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ORIGINAL RESEARCH ARTICLE

Identification of sex-specific biomarkers predicting new-onset heart failure

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Abstract

Aims Heart failure (HF) is common in both men and women, yet disease pathophysiology, presentation, and progression differ between sexes. Studies addressing whether biomarkers predict new onset HF sex-specifically are scarce. This study therefore aims to test the sex-specificity of 252 protein biomarkers for new-onset HF.

Methods and results A matched case–control design in patients selected from cohorts within the HOMAGE consortium was used. Cases (new-onset HF, n = 562) and controls (n = 780) were matched for cohort (PREDICTOR, HEALTH-ABC, & PROSPER), follow-up time (defined as time from entry to incident HF), and age. Incident HF was defined as first hospitalization for HF. Targeted plasma proteins (n = 252) were measured using Proximity Extension Assay technology from O-link. To look for sex differences for new onset HF, we adjusted for cohort, age, and baseline clinical parameters. At baseline, women had a biomarker profile reflecting activated metabolism and immune responses. However, none of the biomarkers had a significant interaction with sex in predicting new onset HF, but four biomarkers had a trend towards sex-specificity ($P < 0.013$). E-selectin and interleukin 1 receptor antagonist were more female-specific, whereas IL17A and CHIT1 tended to be male sex-specific for incident HF.

Conclusions The majority of biomarkers associated with incident HF did not significantly differ between women and men, despite clear differences in biomarkers at baseline.

Keywords Proteomics; Incident heart failure; Sex differences

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Introduction

Heart failure (HF) is a major cause of morbidity and mortality worldwide and the most frequent cause of hospitalization for patients over 65 years of age.[1] Although life-time risk for HF is substantial and similar in both men and women, its onset and phenotype are significantly different between sexes.[2–4] In general, women usually develop HF at an older age, experience a lower quality of life, are more symptomatic, but have a better prognosis and respond better to therapy.[3,5] For example, it has been suggested that women may gain greater benefit from spironolactone[6] or respond differently to cardiac resynchronization therapy.[7] Women have more often a non-ischaemic aetiology and HF with preserved ejection fraction (HFpEF), whereas men are more likely to have ischemic HF and HF with reduced ejection fraction.[3,5] Also, in other cardiovascular diseases (CVD) such as coronary artery disease (CAD), atrial fibrillation, and stroke, sex differences exist.[8,9] The exact mechanisms behind these sex differences are not completely understood, but it is thought that hormonal status and a different profile of comorbidities such as hypertension, inflammation, and diabetes mellitus (DM) are likely to play a major role.[2,9] Studies investigating sex differences in biomarkers predicting incident HF are scarce, and although absolute levels of biomarkers may be different in men and women, predictive value does not seem to differ.[10] Deeper insight in sex-specific biomarker profiles may help to improve sex-specific prediction of incident HF and may also help to understand the underlying pathophysiology of those sex-differences. In contrast to previous studies investigating sex-differences in biomarkers associated with HF, we used a total of 252 biomarkers in this study. The current study reports on (i) sex-specific associations with a broad panel of biomarkers at baseline, irrespective of HF development, and (ii) sex-specific biomarker interactions with new-onset HF.

Methods

Study population

The Heart OMics in AGEing consortium (HOMAGE; *NCT02556450*) is an EU-funded programme that aims to identify and validate biomarkers associated with incident HF in order to develop new and personalized preventive strategies. The HOMAGE consortium collected data from 20 completed and ongoing European studies enrolling healthy subjects and patients at high risk of, or with, CVD. We identified cohorts in whom individuals had been followed-up until first hospitalization for HF and patients at high risk of CVD, and these data were merged in a common database.[11] We selected individuals who were hospitalized for HF without prior diagnosis of HF. Cases of incident HF were identified from two suitable cohorts (PREDICTOR &[12] HEALTH-ABC[13]) and one clinical trial (PROSPER[11,14]). We used a case–control design to identify a control group matched for cohort, follow-up time (defined as time between incident HF and entry to the cohort), age, and sex as was performed in a previous study.[15] The study had two independent phases in which plasma protein assays were performed: a discovery phase (phase 1a) and a replication phase (phase 1b) with patient selection as previously described.[15] For phase 1a, cases and controls were randomly selected in a 2:1 proportion. For phase 1b, cases and controls were randomly selected in a 1:1 proportion. After exclusion of poor-quality samples, the final match was 562 HF cases and 780 controls (pooled data), divided over 286 cases and 515 controls in *phase 1a* and 276 cases and 265 controls in *phase 1b*. Clinical data include baseline characteristics, previous medical history, and routine haematological and biochemical measurements. The study was conducted in accordance with the Declaration of Helsinki and approved by each site's ethics committees. All participants provided written informed consent.

