Original Article

Inflammatory Biomarkers Predict Heart Failure Severity and Prognosis in Patients With Heart Failure With Preserved Ejection Fraction

A Holistic Proteomic Approach

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Background—Underlying mechanisms in heart failure (HF) with preserved ejection fraction remain unknown. We investigated cardiovascular plasma biomarkers in HF with preserved ejection fraction and their correlation to diastolic dysfunction, functional class, pathophysiological processes, and prognosis.

Methods and Results—In 86 stable patients with HF and EF ≥45% in the Karolinska Rennes (KaRen) biomarker substudy, biomarkers were quantified by a multiplex immunoassay. Orthogonal projection to latent structures by partial least square analysis was performed on 87 biomarkers and 240 clinical variables, ranking biomarkers associated with New York Heart Association (NYHA) Functional class and the composite outcome (all-cause mortality and HF hospitalization). Biomarkers significantly correlated with outcome were analyzed by multivariable Cox regression and correlations with echocardiographic measurements performed. The orthogonal partial least square outcome-predicting biomarker pattern was run against the Ingenuity Pathway Analysis (IPA) database, containing annotated data from the public domain. The orthogonal partial least square analyses identified 32 biomarkers correlated with NYHA class and 28 predicting outcomes. Among outcome-predicting biomarkers, growth/differentiation factor-15 was the strongest and an additional 7 were also significant in Cox regression analyses when adjusted for age, sex, and N-terminal probrain natriuretic peptide: adrenomedullin (hazard ratio per log increase 2.53), agouti-related protein; (1.48), chitinase-3–like protein 1 (1.35), C–C motif chemokine 20 (1.35), fatty acid–binding protein (1.33), tumor necrosis factor receptor 1 (2.29), and TNF-related apoptosis-inducing ligand (0.34). Twenty-three of them correlated with diastolic dysfunction (E/e′) and 5 with left atrial volume index. The IPA suggested that increased inflammation, immune activation with decreased necrosis and apoptosis preceded poor outcome.

Conclusions—In HF with preserved ejection fraction, novel biomarkers of inflammation predict HF severity and prognosis that may complement or even outperform traditional markers, such as N-terminal probrain natriuretic peptide. These findings lend support to a hypothesis implicating global systemic inflammation in HF with preserved ejection fraction.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov; Unique identifier: NCT00774709.

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Heart failure (HF) with preserved ejection fraction (HFpEF) is as common and associated with as much morbidity and mortality as HF with reduced ejection fraction (HFrEF).[1]

See Clinical Perspective

Exploration of a wide range of biomarkers has contributed fundamentally to the understanding of underlying mechanisms, pathophysiology and prognosis in HFrEF,[2] which, in turn, has contributed to the development of and successful trials of neurohormonal antagonist drugs. Despite the convincing evidence of prognostic benefit in HFrEF, these pharmacological agents have not been shown to improve outcomes in HFpEF,[3–6] suggesting a different underlying pathophysiology and greater heterogeneity of phenotypes in HFpEF than that in HFrEF.

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HFpEF is characterized by cardiac hypertrophy and fibrosis, leading to diastolic dysfunction and impaired systolic strain, increased filling pressures, and classic signs and symptoms of HF, indistinguishable from those in HFrEF. However, patients with HFpEF are older with more noncardiac comorbidities such as diabetes mellitus, obesity, hypertension, and chronic kidney disease, which are associated with and may drive the poor outcomes.[7] Indeed a new paradigm has emerged proposing noncardiac comorbidities driving HFpEF by inducing a proinflammatory state causing immune cell recruitment, coronary microvascular, and endothelial inflammation resulting in depressed nitric oxide bioavailability and low protein kinase G activity, in turn leading to interstitial fibrosis, remodeling, and high diastolic left ventricular stiffness.[8]

In this context, biomarkers associated with matrix remodeling, inflammation, fibrosis, apoptosis, and cell turnover reflecting potentially key drivers of HFpEF may be of particular interest.[9–11] In HFpEF, associations with diastolic dysfunction and prognosis have been suggested for several markers of fibrosis and neurohormonal activation, but we are not aware of any comprehensive proteomics approach.[12–17]

Analyzing a large set of biomarkers in a holistic manner offers a powerful way of contextualizing the biomarkers and improving the understanding of disease-related pathways. We used orthogonal projection to latent structures by partial least square (OPLS) analysis,[18] which identifies the quantitative relationship between all variables in a data matrix (X) and outcome (Y), but also filters out systematic unrelated variation to Y. Furthermore, we combined OPLS analyses of clinical variables and multiplexed biomarkers, with a semiautomated literature search of the scientific literature to identifiy biomarker patterns predicting HF severity and outcome, as well as potential pathophysiological mechanisms preceding outcome in HFpEF.

Materials and Methods

The Karolinska Rennes (KaRen) was a prospective observational multicenter study characterizing patients with HFpEF, described in detail elsewhere.[19] In brief, 539 patients presented to the hospital with acute signs and symptoms of HF according to the Framingham criteria, N-terminal probrain natriuretic peptide (NT-proBNP) of >300 ng/L and a left ventricular ejection fraction (LVEF) of ≥45% were enrolled in French and Swedish centers. The prespecified KaRen biochemistry substudy enrolled 86 patients between 21 May, 2007 and 29 December, 2011 at Karolinska University Hospital, Stockholm, Sweden. Patients returned to the hospital in stable condition 4 to 8 weeks after enrollment for a follow-up visit, including blood sampling and detailed echocardiography, and were thereafter followed until 30 September, 2012 when vital status was assessed by telephone contact or by the Swedish National Patient Register and Population Register. The primary outcome was defined as time to mortality from any cause or first hospitalization due to HF. All HF hospitalizations were adjudicated and defined according to clinical judgment by the local investigator and additionally centrality validated to confirm the presence of heart failure at hospitalization.

The echocardiographic assessment was performed on a ViVid 7 echo-platform (GE VingMed, Horten, Norway) and analyzed in a dedicated core center in Hôpital Pontchaillou, Rennes, France. Each examination was interpreted once and measurements were performed 3× and averaged by an echocardiographist (E.D.) blinded to the specific clinical history of the patient.

At the follow-up visit, blood samples were collected in a fasting state in the morning in EDTA tubes, centrifuged, and plasma was aliquoted and stored in −70°C until analysis.

Biomarkers were quantified from single samples by a multiplex immunoassay (Proseek Multiplex$^{96\times96}$ CVD I v1; Olink Bioscience, Uppsala, Sweden), which is a 96-plex immunoassay (92 analytes plus internal controls) based on proximity extension assay. Proximity extension assay uses target-specific antibody pairs that are linked to DNA strands that, upon simultaneous binding to the target analyte, creates a real-time polymerase chain reaction amplicon in a proximity-dependent manner enabled by the action of a DNA polymerase.[20] The intra-assay coefficient of variation (CV) ranges between 5% and 13% (mean 8%), and the interassay CV ranges between 9% and 39% (mean 15%). Further information about reproducibility and validation is found at http://www.olink.com.

NT-proBNP was analyzed by proBNPII (Roche Diagnostics, Bromma, Sweden). Creatinine clearance was calculated according to the Cockcroft–Gault formula.

Statistics

Figure 1 shows an overview of the analyses performed. The respective statistical methods are described below.

