ng/mL versus 3.2 ng/mL, $P=0.009$). Levels of cardiac stretch markers NT-proBNP (2988 pg/mL versus 1948 pg/mL, $P<0.001$) and proANP (21.9 pg/mL versus 17.0 pg/mL) were higher in HFrEF. Additionally, the angiogenesis-specific marker VEGFR (0.8 ng/mL versus 0.7 ng/mL, $P=0.009$) was higher in HFrEF. After adjusting for multiple comparisons, levels of hs-CRP ($P=0.022$) remained significantly higher in HFpEF, while the cardiac stretch markers NT-proBNP ($P<0.001$) and proANP ($P=0.042$) remained significantly higher in HFrEF.

Biomarker associations with HFrEF and HFpEF are shown in Table S3. When correcting for clinical covariates (age, sex, estimated glomerular filtration rate, systolic blood pressure, a history of myocardial infarction; diabetes mellitus; atrial fibrillation and anemia), higher levels of hs-CRP (odds ratio: 1.29; 95% CI 1.09–1.52, $P=0.003$) remained associated with HFpEF, while higher levels of NT-proBNP (odds ratio: 0.68; 95% CI 0.57–0.82, $P<0.001$) and proANP (odds ratio: 0.69; 95% CI 0.53–0.88, $P=0.003$) remained associated with HFrEF. After additionally correcting for β-blocker and angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use, the statistical associations for these 3 markers remained (Table S3).

Biomarker Associations and Network Analysis

Heatmaps for the association between biomarkers in HFrEF and HFpEF are depicted in Figure 1. Figure 2 shows the graphical depiction of biomarker networks in HFrEF and HFpEF. Results from the correlation analysis and associated heatmaps reveal that correlations between biomarkers in HFpEF are more associated with remodeling and inflammation, while in HFrEF angiogenesis is a more prominent feature (Figure 1). Network analysis further showed myeloperoxidase to be involved in interactions in both HFrEF and HFpEF. Additionally, renal marker neutrophil gelatinase-associated lipocalin and blood urea nitrogen as well as inflammation marker receptor of advanced glycation end-products were involved in biomarker associations in HFpEF.

When examining the exclusive interactions between biomarkers in HFrEF and HFpEF, HFpEF revealed interactions, which were mainly associated with inflammation (interleukin-6; pentraxin-3; Table 3, corrected *P*-value for difference <0.05). In contrast, HFrEF showed exclusive interactions that were NT-proBNP mediated (Table 3), indicating that biomarker interactions are more associated with cardiac stretch in HFrEF and inflammation in HFpEF. In sensitivity analysis with a definition of HFrEF at LVEF ≤40% and a definition of HFpEF at LVEF ≥50%, exclusive associations in HFpEF remained inflammation mediated, while NT-proBNP mediated associations in HFrEF (Table S4).

Outcome

Of the total cohort, 41% reached the clinical end point of death and/or HF rehospitalization (41% HFrEF versus 44.8% HFpEF, $P=0.659$, Figure S2). NT-proBNP was found to be equally predictive in HFrEF and HFpEF (Table S5). A significant interaction in both univariable and multivariable analysis was found for HF status and neuropilin as well as osteopontin (both $P<0.05$). Both biomarkers were found only to be predictive in HFpEF (Figures 3 and 4, Table S5). Interaction between neuropilin ($P=0.007$) and osteopontin ($P=0.018$) and HF status for the primary end point remained following sensitivity analysis for a definition of HFpEF of LVEF ≥50%. After bootstrapping with 1000 iterations, the interaction with HF status for the primary end point stayed significant for both osteopontin ($P=0.002$) and neuropilin ($P=0.011$) in univariable analyses. Also in multivariable analyses, the interaction remained significant for osteopontin ($P=0.016$) and neuropilin ($P=0.015$).

When examining the relationship with HF rehospitalizations and all-cause mortality separately in univariable analysis, we see that osteopontin is predictive for both HF rehospitalizations ($P=0.007$) and all-cause mortality ($P=0.031$) separately, but not in HFrEF (Figures S3 and S4). Neuropilin was predictive in univariable analysis for all-cause mortality in both HFrEF ($P=0.003$) and HFpEF ($P=0.023$). However, neuropilin was only predictive of HF rehospitalizations in HFpEF ($P=0.026$) and not in HFrEF ($P=0.026$) (Figures S5 and S6).

Discussion

In this study, we demonstrate a distinct biomarker profile for HFpEF and HFrEF patients by using a novel approach employing network analysis to identify exclusive interactions within the 2 disease entities. Higher levels of Hs-CRP and

