ESC HEART FAILURE
ESC Heart Failure 2023; **10**: 200–210
Published online 2 October 2022 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.14167

ORIGINAL ARTICLE

Biomarker profiles in heart failure with preserved vs. reduced ejection fraction: results from the DIAST-CHF study

Abass Eidizadeh[1], Moritz Schnelle[1,2], Andreas Leha[2,3], Frank Edelmann[4,5,6], Kathleen Nolte[7], Stefanie Maria Werhahn[7], Lutz Binder[1,2] and Rolf Wachter[2,7,8]*

[1]*Institute for Clinical Chemistry/Interdisciplinary UMG Laboratory, University Medical Center Göttingen, Göttingen, Germany;* [2]*DZHK (German Centre for Cardiovascular Research), Partner Site Göttingen, Göttingen, Germany;* [3]*Department of Medical Statistics, University Medical Center Göttingen, Göttingen, Germany;* [4]*Department of Internal Medicine and Cardiology, Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum, Berlin, Germany;* [5]*DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, Berlin, Germany;* [6]*Berlin Institute of Health, Berlin, Germany;* [7]*Clinic of Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany; and* [8]*Clinic and Policlinic for Cardiology, University Hospital Leipzig, Leipzig, Germany*

Abstract

Aims Chronic heart failure (HF) is a common disease and one of the leading causes of death worldwide. Heart failure with preserved ejection fraction (HFpEF) and with reduced ejection fraction (HFrEF) are different diseases with distinct as well as comparable pathophysiologies and diverse responses to therapeutic agents. We aimed to identify possible pathobiochemical signalling pathways and biomarkers in HFpEF and HFrEF by using a broad proteomic approach.

Methods and results A total of 180 biomarkers in the plasma of a representative subgroup (71 years old) of HFpEF (70% female) with a left ventricular ejection fraction (LVEF) $\geq$ 50% and HFrEF (18% female) with an LVEF $\leq$ 40% patients (n = 127) from the Prevalence and Clinical Course of Diastolic Dysfunction and Diastolic Heart Failure (DIAST-CHF) trial were examined and compared with a healthy control group (n = 40; 48% female). We were able to identify 35 proteins that were expressed significantly different in both HF groups compared with the control group. We determine 29 unique proteins expressed in HFpEF and 33 unique proteins in HFrEF. Significantly up-regulated trefoil factor 3 (TFF3) and down-regulated contactin-1 could be identified as previously unknown biomarkers for HF. However, TFF3 is also a predictive factor for the occurrence of a cardiovascular event in HFpEF patients. In HFpEF, serine protease 27 was found at reduced levels for the first time, which could offer a new therapeutic target. Additionally, network analyses showed a special role of platelet-derived growth factor subunit A, Dickkopf-related protein 1, and tumour necrosis factor receptor superfamily member 6 in HFpEF patients, whereas perlecan and junctional adhesion molecule A stood out in the HFrEF group. Overall, signalling pathways of metabolic processes, cellular stress, and iron metabolism seemed to be important for HFrEF, whereas for HFpEF, oxygen stress, haemostasis, cell renewal, cell migration, and cell proliferation are in the foreground.

Conclusions The identified proteins and signalling pathways offer new therapeutic and diagnostic approaches for patients with chronic HF.

Keywords Heart failure with preserved ejection fraction; Heart failure with reduced ejection fraction; Plasma biomarkers; Proteomic; Healthy volunteers; Network analysis; Pathophysiology; Pathways; HFpEF; HFrEF

Received: 3 March 2022; Revised: 29 July 2022; Accepted: 15 September 2022
**Correspondence to: Rolf Wachter, DZHK (German Centre for Cardiovascular Research), Partner Site Göttingen, Göttingen, Germany.*
Email: wachter@med.uni-goettingen.de
Lutz Binder and Rolf Wachter have equal contribution.

Introduction

Heart failure (HF) affects 1–3% of the adult population worldwide.[1] An increase in prevalence is to be expected due to an increasing life span and associated increases in cardiovascular comorbidities and risk factors. HF can be classified according to the left ventricular ejection fraction (LVEF) in HF with preserved ejection fraction (HFpEF) with an LVEF

of ≥50%, HF with mildly reduced ejection fraction (HFmrEF) with an LVEF between 41% and 49%, or HF with reduced ejection fraction (HFrEF) with an LVEF ≤ 40%.[2] Nearly 50% of all HF patients present with HFpEF, which in turn is accompanied by a high hospitalization rate.[3,4]

HFpEF and HFrEF represent two distinct disease entities with different pathophysiologies.[5,6] Whereas HFrEF is characterized by systolic dysfunction and a strong involvement of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system,[7] HFpEF is typically associated with left ventricular (LV) hypertrophy, fibrosis, and a diastolic dysfunction.[8] This might arise from a number of comorbidities (age, obesity, hypertension, diabetes mellitus, chronic kidney disease, sleep apnoea, and atrial fibrillation), which generate a pro-inflammatory status that leads to microvascular endothelial dysfunction, cardiometabolic and structural abnormalities, and oxidative stress in the heart.[5,9–11] Still, the pathophysiology of HFpEF is not fully understood, in part because of the heterogeneity of the underlying causes. In addition, HFpEF affects more women than men.[12] Also, different therapy strategies are needed: Although established pharmacological agents and devices show an improvement in symptoms and prognosis in HFrEF, this is only the case for inhibition of the sodium glucose cotransporter II in HFpEF.[13–15] Likewise, no HFpEF-specific suitable biomarkers are known for monitoring diagnosis, course, and prognosis in this pathological condition.[16] HFpEF is thus a very heterogeneous, multifactorial disease with a complex pathophysiology that is determined in particular by its comorbidities. Proteomic studies were carried out in HFpEF patients in order to gain a more detailed insights into the pathophysiology and to develop possible biomarkers or therapeutic approaches, but depending on the method used, the results were often conflicting.[6,11,17–19]

The Prevalence and Clinical Course of Diastolic Dysfunction and Diastolic Heart Failure (DIAST-CHF) study is a multicentre, non-interventional, observational study with a follow-up of 10 years and detailed information about the clinical course, medical history, and echocardiographic data of the patients.[20] The aim of our study was to use biomarker profiling in HFpEF and HFrEF patients in comparison with a healthy control group to identify unique biomarkers and in turn potentially involved pathophysiological pathways that could possibly represent diagnostic approaches and may help to develop novel therapeutic ways. In a subgroup of the DIAST-CHF cohort, we measured a large set of biomarkers in plasma samples of HFpEF and HFrEF patients, as well as in healthy volunteers, and compared these using network, pathway, and comparative analyses.

