



HAL
open science

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

Anne Raafs, Job Verdonschot, João Pedro Ferreira, Ping Wang, Timothy Collier, Michiel Henkens, Jens Björkman, Alessandro Boccanelli, Andrew L Clark, Christian Delles, et al.

► **To cite this version:**

Anne Raafs, Job Verdonschot, João Pedro Ferreira, Ping Wang, Timothy Collier, et al.. Identification of sex-specific biomarkers predicting new-onset heart failure. ESC Heart Failure, 2021, 10.1002/ehf2.13476 . hal-03273973

HAL Id: hal-03273973

<https://hal.univ-lorraine.fr/hal-03273973>

Submitted on 29 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

Anne Raafs¹, Job Verdonschot^{1,2}, João Pedro Ferreira³, Ping Wang², Timothy Collier⁴, Michiel Henkens¹, Jens Björkman⁵, Alessandro Boccanelli⁶, Andrew L. Clark⁷, Christian Delles⁸, Javier Diez^{9,10,11,12}, Arantxa González^{9,10,11}, Nicolas Girerd³, J. Wouter Jukema^{13,14}, Florence Pinet¹⁵, Patrick Rossignol³, Thomas Thum^{16,17}, Nicolas Vodovar¹⁸, Rudolf A. de Boer¹⁹, Vanessa van Empel¹, Jan A. Staessen^{20,21}, Mark Hazebroek¹, John Cleland^{22,23}, Faiez Zannad³ and Stephane Heymans^{1,14,24*}

¹Department of Cardiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre, P. Debyelaan 25, Maastricht, 6229 HX, The Netherlands; ²Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands; ³INSERM, Centre d'Investigations Cliniques-Plurithématique 14-33, CHRU Nancy, and INSERM U1116, CHRU, F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Université de Lorraine, Nancy, France; ⁴Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK; ⁵Tataa Biocenter AB, Gothenburg, Sweden; ⁶Casa di Cura Quisisana, Rome, Italy; ⁷Hull University Teaching Hospitals NHS Trust, Castle Hill Hospital, Cottingham, UK; ⁸Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; ⁹Program of Cardiovascular Diseases, CIMA Universidad de Navarra, Pamplona, Spain; ¹⁰CIBERCV, Carlos III Institute of Health, Madrid, Spain; ¹¹Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; ¹²Departments of Nephrology, and Cardiology and Cardiac Surgery, University of Navarra Clinic, Pamplona, Spain; ¹³Department of Cardiology, Leiden University Medical Centre, Leiden, The Netherlands; ¹⁴Netherlands Heart Institute, Utrecht, The Netherlands; ¹⁵Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Facteurs de risque et déterminants moléculaires des maladies liées au vieillissement, F-59000 Lille, and F-CRIN INI-CRCT, Univ. Lille, Lille, France; ¹⁶Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany; ¹⁷Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hanover, Germany; ¹⁸Inserm UMR-S 942, F-CRIN INI-CRCT, Department of Anaesthesiology and Intensive Care, Hôpital Lariboisière, Université de Paris, Paris, France; ¹⁹Department of Cardiology, University of Groningen, University Medical Centre Groningen, Groningen, The Netherlands; ²⁰Non-Profit Research Institute Alliance for the Promotion of Preventive Medicine, Mechelen, Belgium; ²¹Biomedical Sciences Group, Faculty of Medicine, University of Leuven, Leuven, Belgium; ²²Robertson Centre for Biostatistics and Clinical Trials, Institute of Health and Wellbeing, Glasgow, UK; ²³National Heart and Lung Institute, Royal Brompton and Harefield Hospitals, Imperial College, University of Glasgow, London, UK; ²⁴Department of Cardiovascular Research, University of Leuven, Leuven, Belgium

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

Received: 26 March 2021; Revised: 20 May 2021; Accepted: 1 June 2021

*Correspondence to: Stephane Heymans, Department of Cardiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre, P. Debyelaan 25, 6229 HX Maastricht, the Netherlands. Tel: +31 43 3875099; Fax: +31 43 3877081. Email: s.heyman@maastrichtuniversity.nl
Anne Raafs and Job Verdonschot contributed equally to this work.

