



**HAL**  
open science

## Pathophysiological Sex Differences in Heart Failure Progression After Acute Coronary Syndrome

Amir Razaghizad, Haya Aziz, Guang K Zhang, João Pedro Ferreira, William B White, Cyrus R Mehta, George L. Bakris, Faiez Zannad, Abhinav Sharma

► **To cite this version:**

Amir Razaghizad, Haya Aziz, Guang K Zhang, João Pedro Ferreira, William B White, et al.. Pathophysiological Sex Differences in Heart Failure Progression After Acute Coronary Syndrome. *Journal of Cardiac Failure*, 2023, 10.1016/j.cardfail.2023.10.474 . hal-04331406

**HAL Id: hal-04331406**

**<https://hal.univ-lorraine.fr/hal-04331406v1>**

Submitted on 8 Dec 2023

**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.

# Pathophysiological Sex-Differences in Heart Failure Progression after Acute Coronary Syndrome: Insights from the EXAMINE Trial

Amir Razaghizad MDCM (c), MSc<sup>1,2,3</sup>; Haya Aziz MDCM<sup>3</sup>; Guang K Zhang MSc<sup>1</sup>; João Pedro Ferreira MD, PhD<sup>4</sup>; William B White MD<sup>5</sup>; Cyrus R Mehta PhD<sup>6</sup>; George L Bakris MD<sup>7</sup>; Faiez Zannad MD, PhD<sup>4</sup>; Abhinav Sharma MD, PhD<sup>1,2,3</sup>

<sup>1</sup>Centre for Outcomes Research and Evaluation, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada

<sup>2</sup>Division of Cardiology, McGill University Health Centre, McGill University, Montreal, Quebec, Canada

<sup>3</sup>DREAM-CV Lab, McGill University Health Centre, McGill University, Montreal, Quebec, Canada

<sup>4</sup>Nancy University, Nancy, France

<sup>5</sup>Cardiology Center, University of Connecticut School of Medicine, Farmington, Connecticut, USA

<sup>6</sup>Harvard School of Public Health, Boston, Massachusetts, USA

<sup>7</sup>University of Chicago Pritzker School of Medicine, Chicago, Illinois, USA

## Word Count

Abstract: 250

Introduction to conclusion: 3,370

## Corresponding Author

Abhinav Sharma MD, PhD

Assistant Professor of Medicine

Divisions of Cardiology and Experimental Medicine

McGill University

1001 Decarie Boulevard,

Montreal, Qc, H4A 3J1

Telephone: 514-934-1934

E-Mail: Abhinav.Sharma@McGill.ca

The Canadian Institute of Health Research (Grant # 175095), Fonds de Recherche Santé Quebec (FRSQ) Junior 1 clinician scholars' program, Alberta Innovates Health Solution, European Society of Cardiology young investigator grant, Roche Diagnostics, Boeringer-Ingelheim, Novartis, and Takeda support Dr. Abhinav Sharma.

## Disclosures

AS reports receiving support from the Canadian Institute of Health Research (Grant #175095), Fonds de Recherche Santé Quebec (FRSQ) Junior 1 clinician scholars' program. Alberta Innovates Health Solution, European Society of Cardiology young investigator grant, Roche Diagnostics, Boeringer-Ingelheim, Novartis, and Takeda. GLG reports research support and/or speaking fees and/or honoraria from Novartis, Bayer, Servier, JAMP Pharma, Duke Clinical Research Institute, Canadian Heart Research Centre, CIHR, Université de Montréal, and the Montreal Heart Institute

Foundation (not related to this work). JPF has received personal fees from Boehringer Ingelheim during the conduct of the study. FZ has received personal fees from Boehringer Ingelheim during the conduct of the study; has received personal fees from Janssen, Novartis, Boston Scientific, Amgen, CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno Pharmaceutical, Applied Therapeutics, Merck, Bayer, and Cellprothera; and has received other fees from CVCT and Cardiorenal, outside the submitted work. The other authors declare that they have no competing interests.

[author block]

### **Word Count**

Abstract: 245

Introduction to conclusion: 3,470

### **Keywords**

Proteomics, Heart Failure, Diabetes, Epidemiology, Sex Differences,

### **Address for Correspondence**

[author block]

The Canadian Institute of Health Research (Grant # 175095), Fonds de Recherche Santé Québec (FRSQ) Junior 1 clinician scholars' program, Alberta Innovates Health Solution, European Society of Cardiology young investigator grant, Roche Diagnostics, Boeringer-Ingelheim, Novartis, and Takeda support [author block].

## **ABSTRACT**

**BACKGROUND.** Therapies can reduce the risk of heart failure (HF) development and progression in type 2 diabetes (T2D); nevertheless, the risk of these outcomes is greater in females than in males.