Material and methods

Study group

The DIAST-CHF study is a non-interventional multicentre observational study and part of the national German Competence Network Heart Failure project,[21] in which over 1935 participants aged between 50 and 85 years were enrolled. Details on the recruitment have been published previously.[20] Outpatients were recruited in six centres from 2004 to 2006, with a follow-up from 2004 to 2016. The majority of patients were enrolled at the University Medical Center Göttingen (UMG) or the Charité-Berlin University of Medicine (Germany). The participants received a 10 year follow-up in which mortality and hospitalizations were recorded and categorized as cardiovascular or non-cardiovascular by two independent cardiologists. Inclusion criteria were the presence of at least one risk factor for chronic heart failure (CHF) (e.g. hypertension, diabetes mellitus, sleep apnoea syndrome, or atherosclerotic disease) or a manifest CHF, according to patient's medical history. Exclusion criteria were logistical reasons for non-participation or refusal to participate in the study. At the beginning of the trial, the subjects received a detailed physical examination, echocardiography, a detailed recording of the medical history and medication, ECG, and physical examination tests [6 min walk test and Short Form-36 (SF-36) score].[22,23] The diagnosis of CHF was determined on the basis of the patient's medical history or based on the Framingham diagnostic criteria.[24] The patients were retrospectively classified according to the echocardiographic results as HFrEF (LVEF ≤ 40%) or HFpEF (LVEF ≥ 50% and echocardiographic findings of LV diastolic dysfunction like left atrial volume index > 34 mL/m^2 or LV mass index ≥ 115 g/m^2 for men or ≥95 g/m^2 for women or E/e' ≥ 13 or mean e' septal and lateral wall < 9 cm/s).[25,26] Patients with HFmrEF (LVEF 41–49%) were excluded from this study. Additionally, a group of older healthy volunteers, also originally recruited in the DIAST-CHF study, was also included in this study as a control group and received the same examinations as mentioned above. Healthy was defined as absence of cardiac symptoms (e.g. dyspnoea on exertion or angina), absence of any cardiac disease (e.g. atrial fibrillation or coronary artery disease), and freedom from hypertension and diabetes.

Peripheral venous blood was drawn after resting for at least 15 min in supine position, centrifuged directly, and stored at −80°C for further analysis. Creatinine measurements were carried out at the UMG. For this study, a subgroup was randomly selected from the existing DIAST-CHF cohort with 89 HFpEF and 38 HFrEF patients. These were compared with a healthy older control group (n = 40) from the DIAST-CHF cohort for all further protein analyses. The DIAST-CHF trial and this retrospective subgroup study were conducted according to the World Medical Association Declaration of Helsinki and approved by the UMG Ethics Committee.

Plasma biomarker measurements

A selection of 184 biomarkers were measured in heparinate plasma of 167 patients belonging to the DIAST-CHF study

DOI: 10.1002/ehf2.14167

using the Olink technology (Olink, Uppsala, Sweden). The biomarkers were part of the cardiovascular II and III panel (CVDII and CVDIII panel), a preselection made by the Olink company of known proteins that have an association with cardiovascular diseases (Olink). The biomarkers were measured from 1 μL of heparinate plasma using a high-throughput multiplex immunoassay using proximity extension assay technology. Here, oligonucleotide-labelled antibodies bind in pairs to the target proteins. The resulting PCR target sequences are then amplified and detected using quantitative real-time PCR, using the Fluidigm BioMark HD System (Fluidigm, South San Francisco, CA, USA). The Ct values are converted into a log2 normalized value, the Normalized Protein eXpression (NPX, an Olink arbitrary unit) using company's own process. The NPX scale is inverted compared with the Ct value, which means that increased NPX values speak for a high protein expression but do not provide absolute protein concentrations. Details about the procedure are available online (www.olink.com). Only samples passing the Olink quality control and measurements exceeding the respective limits of detection were included in our analysis. A total of 180 proteins above the detection limit could be found in our samples, which were further analysed in the study. Four proteins with more than 60% missing values have been discarded from our analysis [SLAM family member 7 (SLAMF7), pappalysin-1 (PAPPA), melusin (ITGB1BP2), and poly [ADP-ribose] polymerase 1 (PARP-1)]. A complete list of all measured proteins (including all abbreviations) and their expression levels can be found in the Supporting Information, *Table S1*.

Statistics

Protein levels below the limit of detection were imputed using Regression on Order Statistics.[27] Only protein levels that were recorded as 'Passed' have been considered in the analysis. Distribution plots for all the proteins were generated and visually examined.

Clinical metadata was summarized per group by absolute and relative frequencies or mean ± SD and median (interquartile range) as appropriate. Protein abundance levels were modelled between the groups and compared using contrast tests. The variance estimation was moderated across proteins using limma.[28] *P*-values have been corrected for multiple testing by Benjamini–Hochberg to control the false discovery rate (FDR).

For the comparisons HFpEF vs. control as well as HFrEF vs. control, overrepresentation analyses for gene ontology (GO) terms were conducted.[29] The overlap of differential proteins in HFpEF vs. control and HFrEF vs. control was visualized in a Venn diagram.

In order to determine possible interactions between biomarkers in the two HF groups, pairwise protein–protein correlations were calculated using Kendall's τ. Only strong ($\tau > 0.6$) and significant and unique protein–protein correlations, which occurred exclusively in HFpEF or HFrEF, without overlapping between the two groups or with the control group, were considered. As significant correlations, only those with *P*-values below a cut-off adjusted for multiple comparisons were retained. Following Tromp *et al.*, the cut-off was determined as $0.05/([PC \times PC - 1]/2)$, where PC = 62 was the number of principal components cumulatively explaining >95% of the observed variance.[19] The retained group-specific correlations are shown in a network diagram.

Cardiovascular event was defined as cardiac death, hospitalization for cardiovascular reasons, acute myocardial infarction, new onset of HF, or stroke. Time to the first cardiovascular event was modelled with death for other reasons as competing risk. For all proteins, cause-specific hazards have been modelled via Cox regression. For both events (cardiovascular event and death of other reason), the resulting hazard ratios are presented with 95% confidence intervals and associated *P*-values. For the cardiovascular events, the *P*-values have been adjusted for multiple testing using Benjamini–Hochberg. This analysis was conducted on the full cohort as well as separately in the HFpEF and HFrEF subgroups.

Due to the screening character of this study, both raw and adjusted *P*-values are reported for the differential protein analyses. The significance level was set to $\alpha = 5\%$ for all statistical tests. All analyses were performed with the statistic software R (Version 3.6.1).

Results

Study group characteristics

Plasma samples of 167 patients from a subgroup of the DIAST-CHF study were used to measure 180 proteins (*Table 1*). A total of 53% of our cohort had HFpEF ($n = 89$) and 23% HFrEF ($n = 38$). The remaining 24% ($n = 40$) belonged to a healthy control group. The median age in the HFpEF group was 71 years (65; 77), in HFrEF 73 (66; 78), and in the control group 69 (67; 73). The mean age was comparable between the groups. The majority of HFpEF patients was female (70%), whereas males made up the majority in the HFrEF group (82% male). In the control group, the gender distribution was balanced (48% women). HFpEF patients by definition showed a higher LVEF (60% vs. 35%) and higher E/e' values (19 vs. 13), but also higher systolic blood pressure (156 mmHg vs. 134 mmHg) as compared with HFrEF patients. New York Heart Association (NYHA) functional class was not significantly different between groups. For details, please see *Table 1*.