Table 2. Baseline Markers Stratified to HFrEF and HFpEF

	Total Cohort (n=460)	HFrEF (n=364)	HFpEF (n=96)	*P* Value	*P* Value*
Inflammation					
hs-CRP, mg/L	2.3 (0.9, 5.2)	2.1 (0.8, 4.7)	3.6 (1.8, 7.0)	0.001†	0.022†
Pentraxin-3, ng/mL	3.7 (2.5, 5.6)	3.9 (2.7, 5.8)	3.2 (2.4, 4.7)	0.009†	0.198
GDF-15, ng/mL	2.8 (1.9, 4.2)	2.8 (1.9, 4.3)	2.6 (1.9, 4.1)	0.670	1.000
RAGE, ng/mL	2.9 (1.9, 4.8)	3.0 (1.9, 4.9)	2.6 (1.7, 4.0)	0.053	1.000
IL-6, pg/mL	7.0 (3.7, 12.2)	6.7 (3.6, 11.3)	8.2 (4.5, 13.6)	0.100	1.000
TNF-α, pg/mL	47.9 (6.2, 119.4)	47.3 (8.1, 109.5)	56.7 (4.8, 194.4)	0.350	1.000
TROY, ng/mL	0.9 (0.7, 1.5)	0.9 (0.7, 1.4)	0.9 (0.6, 1.6)	0.540	1.000
TNF-α-R1a, ng/mL	3.0 (2.1, 4.5)	3.0 (2.1, 4.4)	3.1 (2.2, 4.9)	0.490	1.000
Oxidative stress					
MPO, ng/mL	20.4 (15.6, 28.2)	20.6 (15.6, 28.4)	19.9 (15.2, 27.2)	0.530	1.000
Remodeling					
Syndecan-1, ng/mL	20.2 (14.1, 27.5)	20.5 (14.1, 28.1)	19.2 (14.0, 24.6)	0.360	1.000
Periostin, ng/mL	4.6 (3.4, 6.6)	4.7 (3.4, 6.6)	4.5 (3.4, 6.6)	0.520	1.000
Galectin-3, ng/mL	19.9 (15.2, 25.7)	20.0 (14.8, 25.9)	19.3 (15.8, 25.3)	0.960	1.000
ST-2, ng/mL	2.5 (1.4, 5.6)	2.4 (1.4, 5.5)	3.1 (1.6, 6.2)	0.140	1.000
Osteopontin, ng/mL	160.1 (108.8, 219.5)	161.2 (108.4, 217.1)	153.8 (110.7, 240.5)	0.980	1.000
TGF-ß, ng/mL	50.6 (34.4, 75.1)	51.4 (35.3, 77.5)	44.3 (30.9, 63.3)	0.069	1.000
Cardiomyocyte stretch					
NT-proBNP, pg/mL	2601 (1398–5989)	2988.8 (1511.0, 6708.9)	1948.0 (855.3, 3827.0)	<0.001†	<0.001†
proANP, ng/mL	20.4 (12.1–33.3)	21.9 (13.2, 35.4)	17.0 (10.0, 28.2)	0.002†	0.042†
cTnI, pg/mL		14.1 (7.3, 29.4)	13.1 (5.8, 34.8)	0.562	1.000
Angiogenesis					
VEGF, pg/mL	62.8 (31.4, 148.7)	62.5 (28.5, 139.9)	63.0 (35.8, 162.9)	0.280	1.000
VEFGR, ng/mL	0.8 (0.6, 1.0)	0.8 (0.6, 1.1)	0.7 (0.5, 1.0)	0.009†	0.255
Angiogenin, μg/mL	5.0 (3.5, 7.4)	5.0 (3.5, 7.5)	5.2 (3.5, 7.3)	0.840	1.000
NT-proCNP, ng/mL	0.024 (0.017, 0.035)	0.023 (0.017, 0.034)	0.024 (0.015–0.037)	0.440	1.000
Neuropilin-1, ng/mL	10.0 (7.1, 13.7)	10.1 (7.1, 14.0)	9.6 (7.0, 13.5)	0.770	1.000
Arteriosclerosis					
ESAM, ng/mL	52.9 (44.5, 64.4)	53.8 (45.3, 64.8)	50.2 (41.1, 63.2)	0.065	1.000
Renal function					
NGAL, ng/mL	84.6 (60.4, 119.9)	84.2 (59.4, 119.2)	84.7 (63.3, 122.3)	0.440	1.000
BUN, mmol/L	11.0 (8.2, 15.5)	10.7 (8.3, 15.6)	11.1 (7.7, 15.1)	0.650	1.000
Hematopoiesis					
EPOa, IU/L	9.6 (5.1, 15.9)	9.5 (5.0, 15.5)	10.3 (5.2, 16.5)	0.560	1.000
Other					
D-Dimer, μg/mL	0.5 (0.2, 1.1)	0.5 (0.2, 1.1)	0.6 (0.2, 1.0)	0.710	1.000
WAP4C, ng/mL	5.7 (3.1, 10.1)	5.8 (3.5, 10.0)	5.3 (3.1, 10.3)	0.910	1.000
Mesothelin, ng/mL	29.4 (22.8, 38.7)	29.8 (22.9, 38.8)	28.3 (22.5, 38.0)	0.380	1.000
PIGR, ng/mL	600.6 (337.4, 952.0)	609.0 (388.7, 952.0)	598.7 (331.5, 943.0)	0.330	1.000
PSAP, ng/mL	68.6 (49.2, 98.5)	68.8 (49.8, 101.0)	67.3 (48.0, 93.6)	0.760	1.000
ET-1, ng/mL	4.5 (3.6, 6.1)	4.5 (3.6, 6.1)	4.5 (3.4, 5.7)	0.430	1.000

BUN indicates blood urea nitrogen; cTNI, cardiac troponin-I; EPOa, erythropoietin; ESAM, endothelial cell-selective adhesion molecule; ET-1, endothelin-1; GDF-15, growth differentiation factor 15; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; hs-CRP, high-sensitive C-reactive protein; IL-6, interleukin 6; MPO, myeloperoxidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NT-proCNP, amino terminal pro-C-type natriuretic peptide; PIGR, polymeric immunoglobulin receptor; proANP, pro-atrial-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; RAGE, receptor of advanced glycation end-products; ST-2, suppression of tumorigenicity 2; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; TNF-α-R1a, tumor necrosis factor α receptor 1a; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WAP4C, WAP 4 disulfide core domain protein.
*Corrected *P*-value. †*P*-value lower than the significance treshhold of 0.05.

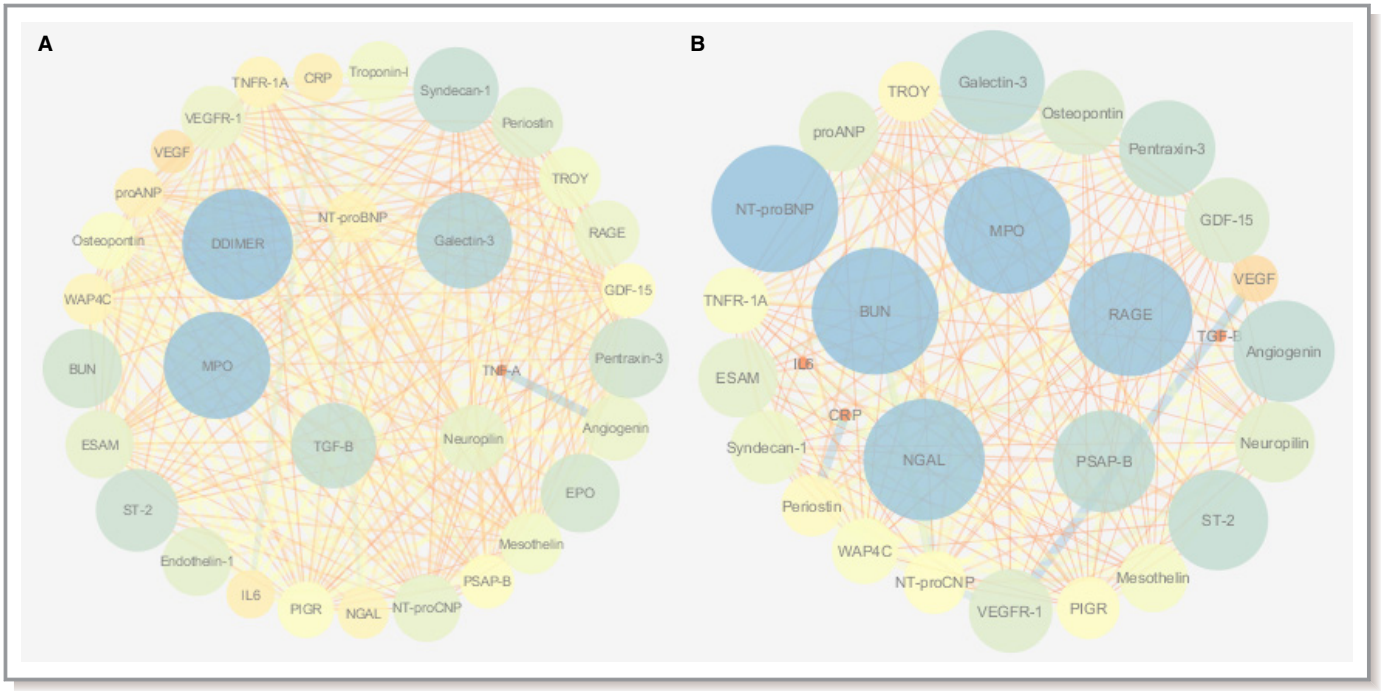

Figure 2. Network analysis depicting associations between biomarkers in HFrEF (A) and HFpEF (B). Associations shown are those that passed the *P*-value cutoff (0.05/21). Node size and color are based on the clustering coefficient. The edge betweenness was used as a criterion for the edges. BUN indicates blood urea nitrogen; CRP, C-reactive protein; EPO, erythropoietin; ESAM, endothelial cell-selective adhesion molecule; GDF-15, growth differentiation factor 15; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IL-6, interleukin 6; MPO, myeloperoxidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NT-proCNP, amino terminal pro-C-type natriuretic peptide; PIGR, polymeric immunoglobulin receptor; proANP, pro-atrial-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; RAGE, receptor of advanced glycation end-products; ST-2, suppression of tumorigenicity 2; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; TNF-α-R1a, tumor necrosis factor α receptor 1a; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WAP4C, WAP 4 disulfide core domain protein.

lower levels of cardiac stretch markers NT-proBNP and pro-ANP are found in HFpEF, which confirm previous studies.[8,18] Furthermore, exclusive interactions between biomarkers in HFpEF were found to be associated with inflammation and angiogenesis. In contrast, HFrEF showed exclusive interactions associated with NT-proBNP. This is the first study reporting on exclusive interactions between biomarkers in HFrEF and HFpEF. Additionally, this study showed for the first time that angiogenesis marker neuropilin and remodeling marker osteopontin have exclusive predictive value for clinical outcome in HFpEF.

