Sex-differences in circulating proteins in heart failure with preserved ejection fraction

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Abstract

Background

Many patients with heart failure with preserved ejection fraction (HFpEF) are women. Exploring mechanisms underlying the sex-differences may improve our understanding of the pathophysiology of HFpEF. Studies focusing on sex-differences in circulating proteins in HFpEF patients are scarce.

Methods

A total of 415 proteins were analyzed in 392 HFpEF patients included in The Metabolic Road to Diastolic Heart Failure: Diastolic Heart Failure study (MEDIA-DHF). Sex-differences in these proteins were assessed using adjusted logistic regression analyses. The associations between candidate proteins and cardiovascular (CV) death or CV hospitalization (with sex interaction) were assessed using Cox regression models.

Results

We found 9 proteins to be differentially expressed between female and male patients. Women expressed more LPL and PLIN1, which are markers of lipid metabolism, more LHB, IGFBP3 and IL1RL2 as markers of transcriptional regulation, and more Ep-CAM as marker of hemostasis. Women expressed less MMP-3, which is a marker associated with extracellular matrix organization, less NRP1, which is associated with developmental processes, and less ACE2, which is related to metabolism. Sex was not associated with the study outcomes (adj. HR 1.48, 95%CI 0.83-2.63), p=0.18).

Conclusion

In chronic HFpEF, assessing sex-differences in a wide range of circulating proteins, led to the identification of 9 proteins that were differentially expressed between female and male patients. These findings may help further investigations into potential pathophysiological processes contributing to HFpEF.

Introduction

Heart failure with preserved ejection fraction (HFpEF) presents an important challenge for clinical practice given its rising incidence, poor prognosis and lack of clear evidence from randomized clinical trials of treatment that can reduce mortality (1-4). Consistent epidemiological data demonstrate that women constitute the majority of patients with HFpEF (5). Although to some extent explained by sex-differences in cardiovascular structure and function, immune system biology and the myocardial response to comorbidities (6), the underlying pathophysiological mechanisms of HFpEF remain incompletely understood.

Given the sex-differences in HFpEF, we sought differences in biomarker profiles possibly linked to underlying pathophysiology. In this study, we therefore assessed sex-differences in 415 circulating proteins in a prospective observational cohort of HFpEF patients (The Metabolic Road to Diastolic Heart Failure (MEDIA-DHF) study; NCT02446327).

Methods

Study population

In MEDIA-DHF, a multicenter, multinational, observational study, a total of 626 HFpEF patients were enrolled between 2012 and 2014 in 10 centers (listed in *Supplemental table 1*). A description of this study population and methods has been published previously (7). To summarize, after standardized echocardiography (*Supplemental data*) and/or local natriuretic peptide measurements, eligible patients with a diagnosis of diastolic dysfunction as established by the 2007 ESC recommendations(8) were included. Three clinical modes of presentations were considered: i) acute decompensated HF patients, ii) patients recently discharged after admission for an acute HF episode (<60 days), or iii) ambulatory chronic disease patients. Data on demographics, clinical parameters, laboratory values (including extensive biomarker measurements), electrocardiography and echocardiography were obtained at inclusion in the study. Follow-up visits took place at 3, 6 and 12 months after inclusion in the study.

Only patients in whom the circulating proteins were measured were included in this substudy (N=392; see also flowchart in *Supplemental figure 1*). No protein measurements were performed in acute decompensated HF patients.

Biomarker measurements

Plasma samples taken at inclusion in the study were analyzed for circulating protein (including natriuretic peptides) using the Olink Proseek Multiplex cardiovascular disease (CVD) II, III, Inflammation, Cardiometabolic and Organ damage panels (Olink Proteomics, Uppsala, Sweden). The assay uses a proximity extension assay (PEA) technology where 92 oligonucleotide-labelled antibody probe pairs per panel are allowed to bind to their respective targets in 1 µL plasma sample. The PEA technology has been described previously (9). In brief, when binding to their correct targets, they give rise to new DNA amplicons with each ID barcoding their respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark HD real-time PCR platform. Data is quality controlled and normalized using an internal extension control and an inter-plate control, to adjust for intra- and inter-run variation. The

extension control is composed of an antibody coupled to a unique pair of DNA-tags that serves as a synthetic control that is added to every sample well. It will adjust for technical variation introduced in the extension step and hence reduce intra-assay variability. The final assay read-out is presented in log2-normalized protein expression (NPX) data where an increase of 1 NPX confers a doubling in concentration of the specific biomarker. This arbitrary unit is therefore a relative quantification of proteins and compares fold changes between groups. All assay validation data for the proteins in the Cardiovascular II, Cardiovascular III, Inflammation, Cardiometabolic and Organ damage panels (detection limits, intra- and inter-assay precision data, accuracy, etc) are available on manufacturer's website (www.olink.com). We excluded proteins that were below the lower limit of detection in more than 50% of the patients (N=45). For the proteins below the LOD in less than 50% of patients, the LOD value was imputed. A total of 415 protein circulating proteins were studied. The abbreviations, full names and respective Olink multiplex panels of all measured proteins are described in the *Supplemental table 2.*

Clinical outcome

The pre-specified endpoint of MEDIA-DHF was a composite of CV death and/or CV hospitalizations (NCT02446327). All endpoints were adjudicated by an independent endpoint committee blinded to the biomarker data.

Network analyses

The FHF-GKB (Fight Heart Failure - Graph Knowledge Box) resource is a customized upgrade of the EdgeBox provided by the EdgeLeap company (available from: https://www.edgeleap.com/edgebox). It extracts data from public data sources hence providing most available public knowledge about human protein-disease, protein-protein and protein-pathway relationships. The FHF-GKB resource enables the study of a total of 20,386 protein nodes imported from Uniprot(10) (including all circulating proteins involved in this study), 28,176 disease nodes from Disease Ontology(11) and DisGenet(12) and 2,222 pathway nodes from Reactome (v65)(13). Protein - protein relationships were retrieved from STRING

(v10.5) (14)or Reactome or WikiPathways(15) or Mentha(16) or BioGrid(17), protein – disease associations from DisGenet (2018-08-24) and protein – pathway relationships from Reactome.

Statistical analyses

Baseline clinical, demographic and echocardiographic characteristics were compared between male and female patients using chi-square, t-test or Mann-Whitney tests, as appropriate.

To study whether biomarker patterns differ between male and female patients, we first identified which clinical variables were associated with female sex using logistic regression analysis ("clinical model"). All variables listed in table 1 were considered in univariate analyses, apart from dyslipidemia as a diagnosis, hemoglobin as a continuous variable, NT-proBNP, and use of aspirin. Variables with ≥20% missing values were excluded from further analyses (pulmonary artery pressure (PASP), left atrial area, ratio of the early (E) to late (A) ventricular filling velocities (E/A), waist circumference, sodium, potassium and CRP). Anemia was defined as hemoglobin <12 g/dL in women and <13 g/dL in men, according to World Health Organization recommendations.

Missing predictor values with <20% of missing values were imputed using linear regression analyses (see *Table 1* for the percentage of missing data for each variable). We imputed missing data 10 times, performed the analysis over all the 10 imputations and averaged results using Rubin's rules (18). Log-linearity of continuous variables was assessed visually by inspecting the shape of the distribution of the beta-estimates vs. the median by quintiles with regard to the outcome of interest ("female sex"). If deemed appropriate based on log-linearity, continuous variables were categorized. Variables with significant p-values (<0.05) in univariate analyses were considered in the multivariate model. The discrimination of the final multivariate model for estimation of clinical end-points was assessed by calculating the area under the curve (AUC). Similar beta-estimates of variables in the multivariate model derived from the multiple imputation datasets were obtained when the pooled mean hemoglobin (N=46), heart rate (N=12) and total cholesterol levels (N=67) were imputed in the original datasets (data not

shown). Further analyses were therefore performed in the original dataset with pooled mean levels for hemoglobin, heart rate and total cholesterol.

To identify differences in proteins between sexes, we studied which proteins were significantly associated with female sex on top of the clinical model. Therefore, all proteins were tested individually on top of the clinical model (i.e., fully adjusted on the clinical variables) in a bivariate logistic regression model, correcting for multiple comparisons using a Bonferroni adjusted p-value of <0.00012 (alpha divided by 415 proteins). We subsequently searched for correlated proteins (>0.3). In order to ascertain independence, we excluded the protein with the largest mean absolute correlation from the two correlated proteins. An additional analysis excluding proteins correlated >0.5 was performed.

The FHF-GKB complex network was queried in order to explore pathways and proteins that could connect together biomarker (BM) nodes of interest. Queries were expressed according to query patterns defining a path structure between two nodes such as BM-BM, BM-pathway-BM, BM-protein-BM, where the BM nodes are taken from a list of interest. The resulting graphs were merged in a figure illustrating all possible paths not longer than two edges, connecting proteins through pathways and proteins.

The association between sex and the composite endpoint of CV death or CV hospitalizations within 1 year after inclusion in the study was studied using Kaplan–Meier estimates and Cox proportional hazards models. In multivariate analyses, adjustment was performed for clinical variables previously found to be independently associated with CV death or CV hospitalizations in MEDIA-DHF (19). These clinical variables were: a history of CAD, pulmonary rales at baseline and age. The proportional hazard assumption was not met and a landmark analysis was performed excluding those patients with an endpoint or being censored within 120 days after inclusion in the study.

Statistical analyses were performed using SPSS 24 (IBM inc., Armonk, New York) and R (The R Foundation for Statistical Computing, Vienna, Austria). This is the first report on sex-differences in clinical features, circulating proteins and outcome in MEDIA-DHF.

Results

Baseline characteristics

There were more female than male patients included in MEDIA-DHF (64% vs. 36%, p<0.001). The distribution of demographic, clinical and laboratory variables according to sex is summarized in *Table 1.* Female patients were older by an average of 2 years, had lower hemoglobin levels (by 4.5%), a higher heart rate (by 3 bpm) and total cholesterol (by 120 mg/dL) when compared to male patients. Male patients were more often current smokers and more likely to have a history of diabetes mellitus, CAD, peripheral arterial disease and prior hospitalizations for HF. Echocardiographic variables are listed in *Table 2.*

Clinical model: variables associated with female sex

Baseline clinical variables that were independently associated with female sex are depicted in *Table 3*. These variables were: higher heart rate, higher total cholesterol, lower hemoglobin, no CAD, no prior HF hospitalizations and no history of smoking.