**METHODS.** To investigate sex-differences in HF development and progression, we compared baseline circulating proteins (Olink® Cardiovascular II panel) in males and females with T2D and recent acute coronary syndrome (ACS) for the outcome of heart failure hospitalization (HFH). Data were from the placebo-controlled Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial. Pathophysiological sex-differences were interpreted with network and pathway overrepresentation analyses.

**RESULTS.** The EXAMINE trial enrolled 5,380 participants (32.1% females) with biomarker data available in 95.4% of individuals. Analyses revealed 43 biomarkers were differentially expressed in HFH, of which 18 were sex-specific. Among these 43 biomarkers, interleukin-6 was identified as a central node for the pathogenesis of HFH in both females and in males. Additional pathway overrepresentation analyses demonstrated that biomarkers associated with inflammatory pathways related to endothelial-dysfunction and cardiac fibrosis were more up-regulated in females than males with HFH. Differential expression of 3 biomarkers (pentraxin-related protein 3, hydroxyacid oxidase 1, and carbonic anhydrase 5A) was independently associated with an increased risk of HFH in females but not in males (interaction  $p < 0.05$ ).

**CONCLUSION.** In males and females with T2DM and ACS, interleukin-6 appears to be central in the pathogenesis of HF. Females exhibit higher levels of circulating proteins related to immunological pathways, reflecting sex-specific differences underlying HF development and progression.

## INTRODUCTION

Heart failure is one of the most common manifestations of cardiovascular disease in people with type 2 diabetes (T2D), and a leading cause of hospitalization and health service utilization.<sup>1</sup> The risk of heart failure development and progression in people with T2D is, moreover, markedly increased early after an acute coronary syndrome.<sup>2</sup> Although guideline-directed medical therapies such as sodium-glucose cotransporter-2 inhibitors (SGLT2i) have been shown to reduce the risk of worsening heart failure in T2D, the excess risk of heart failure persists with treatment, and is significantly greater in females than in males.<sup>3</sup> Furthermore, females are more predisposed to heart failure with persevered ejection fraction (HFpEF) and poorer quality of quality of life. Identifying mechanistic pathways that contribute to sex differences in heart failure in T2D may aid in distinguishing clinical phenotypes and identifying novel molecular targets for treatment.<sup>4</sup>

Over the past several years, numerous studies have investigated the prognostic value of circulating biomarkers (e.g., natriuretic peptides) to develop clinical tools for risk prediction. While numerous tools have demonstrated strong prognostic performance, analyses of circulating biomarkers in disease states have been shown to provide additional value beyond prognostication.<sup>5</sup> Biomarkers reflect biological processes and can therefore contribute to the understanding of pathophysiological mechanisms. To that end, knowledge-based proteomic network analyses have been shown to be a well-validated method for investigating associations between biomarkers and molecular pathways as a means of differentiating distinct pathophysiological phenotypes in disease. In line with this approach, the present study aimed to study baseline circulating proteins in patients from the Examination of Cardiovascular Outcomes with Alogliptin vs. Standard of Care (EXAMINE) trial to investigate sex-differences in the development and progression of heart failure in people with T2D.

## **METHODS**

### **Study Cohort**

Circulating proteins in patients from the EXAMINE trial were studied in this analysis. The details of the EXAMINE trial have been previously reported.<sup>6</sup> In brief, the trial enrolled patients across 49 countries and 898 centres. Patients had T2D, were receiving antidiabetic therapy (minus a dipeptidyl peptidase-4 inhibitor or glucagon-like peptide-1 receptor agonist analogue) and had had a recent acute coronary syndrome (i.e., acute myocardial infarction or unstable angina requiring hospitalization) 15 to 90 days prior to randomization. Here, T2D was defined by having a glycated hemoglobin level between 6.5 to 11.0%, or 7.0 to 11.0% if the patient was receiving antidiabetic therapy with prescribed insulin. Exclusion criteria included dialysis within 14 days, type 1 diabetes, and unstable cardiac disorders, which included class IV heart failure as defined by

the New York Heart Association, critical valvular heart disease, uncontrolled hypertension, and refractory angina. All patients received standard antidiabetic therapy according to regional guidelines in addition to the investigational product. Patients had outpatient visits 1, 3, 6, 9, and 12 months after randomization then subsequently every 4 months until the end of the study. Patients were followed for a maximum of 40 months (median, 18 months). The EXAMINE trial was authorized by the applicable national and institutional regulatory authorities and ethical committees, and all participants provided written informed consent.