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 spironolactone⁶ or respond differently to cardiac resynchronization therapy.⁷ Women have more often a non-ischæmic aetiology and HF with preserved ejection fraction (HFpEF), whereas men are more likely to have ischæmic 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-ABC¹³) 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 ($p_{\text{int}} < 0.05$), 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) | | | All women (n = 607) | | | Case (n = 251) | | | Control (n = 356) | | |
| | Case (n = 311) | Control (n = 424) | P value** | Case (n = 251) | Control (n = 356) | P value** | Case (n = 251) | Control (n = 356) | P value** | Case (n = 251) | Control (n = 356) | P value** |
| Age (year) | 75 ± 4 | 75 ± 4 | 0.78 | 75 ± 4 | 75 ± 4 | 0.83 | 75 ± 4 | 75 ± 4 | 0.83 | 75 ± 4 | 75 ± 4 | 0.47 |
| Cohort, n (%) | 418 (57) | 243 (57) | 0.96 | 149 (59) | 208 (58) | 0.81 | 149 (59) | 208 (58) | 0.81 | 149 (59) | 208 (58) | 0.63 |
| HEALTH-ABC | 53 (7) | 30 (7) | | 21 (8) | 26 (7) | | 21 (8) | 26 (7) | | 21 (8) | 26 (7) | |
| PREDICTOR | 264 (36) | 151 (36) | | 81 (32) | 122 (34) | | 81 (32) | 122 (34) | | 81 (32) | 122 (34) | |
| PROSPER | 5.5 ± 4.2 | 5.6 ± 4.3 | 0.37 | 5.3 ± 4.2 | 5.7 ± 4.4 | 0.38 | 5.3 ± 4.2 | 5.7 ± 4.4 | 0.38 | 5.3 ± 4.2 | 5.7 ± 4.4 | 1.00 |
| Time to incident HF (years) | 5.4 ± 4.1 | 5.6 ± 4.3 | 0.59 | 5.3 ± 4.2 | 5.7 ± 4.4 | 0.69 | 5.3 ± 4.2 | 5.7 ± 4.4 | 0.69 | 5.3 ± 4.2 | 5.7 ± 4.4 | <0.01 |
| Smoking status, n (%) | | | | | | | | | | | | |
| Never | 335 (46) | 197 (47) | | 158 (63) | 235 (66) | | 158 (63) | 235 (66) | | 158 (63) | 235 (66) | |
| Current | 116 (16) | 62 (15) | | 33 (13) | 46 (13) | | 33 (13) | 46 (13) | | 33 (13) | 46 (13) | |
| Past | 284 (39) | 165 (39) | | 60 (24) | 75 (21) | | 60 (24) | 75 (21) | | 60 (24) | 75 (21) | |
| Body mass index (kg/m ²) | 27.0 ± 3.6 | 26.8 ± 3.6 | 0.06 | 27.5 ± 5.3 | 26.8 ± 4.8 | <0.01 | 28.5 ± 5.8 | 26.8 ± 4.8 | <0.01 | 28.5 ± 5.8 | 26.8 ± 4.8 | 0.07 |
| Systolic blood pressure (mmHg) | 143 ± 22 | 142 ± 22 | 0.18 | 144 ± 24 | 143 ± 24 | 0.16 | 146 ± 24 | 143 ± 24 | 0.16 | 146 ± 24 | 143 ± 24 | 0.31 |
| Diastolic blood pressure (mmHg) | 77 ± 13 | 76 ± 12 | 0.21 | 76 ± 13 | 76 ± 13 | 0.59 | 76 ± 14 | 76 ± 13 | 0.59 | 76 ± 14 | 76 ± 13 | 0.18 |
| Heart rate (bpm) | 65 ± 12 | 64 ± 12 | <0.01 | 67 ± 11 | 66 ± 10 | 0.05 | 68 ± 11 | 66 ± 10 | 0.05 | 68 ± 11 | 66 ± 10 | <0.01 |
| Serum creatinine (μmol/L) | 106 ± 24 | 103 ± 19 | <0.01 | 86 ± 21 | 85 ± 21 | 0.06 | 88 ± 22 | 85 ± 21 | 0.06 | 88 ± 22 | 85 ± 21 | <0.01 |
| HDL-cholesterol (mmol/L) | 1.2 ± 0.3 | 1.2 ± 0.3 | 0.25 | 1.5 ± 0.4 | 1.5 ± 0.4 | <0.01 | 1.4 ± 0.4 | 1.5 ± 0.4 | <0.01 | 1.4 ± 0.4 | 1.5 ± 0.4 | <0.01 |
| LDL-cholesterol (mmol/L) | 3.3 ± 0.8 | 3.3 ± 0.8 | 0.81 | 3.5 ± 1.0 | 3.5 ± 1.0 | 0.56 | 3.5 ± 1.0 | 3.5 ± 1.0 | 0.56 | 3.5 ± 1.0 | 3.5 ± 1.0 | <0.01 |
| Comorbidities, n (%) | | | | | | | | | | | | |
| Hypertension | 436 (59) | 236 (56) | 0.02 | 397 (65) | 207 (58) | <0.01 | 190 (76) | 207 (58) | <0.01 | 190 (76) | 207 (58) | 0.02 |
| Diabetes mellitus | 111 (15) | 53 (13) | 0.03 | 83 (14) | 36 (10) | <0.01 | 47 (19) | 36 (10) | <0.01 | 47 (19) | 36 (10) | 0.48 |
| CAD | 268 (37) | 127 (30) | <0.01 | 132 (22) | 55 (15) | <0.01 | 77 (31) | 55 (15) | <0.01 | 77 (31) | 55 (15) | <0.01 |