Association between circulating proteins and female sex

For all individual proteins (including NT-proBNP), associations with female sex were assessed. A total of 17 were identified of which 9 proteins were not correlated (r <0.3) with other proteins. Of these 9 proteins, 6 were positively associated with female sex (insulin-like growth factor-binding protein 3 (IGFBP3), interleukin-1 receptor-like 2 (IL1RL2), lipoprotein lipase (LPL), epithelial cell adhesion molecule (Ep-CAM), perilipin-1 (PLIN1) and lutropin subunit beta (LHB)) and 3 were negatively associated (neuropilin-1 (NRP1), matrix metalloproteinase-3 (MMP-3), angiotensin-converting enzyme 2 (ACE2)) with female sex (*Table 3; Supplemental table 2*). Proteins with moderate correlations (r <0.5) are shown in *Supplemental table 3*.

Network analyses

The visualization of the interactions between circulating proteins and pathways is depicted in the *Central Figure.* The 6 proteins that were higher expressed in female vs. male patients were linked to pathways involved in lipid metabolism, transcriptional regulation and hemostasis. The proteins that were higher expressed in male vs. female patients pointed to pathways related to extracellular matrix organization and developmental processes. Common pathways between the "female" and "male" proteins were signal transduction cascades, protein metabolism and cytokine signaling.

Outcome

There was no difference in the rates of the composite endpoint of CV death or CV hospitalization between female and male patients after 1 year of follow-up (event rates: 41/250 (16.4%) female patients vs. 19/142 (13.4%) male patients, log rank test: p=0.46; *Fig.1*). Crude and adjusted hazard ratios for the composite endpoint are depicted in *Table 4* (adjusted HR 1.48, 95% CI 0.83-2.63, p=0.18).

Discussion

In this study, we investigated sex-differences in circulating proteins measured in a cohort of 392 HFpEF patients. From over 400 proteins measured, we found 9 proteins to be significantly and independently associated with female sex and HFpEF. Females expressed more IGFBP3, IL1RL2, LPL, Ep-CAM, PLIN1 and LHB which are markers of lipid metabolism, transcriptional regulation and hemostasis; whereas they expressed less NRP1, MMP-3 and ACE2 which are markers associated with extracellular matrix organization and developmental processes.

Sex-differences: clinical features

We found several clinical variables associated with female subjects with HFpEF: higher heart rate, higher total cholesterol, lower hemoglobin, absence of CAD, no prior HF hospitalizations and not smoking. These findings are in agreement with previous studies (6, 20, 21). Although previously shown for acute HF patients(22), an association between female sex and higher cholesterol levels in HFpEF has thus far not been reported. Although females were slightly older than male subjects, there was no significant association between age and female sex in this cohort. Interestingly, females were 14% less likely to receive statin therapy at baseline in MEDIA-DHF compared to males. A possible explanation for the higher total cholesterol levels but lower rates of CAD in females compared to males may be that females tend to have microvascular lesions and endothelial dysfunction whereas males are more likely to have obstructive coronary lesions(23), which may potentially lead to an underdiagnosis of coronary artery disease in females.

Sex-differences: circulating proteins

In this study, plasma levels of 9 proteins were significantly differentially expressed between female and male patients. Of these 9 proteins, 6 were higher expressed in female compared to male patients (IGFBP3, IL1RL2, LPL, Ep-CAM, PLIN1 and LHB) whereas the other 3 proteins were higher expressed in males (NRP1, ACE2 and MMP-3). Network analysis revealed that the proteins that were higher expressed in female vs. male patients may be linked to pathways involved in lipid metabolism, transcriptional regulation and hemostasis. The proteins that were higher expressed in males vs. females pointed more towards pathways involved in extracellular matrix organization and developmental processes. Common pathways were signal transduction cascades, protein metabolism and cytokine signaling.

Proposed mechanistic actions and previous studies investigating the differently expressed circulating proteins in HFpEF are described next and summarized in *Table 5*. IGFBP3 is the most abundant carrier protein for insulin-like growth factor 1 (IGF-1) which is known to regulate proliferation, differentiation, metabolism, and cell survival in various tissues. It has been linked to hypertension, obesity, cardiovascular disease and many cancers (24, 25). After an acute myocardial infarction, IGFBP-3 and IGF-1 levels significantly increased, which was associated with improved outcomes and echocardiographic parameters (LV dimensions, mass and ejection fraction)(26). A recent study in 84 HFpEF patients demonstrated a high prevalence of anabolic hormonal deficiencies (including IGF-1) and an low IGF-1 serum levels were associated with increased left atrial size and volume (27). Although IGFBP-3 levels were numerically higher in female compared to male participants in the Cardiovascular Health Study(28), to our knowledge, there have been no reports, so far, on sex-differences in HFpEF, or other cardiovascular diseases.

IL1RL2 (or IL-36 receptor) activates pro-inflammatory pathways upon binding to IL-36 (29). Accumulating data indicates that IL1RL2 is involved in inflammatory diseases such as psoriasis, inflammatory bowel disease and rheumatoid arthritis(29). No previous studies have reported on IL1RL2 in HFpEF or other cardiovascular disease.

The transmembrane glycoprotein expressed in epithelium, Ep-CAM, is involved in various processes such as cell signaling, cell-cell adhesion, proliferation and differentiation, tumorigenesis and metastasis of carcinomas and has been associated with inflammatory bowel disease(30). Ep-CAM was not associated with adverse cardiovascular outcomes in a registry of 263 chronic heart failure patients(31). To our knowledge, no studies have investigated sex-differences in Ep-CAM, in healthy subjects or in patients.

LPL is an enzyme widely expressed in the heart which catalyzes the hydrolysis of triglyceride-rich lipoproteins to fatty acids, as fuel for cardiomyocyte metabolism (32). It plays an important role in atherogenesis and has been associated with coronary heart disease, Alzheimer disease and chronic lymphocytic leukemia(33). LPL deficiency leads to hypertriglyceridemia(34) whereas overexpression of LPL in a mice model resulted in insulin resistance and obesity (35, 36). Influencing cardiac LPL in diabetic mouse models (by either overexpression or downregulation) resulted in impaired cardiac function (37).

Another enzyme involved in lipid metabolism is PLIN1 which is a surface protein of adipocyte lipid droplets that regulates storage and hydrolysis of adipose triglycerides. It has been associated with metabolic diseases such as diabetes, obesity, hepatic steatosis, certain cancers and cardiovascular disease (38). Downregulation of PLIN1 in mice led to excessive cardiac hypertrophy and failure (39). A previous study demonstrated that PLIN1, as a marker of myocardial steatosis, was higher expressed in the right atria of patients with CAD compared to those without (40).

LHB (the beta subunit of luteinizing hormone) promotes spermatogenesis and ovulation by stimulating the testes and ovaria to synthesize steroids. A previous study measured LH in men and postmenopausal women with CAD but also in controls and demonstrated that LH levels were higher in females compared to males but that there was no difference between CAD and control subjects (41). It is therefore possible that our finding of higher LHB levels in female compared to male HFpEF patients may be explained by higher LHB in females in general and that it may not be HFpEF-specific.

Proteins with higher expression in male compared to female HFpEF patients were NRP, ACE2 and MMP-3. NRP1 is a transmembrane receptor for class III semaphorins and for members of the vascular endothelial growth factor family(42). It plays a role in neuronal and vascular development during embryogenesis, angiogenesis and maintenance of vascular integrity (43). In a murine model of cardiac pressure overload, animals that were heterozygous for neuropilin showed higher mortality rates (44). Additionally, Tromp *et al.* demonstrated that NRP1 was associated with poor outcome in HFpEF but not in HFrEF patients (45).

ACE2 hydrolyses angiotensin I and angiotensin II generating angiotensin[1-9] and angiotensin [1-7], respectively. Both angiotensin[1-9] and angiotensin[1-7] are believed to possess direct protective effects against cardiac remodeling and it is hypothesized that the failing heart overexpresses ACE2 to protect itself against the deleterious effects of angiotensin II (46). There is however conflicting data on sex differences in ACE2 expression. One study demonstrated similar ACE2 levels between healthy male and female subjects (47), while another study demonstrated that male controls had significantly higher ACE2 levels compared to females (48). Although still significantly higher in male compared to female patients, the latter study demonstrated also that ACE2 was significantly higher in patients with type 1 diabetes and coronary heart disease compared to controls (48). In line with this, a recent study in heart failure with reduced ejection fraction patients also showed that ACE2 was higher in male than female patients, independent of the use of pharmacological therapies targeting the renin-angiotensin-aldosterone system(49) . It is clear that further study to sex-differences in ACE2 is warranted.

MMP-3 (or stromelysin-1) is an enzyme involved in the breakdown of extracellular matrix proteins and tissue remodeling in physiological (e.g. embryogenesis) and pathophysiological (e.g. tumor metastasis and atherosclerosis) processes(50). High MMP-3 levels have been described in atherosclerotic plaques (51). In contrast, a common mutation in the MMP-3 promoter (which results in decreased MMP-3 expression) was associated with atherosclerosis development (52). Also, in patients with advanced dilated cardiomyopathy, MMP-3 levels were undetectable suggesting that lower levels are associated with worse

cardiac function (53). However, in a study of post-MI patients, MMP-3 levels were higher in male compared to female patients and higher MMP-3 levels were associated with poor outcome in MI (54). In our study, we found that MMP-3 levels were higher in male than in female HFpEF patients. It is obvious that the exact role of MMP-3 remains unclear and warrants further investigation.

It must be noted that one of the circulating proteins that was excluded from the final selection because of being correlated to a different protein (PLIN1 in this case), was leptin. Leptin is an hormone that is released by adipocytes when they are overfilled with lipids, causing reduced food intake and increased energy expenditure (55). It activates the sympathetic nervous system resulting in unfavorable neurohormonal changes such as an increase in blood pressure and cardiac hypertrophy(56). A role in the pathophysiology of HFpEF was also suggested In HF patients (both HFrEF as HFpEF), leptin levels were higher as compared to healthy controls (57). Interestingly, the actions of leptin on the autonomic nervous system appear to be particularly marked in women(58). In our study, female HFpEF patients had higher leptin levels compared to male patients. Although it has been previously hypothesized that leptin may play a role in the pathophysiology of HFpEF (59), the role of sex-differences herein are not yet fully understood.