Levels of hs-CRP were found to be higher in HFpEF patients compared to HFrEF patients. Overall, reports with regard to differences in association of CRP between HFrEF and HFpEF have lacked consensus.[8,19–21] Yet, patients included in the previous studies were older and had relatively low levels of NT-proBNP.[8,19,20,22] Regardless of the difference in levels, predictive value for hs-CRP was found to be limited in both HFrEF and HFpEF after correction for a risk model in both this and an earlier study.[21] The cardiac stretch markers proANP and NT-proBNP were found to be lower in HFpEF. This is the first study reporting differential levels of proANP in HFrEF and HFpEF. The difference in levels of NTproBNP between HFrEF and HFpEF confirms earlier reports.[8,18,23]

A recent study used a similar network analysis approach.[8] However, the number of biomarkers studied was limited and no exclusive correlations were identified. When examining exclusive correlations in HFpEF and HFrEF between biomarkers, we identified correlations that were inflammation and angiogenesis associated in HFpEF, while correlations were associated with NT-proBNP in HFrEF. The relatively strong correlations between markers in both HFrEF and HFpEF provide putative insights into possible differences at the pathophysiological pathway level. For HFpEF, correlations were found to be associated with interleukin-6 and pentraxin-3. This is in line with earlier suggestions, in which a pro-inflammatory state was proposed to underlie the pathophysiology of HFpEF.[24–29] In contrast, exclusive interactions in HFrEF were associated with NT-proBNP. As such, the pathophysiology of HFrEF seems to be more associated with cardiac stretch and oxidative stress.[24] However, using network analysis for determining underlying pathophysiological differences between disease entities using biomarkers is a relatively novel approach. Future studies should confirm

Table 3. Interaction Within HFrEF and HFpEF

Biomarker		HFpEF		HFrEF		*P* Value (Difference)	*P* Value* (Difference)
		R	*P* Value*	*R*	*P* Value*		
HFpEF							
IL-6	D-Dimer	0.365	0.030†	0.149	1.000	0.001†	0.021†
Pentraxin-3	VEGF	−0.344	0.029†	−0.154	1.000	0.002†	0.043†
Periostin	VEGF	−0.438	0.001†	−0.112	1.000	<0.001†	0.001†
NGAL	PSAP-B1	0.396	0.010†	0.138	1.000	<0.001†	0.007†
HFrEF							
NT-proBNP	IL-6	0.135	1.000	0.363	<0.001†	0.001†	0.023†
NT-proBNP	EPO-A	0.147	1.000	0.36	<0.001†	0.001†	0.025†

EPO-A indicates erythropoietin; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; IL-6, interleukin 6; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; VEGF, vascular endothelial growth factor.
*Corrected *P*-value.
†*P*-value lower than the significance treshhold of 0.05.

these findings as well as combine them with data from experimental studies to examine whether the pathophysiological relationships found in clinical data also translate to pathophysiological differences in an experimental setting. Furthermore, most biomarkers are not cardiac exclusive.[5] This makes it relatively difficult to discern whether biomarker differences found in a clinical study are the cause or consequence of HF. To optimize interpretability of biomarker studies, future studies should be focused on biomarkers that are highly cardiac specific. Secondly, when biomarker differences are found, experimental studies should validate the findings and discern possible underlying pathophysiological processes.

This study also showed differential association with outcome of angiogenesis markers neuropilin and remodeling marker osteopontin, which were both found to be more predictive in HFpEF. Results with regard to differential association with outcome should be interpreted in an explanatory context of the pathophysiology, in which an increase in levels of a certain biomarker can be detrimental in 1 disease entity and not necessarily in the other through biological involvement or reflecting an underlying pathway. Indeed, osteopontin was reported earlier to be involved in prognosis in HF.[30] However, a differential involvement between HFrEF and HFpEF has not been previously reported. Earlier experimental studies found a direct involvement of osteopontin and cardiac remodeling, which in turn was found to cause diastolic dysfunction.[31]

Neuropilin is identified as a coreceptor of vascular endothelial growth factor receptor 2 (VEGFR-2).[32] In a murine model of cardiac pressure overload, animals that were heterozygous for neuropilin showed higher mortality rates.[33] This is the first study reporting the predictive value of neuropilin in HF for the combined end point. Here, we found

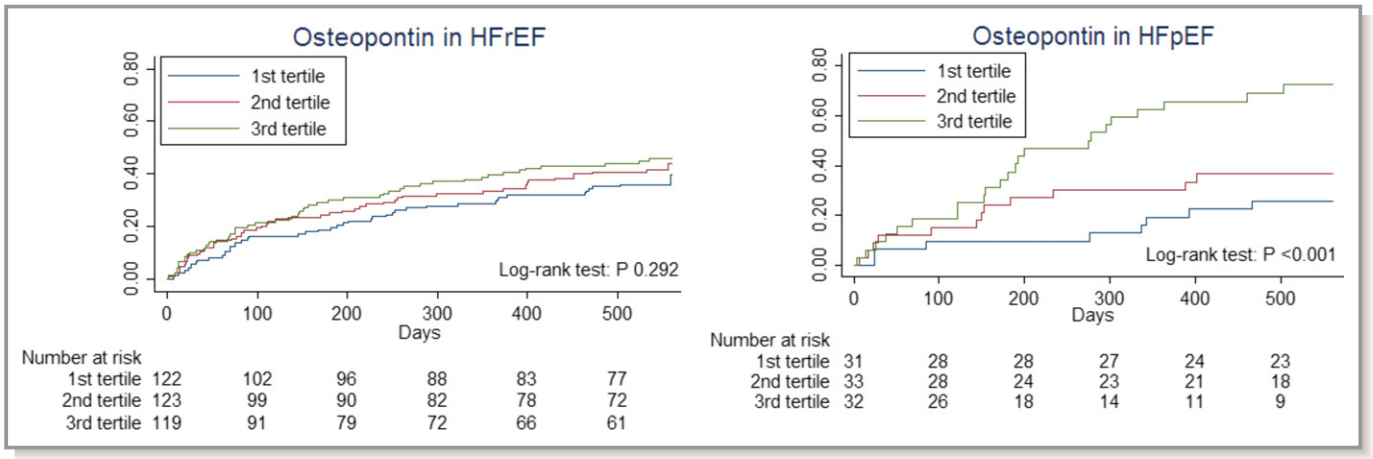

Figure 3. Kaplan–Meier curves depicting the relationship with outcome of osteopontin in tertiles, stratified to HFrEF and HFpEF. HFpEF indicates heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

