

# **Supplemental Material**

**Table S1. Differences between entire cohort and subcohort**

	Total cohort (n=1023)	Sub cohort (n=460)
<b>Treatment allocation</b>		
Control group	33	32,2
Basic support	33,3	32,4
Intensive support	33,6	35,4
<b>Demographics and clinical signs</b>		
Age (years)	70.8 ± 11	70.6 ± 11.1
Female sex (%)	37,5	37,4
BMI (kg/m <sup>2</sup> )	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
<b>Medical history (%)</b>		
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
<b>Laboratory</b>		
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/1.73m <sup>2</sup> )	55.2 ± 21.1	54.9 ± 20.5
BUN (mmol/L)	10.7 (8.1 - 15.2)	11.0 (8.2 - 15.5)
<b>Treatment at discharge (%)</b>		
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		Low Cutoff	High Cutoff	Units
		%CV				
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

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

**Table S3. Logistic regression correcting for clinical confounders**

<b>Marker</b>	<b>Odds ratio(95%CI)</b>	<b>P-value</b>
Hs-CRP (doubling)	1.25 (1.08-1.46)	<b>0.003</b>
Model 1	1.29 (1.09-1.52)	<b>0.003</b>
Model 2	1.28 (1.08-1.51)	<b>0.004</b>
Pentraxin-3 (doubling)	0.74 (0.56-0.98)	<b>0.037</b>
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)	<b>&lt;0.001</b>
Model 1	0.68 (0.57-0.82)	<b>&lt;0.001</b>
Model 2	0.74 (0.62-0.88)	<b>0.001</b>
proANP (doubling)	0.72 (0.58-0.89)	<b>0.002</b>
Model 1	0.66 (0.51-0.85)	<b>0.001</b>
Model 2	0.69 (0.54-.0.89)	<b>0.004</b>
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-sensitive 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	
<i>Biomarker</i>		<i>R</i>	<i>p-value*</i>	<i>R</i>	<i>p-value*</i>
<b>HFpEF</b>					
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	<b>&lt;0.001</b>	-0.102	1.000
NGAL	PSAP-B1	0.381	0.21	0.147	1.000
<b>HFrEF</b>					
NT-proBNP	IL6	0.204	1.000	0.378	<b>&lt;0.001</b>
NT-proBNP	EPO-A	0.315	1.000	0.360	<b>&lt;0.001</b>

**\*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.

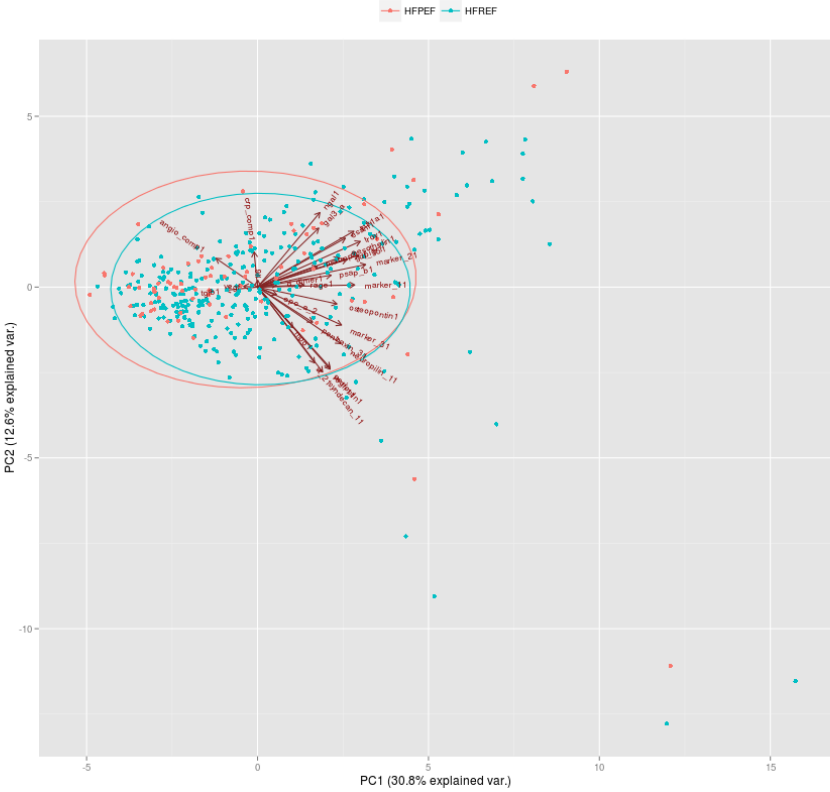
**Table S5. Relationship with outcome of biomarkers.**