As can be appreciated above, for most of the circulating proteins that were differentially expressed between female and male HFpEF patients in our study, we could not find previous studies on sex-differences. However, there have been reports on sex-differences for some of the observed pathways. Body fat distribution is different between sexes where women have more subcutaneous and men more visceral fat. Visceral adiposity, as compared to subcutaneous adiposity, is associated with increased rates of lipolysis and inflammation, hence resulting in an increased susceptibility for metabolic complications (as reviewed in (60)). In HFpEF, it is hypothesized comorbidities such as diabetes and obesity induce a low-grade systemic pro-inflammatory state(61). Whether sex-differences in lipid metabolism are of importance in the pathophysiology of HFpEF warrants further investigation.

Moreover, there is an increasing body of evidence suggesting sex-differences in primary and secondary hemostasis, and fibrinolysis. It has been demonstrated, for example, that women have higher platelet counts and activity and generate more thrombin compared to men (as reviewed in (62)).

Lastly, sex-differences in extracellular matrix turnover have also been previously reported. Women demonstrate a smaller fibrotic response compared to men as reflected by a lower expression of TGF-beta (a highly pro-fibrotic growth-factor) in women (as reviewed in (63)). Moreover, in a mouse model, matrix metalloproteinase activity was higher in males compared to females after an acute MI, leading to poorer outcome in males (64). Although cardiac fibrosis has been hypothesized by Paulus *et al.* to be one of the key players in the pathophysiology of HFpEF(61), the role of sex-differences herein has yet to be identified. It is also possible that our finding of more activated extracellular matrix pathways in male compared to female HFpEF patients simply reflects the presence of more CAD in males (as observed in our study) and subsequent myocardial injury-induced extracellular matrix alterations.

Sex-differences: outcome

Female sex was not associated with cardiovascular death and/or cardiovascular hospitalizations in our study. Our findings are in line with a recent post-hoc analysis from PARAGON demonstrating similar rates of the composite of heart failure hospitalizations or death for cardiovascular reasons(65), but in conflict with previous other studies. Lam *et al.* observed in a post-hoc analysis of I-PRESERVE that female sex was associated with better prognosis, although the effect was moderated by 4 common baseline characteristics which were atrial fibrillation, renal dysfunction, stable angina pectoris and NYHA class III/IV (21). In the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) meta-analysis, women had lower rates of all-cause mortality over 3 years compared to men, irrespective of ejection fraction (66). Our findings and those of Lam *et al.* suggest that the relation between sex and outcome in

HFpEF is confounded to some extent by comorbidities or other patient characteristics. Future studies need to determine whether a sex-difference in prognosis exists.

Perspectives and Significance

For the majority of the circulating proteins that were differently expressed between female and male HFpEF patients in our study, sex-differences in a healthy population have not been studied. A recent population-based study compared 30 cardiometabolic biomarkers (not including the proteins that were measured in MEDIA-DHF) between female and male participants and found that biomarker profiles significantly differed (67). Whether the sex-differences in circulating proteins in our cohort of HFpEF patients reflect pathophysiological or physiological processes warrants further study. Our findings of different pathways being activated in female compared to male HFpEF patients may be the basis of investigating therapies specifically targeting the identified pathways, potentially remediating the poor track record of past HFpEF large outcome trials (68). In addition, they may be of help in the search for biologically plausible explanations for the sex-differences in treatment response as reported for neprilysin inhibition (69), mineralocorticoid receptor antagonists (70), and cardiac resynchronization therapy (71).

Limitations

The most important limitation of this study is that we could not externally validate our findings due to the absence of other HFpEF cohorts in which similar proteins were measured. It is clear that other studies need to confirm our findings. Second, as already mentioned before, sex-differences have not been studied in a general population for the majority of circulating proteins tested in our study. Moreover, in this study only HFpEF patients were included and a 'healthy' control group (including reference values for the measured proteins) was thus absent. It is therefore not clear whether the observed differences in biomarker expression reflects pathophysiological and/or physiological processes. Third, at the time of enrollment,

HFpEF was diagnosed following 2007 ESC diagnostic recommendations. Since the 2007 criteria, other criteria have been proposed and it has recently been shown that large variations in prevalence of diastolic dysfunction may be expected according to which criterion is used (72). However, when applying the H_2FPEF score by Reddy and colleagues(73) to the MEDIA-DHF cohort, only 2% of patients had a low probability of HFpEF whereas 58% and 40% of patients had an intermediate or high probability, respectively. Fourth, although the biomarker assay in this study covers a wide variety of disease domains, the possibility exists that other, now unmeasured, proteins may (also) play a role in the pathophysiology of HFpEF. Fifth, the biomarker assay does not provide standard concentration units, making comparisons with clinically applied cut-offs difficult.

Conclusion

Assessing sex-differences in >400 circulating proteins analyzed in a large cohort of HFpEF patients led to the identification of 9 uncorrelated sex-specific proteins. Females expressed more markers associated with lipid metabolism, transcriptional regulation and hemostasis, whereas they expressed less proteins associated with extracellular matrix organization and developmental processes. These findings may help further investigations into potential pathophysiological processes contributing to HFpEF.

Declarations

Ethics approval and consent to participate

The study protocol complied with the Declaration of Helsinki and was approved by the respective Ethics Committees of the participating institutions. Written informed consent was provided by all patients. (ClinicalTrials.gov Identifier: NCT02446327)

Consent for publication

Not applicable

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interest and funding

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Authors' contributions

SS analysed and interpreted the data and drafted the manuscript. JPF made substantial contributions to the design and analysis, and drafting of the manuscript. MK analysed the data and revised the manuscript. DD made substantial contributions to the conception of the data and revised the manuscript. GP, JLM and KD developed the software used for the analysis and revised the manuscript. KD developed the software used for the analysis and revised the manuscript. EB and MDD performed the complex network analyses and revised the manuscript. NLA helped interpreting the data and revised the manuscript. SA, GA, HFBLR, RFC, AF, LvH, GdK, PM, KM, AM, ZP, RR, CT, WJP made substantial contributions to the conception of the data and revised the manuscript. NG, FZ and PR made substantial contributions to the conception of the data, the design of the study, and substantial revisions to the manuscript. All co-authors have approved

the submitted version and both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Table 1 Baseline characteristics of patients in the MEDIA-DHF cohort according to sex

	Global (N=392)	Male (N=142)	Female (N=250)	p-value	% of missing values
Age, years, median (IQR)	74.0 (67.5 - 80.0)	73.0 (67.0 - 79.0)	75.0 (68.0 - 81.0)	0.028	0%
Recently decompensated HF, n (%)	60 (15.3 %)	25 (17.6 %)	35 (14.0 %)	0.34	0%
Smoking status, n (%)					
Never	205 (52.7 %)	39 (27.9 %)	166 (66.7 %)	<0.001	1%
Former	150 (38.6 %)	84 (60.0 %)	66 (26.5 %)		
Current	34 (8.7 %)	17 (12.1 %)	17 (6.8 %)		
BMI, kg/m^2, mean±SD	30.6 ± 6.2	30.5 ± 5.6	30.6 ± 6.5	0.79	1%
Alcohol status, n (%)					
Non-consumer	245 (63.3 %)	73 (52.1 %)	172 (69.6 %)	0.001	1%
1-2 drinks/day	124 (32.0 %)	56 (40.0 %)	68 (27.5 %)		
>2 drinks/day	18 (4.7 %)	11 (7.9 %)	7 (2.8 %)		
Waist circumference, cm, mean±SD	104.8 ± 14.1	108.2 ± 13.8	102.9 ± 13.9	0.001	21%
SBP, mmHg, mean±SD	137.2 ± 23.1	137.5 ± 22.3	137.0 ± 23.5	0.83	2%
DBP, mmHg, mean±SD	74.0 ± 11.6	74.7 ± 11.1	73.7 ± 11.9	0.41	2%
Pulmonary rales, n (%)	89 (23.0 %)	33 (23.4 %)	56 (22.8 %)	0.89	1%
NYHA class III/IV, n (%)	73 (18.7 %)	23 (16.2 %)	50 (20.1 %)	0.34	0%
Peripheral edema, n (%)	178 (45.4 %)	74 (52.1 %)	104 (41.6 %)	0.045	1%
Elevated JVP, n (%)	24 (6.3 %)	12 (8.7 %)	12 (4.9 %)	0.15	3%
Hepatomegaly, n (%)	13 (3.6 %)	6 (4.5 %)	7 (3.1 %)	0.68	7%
Fatigue at exertion, n (%)	305 (79.2 %)	105 (75.5 %)	200 (81.3 %)	0.18	2%
Heart rate, bpm, mean±SD	69.4 ± 14.4	67.1 ± 12.9	70.8 ± 15.0	0.016	3%
Hypertension n (%)	342 (87.7 %)	121 (86.4 %)	221 (88.4 %)	0.57	1%
Atrial fibrillation, n (%)	117 (31%)	49 (36%)	68 (28%)	0.13	3%
Diabetes mellitus n (%)	154 (39.3 %)	67 (47.2 %)	87 (34.8 %)	0.016	0%
Dyslipidemia n (%)	226 (58.2 %)	75 (53.6 %)	151 (60.9 %)	0.16	1%
Previous HF hospitalization, n (%)	137 (35.5 %)	65 (46.8 %)	72 (29.1 %)	<0.001	2%
CAD, n (%)	128 (33.7 %)	73 (52.9 %)	55 (22.7 %)	<0.001	3%
Stroke or TIA, n (%)	44 (11.3 %)	14 (10.0 %)	30 (12.0 %)	0.54	1%
Peripheral artery disease, n (%)	34 (8.9 %)	18 (13.0 %)	16 (6.5 %)	0.030	2%
COPD, n (%)	71 (18.4 %)	30 (21.4 %)	41 (16.7 %)	0.25	2%
Laboratory values, mean±SD					
LDL, mg/dL	99.8 ± 37.1	89.8 ± 30.2	104.5 ± 39.1	<0.001	20%
HDL, mg/dL	53.7 ± 19.7	47.3 ± 15.5	56.8 ± 20.8	<0.001	19%
Total cholesterol, mg/dL	176.1 ± 43.1	160.2 ± 35.1	183.9 ± 44.6	<0.001	17%