### **Biomarker Panel**

Blood samples and a thorough physical examination and medical history were taken at baseline. Circulating proteins were analyzed with the Olink® Cardiovascular II panel, which measures 92 biomarkers associated with cardiovascular disease and immunological and inflammatory processes.<sup>7</sup> The 92-biomarker panel reflects biological processes which include angiogenesis, blood vessel morphogenesis, catabolic processes, cell adhesion, coagulation, heart development, immune response, inflammatory response, MAPK cascade, platelet activation, proteolysis, blood pressure regulation, response to hypoxia, wound healing, and peptide hormone response. The 92-biomarker panel also includes exploratory biomarkers potentially involved in the pathophysiology of cardiovascular disease. The names and Uniprot identification codes of the 92 biomarkers are available in **Supplemental Table S1**.

### **Outcome**

The primary endpoint was heart failure hospitalization which served as a proxy for heart failure development and progression. The EXAMINE trial defined heart failure hospitalization as any hospital stay or emergency department visit with clinical manifestations of heart failure lasting for more than 12 hours. Manifestations of heart failure included bibasilar rales on pulmonary

examination, new or worsening dyspnea, orthopnea, peripheral edema, jugular venous distention, radiographic evidence of heart failure, paroxysmal nocturnal dyspnea, new third heart sound, ultrafiltration or dialysis, parenteral diuretic use, inotropic or vasodilator therapy, or any mechanical or surgical intervention including heart transplantation.<sup>8</sup> A central adjudication committee adjudicated all the recorded cardiovascular events and deaths in EXAMINE.

### **Biomarker Quantification**

The measurement and quantification of the circulating biomarkers was done utilizing a high-throughput multiplex immunoassay that simultaneously measures the target biomarkers. The technology is based on a proximity extension assay.<sup>9</sup> That is, pairs of antibodies attached to oligonucleotides-labeled probes are introduced into microliter samples of plasma to bind to biomarker antigen targets. Upon successful antigen-antibody binding, paired oligonucleotide-based probes come within proximity, anneal together, and produce DNA amplicons barcoding their respective biomarker. A Fluidigm BioMark™ HD real-time PCR platform is then used to quantify the DNA amplicons. The platform yields Log<sub>2</sub> normalized protein expression (NPX) data, whereby large values indicate a high biomarker concentration. NPX data do not represent an absolute quantification of circulating biomarker concentrations. Additional information on the Olink® Cardiovascular II panel is available in the Supplemental Olink® addenda.

### **Sample Handling**

Plasma was isolated and kept at -20°C to -80°C after participant blood was taken into EDTA-anticoagulated polypropylene tubes at local sites. The frozen samples were then delivered to the Thrombolysis in Myocardial Infarction Clinical Trials Laboratory (Brigham and Women's Hospital [Boston, MA]) where they were kept at -80°C or colder prior to analysis. To minimize degradation, the EXAMINE trial took precautions to ensure proper sample handling and storage.

Sample handling precautions included minimizing sample freeze-thaw cycles and implementing standardized protocols for sample collection, storage, and transportation to mitigate potential variations in sample integrity.

### **Statistical Analysis**

Clinical characteristics of patients with and without heart failure and T2D were presented as means and standard deviations for continuous variables and as frequencies and percentages for categorical variables. Clinical differences were evaluated with the independent sample t-tests for continuous variables and chi-square test for categorical variables.<sup>10</sup> To investigate pathophysiological sex-differences, we first evaluated the differential expression of cardiovascular biomarkers in males and females with and without heart failure hospitalization, respectively. That is, we separately evaluated which biomarkers were over- or under-expressed in males and females experiencing recurrent or incident heart failure hospitalization. This was done utilizing linear models for microarray data analysis (limma version 3.35.2) that were corrected for test multiplicity with the Benjamini and Hochberg method.<sup>11,12</sup> Adjustments for covariates such as age, ethnicity, or BMI were not included in order to reflect natural differences occurring in the progression of heart failure after ACS in individuals with T2D. Biomarkers passing an absolute  $\log_2$  fold-change criterion of 0.2 and a false discovery rate-corrected *p-value* less than 0.05 were judged as pathophysiological biomarkers.<sup>13</sup> To account for inherent sex-differences, we also calculated  $\log_2$  fold-change differences in biomarker in females versus males. This measure quantifies the difference in differential expression of biomarkers in females with versus without heart failure hospitalization, as well as in males with and without heart failure hospitalization, using  $\log_2$  fold-change units.