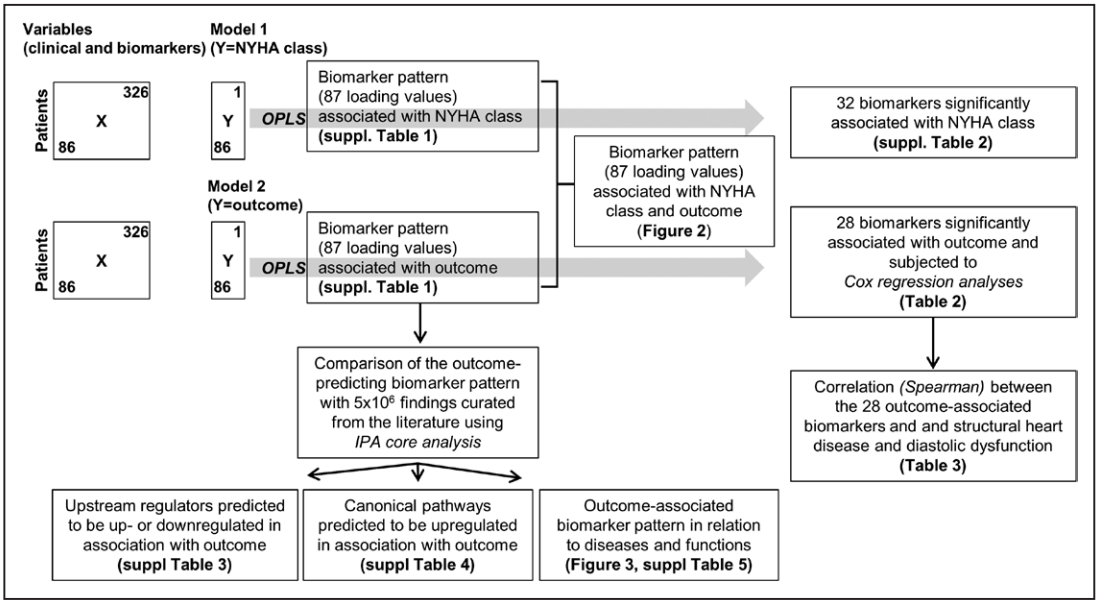

Figure 1. Outline of the analyses performed and the corresponding Tables and Figures. OPLS indicates orthogonal partial least square analysis.

Table 1. Baseline Characteristics Divided According to Outcome (Heart Failure Hospitalization or Death From Any Cause)

Variable	All, n=86 n (%)	Outcome, n=36 n (%)	No Outcome, n=50 n (%)	*P* Value
Demographics				
Age; median (Q1; Q3)	73 (66; 78)	74 (66; 79)	73 (66; 81)	0.743
Sex (females)	44 (51)	20 (57)	24 (48)	0.519
Medical history				
Hypertension	68 (79)	29 (81)	39 (78)	1.000
COPD	14 (16)	9 (25)	5 (10)	0.080
T2DM	27 (31)	15 (42)	12 (24)	0.102
Coronary heart disease	29 (34)	15 (42)	14 (28)	0.248
Stroke	9 (10)	4 (11)	5 (10)	1.000
Clinical data at 4–8 wk stable state (baseline)				
Atrial fibrillation	49 (57)	20 (56)	29 (58)	0.829
NYHA class I	19 (22)	4 (21)	15 (30)	0.020
NYHA class II	47 (55)	19 (53)	28 (56)	
NYHA class III	20 (23)	13 (36)	7 (14)	
Measurements				
Weight (kg)	83.5 (72; 98)	88 (73; 99)	82.5 (70; 95)	0.356
BMI (kg/m^2)	28.5 (25.0; 32.9)	30.2 (26; 34)	27.0 (24; 32)	0.130
Systolic blood pressure (mm Hg)	140 (90; 210)	140 (128; 150)	145 (130; 160)	0.214
Diastolic blood pressure (mm Hg)	80 (70; 85)	78 (70; 83)	80 (75; 85)	0.080
Heart rate (beats per minute)	70 (60; 80)	71 (62; 81)	69 (58; 80)	0.480
Treatment				
ARB	28 (33)	12 (33)	16 (32)	1.000
ACE-inhibitor	42 (49)	20 (56)	22 (44)	0.382
Tiazid diuretics	14 (16)	7 (19)	7 (14)	0.562
Potassium sparing diuretics	18 (21)	8 (22)	10 (20)	0.796
Loop diuretics	63 (73)	33 (92)	30 (60)	0.001
Calcium channel blocker	27 (31)	10 (28)	17 (34)	0.640
β-blocker	69 (80)	27 (75)	42 (84)	0.411
Anticoagulants	47 (55)	18 (50)	29 (58)	0.515
Antiplatelet	29 (34)	12 (33)	17 (34)	1.000
Statins	38 (44)	20 (56)	18 (36)	0.082
Nitroglycerine	12 (14)	7 (19)	5 (10)	0.169
Pacemaker	20 (23)	10 (27)	10 (20)	0.445
Echocardiographic measurements				
LVEF (%)	64 (58; 68)	63 (57; 67)	64 (60; 68)	0.279
LAVI (mL/m^2)	43.3 (37.2; 52.8)	41.7 (36; 55)	43.3 (38; 53)	0.654
LA volume (mL)	86.5 (75; 104)	86.5 (72; 102)	86.5 (77; 105)	0.619
Left ventricular mass index (g/m^2)	115 (95; 142)	123 (99; 148)	107 (95; 141)	0.525
Males	125 (102; 157)	154 (121; 157)	110 (85; 157)	0.362
Females	109 (94; 136)	115 (92; 138)	102 (95; 133)	0.764

(*Continued*)

Table 1. Continued

Variable	All, n=86 n (%)	Outcome, n=36 n (%)	No Outcome, n=50 n (%)	*P* Value
LVEDd (mm)	47 (43; 53)	49 (44; 53)	46 (43; 52)	0.341
E/A ratio	1.3 (0.9; 2.5)	1.7 (1.2; 3.3)	1.1 (0.8; 1.4)	0.003
E/e´ ratio	10.8 (8.3; 14.0)	13.4 (10; 18)	9.5 (7.4; 13.5)	0.022
E´	8.0 (7.0; 10.0)	7.5 (5.5; 10.5)	8.0 (7.0; 10.0)	0.321
IVRT (diastole)	94 (77; 113)	80 (68; 108)	102 (82; 115)	0.050
Mitral VTI	23 (16; 30)	27 (20; 34)	22 (15; 28)	0.012
E-wave deceleration time (ms)	203 (156; 228)	203 (158; 228)	202 (151; 222)	0.768
Biochemistry				
NT-proBNP (ng/L)	1000 (469; 2330)	1375 (574; 2565)	819 (429; 1820)	0.062
Glucose fasting (mmol/L)	5.6 (5.1; 7.5)	6.0 (5.3; 7.5)	5.6 (5.1; 7.5)	0.359
Creatinine (μmol/L)	84 (73; 107)	90 (74; 121)	80 (70; 102)	0.141
Creatinine clearance (mL/min)	70 (51; 92)	65 (46; 85)	74 (53; 94)	0.330

Continuous variables are presented as median and lower and upper quartiles (Q1;Q3) and categorical variables as numbers (n) and percentages. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; COPD; chronic obstructive pulmonary disease; LA, left atrium; LAVI, left atrial volume index; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; IVRT, isovolumteric relaxation time; NT-proBNP, N-terminal probrain natriuretic peptide; VTI, velocity-time integral; and T2DM, type 2 diabetes mellitus.

Descriptive data in Table 1 were expressed as median and quartiles (Q1; Q3) or number (%) and divided according to the presence of the composite end point and compared by the Wilcoxon rank-sum test and Fisher's exact tests as appropriate. The difference in biomarker levels was determined on log2-values (ProSeek biomarkers) or log10-values (NT-proBNP levels by ELISA) by 2-sided *t* test using GraphPad Prism 6. For visualization (Figure I in the Data Supplement), the data were transformed from linear values. The concentrations of the ProSeek biomarkers are expressed as arbitrary units because of the lack of specific calibrators for the assays.

Analyses of Outcome

Associations with the outcome was determined with Cox proportional hazards models and presented as hazard ratio and 95% confidence interval. In the final multivariable Cox regression model, three clinically significant covariates, age, sex, and NT-proBNP, were included together with each biomarker. All biomarkers were analyzed in log-transformed format.

P values were 2-sided, and statistical significance was set at 0.05. Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC, USA).

OPLS Analyses Correlating Biomarker Patterns With NYHA Class and Outcome

A total of 240 clinical and medication variables were collected at enrollment and at the 4- to 8-week follow-up (excluding repeated measures), and outcome data were merged with the 87 biomarker data set (5 of the biomarkers in the 92-plex yielded no or noninformative values and were excluded from the analysis). This generated a data matrix consisting of 327 variables in 86 patients who were subjected to OPLS analyses using Simca 13 (Umetrics, Umeå, Sweden). All data were scaled to unit variance and mean centered. In addition, variables with a max/mean ratio >10 were log-transformed to increase equal leverage of all variables. The OPLS analysis yields a model, in which the contribution of each variable is represented by a loading value (herein normalized to a correlation coefficient in between −1 and 1) to what is predicted. Two OPLS models were generated, each one represented by 326 X-variables predicting 1 Y-variable: In model 1, New York Heart Association (NYHA) Functional class (4 levels) at the 4- to 8-week follow-up was set as the Y-variable, and in model 2, the binary composite outcome variable "heart failure hospitalization or all-cause death" was set as the Y-variable. The validity of the models (Q2 values) was assessed by cross-validation, leaving out and predicting a seventh of the data set in an iterative manner. The cross-validation also yielded 95% confidence intervals for the contribution of each of the variables for the respective Y-variable, as implicated in the Simca software. The OPLS model predicting NYHA class explained 70% (R2Y=0.70) of the variation of NYHA class in the data set, with a predictive (Q2 value) of 0.24 after cross-validation. The corresponding values for outcome were 0.96 (R2Y) and 0.37 (Q2). The confidence intervals were used to determine whether a biomarker significantly predicted the Y-variable (NYHA class and outcome, respectively), adding up to 32 significant biomarkers for NYHA class and 28 significant biomarkers for outcome.

