

Supplementary information

Title: Systemic oxidative stress associates with disease severity and outcome in patients with new-onset or worsening heart failure

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Journal: Clinical Research in Cardiology

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Supplementary Table 1 – Baseline characteristics of the BIOSTAT-CHF index cohort according to tertiles of serum free thiols

	1st tertile n=726	2nd tertile n=725	3rd tertile n=725	P-value
Serum free thiols (µmol/L)	227 [190;258] Full range (38-281)	326 [304;348] Full range (281-369)	419 [392;458] Full range (369-799)	
<i>Demographics</i>				
Age (years)	74.2 [65.7;79.8]	70.7 [62.1;78.8]	65.2 [56.6;74.1]	<0.001
Female sex	237 (33%)	208 (29%)	142 (20%)	<0.001
BMI (kg/m ²)	26.7 [23.6;30.5]	27.2 [23.9;30.5]	27.2 [24.5;30.9]	0.198
HF type				<0.001
HF _r EF	503 (78%)	521 (80%)	549 (84%)	
HF _{mr} EF	82 (13%)	81 (13%)	78 (12%)	
HF _p EF	64 (10%)	46 (7%)	24 (4%)	
Months since HF diagnosis	1 [0;12]	6 [0;46]	3 [0;53]	0.305
Ischaemic etiology	304 (43%)	330 (46%)	319 (45%)	0.421
Inpatient enrolment	604 (83%)	520 (72%)	423 (58%)	<0.001
NYHA class				<0.001
I/II	207 (30%)	269 (38%)	332 (47%)	
III/VI	494 (71%)	431 (62%)	379 (53%)	
Systolic BP (mmHg)	120 [110;135]	120 [110;140]	120 [110;136]	0.019
Diastolic BP (mmHg)	70 [64;80]	73 [66;82]	76.0 [70;85]	<0.001
Heart rate (bpm)	77 [66;90]	76 [68;90]	75 [67;88]	0.854
LVEF (%)	30 [25;38]	30 [25;36]	30 [25;35]	0.038
<i>Signs and symptoms</i>				
Peripheral oedema	433 (67%)	352 (60%)	288 (51%)	<0.001
Elevated JVP	208 (39%)	169 (32%)	116 (23%)	<0.001
Hepatomegaly	127 (18%)	101 (14%)	81 (11%)	0.002
Pulmonary congestion	444 (63%)	393 (56%)	301 (43%)	<0.001
<i>Medical history</i>				
Anaemia	304 (43%)	240 (35%)	220 (32%)	<0.001
Atrial fibrillation	369 (51%)	340 (47%)	282 (39%)	<0.001
Diabetes Mellitus	233 (32%)	235 (32%)	234 (32%)	0.991
COPD	139 (19%)	124 (17%)	112 (15%)	0.175
CKD	282 (39%)	186 (26%)	144 (20%)	<0.001
Hypertension	460 (63%)	459 (63%)	435 (60%)	0.318
PAVD	84 (12%)	81 (11%)	81 (11%)	0.963
Stroke	78 (11%)	73 (10%)	54 (7%)	0.076
PCI	128 (18%)	140 (19%)	179 (25%)	0.002
CABG	134 (19%)	133 (18%)	105 (15%)	0.073
<i>Medication use at baseline</i>				
Loop diuretics	723 (99%)	722 (99%)	719 (99%)	0.585
Loop diuretic dose (mg furosemide equivalent)	40 [40;125]	40 [40;100]	40 [40;80]	<0.001
ACEi/ARB	491 (68%)	517 (71%)	556 (77%)	0.001
Beta-blocker	595 (82%)	604 (83%)	608 (84%)	0.609
Aldosteron antagonist	376 (52%)	382 (53%)	383 (53%)	0.912
<i>Laboratory</i>				
Haemoglobin (g/dL)	12.9 [11.6;14.1]	13.4 [12.0;14.5]	13.6 [12.2;14.8]	<0.001
Leukocytes (10 ⁹ /L)	7.8 [6.3;9.8]	7.9 [6.6;9.6]	7.8 [6.4;9.4]	0.581
Sodium (mmol/L)	139 [136;142]	140 [137;142]	140 [137;142]	0.143
Potassium (mmol/L)	4.2 [3.8;4.6]	4.2 [3.9;4.6]	4.3 [3.9;4.6]	0.092
Urea (mmol/L)	13.6 [8.6; 22.5]	10.5 [7.3; 16.1]	9.8 [7.0; 15.3]	<0.001
Serum creatinin (µmol/L)	113 [90;150]	103 [82;128]	97 [80;116]	<0.001
eGFR (mL/min/1.73 m ²)	51 [35;68]	59 [45;77]	68 [52;84]	<0.001
NT-proBNP (ng/L)	3890 [1836; 8325]	2858 [1329;5386]	1910 [851;4052]	<0.001
Albumin (g/L)	29 [24;34]	32 [27;37]	36 [31;41]	<0.001