Table 1 Baseline characteristics of study group

Parameter	Level	HFpEF	HFrEF	Control
n		89	38	40
Age (years)	Median (IQR)	71 (65; 77)	73 (66; 78)	69 (67; 73)
Sex	Female	62 (69.7%)	7 (18.4%)	19 (47.5%)
BMI (kg/m^2)	Mean ± SD	30 ± 5	29 ± 6	26 ± 4
Creatinine (mg/dL)	Mean ± SD	0.97 ± 0.23	1.2 ± 0.43	0.95 ± 0.17
	Missing	1	0	36
LVEF (%)	Mean ± SD	60 ± 6.6	34 ± 5.7	60 ± 5.6
Blood pressure systolic (mmHg)	Mean ± SD	156 ± 23	134 ± 21	135 ± 17
Blood pressure diastolic (mmHg)	Mean ± SD	81 ± 12	75 ± 10	74 ± 12
Heart rate (b.p.m.)	Mean ± SD	70 ± 12	71 ± 14	72 ± 12
Diabetes mellitus	Yes	27 (30.3%)	14 (36.8%)	0 (0.0%)
CAD	Yes	19 (21.3%)	21 (55.3%)	0 (0.0%)
Arterial hypertension	Yes	81 (91.0%)	32 (84.2%)	1 (2.5%)
Atrial fibrillation	Yes	8 (9.0%)	9 (23.7%)	0 (0.0%)
NYHA class	I	1	8	NA
	II	2	11	NA
	III	2	6	NA
	IV	0	1	NA
	Missing	84	12	40
E/e′	Mean ± SD	19 ± 4.4	13 ± 7.7	9.1 ± 3.4

BMI, body mass index; CAD, coronary artery disease; E/e′, ratio of mitral early diastolic flow velocity over tissue Doppler lateral mitral annular lengthening velocity; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; LVEF, left ventricular ejection fraction; NA, not applicable; NYHA, New York Heart Association.
Baseline characteristics of the study group (89 HFpEF, 38 HFrEF, and 40 healthy patients), a subgroup of the Prevalence and Clinical Course of Diastolic Dysfunction and Diastolic Heart Failure (DIAST-CHF) trial, are presented.

Biomarker profile in heart failure with preserved ejection fraction and with reduced ejection fraction vs. control

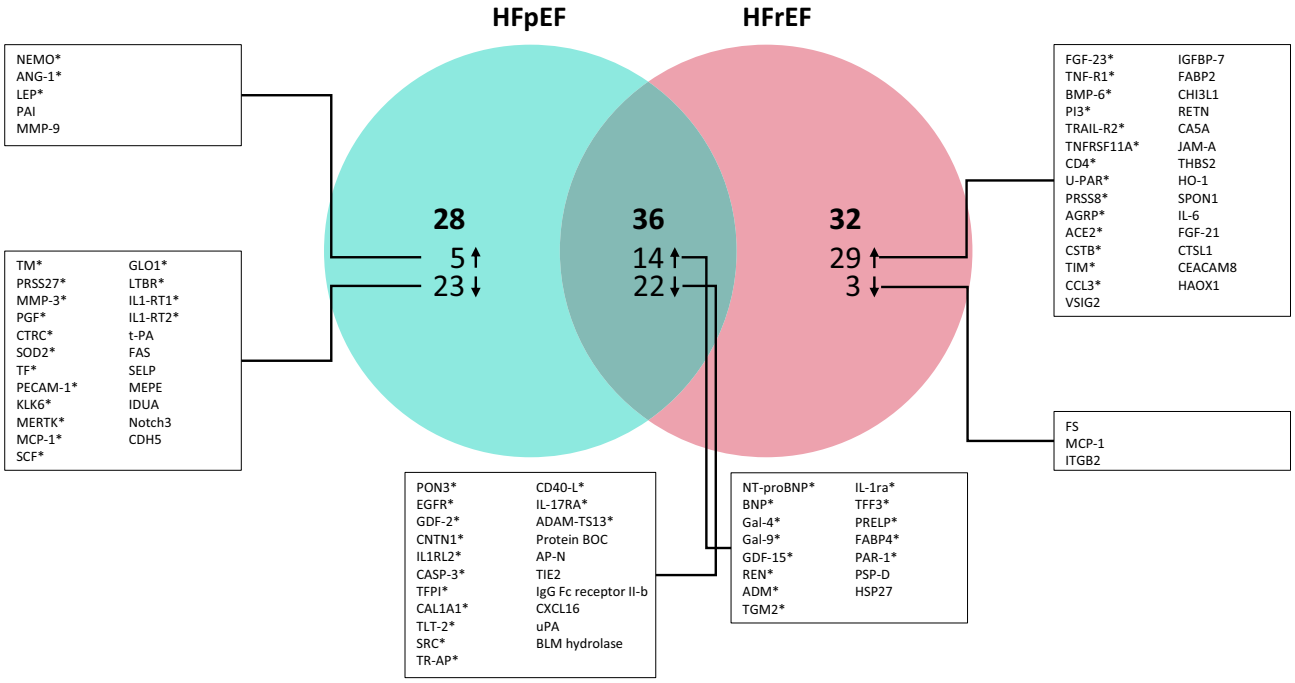
The biomarker profile was compared between HFpEF and HFrEF patients against the control group (*Figure 1*). In both HF groups combined, 36 proteins showed a significantly differential expression vs. the control group.

Of these, 14 proteins were significantly up-regulated compared with the control group and 22 were down-regulated. After adjusting for multiple testing, 13 proteins remained significant in the up-regulated group and 14 in the down-regulated group. The proteins with the highest expression difference vs. control were N-terminal prohormone brain natriuretic peptide (NT-proBNP), brain natriuretic peptide (BNP), galectin-4 (Gal-4), galectin-9 (Gal-9), and growth differentiation factor 15 (GDF-15). In contrast, the down-regulated proteins were paraoxonase (PON3), epidermal growth factor receptor (EGFR), growth differentiation factor 2 (GDF-2), contactin-1 (CNTN1), and interleukin-1 receptor-like 2 (IL1RL2).

In the next step, we focused on proteins exclusively expressed in both HF entities separately compared with the control group. The HFpEF patients had 28 unique proteins that showed different expression levels to the control group and the HFrEF patients had 32. In the HFpEF group, 5 of these were significantly overexpressed [NF-κB essential modulator (NEMO), angiopoietin-1 (ANG-1), leptin (LEP), plasminogen activator inhibitor 1 (PAI), and matrix metalloproteinase 9 (MMP-9)] and 23 were down-regulated. After adjustment, 3 proteins (NEMO, ANG-1, and LEP) remained significantly overexpressed and 16 showed lower expression. The most significantly less expressed were thrombomodulin (TM), serine protease 27 (PRSS27), matrix metalloproteinase 3 (MMP-3), and placenta growth factor (PGF). In contrast, 29 unique proteins showed higher expression in HFrEF compared with the control group and only 3 were down-regulated [follistatin (FS), monocyte chemotactic protein 1 (MCP-1), and integrin beta-2 (ITGB2)]. After adjustment, 14 proteins remained significantly overexpressed and none were significantly down-regulated. The most signifi-

DOI: 10.1002/ehf2.14167

Figure 1 Venn diagram of significantly altered protein expressions in heart failure with preserved ejection fraction (HFpEF) and with reduced ejection fraction (HFrEF) compared with the control group. A total of 180 biomarkers expression levels are measured in the HFpEF, HFrEF, and healthy control groups. Thirty-six proteins are significantly ($P < 0.05$) changed in both HFpEF and HFrEF. From these, 14 proteins are higher and 22 are lower expressed compared with control. Twenty-eight proteins are significantly chanced to the control group exclusively in HFpEF (5 with higher and 23 with lower expression). Thirty-two proteins are significantly chanced to the control group exclusively in HFrEF (29 with higher and 3 with lower expression). The proteins are sorted in descending order according to their level of significance. For a detailed list of protein expression levels and abbreviations, see Supporting Information, *Table S1*. *Significant after adjustment for multiple testing.



cantly up-regulated were fibroblast growth factor 23 (FGF-23), tumour necrosis factor receptor 1 (TNF-R1), bone morphogenetic protein 6 (BMP-6), elafin (PI3), and TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2).

