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RESEARCH ARTICLE

Biomarker signature and pathophysiological pathways in patients with chronic heart failure and metabolic syndrome

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Aim

The comorbidities that collectively define metabolic syndrome are common in patients with heart failure. However, the role of metabolic syndrome in the pathophysiology of heart failure is not well understood. We therefore investigated the clinical and biomarker correlates of metabolic syndrome in patients with heart failure.

Methods and results

In 1103 patients with heart failure, we compared the biomarker expression using a panel of 363 biomarkers among patients with (n = 468 [42%]) and without (n = 635 [58%]) metabolic syndrome. Subsequently, a pathway overrepresentation analysis was performed to identify key biological pathways. Findings were validated in an independent cohort of 1433 patients with heart failure of whom 615 (43%) had metabolic syndrome. Metabolic syndrome was defined as the presence of three or more of five criteria, including central obesity, elevated serum triglycerides, reduced high-density lipoprotein cholesterol, insulin resistance and hypertension. The most significantly elevated biomarkers in patients with metabolic syndrome were leptin (log2 fold change 0.92, $p = 5.85 \times 10^{-21}$), fatty acid-binding protein 4 (log2 fold change 0.61, $p = 1.21 \times 10^{-11}$), interleukin-1 receptor antagonist (log2 fold change 0.47, $p = 1.95 \times 10^{-13}$), tumour necrosis factor receptor superfamily member 11a (log2 fold change 0.35, $p = 4.16 \times 10^{-9}$), and proto-oncogene tyrosine-protein kinase receptor Ret (log2 fold change 0.31, $p = 4.87 \times 10^{-9}$). Network analysis identified 10 pathways in the index cohort and 6 in the validation cohort, all related to inflammation. The primary overlapping pathway in both the index and validation cohorts was up-regulation of the natural killer cell-mediated cytotoxicity pathway.

Conclusion

Metabolic syndrome is highly prevalent in heart failure and is associated with biomarkers and pathways relating to obesity, lipid metabolism and immune responses underlying chronic inflammation.

Keywords Chronic heart failure • Metabolic syndrome • Biomarkers

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Introduction

Metabolic syndrome represents a collection of cardiometabolic risk factors, including central obesity, hypertension, insulin resistance, and dyslipidaemia.[1] Metabolic syndrome and heart failure are both highly prevalent and gradually increasing conditions in the general population.[2] The presence of metabolic syndrome is associated with a two-fold higher risk of developing heart failure.[3] Additionally, adverse progression of metabolic syndrome significantly influences the therapeutic management of heart failure.[1] The increased risk of heart failure seems intuitive because of the combination of factors that define metabolic syndrome.[1] Interestingly, the rising incidence of heart failure, as well as cases of metabolic syndrome have been linked to the alarming growth of obesity rates worldwide, particularly among younger populations.[1,4,5] It has been debated whether the high prevalence of metabolic syndrome in patients with heart failure is merely a reflection of the commonalities in risk factors.[1] Therefore, research is necessary to gain more insight into the pathophysiology associated with metabolic syndrome in heart failure. We thus aimed to investigate the clinical characteristics and biomarker profiles of patients with heart failure and metabolic syndrome to understand the underlying intricate pathophysiological pathways defining both heart failure and metabolic syndrome.

Methods

Patient population and study design

In this retrospective post-hoc analysis, data from the BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) cohort were studied. The BIOSTAT-CHF cohort is a multicentre, prospective, observational study consisting of patients with worsening or new-onset heart failure.[6] BIOSTAT-CHF compromises two cohorts, an index cohort with 2516 patients from 11 European countries and a validation cohort with 1738 patients from Scotland.[6]

Study group definition

Due to missing variables in the database, we used modified criteria for the definition of metabolic syndrome in the index and validation cohorts. In principle, we based these on the current unified metabolic syndrome criteria as defined by the International Diabetes Federation (online supplementary *Table S1*).

In the index cohort, patients meeting three or more of the following five criteria were defined as having metabolic syndrome:

1. Central obesity, defined as body mass index (BMI) ≥28.1 kg/m^2 in men and ≥27.5 kg/m^2 in women
2. Elevated serum triglycerides, defined as triglyceride levels ≥1.7 mmol/L
3. Reduced high-density lipoprotein cholesterol (HDL), defined as HDL <1 mmol/L in men and <1.3 mmol/L in women
4. Insulin resistance, defined as either a history of diabetes or the use of antidiabetic drug treatment
5. Hypertension, defined as either a systolic blood pressure ≥130 mmHg and/or a diastolic blood pressure ≥85 mmHg or a history of hypertension

In the validation cohort, patients meeting three or more of the following four criteria were defined as having metabolic syndrome:

1. Central obesity, defined as a waist circumference ≥94 cm in men and ≥80 cm in women
2. Reduced HDL, defined as HDL <1 mmol/L in men and <1.3 mmol/L in women
3. Insulin resistance, defined as either a history of diabetes or the use of antidiabetic drug treatment
4. Hypertension defined as either a systolic blood pressure ≥130 mmHg and/or a diastolic blood pressure ≥85 mmHg or a history of hypertension

After applying the modified inclusion criteria for metabolic syndrome and excluding patients with missing data of one or more of the variables, a total of 1033 patients in the index cohort and 1433 patients in the validation cohort were selected for this study.

Protein biomarkers

In this study, 363 protein biomarkers, measured with four multiplex immunoassay panels, were analysed. The four panels were provided by Olink Bioscience analysis service and included the Cardiovascular 2, Cardiovascular 3, Immune response, and Oncology panels. The different panels contain carefully selected protein biomarkers based on their established or putative participation in distinct biological processes involved in cardiovascular diseases (CVDs), inflammation, immune response processes, or the initiation and progression of cancer.[7]

Statistical analysis

The differences in clinical characteristics of patients with heart failure with versus without metabolic syndrome were compared. Unpaired *t*-test was used for normally distributed continuous variables, displayed as mean ± standard deviation.[8] The Mann–Whitney test was used for non-normally distributed continuous variables, displayed as median with the first and third interquartile range (Q1–Q3).[8] The Pearson Chi-square test was used for categorical variables, presented as numbers with percentages.[8] A two-sided *p*-value <0.05 was considered statistically significant. The analyses were conducted using R (version 4.1.1); a software environment for statistical computing and graphics.[9]

Univariable and multivariable logistic regression analyses were performed to study the clinical predictors of metabolic syndrome. Variables with a *p*-value <0.10 in the univariable regression analysis were included in multivariable regression analysis. Variables from the inclusion criteria of metabolic syndrome and the variables (i.e. serum glucose levels and waist circumference) strongly correlated (correlation coefficient >0.7) to diabetes and BMI were excluded. Logistic regression assumption of linearity between the independent variables and logit was checked, and log transformation using natural logarithm was applied where necessary. Multivariable logistic regression analysis was performed using the backward stepwise exclusion method, and the remaining variables with a *p*-value <0.05 were considered to have a significant association with the presence of metabolic syndrome.