LDL-cholesterol (mmol/L)	2.2 [1.7; 3.0]	2.6 [1.9; 3.3]	2.6 [2.0; 3.3]	<0.001
HDL-cholesterol (mmol/L)	1.02 [0.83;1.30]	1.06 [0.88;1.32]	1.04 [0.85;1.30]	0.753
Glucose (mmol/L)	6.3 [5.4;7.8]	6.4 [5.4;8.0]	6.2 [5.4;8.0]	0.539

Data shown as median [IQR] or n (%). Significant *P*-values are bold-printed.
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; HF, heart failure; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrfEF, heart failure with reduced ejection fraction; JVP, jugular venous pressure; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PAVD, peripheral arterial vascular disease; PCI, percutaneous coronary intervention.

Supplementary Table 2 – Baseline characteristics of the BIOSTAT-CHF validation cohort according to tertiles of serum free thiols

	1st tertile n=542	2nd tertile n=542	3rd tertile n=542	P-value
Serum free thiols (µmol/L)	269 [236;296] Full range (59-317)	353 [337;371] Full range (317-388)	431 [406;461] Full range (388-645)	
<i>Demographics</i>				
Age (years)	78.2 [70.6;83.9]	75.9 [68.3;81.6]	70.8 [64.5;78.0]	<0.001
Female sex	215 (40%)	198 (37%)	146 (27%)	<0.001
BMI (kg/m ²)	27.5 [23.9;32.1]	28.0 [24.8;32.7]	28.5 [25.1;32.7]	0.051
HF type				0.580
HF _r EF	219 (44%)	234 (48%)	220 (46%)	
HF _{mr} EF	130 (26%)	123 (25%)	133 (28%)	
HF _p EF	150 (30%)	131 (27%)	127 (27%)	
Months since HF diagnosis	11 [0.2;48]	20 [2;63]	16 [2;58]	<0.001
Inpatient enrolment	377 (70%)	281 (52%)	216 (40%)	<0.001
Ischaemic etiology	354 (94%)	360 (94%)	339 (94%)	0.935
NYHA class				<0.001
I/II	163(30%)	221 (41%)	298 (55%)	
III/VI	379 (70%)	321 (59%)	243 (45%)	
Systolic BP (mmHg)	118 [106;136]	126 [113;141]	125 [112;144]	<0.001
Diastolic BP (mmHg)	65 [57;74]	69 [60;78]	70 [63;79]	<0.001
Heart rate (bpm)	73 [63;85]	70 [60;84]	72 [63;83]	0.210
LVEF (%)	41 [35;50]	40 [33;50]	40 [35;50]	0.269
<i>Signs and symptoms</i>				
Peripheral oedema	368 (76%)	300 (61%)	229 (49%)	<0.001
Elevated JVP	170 (37%)	131 (28%)	113 (25%)	<0.001
Hepatomegaly	24 (5%)	14 (3%)	16 (3%)	0.202
Pulmonary congestion	297 (58%)	230 (44%)	160 (31%)	<0.001
<i>Medical history</i>				
Anemia	274 (51%)	199 (37%)	130 (24%)	<0.001
Atrial fibrillation	244 (45%)	238 (44%)	229 (43%)	0.716
Diabetes Mellitus	190 (35%)	175 (32%)	166 (31%)	0.278
COPD	113 (21%)	95 (18%)	85 (16%)	0.079
CKD	319 (59%)	258 (48%)	161 (31%)	<0.001
Hypertension	319 (59%)	317 (59%)	306 (57%)	0.673
PAVD	103 (20%)	111 (21%)	137 (26%)	0.033
Stroke	114 (21%)	98 (18%)	83 (16%)	0.052
PCI	92 (17%)	102 (19%)	113 (21%)	0.265
CABG	78 (14%)	99 (18%)	106 (20%)	0.067
<i>Medication use at baseline</i>				
Loop diuretics	535 (99%)	535 (99%)	537 (99%)	0.808
Loop diuretic dose (mg furosemide equivalent)	80 [40;100]	40.0 [40;80]	40 [40;80]	<0.001
ACEi/ARB	359 (66%)	374 (69%)	421 (78%)	<0.001
Beta-blocker	372 (69%)	394 (73%)	418 (77%)	0.007
Aldosteron antagonist	170 (31%)	179 (33%)	175 (32%)	0.842
<i>Laboratory</i>				
Haemoglobin (g/dL)	12.5 [11.1;13.8]	13.2 [11.8;14.5]	13.9 [12.6;15.1]	<0.001
Leukocytes (10 ⁹ /L)	7.7 [6.1;9.6]	7.4 [6.1;9.1]	7.3 [6.1;8.9]	0.019
Sodium (mmol/L)	139 [136;141]	140 [137;141]	140 [138;141]	<0.001
Potassium (mmol/L)	4.3 [4.0;4.6]	4.3 [4.0;4.6]	4.3 [4.0;4.6]	0.857
Urea (mmol/L)	10.0 [7.5;14.9]	8.6 [6.8;11.6]	7.5 [5.9;9.8]	<0.001
Serum creatinin (µmol/L)	108 [84;142]	96 [80;124]	89 [76;111]	<0.001
eGFR (mL/min/1.73 m ²)	54 [38;60]	60 [44;60]	60 [55;60]	<0.001
NT-proBNP (pg/mL)	2448 [958;6447]	1473 [554;3210]	828 [294;1863]	<0.001
Albumin (g/L)	35 [31;39]	39 [35;43]	39 [36;43]	<0.001