Hemoglobin, g/dL	13.0 ± 1.6	13.4 ± 1.8	12.8 ± 1.5	<0.001	12%
Anemia, n (%)	106 (30.6 %)	46 (39.0 %)	60 (26.3 %)	0.019	12%
eGFR, mL/min/1.73m2	66.4 ± 23.1	66.4 ± 23.7	66.4 ± 22.8	1.00	6%
NT-proBNP (in NPX)	4.2 ± 1.3	4.4 ± 1.4	4.1 ± 1.3	0.042	0%
Medication prescription rates, n (%)					
ACEi or ARB	319 (81.4 %)	114 (80.3 %)	205 (82.0 %)	0.67	0%
Betablockers	286 (73.0 %)	111 (78.2 %)	175 (70.0 %)	0.080	0%
Thiazide diuretics	87 (22.3 %)	22 (15.5 %)	65 (26.1 %)	0.015	0%
Loop diuretics	235 (59.9 %)	89 (62.7 %)	146 (58.4 %)	0.41	0%
MRA	43 (11.0 %)	16 (11.3 %)	27 (10.8 %)	0.87	0%
Aspirin	159 (40.6 %)	64 (45.1 %)	95 (38.0 %)	0.17	0%
Insulin	52 (13.3 %)	26 (18.3 %)	26 (10.4 %)	0.028	0%
Statin	241 (61.5 %)	96 (67.6 %)	145 (58.0 %)	0.060	0%
Oral anticoagulants	161 (41.1 %)	57 (40.1 %)	104 (41.6 %)	0.78	0%

Legend: HF, heart failure; BMI, body mass index; DM, diabetes mellitus; CAD, coronary artery disease; PAD, peripheral artery disease; COPD, chronic obstructive pulmonary disease; OSAS, obstructive sleep apnea syndrome; TIA, transient ischemic attack; DBP, diastolic blood pressure; SBP, systolic blood pressure; NYHA, New York Heart Association; JVP, jugular venous pressure; eGFR, estimated glomerular filtration rate, BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; NPX, normalized protein expression; ASA, acetylsalicylic acid; ACEi, ACE-inhibitor; ARB, angiotensin receptor blocker, BB, betablocker; MRA, mineralocorticoid receptor antagonist; LVEF, left ventricular ejection fraction; E/e', the ratio of mitral inflow velocity and early mitral annulus velocity, PASP, pulmonary artery systolic pressure; TAPSE, Tricuspidal Annular Plane Systolic Excursion; ; E/A, ratio of the early (E) to late (A) ventricular filling velocities; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LAVI, left atrial volume index; IQR, interquartile range; SD, standard deviation.

Table 2 Echocardiography characteristics of patients in the MEDIA-DHF cohort according to sex

	Global (N=392)	Male (N=142)	Female (N=250)	p-value	% of missing values
Echocardiographic variables, mean±SD					
LVEF, %,	60.8 ± 7.0	60.5 ± 6.9	61.0 ± 7.0	0.54	0%
E/e'	13.3 ± 5.2	12.6 ± 4.6	13.7 ± 5.4	0.057	7%
PASP, mmHg	34.8 ± 12.7	34.9 ± 15.0	34.8 ± 11.4	0.97	25%
TAPSE, cm	20.5 ± 4.8	21.0 ± 5.4	20.2 ± 4.4	0.13	10%
E/A	1.3 ± 0.9	1.4 ± 1.0	1.2 ± 0.8	0.033	30%
LDEVi	43.8 ± 14.0	48.3 ± 14.4	41.1 ± 13.1	<0.001	4%
LDESi	17.4 ± 7.3	19.6 ± 7.5	16.2 ± 6.7	<0.001	6%
LAVI, ml/m²	43.8 ± 15.8	45.0 ± 16.6	43.1 ± 15.4	0.27	3%

Legend: LVEF, left ventricular ejection fraction; E/e', the ratio of mitral inflow velocity and early mitral annulus velocity, PASP, pulmonary artery systolic pressure; TAPSE, Tricuspidal Annular Plane Systolic Excursion; ; E/A, ratio of the early (E) to late (A) ventricular filling velocities; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LAVI, left atrial volume index; IQR, interquartile range; SD, standard deviation.

Table 3 Adjusted and multiple testing-corrected circulating proteins associated with female sex

	Clinical model (AUC 0.82)		**Proteins**	
	OR (95% CI) for female sex association	*p-value*	*OR (95% CI) for female sex association*	*p-value*
Heart rate, per 10 bpm increase	1.22 (1.00-1.48)	0.049		
Total cholesterol, per 10 mg/dL increase	1.13 (1.03-1.02)	0.010		
Smoking status				
Never	Ref.	-		
Former	0.21 (0.12-0.36)	<0.001		
Current	0.33 (0.14-0.80)	0.014		
CAD	0.36 (0.21-0.62)	<0.001		
Previous HF hospitalization	0.47 (0.27-0.82)	0.008		
Hb, per 1 g/dL increase	0.69 (0.57-0.82)	<0.001		
Higher expression in females vs. males				
IL1RL2			4.13 (2.22-7.68)	<0.001
LPL			4.08 (2.44-6.82)	<0.001
LHB			3.15 (2.20-4.52)	<0.001
IGFBP3			2.83 (1.74-4.58)	<0.001
PLIN1			2.44 (1.62-3.66)	<0.001
Ep-CAM			1.87 (1.41-2.48)	<0.001
Less expression in females vs. males				
NRP1			0.05 (0.01-0.19)	<0.001
MMP-3			0.22 (0.14-0.35)	<0.001
ACE2			0.41 (0.28-0.61)	<0.001

Legend: eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval; AUC, area under the curve; bpm, beats per minute; CAD, coronary artery disease; HF, heart failure; Hb, hemoglobin; IGFBP3, insulin-like growth factor-binding protein 3; NRP1, neuropilin 1; IL1RL2, interleukin-1 receptor-like 2; LPL, lipoprotein lipase; ACE2, angiotensin-converting-enzyme 2; Ep_CAM, epithelial cell adhesion molecule; CA14, carbonic anhydrase 14; PLIN1, perilipin-1; LHB, lutropin subunit beta.

Each OR unit increase represents doubling in the NPX values

Table 4 **Crude and adjusted hazard ratios for the prediction of cardiovascular death and/or cardiovascular hospitalization during 1 year of follow-up according to sex**

	Crude HR (95%CI)	Adj. HR (95%CI)*
Complete cohort		
Female vs. Male	1.26 (0.73-2.16), p=0.41	1.48 (0.83-2.63), p=0.18
Landmark analysis (>120 days)		
Female vs. Male	1.70 (0.72-4.03), p=0.23	1.89 (0.77-4.65), p=0.17

*adjusted on coronary artery disease, pulmonary rales at baseline and age. Since our aim was to assess the association of female sex on top of this clinical risk score and female sex was already incorporated in the prognostic risk score, sex was not considered in the prognostic risk score used for these analyses.

The proportional hazard assumption was not met and a landmark analysis was performed excluding those patients with an endpoint or being censored within 120 days after inclusion in the study.

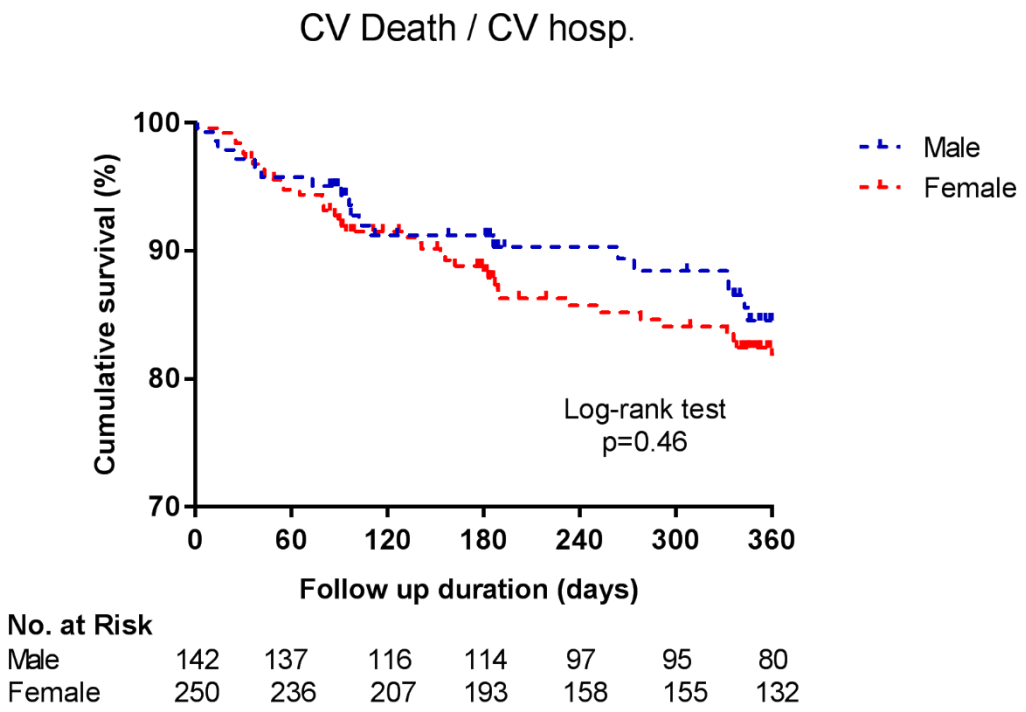
Table 5 Overview of the proteins differentially expressed between sexes