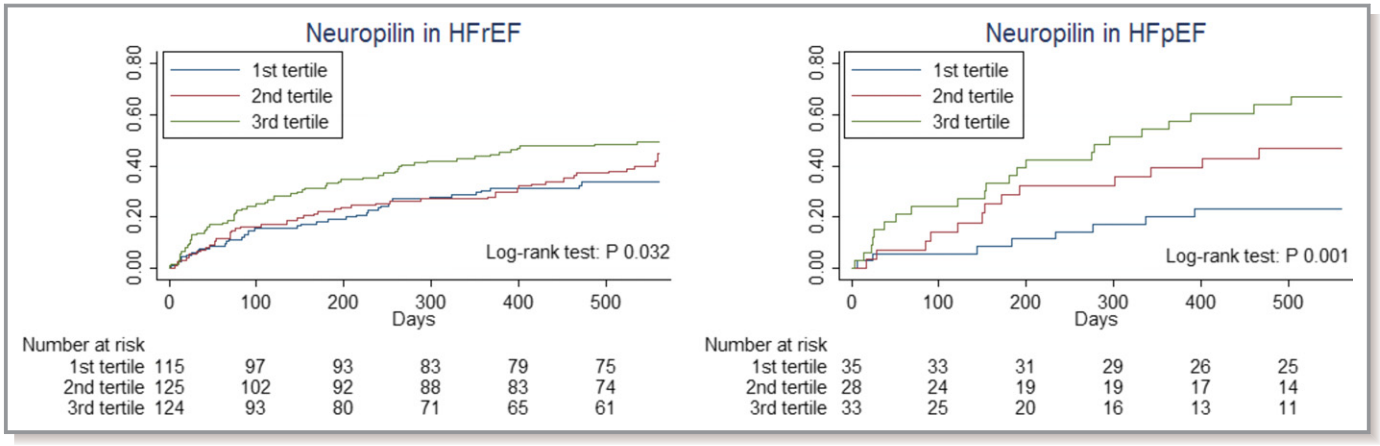

Figure 4. Kaplan–Meier curves depicting the relationship with outcome of neuropilin in tertiles, stratified to HFrEF and HFpEF. HFpEF indicates heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

that neuropilin was predictive of HF rehospitalizations in HFpEF. Additionally, in multivariable analysis, neuropilin only held predictive power in HFpEF. This suggests that neuropilin is more reflective of HF severity in HFpEF and not in HFrEF. Essentially, neuropilin is associated with angiogenesis. This again emphasizes the importance of angiogenesis markers in HFpEF compared to HFrEF.[24]

In earlier studies a significant association between outcome and HF status was found for end-terminal pro c-type natriuretic peptide and galectin-3 with a definition of HFpEF of LVEF >40%.[34,35] These findings were confirmed in this study. Additionally, an earlier publication found significant predictive value of syndecan-1 in HFpEF but not in HFrEF.[6] The fact that no significant interaction was found in this study for syndecan-1 and the primary end point can potentially be explained by the limited power of this study for HFpEF patients at a definition of LVEF >45%, and the previous publication for syndecan-1 corrected for a stepwise based model for syndecan-1 instead of the COACH risk model.

The clinical implications of this study are 2-fold. First, this study characterizes the underlying pathophysiology of patients with HFpEF to be associated with inflammation and endothelial function. This confirms earlier studies with regard to HFpEF and endorses the earlier proposed theory by Paulus et al.[24] Secondly, this study propagates a novel method for utilizing network analysis to analyze a wide array of biomarkers in discerning the underlying pathophysiology of disease entities in HF.[8] This methodology provides a possible step forward in dissecting the HF syndrome.[5,36]

Strengths and Limitations

The strengths of this study are the relatively high levels of NT-proBNP of both the HFrEF and HFpEF patients and the large number of available biomarkers. By having relatively high NT-proBNP levels, the HFpEF patients in this study represent true HF patients and have a relatively low number of false positives. Secondly, the large number of biomarkers from different disease domains available in this study provide for a more unbiased approach towards discerning underlying pathophysiological pathways.

However, the current analysis is a post-hoc analysis, leading to a possible selection bias. Secondly, since patients included are of European descent and relatively old, this limits extrapolation to patients of different age and origin. Also, pharmacological treatment during hospitalization might have influenced biomarker levels and associations between HFrEF and HFpEF. Furthermore, the choice for biomarkers was restricted by limited baseline sample availability, with the result that several interesting markers could not be studied. Therefore, this study is not an exhaustive study of biomarker-level differences in HFrEF and HFpEF and should be considered exploratory and hypothesis generating. Also, some of the biomarkers measured had relatively high coefficients of variation. Therefore, some possible interesting interactions and differences between biomarkers in HFrEF and HFpEF may have been missed. Most importantly, results from this study should be validated in a separate cohort.

The sampling of patients in COACH was performed at discharge after recompensation. Since no data are available on treatment during admission for HF previous to discharge, this might confound some of the reported findings. In this context, patients in the COACH trial cover a gray area between acute decompensated and chronic HF patients. The findings in this study should be regarded as explanatory in the context of the pathophysiology of HFpEF and HFrEF, acting as a stepping-stone for further research.

Conclusions

Biomarker levels differ in HFpEF and HFrEF, mainly in the domains of cardiac stretch and inflammation. Interactions in

HFpEF were found to be associated with inflammation and angiogenesis, while interactions in HFrEF were associated with cardiac stretch. The angiogenesis marker neuropilin and remodeling marker osteopontin were found to only hold predictive value in HFpEF, possibly reflecting underlying pathophysiological processes. Results of this study should be confirmed in prospective biomarker studies.

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Disclosures

Tromp, Khan, Klip, Meyer, de Boer, Jaarsma, Hillege, van Veldhuisen, and van der Meer have nothing to disclose with regard to this manuscript. Voors received research grants from Alere, Singulex, and Sphingotec.

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Supplemental Material

Table S1. Differences between entire cohort and subcohort

	Total cohort (n=1023)	Sub cohort (n=460)
Treatment allocation		
Control group	33	32,2
Basic support	33,3	32,4
Intensive support	33,6	35,4
Demographics and clinical signs		
Age (years)	70.8 ± 11	70.6 ± 11.1
Female sex (%)	37,5	37,4
BMI (kg/m2	26.9 ± 5.3	27.0 ± 5.6
Systolic BP (mmHg)	118.3 ± 21.0	117.9 ± 21.3
Heart rate (bpm)	74.6 ± 13.4	74.2 ± 13.4
LVEF (%)	33.7 ± 14.4	32.5 ± 14.0
Previous HF hospitalization	32,7	33,7
NYHA class, II/III/IV (%)	50.9/45.7/3.4	44/52/4
Medical history (%)		
Myocardial infarction	42,6	40,7
Stroke	16	14,8
Hypertension	42,9	41,5
Atrial fibrillation of flutter	44	45,4
Diabetes	29,3	29,3
COPD	26,2	28,3
Laboratory		
Hemoglobin (g/dL)	13.1 ± 2.0	13.2 ± 2.1
Sodium (mmol/L)	139 ± 4	138.6 ± 4.3
Creatinine (µmol/L)	125.0 ± 53	125.7 ± 52.8
eGFR (mL/min/1.73m2)	55.2 ± 21.1	54.9 ± 20.5
BUN (mmol/L)	10.7 (8.1 - 15.2)	11.0 (8.2 - 15.5)
Treatment at discharge (%)		
ACE inhibitor or ARB	82,8	82,2
Beta blocker	66,2	67,8
Diuretic	95,8	95,7
MRA	54,1	56,3
Statin	37,9	39,8
Digoxin	30,2	33,7

Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; BP, blood pressure; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HF, heart failure; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; NYHA, New York heart association.