LDL-cholesterol (mmol/L)	1.9 [1.5;2.3]	2.0 [1.5;2.5]	2.0 [1.5;2.6]	0.080
HDL-cholesterol (mmol/L)	1.09 [0.86;1.37]	1.09 [0.90;1.35]	1.10 [0.89;1.40]	0.652
Glucose (mmol/L)	6.7 [5.5;8.7]	6.2 [5.2;8.3]	5.9 [5.1;8.2]	0.001

Data shown as median [IQR] or n (%). Significant *P*-values are bold-printed.
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index;
BP, blood pressure; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease;
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin;
HDL, high-density lipoprotein; HF, heart failure; HFmrEF, heart failure with mid-range ejection fraction;
HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction;
JVP, jugular venous pressure; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NT-
proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PAVD, peripheral
arterial vascular disease; PCI, percutaneous coronary intervention.

Supplementary Table 1 - Risk model-adjusted hazard ratios of serum free thiols in predicting the composite endpoint (all-cause mortality or heart failure hospitalization) and all-cause mortality in 2 years across pre-specified subgroups

Variable	<i>Composite endpoint^c</i>			<i>All-cause mortality^d</i>	
	<i>n</i>	HR per SD decrease (95% CI)	<i>P</i> for interaction	HR per SD decrease (95% CI)	<i>P</i> for interaction
Age			0.34		0.25
≤ 70	1592	0.991 (0.904 – 1.086)		1.218 (1.079 – 1.376)	
> 70	2203	1.101 (1.028 – 1.179)		1.221 (1.124 – 1.326)	
Sex			0.62		0.79
Male	2650	1.065 (0.998 – 1.137)		1.265 (1.168 – 1.370)	
Female	1145	1.038 (0.934 – 1.154)		1.222 (1.075 – 1.390)	
HF groups					
HFrEF	2246	1.045 (0.972 – 1.123)		1.284 (1.174 – 1.404)	
HFmrEF	627	1.097 (0.951 – 1.265)	0.76 ^a	1.095 (0.924 – 1.299)	0.16 ^a
HFpEF	542	1.027 (0.891 – 1.182)	0.48 ^b	1.241 (1.048 – 1.468)	0.49 ^b
Ischemic etiology			0.82		0.71
Yes	2000	1.081 (1.002 – 1.166)		1.323 (1.209 – 1.449)	
No	1251	1.046 (0.954 – 1.147)		1.166 (1.034 – 1.314)	
NYHA class			0.18		0.29
I-II	1488	1.071 (0.961 – 1.193)		1.251 (1.091 – 1.435)	
III-IV	2242	1.054 (0.988 – 1.124)		1.218 (1.125 – 1.317)	
History of renal disease			0.71		0.64
Yes	1347	1.079 (0.993 – 1.173)		1.282 (1.161 – 1.417)	
No	2425	1.042 (0.968 – 1.121)		1.147 (1.043 – 1.262)	

^a HFmrEF compared to HFrEF

^b HFpEF compared to HFrEF

^c BIOSTAT-CHF risk model for composite endpoint (all-cause mortality & HF hospitalization): age, heart failure hospitalization in the year before inclusion, oedema, N-terminal pro-B-type natriuretic peptide, systolic blood pressure, haemoglobin, high-density lipoprotein levels, serum sodium concentration and failure to prescribe a beta-blocker.

^d BIOSTAT-CHF risk model for predicting all-cause mortality: age, blood urea nitrogen, NT-proBNP, haemoglobin and the use of a beta-blocker at time of inclusion.

CI, confidence interval; HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; NYHA, New York Heart Association; SD standard deviation.