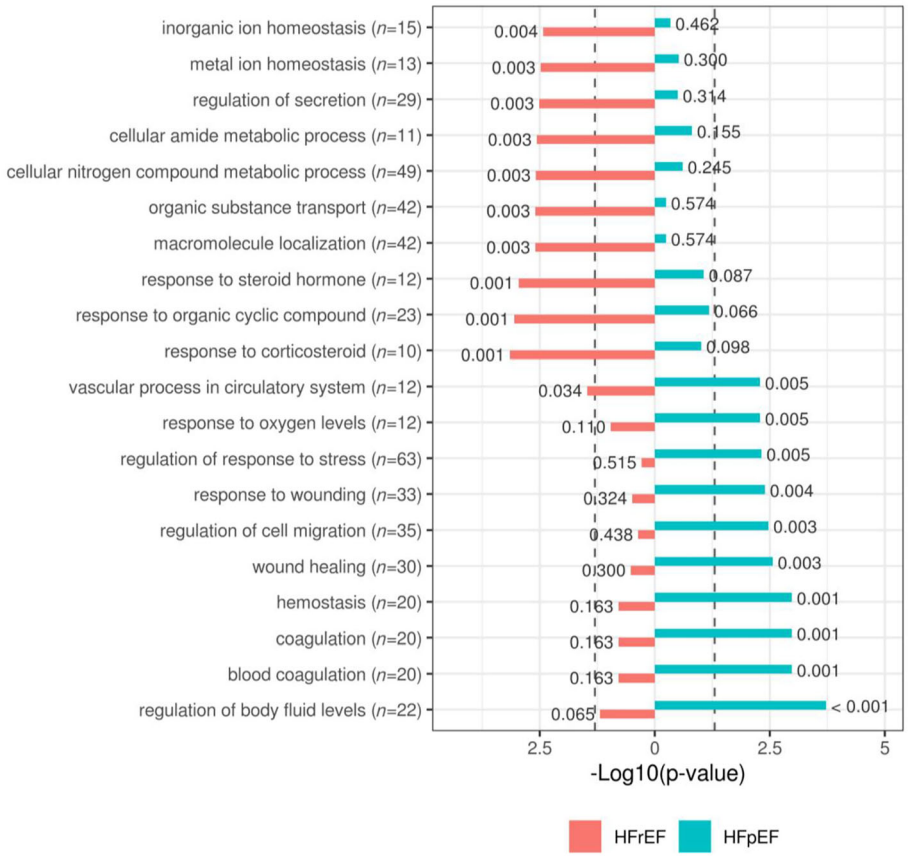
A GO term (biological processes) overrepresentation analysis of associated proteins showed in HFpEF these following terms as the top 10 significantly overrepresented (*Figure 2*): negative regulation of biological processes, regulation of cell death, regulation of cell migration, negative regulation of cellular processes, response to oxygen levels, haemostasis, and regulation of apoptotic processes and cell migration. The top 10 GO terms overrepresented in HFrEF were regulation of system processes, morphogenesis of branching structure, ion homeostasis, response to corticosteroid, cellular nitrogen compound metabolic processes, and cellular amide metabolic processes.

In conclusion, processes of cell renewal, cell death, and cell movement were represented in patients with HFpEF, whereas metabolic, growth-promoting, and other cell growth processes were signified in HFrEF, but also processes of cell stress and iron metabolism (*Figure 2*).

Protein–protein correlations

In order to determine possible interactions between biomarkers in the two HF groups, pairwise protein–protein correlations were conducted. Significant unique protein–protein correlations, which occurred exclusively in HFpEF or HFrEF, without overlapping between the two groups or with the control group, were shown in a network analysis (*Figure 3*). The significant protein–protein correlations, exclusive to the HFpEF group, show an important role for the following proteins: platelet-derived growth factor subunit A (PDGF-A) and Dickkopf-related protein 1 (Dkk-1), which are correlated in one network and tumour necrosis factor receptor superfamily member 6 (FAS), which forms another main hub. In the HFrEF group, PDGF-A seems to be correlated with C-X-C motif chemokine 1 (CXCL1). Perlecan (PLC), adrenomedullin (ADM), and TRAIL-R2 were identified to be key proteins that form a close correlation. Another main hub is the junctional adhesion molecule A (JAM-A), together with tumour necrosis factor receptor superfamily member 14 (TNFRSF14) and trefoil factor 3 (TFF3). There is a significant connection between

Figure 2 Gene ontology (GO) term overrepresentation in heart failure with preserved ejection fraction (HFpEF) and with reduced ejection fraction (HFrEF) compared with control. The top 10 overrepresented GO terms (biological processes) in either HFrEF (red) vs. control or HFpEF (blue) vs. control. X-axis shows −log10 of the *P*-value, the text next to the bars gives the *P*-value, and the dashed lines indicate a *P*-value of 0.05.



the networks via a JAM-A and PLC protein–protein correlation.

Predictive biomarkers in heart failure with preserved ejection fraction and with reduced ejection fraction for a cardiovascular event

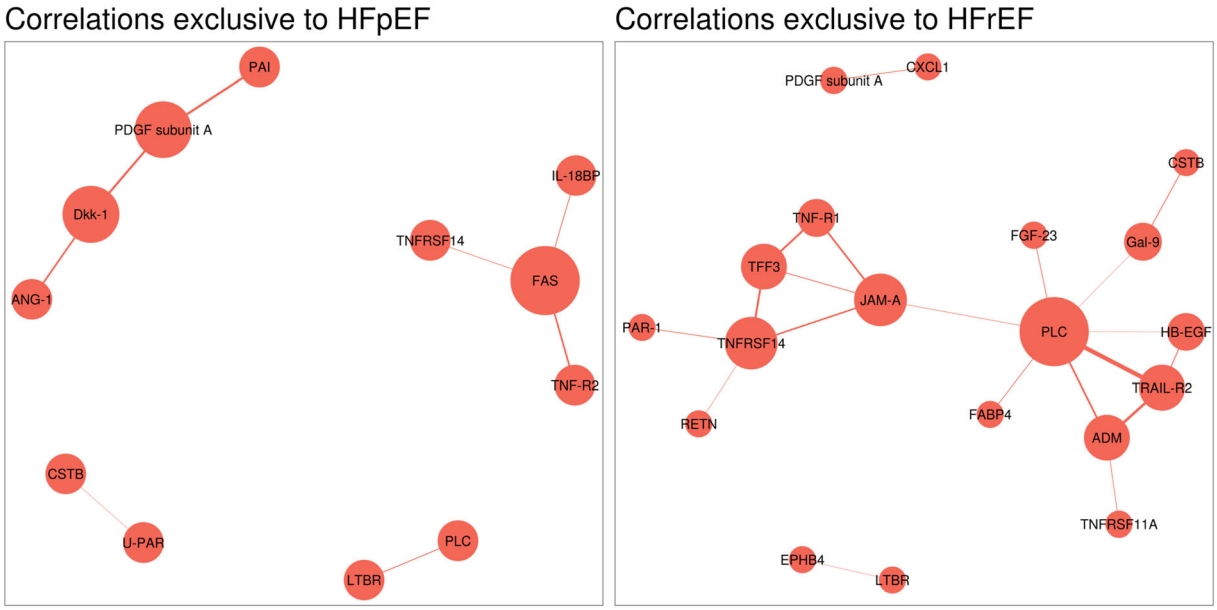
Using the 10 year follow-up data, the measured biomarkers could also be analysed with regard to their predictive ability for a cardiovascular event. Considered in all groups, 19 biomarkers could be identified that were significantly associated with the occurrence of a cardiovascular event in the course of the study. After *P*-value adjustment, only Gal-4 was significant. However, we then analysed the biomarkers in the respective subgroups. In HFpEF, 17 biomarkers were associated with the occurrence of a cardiovascular event. After *P*-value adjustment, five biomarkers remained significant: TFF3, Gal-4, proprotein convertase subtilisin/kexin type 9 (PCSK9), urokinase-type plasminogen activator (uPA), and superoxide mitochondrial dismutase (SOD2). In contrast, in HFrEF, 18 biomarkers were predictive of a cardiovascular event, but after statistical adjustment, no significant protein remained.