Plasma protein assays

A total of 252 targeted plasma proteins were assessed in baseline samples by TATAA Biocenter (Gothenburg, Sweden) using PEA chemistry and three pre-designed panels from Olink Proteomics (Uppsala, Sweden). The methods of the assays have been published.[15] Abbreviations, full names and respective Olink® multiplex panels of the studied proteins are described in Supporting Information, *Table S1*. The samples were randomized before measurement. The concentrations of proteins were expressed in Olink's arbitrary unit (NPX), which is in a Log2 scale.

Statistical analyses

Continuous variables are expressed as means ± standard deviation. Categorical variables are presented as frequencies and percentages. Clinical characteristics were compared between men and women, and between cases and controls, using independent sample *t*-tests for continuous variables and χ^2 tests for categorical variables. Outliers in the plasma protein data were detected by the Tukey's fences method were manually checked and removed before analyses.

Association between biomarkers and sex

To assess the relation of each plasma protein with sex, we adjusted all analyses for cohort (PREDICTOR,[12] HEALTH-ABC,[13] or PROSPER[11,14]), age, and the pre-specified clinical HOMAGE risk model for HF (smoking, DM, history of CAD, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate).[16] For each biomarker, the standardized beta-coefficient of sex is

reported, with a positive value implying higher levels in women and a negative value implying higher levels in men. We corrected for multiple testing using the false discovering rate of 5% (q value < 0.05). All analyses were performed in the pooled data and replicated both in phase 1a and phase 1b independently.

Biomarker interactions with sex in the association with new-onset heart failure

Adjusted Cox linear regression models were performed to examine associations of individual biomarkers with incident HF. Next, we tested for *biomarker*sex* interactions in the adjusted model for incident HF. In the adjusted models, we adjusted for age, cohort, and the prespecified clinical HOMAGE risk model for HF. We corrected for multiple testing using the false discovering rate of 5%. All analyses were performed in the pooled data and replicated both in phase 1a and phase 1b independently. We defined a *suggestive* interaction as having (i) a significant interaction with sex with $P < 0.05$ in the pooled data, (ii) $P < 0.1$ in phase 1a and 1b, and (iii) the coefficients were in the same direction in all phases. All analyses were conducted in R environment Version 3.5.

Bio-informatical analysis

We performed gene-enrichment in pathway analysis using all biomarkers that were significantly enriched in male vs. female analyses at baseline. The overrepresentation analysis was performed using Gene Ontology (GO) processes, providing a computational representation of biological processes and molecular functions enriched by the significant biomarkers against proteins on the OLINK panels, introducing an adjustment for the clustering and correlation of proteins on the panels, further consolidating the results.[17,18] Clustering of a biomarker within a biological function (inflammation, metabolism, extracellular matrix, and metalloendopeptidase activity) is based on the main association of the biomarker using GO-biological processes, UniProt, and GeneCards.

Results

Study population

The clinical characteristics in men and women in both HF cases and controls are depicted in *Table 1*. Cases and controls were matched for age, sex, cohort, and follow-up time as described and performed previously (*Table S2*).[15] Mean follow-up time (time to incident HF) was 5.5 ± 4.3 years. Women had higher concentrations of both LDL and HDL, lower creatinine, were more often non- or ex-smokers, and had a lower prevalence of CAD. In both men and women, HF cases were more likely to have hypertension, diabetes, and CAD at baseline. Body mass index and LDL were significantly higher in female cases compared with controls, but not in men. Male cases had a higher creatinine concentration compared with male controls.

Sex-specific associations of biomarkers irrespective of new-onset heart failure

A total of 94 biomarkers were significantly different between sexes after adjusting for cohort, age, smoking, diabetes mellitus, history of CAD, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate in the pooled data and replicated in both phases independently (P value < 0.05; *Table S3*). More biomarkers were higher in women (n = 89) compared with men (n = 5) (*Figure 1*). Matrix metalloproteinase-3 (MMP-3) had the strongest association with male sex (effect size −0.37, $P < 0.0001$, *Table S3*, *Figure 1*). Leptin, fatty acid-binding protein 4, trefoil factor 3, and low-density lipoprotein receptor were the biomarkers strongly increased in women (effect size 0.51, 0.48, 0.30, and 0.26, respectively, all $P < 0.0001$; *Table S3*, *Figure 1*). The male bio profile was most strongly associated with *metalloendopeptidase activity* (GO:0004222), and the female profile was dominated by *immune response* (GO:0006955) and *regulation of metabolic processes* (GO:0019222), irrespective of HF development (*Figure 1*).