Cox regression analysis was performed to investigate the association of metabolic syndrome with clinical endpoints of mortality and

heart failure hospitalization, first in a univariable model and secondly corrected for the BIOSTAT-CHF risk model, which consists of the strongest predictors of each clinical outcome.[10] Cox regression models were checked based on the proportional hazards assumption with the use of the Survival and Survminer package in R to test and plot the Schoenfeld residuals.

Subsequently, differential expression analysis of the 363 protein biomarkers between patients with heart failure with and without metabolic syndrome was performed using the linear models for microarray data analysis (Limma version 3.50.0) package in R.[11] Biomarkers were considered differentially expressed at a log2 fold-change cut-off of 0.2, and p-value and false discovery rate <0.05. The p-values were corrected for multiple testing according to the Benjamini–Hochberg procedure.[12] The overlapping common elevated protein biomarkers between the index and validation cohort were selected and further investigated in a pathway overrepresentation analysis, performed using the ClueGo plug-in app (version 2.5.8) in Cytoscape (version 3.9.0), a software platform for visualizing biomolecular networks.[13,14] The elevated protein biomarkers were assessed in pathways from the databases gene ontology (GO) biological processes, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathways.[15] The statistical parameters applied were the hypergeometric test and the default Bonferroni step-down method for multiple testing corrections. The whole annotation option was used as a reference set, and only pathways with a corrected p-value ≤0.05 were considered significant.[16]

Results

Baseline characteristics

Metabolic syndrome was present in 468 of 1103 patients (42.4%) in the index cohort and 615 of 1433 (42.9%) patients in the validation cohort. A comparison of the baseline characteristics between patients with and without metabolic syndrome is shown in *Table 1*.

In the index cohort, patients with heart failure and metabolic syndrome were younger (67 years; IQR 59–75 vs. 70 years; IQR 60–78, $p = 0.003$), had a higher BMI (30.93 ± 4.81 kg/m^2 vs. 25.85 ± 4.64 kg/m^2, $p < 0.001$) and had a more extensive medical history compared to patients with heart failure and without metabolic syndrome. Additionally, they had more signs and symptoms of congestion but lower concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP, 3484 ng/L; IQR 2011–7220 vs. 4619 ng/L; IQR 2559–8862, $p = 0.003$).

Differences in clinical characteristics between patients with and without metabolic syndrome in the validation cohort yielded similar findings to the index cohort (*Table 1*). Additionally, in the validation cohort patients with metabolic syndrome more often had a higher New York Heart Association class (NYHA class IV: 15.8% vs. 10.9%, $p < 0.001$) and higher concentrations of the biomarkers bioactive adrenomedullin (Bio-ADM, 32 pg/ml; IQR 22.50–48.58 vs. 23 pg/ml; IQR 16.33–34.10, $p < 0.001$) and growth differentiation factor-15 (GDF-15, 3305.5 pg/ml; IQR 2059–5350.5 vs. 2549 pg/ml; IQR 1685–4019, $p < 0.001$) compared to patients without metabolic syndrome. They had similar concentrations of NT-proBNP (1162 ng/L; IQR 512–3347 vs. 1319 ng/L; IQR 437–3176, $p = 0.739$).

Predictors of metabolic syndrome

Variables associated with metabolic syndrome ($p < 0.10$) in univariable logistic regression analysis are presented in online supplementary *Table S2*.

Multivariable logistic regression analysis in the index cohort showed that higher glucose concentrations (odds ratio [OR] per log increase 3.50, 95% confidence interval [CI] 2.02–6.12; $p < 0.001$), higher Bio-ADM concentrations (OR per log increase 1.58, 95% CI 1.30–1.92; $p < 0.001$), and lower NT-proBNP concentrations (OR per log increase 0.73, 95% CI 0.63–0.84; $p < 0.001$) were significantly associated with the presence of metabolic syndrome (*Table 2*).

Results from multivariable logistic regression analysis in the validation cohort showed that a history of percutaneous coronary intervention (OR 1.69, 95% CI 1.12–2.56; $p = 0.013$), higher glucose (OR per log increase 11.30, 95% CI 6.78–18.82; $p < 0.001$), lower low-density lipoprotein (LDL) (OR per mmol/L increase 0.68, 95% CI 0.56–0.84; $p < 0.001$), and Bio-ADM (OR per log increase 1.43, 95% CI 1.19–1.70; $p < 0.001$) were significant and independent predictors of the presence of metabolic syndrome (*Table 2*).

Outcomes of metabolic syndrome

Kaplan–Meier survival curves (index cohort online supplementary *Figure S3* and validation cohort online supplementary *Figure S4*, log-rank p for both >0.05) and Cox regression analysis (online supplementary *Table S5*) showed no significant association between the presence of metabolic syndrome and risk of mortality and heart failure hospitalization. Analyses of the association between the separate components of metabolic syndrome and mortality or heart failure hospitalization are included in online supplementary *Table S6*. Lastly, in patients with metabolic syndrome there was no significant interaction with clinical outcomes between heart failure with a preserved ejection fraction and heart failure with a reduced ejection fraction and between men and women.

Differential protein expression analysis

In the index cohort, 48 biomarkers were differentially expressed, of which 43 were elevated, and five were decreased in patients with metabolic syndrome. The validation cohort showed 58 elevated, and three decreased biomarkers. A total of 29 biomarkers were found to be elevated in both the index and validation cohort (online supplementary *Figure S7*). The volcano plots of the elevated and decreased biomarkers in the index and validation cohort are presented in *Figure 1*.