Furthermore, biomarkers displaying distinct expression patterns, such as over-expression in females but non-differential or under-expression in males, and vice versa, were considered as indicative of sex-specific differences. To evaluate the association between these sex-specific biomarkers and heart failure hospitalization, interaction analyses for sex were performed with Fine-Gray regression models.<sup>14,15</sup> These models were adjusted for the competing risk of death and well-validated risk factors for heart failure hospitalization including age, HbA1c, body mass index (kg/m<sup>2</sup>), systolic blood pressure, prior heart failure hospitalization, duration of diuretic use, brain natriuretic peptide, history of myocardial infarction, hypertension, smoking status, and estimated glomerular filtration rate.<sup>16</sup> To avoid issues with multicollinearity with history of myocardial infarction, type of ACS and history of coronary bypass grafting were not included in the multivariable models.

### **Functional Enrichment and Network Analyses**

To elucidate pathophysiological sex-differences in heart failure, biological context and functional designations were ascribed to the biomarkers of interest. This functional enrichment was done utilizing the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, which draws functional designations from several electronic sources including Gene Ontology biologic processes, Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome pathways, and WikiPathways.<sup>17</sup> In addition, the STRING database was used to build a knowledge-based network analysis evaluating the association between the differentially expressed biomarkers in males and females with vs. without heart failure hospitalization. The knowledge-based network analysis is based on available knowledge of protein and gene interactions. Low-confidence interactions were excluded from the network analysis. Cytoscape, version 3.8.2, was used to analyze and organize the resultant network, and to calculate betweenness centrality, a measure

quantifying the centrality of nodes within a network.<sup>18</sup> Furthermore, to understand how proteins fit within broader biological contexts, we utilized Enrichr to conduct sex-specific pathway overrepresentation analyses using the Gene Ontology Biological Processes 2023 database.<sup>19–21</sup> These analyses were conducted using the backdrop of the 92 Olink biomarkers, allowing us to discern ontological associations among significant biomarkers.

## RESULTS

### Clinical Characteristics

In total, 5,380 people were enrolled in the EXAMINE trial and females represented 32.1% of the study cohort. Biomarker data was available for 95.4% (N=5,131) of the study population. The average age of participants was  $61 \pm 10$ , and the average duration of T2D was 7.1 (interquartile range 2.7-13.7) years with an average HbA1c of  $8.0\% \pm 1.1$ . From the start of recruitment (October 2009) to the date of the last patient visit (June 2013), 117 males and 78 females experienced heart failure hospitalization.

### Sex-Based Differences

Males and females who developed heart failure hospitalization during the trial were significantly more likely to have a longer duration of T2D (men,  $13.1 \pm 10.8$  vs.  $8.3 \pm 7.6$  years; females,  $13.8 \pm 11.2$  vs.  $10.6 \pm 8.6$  years) and history of coronary artery bypass grafts (men, 29.1 vs. 13.6%; females 24.4 vs. 9.4%), congestive heart failure (men, 62.4 vs. 24.3%; females, 70.5 vs. 33.1%), and peripheral arterial disease (men, 22.2 vs. 8.8%; females, 23.1 vs. 9.6%). Females who experienced heart failure hospitalization had higher body mass indices ( $32.0 \pm 8.6$  vs.  $30.1 \pm 6.0$  kg/m<sup>2</sup>) compared to females who did not. Conversely, males who developed heart failure hospitalization tended to be older ( $63.5 \pm 9.8$  vs.  $59.6 \pm 9.7$  years) compared males who did not. Results are presented in Table 1.

### Differential Biomarker Expression

**Figure 1A** presents the differential expression of biomarkers in individuals with versus without heart failure hospitalization as stratified by sex. In males with versus without heart failure hospitalization, 28 circulating biomarkers were significantly up-regulated and 1 biomarker was significantly down-regulated. In females with versus without heart failure hospitalization, 39 biomarkers were significantly up-regulated. After addressing for overlap in differential expression, 25 non-sex-specific biomarkers and 18 sex-specific biomarkers were associated with heart failure hospitalization, yielding a total of 43 biomarkers of interest (**Figure 1B; Supplemental Table S2**). The most differentially expressed biomarkers in females versus males with heart failure hospitalization were hydroxyacid oxidase 1 (HAOX1;  $\text{Log}_2$  fold-change difference: 0.69), growth hormone 1 (GH1;  $\text{Log}_2$  fold-change difference: -0.56), and carbonic anhydrase (CA5A;  $\text{Log}_2$  fold-change difference: 0.52; **Figure 2**).

### Knowledge-Based Network Analysis

To investigate the biological context of the differentially expressed biomarkers a network analysis was performed with k-means clustering for the 43 differentially expressed biomarkers (**Figure 3**). The network analysis revealed that the main pathophysiological drivers of heart failure hospitalization related to inflammation, lipid metabolism, renal and cardiac homeostasis, hemostasis, angiogenesis, and cytokine and interleukin signaling. Among the analyzed biomarkers, cluster of differentiation 4 (CD4), interleukin 6, and renin emerged as the most central biological nodes in the clusters related to cytokine and interleukin signalling, inflammation, and renal and cardiac homeostasis. These biomarkers exhibited high betweenness centrality values, indicating their significant role in connecting various components within the biological network.