	HFrfEF (n = 364)		HFpEF (n = 96)		p-value <sup>1</sup>	p-value <sup>2</sup>
	HR (95%CI)	p-value	HR (95%CI)	p-value		
<b>Inflammation</b>						
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)	<b>0.014</b>	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)	<b>0.008</b>	0.136	<b>0.014</b>
TNF- $\alpha$ , (doubling)	1.01 (0.96-1.06)	0.707	0.97 (0.88-1.07)	0.580	0.282	0.610
TNF- $\alpha$ -R1a, (doubling)	1.31 (0.99-1.73)	0.057	1.47 (0.90-2.39)	0.120	0.666	0.653
<b>Oxidative stress</b>						
MPO, (doubling)	0.89 (0.74-1.08)	0.243	1.12 (0.70-1.79)	0.644	0.505	0.276
<b>Remodelling</b>						
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)	<b>0.016</b>	<b>0.070</b>	<b>0.026</b>
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	<b>0.004</b>	<b>0.009</b>
TGF- $\beta$ , (doubling)	1.01 (0.91-1.13)	0.834	1.07 (0.89-1.28)	0.465	0.702	0.466
<b>Cardiomyocyte stretch</b>						
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
<b>Angiogenesis</b>						
VEGF, (doubling)	0.88 (0.81-0.96)	0.004	1.13 (0.88-1.47)	0.326	0.273	0.080
VEGFR (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)	<b>0.026</b>	0.139	0.156
NT-proCNP, (doubling)	0.96 (0.73-1.25)	0.749	1.69 (1.15-2.49)	<b>0.007</b>	0.232	<b>0.042</b>
Neuropilin-1 (doubling)	1.12 (0.85-1.48)	0.425	2.34 (1.40-3.90)	<b>0.001</b>	<b>0.017</b>	<b>0.024</b>
<b>Arteriosclerosis</b>						
ESAM, (doubling)	1.35 (0.79-2.29)	0.268	1.77 (0.85-3.71)	0.127	0.571	0.528
<b>Renal function</b>						
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
<b>Haematopoiesis</b>						
EPOa, (doubling)	1.09 (0.96-1.24)	0.197	1.27 (1.00-1.62)	0.049	0.745	0.129
<b>Other</b>						
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;