Based on the p-value the most significantly elevated biomarkers that were common between the index and validation cohort were leptin (log2 fold change 0.92, $p = 5.85 \times 10^{-21}$), fatty acid-binding protein 4 (FABP-4; log2 fold change 0.61, $p = 1.21 \times 10^{-11}$), interleukin-1 receptor antagonist (IL-1ra; log2 fold change 0.47, $p = 1.95 \times 10^{-13}$), tumour necrosis factor receptor superfamily member 11a (TNFRSF11a; log2 fold change 0.35, $p = 4.16 \times 10^{-9}$),

Table 1 Baseline characteristics of metabolic syndrome

Variables	Index cohort (n = 1103)			Validation cohort (n = 1433)		
	No metabolic syndrome (n = 635)	Metabolic syndrome (n = 468)	p-value	No metabolic syndrome (n = 818)	Metabolic syndrome (n = 615)	p-value
Clinical characteristics						
Age, years	70.00 [60.00–78.00]	67.50 [59.00–75.00]	0.003*	75.00 [66.00–81.00]	74.00 [67.00–80.00]	0.221
Sex, male, *n* (%)	478 (75.3)	332 (70.9)	0.123	568 (69.4)	402 (65.4)	0.115
Duration of heart failure, years	0.05 [0.00–3.56]	0.28 [0.01–4.30]	0.440	1.61 [0.20–5.07]	1.43 [0.09–4.63]	0.318
Inpatient hospitalization, *n* (%)	421 (66.3)	322 (68.8)	0.166	371 (45.4)	331 (53.8)	0.006*
BMI, kg/m^2	25.85 (± 4.64)	30.93 (± 4.81)	<0.001*	27.40 (± 5.88)	30.99 (± 5.99)	<0.001*
Waist circumference, cm	–	–	–	99.00 (± 14.68)	109.23 (± 14.01)	<0.001*
Systolic blood pressure, mmHg	123.39 (± 22.39)	129.54 (± 20.03)	<0.001*	123.20 (± 21.94)	129.61 (± 21.63)	<0.001*
Diastolic blood pressure, mmHg	74.33 (± 13.94)	76.77 (± 12.82)	0.003*	69.40 (± 12.77)	69.42 (± 13.19)	0.973
Heart rate, bpm	77.00 [66.50, 90.00]	76.00 [68.00, 88.00]	0.658	73.39 (± 16.38)	73.41 (± 15.52)	0.981
LVEF, %	30.18 (± 10.61)	31.61 (± 10.24)	0.029*	40.13 (± 12.87)	41.78 (± 13.11)	0.021*
Type of HF, *n* (%)			0.040*			0.088
HFpEF	39 (6.5)	23 (5.2)		199 (26.1)	167 (29.0)	
HFmrEF	16 (2.7)	25 (5.6)		126 (16.5)	113 (19.6)	
HFrEF	542 (90.8)	398 (89.2)		437 (57.3)	296 (51.4)	
LVEDD, mm	61.32 (±9.74)	61.75 (±9.08)	0.478	55.05 (± 9.48)	54.79 (± 8.82)	0.643
NYHA class, *n* (%)			0.063			<0.001*
I	13 (2.1)	10 (2.2)		11 (1.3)	3 (0.5)	
II	254 (41.0)	153 (33.0)		381 (46.6)	226 (36.7)	
III	274 (44.2)	234 (50.4)		336 (41.1)	289 (47.0)	
IV	79 (12.7)	67 (14.4)		89 (10.9)	97 (15.8)	
Medical history, *n* (%)						
Myocardial infarction	210 (33.1)	203 (43.4)	0.001*	393 (48.1)	322 (52.4)	0.123
Coronary artery bypass graft	91 (14.3)	97 (20.7)	0.007*	141 (17.3)	120 (19.5)	0.306
Valvular surgery	51 (8.0)	33 (7.1)	0.623	71 (8.7)	28 (4.6)	0.003*
Percutaneous coronary intervention	114 (18.0)	102 (21.8)	0.130	138 (17.0)	133 (21.7)	0.031*
Atrial fibrillation	283 (44.6)	196 (41.9)	0.408	347 (42.6)	285 (46.8)	0.130
Stroke	56 (8.8)	44 (9.4)	0.820	130 (16.0)	123 (20.2)	0.052
Peripheral arterial disease	50 (7.9)	70 (15.0)	<0.001*	164 (20.6)	166 (27.9)	0.002*
Hypertension	311 (49.0)	383 (81.8)	<0.001*	342 (41.8)	488 (79.3)	<0.001*
Diabetes mellitus	88 (13.9)	276 (59.0)	<0.001*	51 (6.2)	397 (64.6)	<0.001*
Chronic obstructive pulmonary disease	107 (16.9)	78 (16.7)	>0.999	150 (18.4)	112 (18.5)	>0.999
Renal disease	149 (23.5)	150 (32.1)	0.002*	324 (40.0)	324 (53.5)	<0.001*
Thyroid disease			0.237			0.312
Not present	576 (90.7)	417 (89.1)		702 (85.8)	509 (83.0)	
Hypothyroidism	54 (8.5)	42 (9.0)		109 (13.3)	96 (15.7)	
Hyperthyroidism	5 (0.8)	9 (1.9)		7 (0.9)	8 (1.3)	
Current malignancy	24 (3.8)	10 (2.1)	0.166	39 (4.8)	25 (4.1)	0.614
Current smoking	105 (16.6)	66 (14.1)	0.516	124 (15.2)	74 (12.1)	0.197
Primary etiology, *n* (%)			<0.001*			0.080
Cardiomyopathy	190 (29.9)	101 (21.6)		6 (0.7)	5 (0.8)	
Hypertensive	47 (7.4)	58 (12.4)		11 (1.3)	13 (2.1)	
Ischaemic heart disease	251 (39.5)	245 (52.4)		519 (63.4)	426 (69.3)	
Valvular disease	59 (9.3)	26 (5.6)		6 (0.7)	5 (0.8)	
Congestion, *n* (%)						
Pulmonary congestion, bibasilar	226 (36.8)	189 (41.2)	0.164	260 (32.7)	235 (39.8)	0.008*
Peripheral oedema			0.019*			0.028*
Not present	267 (48.6)	175 (42.5)		314 (42.6)	201 (35.8)	
Ankle	162 (29.5)	111 (26.9)		230 (31.2)	174 (31.0)	
Below knee	94 (17.1)	94 (22.8)		157 (21.3)	154 (27.5)	
Above knee	26 (4.7)	32 (7.8)		36 (4.9)	32 (5.7)	
Elevated JVP, *n* (%)			0.454			0.554
No	322 (67.5)	231 (65.6)		522 (71.5)	370(68.8)	
Yes	131 (27.5)	96 (27.3)		205 (28.1)	165 (30.7)	
Uncertain	24 (5.0)	25 (7.1)		3 (0.4)	3 (0.6)	
Hepatomegaly, *n* (%)	79 (12.5)	53 (11.4)	0.643	21 (2.8)	29 (5.2)	0.032*
Orthopnoea, *n* (%)	205 (32.3)	175 (37.5)	0.088	–	–	–
Dyspnoea VAS score	50.00 [31.25–70.00]	56.50 [30.00–70.00]	0.995	2.00 [1.00–3.00]	2.00 [2.00–3.00]	<0.001*
X-ray, *n* (%)						
Congestion present	153 (33.0)	85 (26.3)	0.052	–	–	–
Cardiomegaly (CTR >0.5)	328 (70.8)	242 (74.9)	0.238	–	–	–
Laboratory values						
Glucose, mmol/L	6.00 [5.30–7.20]	6.80 [5.60–9.20]	<0.001*	5.60 [5.00–6.90]	7.80 [5.90–11.17]	<0.001*
HDL, mmol/L	1.22 (± 0.40)	0.95 (± 0.28)	<0.001*	1.35 (± 0.46)	0.99 (± 0.33)	<0.001*
LDL, mmol/L	2.60 (± 0.99)	2.54 (± 1.15)	0.315	2.23 (± 0.87)	1.87 (± 0.78)	<0.001*
Total cholesterol, mmol/L	4.30 (± 1.21)	4.26 (± 1.51)	0.586	–	–	–
Triglycerides, mmol/L	1.10 [0.84–1.38]	1.52 [1.06–2.08]	<0.001*	–	–	–