Numbers are mean (SD) for numeric variables, and counts (%) for categorical variables.

ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; 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.

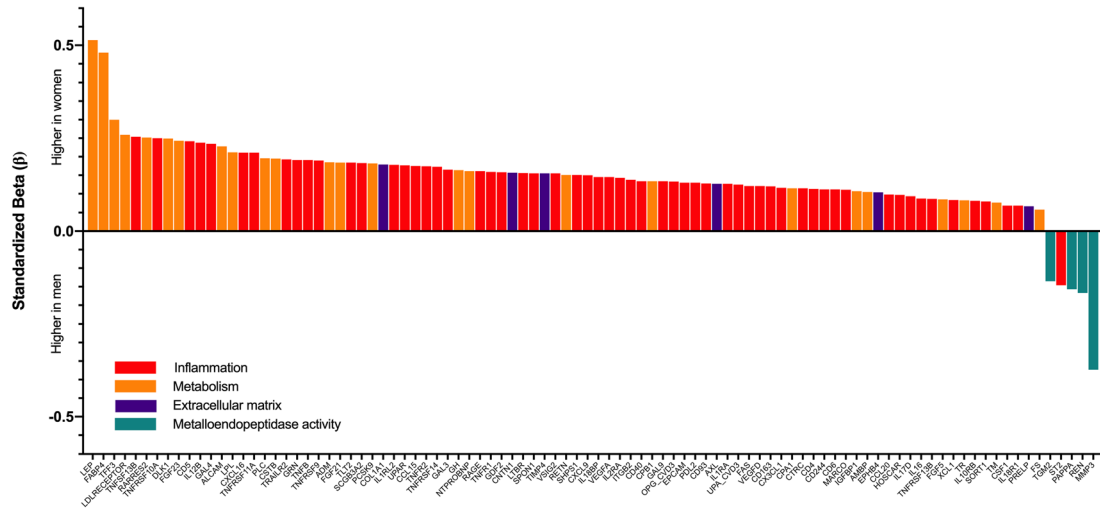


Table 2 Association of biomarkers with incident heart failure: men vs. women

| Biomarker | Men | | Women | | Interaction <i>p</i> _{int} |
|-----------|------------------|----------------|------------------|------------------|--|
| | HR (95% CI) | <i>P</i> value | HR (95% CI) | <i>P</i> value | |
| 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.

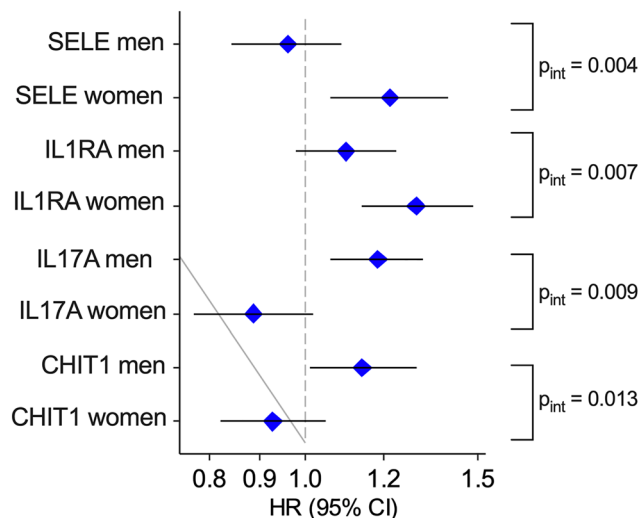
Discussion

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.

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 potential 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.