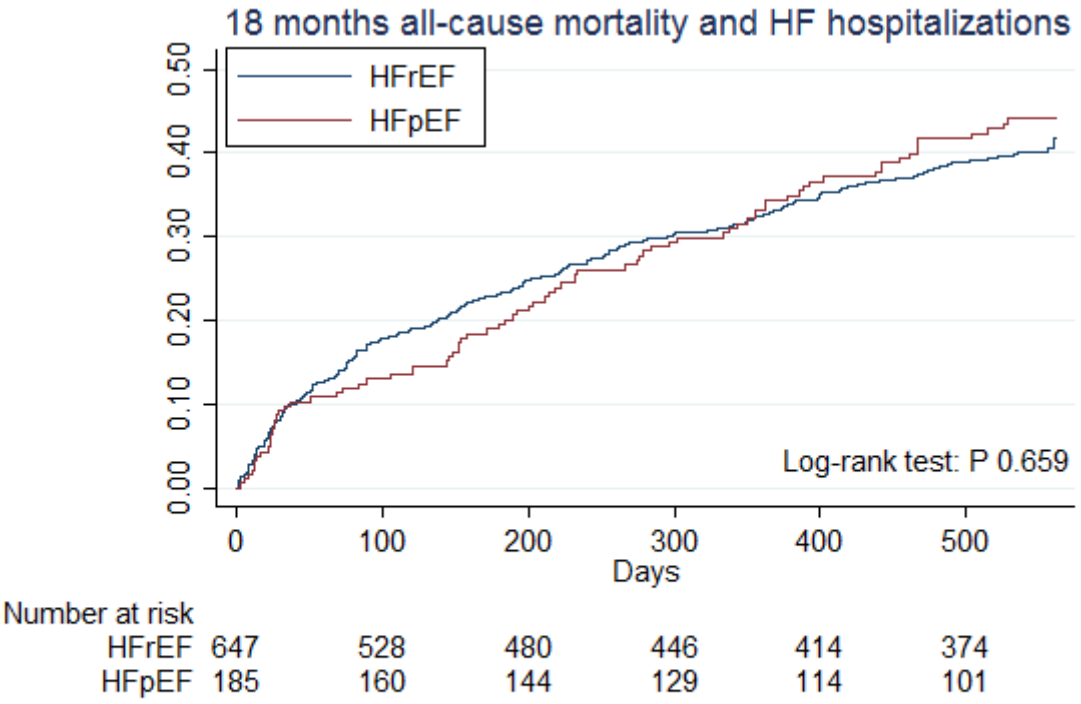
Figure S1. PCA analysis



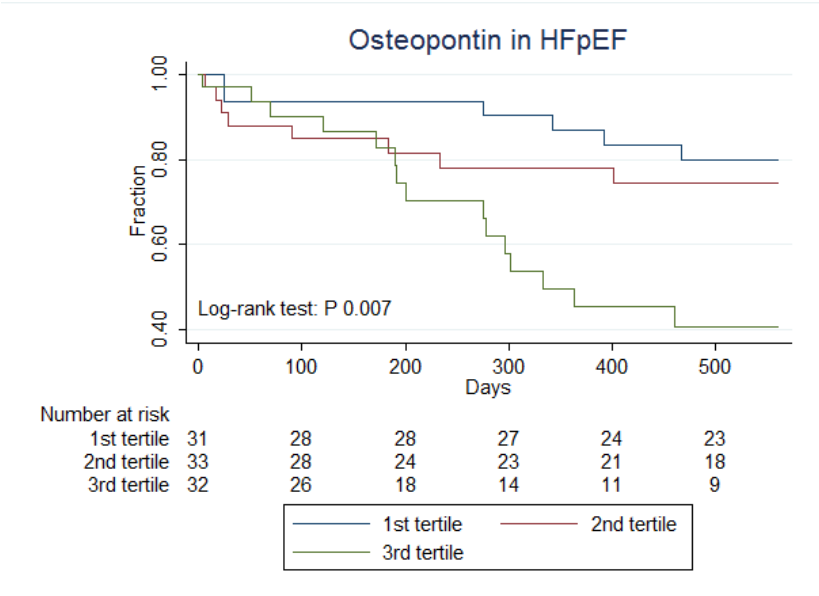
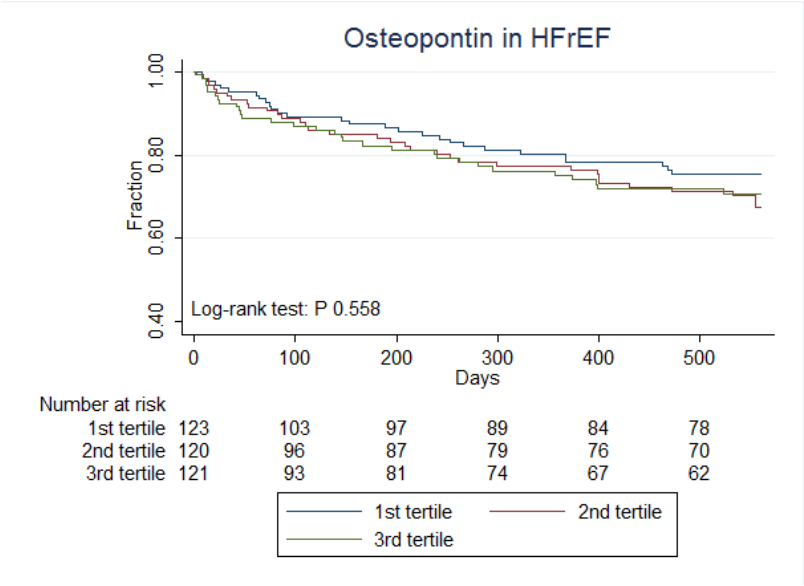
**Principal Component Analysis** – PCA plot illustrating the first two principal components, collectively accounting for 43.4% (PC1 accounting for 30.8%, and PC2 for 12.6%) of the overall variance in the combined HFpEF and HFrEF biomarker measurements. The PCA was performed using HFpEF and HFrEF as categorical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