Biomarker interaction with sex in the association with incident heart failure

A total of 93 biomarkers were significantly associated with incident HF in the pooled data, after adjustment for age, cohort, and the prespecified clinical HOMAGE risk model for incident HF ($q < 0.05$; *Table S4*). None of the biomarkers showed a significant interaction with sex for incident HF after adjustment for multiple testing. Four biomarkers showed a suggestive interaction with sex for incident HF in the pooled data ($p_{int} < 0.05$), and consistent hazard ratios (HR) in the same direction in both phases (*Tables 2* & *S5*). In SELE and IL1RA, higher values were associated with incident HF only in women [HR: 1.13; 95% confidence interval (CI): 1.05–1.40 and HR: 1.30; 95% CI: 1.14–1.48 respectively], whereas higher values of IL17A and CHIT1 are associated with incident HF only in men (HR: 1.19; 95% CI: 1.06–1.32 and HR: 1.14; 95% CI: 1.01–1.30, respectively; *Figure 2*; *Table 2*). However, the suggestive interaction of IL1RA was the only biomarker remaining after validating in both phases (phases 1a and 1b), indicating no strong interaction with sex in biomarkers for the development of HF in this population (*Table S5*).

Table 1 Characteristics of the study population for both men and women

Characteristic *N* = 1343	Men				Women				
	All men (*n* = 735)	Case (*n* = 311)	Control (*n* = 424)	*P* value**	All women (*n* = 607)	Case (*n* = 251)	Control (*n* = 356)	*P* value**	*P* value*
Age (year)	**75 ± 4**	75 ± 3	75 ± 4	0.78	**75 ± 4**	75 ± 4	75 ± 4	0.83	**0.47**
Cohort, *n* (%)				0.96				0.81	**0.63**
HEALTH-ABC	**418 (57)**	175 (56)	243 (57)		**357 (59)**	149 (59)	208 (58)		
PREDICTOR	**53 (7)**	23 (7)	30 (7)		**47 (8)**	21 (8)	26 (7)		
PROSPER	**264 (36)**	113 (36)	151 (36)		**203 (33)**	81 (32)	122 (34)		
Time to incident HF (years)	**5.5 ± 4.2**	5.4 ± 4.1	5.6 ± 4.3	0.37	**5.5 ± 4.3**	5.3 ± 4.2	5.7 ± 4.4	0.38	**1.00**
Smoking status, *n* (%)				0.59				0.69	<**0.01**
Never	**335 (46)**	138 (44)	197 (47)		**393 (65)**	158 (63)	235 (66)		
Current	**116 (16)**	54 (17)	62 (15)		**79 (13)**	33 (13)	46 (13)		
Past	**284 (39)**	119 (38)	165 (39)		**135 (22)**	60 (24)	75 (21)		
Body mass index (kg/m^2)	**27.0 ± 3.6**	27.3 ± 3.7	26.8 ± 3.6	0.06	**27.5 ± 5.3**	28.5 ± 5.8	26.8 ± 4.8	<0.01	**0.07**
Systolic blood pressure (mmHg)	**143 ± 22**	144 ± 23	142 ± 22	0.18	**144 ± 24**	146 ± 24	143 ± 24	0.16	**0.31**
Diastolic blood pressure (mmHg)	**77 ± 13**	77 ± 14	76 ± 12	0.21	**76 ± 13**	76 ± 14	76 ± 13	0.59	**0.18**
Heart rate (bpm)	**65 ± 12**	67 ± 12	64 ± 12	<0.01	**67 ± 11**	68 ± 11	66 ± 10	0.05	<**0.01**
Serum creatinine (μmol/L)	**106 ± 24**	111 ± 28	103 ± 19	<0.01	**86 ± 21**	88 ± 22	85 ± 21	0.06	<**0.01**
HDL-cholesterol (mmol/L)	**1.2 ± 0.3**	1.2 ± 0.3	1.2 ± 0.3	0.25	**1.5 ± 0.4**	1.4 ± 0.4	1.5 ± 0.4	<0.01	<**0.01**
LDL-cholesterol (mmol/L)	**3.3 ± 0.8**	3.2 ± 0.8	3.3 ± 0.8	0.81	**3.5 ± 1.0**	3.5 ± 1.0	3.5 ± 1.0	0.56	<**0.01**
Comorbidities, *n* (%)									
Hypertension	**436 (59)**	200 (64)	236 (56)	0.02	**397 (65)**	190 (76)	207 (58)	<0.01	**0.02**
Diabetes mellitus	**111 (15)**	58 (19)	53 (13)	0.03	**83 (14)**	47 (19)	36 (10)	<0.01	**0.48**
CAD	**268 (37)**	141 (45)	127 (30)	<0.01	**132 (22)**	77 (31)	55 (15)	<0.01	<**0.01**