Correlation Analyses With Echocardiographic Measurements

Correlations between the 28 biomarkers predictive of outcome according to the OPLS analysis and the 3 guidelines-specified echocardiographic parameters for the diagnosis of HFpEF; E/e´ >15, left atrial volume index (LAVI) >34 mL/m^2, and left ventricular hypertrophy defined as left ventricular mass index (LVMI) ≥95 g/m^2 in women and ≥115 g/m^2 in men, respectively,[21] were determined using Spearman's correlation coefficient. Statistical analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC, USA).

Identification of Pathophysiological Mechanisms and Pathways Preceding Outcome

To investigate which pathophysiological mechanisms were involved in patients with an event, the outcome-predictive biomarker pattern (the 87 loading scores expressed as correlation coefficients) was compared with annotated data from the public domain using QIAGEN's Ingenuity Pathway Core Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity). The IPA Core analysis compares a biomarker pattern with annotated findings, listing the likelihood of

involvement of different upstream regulators, canonical pathways, effector mechanisms, and similarities to annotated mechanisms and diseases. The correlation coefficients were imported into IPA and denoted as log expression ratios and annotated with the official gene symbol for each biomarker. NT-proBNP was not included in analysis as it represents an N-terminal cleavage product of BNP and not a gene.

The Ingenuity Knowledge Base was used as reference set and both direct and indirect experimentally confirmed relationships from all species were included. The analysis as implicated in the software calculates a *P* value (Fisher exact test and right tailed), quantifying the overlap, and a *Z* score, quantifying the likelihood and direction (up or downregulated), between the biomarker pattern and published canonical pathways, diseases, and mechanisms as well as upstream regulators. *Z* scores of >2 or <−2 were considered significant. Details about the statistics used can be found at the following website: http://www.ingenuity.com/products/ipa#/?tab=resources.

Ethical Approval

The KaRen study was conducted according to International Conference on Harmonization and Good Clinical Practice guidelines and the Declaration of Helsinki, and the study was approved by the ethical review board at Karolinska Institutet. Written informed consent was obtained from all patients.

Results

Patient Characteristics

At the follow-up visit, in stable state 4 to 8 weeks after hospital discharge (baseline for this substudy), patients were median 73 (interquartile range [IQR] 66; 79) years old and 51% were female. NT-proBNP was 1000 (469–2330) ng/L, and cardiac function assessed as ejection fraction was 64% (58; 68), E/e′ ratio was 10.8 (8.3; 14.0), and LAVI was 43.3 mL/m^2 (37.2; 53.8). Of the 86 patients, 23% had E/e′>15, 67% had e′<9, 89% had LAVI>34 mL/m^2, and 61% had left ventricular hypertrophy. Median follow-up time was 579 days (Q1; Q3: 276; 1178), and no patient was lost to follow-up. The composite end point occurred in 36 patients of which 11 were death.

Characteristics of all 86 patients divided by the presence of the composite end point of HF hospitalization or all-cause death are presented in Table 1. Among patients experiencing an end point, the NT-proBNP was 1375 (574–2565), and 40% had an E/e′>15, 65% had e′<9, 87% had LAVI>34 mL/m^2, and 69% had left ventricular hypertrophy, and in surviving patients free of HF hospitalization, NT-proBNP was 819 (429–1820) and 13% had an E/e′>15, 68% had e′<9, 91% had LAVI>34 mL/m^2, and 56% had left ventricular hypertrophy.

Correlation Between Biomarker Patterns With NYHA Class and Outcome by OPLS Analyses

In total, 92 biomarkers (Table I in the Data Supplement) were analyzed, 5 of which generated no or low values (Pappalysin-1, β-nerve growth factor, P-selectin glycoprotein ligand 1, Melusin, and interleukin-4 [IL-4]) and were, thus, not included in the analyses. Of the remaining 87 biomarkers, the OPLS analysis model 1 identified 32 significantly correlated with NYHA class (30 positively and 2 negatively; Table II in the Data Supplement). The OPLS model 2 identified 28 biomarkers significantly correlated with the composite outcome (24 positively and 4 negatively) presented in Table 2 together with OPLS loading values and their impact on the composite end point according to Cox regression analysis (levels of arbitrary units presented in Figure I in the Data Supplement). The biomarkers having the highest (positively correlated) and the lowest (negatively correlated) loading values for the composite outcome were growth/differentiation factor (GDF)-15 and TNF-related apoptosis-inducing ligand (TRAIL), respectively. To illustrate the relationship between the NYHA class- and outcome-predicting biomarker patterns, the respective loading scores of all 326 variables from the 2 OPLS models were plotted against each other (Figure 2). Given their position in the upper right quadrant, biomarkers such as GDF-15 and chitinase-3–like protein 1 positively correlated with higher NYHA class (ie, worse disease state) and with increased risk of outcome, that is, the levels of these biomarkers increased with NYHA class and were also elevated in patients eventually experiencing the composite end point. In the lower left quadrant, the biomarkers such as TRAIL, TRANCE, and GAL were all negatively associated with NYHA class and the composite end point. KLK6, however, was negatively associated with outcome but noninformative in relation to NYHA class.

Correlations With Echocardiographic Measurements

Correlations by Spearman between biomarkers predicting outcome according to the OPLS analysis and measurements of diastolic dysfunction and structural heart disease, assessed as E/e′, LAVI and left ventricular mass index (LVMI), respectively, are presented in Table 3. The majority of the biomarkers correlated with E/e′, with the soluble receptors tumor necrosis factor receptor 1 (TNF-R1), TRAIL-R2, and U-PAR and the cytokines SPON1, VEGF-A, and the protease matrix metalloproteinase-12 being the most strongly correlated. Four of the biomarkers (C–C motif chemokine 20, prolactin, fatty acid–binding protein, and interleukin 1 receptor like 1 [ST2]) and NT-proBNP measured by ELISA correlated significantly to LAVI, whereas none of the biomarkers correlated to LVMI.

Pathway Analysis and Pathophysiological Mechanisms Preceding Outcome

To identify mechanisms preceding poor outcome, the biomarker pattern (represented by the list of OPLS correlation coefficients of the 87 biomarkers predicting outcome) was subjected to an IPA Core Analysis. Such an analysis summarizes the similarity between a pattern of biomarkers with annotated results published in the scientific literature, yielding probability scores that certain upstream regulators are up or downregulated, whether certain signaling (canonical) pathways are likely to be up or downregulated and whether the pattern resembles other functions or diseases. The analysis suggested that the outcome-predictive biomarker pattern was consistent with reported data from 115 different upstream regulators (Table III in the Data Supplement) of which the most prominent (combined *P* value and *Z* score) activators were the endotoxin lipopolysaccharide, IL-1β and TNF. Similarly, typical inflammatory signaling pathways, such as IL-6–signaling, IL-8–signaling, and the coagulation system canonical pathways, were predicted to be upregulated (Table IV in the Data Supplement). The comparison to other functions and diseases is illustrated as a heatmap in Figure 3 (see Table V in the Data Supplement for complete list) where the pattern of outcome-predicting biomarkers is

Table 2. Biomarkers Significantly Associated With Outcome According to the OPLS (Model 2)