Table 1 (Continued)

Variables	Index cohort (n = 1103)			Validation cohort (n = 1433)		
	No metabolic syndrome (n = 635)	Metabolic syndrome (n = 468)	p-value	No metabolic syndrome (n = 818)	Metabolic syndrome (n = 615)	p-value
Haemoglobin, g/dl	13.38 (± 1.83)	13.27 (±1.96)	0.320	15.40 (± 15.92)	13.61 (± 9.88)	0.014*
Serum creatinine, µmol/L	108.06 (± 45.07)	116.06 (±46.60)	0.004*	104.51 (± 44.68)	115.15 (± 50.83)	<0.001*
ASAT, U/L	26.00 [20.00–36.00]	24.00 [18.00–33.00]	<0.001*	24.00 [19.00–31.00]	23.00 [17.00–31.00]	0.015*
ALAT, U/L	25.00 [17.00–37.29]	24.00 [16.00–36.00]	0.147	22.00 [17.00–32.00]	22.00 [17.00–33.00]	0.547
Total bilirubin, µmol/L	18.77 (± 16.45)	23.56 (± 106.43)	0.370	11.00 [8.00–15.00]	10.00 [7.00–14.00]	0.001*
Gamma-GT, U/L	50.00 [29.00–101.00]	48.50 [25.00–97.75]	0.349	42.00 [25.00–86.00]	47.00 [30.00–90.50]	0.012*
Alkaline phosphatase, µg/L	83.45 [64.00–110.75]	82.00 [63.76–117.32]	0.887	88.00 [71.00–113.00]	91.00 [71.25–120.00]	0.154
Renal function						
eGFR, ml/min/1.73 m^2	63.60 (± 21.86)	59.41 (± 22.92)	0.002*	60.00 [49.00–60.00]	57.00 [42.00–60.00]	<0.001*
Aldosterone, pg/ml	101.00 [48.00–215.00]	100.00 [50.00–214.00]	0.969	–	–	–
Renin, µIU/ml	81.54 [24.73–249.12]	85.32 [26.10–247.23]	0.713	–	–	–
Urea, mmol/L	10.20 [7.30–16.42]	12.10 [7.80–18.92]	0.004*	8.20 [6.40–10.70]	9.00 [6.60–12.85]	<0.001*
Urinary creatinine, mmol/L	5.90 [2.70–10.25]	5.65 [3.00–9.83]	0.955	3.70 [2.00–7.30]	4.30 [2.30–7.40]	0.043*
UACR, mg/gCr	21.20 [6.96–79.66]	29.47 [7.37–146.30]	0.011*	12.63 [5.77–44.20]	23.54 [7.79–93.31]	<0.001*
Urinary KIM-1, pg/ml	1170.73 [473.25–2832.11]	1227.07 [420.42–2685.12]	0.862	–	–	–
Urinary NGAL, pg/ml	20 153.05 [8546.27–43 995.51]	18 125.19 [8583.89–44 438.65]	0.739	–	–	–
Biomarkers						
CRP, ng/ml	12 994.10 [5543.65–24 733.83]	13 557.90 [6583.30–26 720.86]	0.225	–	–	–
FGF-23, RU/ml	198.69 [107.08–506.39]	207.77 [114.49–501.61]	0.575	–	–	–
Bio-ADM, pg/ml	28.51 [19.26–45.08]	35.94 [26.09–56.77]	<0.001*	23.00 [16.33–34.10]	32.00 [22.50–48.58]	<0.001*
NT-proBNP, ng/l	4619.00 [2559.00–8862.00]	3484.00 [2011.00–7220.00]	0.003*	1319.50 [437.50–3176.25]	1162.00 [512.00–3347.00]	0.739
GDF-15, pg/ml	2465.00 [1571.50–3976.75]	2644.00 [1724.25–4675.75]	0.017*	2549.00 [1685.00–4019.00]	3305.50 [2059.00–5350.50]	<0.001*
IL-6, pg/ml	5.10 [2.60–10.12]	5.10 [2.80–9.20]	0.689	–	–	–
CA-125, U/ml	45.90 [15.90–130.15]	29.80 [14.88–105.55]	0.045*	23.70 [13.72–55.00]	25.30 [14.30–64.10]	0.130
Medication, n (%)						
ACEi/ARB	440 (69.3)	333 (71.2)	0.548	583 (71.3)	429 (69.8)	0.572
BB	511 (80.5)	382 (81.6)	0.686	597 (73.0)	443 (72.0)	0.734
MRA	326 (51.3)	252 (53.8)	0.445	250 (30.6)	216 (35.1)	0.077
Loop diuretic	631 (99.4)	465 (99.4)	>0.999	809 (98.9)	607 (98.7)	0.920

Values are median [interquartile range] or mean (± standard deviation), unless otherwise indicated.