Discussion

We were able to identify 35 proteins that were expressed significantly different in both HF groups compared with the control group. We were also able to determine 29 unique proteins expressed in HFpEF and 33 unique proteins in HFrEF. Network analyses showed a special role of PDGF-A, Dkk-1, and FAS in the HFpEF, whereas PLC and JAM-A stood out in the HFrEF. Overall, signalling pathways of metabolic processes, cellular stress, and iron metabolism seemed to be important for HFrEF, whereas for HFpEF, oxygen stress, haemostasis, cell renewal, cell migration, and cell proliferation are in the foreground. Predictive biomarkers for a cardiovascular event for HFpEF could also be identified.

We found similarly altered proteins in both HF groups compared with the control group. They seem to be relevant in the pathology of both HF entities and could generally be used for

Figure 3 Network analysis of exclusive protein–protein correlations in heart failure with preserved ejection fraction (HFpEF) and with reduced ejection fraction (HFrEF). Protein–protein correlations in the HFpEF, HFrEF, and control groups are analysed in a network analysis. Significant correlations exclusive in HFpEF and HFrEF are shown. The size of the node represents the importance of the node in the network. The thickness of the lines between the nodes represents the correlation coefficient.



the diagnosis or prognosis of CHF. Some of these biomarkers that we found higher expressed in both HF groups are already known and described (like BNP, NT-proBNP, galectins, or GDF-15).[20,30–33] However, we were also able to identify possible new biomarkers: TFF3 was identified as significantly increased in HF. So far, only one other proteomic-based study has identified TFF3 as a possible biomarker for HF and associated it with the outcome.[34] We also confirm that TFF3 is predictive of outcome, although interestingly in our study, this was only true for the HFpEF patients, emphasizing the importance of TFF3 as a new biomarker. TFF3 is a peptide that is responsible for cell repair and is released through tissue damage, such as in gastrointestinal tissue and skeletal muscles.[35] Serum TFF3 is already being discussed as a biomarker in other diseases, such as colorectal cancer.[36]

Remarkably, it was also possible to identify probable new cardioprotective proteins that were present in HF in lower expression rates than in the control group. For example, PON3 overexpressed mice were protected against cardiac hypertrophy, and therefore, PON3 could represent a therapeutic target.[37] Furthermore, increased PON3 concentrations in the total cohort and in the HFpEF patients were associated (only without statistical adjustment) with the occurrence of a cardiovascular event in our study. Likewise, GDF-2 is also known as bone morphogenetic protein 9 (BMP9). It is known that GDF-2 reduces cardiac fibrosis and can improve cardiac function in HF and is being discussed as a therapeutic agent.[38] CNTN1 could be identified as a new biomarker in reduced expression levels in HF, and as far as we know, no studies regarding CNTN1 and cardiovascular diseases have been carried out yet.

In addition, we identify biomarkers that were solitary significantly changed in either HFpEF or HFrEF compared with the control group and that could offer therapeutic or diagnostic aims in particular for HFpEF. In HFpEF, only five proteins were increased; those are linked to inflammation (NEMO and LEP), regeneration, and fibrosis (PAI, MMP-9, and ANG-1). NEMO is involved in the NF-κB signalling pathway, and because inflammation plays an important role in the pathogenesis of HFpEF,[39] this could be a new possible therapeutic approach for inhibition.[40] However, MMP-9, PAI, and ANG-1 are more involved in endothelial dysfunction and fibrotic remodelling processes,[41–43] and because these are also decisive in the pathology of HFpEF,[5,44] they could be possible biomarkers for diagnosis and severity assessment.

In contrast, 23 of our investigated proteins were down-regulated in the HFpEF patients. For example, TM was clearly down-regulated, although it has already been shown that TM, as a marker for endothelial damage and dysfunction, is rather elevated in HF.[45] PRSS27 was also down-regulated, whereof little is known and its significance in HFpEF is completely unclear. This could be a possible new target for further research.

Interestingly, proteins could also be found that were predictive for the occurrence of a cardiovascular event in HFpEF patients and speak for a poorer outcome: TFF3, Gal-4, PCSK9,

and uPA. Although the predictive power of Gal-4[31] and uPA[46] is already known and that increased PCSK9 concentrations driving HFpEF formation,[47] to our knowledge, little has been published on the biomarkers only in the context of HFpEF. It can be said that for uPA, Gal-4, PCSK9, and TFF3 in particular, a direct function as a predictive biomarker for a poorer outcome and the occurrence of a cardiovascular event, especially in HFpEF patients, could probably be shown for the first time.

Unique protein expressions were also found in HFrEF, with 29 showing higher levels and only 3 lower levels compared with the control. For example, ITGB2 were down-regulated. ITGB2 appears to be involved in the pathogenesis of cardiac hypertrophy[48] and in chronic inflammatory processes and endothelial dysfunction.[49] Interestingly, Tromp *et al*. identified ITGB2 as a specific marker for the HFpEF,[19] but we only see it down-regulated in the HFrEF. In contrast to Tromp *et al*., however, we did not identify ITGB2 through a knowledge-based network analysis but rather evaluated it directly from our data.

In contrast, FGF-23 and PI3 were expressed significantly higher in the HFrEF group than in the control. FGF-23 is known as a biomarker of HFpEF: Roy *et al*. found FGF-23 strongly expressed in HFpEF patients, correlated with fibrosis on magnetic resonance imaging (MRI) diagnosis, and as a predictor of poor outcome.[50] In our case, FGF-23 was only overexpressed in the HFrEF, which reduces the diagnostic value of FGF-23 for HFpEF. PI3, in turn, is a protease inhibitor with anti-inflammatory potential, which has already been researched as a therapeutic agent for various inflammatory diseases and shown to protect against ischaemia–reperfusion injury during a coronary artery bypass graft.[51,52] Until now, there were no data to PI3 in HF.

Next, we looked at the protein–protein correlations within the groups by performing a network analysis and identified specific biomarker interactions exclusively for HFpEF or HFrEF compared with the control group with different proteins acting as main hubs in the network. At the HFrEF, these were especially PLC, JAM-A, and TRAIL-R2, whereas at the HFpEF, these were PDGF-A, Dkk-1, and FAS. FAS is an expression of a strongly inflammatory process, aggravates HF, and has already been discussed as a therapeutic objective,[53,54] which fits with the pathophysiology of HFpEF. Dkk-1 is an important regulator in the Wnt signalling pathway and is involved in bone remodelling processes,[55] rheumatic diseases, and cancer.[56] It could be shown that Dkk-1 correlates with arterial stiffness,[57] which in turn is an important factor in the pathophysiology of HFpEF.[58] Otherwise, Dkk-1 could be a new target for further research on biomarkers or therapeutic intervention, like an inhibition by monoclonal antibodies. Rashid *et al*. were able to show that PDGF-A overexpression in rats after transient coronary artery occlusion showed better wound healing and improved LV function.[59] As a strong growth factor, PDGF-A is an important promoter of cardiac fibrosis and hypertrophy[60,61] and has therefore been researched in a therapeutic context.[62] Interestingly, in one study, PDGF-A was found to be significantly expressed in patients with HFpEF and atrial fibrillation, in contrast to HFpEF with sinus rhythm.[11]

In addition, we wanted to use the protein expression levels found to make statements about possible signal pathways involved in the two HF entities, especially in HFpEF. Searching for overrepresentation in GO terms for biological processes among the differentially expressed proteins showed that processes of cell renewal, cell death, and cell movement were affected in patients with HFpEF, whereas metabolic and growth-promoting processes of cell stress and iron metabolism dominated in HFrEF.