Numbers are mean (SD) for numeric variables, and counts (%) for categorical variables.
ACE, angiotensin-converting enzyme; ARBs, angiotensin receptors diseases; CAD, coronary artery disease; CCB, calcium channel blockers; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAD, peripheral arterial diseases.
*Difference between all male and all female participants.
**Difference between cases and controls.

Figure 1 Sex-specific associations of biomarkers irrespective of new-onset HF. Overview of the 94 biomarkers that are significantly different between sexes. Positive beta-values indicate higher values in women, negative beta-values indicate higher values in men. Clustering of a biomarker within a biological function is based on the main association of the biomarker using GO-biological processes, UniProt, and GeneCards.

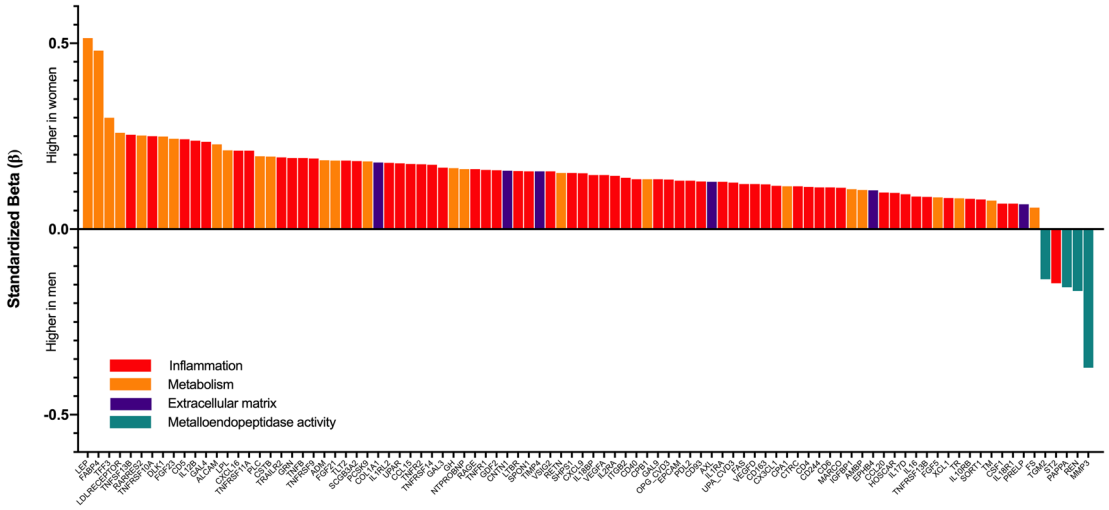

Table 2 Association of biomarkers with incident heart failure: men vs. women

Biomarker	Men		Women		Interaction
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	p_{int}
SELE	0.96 (0.84–1.09)	0.55	1.22 (1.05–1.40)	**0.005**	**0.004**
IL1RA	1.10 (0.98–1.24)	0.11	1.30 (1.14–1.48)	**<0.001**	**0.007**
IL17A	1.19 (1.06–1.32)	**0.002**	0.89 (0.77–1.02)	0.091	**0.009**
CHIT1	1.14 (1.01–1.30)	**0.037**	0.93 (0.82–1.05)	0.24	**0.013**

All associations were adjusted for cohort, age, smoking, diabetes mellitus, history of coronary artery disease, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication and heart failure.
CI, confidence interval; HR, hazard ratio.