Biomarker	Short Name	Gene Symbol	Uniprot No.	HR (95% CI) Per Log Increase	*P* Value for HR	HR (95% CI) per Log Increase, Adjusted*	*P* Value for Adjusted HR	*P* (Corr)	Type
Growth/differentiation factor 15	GDF-15	GDF-15	Q99988	1.73 (1.15–2.59)	0.009	1.53 (0.97–2.43)	0.068	0.298	Growth factor
Chitinase-3-like protein 1	CHI3L1	CHI3L1	P36222	1.37 (1.07–1.78)	0.014	1.35 (1.03–1.77)	0.033	0.297	Enzyme
C–C motif chemokine 20	CCL20	CCL20	P78556	1.43 (1.11–1.84)	0.008	1.35 (1.02–1.78)	0.035	0.276	Cytokine
Prolactin	PRL	PRL	P01236	1.56 (1.04–2.34)	0.030	1.33 (0.87–2.06)	0.191	0.254	Cytokine
Spondin-1	SPON1	SPON1	Q9HCB6	2.84 (1.30–6.18)	0.009	2.31 (0.95–5.58)	0.064	0.254	Other
Agouti-related protein	AGRP	AGRP	O00253	1.46 (1.03–2.06)	0.035	1.48 (1.01–2.16)	0.043	0.249	Other
Adrenomedullin	AM	ADM	P35318	3.05 (1.34–6.96)	0.008	2.53 (1.03–6.21)	0.044	0.247	Other
Fatty acid–binding protein	FABP4	FABP4	P15090	1.46 (1.06–2.03)	0.022	1.33 (0.92–1.93)	0.127	0.246	Transporter
Fibroblast growth factor 23	FGF-23	FGF23	Q9GZV9	1.27 (1.04–1.56)	0.020	1.18 (0.94–1.48)	0.160	0.239	Growth factor
Tumor necrosis factor receptor 1	TNF-R1	TNFRSF1A	P19438	2.37 (1.13–4.98)	0.022	2.29 (1.10–4.79)	0.027	0.238	Transmembrane receptor
Interleukin-8	IL-8	CXCL8	P10145	1.58 (1.08–2.30)	0.017	1.49 (0.98–2.27)	0.065	0.235	Cytokine
Interleukin-6	IL-6	IL-6	P05231	1.40 (1.07–1.84)	0.015	1.34 (0.99–1.82)	0.061	0.231	Cytokine
TNF receptor superfamily member 5	CD40	CD40	P25942	1.99 (1.10–3.60)	0.024	1.61 (0.85–3.04)	0.144	0.227	Transmembrane receptor
TNF-related apoptosis-inducing ligand receptor 2	TRAIL-R2	TNFRSF10B	O14763	1.70 (1.05–2.76)	0.030	1.46 (0.85–2.49)	0.169	0.222	Transmembrane receptor
Galectin-3	Gal-3	LGALS3	P17931	2.16 (1.07–4.33)	0.031	1.70 (0.79–3.68)	0.179	0.213	Other
Urokinase plasminogen activator surface receptor	U-PAR	PLAUR	Q03405	2.89 (1.11–7.53)	0.030	2.01 (0.68–5.94)	0.208	0.199	Transmembrane receptor
ST2 protein	ST2	IL1RL1	Q01638	1.98 (1.14–3.45)	0.016	1.78 (0.98–3.26)	0.060	0.199	Transmembrane receptor
Proteinase-activated receptor 1	PAR-1	F2R	P25116	2.20 (1.02–4.73)	0.043	1.79 (0.83–3.85)	0.137	0.194	G-protein–coupled receptor
NT-proBNP (ELISA)†	NT-proBNP†			1.49 (1.05–2.12) †	0.027†	1.49 (1.02–2.17)†‡	0.038†	0.188†	
Matrix metalloproteinase-12	MMP-12	MMP12	P39900	1.52 (1.02–2.24)	0.038	1.42 (0.93–2.16)	0.105	0.186	Peptidase
Vascular endothelial growth factor A	VEGF-A	VEGFA	P15692	1.69 (0.88–3.24)	0.114	NA	NA	0.161	Growth factor
Matrix metalloproteinase-7	MMP-7	MMP7	P09237	1.75 (1.01–3.02)	0.046	1.52 (0.85–2.69)	0.155	0.152	Peptidase
Tissue factor	TF	F3	P13726	1.68 (0.68–4.14)	0.259	NA	NA	0.149	Transmembrane receptor
Cystatin-B	CSTB	CSTB	P04080	1.27 (0.85–1.90)	0.247	NA	NA	0.136	Peptidase
Resistin	RETN	RETN	Q9HD89	1.21 (0.73–2.03)	0.462	NA	NA	0.132	Other
Kallikrein-6	KLK6	KLK6	Q92876	0.70 (0.35–1.39)	0.309	0.52 (0.26–1.07)	0.074	–0.153	Peptidase
TNF-related activation-induced cytokine	TRANCE	TNFSF11	O14788	0.69 (0.39–1.22)	0.201	0.71 (0.40–1.27)	0.250	–0.211	Cytokine
Galanin peptides	GAL	GAL	P22466	0.67 (0.45–1.00)	0.052	0.71 (0.48–1.05)	0.086	–0.238	Other
TNF-related apoptosis-inducing ligand	TRAIL	TNFSF10	P50591	0.50 (0.1–1.20)	0.154	0.34 (0.12–0.96)	0.041	–0.292	Cytokine

CI indicates confidence interval; HR, hazard ratio; NA, not applicable; NT-proBNP, N-terminal probrain natriuretic peptide; and OPLS, orthogonal partial least square.
*Adjusted NT-proBNP, age, sex.
‡Adjusted age, sex. Analyzed by ELISA.

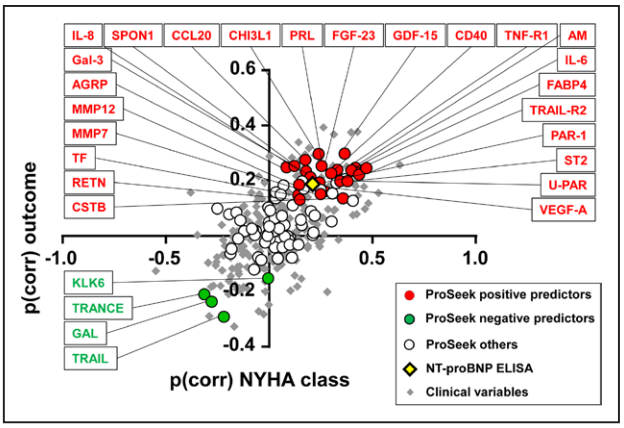

Figure 2. The relationship between the NYHA class- and outcome-associated biomarker patterns. The graph shows the loadings, expressed as correlation coefficients, *P*(corr), for the orthogonal partial least square (OPLS) model predicting NYHA class and the OPLS predicting outcome. The clinical variables are indicated as gray diamonds and the ProSeek biomarkers as circles. Colored circles indicate that the biomarker has a significant predictive value for outcome. The biomarker short name for the 24 positive predictors (red) and the 4 negative predictors (green) are outlined in the upper and the lower left quadrants, respectively. The complete list of individual *P*(corr) values are listed in Table I in the Data Supplement. The *P*(corr) for the N-terminal probrain natriuretic peptide (NT-proBNP) data analyzed by ELISA is illustrated by a yellow diamond. AGRP indicates agouti-related protein; AM, adrenomedullin; CCL20, C–C motif chemokine 20; CHI3L1, chitinase-3-like protein 1; CSTB, cystatin-B; FABP4, fatty acid–binding protein 4; FGF-23, fibroblast growth factor 23; Gal-3, galectin-3; GDF-15, growth/differentiation factor 15; IL, interleukin; KLK6, kallikrein-6; MMP, matrix metalloproteinase; PAR, proteinase-activated receptor; PRL, prolactin; RETN, resistin; SPON1, spondin-1; TF, tissue factor; TNF-R, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; TRANCE, TNF-related activation-induced cytokine; U-PAR, urokinase plasminogen activator surface receptor; and VEGF-A, vascular endothelial growth factor A.

compared with annotated mechanisms and diseases. The pattern highlighted increased immunologic and inflammatory processes as main mechanisms associated with outcome. There was also an association between worse outcome and reduced apoptosis and reduced cell death, as illustrated by the inverted (blue) correlation with these features in the "Cell death and Survival"-annotated square.

Discussion

In this proteomics approach, numerous novel individual biomarkers and biomarker clusters representing inflammation, immune activation, growth factors, and inhibition of apoptosis independently associated with HFpEF severity and outcome, were identified. These may represent pathophysiological pathways implicated in global inflammation and may for prognostication also complement or even be more important than traditional markers, such as NT-proBNP.