Individual biomarker	**Mechanistic significance**	**Previous relevant reports**		**Reports on sex differences**	
		Basic reports	*Clinical reports*	*Healthy population*	*Heart disease*
More highly expressed in females					
IL1RL2	Activates pro-inflammatory pathways upon binding of IL-36 (29)		Involved in inflammatory diseases such as psoriasis, inflammatory bowel disease and rheumatoid arthritis(29)	No	No
LPL	Enzyme that catalyzes the hydrolysis of triglycerides	Overexpression or downregulation of cardiac LPL in diabetic mouse models resulted in impaired left ventricular function (37).	Associated with coronary heart disease, Alzheimer disease and chronic lymphocytic leukemia(33)	No	No
LHB	Promotes spermatogenesis and ovulation by stimulating the testes and ovaria to synthesize steroids		Levels not different between CAD and control subjects (41).	Yes (higher in females vs. males (41))	Yes (higher in female vs. male CAD patients(41))
IGFBP3	Most abundant carrier protein for insulin-like growth factor 1 (IGF-1) which is known to play a major role in metabolism	The IGF system (including IGFBP3) has been previously associated with cardiovascular disease and many cancers (24, 25)	In HFpEF patients, low IGF-1 and IGFBP3 were associated with increased parameters of left atrial size and volume (27) Higher IGF-1 after an acute MI was associated with improved clinical outcomes and echocardiographic measures (LV dimensions, mass and ejection fraction)(26)	Yes (numerically higher in female vs. male but no formal comparison made(28))	No
PLIN1	Surface protein of adipocyte lipid droplets that regulates	Linked to endocrine metabolism disease	Higher expressed in the right atria of patients with CAD compared to	No	No

	storage and hydrolysis of adipose triglycerides(74)	(diabetes, obesity etc.), cancers and cardiovascular disease (38)	those without (40).		
Ep-CAM	Transmembrane glycoprotein expressed in epithelium	Involved in various processes such as cell signaling, cell-cell adhesion, proliferation and differentiation, tumorigenesis and metastasis of carcinomas(30)	Not associated with adverse cardiovascular outcomes in a registry of 263 chronic heart failure patients(31) Associated with inflammatory bowel disease(30)	No	No
Less expressed in females					
NRP1	Transmembrane receptor for class III semaphorins and for members of the vascular endothelial growth factor family(42)	Neuronal and vascular development during embryogenesis, angiogenesis and maintenance of vascular integrity (43)	Associated with poor outcome in HFpEF but not in HFrEF patients (45)	No	No
MMP-3	Enzyme involved in the breakdown of extracellular matrix proteins	Involved in physiological (e.g. embryogenesis) and pathophysiological (e.g. tumor metastasis and atherosclerosis) processes(50)	Conflicting data: High levels have been described in atherosclerotic plaques (51) and associated with poor outcome after MI(54). However, a common mutation in the MMP-3 promoter (which results in decreased MMP-3 expression) was associated with atherosclerosis development (52).	No	Yes (higher in male vs. female patients post-MI (54))
ACE2	Hydrolyses angiotensin I and angiotensin II generating angiotensin[1-9] and angiotensin [1-7], respectively.		ACE2 levels were higher in patients with type 1 diabetes and coronary heart disease vs. controls(48).	Yes (conflicting data: similar levels in male and females (47) or higher in male vs. female (48)	Yes (higher in males vs. females with type 1 DM and CAD (48))

				)	

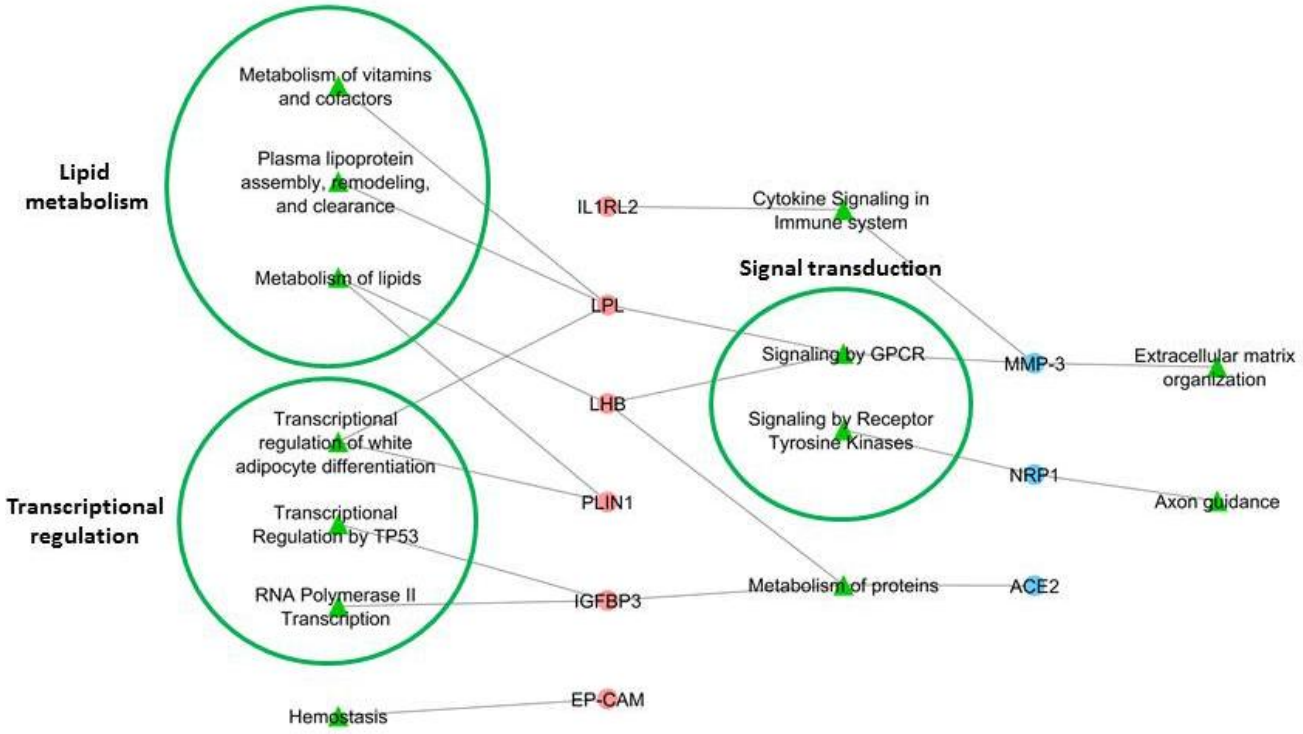
Figure 1 **Primary outcome according to sex**



Legend: CV, cardiovascular; No., number.

Central Figure

Network analyses for the visualization of pathways and protein-interactions between circulating proteins associated with sex in MEDIA-DHF. The proteins that were higher expressed in female vs. male patients may be linked to pathways involved in lipid metabolism, transcriptional regulation and hemostasis. The proteins that were higher expressed in male vs. female patients may be linked to pathways such as extracellular matrix organization and developmental processes. Common pathways between the proteins seem signal transduction, protein metabolism and cytdfauokine signaling.



The FHF-GKB complex network was queried in order to explore pathways and proteins that could connect together biomarker nodes of interest. Queries were expressed according to query patterns defining a path structure between two nodes such as BM-BM, BM-pathway-BM, and BM-pathway, where the BM nodes are taken from a list of interest. The resulting graphs were merged in a figure illustrating all possible paths not longer than two edges, connecting proteins through pathways and proteins. This graph depicts biomarker-pathway—protein interactions. There were no direct interactions between proteins. Blue: proteins higher expressed in males; Pink: proteins higher expressed in females. Green: pathways.

Supplemental data

Echocardiography

All patients underwent echocardiography and images were stored in a digital cine-loop format for off-line analysis according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging relative to the cardiac chamber and right heart measurements[1]

LV structure and function were evaluated from standard 2D views, including apical four-chamber, apical two-chamber and parasternal long- and short-axis views. Ventricular dimensions, wall thickness, mass and geometry were determined from 2D parasternal short- and long-axis views. LV volumes, stroke volume and ejection fraction were calculated using the biplane method of disks summation (modified Simpson's rule). All cardiac chamber volumes and mass measurements were indexed to body surface area.

Left atrial volume was assessed by the biplane area-length method from apical 2- and 4-chamber views at end-systole and was indexed to body surface area (LA volume index, LAVi). Left atrial area (cm^2) was estimated from the apical views. If LA volume was not available, LA area was used to evaluate remodeling. Patients with either LAVi>40ml/m^2 or LA area>20cm^2 were considered to have dilated LA.

Peak velocity of early (E) and late (A) wave of transmitral flow and E-wave deceleration time (DT) were measured from the pulsed-wave Doppler obtained at the tip of mitral leaflets. The average of septal and lateral annular velocities (e') was obtained by tissue Doppler imaging

[1] Lang RM *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015; 16(3):233-70.

(TDI). The E/e′ ratio was calculated using the peak E-wave velocity and the average of septal and lateral. LV outflow tract (LVOT) time velocity integral, isovolumic relaxation time (IVRT) and color M-mode of early diastolic mitral inflow into the left ventricle (Flow propagation velocity, Vp) were acquired from apical four- and five-chamber views. Pulmonary venous flow (PVF) was sampled using pulsed-wave Doppler at 1 cm into the orifice of the right upper pulmonary vein.

Right ventricular function was estimated by measuring TAPSE (mm) and pulmonary arterial systolic pressure (PASP) was calculated using the peak velocity of tricuspid regurgitation (TR) and the maximum IVC diameter (IVC baseline) and respiratory variation (Ratio IVC inspiration / IVC baseline) measured 3 cm before merger with the right atrium.

Overall, echocardiographic data were complete in >75% of patients, except for the following variables: A (and E/A ratio), S/D, Ard-Ad, E/Vp (which were available in 70%, 61%, 44% and 62% of the population, respectively).