Discussion

In this study, we investigated sex-specificity of protein biomarkers both at baseline levels, and in association with incident HF in a matched case–control design with 562 cases (new-onset HF) and 780 controls selected out of different international cohorts within the HOMAGE consortium.[11] Our principal findings are as follows: (i) several circulating protein biomarkers are significantly different between sexes at baseline, suggesting the presence of biological differences; (ii) a majority of biomarkers was strongly associated with incident HF in both sexes; and (iii) no robust sex-specific association between a biomarker and incident HF was observed, although subtle sex-related differences were found in four biomarkers (SELE, IL1RA, IL17, and CHIT1). We speculate that the biological sex-specific differences may overshadow sex-specific associations of biomarkers related to incident HF development.

Biological sex differences in protein biomarkers

Sex is known to have a large influence on biomarker levels in the blood. Differences in biomarkers can reflect both distinct physiological and pathophysiological pathways in men and women, which could be associated with incident HF.[19] Sex differences have been detected in proteomic as well as transcriptomic profiles in healthy populations,[20] in which the differentially expressed proteins and transcripts seem to underlie the biological differences in circulating biomarkers.[21] Sex hormones, accompanied by differences in fat distribution among men and women, play a major role in the specific proteomic profiles.[21,22] Alterations in circulating biomarkers associated with cardiometabolic risk can already be measured in asymptomatic women, which showed the influence of body composition and cardiac morphology on CVD risk.[19,23] We also detected differences in bioprofiles between men and women irrespective of disease and characterized a bio profile of immuno-metabolic risk in women.

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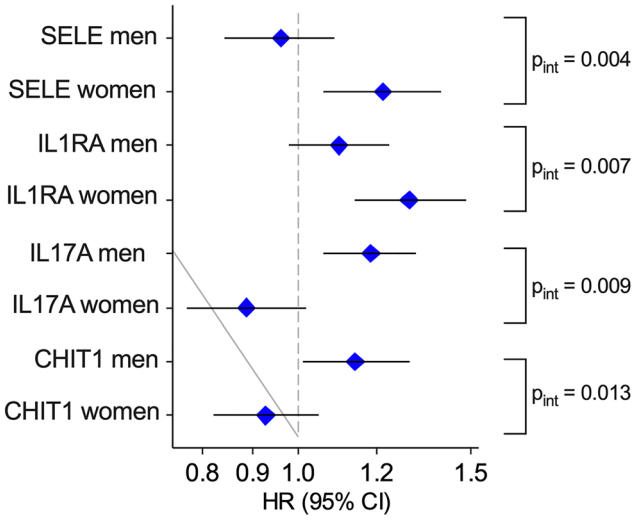
Figure 2 Association of biomarkers with incident heart failure: men vs. women. Hazard ratios with 95% confidence interval of the biomarkers that had a suggestive interaction with sex in the association with incident HF. All associations were adjusted for the prespecified clinical HOMAGE risk model: smoking, DM, history of coronary artery disease (CAD), serum creatinine, body mass index (BMI), systolic blood pressure (SBP), use of antihypertensive medication, and heart rate (HR). The interaction *P* value (P_{int}) denotes the sex*biomarker interaction. CHIT1, chitotriosidase 1; IL1RA, interleukin 1 receptor antagonist; IL17A, interleukin 17A; SELE, E-selectin.



Sex-specific biomarkers in heart failure

Male and female HF patients have different biomarker profiles, both on transcriptomic and proteomic levels.[24,25] Most literature focuses on sex differences in biomarkers that are used in HF management, such as N-terminal pro brain natriuretic peptide and cardiac troponins. For example, N-terminal pro brain natriuretic peptide levels tend to be higher in women with HF compared with men.[19,23] We did not find a sex-specific association for any biomarker with incident HF, in line with a previous study investigating 10 biomarkers.[10] It is possible that the biological differences are of such magnitude that they overshadow sex-specific associations related to HF development in this population at risk.[25,26]

Associations between biomarkers and sex with incident heart failure

We observed a baseline increase in proteins of the immune response in women. Endothelial inflammation has been associated with HF in women, which could explain the increase in markers for endothelial leukocyte migration (SELE and IL1RA), both involved in immunoregulatory and inflammatory processes. Up-regulation of SELE mediates the interaction and adhesion of leukocytes—especially neutrophils—and cytokine-activated endothelium. It controls inflammation in many diseases including atherosclerosis and HF.[27–29] Increased circulating levels of SELE are associated with an increased risk of developing essential hypertension and metabolic syndrome in women, which is an important risk factor for the development of HFpEF, a HF phenotype that is predominantly present in women.[30–32] IL1RA is a member of the interleukin-1 family and is also involved in immunological and inflammatory processes.[33] IL1RA inhibits the activity of interleukin-1 by binding to the IL1-receptor.[34] A meta-analysis of six population-based cohorts showed that IL-1RA levels are associated with a higher risk of cardiovascular disease, as it is induced by endothelial activation, oxidative stress, and subclinical inflammation.[35] The role of both SELE and IL1RA in the inflammatory response and their association with cardiovascular disease might make them promising HF biomarkers in women, clearly warranting further investigation.