ACEi, angiotensin-converting enzyme inhibitor; ALAT, alanine transaminase; ARB, angiotensin receptor blocker; ASAT, aspartate aminotransferase; BB, beta-blocker; Bio-ADM, bioactive adrenomedullin; BMI, body mass index; CA-125, cancer antigen-125; CTR, cardiothoracic ratio; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; GDF-15, growth differentiation factor-15; GT, glutamyltransferase; HDL, high-density lipoprotein; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IL-6, interleukin-6; JVP, jugular venous pressure; KIM-1, kidney injury molecule-1; LDL, low-density lipoprotein; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; UACR, urine albumin-to-creatinine ratio; VAS, visual analogue scale.

*$p < 0.05$.

and proto-oncogene tyrosine-protein kinase receptor Ret (RET; Rearranged during Transfection; log2 fold change 0.31, $p = 4.87 \times 10^{-9}$). B-type natriuretic peptide (BNP; log2 fold change -0.43, $p = 5.66 \times 10^{-5}$) was most significantly decreased in the index cohort, while paraoxonase-3 (PON-3; log2 fold change −0.46, $p = 6.88 \times 10^{-21}$) was most significantly decreased in the validation cohort.

A detailed description of the log2 fold changes, adjusted p-values, and annotations of the differentially expressed biomarkers, are included in online supplementary *Tables S9* and *S10*.

Pathway overrepresentation analysis

Pathway overrepresentation analysis of the 29 overlapping elevated biomarkers between the index and validation cohort identified the following seven pathways ($p \leq 0.01$, details in online supplementary *Table S11*): 'interleukin-10 signalling'; 'natural killer cell-mediated cytotoxicity'; 'mammary gland alveolus development'; 'positive regulation of receptor-mediated endocytosis'; 'regulation of endothelial cell apoptotic process'; 'lipid export from cell' and 'regulation of neuroinflammatory response' (*Figure 2*).

Pathway overrepresentation analysis of the individual elevated biomarkers in the index cohort identified 10 pathways ($p \leq 0.010$, details in online supplementary *Table S12* and *Figure S13*). In the validation cohort six pathways were identified ($p \leq 0.010$, details in online supplementary *Table S14* and *Figure S15*).

When comparing the 10 and six individual pathways from the index and validation cohort, respectively, the only common pathway was 'natural killer cell-mediated cytotoxicity'. This pathway also appeared to be significant in the network constructed using solely the 29-overlapping elevated biomarkers between both cohorts, illustrated in *Figure 2*.

To account for obesity as a predominant confounder in metabolic syndrome, we repeated our analysis in patients with both obesity and metabolic syndrome versus patients with obesity and without metabolic syndrome (online supplementary *Tables S16–S19*). Comparing and combining the clinical outcomes

Table 2 Multivariable logistic regression analysis of metabolic syndrome

Index cohort (excluding BMI, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, HDL, triglycerides)[a]				Validation cohort (excluding BMI, waist circumference, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, HDL)[b]			
Variables	**Odds ratio**	**95% CI**	***p*-value**	**Variables**	**Odds ratio**	**95% CI**	***p*-value**
Log glucose	3.50	2.02–6.12	<0.001*	Percutaneous coronary intervention	1.69	1.12–2.56	0.013*
Log2 (Bio-ADM)	1.58	1.30–1.92	<0.001*	Log glucose	11.30	6.78–18.82	<0.001*
Log2 (NT-proBNP)	0.73	0.63–0.84	<0.001*	LDL	0.68	0.56–0.84	<0.001*
				Log2 (Bio-ADM)	1.43	1.19–1.70	<0.001*

Bio-ADM, bioactive adrenomedullin; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-B-type natriuretic peptide.
[a]The final model in the index cohort excluded the inclusion criteria of metabolic syndrome.
[b]The final model in the validation cohort excluded BMI in addition to the inclusion criteria of metabolic syndrome.
*$p < 0.05$.

of the index and validation cohort, some notable findings in patients with obesity and metabolic syndrome included a higher left ventricular ejection fraction, higher NYHA class, more signs of congestion, a more extensive medical history, worse renal function, and higher concentrations of the measured biomarkers Bio-ADM, cancer antigen-125 and GDF-15 compared to those with obesity and without metabolic syndrome (online supplementary *Tables S16* and *S17*). Differential expression analysis of patients with obesity and metabolic syndrome showed that serine protease-8 (PRSS-8) and CD-27 were most significantly elevated in the index cohort, while signalling threshold-regulating transmembrane adapter-1 (SIT-1) and TNFRSF11A were most significantly elevated in the validation cohort. There were no significantly decreased biomarkers in the index cohort; however, in the validation cohort PON-3 was significantly decreased (online supplementary *Figure S20*).

Discussion

In the present study, the prevalence of metabolic syndrome was around 42% in patients with chronic heart failure. Patients with metabolic syndrome were younger and had a higher BMI, with higher serum glucose concentrations and lower absolute plasma NT-proBNP concentrations in the index cohort. Biomarker profiles showed an elevation of biomarkers related to obesity, lipid metabolism, and inflammation and a decrease of BNP and PON-3. Network analyses of the elevated biomarkers revealed pathophysiological pathways related to inflammation, in particular natural killer cell-mediated cytotoxicity.

Clinical profile of metabolic syndrome

Although the prevalence of metabolic syndrome in CVD is highly dependent on the definition, demographics, ethnicity, and gender, it is widely known that this patient group has a considerably higher risk of CVD and heart failure.[3,17] Our study confirms the high prevalence of metabolic syndrome in the population of patients with chronic heart failure. These patients also appeared to have a more extensive cardiovascular medical history evident by the significantly higher incidences of myocardial infarction, coronary artery bypass graft, peripheral arterial disease, and percutaneous coronary interventions. This observation could further be explained by the individual constituents that define metabolic syndrome, including central obesity, hyperinsulinaemia, hypertension, and dyslipidaemia, which are also risk factors for CVD.