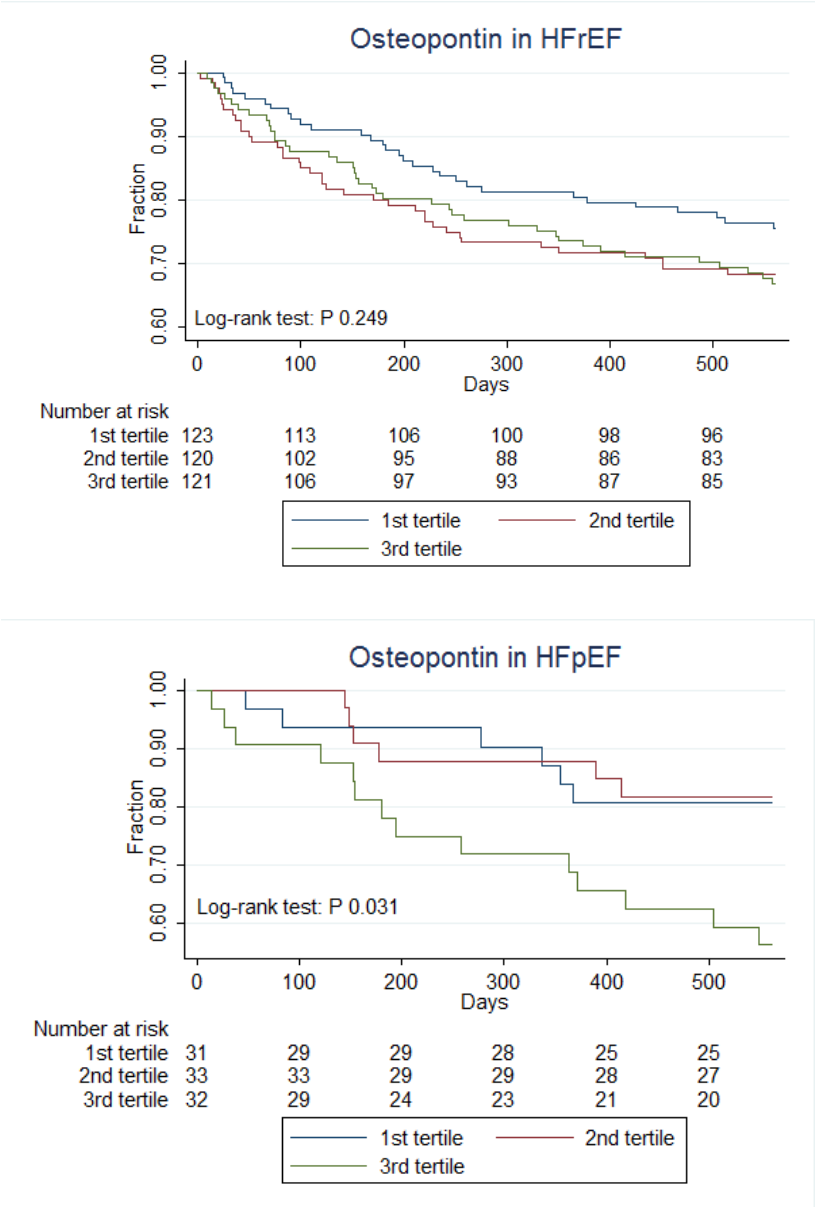
Figure S2. Survival stratified according to HFrEF and HFpEF