Table S2. Biomarker assay data.

Biomarker	Intra Assay %CV	Inter Assay %CV	Low Cutoff	High Cutoff	Units
LTBR	13%	13%	0,028	45	ng/mL
Mesothelin	12%	12%	6,1	120	ng/mL
MPO	15%	14%	2	800	ng/mL
Neuropilin 1	14%	15%	1	900	ng/mL
Osteopontin	21%	22%	2,5	2500	ng/mL
Pentraxin 3	10%	11%	0,07	150	ng/mL
Periostin	12%	12%	2,3	1921	ng/mL
PIGR	16%	16%	12	2341	ng/mL
PSAP-B	14%	16%	2	530	ng/mL
ST-2	9%	10%	0,28	380	ng/mL
Syndecan-1	25%	24%	2,4	393	ng/mL
TNFR1A	11%	13%	0,025	68	ng/mL
Troy	15%	14%	0,044	87	ng/mL
RAGE	9%	10%	0,019	85	ng/mL
VEGFR1	13%	12%	0,38	195	ng/mL
NTProCNP	11%	12%	0,003	9	ng/mL
WAP4C	14%	14%	0,16	130	ng/mL
ANP propeptide	29%	28%	1600	110000	pg/mL
D-Dimer	9%	10%	0,028	26	ug/mL
ESAM	9%	9%	0,5	110	ng/mL
GDF-15	9%	10%	0,014	6,4	ng/mL
Angiogenin	18%	18%	170	40000	ng/mL
CRP	17%	16%	0,065	33	ug/mL
NGAL	19%	21%	7,5	1500	ng/mL

Biomarker	Low cut off	high cut off	Inter assay coefficient of variation (%)
IL-6	0.10	0.88	13
cTNI	0.20	1000	10
ET1	0.5	250	7

BG medicine	Total Imprecision		Detection limit			
	Intra-assay variability (%)	Intra-assay variability (%)	LoB	LoD	LoQ	Measuring range
Galectin-3	3,2	5,6	0,86	1,13	1,32	1,4-94,8

Table S3. Logistic regression correcting for clinical confounders

Marker	Odds ratio(95%CI)	P-value
Hs-CRP (doubling)	1.25 (1.08-1.46)	**0.003**
Model 1	1.29 (1.09-1.52)	**0.003**
Model 2	1.28 (1.08-1.51)	**0.004**
Pentraxin-3 (doubling)	0.74 (0.56-0.98)	**0.037**
Model 1	0.81 (0.60-1.09)	0.165
Model 2	0.83 (0.62-1.12)	0.212
NT-proBNP (doubling)	0.75 (0.65-0.87)	**<0.001**
Model 1	0.68 (0.57-0.82)	**<0.001**
Model 2	0.74 (0.62-0.88)	**0.001**
proANP (doubling)	0.72 (0.58-0.89)	**0.002**
Model 1	0.66 (0.51-0.85)	**0.001**
Model 2	0.69 (0.54-.0.89)	**0.004**
VEGF (doubling)	1.09 (0.97-1.23)	0.159
Model 1	1.03 (0.90-1.18)	0.639
Model 2	1.05 (0.92-1.20)	0.442

Model 1: age, sex, eGFR, systolic blood pressure, a history of myocardial infarction; diabetes; atrial fibrillation and anemia
Model 2: Model 1+ ACE-inhibitors/ARB & Beta-blocker usage

Abbreviations: Hs-CRP, high-senstive C-reactive protein; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; Pro-ANP, pro-atrial-type natriuretic peptide; VEGF, vascular endothelial growth factor

Table S4. Sensitivity analysis exclusive interactions

		HFpEF		HFrEF	
Biomarker		*R*	*p-value**	*R*	*p-value**
HFpEF					
IL6	D-Dimer	0.361	0.63	0.158	0.840
Pentraxin-3	VEGF	-0.388	0.21	-0.157	1.000
Periostin	VEGF	-0.476	**<0.001**	-0.102	1.000
NGAL	PSAP-B1	0.381	0.21	0.147	1.000
HFrEF					
NT-proBNP	IL6	0.204	1.000	0.378	**<0.001**
NT-proBNP	EPO-A	0.315	1.000	0.360	**<0.001**

***corrected p-value**

Abbreviations: EPO-A, erythropoietin; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; IL-6, Interleukin 6; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; VEGF, vascular endothelial growth factor.

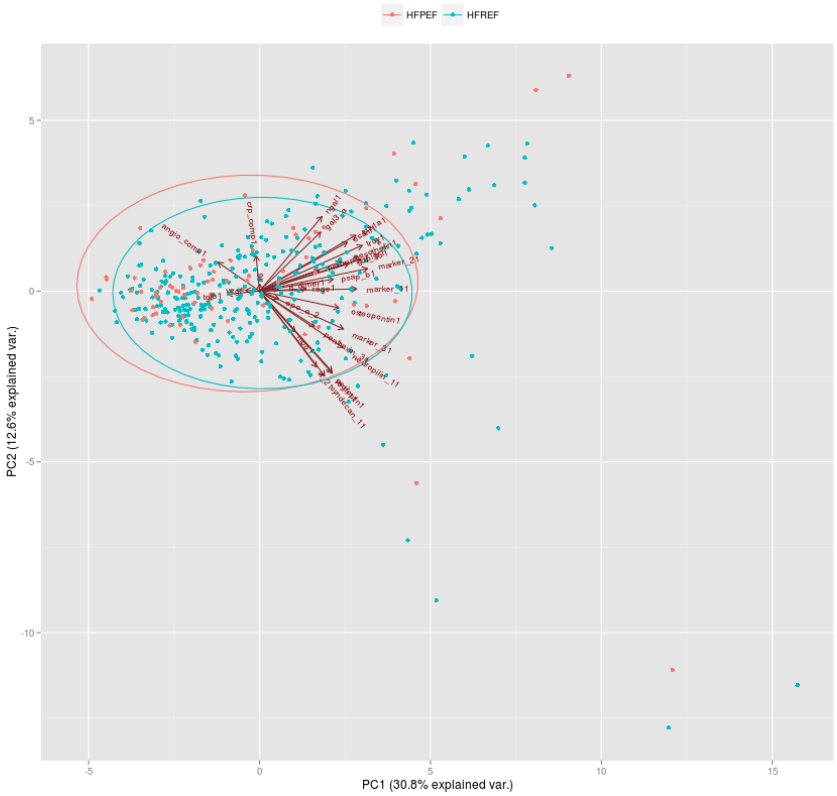
Table S5. Relationship with outcome of biomarkers.