Obesity, an important constituent of metabolic syndrome, is a well-known risk factor of CVD and is strongly associated with a spectrum of heart failure phenotypes.[18,19] The drastically rising cases of obesity in younger populations may also explain why patients with metabolic syndrome were younger.[4] To account for the possibility that obesity might have been the main driver of our results we compared patients with obesity and metabolic syndrome to those with only obesity, which resulted in different clinical and biomarker profiles. Notably, patients with obesity and metabolic syndrome had a higher NYHA class, broader medical history, more cases of diabetes and more signs of congestion, reflecting a worse prognostic state compared to patients with obesity and without metabolic syndrome. These findings indicate that the presence of metabolic syndrome has detrimental effects on the progression of heart failure beyond those found in obesity.

Although patients with metabolic syndrome had more signs of congestion and a higher NYHA class, reflecting a more severe disease state, they still had lower concentrations of NT-proBNP.[20] The lower NT-proBNP concentrations may be explained by the obesity rates in patients with metabolic syndrome, as further evidenced by the fact that no differences were found in NT-proBNP concentrations in patients with obesity and metabolic syndrome versus those with obesity alone.[21] Furthermore, when comparing patients with metabolic syndrome and obesity versus those without obesity, NT-proBNP concentrations remain significantly lower in patients with obesity and metabolic syndrome compared to patients without obesity and metabolic syndrome (online supplementary *Tables S21* and *S22*). From these observations we may denote that metabolic syndrome itself is not associated with lower

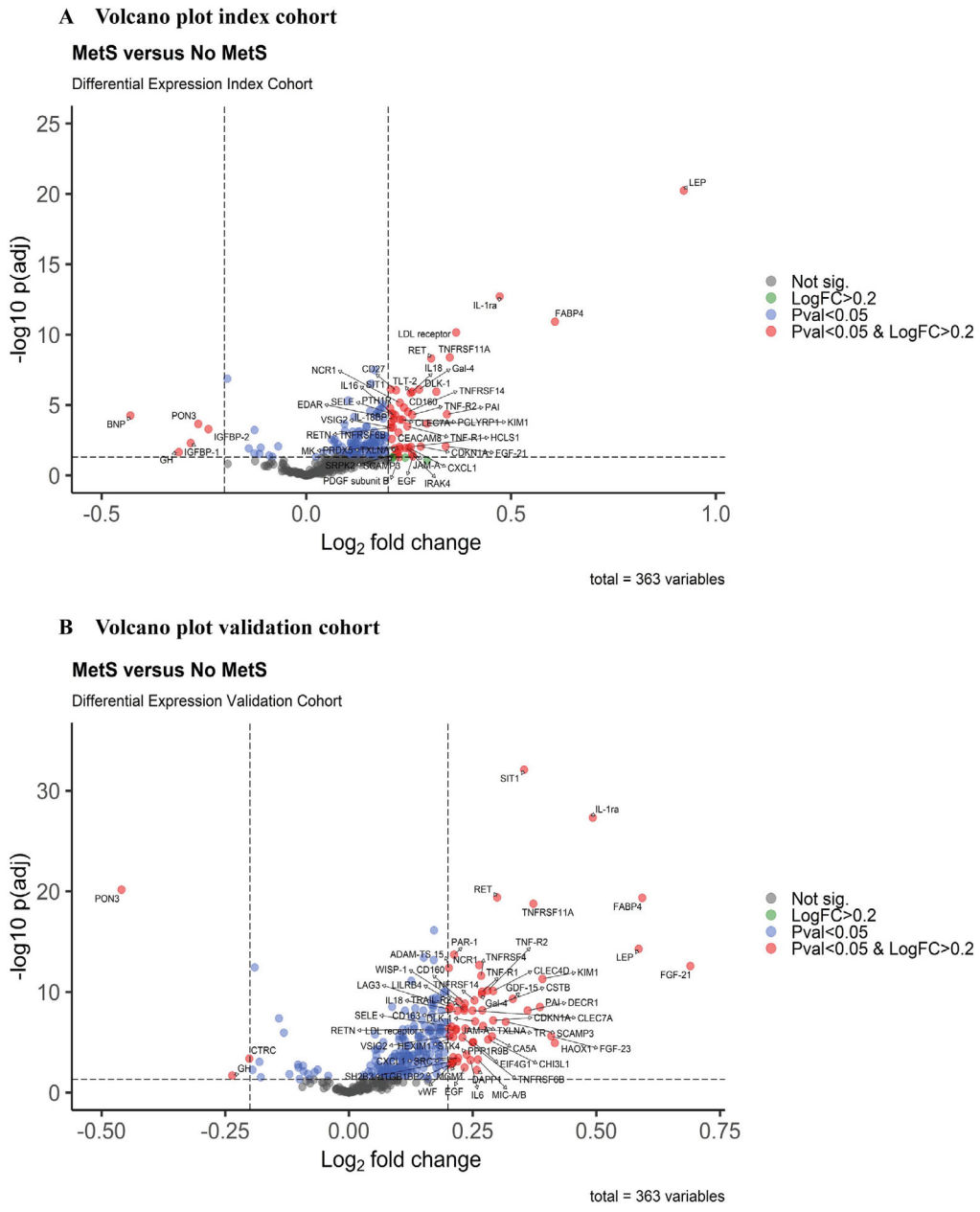

Figure 1 Volcano plots of the differentially expressed biomarkers in patients with chronic heart failure with metabolic syndrome (MetS) versus without metabolic syndrome (No MetS) in the BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) index (*A*) and validation (*B*) cohort. *Y*-axis: significance plotted as −log10 *p*-value (adjusted) with a cut-off of 0.05. *X*-axis: effect size plotted as log2 fold change (FC) with a cut-off of −0.2 (negative = decreased) and 0.2 (positive = elevated). Significant differentially expressed biomarkers are labelled. Abbreviations of the labelled biomarkers are included in online supplementary *Table S8*.