### Sex-Specific Knowledge-Based Network Analysis

To further differentiate pathophysiological sex-differences in heart failure hospitalization, we conducted sex-specific network analyses. The female biomarker profile had a phenotype primarily characterized by up-regulated inflammatory activity and cytokine and interleukin signaling, while the male biomarker profile was characterized by up-regulated metabolic, renal, and cardiac homeostasis (**Figure 2**). Within the sex-specific networks (**Supplemental Figure S2**), chemokine ligand 3 and thrombomodulin exhibited high betweenness centrality values in females. However, in the male-specific network, there were no identified central biological nodes.

### **Pathway Overrepresentation Analysis**

Ontological pathways associated with the differentially expressed biomarkers in males and females with heart failure hospitalization are shown in **Supplemental Figure S3**. Compared to males, females experiencing heart failure hospitalization displayed a greater degree of overrepresentation in certain pathways. These included increased positive regulation of lymphocyte chemotaxis (GO::0140131) and regulation of natural killer cells (GO::2000501) and eosinophils (GO::0048245). Conversely, males showed a greater degree of representation in pathways associated with proteolysis, encompassing membrane protein ectodomain proteolysis (GO:0006509), membrane protein intracellular domain proteolysis (GO:0031293), and membrane protein proteolysis (GO:0033619). However, these findings were primarily linked to metalloprotease 7, which exhibited minor differential expression in females compared to males (**Figure 2**).

### **Biomarker Interaction with Sex and Heart Failure Hospitalization**

After multivariable adjustment that included the competing risk of cardiovascular death, 3 biomarkers demonstrated a significant interaction with sex for the outcome heart failure hospitalization (**Figure 4**). Specifically, overexpression of pentraxin-related protein and carbonic anhydrase 5A was associated with an increased incidence of heart failure hospitalization in females

(hazard ratio [HR]: 2.47, 95% confidence interval [95% CI] 1.62–3.77 and HR: 1.27, 95% CI 1.06–1.52, respectively), but not in males (HR: 1.09, 95% CI 0.76–1.56 and HR: 0.93, 95% CI 0.76–1.15; all P-interaction values < 0.05). On the other hand, overexpression of hydroxyacid oxidase 1 was associated with a reduced incidence of heart failure hospitalization in males (HR: 0.84, 95% CI 0.73–0.96) but not in females (HR: 1.07, 95% CI 0.93–1.24; P-interaction value: 0.004).

## DISCUSSION

In this study we explored sex differences in the development and progression of heart failure hospitalization in females and males with T2D and recent acute coronary syndrome. By analyzing the differential expression of biomarkers in males and females, several key differences were observed. First, the data showed significant overlap in the proteomic pathogenesis of heart failure hospitalization in males and females. Second, the data showed that compared to males, females developing heart failure hospitalization had unique biomarkers which were differentially regulated. Specifically, pathway overrepresentation analyses showed that immunological pathways (e.g., leukocyte chemotaxis) were more associated with biomarkers up-regulated in females than males. We postulate that these findings may contribute to phenotypic sex differences in heart failure and provide insights for the prevention of heart failure development and progression following acute coronary syndrome in T2D.

### **Differentially Expressed Biomarkers & the Central Role of Interleukin-6**

A key finding of our proteomic analyses was the similarity in differential biomarker expression between males and females, indicating consistent and overlapping proteomic changes in both groups. Notably, of the 43 biomarkers that were found to be differentially expressed in males and females developing heart failure hospitalization, 25 were found to be up-regulated in

both sexes. Knowledge-based network analyses of the 43 biomarkers provided additional information on these proteins and how they interact in biological systems. A key finding here was the fact that interleukin-6 was identified as a central hub in the pathogenesis of heart failure hospitalization in both males and females. Interestingly, interleukin-6 has been shown to be independently associated with the development heart failure with preserved ejection fraction (HFpEF)<sup>22(p6),23</sup>. The use of interleukin-6 inhibitors are also currently being evaluated as potential treatments for patients with cardiovascular disease.<sup>25</sup> These facts serve to validate our ontological findings and emphasize the importance of interleukin-6 as a potential therapeutic target in heart failure. Furthermore, the results underscore the potential involvement of interleukin-6 in the pathogenesis of heart failure following acute coronary syndromes, particularly in females with T2D, who exhibited increased up-regulation of this biomarker compared to males.