	HFrEF (n = 364)	HFpEF (n = 96)	p-value[1]	p-value[2]
Inflammation	HR (95%CI) p-value	HR (95%CI) p-value		
hs-CRP, (doubling)	0.98 (0.88-1.08) 0.684	1.00 (0.77-1.30) 0.982	0.615	0.638
Pentraxin-3, (doubling)	0.89 (0.71-1.12) 0.336	1.14 (0.73-1.77) 0.569	0.074	0.277
GDF-15, (doubling)	1.09 (0.81-1.48) 0.563	2.06 (1.16-3.65) **0.014**	0.180	0.064
RAGE, (doubling)	1.05 (0.86-1.28) 0.629	1.34 (0.90-1.98) 0.147	0.174	0.451
Interleukin 6, (doubling)	1.00 (0.87-1.16) 0.943	1.68 (1.14-2.48) **0.008**	0.136	**0.014**
TNF-α, (doubling)	1.01 (0.96-1.06) 0.707	0.97 (0.88-1.07) 0.580	0.282	0.610
TNF-α-R1a, (doubling)	1.31 (0.99-1.73) 0.057	1.47 (0.90-2.39) 0.120	0.666	0.653
Oxidative stress				
MPO, (doubling)	0.89 (0.74-1.08) 0.243	1.12 (0.70-1.79) 0.644	0.505	0.276
Remodelling				
Syndecan-1, (doubling)	1.01 (0.82-1.24) 0.955	1.38 (0.99-1.93) 0.059	0.244	0.163
Periostin, (doubling)	1.03 (0.80-1.33) 0.824	1.17 (0.76-1.79) 0.485	0.798	0.849
Galectin-3, (doubling)	0.86 (0.59-1.25) 0.425	2.57 (1.19-5.53) **0.016**	**0.070**	**0.026**
ST-2, (doubling)	0.98 (0.86-1.11) 0.694	1.27 (0.96-1.67) 0.092	0.268	0.219
Osteopontin, (doubling)	0.90 (0.72-1.14) 0.398	1.60 (0.98-2.62) 0.062	**0.004**	**0.009**
TGF-ß, (doubling)	1.01 (0.91-1.13) 0.834	1.07 (0.89-1.28) 0.465	0.702	0.466
Cariomyocyte stretch				
NT-proBNP, (doubling)	1.28 (1.14-1.43) <0.001	1.42 (1.10-1.84) 0.007	0.417	0.605
proANP, (doubling)	1.02 (0.82-1.27) 0.840	1.23 (0.85-1.76) 0.268	0.364	0.437
TnI, (doubling)	1.16 (1.06-1.28) 0.001	1.07 (0.86-1.33) 0.532	0.347	0.269
Angiogenesis				
VEGF, (doubling)	0.88 (0.81-0.96) 0.004	1.13 (0.88-1.47) 0.326	0.273	0.080
VEFGR (doubling)	1.19 (0.94-1.51) 0.156	1.37 (0.93-2.01) 0.106	0.918	0.603
Angiogenin, (doubling)	0.89 (0.74-1.06) 0.195	0.67 (0.47-0.95) **0.026**	0.139	0.156
NT-proCNP, (doubling)	0.96 (0.73-1.25) 0.749	1.69 (1.15-2.49) **0.007**	0.232	**0.042**
Neuropilin-1 (doubling)	1.12 (0.85-1.48) 0.425	2.34 (1.40-3.90) **0.001**	**0.017**	**0.024**
Arteriosclerosis				
ESAM, (doubling)	1.35 (0.79-2.29) 0.268	1.77 (0.85-3.71) 0.127	0.571	0.528
Renal function				
NGAL, (doubling)	0.95 (0.69-1.29) 0.729	0.84 (0.46-1.53) 0.569	0.702	0.884
BUN, (doubling)	0.91 (0.63-1.33) 0.632	1.03 (0.49-2.19) 0.929	0.673	0.816
Haematopoiesis				
EPOa, (doubling)	1.09 (0.96-1.24) 0.197	1.27 (1.00-1.62) 0.049	0.745	0.129
Other				
D-Dimer, (doubling)	1.09 (0.97-1.22) 0.132	1.31 (0.99-1.75) 0.056	0.452	0.331
WAP4C (doubling)	1.18 (0.93-1.50) 0.164	1.61 (1.09-2.37) 0.016	0.413	0.181
Mesothelin, (doubling)	1.19 (0.88-1.61) 0.258	0.93 (0.46-1.89) 0.841	0.569	0.803
PIGR (doubling)	1.03 (0.79-1.34) 0.825	1.79 (1.13-2.83) 0.013	0.207	0.101
PSAP (doubling)	1.24 (0.96-1.59) 0.099	1.26 (0.79-2.01) 0.324	0.786	0.844
ET-1, (doubling)	1.30 (0.93-1.80) 0.120	0.99 (0.46-2.13) 0.972	0.746	0.859
TROY (doubling)	0.98 (0.73-1.31) 0.868	1.63 (1.05-2.54) 0.030	0.351	0.101

1. Univariable interaction p-value
2. Multivariable interaction p-value

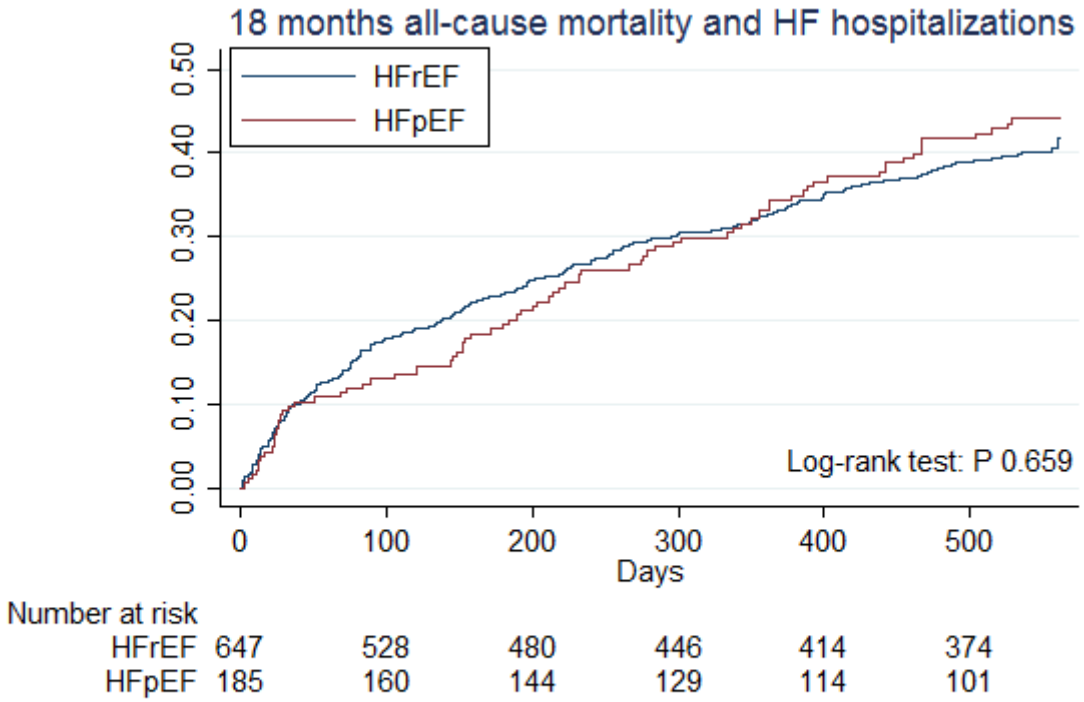
Abbreviations: BUN, blood urea nitrogen; cTNI, cardiac troponin-I; EPOa, erythropoietin; ESAM, endothelial cell-selective adhesion molecule; ET-1, endothelin-1; GDF-15, growth differentiation factor 15; HFrEF, heart failure with a reduced ejection fraction ; HFpEF, heart failure with a preserved ejection fraction; hs-CRP, high-sensitive C-reactive protein; IL-6, Interleukin 6; MPO, myeloperoxidase; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NT-proCNP, amino terminal pro-C-type natriuretic peptide; PIGR, polymeric immunoglobulin receptor; Pro-ANP, pro-atrial-type natriuretic peptide; PSAP, prostate-specific acid phosphatase; RAGE, receptor of advanced glycation end-products; TGF-b, transforming growth factor beta; TNF-a, tumor necrosis factor alpha; TNF-aR1a, tumor necrosis factor alpha receptor 1a; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WAP4C, WAP 4 disulfide core domain protein;