Biomarkers of Inflammation as Predictors of Prognosis

Systemic inflammation is proposed to be one potential major driver of HFpEF, causing coronary microvascular endothelial inflammation and the development of diastolic dysfunction.[8,22–25] Indeed, numerous single biomarkers of

Table 3. Correlation Between the 28 Biomarkers Predictive of the Composite Outcome According to the OPLS Analysis and Echocardiographic Markers of Diastolic Dysfunction and Structural Heart Disease

Biomarker Short Name	E/e´		LAVI		LVMI	
	r	*P* Value	*r*	*P* Value	*r*	*P* Value
Positive predictors						
GDF-15	0.321*	0.009*	0.183	0.119	0.0145	0.361
CHI3L1	0.091	0.469	–0.008	0.943	0.270	0.088
CCL20	0.292*	0.018*	0.316*	0.006*	0.034	0.833
PRL	0.197	0.115	0.317*	0.006*	0.064	0.689
SPON1	0.473*	<0.001*	0.075	0.527	0.077	0.632
AGRP	0.268*	0.031*	0.013	0.914	0.146	0.364
AM	0.389*	0.001*	0.088	0.456	–0.084	0.603
FABP4	0.352*	0.004*	0.397*	0.001*	0.072	0.655
FGF-23	0.276	0.026*	0.219	0.061	0.130	0.416
TNF-R1	0.427*	<0.001*	0.038	0.746	0.191	0.231
IL-8	0.250*	0.044*	0.008	0.944	–0.121	0.451
IL-6	0.265*	0.033*	0.192	0.101	0.104	0.517
CD40	0.343*	0.005*	–0.033	0.778	0.039	0.808
TRAIL-R2	0.424*	<0.001*	0.014	0.907	0.056	0.730
Gal-3	0.393*	0.001*	0.026	0.915	0.022	0.892
U-PAR	0.442*	<0.001*	0.023	0.847	0.036	0.823
ST2	0.296*	0.017*	0.271*	0.020*	0.119	0.459
PAR-1	0.342*	0.005*	–0.080	0.501	0.076	0.638
NT-proBNP (ELISA)	0.151	0.233	0.307*	0.008*	–0.088	0.583
MMP-12	0.461*	<0.001*	–0.030	0.798	–0.077	0.634
VEGF-A	0.411*	<0.001*	0.078	0.511	0.153	0.340
MMP-7	0.392*	0.001*	0.033	0.783	0.096	0.549
TF	0.351*	0.004*	0.044	0.709	–0.135	0.400
CSTB	0.313*	0.011*	–0.024	0.842	–0.041	0.797
RETN	0.265*	0.033*	–0.173	0.140	0.192	0.230
Negative predictors						
KLK6	0.301*	0.015*	0.046	0.695	–0.211	0.186
TRANCE	0.003	0.984	–0.041	0.724	0.239	0.133
GAL	0.002	0.988	–0.064	0.587	–0.102	0.525
TRAIL	0.101	0.424	–0.003	0.828	–0.034	0.831

AGRP indicates agouti-related protein; AM, adrenomedullin; CCL20, C–C motif chemokine 20; CHI3L1, chitinase-3–like protein 1; CSTB, cystatin-B; FABP4, fatty acid–binding protein; FGF, fibroblast growth factor; Gal-3, galectin-3; GDF, growth/differentiation factor; IL, interleukin; KLK6, kallikrein-6; LAVI, left atrial volume index; LVMI, Left ventricular mass index; MMP, matrix metalloproteinase; NT-proBNP, N-terminal probrain natriuretic peptide; OPLS, orthogonal partial least square; PAR, proteinase-activated receptor; PRL, prolactin; RETN, resistin; SPON1, spondin-1; TF, tissue factor; TNF, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; TRANCE, TNF-related activation-induced cytokine; U-PAR, urokinase plasminogen activator surface receptor; and VEGF-A, vascular endothelial growth factor A.

1. Cardiovascular system and development
2. Cardiovascular disease
3. Inflammatory disease
4. Hematopoiesis
5. Cellular function and maintenance
6. Organismal development
7. Gastrointestinal disease
8. Molecular transport
9. Tumor morphology
10. Lymphoid tissue structure and development
11. Lipid metabolism
12. Tissue morphology
13. Organ development
14. Immunological disease
15. Reproductive system disease
16. Skeletal and muscular disorders
17. Infection disease
18. Endocrine system disorders
19. Skeletal and muscular system development and function
20. Hepatic system disease
21. Small molecule biochemistry
22. Respiratory disease
23. Embryonic development

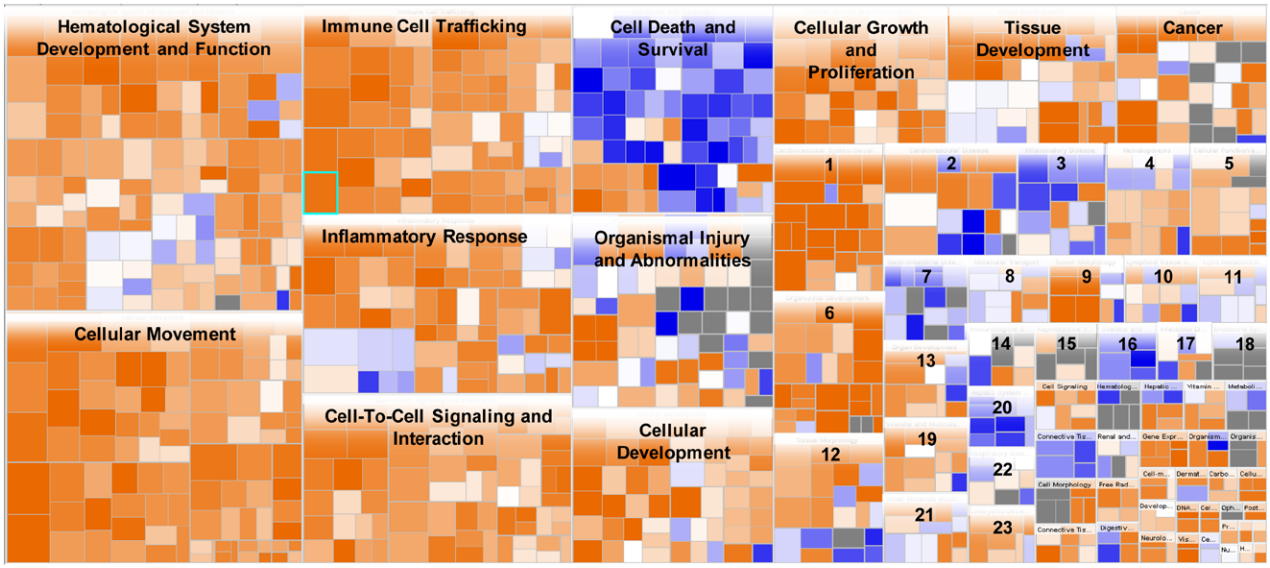

Figure 3. Heatmap illustrating the similarity between the outcome-predicting biomarker pattern with other functions and diseases in the scientific literature. Each square represents an annotated mechanism or disease, grouped by the outlined functions/diseases. The size of the squares represent how many of the biomarkers in the panel are represented in the implicated function (*P* value of overlap). The color intensity represents the *Z* score, which is used to infer the grade of activation states ("increased" or "decreased") of implicated biological functions, with positive *Z* score (red) indicative of activation, and negative *Z* score (blue) indicative of inhibition, in subjects eventually experiencing an outcome. In the "Cell Death and Survival" group, the blue color represents apoptosis and necrosis and red color represents cell viability. The mechanisms represented by the 30 smallest squares are not outlined in the numbered list. The complete list is found in Table IV in the Data Supplement.

inflammation have previously (eg, GDF-15, galectin-3, ST2, IL-6, and IL-8[13,16,17,26,27]) and now, to our knowledge, for the first time (eg, chitinase-3–like protein 1 and C–C motif chemokine 20) been found to be associated with HFpEF severity and outcome. GDF-15 was the strongest predictor of outcome among 87 different biomarkers in this study, consistent with previous analyses.[26,28] In addition, we present novel prognostic information on the pseudochitinase chitinase-3–like protein 1 (YKL-40), present in inflammatory cells, such as in granules of neutrophils, which has been associated with the increased risk of atrial fibrillation,[29] a common comorbidity in HFpEF. Chitinase-3–like protein 1 is a marker of disease severity in many systemic inflammatory diseases, including asthma,[30] and is also a predictor of mortality in HFrEF.[31]