### **Targeting Sex-Specific Hubs & Pathological Pathways**

To better understand phenotypic sex variations in heart failure, we also performed sex-specific network analyses of biomarkers that were uniquely up- or down-regulated in males and females developing heart failure hospitalization. C-C Motif Chemokine Ligand 3 (CCL3) was identified as a central hub in the proteomic pathogenesis of heart failure hospitalization in females. Interestingly, CCL3 has been associated to chemokinetic actions (e.g., monocyte and leukocyte chemotaxis) and mitogenic pathways (e.g., mitogen-associated protein kinase [MAPK] cascades and extracellular signal-regulated kinases [ERK] signaling). These facts are consistent with our pathway over-representation analyses, which showed increased over-representation specifically chemokinetic pathways in females developing heart failure hospitalization compared to males. The observation that the pathogenesis of heart failure hospitalization in females seems to be primarily mediated by such an inflammatory-chemokinetic phenotype aligns with research in this area.

Consequently, CCL3 emerges as potentially an interesting druggable target. Moreover, recent clinical trials of colchicine, which inhibits chemotaxis, have shown favorable cardiovascular effects in people following myocardial infarction.<sup>26</sup> Studies conducted in rat models of HFpEF have also shown improvements in survival and mitigation of cardiac dysfunction with the use of colchicine.<sup>27</sup> Therefore, our results emphasize the potential benefits of these therapies in the context of preventing the development and progression of heart failure following acute coronary syndrome in individuals with T2D.

### **Sex-Specific Biomarkers and Heart Failure Development and Progression**

Among the differentially expressed biomarkers, three were associated with a significantly increased risk of heart failure hospitalization in females but not in males. These biomarkers were hydroxyacid oxidase (glycolate oxidase) 1 (HAOX1), pentraxin 3 (PTX3), and carbonic anhydrase (CA5A). Notably, CA5A demonstrated a substantial difference in up-regulation between females and males experiencing heart failure hospitalization (Log<sub>2</sub> fold-change difference: 1.4). Interestingly, recent studies of CA5A inhibitors have reported clinical benefits, particularly in increasing the efficacy of decongestion in acute decompensated heart failure with volume overload.<sup>28</sup> Although these studies have not demonstrated significant sex differences in treatment effects, numerically increased benefit has been reported in males. The combination of these findings and the observed differences in CA5A regulation may consequently suggest the existence of sex-related variations in fluid homeostasis, which may warrant future investigation.

Pentraxin-3 has conversely been implicated in fibroblast differentiation, left ventricular hypertrophy, and myocardial fibrosis. These findings are supported by previous research showing that females with T2D and heart failure often demonstrate more pronounced adverse left ventricular remodelling compared to males, as demonstrated by increased left ventricular mass,

left ventricular wall thickness, and relative wall thickness when adjusted for height.<sup>31,32,33</sup> Studies have shown elevated levels of PTX3 in patients with T2D and acute heart failure.<sup>34</sup> As a result, inhibitors of PTX3 may also constitute a potential target for preventing heart failure development and progression after acute coronary syndromes in T2D. For instance, inhibiting the interaction of PTX3 with its endogenous immunoglobulin  $\gamma$  Fc receptors could prevent heart failure progression through the inhibition of leukocyte-mediated phagocytosis and pro-inflammatory cytokine release.<sup>36</sup>

Lastly, HAOX1, a known mediator of peroxisome degradation and fatty acid alpha-oxidation, emerged as the most differentially expressed biomarker between females and males with heart failure hospitalization with significantly lower expression in males (Log2 fold-change difference: 1.6). This is particularly interesting given previous research linking diabetic cardiomyopathy with lipid accumulation and lipotoxicity. Moreover, studies have indicated that impaired fatty acid alpha-oxidation, specifically in cardiomyocytes, can contribute to the development of lipotoxicity.<sup>37</sup> Together, these results suggest that impaired lipid metabolism may play a significant role in heart failure hospitalization in males compared to females. Furthermore, HAOX1 has been implicated in the metabolism of medications such as Losartan, an angiotensin receptor blocker that has demonstrated differential dose response in females and males with heart failure, with females responding more favorably to low-dose therapy.<sup>38,39</sup> Therefore, future studies investigating the interplay between HAOX1, drug metabolism, and treatment response could contribute to the development of personalized treatment approaches for heart failure patients.

### **Limitations**

Our study had several limitations. First, the EXAMINE trial was not designed to elucidate sex-specific differences in the expression of biomarkers in males and females progressing to heart



failure. Therefore, the results of this study should be primarily considered hypothesis generating as the results do not allow causal inference. Second, the primary endpoint for this study, heart failure progression, was defined as incident or recurrent heart failure hospitalization. Third, clinical data were not available on patients' ejection fractions which limited the possibility of conducting analyses stratified to preserved or reduction ejection fraction. Fourth, there were likely some highly correlated biomarkers in our dataset. Therefore, the use of the Benjamini-Hochberg procedure may have led to modest reduction in statistical power. Finally, circulating proteins were analyzed with the Olink® Cardiovascular II panel, which contains 92 proteins potentially associated with cardiovascular disorders. As a result, this may be considered limited when compared to broader proteomic analyses.