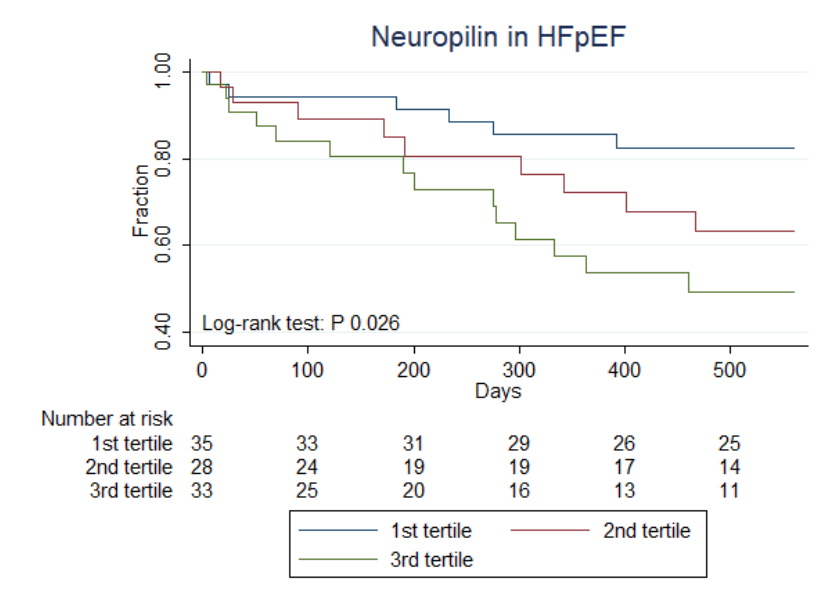
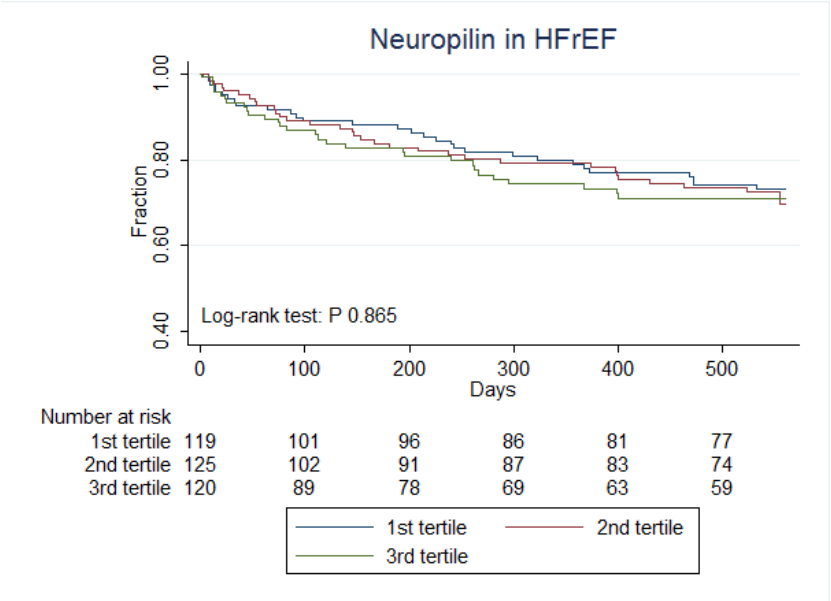
**Figure S3. Osteopontin in HFrEF and HFpEF for HF related hospitalizations at 18 months.**



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



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



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