Supplemental table 1 Participating centers MEDIA-DHF

Nancy Clinical Investigation Centre, University of Lorraine, France (and EDDH European Drug Development Hub, Nancy, France as project manager)
Lariboisière Hospital, Paris, France
Free University Medical Centre, Amsterdam, The Netherlands
CHARITE, universitätsmedizin Berlin, Berlin Germany
University of Porto, Porto, Portugal
University of Maastricht, Maastricht, The Netherlands
University of Antwerp, Antwerp, Belgium
Cardiff University, Cardiff, United Kingdom
Foundation for Applied Medical Research, Pamplona, Spain
University of Eastern PIEMONTE Medical School, Novara, Italy
University of Brescia, Brescia, Italy
University of Perugia, Perugia, Italy
University College Dublin, National University of Ireland, Dublin, Ireland
University Debrecen, Debrecen, Hungary
Oslo University Hospital

Supplemental table 2 Overview of circulating proteins measured at baseline in MEDIA-DHF

Organ damage panel	
5'-AMP-activated protein kinase subunit beta-1 (PRKAB1) Q9Y478	Kidney Injury Molecule (KIM1) Q96D42
Adhesion G-protein coupled receptor G1 (ADGRG1) Q9Y653	Leukotriene A-4 hydrolase (LTA4H) P09960
Aldehyde dehydrogenase, dimeric NADP-preferring (ALDH3A1) P30838	Linker for activation of T-cells family member 2 (LAT2) Q9GZY6
Anterior gradient protein 2 homolog (AGR2) O95994	Lutropin subunit beta (LHB) P01229
Apoptosis-inducing factor 1, mitochondrial (AIFM1) O95831	Macrophage erythroblast attacher (MAEA) Q7L5Y9
B-cell scaffold protein with ankyrin repeats (BANK1) Q8NDB2	Macrophage-capping protein (CAPG) P40121
BH3-interacting domain death agonist (BID) P55957	Melanoma-associated antigen D1 (MAGED1) Q9Y5V3
BMP and activin membrane-bound inhibitor homolog (BAMBI) Q13145	Methionine aminopeptidase 1 (METAP1) P53582
Calcitonin (CALCA) P01258	Mevalonate kinase (MVK) Q03426
Calreticulin (CALR) P27797	Mitogen-activated protein kinase kinase kinase kinase 5 (MAP4K5) Q9Y4K4
Carbonic anhydrase 12 (CA12) O43570	Mothers against decapentaplegic homolog 1 (SMAD1) Q15797
Carbonic anhydrase 14 (CA14) Q9ULX7	NAD-dependent protein deacylase sirtuin-5, mitochondrial (SIRT5) Q9NXA8
Casein kinase I isoform delta (CSNK1D) P48730	NEDD8 ultimate buster 1 (NUB1) Q9Y5A7
Claspin (CLSPN) Q9HAW4	Neutrophil cytosol factor 2 (NCF2) P19878
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-	Nibrin (NBN) O60934
sialyltransferase 1 (ST3GAL1)	Nitric oxide synthase, endothelial (NOS3) P29474
Q11201	Nucleobindin-2 (NUCB2) P80303
Cocaine esterase (CES2) O00748	Parvalbumin alpha (PVALB) P20472
Contactin-2 (CNTN2) Q02246	Paxillin (PXN) P49023
Corticoliberin (CRH) P06850	Peptidyl-prolyl cis-trans isomerase FKBP1B (FKBP1B) P68106
C-type lectin domain family 1 member A (CLEC1A) Q8NC01	Perilipin-1 (PLIN1) O60240
C-type natriuretic peptide (NPPC) P23582	Placenta growth factor (PGF) P49763
Desmoglein-4 (DSG4) Q86SJ6	Platelet-derived growth factor C (PDGFC) Q9NRA1
Dipeptidyl aminopeptidase-like protein 6 (DPP6) P42658	Pleiotrophin (PTN) P21246
DNA topoisomerase 2-beta (TOP2B) Q02880	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2 (INPPL1) O15357
Ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2) Q9Y5L3	Plexin domain-containing protein 1 (PLXDC1) Q8IUK5
Ectonucleoside triphosphate diphosphohydrolase 6 (ENTPD6) O75354	Polypeptide N-acetylgalactosaminyltransferase 10 (GALNT10) Q86SR1

EGF-like repeat and discoidin I-like domain-containing protein 3 (EDIL3) O43854	Probetacellulin (BTC) P35070
Enteropeptidase (TMPRSS15) P98073	Programmed cell death protein 1 (PDCD1) Q15116
Epidermal growth factor-like protein 7 (EGFL7) Q9UHF1	Prolow-density lipoprotein receptor-related protein 1 (LRP1) Q07954
Erbin (ERBIN) Q96RT1	Proteasome subunit alpha type-1 (PSMA1) P25786
Erythropoietin (EPO) P01588	Protein amnionless (AMN) Q9BXJ7
Fatty acid-binding protein 9 (FABP9) Q0Z7S8	Protein fosB (FOSB) P53539
Forkhead box protein O1 (FOXO1) Q12778	Protein max (MAX) P61244
Fructose-2,6-bisphosphatase TIGAR (TIGAR) Q9NQ88	Protein phosphatase 1B (PPM1B) O75688
Hematopoietic prostaglandin D synthase (HPGDS) O60760	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1,
Inactive tyrosine-protein kinase 7 (PTK7) Q13308	mitochondrial (PDP1) Q9P0J1
Integrin beta-1-binding protein 1 (ITGB1BP1) O14713	Ras association domain-containing protein 2 (RASSF2) P50749
Interferon-inducible double-stranded RNA-dependent protein	Ras GTPase-activating protein 1 (RASA1) P20936
kinase activator A (PRKRA) O75569	Receptor-type tyrosine-protein phosphatase eta (PTPRJ) Q12913
Renin receptor (ATP6AP2) O75787	EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) Q12805
REST corepressor 1 (RCOR1) Q9UKL0	Endoglin (ENG) P17813
Retinoic acid receptor responder protein 1 (RARRES1) P49788	Fetuin-B (FETUB) Q9UGM5
Ribonucleoside-diphosphate reductase subunit M2 B (RRM2B) Q7LG56	Ficolin-2 (FCN2) Q15485
Serpin A9 (SERPINA9) Q86WD7	Glutaminyl-peptide cyclotransferase (QPCT) Q16769
Serum paraoxonase/arylesterase 2 (PON2) Q15165	Granulysin (GNLY) P22749
Syntaxin-8 (STX8) Q9UNK0	Growth arrest-specific protein 6 (GAS6) Q14393
Syntaxin-binding protein 3 (STXBP3) O00186	Hepatocyte growth factor receptor (MET) P08581
Troponin I, cardiac muscle (TNNI3) P19429	Ig lambda-2 chain C regions (IGLC2) P0CG05
Tyrosine-protein kinase Fes/Fps (FES) P07332	Insulin-like growth factor-binding protein 3 (IGFBP3) P17936
Tyrosine-protein kinase Fgr (FGR) P09769	Insulin-like growth factor-binding protein 6 (IGFBP6) P24592
Tyrosine-protein kinase Yes (YES1) P07947	Integrin alpha-M (ITGAM) P11215
Vascular endothelial growth factor C (VEGFC) P49767	Intercellular adhesion molecule 1 (ICAM1) P05362
Vasohibin-1 (VASH1) Q7L8A9	Intercellular adhesion molecule 3 (ICAM3) P32942
Wiskott-Aldrich syndrome protein (WAS) P42768	Interleukin-7 receptor subunit alpha (IL7R) P16871
	Latent-transforming growth factor beta-binding protein 2 (LTBP2) Q14767
Cardiometabolic panel	Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) Q8NHL6

Angiogenin (ANG) P03950	Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2) Q8N423
Angiopoietin-related protein 3 (ANGPTL3) Q9Y5C1	Leukocyte immunoglobulin-like receptor subfamily B member 5 (LILRB5) O75023
Apolipoprotein M (APOM) O95445	Lithostathine-1-alpha (REG1A) P05451
Beta-Ala-His dipeptidase (CNDP1) Q96KN2	Liver carboxylesterase 1 (CES1) P23141
Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) P15907	Low affinity immunoglobulin gamma Fc region receptor II-a (FCGR2A) P12318
Cadherin-1 (CDH1) P12830	Low affinity immunoglobulin gamma Fc region receptor III-B (FCGR3B) O75015
Carbonic anhydrase 1 (CA1) P00915	L-selectin (SELL) P14151
Carbonic anhydrase 3 (CA3) P07451	Lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1) Q9Y5Y7
Carbonic anhydrase 4 (CA4) P22748	Lysosomal Pro-X carboxypeptidase (PRCP) P42785
Cartilage acidic protein 1 (CRTAC1) Q9NQ79	Mannose-binding protein C (MBL2) P11226
Cartilage oligomeric matrix protein (COMP) P49747	Mast/stem cell growth factor receptor Kit (KIT) P10721
C-C motif chemokine 5 (CCL5) P13501	Membrane cofactor protein (CD46) P15529
C-C motif chemokine 14 (CCL14) Q16627	Membrane primary amine oxidase (AOC3) Q16853
C-C motif chemokine 18 (CCL18) P55774	Metalloproteinase inhibitor 1 (TIMP1) P01033
CD59 glycoprotein (CD59) P13987	Microfibrillar-associated protein 5 (MFAP5) Q13361
Coagulation factor VII (F7) P08709	Multiple epidermal growth factor-like domains protein 9 (MEGF9) Q9H1U4
Coagulation factor XI (F11) P03951	Neural cell adhesion molecule 1 (NCAM1) P13591
Collagen alpha-1(XVIII) chain (COL18A1) P39060	Neural cell adhesion molecule L1-like protein (CHL1) O00533
Complement C1q tumor necrosis factor-related protein 1 (C1QTNF1) Q9BXJ1	Neurogenic locus notch homolog protein 1 (NOTCH1) P46531
Complement C2 (C2) P06681	Neuropilin-1 (NRP1) O14786
Complement factor H-related protein 5 (CFHR5) Q9BXR6	Neutrophil defensin 1 (DEFA1) P59665
Complement receptor type 2 (CR2) P20023	Neutrophil gelatinase-associated lipocalin (LCN2) P80188
Cystatin-C (CST3) P01034	Nidogen-1 (NID1) P14543
Dipeptidyl peptidase 4 (DPP4) P27487	Oncostatin-M-specific receptor subunit beta (OSMR) Q99650
Peptidyl-glycine alpha-amidating monooxygenase (PAM) P19021	C-C motif chemokine 17 (CCL17) Q92583
Phospholipid transfer protein (PLTP) P55058	CD40 ligand (CD40-L) P29965
Plasma serine protease inhibitor (SERPINA5) P05154	Chymotrypsin C (CTRC) Q99895
Platelet glycoprotein Ib alpha chain (GP1BA) P07359	C-X-C motif chemokine 1 (CXCL1) P09341
Platelet-activating factor acetylhydrolase (PLA2G7) Q13093	Decorin (DCN) P07585