NT-proBNP, but rather that obesity remains to be associated with 'BNP deficiency' as previously described.[21]

We additionally tried to address whether our findings of the differential expression analysis between patients with or without metabolic syndrome were mainly driven by obesity. The significant difference in biomarker profiles between patients with obesity with versus without metabolic syndrome demonstrated that processes beyond those involved in obesity likely play a role in the pathophysiology of patients with heart failure and metabolic syndrome. The question remains whether metabolic syndrome is an independent risk factor for heart failure or if one of the other constituents, namely insulin resistance, dyslipidaemia, or hypertension, predominantly drove the outcomes of this study. Concluding, the role of the various constituent of metabolic syndrome should

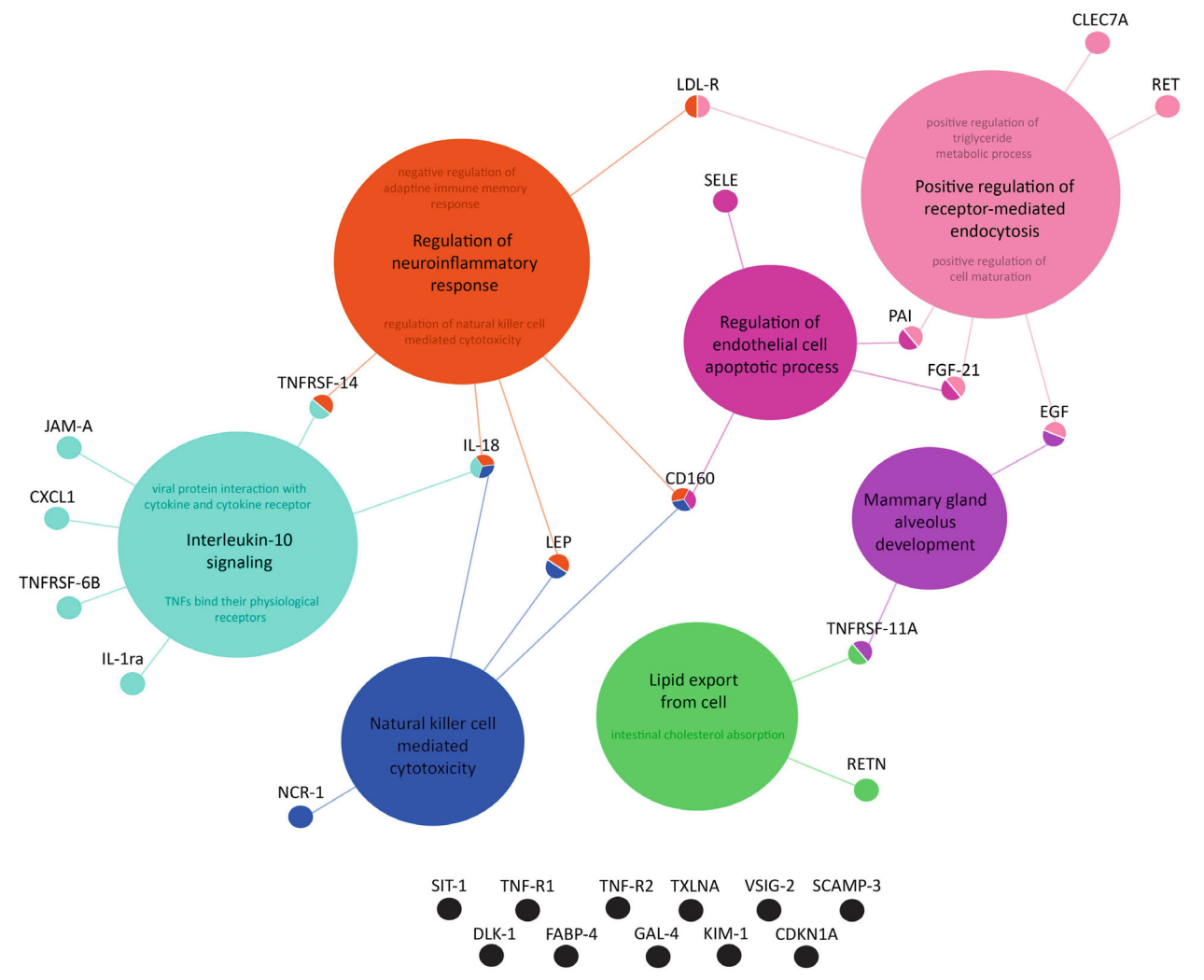

Figure 2 Results of pathway overrepresentation analysis of patients with chronic heart failure with versus without metabolic syndrome using the 29 common elevated biomarkers between the BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) index and validation cohort. The small nodes represent the 29 significantly elevated biomarkers. The big nodes represent the pathophysiological pathways involved with these biomarkers. The seven pathways are labelled based on the most significant group term. The black nodes below the figure are biomarkers that were not found to be involved in a specific pathway based on current knowledge. Abbreviations of the labelled biomarkers are included in online supplementary *Table S8*.

be analysed independently to evaluate whether any one of them influenced our results.

Biomarker profile of metabolic syndrome

The differentially expressed biomarkers we found in patients with metabolic syndrome strongly relate to the clinical profile of obesity in this patient group and may be explained by the underlying chronic systemic inflammation. The five most significantly elevated biomarkers derived from both the index and validation cohorts were leptin, FABP-4, IL-1ra, TNFRSF11A, and RET. Adipose tissue functions as an endocrine organ and, especially in the presence of obesity, is known for secreting various pro-and anti-inflammatory by-products, among which are the ones found elevated in our population.[22] These could be of interest in the pathophysiology of heart failure.

Leptin, the most significantly elevated biomarker, is an energy-balancing hormone mainly secreted by adipocytes.[23] Leptin suppresses the appetite to manage body weight and energy homeostasis; however, chronic increase in leptin levels, as found in severe adiposity, may lead to hyperleptinaemia and eventually result in leptin resistance.[23,24] The impaired regulation of appetite in leptin-resistant individuals plays an important role in the progression of obesity and other metabolic disorders.[24] Leptin has been correlated with all the criteria of metabolic syndrome,

independently of obesity.[25] Furthermore, leptin has shown to be an independent risk factor for CVD, which may further explain the link with heart failure.[25]

The hyperleptinaemic state and insulin resistance found in obesity appear to induce IL-1ra and TNFRSF11A expression, explaining the elevation of these biomarkers in metabolic syndrome in our data.[26,27] IL-1ra has been shown to inhibit leptin function, thereby contributing to leptin resistance and promoting a vicious cycle with obesity.[28] Furthermore, IL-1ra reduces the proinflammatory effect of IL-1, and a disbalance between these molecules has been associated with metabolic diseases.[30]