Figure S1. PCA analysis



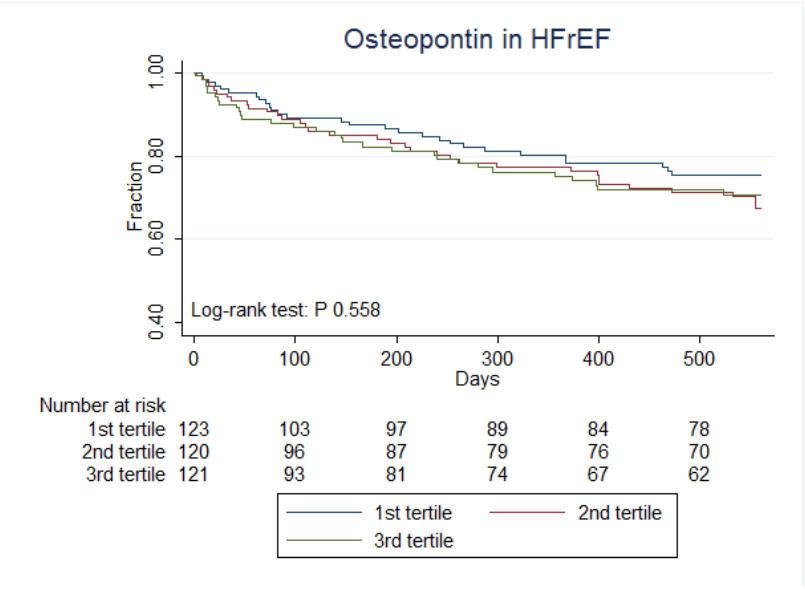
Principal Component Analysis – PCA plot illustrating the first two principal components, collectively accounting for 43.4% (PC1 accounting for 30.8%, and PC2 for 12.6%) of the overall variance in the combined HFpEF and HFrEF biomarker measurements. The PCA was performed using HFpEF and HFrEF as categorical variables, where biomarker levels are displayed as red and blue for patients with HFpEF and HFrEF respectively.

Figure S2. Surival stratified according to HFrEF and HFpEF



Number at risk						
HFrEF	647	528	480	446	414	374
HFpEF	185	160	144	129	114	101

Figure S3. Osteopontin in HFrEF and HFpEF for HF related hospitalizations at 18 months.



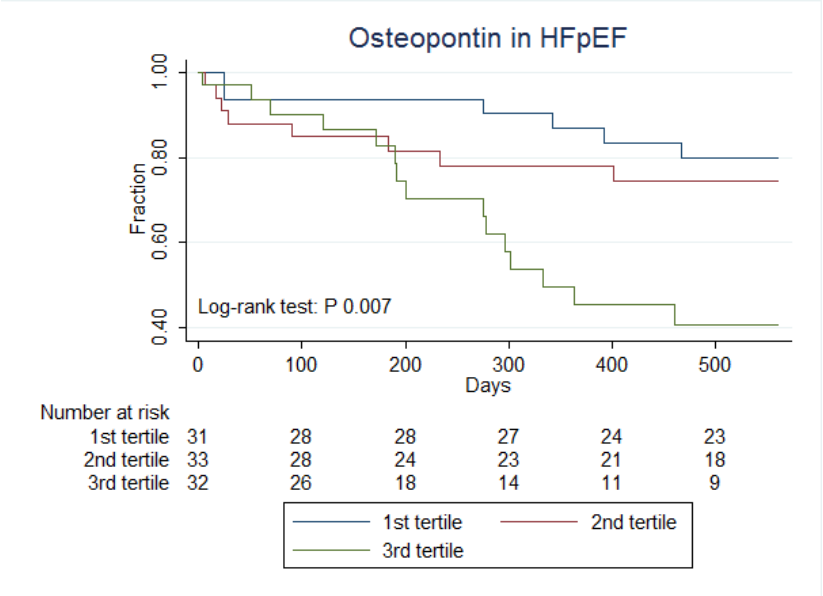
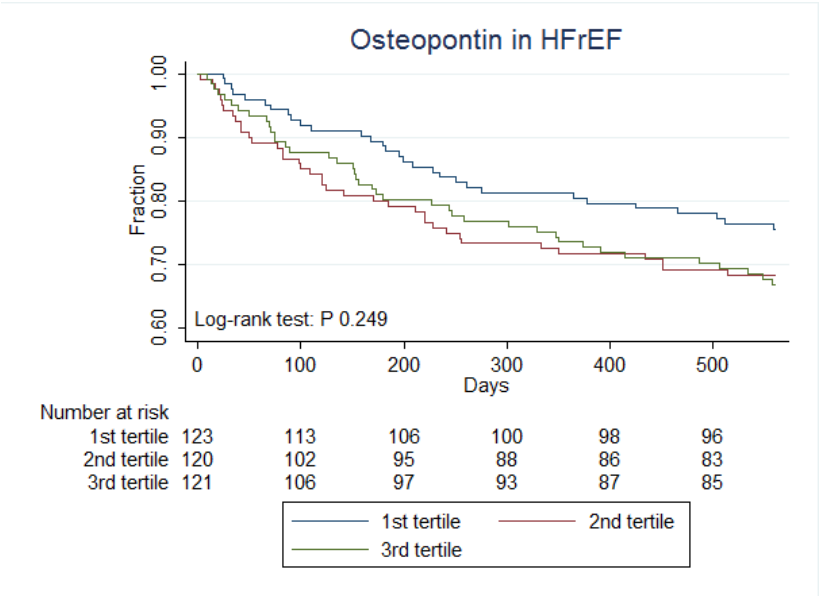


Figure S4. Osteopontin in HFrEF and HFpEF for all-cause mortality at 18 months.