Plexin-B2 (PLXNB2) O15031	Dickkopf-related protein 1 (Dkk-1) O94907
Procollagen C-endopeptidase enhancer 1 (PCOLCE) Q15113	Fatty acid-binding protein, intestinal (FABP2) P12104
Prolyl endopeptidase FAP (FAP) Q12884	Fibroblast growth factor 21 (FGF-21) Q9NSA1
Receptor-type tyrosine-protein phosphatase S (PTPRS) Q13332	Fibroblast growth factor 23 (FGF-23) Q9GZV9
Regenerating islet-derived protein 3-alpha (REG3A) Q06141	Follistatin (FS) P19883
Serum amyloid A-4 protein (SAA4) P35542	Galectin-9 (Gal-9) O00182
SPARC-like protein 1 (SPARCL1) Q14515	Gastric intrinsic factor (GIF) P27352
Superoxide dismutase [Cu-Zn] (SOD1) P00441	Gastrotropin (GT) P51161
T-cell immunoglobulin and mucin domain-containing protein 4 (TIMD4) Q96H15	Growth hormone (GH) P01241
Tenascin (TNC) P24821	Growth/differentiation factor 2 (GDF-2) Q9UK05
Tenascin-X (TNXB) P22105	Heat shock 27 kDa protein (HSP 27) P04792
Thrombospondin-4 (THBS4) P35443	Heme oxygenase 1 (HO-1) P09601
Thyroxine-binding globulin (SERPINA7) P05543	Hydroxyacid oxidase 1 (HAOX1) Q9UJM8
Transcobalamin-2 (TCN2) P20062	Interleukin-1 receptor antagonist protein (IL-1ra) P18510
Transforming growth factor beta receptor type 3 (TGFBR3) Q03167	Interleukin-1 receptor-like 2 (IL1RL2) Q9HB29
Transforming growth factor-beta-induced protein ig-h3 (TGFBI) Q15582	Interleukin-4 receptor subunit alpha (IL-4RA) P24394
Trypsin-2 (PRSS2) P07478	Interleukin-6 (IL6) P05231
Tyrosine-protein kinase receptor Tie-1 (TIE1) P35590	Interleukin-17D (IL-17D) Q8TAD2
Uromodulin (UMOD) P07911	Interleukin-18 (IL-18) Q14116
Vascular cell adhesion protein 1 (VCAM1) P19320	Interleukin-27 (IL-27) Q8NEV9,
Vasorin (VASN) Q6EMK4	Q14213
Vitamin K-dependent protein C (PROC) P04070	Kidney Injury Molecule (KIM1) Q96D42
	Lactoylglutathione lyase (GLO1) Q04760
Cardiovascular II panel	Lectin-like oxidized LDL receptor 1 (LOX-1) P78380
2,4-dienoyl-CoA reductase, mitochondrial (DECR1) Q16698	Leptin (LEP) P41159
A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAM-TS13) Q76LX8	Lipoprotein lipase (LPL) P06858
ADM (ADM) P35318	Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b) P31994
Agouti-related protein (AGRP) O00253	Lymphotactin (XCL1) P47992
Alpha-L-iduronidase (IDUA) P35475	Macrophage receptor MARCO (MARCO) Q9UEW3
Angiopoietin-1 (ANG-1) Q15389	Matrix metalloproteinase-7 (MMP-7) P09237
Angiopoietin-1 receptor (TIE2) Q02763	Matrix metalloproteinase-12 (MMP-12) P39900

Angiotensin-converting enzyme 2 (ACE2) Q9BYF1	Melusin (ITGB1BP2) Q9UKP3
Bone morphogenetic protein 6 (BMP-6) P22004	Natriuretic peptides B (BNP) P16860
Brother of CDO (Protein BOC) Q9BWV1	NF-kappa-B essential modulator (NEMO) Q9Y6K9
Carbonic anhydrase 5A, mitochondrial (CA5A) P35218	Osteoclast-associated immunoglobulin-like receptor (hOSCAR) Q8IYS5
Carcinoembryonic antigenrelated cell adhesion molecule 8 (CEACAM8) P31997	Pappalysin-1 (PAPPA) Q13219
Cathepsin L1 (CTSL1) P07711	Pentraxin-related protein PTX3 (PTX3) P26022
C-C motif chemokine 3 (CCL3) P10147	Placenta growth factor (PGF) P49763
Platelet-derived growth factor subunit B (PDGF subunit B) P01127	Caspase-3 (CASP-3) P42574
Poly [ADP-ribose] polymerase 1 (PARP-1) P09874	Cathepsin D (CTSD) P07339
Polymeric immunoglobulin receptor (PIgR) P01833	Cathepsin Z (CTSZ) Q9UBR2
Programmed cell death 1 ligand 2 (PD-L2) Q9BQ51	C-C motif chemokine 15 (CCL15) Q16663
Proheparin-binding EGF-like growth factor (HB-EGF) Q99075	C-C motif chemokine 16 (CCL16) O15467
Pro-interleukin-16 (IL16) Q14005	Note: New assay under development N/A
Prolargin (PRELP) P51888	C-C motif chemokine 24 (CCL24) O00175
Prostasin (PRSS8) Q16651	CD166 antigen (ALCAM) Q13740
Protein AMBP (AMBP) P02760	Chitinase-3-like protein 1 (CHI3L1) P36222
Proteinase-activated receptor 1 (PAR-1) P25116	Chitotriosidase-1 (CHIT1) Q13231
Protein-glutamine gamma-glutamyltransferase 2 (TGM2) P21980	Collagen alpha-1(I) chain (COL1A1) P02452
Proto-oncogene tyrosine-protein kinase Src (SRC) P12931	Complement component C1q receptor (CD93) Q9NPY3
P-selectin glycoprotein ligand 1 (PSGL-1) Q14242	Contactin-1 (CNTN1) Q12860
Receptor for advanced glycosylation end products (RAGE) Q15109	C-X-C motif chemokine 16 (CXCL16) Q9H2A7
Renin (REN) P00797	Cystatin-B (CSTB) P04080
Serine protease 27 (PRSS27) Q9BQR3	Elafin (PI3) P19957
Serine/threonine-protein kinase 4 (STK4) Q13043	Ephrin type-B receptor 4 (EPHB4) P54760
Serpin A12 (SERPINA12) Q8IW75	Epidermal growth factor receptor (EGFR) P00533
SLAM family member 5 (CD84) Q9UIB8	Epithelial cell adhesion molecule (Ep-CAM) P16422
SLAM family member 7 (SLAMF7) Q9NQ25	E-selectin (SELE) P16581
Sortilin (SORT1) Q99523	Fatty acid-binding protein, adipocyte (FABP4) P15090
Spondin-2 (SPON2) Q9BUD6	Galectin-3 (Gal-3) P17931
Stem cell factor (SCF) P21583	Galectin-4 (Gal-4) P56470
Superoxide dismutase [Mn], mitochondrial (SOD2) P04179	Granulins (GRN) P28799

T-cell surface glycoprotein CD4 (CD4) P01730	Growth/differentiation factor 15 (GDF-15) Q99988
Thrombomodulin TM P07204	Insulin-like growth factor-binding protein 1 (IGFBP-1) P08833
Thrombopoietin (THPO) P40225	Insulin-like growth factor-binding protein 2 (IGFBP-2) P18065
Thrombospondin-2 (THBS2) P35442	Insulin-like growth factor-binding protein 7 (IGFBP-7) Q16270
Tissue factor (TF) P13726	Integrin beta-2 (ITGB2) P05107
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) O14763	Intercellular adhesion molecule 2 (ICAM-2) P13598
Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A) O00220	Interleukin-1 receptor type 1 (IL-1RT1) P14778
Tumor necrosis factor receptor superfamily member 11A (TNFRSF11A) Q9Y6Q6	Interleukin-1 receptor type 2 (IL-1RT2) P27930
Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B) O14836	Interleukin-2 receptor subunit alpha (IL2-RA) P01589
Tyrosine-protein kinase Mer (MERTK) Q12866	Interleukin-6 receptor subunit alpha (IL-6RA) P08887
Vascular endothelial growth factor D (VEGFD) O43915	Interleukin-17 receptor A (IL-17RA) Q96F46
V-set and immunoglobulin domain-containing protein 2 (VSIG2) Q96IQ7	Interleukin-18-binding protein (IL-18BP) O95998
Cardiovascular III panel	Junctional adhesion molecule A (JAM-A) Q9Y624
Aminopeptidase N (AP-N) P15144	Kallikrein-6 (KLK6) Q92876
Azurocidin (AZU1 P20160	Low-density lipoprotein receptor (LDL receptor) P01130
Bleomycin hydrolase (BLM hydrolase) Q13867	Lymphotoxin-beta receptor (LTBR) P36941
Cadherin-5 (CDH5) P33151	Matrix extracellular phosphoglycoprotein (MEPE) Q9NQ76
Carboxypeptidase A1 (CPA1) P15085	Matrix metalloproteinase-2 (MMP-2) P08253
Carboxypeptidase B (CPB1) P15086	Matrix metalloproteinase-3 (MMP-3) P08254
Matrix metalloproteinase-9 (MMP-9) P14780	von Willebrand factor (vWF) P04275
Metalloproteinase inhibitor 4 (TIMP4) Q99727	
Monocyte chemotactic protein 1 (MCP-1) P13500	**Inflammation panel**
Myeloblastin (PRTN3) P24158	Adenosine Deaminase (ADA) P00813
Myeloperoxidase (MPO) P05164	Artemin (ARTN) Q5T4W7
Myoglobin (MB) P02144	Axin-1 (AXIN1) O15169
Neurogenic locus notch homolog protein 3 (Notch 3) Q9UM47	Beta-nerve growth factor (Beta-NGF) P01138
N-terminal prohormone brain natriuretic peptide (NT-proBNP) NA	Caspase-8 (CASP-8) Q14790
Osteopontin (OPN) P10451	C-C motif chemokine 3 (CCL3) P10147
Osteoprotegerin (OPG) O00300	C-C motif chemokine 4 (CCL4) P13236
Paraoxonase (PON3) Q15166	C-C motif chemokine 19 (CCL19) Q99731