Funding

The research leading to these results has received funding from the European Union Commission's Seventh Framework Programme under grant agreement 305507 [HOMAGE (Heart Omics in Ageing consortium)]. We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with the support of the Dutch Heart Foundation CVON2016-Early HFPEF, and CVON 2017-21, SHE-PREDICTS-HF. JPF, NG, PR, and FZ are supported by the Contrat de Plan Etat-Lorraine and FEDER Lorraine, and a public grant overseen by the French National Research Agency (ANR) as part of the second "Investissements d'Avenir" programme FIGHT-HF (reference: ANR-15-RHU-0004) and by the French PIA project "Lorraine Université d'Excellence", reference ANR-15-IDEX-04-LUE. They thank the CRB lorrain for biobanking activities. JAS is supported by the Non-Profit Research Institute Alliance for the Promotion of Preventive Medicine (URL: <http://www.appremed.org>), Mechelen, Belgium received a non-binding research grant from OMRON Healthcare Co., Ltd., Kyoto, Japan.

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.

References

1. Conrad N, Judge A, Tran J, Mohseni H, Hedgecott D, Crespillo AP, Allison M, Hemingway H, Cleland JG, McMurray JJV, Rahimi K. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *Lancet* 2018; **391**: 572–580.
2. Beale AL, Meyer P, Marwick TH, Lam CSP, Kaye DM. Sex differences in cardiovascular pathophysiology: why women are overrepresented in heart failure with preserved ejection fraction. *Circulation* 2018; **138**: 198–205.