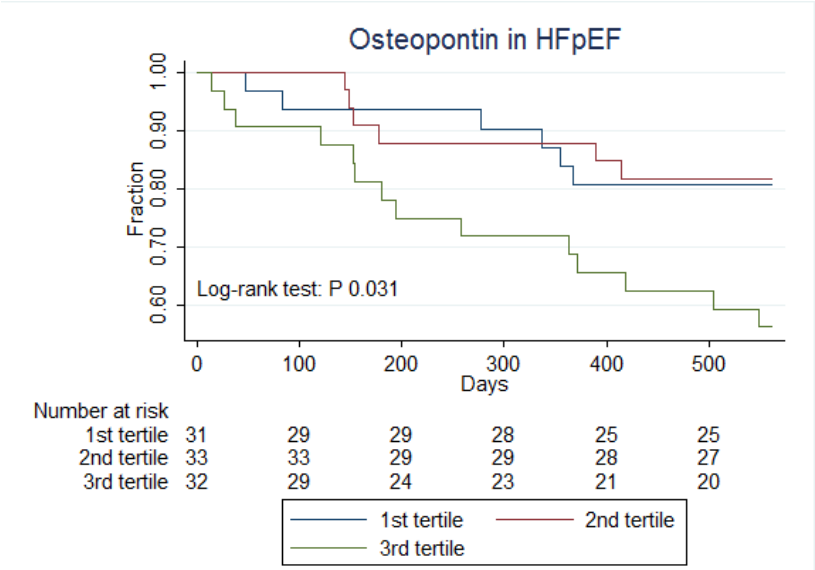
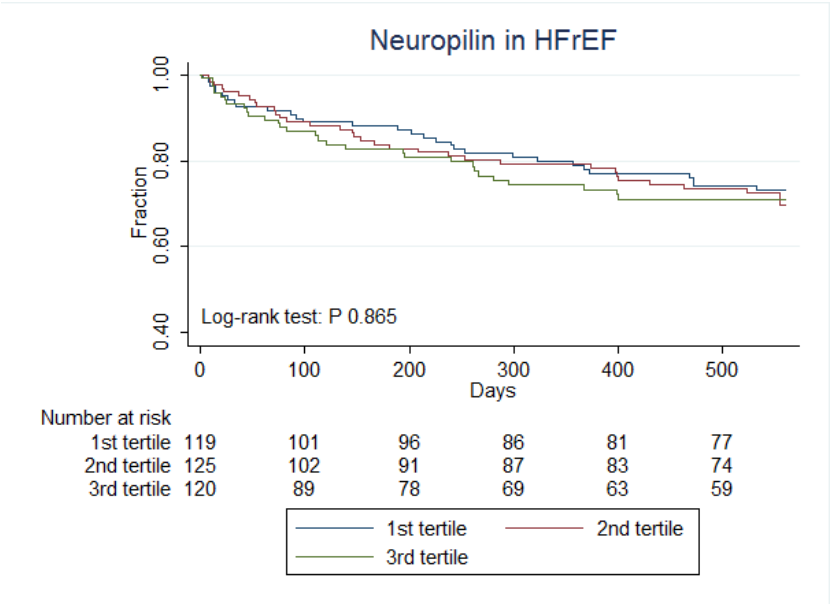


Figure S5. Neuropilin in HFrEF and HFpEF for HF related hospitalizations at 18 months.



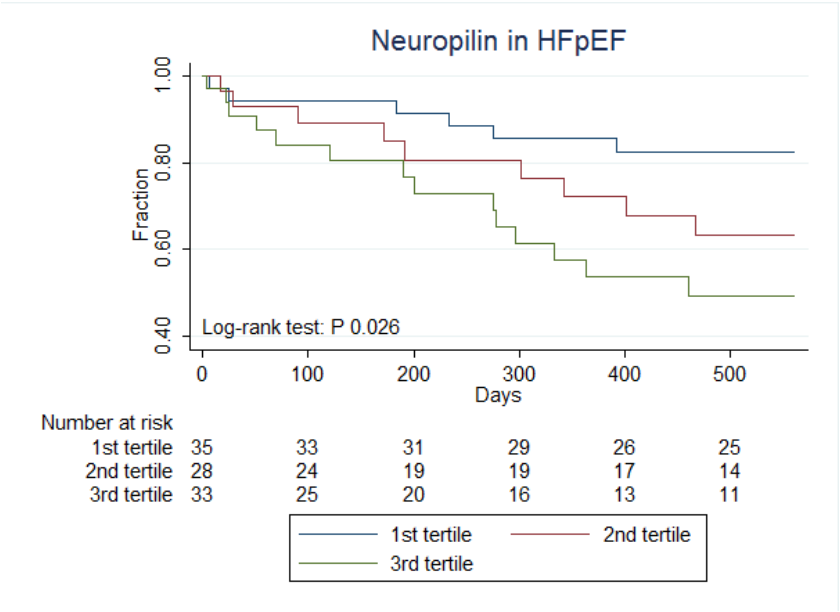
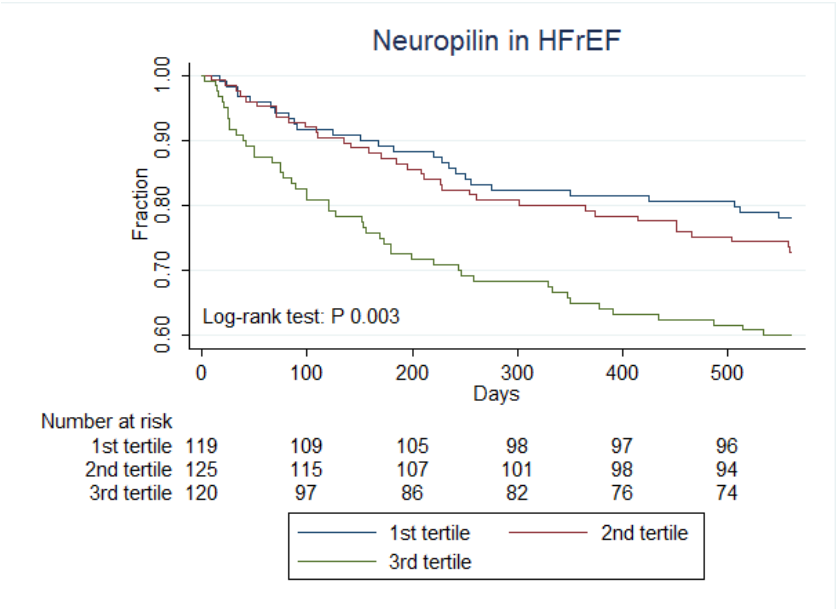
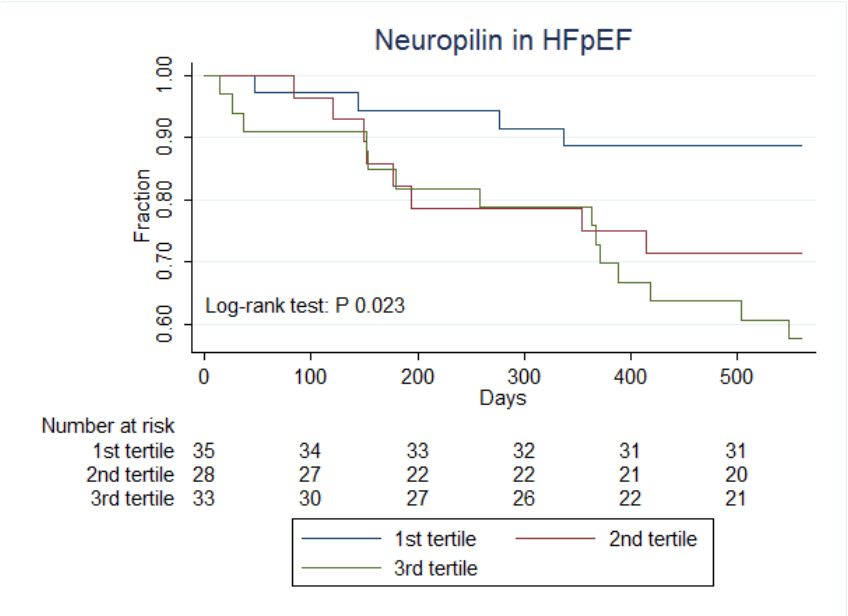


Figure S6. Neuropilin in HFrEF and HFpEF for all-cause mortality at 18 months.





Biomarker Profiles in Heart Failure Patients With Preserved and Reduced Ejection Fraction

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