Two biomarkers had a suggestive interaction with male sex in the association with incident HF. CHIT1 is mainly produced and secreted by activated human macrophages and plays a role in the inflammatory processes of atherosclerosis. Activated macrophages in atherosclerotic plaques induce the formation of foam cells, which are a landmark for the development of atherosclerosis and myocardial infarction.[36,37] Serum levels of CHIT1 are found to be elevated in patients with ischemic HF, which is especially common in men, as result of atherosclerosis.[3,38] IL17 is a proinflammatory cytokine produced by activated T-cells (T-helper 17 cells).[39] Binding to the IL17 receptor leads to induction of chemokines that recruit immune cells such as monocytes and neutrophils.[40]

The finding of sex-specific associations of circulating biomarkers with incident HF could improve our understanding of sex-specific mechanisms underlying HF development. Most biomarkers measured in this study were higher in women compared with men at baseline; however, most of these biomarkers reflect biological sex-based differences and to a lesser extent discriminative between HF cases and controls. The study design and patient population can be an important determining factor, as the blood was sampled at time of inclusion, which was around 5 years[2–9] before participants developed HF. Therefore, the blood sampling might be performed in a too early stage in which the biological differences overshadow biomarkers related to HF development, Still, these results are in line with a recent study, investigating sex-specific biomarkers in 22 765 participants (2095 HF cases and 20 670 controls). This study showed that biomarkers only have limited value in improving a clinical HF risk prediction model.[10] In line, biological sex-differences are primarily present in healthy individuals, and less pronounced in HF patients, suggesting that the biological differences in biomarkers might become overshadowed due to HF processes in a later stage.[25]

Limitations

The combination of this case–control study design and the used statistical and bioinformatical analyses does not allow causality assessment. Therefore, we acknowledge the exploratory nature of our study and emphasize the descriptive and hypothesis generating nature of this study. Incident HF was defined as first HF hospitalization, neglecting the fact that patients might be diagnosed with HF before their first hospitalization. Also, detailed clinical data such as ejection fraction, symptoms, and electrocardiogram parameters were not available, which limited the ability to stratify patients based on HF with preserved or reduced ejection fraction or other clinical parameters. Finally, three Olink panels with pre-selected proteins were measured (CVD II, CVD III, and inflammation). These panels are mainly focused on circulating proteins that are previously found to be associated with cardiovascular and inflammatory diseases. Other potentional pathways involved in HF development are not included but could have enriched the analyses.

Conclusions

The majority of biomarkers associated to incident HF were broadly similar in women and men. Circulating biomarkers are predominantly higher in women and reflect biological sex differences. The at-risk population might be in an early stage in which biological differences overshadow biomarkers related to HF development.

Conflict of interest

TT has filed and licensed patents in the field of noncoding RNAs. TT is founder and shareholder of Cardior Pharmaceuticals GmbH. TT reports speaker fees or other support from Boehringer Ingelheim, Novo Nordisk, Amicus Therapeutics, Sanofi-Genzyme and Takeda (outside the scope of this article). The UMCG, which employs RdB has received research grants and/or fees from AstraZeneca, Abbott, Boehringer Ingelheim, Cardior Pharmaceuticals Gmbh, Ionis Pharmaceuticals, Inc., Novo Nordisk, and Roche. RdB received speaker fees from Abbott, AstraZeneca, Bayer, Novartis, and Roche. NV has received speaker fees from Siemens, Abbot and Roch Diagnostics outside the scope of this study.

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Supporting information

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

Table S1. Protein names and respective Olink® panel sorted in alphabetical order.
Table S2. Characteristics of the Study Population for cases and controls.
Table S3. Biological sex differences in protein biomarkers.
Table S4. Biomarkers that are significantly associated with incident HF in pooled data.
Table S5. Association of biomarkers with incident heart failure: men versus women in pooled data, phase 1a and 1b.

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