3. Aimo A, Vergaro G, Barison A, Maffei S, Borrelli C, Morrone D, Cameli M, Palazzuoli A, Ambrosio G, Coiro S, Savino K, Cerbai E, Marcucci R, Pedrinelli R, Padeletti L, Passino C, Emdin M. Sex-related differences in chronic heart failure. *Int J Cardiol* 2018; **255**: 145–151.
4. Lam CSP, Arnott C, Beale AL, Chandramouli C, Hilfiker-Kleiner D, Kaye DM, Ky B, Santema BT, Sliwa K, Voors AA. Sex differences in heart failure. *Eur Heart J* 2019; **40**: 3859–68c.
5. O'Meara E, Clayton T, McEntegart MB, McMurray JJ, Pina IL, Granger CB, Ostergren J, Michelson EL, Solomon SD, Pocock S, Yusuf S, Swedberg K, Pfeffer MA. Sex differences in clinical characteristics and prognosis in a broad spectrum of patients with heart failure: results of the Candesartan in Heart failure: assessment of reduction in mortality and morbidity (CHARM) program. *Circulation* 2007; **115**: 3111–3120.
6. Merrill M, Sweitzer NK, Lindenfeld J, Kao DP. Sex differences in outcomes and responses to spironolactone in heart failure with preserved ejection fraction: a secondary analysis of TOPCAT trial. *JACC: Heart Failure* 2019; **7**: 228–238.
7. Goldenberg I, Moss AJ, Hall WJ, Foster E, Goldberger JJ, Santucci P, Shinn T, Solomon S, Steinberg JS, Wilber D, Barsheshet A, McNitt S, Zareba W, Klein H, Committee M-CE. Predictors of response to cardiac resynchronization therapy in the Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT). *Circulation* 2011; **124**: 1527–1536.
8. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, Gillum RF, Kim YH, McAnulty JH Jr, Zheng ZJ, Forouzanfar MH, Naghavi M, Mensah GA, Ezzati M, Murray CJ. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation* 2014; **129**: 837–847.
9. Mehta LS, Beckie TM, DeVon HA, Grines CL, Krumholz HM, Johnson MN, Lindley KJ, Vaccarino V, Wang TY, Watson KE, Wenger NK. Acute myocardial infarction in women: a scientific statement from the American Heart Association. *Circulation* 2016; **133**: 916–947.
10. Suthahar N, Lau ES, Blaha MJ, Paniagua SM, Larson MG, Psaty BM, Benjamin EJ, Allison MA, Bartz TM, Januzzi JL Jr, Levy D, Meems LMG, Bakker SJL, Lima JAC, Cushman M, Lee DS, Wang TJ, deFilippi CR, Herrington DM, Naylor M, Vasani RS, Gardin JM, Kizer JR, Bertoni AG, Allen NB, Gansevoort RT, Shah SJ, Gottdiener JS, Ho JE, de Boer RA. Sex-specific associations of cardiovascular risk factors and biomarkers with incident heart failure. *J Am Coll Cardiol* 2020; **76**: 1455–1465.
11. Jacobs L, Thijs L, Jin Y, Zannad F, Mebazaa A, Rouet P, Pinet F, Bauters C, Pieske B, Tomaschitz A, Mamas M, Diez J, McDonald K, Cleland JG, Brunner-La Rocca HP, Heymans S, Latini R, Masson S, Sever P, Delles C, Pocock S, Collier T, Kuznetsova T, Staessen JA. Heart 'omics' in AGEing (HOMAGE): design, research objectives and characteristics of the common database. *J Biomed Res* 2014; **28**: 349–359.
12. Mureddu GF, Agabiti N, Rizzello V, Forastiere F, Latini R, Cesaroni G, Masson S, Cacciato G, Colivicchi F, Ugucconi M, Perucci CA, Boccanelli A. Prevalence of preclinical and clinical heart failure in the elderly. A population-based study in Central Italy. *Eur J Heart Fail* 2012; **14**: 718–729.
13. Beavers KM, Hsu FC, Houston DK, Beavers DP, Harris TB, Hue TF, Kim LJ, Koster A, Penninx BW, Simonsick EM, Strotmeyer ES, Kritchevsky SB, Nicklas BJ. The role of metabolic syndrome, adiposity, and inflammation in physical performance in the Health ABC Study. *J Gerontol A Biol Sci Med Sci* 2013; **68**: 617–623.
14. Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, Ford I, Jukema JW, Hyland M, Gaw A, Lagaay AM, Perry LJ, Macfarlane PW, Meinders AE, Sweeney BJ, Packard CJ, Westendorp RG, Twomey C, Stott DJ. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROSpective Study of Pravastatin in the Elderly at Risk. *Am J Cardiol* 1999; **84**: 1192–1197.
15. Ferreira JP, Verdonschot J, Collier T, Wang P, Pizard A, Bar C, Bjorkman J, Boccanelli A, Butler J, Clark A, Cleland JG, Delles C, Diez J, Girerd N, Gonzalez A, Hazebroek M, Huby AC, Jukema W, Latini R, Leenders J, Levy D, Mebazaa A, Mischak H, Pinet F, Rossignol P, Sattar N, Sever P, Staessen JA, Thum T, Vodovar N, Zhang ZY, Heymans S, Zannad F. Proteomic bioprofiles and mechanistic pathways of progression to heart failure. *Circ Heart Fail* 2019; **12**: e005897.
16. Jacobs L, Efremov L, Ferreira JP, Thijs L, Yang WY, Zhang ZY, Latini R, Masson S, Agabiti N, Sever P, Delles C, Sattar N, Butler J, Cleland JGF, Kuznetsova T, Staessen JA, Zannad F. Risk for incident heart failure: a subject-level meta-analysis from the heart "OMics" in AGEing (HOMAGE) study. *J Am Heart Assoc* 2017; **6**: e005231.
17. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**: 25–29.
18. Carbon S, Douglass E, Good BM, Unni DR, Harris NL, Mungall CJ, Basu S, Chisholm RL, Dodson RJ, Hartline E, Fey P. The Gene Ontology resource: enriching a GOLD mine. *Nucleic Acids Res* 2021; **49**: D325–D334.
19. Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, Gore MO, Neeland I, Berry JD, Khera A, Rohatgi A, de Lemos JA. Sex-based differences in cardiometabolic biomarkers. *Circulation* 2017; **135**: 544–555.
20. Lehallier B, Gate D, Schum N, Nanasi T, Lee SE, Yousef H, Moran Losada P, Berdnik D, Keller A, Verghese J, Sathyan S, Franceschi C, Milman S, Barzilai N, Wyss-Coray T. Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med* 2019; **25**: 1843–1850.
21. Arnold AP, Cassis LA, Eghbali M, Reue K, Sandberg K. Sex hormones and sex chromosomes cause sex differences in the development of cardiovascular diseases. *Arterioscler Thromb Vasc Biol* 2017; **37**: 746–756.
22. Mongraw-Chaffin ML, Anderson CA, Allison MA, Ouyang P, Szklo M, Vaidya D, Woodward M, Golden SH. Association between sex hormones and adiposity: qualitative differences in women and men in the multi-ethnic study of atherosclerosis. *J Clin Endocrinol Metab* 2015; **100**: E596–E600.
23. Suthahar N, Meijers WC, Ho JE, Gansevoort RT, Voors AA, van der Meer P, Bakker SJL, Heymans S, van Empel V, Schroen B, van der Harst P, van Veldhuisen DJ, de Boer RA. Sex-specific associations of obesity and N-terminal pro-B-type natriuretic peptide levels in the general population. *Eur J Heart Fail* 2018; **20**: 1205–1214.
24. Melé M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, Young TR, Goldmann JM, Pervouchine DD, Sullivan TJ, Johnson R, Segrè AV, Djebali S, Niarchou A, Wright FA, Lappalainen T, Calvo M, Getz G, Dermitzakis ET, Ardlie KG, Guigó R. Human genomics. The human transcriptome across tissues and individuals. *Science* 2015; **348**: 660–665.
25. Suthahar N, Meems LMG, Ho JE, de Boer RA. Sex-related differences in contemporary biomarkers for heart failure: a review. *Eur J Heart Fail* 2020; **22**: 775–788.
26. Duca F, Zotter-Tufaro C, Kammerlander AA, Aschauer S, Binder C, Mascherbauer J, Bonderman D. Gender-related differences in heart failure with preserved ejection fraction. *Sci Rep* 2018; **8**: 1080.
27. Vargas-Alarcon G, Perez-Mendez O, Herrera-Maya G, Posadas-Romero C, Posadas-Sanchez R, Ramirez-Bello J, Escobedo G, Fragoso JM. The rs1805193, rs5361, and rs5355 single nucleotide polymorphisms in the E-selectin gene (SEL-E) are associated with subclinical atherosclerosis: the Genetics of Atherosclerotic Disease (GEA) Mexican study. *Immunobiology* 2019; **224**: 10–14.