Tromp *et al*. examined 92 biomarkers in a similar cohort with 809 HFpEF and HFrEF patients and saw mainly processes of cell adhesion, leukocyte migration, inflammation, and extracellular matrix organization in HFpEF and DNA regulation, smooth muscle proliferation, and nitric oxide biosynthesis regulated in HFrEF.[19] Which is largely comparable with our data, whereby we compared the groups against a healthy control group, which means that the signalling pathways found are clearly due to the disease and possibly comorbidities. In another study, HFpEF patients were measured for 248 biomarkers in the plasma, and primarily, inflammation markers were found to be relevantly changed, with the hypothesis that they could act as a mediator between the comorbidities and echocardiographic indicators of right ventricular dysfunction.[18] Furthermore, Sanders-van Wijk *et al*. show signal pathways of leukocyte degranulation (e.g. via GDF-15 and PLC), adherens junction organization, and platelet activation (e.g. via PDGF-A) as relevant in the HFpEF. Our data, both the biomarkers presented above and the signalling pathway analyses, support the hypothesis of the involvement of matrix remodelling, inflammation, fibrosis, apoptosis, and cell turnover as key drivers in HFpEF.[63–65]

Some limitations of our study should be mentioned: We only performed a subgroup analysis of the DIAST-CHF study. Although the patient groups are representative of the diseases and the control group has no comorbidities, the group sizes are relatively small. The limited sample size could therefore reduce the validity of the proteomic analysis. In the following studies, the results should be evaluated in larger groups. Especially because more HFpEF patients were examined than HFrEF patients, this could cause a bias in the jointly changed biomarkers. Besides, only HFpEF and HFrEF patients were examined in this study, HFmrEF patients were excluded. HFmrEF is also a vulnerable group that should receive more attention in further studies. Furthermore, there is an imbalance between the sexes, as we have significantly more women in the HFpEF group than in the HFrEF group. This, in turn, is characteristic of HFpEF, as it generally occurs more in the female sex.[12] It should also be noted that some biomarkers could present a gender-specificity regulation, which

was not taken into account here but was recently shown in one study.[66] Another limitation is the assortment of the examined biomarkers, because commercial panels were used here, which only represent a selection of possible pathophysiological pathways.

Overall, in our study, we were able to identify distinct as well as overlapping protein expression patterns and signalling pathways in HFpEF and HFrEF compared with a healthy control group. This may offer the potential for new diagnostic and therapeutic approaches for patients with CHF. Our data thus provide a basis for further investigations on specific biomarkers for HFpEF and HFrEF.

Acknowledgements

Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest

The authors declare no conflict of interest.

Funding

DIAST-CHF is funded by the Bundesministerium für Bildung und Forschung within the Competence Network Heart Failure.

Supporting information

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

Table S1. Full list of all measured plasma biomarkers and expression levels of the HFrEF, HFpEF and control group. All plasma biomarkers full names, abbreviations and expression levels (NPX values) are given for HFpEF, HFrEF and control group. Also *p*-values for statistical testing for HFpEF vs. HFrEF, HFpEF vs. control and HFrEF vs. control are given.

Table S2. All plasma biomarkers significantly associated with the occurrence of a cardiovascular event in all groups. Protein name, hazard ratio, confidence interval, *p*-value and adjusted *p*-value are given. A total of 19 biomarkers is significant as a potential risk factor for the occurrence of a cardiovascular event; after *p*-value adjustment, only one biomarker is significant.

Table S3. All plasma biomarkers significantly associated with the occurrence of a cardiovascular event in HFpEF. Protein name, hazard ratio, confidence interval, *p*-value and adjusted *p*-value are given. A total of 17 biomarkers is significant as a potential risk factor for the occurrence of a cardiovascular event; after *p*-value adjustment, only five biomarkers are significant.

Table S4. All plasma biomarkers significantly associated with the occurrence of a cardiovascular event in HFrEF. Protein name, hazard ratio, confidence interval, *p*-value and adjusted *p*-value are given. A total of 18 biomarkers is significant as a potential risk factor for the occurrence of a cardiovascular event; after *p*-value adjustment, no biomarker is significant.

References

1. Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol*. 2017; **14**: 591–602.
2. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, JGF C, AJS C, Crespo-Leiro MG, Farmakis D, Gilard M, Heymans S, Hoes AW, Jaarsma T, Jankowska EA, Lainscak M, CSP L, Lyon AR, McMurray JJV, Mebazaa A, Mindham R, Muneretto C, Francesco Piepoli M, Price S, Rosano GMC, Ruschitzka F, Kathrine Skibelund A, ESC Scientific Document Group. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021; **2021**: 3599–3726.
3. Ather S, Chan W, Bozkurt B, Aguilar D, Ramasubbu K, Zachariah AA, Wehrens XHT, Deswal A. 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.
4. 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.
5. 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.
6. Tromp J, Khan MAF, Klip IT, Meyer S, de Boer RA, Jaarsma T, Hillege H, van Veldhuisen DJ, van der Meer P, Voors AA. Biomarker profiles in heart failure patients with preserved and reduced ejection fraction. *J Am Heart Assoc*. 2017; **6**: e003989.
7. Schwinger RHG. Pathophysiology of heart failure. *Cardiovasc Diagn Ther*. 2021; **11**: 263–276.
8. Gevaert AB, Boen JRA, Segers VF, Van Craenenbroeck EM. Heart failure with preserved ejection fraction: a review of cardiac and noncardiac pathophysiology. *Front Physiol*. 2019; **10**: 638.
9. Bayes-Genis A, Bisbal F, Núñez J, Santas E, Lupón J, Rossignol P, Paulus W. Transitioning from preclinical to clinical heart failure with preserved ejection fraction: a mechanistic approach. *J Clin Med*. 2020; **9**: E1110.
10. Mentz RJ, Kelly JP, von Lueder TG, Voors AA, Lam CSP, Cowie MR, Kjeldsen