Peptidoglycan recognition protein 1 (PGLYRP1) O75594	C-C motif chemokine 20 (CCL20) P78556
Perlecan (PLC) P98160	C-C motif chemokine 23 (CCL23) P55773
Plasminogen activator inhibitor 1 (PAI) P05121	C-C motif chemokine 25 (CCL25) O15444
Platelet endothelial cell adhesion molecule (PECAM-1) P16284	C-C motif chemokine 28 (CCL28) Q9NRJ3
Platelet-derived growth factor subunit A (PDGF subunit A) P04085	CD40L receptor (CD40) P25942
Proprotein convertase subtilisin/kexin type 9 (PCSK9) Q8NBP7	CUB domain-containing protein 1 (CDCP1) Q9H5V8
Protein delta homolog 1 (DLK-1) P80370	C-X-C motif chemokine 1 (CXCL1) P09341
P-selectin (SELP) P16109	C-X-C motif chemokine 5 (CXCL5) P42830
Pulmonary surfactant-associated protein D (PSP-D) P35247	C-X-C motif chemokine 6 (CXCL6) P80162
Resistin (RETN) Q9HD89	C-X-C motif chemokine 9 (CXCL9) Q07325
Retinoic acid receptor responder protein 2 (RARRES2) Q99969	C-X-C motif chemokine 10 (CXCL10) P02778
Scavenger receptor cysteine-rich type 1 protein M130 (CD163) Q86VB7	C-X-C motif chemokine 11 (CXCL11) O14625
Secretoglobin family 3A member 2 (SCGB3A2) Q96PL1	Cystatin D (CST5) P28325
Spondin-1 (SPON1) Q9HCB6	Delta and Notch-like epidermal growth factor-related receptor (DNER) Q8NFT8
ST2 protein (ST2) Q01638	Eotaxin (CCL11) P51671
Tartrate-resistant acid phosphatase type 5 (TR-AP) P13686	Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) Q13541
Tissue factor pathway inhibitor (TFPI) P10646	Fibroblast growth factor 21 (FGF-21) Q9NSA1
Tissue-type plasminogen activator (t-PA) P00750	Fibroblast growth factor 23 (FGF-23) Q9GZV9
Transferrin receptor protein 1 (TR) P02786	Fibroblast growth factor 5 (FGF-5) Q8NF90
Trefoil factor 3 (TFF3) Q07654	Fibroblast growth factor 19 (FGF-19) O95750
Trem-like transcript 2 protein (TLT-2) Q5T2D2	Fms-related tyrosine kinase 3 ligand (Flt3L) P49771
Tumor necrosis factor ligand superfamily member 13B (TNFSF13B) Q9Y275	Fractalkine (CX3CL1) P78423
Tumor necrosis factor receptor 1 (TNF-R1) P19438	Glial cell line-derived neurotrophic factor (GDNF) P39905
Tumor necrosis factor receptor 2 (TNF-R2) P20333	Hepatocyte growth factor (HGF) P14210
Tumor necrosis factor receptor superfamily member 6 (FAS) P25445	Interferon gamma (IFN-gamma) P01579
Tumor necrosis factor receptor superfamily member 10C (TNFRSF10C) O14798	Interleukin-1 alpha (IL-1 alpha) P01583
Tumor necrosis factor receptor superfamily member 14 (TNFRSF14) Q92956	Interleukin-2 (IL-2) P60568
Tyrosine-protein kinase receptor UFO (AXL) P30530	Interleukin-2 receptor subunit beta (IL-2RB) P14784
Tyrosine-protein phosphatase non-receptor type substrate 1 (SHPS-1) P78324	Interleukin-4 (IL-4) P05112

Urokinase plasminogen activator surface receptor (U-PAR) Q03405	Interleukin-5 (IL5) P05113
Urokinase-type plasminogen activator (uPA) P00749	Interleukin-6 (IL6) P05231
Interleukin-7 (IL-7) P13232	Thymic stromal lymphopoietin (TSLP) Q969D9
Interleukin-8 (IL-8) P10145	TNF-related activation-induced cytokine (TRANCE) O14788
Interleukin-10 (IL10) P22301	TNF-related apoptosis-inducing ligand (TRAIL) P50591
Interleukin-10 receptor subunit alpha (IL-10RA) Q13651	Transforming growth factor alpha (TGF-alpha) P01135
Interleukin-10 receptor subunit beta (IL-10RB) Q08334	Tumor necrosis factor (Ligand) superfamily, member 12(TWEAK) O43508
Interleukin-12 subunit beta (IL-12B) P29460	Tumor necrosis factor (TNF) P01375
Interleukin-13 (IL-13) P35225	Tumor necrosis factor ligand superfamily member 14 (TNFSF14) O43557
Interleukin-15 receptor subunit alpha (IL-15RA) Q13261	Tumor necrosis factor receptor superfamily member 9(TNFRSF9) Q07011
Interleukin-17A (IL-17A) Q16552	Urokinase-type plasminogen activator (uPA) P00749
Interleukin-17C (IL-17C) Q9P0M4	Vascular endothelial growth factor A (VEGF-A) P15692
Interleukin-18 (IL-18) Q14116	
Interleukin-18 receptor 1 (IL-18R1) Q13478	
Interleukin-20 (IL-20) Q9NYY1	
Interleukin-20 receptor subunit alpha (IL-20RA) Q9UHF4	
Interleukin-22 receptor subunit alpha-1 (IL-22 RA1) Q8N6P7	
Interleukin-24 (IL-24) Q13007	
Interleukin-33 (IL-33) O95760	
Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1) P01137	
Leukemia inhibitory factor (LIF) P15018	
Leukemia inhibitory factor receptor (LIF-R) P42702	
Macrophage colony-stimulating factor 1 (CSF-1) P09603	
Matrix metalloproteinase-1 (MMP-1) P03956	
Matrix metalloproteinase-10 (MMP-10) P09238	
Monocyte chemotactic protein 1 (MCP-1) P13500	
Monocyte chemotactic protein 2 (MCP-2) P80075	
Monocyte chemotactic protein 3 (MCP-3) P80098	
Monocyte chemotactic protein 4 (MCP-4) Q99616	
Natural killer cell receptor 2B4 (CD244) Q9BZW8	

Neurotrophin-3 (NT-3) P20783	
Neurturin (NRTN) Q99748	
Oncostatin-M (OSM) P13725	
Osteoprotegerin (OPG) O00300	
Programmed cell death 1 ligand 1 (PD-L1) Q9NZQ7	
Protein S100-A12 (EN-RAGE) P80511	
Signaling lymphocytic activation molecule (SLAMF1) Q13291	
SIR2-like protein 2 (SIRT2) Q8IXJ6	
STAM-binding protein (STAMBP) O95630	
Stem cell factor (SCF) P21583	
Sulfotransferase 1A1 (ST1A1) P50225	
T cell surface glycoprotein CD6 isoform (CD6) Q8WWJ7	
T-cell surface glycoprotein CD5 (CD5) P06127	
T-cell surface glycoprotein CD8 alpha chain (CD8A) P01732	
TNF-beta (TNFB) P01374	

Supplemental table 2 Circulating protein concentrations (in NPX) according to sex

	Global (N=392)	Male (N=142)	Female (N=250)	p-value
IGFBP3	4.4 ± 0.6	4.2 ± 0.6	4.6 ± 0.5	<0.001
IL1RL2	3.2 ± 0.4	3.1 ± 0.4	3.3 ± 0.4	<0.001
LPL	9.8 ± 0.6	9.5 ± 0.6	9.9 ± 0.5	<0.001
Ep-CAM	4.8 ± 1.0	4.5 ± 0.8	5.0 ± 1.0	<0.001
PLIN1	2.2 ± 0.8	1.9 ± 0.6	2.3 ± 0.8	<0.001
LHB	1.6 ± 1.1	1.1 ± 1.1	2.0 ± 1.0	<0.001
NRP1	2.2 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	<0.001
MMP_3	5.9 ± 0.8	6.3 ± 0.7	5.6 ± 0.7	<0.001
ACE2	3.8 ± 0.7	4.1 ± 0.8	3.7 ± 0.6	<0.001

Legend: IGFBP3, insulin-like growth factor-binding protein 3; NRP1, neuropilin 1; IL1RL2, interleukin-1 receptor-like 2; LPL, lipoprotein lipase; ACE2, angiotensin-converting-enzyme 2; Ep_CAM, epithelial cell adhesion molecule; CA14, carbonic anhydrase 14; PLIN1, perilipin-1; LHB, lutropin subunit beta.

Supplemental Table 3 ***Circulating protein concentrations (in NPX) according to sex (after exclusion of proteins with correlation > 0.5)***

	Global (N=392)	Male (N=142)	Female (N=250)	p-value
IGFBP3	4.4 ± 0.6	4.2 ± 0.6	4.6 ± 0.5	<0.0001
NRP1	2.2 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	<0.0001
IL1RL2	3.2 ± 0.4	3.1 ± 0.4	3.3 ± 0.4	<0.0001
LPL	9.8 ± 0.6	9.5 ± 0.6	9.9 ± 0.5	<0.0001
ACE2	3.8 ± 0.7	4.1 ± 0.8	3.7 ± 0.6	<0.0001
LEP	5.8 ± 1.1	5.1 ± 1.1	6.2 ± 0.9	<0.0001
Gal_3	5.2 ± 0.4	5.0 ± 0.4	5.2 ± 0.4	<0.0001
Ep_CAM	4.8 ± 1.0	4.5 ± 0.8	5.0 ± 1.0	<0.0001
MB	7.2 ± 0.7	7.4 ± 0.7	7.0 ± 0.7	<0.0001
MMP_3	5.9 ± 0.8	6.3 ± 0.7	5.6 ± 0.7	<0.0001
CA14	1.2 ± 0.4	1.3 ± 0.4	1.1 ± 0.4	<0.0001
PLIN1	2.2 ± 0.8	1.9 ± 0.6	2.3 ± 0.8	<0.0001
CALCA	4.7 ± 1.0	5.3 ± 1.0	4.4 ± 0.9	<0.0001
LHB	1.6 ± 1.1	1.1 ± 1.1	2.0 ± 1.0	<0.0001

Legend: IGFBP3, insulin-like growth factor-binding protein 3; NRP1, neuropilin 1; IL1RL2, interleukin-1 receptor-like 2; LPL, lipoprotein lipase; ACE2, angiotensin-converting-enzyme 2; LEP, leptin; Gal_3, galectin 3; Ep_CAM, epithelial cell adhesion molecule; MB, myoglobin, MMP_3, matrix metalloproteinase 3; CA14, carbonic anhydrase 14; PLIN1, perilipin 1; CALCA, calcitonin; LHB, lutropin subunit beta.