28. Tsoref O, Tyomkin D, Amit U, Landa N, Cohen-Rosenboim O, Kain D, Golan M, Naftali-Shani N, David A, Leor J. E-selectin-targeted copolymer reduces atherosclerotic lesions, adverse cardiac remodeling, and dysfunction. *J Control Release* 2018; **288**: 136–147.
29. Collins T, Williams A, Johnston GI, Kim J, Eddy R, Shows T, Gimbrone MA Jr, Bevilacqua MP. Structure and chromosomal location of the gene for endothelial-leukocyte adhesion molecule 1. *J Biol Chem* 1991; **266**: 2466–2473.
30. Srivastava K, Chandra S, Narang R, Bhatia J, Saluja D. E-selectin gene in essential hypertension: a case-control study. *Eur J Clin Invest* 2018; **48**: e12868.
31. Lee CH, Kuo FC, Tang WH, Lu CH, Su SC, Liu JS, Hsieh CH, Hung YJ, Lin FH. Serum E-selectin concentration is associated with risk of metabolic syndrome in females. *PLoS ONE* 2019; **14**: e0222815.
32. Franssen C, Chen S, Unger A, Korkmaz HI, De Keulenaer GW, Tschöpe C, Leite-Moreira AF, Musters R, Niessen HW, Linke WA, Paulus WJ, Hamdani N. Myocardial microvascular inflammatory endothelial activation in heart failure with preserved ejection fraction. *JACC Heart Fail* 2016; **4**: 312–324.
33. Patterson D, Jones C, Hart I, Bleskan J, Berger R, Geyer D, Eisenberg SP, Smith MF, Arend WP. The human interleukin-1 receptor antagonist (IL1RN) gene is located in the chromosome 2q14 region. *Genomics* 1993; **15**: 173–176.
34. Yazdi AS, Ghoreschi K. The interleukin-1 family. *Adv Exp Med Biol* 2016; **941**: 21–29.
35. Herder C, de Las Heras Gala T, Carstensen-Kirberg M, Huth C, Zierer A, Wahl S, Sudduth-Klinger J, Kuulasmaa K, Peretz D, Ligthart S, Bongaerts BWC, Dehghan A, Ikram MA, Jula A, Kee F, Pietilä A, Saarela O, Zeller T, Blankenberg S, Meisinger C, Peters A, Roden M, Salomaa V, Koenig W, Thorand B. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of six population-based cohorts. *Arterioscler Thromb Vasc Biol* 2017; **37**: 1222–1227.
36. Kanneganti M, Kamba A, Mizoguchi E. Role of chitotriosidase (chitinase 1) under normal and disease conditions. *J Epithel Biol Pharmacol* 2012; **5**: 1–9.
37. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. *Circulation* 1994; **90**: 775–778.
38. Artieda M, Cenarro A, Gañán A, Jericó I, Gonzalvo C, Casado JM, Vitoria I, Puzo J, Pocoví M, Civeira F. Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1645–1652.
39. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med* 2009; **361**: 888–898.
40. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev* 2003; **14**: 155–174.