K, Jankowska EA, Atar D, Butler J, Fiuzat M, Zannad F, Pitt B, O'Connor CM. Noncardiac comorbidities in heart failure with reduced versus preserved ejection fraction. *J Am Coll Cardiol*. 2014; **64**: 2281–2293.
11. Santema BT, Kloosterman M, Van Gelder IC, Mordi I, Lang CC, Lam CSP, Anker SD, Cleland JG, Dickstein K, Filippatos G, Van der Harst P, Hillege HL, Ter Maaten JM, Metra M, Ng LL, Ponikowski P, Samani NJ, Van Veldhuisen DJ, Zwinderman AH, Zannad F, Damman K, Van der Meer P, Rienstra M, Voors AA. Comparing biomarker profiles of patients with heart failure: atrial fibrillation vs. sinus rhythm and reduced vs. preserved ejection fraction. *Eur Heart J*. 2018; **39**: 3867–3875.
12. Lam CSP, Donal E, Kraigher-Krainer E, Vasan RS. Epidemiology and clinical course of heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2011; **13**: 18–28.
13. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, Brunner-La Rocca H-P, Choi D-J, Chopra V, Chuquiure-Valenzuela E, Giannetti N, Gomez-Mesa JE, Janssens S, Januzzi JL, Gonzalez-Juanatey JR, Merkely B, Nicholls SJ, Perrone SV, Piña IL, Ponikowski P, Senni M, Sim D, Spinar J, Squire I, Taddei S, Tsutsui H, Verma S, Vinereanu D, Zhang J, Carson P, Lam CSP, Marx N, Zeller C, Sattar N, Jamal W, Schnaidt S, Schnee JM, Brueckmann M, Pocock SJ, Zannad F, Packer M, EMPEROR-Preserved Trial Investigators. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med*. 2021; **385**: 1451–1461.
14. Senni M, Paulus WJ, Gavazzi A, Fraser AG, Díez J, Solomon SD, Smiseth OA, Guazzi M, Lam CSP, Maggioni AP, Tschöpe C, Metra M, Hummel SL, Edelmann F, Ambrosio G, Stewart Coats AJ, Filippatos GS, Gheorghiade M, Anker SD, Levy D, Pfeffer MA, Stough WG, Pieske BM. New strategies for heart failure with preserved ejection fraction: the importance of targeted therapies for heart failure phenotypes. *Eur Heart J*. 2014; **35**: 2797–2815.
15. Wintrich J, Kindermann I, Ukena C, Selejan S, Werner C, Maack C, Laufs U, Tschöpe C, Anker SD, Lam CSP, Voors AA, Böhm M. Therapeutic approaches in heart failure with preserved ejection fraction: past, present, and future. *Clin Res Cardiol*. 2020; **109**: 1079–1098.
16. Carnes J, Gordon G. Biomarkers in heart failure with preserved ejection fraction: an update on progress and future challenges. *Heart Lung Circ*. 2020; **29**: 62–68.
17. Michelhaugh SA, Januzzi JL. Finding a needle in a haystack: proteomics in heart failure. *JACC Basic Transl Sci*. 2020; **5**: 1043–1053.
18. Sanders-van Wijk S, Tromp J, Beussink-Nelson L, Hage C, Svedlund S, Saraste A, Swat SA, Sanchez C, Njoroge J, Tan R-S, Fermer ML, Gan L-M, Lund LH, Lam CSP, Shah SJ. Proteomic evaluation of the comorbidity-inflammation paradigm in heart failure with preserved ejection fraction: results from the PROMIS-HFpEF study. *Circulation*. 2020; **142**: 2029–2044.
19. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P, Voors AA. Identifying pathophysiological mechanisms in heart failure with reduced versus preserved ejection fraction. *J Am Coll Cardiol*. 2018; **72**: 1081–1090.
20. Stahrenberg R, Edelmann F, Mende M, Kockskämper A, Düngen H-D, Lüers C, Binder L, Herrmann-Lingen C, Gelbrich G, Hasenfuss G, Pieske B, Wachter R. The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur J Heart Fail*. 2010; **12**: 1309–1316.
21. Mehrhof F, Löffler M, Gelbrich G, Ozcelik C, Posch M, Hense H-W, Keil U, Scheffold T, Schunkert H, Angermann C, Ertl G, Jahns R, Pieske B, Wachter R, Edelmann F, Wollert KC, Maisch B, Pankuweit S, Erbel R, Neumann T, Herzog W, Katus H, Müller-Tasch T, Zugck C, Düngen H-D, Regitz-Zagrosek V, Lehmkuhl E, Störk S, Siebert U, Wasem J, Neumann A, Göhler A, Anker SD, Köhler F, Möckel M, Osterziel K-J, Dietz R, Rauchhaus M. A network against failing hearts—introducing the German "competence network heart failure". *Int J Cardiol*. 2010; **145**: 135–138.
22. Rostagno C, Gensini GF. Six minute walk test: a simple and useful test to evaluate functional capacity in patients with heart failure. *Intern Emerg Med*. 2008; **3**: 205–212.
23. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care*. 1992; **30**: 473–483.
24. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med*. 1971; **285**: 1441–1446.
25. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbély A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. *Eur Heart J*. 2007; **28**: 2539–2550.
26. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola V-P, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P, ESC Scientific Document Group. 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; **2016**: 2129–2200.
27. Lee L, Helsel D. Statistical analysis of water-quality data containing multiple detection limits: S-language software for regression on order statistics. *Comput Geosci*. 2005; **31**: 1241–1248.
28. Phipson B, Lee S, Majewski IJ, Alexander WS, Smyth GK. Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Ann Appl Stat*. 2016; **10**: 946–963.
29. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS: A J Integr Biol*. 2012; **16**: 284–287.
30. Fonarow GC, Peacock WF, Phillips CO, Givertz MM, Lopatin M, ADHERE Scientific Advisory Committee and Investigators. Admission B-type natriuretic peptide levels and in-hospital mortality in acute decompensated heart failure. *J Am Coll Cardiol*. 2007; **49**: 1943–1950.
31. Rullman E, Melin M, Mandić M, Gonon A, Fernandez-Gonzalo R, Gustafsson T. Circulatory factors associated with function and prognosis in patients with severe heart failure. *Clin Res Cardiol*. 2020; **109**: 655–672.
32. Trippel TD, Mende M, Düngen H-D, Hashemi D, Petutschnigg J, Nolte K, Herrmann-Lingen C, Binder L, Hasenfuss G, Pieske B, Wachter R, Edelmann F. The diagnostic and prognostic value of galectin-3 in patients at risk for heart failure with preserved ejection fraction: results from the DIAST-CHF study. *ESC Heart Fail*. 2021; **8**: 829–841.
33. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masoudi FA, McBride PE, Peterson PN, Stevenson LW, Westlake C. 2017 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation*. 2017; **136**: e137–e161.
34. Brankovic M, Martijn Akkerhuis K, Mouthaan H, Constantinescu A, Caliskan K, van Ramshorst J, Germans T, Umans V, Kardys I. Utility of temporal