Previous studies have analyzed single or few biomarkers and because of complex relationships between numerous biomarkers, independent roles of the relevant biomarkers have not been established. In this study, our methodology allows simultaneous and comprehensive assessment of a large number of biomarkers as we used the OPLS approach to simultaneously contextualize a large set of biomarker data in relation to all clinical variables recorded. The OPLS yields a pattern of correlation coefficients of all variables represented in X, which in the case of biomarkers can be viewed as a fingerprint for the analyzed diseased state or outcome. The model had a high degree of explanatory power, 96%, as a result of the integration of all the clinical variables in the analysis. There were for instance a handful of variables related to outcome (eg, death and time to death) that contributed to the strong explanation of the model. Removal of these "outcome-related" variables reduced R2Y to 0.41 and Q2 to 0.013. By keeping all available variables in the OPLS, we minimized the risk of bias that could have been a result by hypothesis-driven exclusion of variables. The principal component analysis is

built on an integrative analysis of the pattern formed by all of the variables, rather than a compilation of univariate analyses. Thus, although there is a statistical chance that a single variable may covary with the outcome-predicting pattern by chance, the chance of such a spurious correlation having an impact on the overall pattern is minimized, inasmuch as it would be "diluted" by all the other variables. By comparing the generated fingerprint with databases containing annotated data from the public domain, insights into disease biology can be gained in an expedited and unbiased manner as compared to traditional literature searches. As a result, the value of the biomarker, and also the interbiomarker relationship, for predicting HFpEF severity and prognosis could be illustrated.

As NT-proBNP was shown to predict prognosis in HFpEF,[32] several biomarkers have been evaluated,[15,27,33,34] but no comprehensive assessments have to our knowledge been performed. When comparing prognostic impact of biomarkers, IL-18 has been suggested to be a superior predictor of mortality in multivariable analyses, including BNP.[35] In this study with more data input, IL-18 quantified by ProSeek did not have any independent predictive value for outcome. Interestingly as many as 21 of the biomarkers, among them several inflammatory markers, outperformed NT-proBNP determined by ELISA (Figure 2; Table 2).

Recent findings have highlighted the information we obtain from markers of inflammation and oxidative stress for the development of the HFpEF syndrome[17,36] and a specific role of TNFα and its receptors and IL-16 correlating with myocardial fibrosis, and stiffening has been demonstrated in patients with HFpEF compared with HFrEF and controls.[37,38] Cytokines signaling inflammation, such as IL-8, IL-6, and PTX3 (a marker for the primary local activation of innate immunity), and low levels of matrix metalloproteinase-1 (indicating a decrease in the major collagenase system) are all suggested to be correlated with diastolic dysfunction in HFpEF.[12,16,17,39] In this study, we confirm correlation with diastolic dysfunction expressed as E/e′ for several inflammatory markers, such as IL-6, GDF-15, and C–C motif chemokine 20. However, when analyzed individually, the correlation with structural heart disease was less prominent, indicating that functional class and prognosis in HFpEF may be better reflected by markers of inflammation and apoptosis, or that the echocardiographic measurements remain poor markers of the severity of the HFpEF syndrome. This is supported in a recent study where miRNA combinations, including lower expression of miR-146a, associated with TNFα and IL6, differentiated HFpEF from HFrEF but did not correlate with LAVI.[40] Inflammatory biomarkers such as cytokines have pleiotropic effects, which depend on signaling, cellular, organ and disease context.[41] When comparing the outcome-predicting biomarker pattern with previously published data compiled in the IPA knowledge base, inflammation, hyper-proliferation, angiogenesis, and anti-apoptotic activity appeared as processes likely to be associated with poor prognosis. This pattern is in agreement with recent findings, suggesting impaired myocardial oxygen delivery possibly relating to microvascular dysfunction as a possible mechanism driving HFpEF.[25,42,43] This hypothesis is also consistent with histopathologic findings of more prominent myocardial fibrosis and coronary capillary rarefaction in this patient group.[44]

Biomarkers as Prognostic Protective Predictors

We found the TNF superfamily member (TRAIL) to be negatively associated with outcome, with a reciprocal pattern of the soluble form of its receptor TRAIL-R2 (which was positively associated with outcome). By activating cell death–related pathways, members of the TNF superfamily can induce a variety of effects through the TNF receptors on cells, for exmple, within the myocardium, including TRAIL-mediated apoptosis. Not only lower levels of the ligand but also increased levels of the decoy receptor were associated with outcome, suggesting a beneficial role of TRAIL signaling in patients with HFpEF. In the myocardium, this would seem plausible given that myocardial hypertrophy is a major component of the HFpEF syndrome.[45] Cardiomyocyte cell death has been associated with worse prognosis in HFrEF[46] but is less prominent in HFpEF. However, although cardiomyocyte apoptosis may be disadvantageous in HFrEF, with reduced contractility, it is conceivable that global systemic apoptosis may be advantageous, for example, by limiting inflammation and downstream remodeling. Notably, higher levels of TRAIL have previously been associated with a better prognosis in HFrEF,[47] as observed here for HFpEF perhaps also reflecting the need for TRAIL-induced apoptosis to resolve inflammation. Another member of the TNF superfamily, TRANCE, was also negatively associated with outcome. Similar to the TRAIL-R2 pattern, the corresponding soluble decoy receptor for TRANCE (OPG) was also positively associated with outcome, although this did not reach statistical significance in the OPLS analysis. TRANCE has previously been suggested to be implicated in myocardial remodeling,[48] and the ratio between OPG and TRAIL has been shown to be associated with HF after myocardial infarction.[49] In addition to being a regulator of calcification and immunity, the TRAIL–OPG axis is also involved in apoptosis, for exmple, by the OPG-binding and neutralization of TRAIL.[50]

Pathophysiological Pathways

Compared to the use of single biomarkers in the characterization of pathophysiology and prognosis, multiplexed analyses combine numerous biomarkers creating patterns of up and downregulated processes to more broadly shed light on potential underlying pathophysiology and to identify the most prominent pathways associated with both severity and prognosis and also potentially with pathophysiology. This methodology is particularly relevant now when large biomarker data sets are readily generated through novel technologies, for example, by next-generation sequencing, by mass cytometry, or by multiplexed protein analysis. Using multivariate projection methods, dissecting patterns of data in large data sets, identifies dimensions describing the majority of data in a data set. Recently, Zordoky et al[51] investigated the metabolomic profile of HFpEF by analyzing 180 different endogenous metabolites. They reported higher serum concentration of long-chain acylcarnitines in patients with HFpEF compared with both patients with HFrEF and non-HF controls and identified a set of 4 biomarkers (2-hydroxybutyrate, octadecenoylcarnitine [C18:1], hydroxyprionylcarnitine [C3–OH], and SM [C24:1]) that without NT-proBNP produced a receiver operating characteristic area under the curve

value of 0.908 to differentiate between HFpEF and HFrEF patients.[51] Our data in HFpEF patients with a poor outcome identified a biomarker pattern consistent with an upregulated innate immune response but also impaired apoptosis. Leukocyte apoptosis is a key process for control of inflammation, which is a regulated rather than a passive process.[52] Examples of resolution factors are lipoxins, the paucity of which results in chronic inflammation.[53] Recently, it was shown that the reduced levels of lipoxins predict poor outcome in chronic HF patients with reduced LVEF, suggesting together with our present data that improperly regulated chronic inflammatory reactions may be a common feature of chronic HF, irrespective of subtype.

Limitations

Caution should be taken when interpreting concentrations of plasma biomarkers in the description of pathophysiological mechanisms in specific tissues as they may not necessarily reflect processes in that tissue, for example, in the myocardium. However, the use of biomarkers provides us with pathophysiological clues on how to proceed and validate our findings further, especially when the evaluation of biomarker patterns are used instead of single biomarker approaches.

Of the 87 analyzed ProSeek biomarkers, there were 3 for which the platform displayed a considerable interassay variability (CV≥25%). One of these was significantly associated with outcome (matrix metalloproteinase-12), whereas the others (matrix metalloproteinase-1 and tumor necrosis factor ligand superfamily member 14) were not. Caution should be taken regarding these biomarkers, inasmuch as the high-assay variability may both yield falsely positive and negative associations.