The consistent and pivotal role of obesity, inflammation and hyperleptinaemia in metabolic syndrome is further demonstrated by the decrease of PON-3. PON proteins have an anti-inflammatory aetiological involvement in several inflammatory-driven diseases, including CVD.[29,30] PON-3 is often found in the circulation associated with HDL and exhibits atheroprotective functions.[30] A study using PON-3 overexpressed mouse models has demonstrated decreased adiposity and circulating leptin levels, providing more evidence of protective properties against obesity and atherosclerosis.[31]

Similar to leptin, FABP-4 secretion by adipocytes is increased in obesity and in this way may contribute to metabolic and inflammatory pathways through lipid signalling.[32] Additionally, previous research has shown that FABP-4 is abundantly produced by epicardial adipose tissue and an important adipocytokine in patients with CVD.[33] These points indicate that FABP-4 may play a role in the pathogenesis of patients with heart failure and metabolic syndrome by contributing to the development of insulin resistance and the chronic low-grade inflammation recognized in adiposity.[34,35]

Finally, we found an elevation of RET, a proto-oncogene. This biomarker may be linked to the clinical characteristic of higher GDF-15 concentrations we found in patients with metabolic syndrome. GDF-15, a stress response cytokine, plays a role in energy homeostasis, weight-related metabolic alterations and has previously been acknowledged as a prognostic marker for heart failure.[36,37] In patients with metabolic syndrome elevated GDF-15 could be associated with a variety of factors including obesity, muscle atrophy and diabetes.[36,37] Seemingly, GDF-15 and RET have a shared receptor signalling pathway in metabolic regulation during stress, which may explain the elevation of RET in patients with heart failure and metabolic syndrome.[38,39]

Natural killer cell-mediated cytotoxicity pathway

Prior research has extensively recognized the underlying inflammation in obesity to play a causal role in the development of heart failure.[19] The interplay between metabolic syndrome, inflammatory mediators of chronic inflammation, including cytokines and immune cells, and lipid metabolic processes are clearly reflected in the pathways we found to be involved with the overexpressed biomarkers. 'Natural killer cell-mediated cytotoxicity' appeared to be an important and recurring pathway in the different networks that were constructed. Studies have enlightened the contribution of natural killer cells to the chronic inflammatory state found in obesity. Natural killer cells are innate lymphoid cells that reside in visceral adipose tissue and mediate cellular cytotoxicity.[38,39] It has been proposed that chronic inflammation is essential in the development and progression of obesity-related diseases.[40] In individuals without obesity, macrophages are the primary immune cells in adipose tissue with a distinct phenotype that regulates anti-inflammatory mediators.[41] In obesity, a transition of macrophage phenotypes results in the recruitment of natural killer cells.[38,41] In turn, natural killer cells produce cytokines and chemokines, further contributing to the inflammatory microenvironment associated with obesity.[38] Furthermore, hyperleptinaemia has also been shown to enhance natural killer cell activation in patients with obesity.[38,39] Additionally, natural killer cells have been associated with the progression of insulin resistance and hypertension; both are relevant factors in the criteria of metabolic syndrome.[38] As shown by our findings, natural killer cells are merely one of the many factors contributing to the systemic inflammation found in obesity.

Taken together, natural killer cells play a role in insulin resistance, hypertension and the inflammatory microenvironment found in obesity, all factors that are strongly represented by metabolic syndrome and risk factors of heart failure.[38,39]

Limitations

Limitations result from the modification of the criteria used to define metabolic syndrome. The modification possibly affected the findings in the validation cohort as there were four instead of five criteria used for selection. In the index cohort, BMI was used to define central obesity instead of the advised criterium of waist circumference. Studies have demonstrated that BMI alone is insufficient to assess cardiometabolic risks associated with obesity.[42] However, studies have also considered that age-related changes in body composition might explain why BMI is a better indicator of obesity in the elderly, which comprised the majority of our study population.[43] Additionally, by modifying the selection criteria, the criteria were not completely the same for the index and validation cohort. Moreover, the two cohorts are not precisely represented by comparable patient populations, evidenced by differences in heart failure duration and NT-proBNP concentrations, which is important when anticipating the impact of chronic cardiac stress on other biomarker levels. It should also be noted that heart failure with a preserved ejection fraction was under-represented in the cohorts. However, the comparable results in both cohorts do potentially indicate that patients were equivalent in terms of metabolic syndrome and that our findings are relevant to a wide range of patients with heart failure. Lastly, the post hoc nature of our study makes it impossible to prove causation between obesity, inflammation, heart failure and metabolic syndrome and results should be interpreted as such.

Future perspectives

The findings of this study have confirmed the importance of the chronic inflammatory state in obesity and metabolic syndrome. The

clinical characteristics and biomarker profiles suggested an essential connection between inflammation and obesity as the underlying pathophysiology of metabolic syndrome in heart failure. Further research is needed to investigate the individual association between obesity and heart failure to confirm to what extent obesity has driven the outcomes of this study, although our preliminary results indicate a role of metabolic syndrome beyond solely the effects of obesity. Additionally, similar analysis is necessary to determine whether any one of the other constituents of metabolic syndrome influenced the outcomes of our study. Important new insights may also be gained by comparing outcomes with healthy controls and between the different subtypes of heart failure. Moreover, research of the complex molecular pathways involved with the elevated and decreased biomarkers, such as leptin, BNP, RET, PON-3, and the inflammatory microenvironment found in obesity, is necessary for a better understanding of their contribution to cardiometabolic diseases. The present data already suggest that anti-inflammatory agents, such as tumour necrosis factor alpha blockers and interleukin inhibitors might be particularly beneficial in patients with heart failure and metabolic syndrome.

Conclusion

Metabolic syndrome is prevalent in patients with heart failure and associated with increased concentration of biomarkers and activation of pathways related to lipid metabolism, obesity, and immune responses associated with chronic inflammation.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Conflict of interest: M.M. reports personal fees of minimal amounts since January 2021 from Actelion, Amgen, Livanova, and Vifor pharma as member of Executive or Data Monitoring Committees of sponsored clinical trials; from AstraZeneca, Bayer, Boehringer Ingelheim, Edwards Lifesciences, Novartis for participation in advisory boards and/or speeches at sponsored meetings. All other authors have nothing to disclose.

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