profiles of new cardio-renal and pulmonary candidate biomarkers in chronic heart failure. *Int J Cardiol*. 2019; **276**: 157–165.
35. Krüger K, Schmid S, Paulsen F, Ignatius A, Klinger P, Hotfiel T, Swoboda B, Gelse K. Trefoil factor 3 (TFF3) is involved in cell migration for skeletal repair. *Int J Mol Sci*. 2019; **20**: E4277.
36. Li Q, Wang K, Su C, Fang J. Serum trefoil factor 3 as a protein biomarker for the diagnosis of colorectal cancer. *Technol Cancer Res Treat*. 2017; **16**: 440–445.
37. Pei J-F, Yan Y-F, Tang X, Zhang Y, Cui S-S, Zhang Z-Q, Chen H-Z, Liu D-P. Human paraoxonase gene cluster overexpression alleviates angiotensin II-induced cardiac hypertrophy in mice. *Sci China Life Sci*. 2016; **59**: 1115–1122.
38. Morine KJ, Qiao X, York S, Natov PS, Paruchuri V, Zhang Y, Aronovitz MJ, Karas RH, Kapur NK. Bone morphogenetic protein 9 reduces cardiac fibrosis and improves cardiac function in heart failure. *Circulation*. 2018; **138**: 513–526.
39. Miranda-Silva D, Lima T, Rodrigues P, Leite-Moreira A, Falcão-Pires I. Mechanisms underlying the pathophysiology of heart failure with preserved ejection fraction: the tip of the iceberg. *Heart Fail Rev*. 2021; **26**: 453–478.
40. Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF-κB in the heart: to be or not to NF-κB. *Circ Res*. 2011; **108**: 1122–1132.
41. Chen S, Guo L, Chen B, Sun L, Cui M. Association of serum angiopoietin-1, angiopoietin-2 and angiopoietin-2 to angiopoietin-1 ratio with heart failure in patients with acute myocardial infarction. *Exp Ther Med*. 2013; **5**: 937–941.
42. Flevaris P, Vaughan D. The role of plasminogen activator inhibitor type-1 in fibrosis. *Semin Thromb Hemost*. 2017; **43**: 169–177.
43. Morishita T, Uzui H, Mitsuke Y, Amaya N, Kaseno K, Ishida K, Fukuoka Y, Ikeda H, Tama N, Yamazaki T, Lee J-D, Tada H. Association between matrix metalloproteinase-9 and worsening heart failure events in patients with chronic heart failure. *ESC Heart Fail*. 2017; **4**: 321–330.
44. Simmonds SJ, Cuijpers I, Heymans S, Jones EAV. Cellular and molecular differences between HFpEF and HFrEF: a step ahead in an improved pathological understanding. *Cell*. 2020; **9**: E242.
45. Chong AY, Freestone B, Patel J, Lim HS, Hughes E, Blann AD, Lip GYH. Endothelial activation, dysfunction, and damage in congestive heart failure and the relation to brain natriuretic peptide and outcomes. *Am J Cardiol*. 2006; **97**: 671–675.
46. van den Berg VJ, Bouwens E, Umans VAWM, de Maat M, Manintveld OC, Caliskan K, Constantinescu AA, Mouthaan H, Cornel J-H, Baart S, Akkerhuis KM, Boersma E, Kardys I. Longitudinally measured fibrinolysis factors are strong predictors of clinical outcome in patients with chronic heart failure: the Bio-SHiFT study. *Thromb Haemost*. 2019; **119**: 1947–1955.
47. Da Dalt L, Castiglioni L, Baragetti A, Audano M, Svecla M, Bonacina F, Pedretti S, Uboldi P, Benzoni P, Giannetti F, Barbuti A, Pellegatta F, Indino S, Donetti E, Sironi L, Mitro N, Catapano AL, Norata GD. PCSK9 deficiency rewires heart metabolism and drives heart failure with preserved ejection fraction. *Eur Heart J*. 2021; **42**: 3078–3090.
48. Liu L, Zhao Q, Kong M, Mao L, Yang Y, Xu Y. Myocardin-related transcription factor A (MRTF-A) regulates integrin beta 2 transcription to promote macrophage infiltration and cardiac hypertrophy in mice. *Cardiovasc Res*. 2021; **118**: cvab110.
49. Ducat A, Doridot L, Calicchio R, Méhats C, Vilotte J-L, Castille J, Barbaux S, Couderc B, Jacques S, Letourneur F, Buffat C, Le Grand F, Laissue P, Miralles F, Vaiman D. Endothelial cell dysfunction and cardiac hypertrophy in the STOX1 model of preeclampsia. *Sci Rep*. 2016; **6**: 19196.
50. Roy C, Lejeune S, Slimani A, de Meester C, Ahn As SA, Rousseau MF, Mihaela A, Ginion A, Ferracin B, Pasquet A, Vancraeynest D, Beauloye C, Vanoverschelde J-L, Horman S, Gruson D, Gerber BL, Pouleur A-C. Fibroblast growth factor 23: a biomarker of fibrosis and prognosis in heart failure with preserved ejection fraction. *ESC Heart Fail*. 2020; **7**: 2494–2507.
51. Alam SR, Lewis SC, Zamvar V, Pessotto R, Dweck MR, Krishan A, Goodman K, Oatey K, Harkess R, Milne L, Thomas S, Mills NM, Moore C, Semple S, Wiedow O, Stirrat C, Mirsadraee S, Newby DE, Henriksen PA. Perioperative elafin for ischaemia-reperfusion injury during coronary artery bypass graft surgery: a randomised-controlled trial. *Heart*. 2015; **101**: 1639–1645.
52. Shaw L, Wiedow O. Therapeutic potential of human elafin. *Biochem Soc Trans*. 2011; **39**: 1450–1454.
53. Chen QM, Tu VC. Apoptosis and heart failure: mechanisms and therapeutic implications. *Am J Cardiovasc Drugs*. 2002; **2**: 43–57.
54. Huby A-C, Turdi S, James J, Towbin JA, Purevjav E. FasL expression in cardiomyocytes activates the ERK1/2 pathway, leading to dilated cardiomyopathy and advanced heart failure. *Clin Sci (Lond)*. 2016; **130**: 289–299.
55. Maroni P, Bendinelli P. Bone, a secondary growth site of breast and prostate carcinomas: role of osteocytes. *Cancers (Basel)*. 2020; **12**: E1812.
56. Huang Y, Liu L, Liu A. Dickkopf-1: current knowledge and related diseases. *Life Sci*. 2018; **209**: 249–254.
57. Fehérvári L, Frigy A, Kocsis L, Szabó IA, Szabo TM, Urkon M, Jakó Z, Nagy EE. Serum osteoprotegerin and carotid intima-media thickness are related to high arterial stiffness in heart failure with reduced ejection fraction. *Diagnostics (Basel)*. 2021; **11**: 764.
58. Reddy YNV, Andersen MJ, Obokata M, Koepp KE, Kane GC, Melenovsky V, Olson TP, Borlaug BA. Arterial stiffening with exercise in patients with heart failure and preserved ejection fraction. *J Am Coll Cardiol*. 2017; **70**: 136–148.
59. Rashid FN, Clayton ZE, Ogawa M, Perdomo J, Hume RD, Kizana E, Chong JJH. Platelet derived growth factor-A (Pdgf-a) gene transfer modulates scar composition and improves left ventricular function after myocardial infarction. *Int J Cardiol*. 2021; **S0167-5273**: 01166–01169.
60. Gallini R, Lindblom P, Bondjers C, Betsholtz C, Andrae J. PDGF-A and PDGF-B induces cardiac fibrosis in transgenic mice. *Exp Cell Res*. 2016; **349**: 282–290.
61. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. *Cell Mol Life Sci*. 2014; **71**: 549–574.
62. Medamana J, Clark RA, Butler J. Platelet-derived growth factor in heart failure. *Handb Exp Pharmacol*. 2017; **243**: 355–369.
63. Hage C, Michaëlsson E, Linde C, Donal E, Daubert J-C, Gan L-M, Lund LH. Inflammatory biomarkers predict heart failure severity and prognosis in patients with heart failure with preserved ejection fraction: a holistic proteomic approach. *Circ Cardiovasc Genet*. 2017; **10**: e001633.
64. Lok DJ, Lok SI, Bruggink-André de la Porte PW, Badings E, Lipsic E, van Wijngaarden J, de Boer RA, van Veldhuisen DJ, van der Meer P. Galectin-3 is an independent marker for ventricular remodeling and mortality in patients with chronic heart failure. *Clin Res Cardiol*. 2013; **102**: 103–110.
65. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JPM, Schroen B, André S, Crijns HJGM, Gabius H-J, Maessen J, Pinto YM. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004; **110**: 3121–3128.
66. Raafs A, Verdonschot J, Ferreira JP, Wang P, Collier T, Henkens M, Björkman J, Boccanelli A, Clark AL, Delles C, Diez J, González A, Girerd N, Jukema JW, Pinet F, Rossignol P, Thum T, Vodovar N, de Boer RA, van Empel V, Staessen JA, Hazebroek M, Cleland J, Zannad F, Heymans S. Identification of sex-specific biomarkers predicting new-onset heart failure. *ESC Heart Fail*. 2021; **8**: 3512–3520.