The NT-proBNP criterion of >300 ng/L was used to select patients with a reasonable likelihood of heart failure and discriminate from other causes of dyspnea. It is increasingly recognized that NT-proBNP may be elevated in the absence of HF in patients with combinations of female sex, higher age, lower body mass index, renal insufficiency, and atrial fibrillation. However, the inclusion criteria were similar to other trials and studies of HFpEF, and the diagnosis was verified by Framingham criteria.

The LVEF inclusion criterion was set at 45%, which was a cutoff used in many of the HFpEF trials.[4,6] In the Karen biomarker substudy presented here, only two patients had an LVEF of <50%, thus the vast majority of the population did in this aspect comply with the present criteria of HFpEF as recommended by the American College of Cardiology Foundation and American Heart Association and the European Society of Cardiology guidelines (LVEF ≥50%).[21,54]

A limitation of this study is that the data are based on a relatively small single cohort, which may represent a bias in terms of geographical and ethnical aspects.

Conclusions

In HFpEF, novel biomarkers reflecting inflammation and hyper-proliferative, proangiogenetic, and anti-apoptotic activity and their interrelation are linked to disease severity and prognosis. This may support the hypotheses of global inflammation, implicating microvascular inflammation, which may be a potential treatment target in patients with HFpEF. Furthermore, the prognostic information may complement or even be more important than for traditional markers, such as NT-proBNP. The validity of these findings for the overall population with HFpEF will require the replication of the data in ethnically and geographically distinct cohorts.

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Disclosures

The extent that findings in Karolinska Rennes (KaRen) may affect the use of heart failure drugs or devices, the authors disclose the following: Dr Hage received consulting fees from Novartis and speaker and honoraria from Merck Sharp & Dohme; Dr Lund received research grants and speaker and honoraria from AstraZeneca, consulting honoraria from Novartis and St. Jude Medical, and research grants from Boston Scientific; Dr Linde was a principal investigator of the Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction (REVERSE) study, a Cardiac Resynchronization Therapy study sponsored by Medtronic research grants, speaker honoraria, and received consulting fees from Medtronic, speaker honoraria and consulting fees from St. Jude Medical; Dr Donal received speaker honoraria and consulting fees from Novartis, AstraZeneca; Dr Daubert received research grants, speaker honoraria and consulting fees from Medtronic and St. Jude Medical; Drs Michaëlsson and Gan are employees at AstraZeneca. The other author reports no conflicts.

References

1. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251–259. doi: 10.1056/NEJMoa052256.
2. Braunwald E. Biomarkers in heart failure. *N Engl J Med*. 2008;358:2148–2159. doi: 10.1056/NEJMra0800239.
3. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJV, et al; CHARM Investigators and Committees. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the charm-preserved trial. *Lancet*. 2003;362:777–781. doi: 10.1016/S0140-6736(03)14285-7.
4. Pitt B, Pfeffer MA, Assmann SF, Boineau R, Anand IS, Claggett B, et al; TOPCAT Investigators. Spironolactone for heart failure with preserved ejection fraction. *N Engl J Med*. 2014;370:1383–1392. doi: 10.1056/NEJMoa1313731.
5. Cleland JG, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J; PEP-CHF Investigators. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. *Eur Heart J*. 2006;27:2338–2345. doi: 10.1093/eurheartj/ehl250.

6. Massie BM, Carson PE, McMurray JJ, Komajda M, McKelvie R, Zile MR, et al; I-PRESERVE Investigators. Irbesartan in patients with heart failure and preserved ejection fraction. *N Engl J Med*. 2008;359:2456–2467. doi: 2410.1056/NEJMoa0805450. doi: 10.1056/NEJMoa0805450.
7. Ather S, Chan W, Bozkurt B, Aguilar D, Ramasubbu K, Zachariah AA, et al. Impact of noncardiac comorbidities on morbidity and mortality in a predominantly male population with heart failure and preserved versus reduced ejection fraction. *J Am Coll Cardiol*. 2012;59:998–1005. doi: 10.1016/j.jacc.2011.11.040.
8. Paulus WJ, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263–271. doi: 10.1016/j.jacc.2013.02.092.
9. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110:3121–3128. doi: 10.1161/01.CIR.0000147181.65298.4D.
10. Januzzi JL Jr. ST2 as a cardiovascular risk biomarker: from the bench to the bedside. *J Cardiovasc Transl Res*. 2013;6:493–500. doi: 10.1007/s12265-013-9459-y.
11. Lok SI, Winkens B, Goldschmeding R, van Geffen AJ, Nous FM, van Kuik J, et al. Circulating growth differentiation factor-15 correlates with myocardial fibrosis in patients with non-ischaemic dilated cardiomyopathy and decreases rapidly after left ventricular assist device support. *Eur J Heart Fail*. 2012;14:1249–1256. doi: 10.1093/eurjhf/hfs120.
12. Westermann D, Lindner D, Kasner M, Zietsch C, Savvatis K, Escher F, et al. Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. *Circ Heart Fail*. 2011;4:44–52. doi: 10.1161/CIRCHEARTFAILURE.109.931451.
13. de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med*. 2011;43:60–68. doi: 10.3109/07853890.2010.538080.
14. van Veldhuisen DJ, Linssen GC, Jaarsma T, van Gilst WH, Hoes AW, Tijssen JG, et al. B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. *J Am Coll Cardiol*. 2013;61:1498–1506. doi: 10.1016/j.jacc.2012.12.044.
15. Gori M, Senni M, Gupta DK, Charytan DM, Kraigher-Krainer E, Pieske B, et al; PARAMOUNT Investigators. Association between renal function and cardiovascular structure and function in heart failure with preserved ejection fraction. *Eur Heart J*. 2014;35:3442–3451. doi: 10.1093/eurheartj/ehu254.
16. Collier P, Watson CJ, Voon V, Phelan D, Jan A, Mak G, et al. Can emerging biomarkers of myocardial remodelling identify asymptomatic hypertensive patients at risk for diastolic dysfunction and diastolic heart failure? *Eur J Heart Fail*. 2011;13:1087–1095. doi: 10.1093/eurjhf/hfr079.
17. Glezeva N, Voon V, Watson C, Horgan S, McDonald K, Ledwidge M, et al. Exaggerated inflammation and monocytosis associate with diastolic dysfunction in heart failure with preserved ejection fraction: evidence of M2 macrophage activation in disease pathogenesis. *J Card Fail*. 2015;21:167–177. doi: 10.1016/j.cardfail.2014.11.004.
18. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). *J Chemometrics*. 2002;16:119–128.
19. Donal E, Lund LH, Linde C, Edner M, Lafitte S, Persson H, et al. Rationale and design of the Karolinska-Rennes (KaRen) prospective study of dyssynchrony in heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2009;11:198–204. doi: 10.1093/eurjhf/hfn025.
20. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9:e95192. doi: 10.1371/journal.pone.0095192.
21. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al; Authors/Task Force Members. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129–2200. doi: 10.1093/eurheartj/ehw128.
22. Maréchaux S, Rusinaru D, Jobic Y, Ederhy S, Donal E, Réant P, et al. Food and Drug Administration criteria for the diagnosis of drug-induced valvular heart disease in patients previously exposed to benfluorex: a prospective multicentre study. *Eur Heart J Cardiovasc Imaging*. 2015;16:158–165. doi: 10.1093/ehjci/jeu212.
23. Matsue Y, Suzuki M, Nagahori W, Ohno M, Matsumura A, Hashimoto Y, et al. Endothelial dysfunction measured by peripheral arterial tonometry predicts prognosis in patients with heart failure with preserved ejection fraction. *Int J Cardiol*. 2013;168:36–40. doi: 10.1016/j.ijcard.2012.09.021.
24. Elesber AA, Redfield MM, Rihal CS, Prasad A, Lavi S, Lennon R, et al. Coronary endothelial dysfunction and hyperlipidemia are independently associated with diastolic dysfunction in humans. *Am Heart J*. 2007;153:1081–1087. doi: 10.1016/j.ahj.2007.03.007.
25. Akiyama E, Sugiyama S, Matsuzawa Y, Konishi M, Suzuki H, Nozaki T, et al. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. *J Am Coll Cardiol*. 2012;60:1778–1786. doi: 10.1016/j.jacc.2012.07.036.
26. Chan MM, Santhanakrishnan R, Chong JP, Chen Z, Tai BC, Liew OW, et al. Growth differentiation factor 15 in heart failure with preserved vs. reduced ejection fraction. *Eur J Heart Fail*. 2016;18:81–88. doi: 10.1002/ejhf.431.
27. Friões F, Lourenço P, Laszczynska O, Almeida PB, Guimarães JT, Januzzi JL, et al. Prognostic value of sST2 added to BNP in acute heart failure with preserved or reduced ejection fraction. *Clin Res Cardiol*. 2015;104:491–499. doi: 10.1007/s00392-015-0811-x.
28. Izumiya Y, Hanatani S, Kimura Y, Takashio S, Yamamoto E, Kusaka H, et al. Growth differentiation factor-15 is a useful prognostic marker in patients with heart failure with preserved ejection fraction. *Can J Cardiol*. 2014;30:338–344. doi: 10.1016/j.cjca.2013.12.010.
29. Marott SC, Benn M, Johansen JS, Jensen GB, Tybjærg-Hansen A, Nordestgaard BG. YKL-40 levels and atrial fibrillation in the general population. *Int J Cardiol*. 2013;167:1354–1359. doi: 10.1016/j.ijcard.2012.04.006.
30. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med*. 2007;357:2016–2027. doi: 10.1056/NEJMoa073600.
31. Harutyunyan M, Christiansen M, Johansen JS, Køber L, Torp-Petersen C, Kastrup J. The inflammatory biomarker YKL-40 as a new prognostic marker for all-cause mortality in patients with heart failure. *Immunobiology*. 2012;217:652–656. doi: 10.1016/j.imbio.2011.11.003.
32. Cleland JG, Taylor J, Tendera M. Prognosis in heart failure with a normal ejection fraction. *N Engl J Med*. 2007;357:829–830. doi: 10.1056/NEJMc076179.
33. Hage C, Lund LH, Donal E, Daubert JC, Linde C, Mellbin L. Copeptin in patients with heart failure and preserved ejection fraction: a report from the prospective KaRen-study. *Open Heart*. 2015;2:e000260. doi: 10.1136/openhrt-2015-000260.
34. Zabarovskaja S, Hage C, Linde C, Daubert JC, Donal E, Gabrielsen A, et al. Adaptive cardiovascular hormones in a spectrum of heart failure phenotypes. *Int J Cardiol*. 2015;189:6–11. doi: 10.1016/j.ijcard.2015.03.381.
35. Sanchez I, Santana S, Escobar C, Santiago JL, Gonzalez A, Ribas N, et al. Clinical implications of different biomarkers in elderly patients with heart failure. *Biomark Med*. 2014;8:535–541. doi: 10.2217/bmm.14.24.
36. Szelényi Z, Fazakas Á, Szénási G, Kiss M, Tegze N, Fekete BC, et al. Inflammation and oxidative stress caused by nitric oxide synthase uncoupling might lead to left ventricular diastolic and systolic dysfunction in patients with hypertension. *J Geriatr Cardiol*. 2015;12:1–10. doi: 10.11909/j.issn.1671-5411.2015.01.001.
37. Putko BN, Wang Z, Lo J, Anderson T, Becher H, Dyck JR, et al; Alberta HEART Investigators. Circulating levels of tumor necrosis factor-alpha receptor 2 are increased in heart failure with preserved ejection fraction relative to heart failure with reduced ejection fraction: evidence for a divergence in pathophysiology. *PLoS One*. 2014;9:e99495. doi: 10.1371/journal.pone.0099495.
38. Tamaki S, Mano T, Sakata Y, Ohtani T, Takeda Y, Kamimura D, et al. Interleukin-16 promotes cardiac fibrosis and myocardial stiffening in heart failure with preserved ejection fraction. *PLoS One*. 2013;8:e68893. doi: 10.1371/journal.pone.0068893.
39. Matsubara J, Sugiyama S, Nozaki T, Sugamura K, Konishi M, Ohba K, et al. Pentraxin 3 is a new inflammatory marker correlated with left ventricular diastolic dysfunction and heart failure with normal ejection fraction. *J Am Coll Cardiol*. 2011;57:861–869. doi: 10.1016/j.jacc.2010.10.018.
40. Watson CJ, Gupta SK, O'Connell E, Thum S, Glezeva N, Fendrich J, et al. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. *Eur J Heart Fail*. 2015;17:405–415. doi: 10.1002/ejhf.244.
41. Kushner I. Semantics, inflammation, cytokines and common sense. *Cytokine Growth Factor Rev*. 1998;9:191–196.
42. Sucato V, Evola S, Novo G, Sansone A, Quagliana A, Andolina G, et al. Angiographic evaluation of coronary microvascular dysfunction in patients with heart failure and preserved ejection fraction. *Microcirculation*. 2015;22:528–533. doi: 10.1111/micc.12223.

43. Franssen C, Chen S, Unger A, Korkmaz HI, De Keulenaer GW, Tschöpe C, et al. Myocardial microvascular inflammatory endothelial activation in heart failure with preserved ejection fraction. *JACC Heart Fail*. 2016;4:312–324. doi: 10.1016/j.jchf.2015.10.007.
44. Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. *Circulation*. 2015;131:550–559. doi: 10.1161/CIRCULATIONAHA.114.009625.
45. Ueland T, Yndestad A, Dahl CP, Gullestad L, Aukrust P. TNF revisited: osteoprotegerin and TNF-related molecules in heart failure. *Curr Heart Fail Rep*. 2012;9:92–100. doi: 10.1007/s11897-012-0088-6.
46. Mughal W, Dhingra R, Kirshenbaum LA. Striking a balance: autophagy, apoptosis, and necrosis in a normal and failing heart. *Curr Hypertens Rep*. 2012;14:540–547. doi: 10.1007/s11906-012-0304-5.
47. Niessner A, Hohensinner PJ, Rychli K, Neuhold S, Zorn G, Richter B, et al. Prognostic value of apoptosis markers in advanced heart failure patients. *Eur Heart J*. 2009;30:789–796. doi: 10.1093/eurheartj/ehp004.
48. Halapas A, Zacharoulis A, Theocharis S, Karavidas A, Korres D, Papadopoulos K, et al. Serum levels of the osteoprotegerin, receptor activator of nuclear factor kappa-B ligand, metalloproteinase-1 (MMP-1) and tissue inhibitors of MMP-1 levels are increased in men 6 months after acute myocardial infarction. *Clin Chem Lab Med*. 2008;46:510–516. doi: 10.1515/CCLM.2008.091.
49. Secchiero P, Corallini F, Beltrami AP, Ceconi C, Bonasia V, Di Chiara A, et al. An imbalanced OPG/TRAIL ratio is associated to severe acute myocardial infarction. *Atherosclerosis*. 2010;210:274–277. doi: 10.1016/j.atherosclerosis.2009.11.005.
50. Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem*. 1998;273:14363–14367.
51. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, et al; Alberta HEART. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PLoS One*. 2015;10:e0124844. doi: 10.1371/journal.pone.0124844.
52. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. *Nat Rev Immunol*. 2013;13:59–66. doi: 10.1038/nri3362.
53. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol*. 2001;2:612–619. doi: 10.1038/89759.
54. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, et al; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2013;62:e147–e239. doi: 10.1016/j.jacc.2013.05.019.

CLINICAL PERSPECTIVE

In heart failure (HF) with reduced ejection fraction exploration of a wide range of biomarkers has contributed fundamentally to the understanding of underlying mechanisms, pathophysiology, and prognosis, which, in turn, has contributed to successful trials and the development of neurohormonal antagonist drugs. These pharmacological agents have not been shown to improve outcomes in HF with preserved ejection fraction (HFpEF), suggesting a different underlying pathophysiology and greater heterogeneity of phenotypes in HFpEF than in HF with reduced ejection fraction. In HFpEF, it is previously known that inflammatory plasma biomarkers are elevated. However, we have novel information on combined biomarker patterns in this complex syndrome. We used orthogonal partial least square and Ingenuity Pathway Analysis core analyses to contextualize a large set of biomarker data in relation to numerous clinical variables. This generated patterns of up and downregulated pathways associated with both severity and prognosis in HFpEF. The identified biomarker pattern reflects inflammation and hyper proliferative, proangiogenetic, and anti-apoptotic activity associated with disease severity and prognosis in HFpEF. The findings implicate inflammation as a driver contributing to the HFpEF syndrome and lend support to the hypothesis on microvascular and endothelial inflammation as a potential treatment target in these patients.