### **CONCLUSION**

The proteomic analysis of biomarkers in individuals with T2D and heart failure hospitalization showed significant pathophysiological sex-differences. While many biomarkers associated with heart failure were similar between males and females, pathways related to immunological and inflammatory processes appear to be significantly more over-represented in females than in males. These findings provide valuable insights into the sex-specific factors contributing to the development and progression of heart failure. They also help explain phenotypic sex-differences in clinical outcomes associated with heart failure which include, among females, poorer quality of life and greater adverse left ventricular remodeling. The identification of these sex-specific differences highlights the need for the development of tailored therapies and guidelines that consider the unique characteristics and needs of both males and females at risk of developing incident or worsening heart failure.

### **CONFLICT OF INTEREST**

[author block].

## REFERENCES

1. Conrad N, Judge A, Tran J, et al. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *The Lancet*. 2018;391(10120):572-580. doi:10.1016/S0140-6736(17)32520-5
2. Sharma A, Cannon CP, White WB, et al. Early and Chronic Dipeptidyl-Peptidase-IV Inhibition and Cardiovascular Events in Patients With Type 2 Diabetes Mellitus After an Acute Coronary Syndrome: A Landmark Analysis of the EXAMINE Trial. *J Am Heart Assoc Cardiovasc Cerebrovasc Dis*. 2018;7(11). doi:10.1161/JAHA.117.007649
3. Ohkuma T, Komorita Y, Peters SAE, Woodward M. Diabetes as a risk factor for heart failure in women and men: a systematic review and meta-analysis of 47 cohorts including 12 million individuals. *Diabetologia*. 2019;62(9):1550-1560. doi:10.1007/s00125-019-4926-x
4. Echouffo-Tcheugui JB, Greene SJ, Papadimitriou L, et al. Population risk prediction models for incident heart failure: a systematic review. *Circ Heart Fail*. 2015;8(3):438-447.
5. Razaghizad A, Oulousian E, Randhawa VK, et al. Clinical Prediction Models for Heart Failure Hospitalization in Type 2 Diabetes: A Systematic Review and Meta-Analysis. *J Am Heart Assoc*. 2022;11(10):e024833. doi:10.1161/JAHA.121.024833
6. White WB, Bakris GL, Bergenstal RM, et al. EXamination of cArdiovascular outcoMes with alogliptIN versus standard of carE in patients with type 2 diabetes mellitus and acute coronary syndrome: a cardiovascular safety study of the dipeptidyl peptidase 4 inhibitor alogliptin in patients with type 2 diabetes with acute coronary syndrome. *Am Heart J*. 2011;162(4):620-626. e1.
7. Olink Target 96 Cardiovascular panels. Olink. Accessed June 14, 2022. <https://www.olink.com/products-services/target/cardiometabolic-panel/>
8. Zannad F, Cannon CP, Cushman WC, et al. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. *The Lancet*. 2015;385(9982):2067-2076. doi:10.1016/S0140-6736(14)62225-X
9. What is Proximity Extension Assay (PEA)? - Olink. Accessed June 14, 2022. <https://www.olink.com/faq/what-is-proximity-extension-assay-pea/>
10. Raafs A, Verdonschot J, Ferreira JP, et al. Identification of sex-specific biomarkers predicting new-onset heart failure. *ESC Heart Fail*. 2021;8(5):3512-3520.
11. Smyth GK. Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. Springer; 2005:397-420.

12. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol.* 1995;57(1):289-300.
13. Tromp J, Voors AA, Sharma A, et al. Distinct Pathological Pathways in Patients With Heart Failure and Diabetes. *JACC Heart Fail.* 2020;8(3):234-242. doi:10.1016/j.jchf.2019.11.005
14. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc.* 1999;94(446):496-509.
15. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. *Bone Marrow Transplant.* 2010;45(9):1388-1395.
16. Razaghizad A, Sharma A, Ni J, et al. External validation and extension of the TIMI risk score for heart failure in diabetes for patients with recent acute coronary syndrome: An analysis of the EXAMINE trial. *Diabetes Obes Metab.* Published online September 9, 2022. doi:10.1111/dom.14867
17. Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 2021;49(D1):D605-D612.
18. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 2003;13(11):2498-2504.
19. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* 2016;44(W1):W90-W97.
20. Xie Z, Bailey A, Kuleshov MV, et al. Gene set knowledge discovery with enrichr. *Curr Protoc.* 2021;1(3):e90.
21. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. *Nat Genet.* 2000;25(1):25-29.
22. Chia YC, Kieneker LM, van Hassel G, et al. Interleukin 6 and Development of Heart Failure With Preserved Ejection Fraction in the General Population. *J Am Heart Assoc.* 2021;10(11):e018549. doi:10.1161/JAHA.120.018549
23. Markousis-Mavrogenis G, Tromp J, Ouwerkerk W, et al. The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study. *Eur J Heart Fail.* 2019;21(8):965-973. doi:10.1002/ejhf.1482
24. Adamstein NH, Cornel JH, Davidson M, et al. Association of Interleukin 6 Inhibition With Ziltivekimab and the Neutrophil-Lymphocyte Ratio: A Secondary Analysis of the RESCUE Clinical Trial. *JAMA Cardiol.* Published online 2022.
25. Novo Nordisk A/S. *ZEUS - Effects of Ziltivekimab Versus Placebo on Cardiovascular Outcomes in Participants With Established Atherosclerotic Cardiovascular Disease,*

*Chronic Kidney Disease and Systemic Inflammation*. clinicaltrials.gov; 2022. Accessed November 13, 2022. <https://clinicaltrials.gov/ct2/show/NCT05021835>

26. Tardif JC, Kouz S, Waters DD, et al. Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *N Engl J Med*. 2019;381(26):2497-2505. doi:10.1056/NEJMoa1912388
27. Shen S, Duan J, Hu J, et al. Colchicine alleviates inflammation and improves diastolic dysfunction in heart failure rats with preserved ejection fraction. *Eur J Pharmacol*. 2022;929:175126. doi:10.1016/j.ejphar.2022.175126
28. Mullens W, Dauw J, Martens P, et al. Acetazolamide in Acute Decompensated Heart Failure with Volume Overload. *N Engl J Med*. 2022;387(13):1185-1195. doi:10.1056/NEJMoa2203094
29. Blumer V, Greene SJ, Wu A, et al. Sex Differences in Clinical Course and Patient-Reported Outcomes Among Patients Hospitalized for Heart Failure. *JACC Heart Fail*. 2021;9(5):336-345. doi:10.1016/j.jchf.2020.12.011
30. Dewan P, Rørth R, Jhund PS, et al. Differential Impact of Heart Failure With Reduced Ejection Fraction on Men and Women. *J Am Coll Cardiol*. 2019;73(1):29-40. doi:10.1016/j.jacc.2018.09.081
31. Galderisi M, Anderson KM, Wilson PW, Levy D. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol*. 1991;68(1):85-89.
32. Lam CSP, Arnott C, Beale AL, et al. Sex differences in heart failure. *Eur Heart J*. 2019;40(47):3859-3868c. doi:10.1093/eurheartj/ehz835
33. Packer M, Carson PE, Frid D. Effect of Amlodipine on Morbidity and Mortality in Severe Chronic Heart Failure. *N Engl J Med*. Published online 1996:8.
34. Sharma A, Demissei BG, Tromp J, et al. A network analysis to compare biomarker profiles in patients with and without diabetes mellitus in acute heart failure. *Eur J Heart Fail*. 2017;19(10):1310-1320. doi:10.1002/ejhf.912
35. Suzuki S, Takeishi Y, Niizeki T, et al. Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure. *Am Heart J*. 2008;155(1):75-81. doi:10.1016/j.ahj.2007.08.013
36. Lu J, Marnell LL, Marjon KD, Mold C, Du Clos TW, Sun PD. Structural recognition and functional activation of FcγR by innate pentraxins. *Nature*. 2008;456(7224):989-992. doi:10.1038/nature07468
37. Haffar T, Bérubé-Simard F, Boussette N. Impaired fatty acid oxidation as a cause for lipotoxicity in cardiomyocytes. *Biochem Biophys Res Commun*. 2015;468(1):73-78. doi:10.1016/j.bbrc.2015.10.162

38. Santema BT, Ouwerkerk W, Tromp J, et al. Identifying optimal doses of heart failure medications in men compared with women: a prospective, observational, cohort study. *The Lancet*. 2019;394(10205):1254-1263. doi:10.1016/S0140-6736(19)31792-1
39. Ferreira JP, Konstam MA, McMurray JJV, et al. Dosing of losartan in men versus women with heart failure with reduced ejection fraction: the HEAAL trial. *Eur J Heart Fail*. 2021;23(9):1477-1484. doi:10.1002/ejhf.2255

Journal Pre-proof

## FIGURE TITLES & LEGENDS

**Fig. 1.** Biomarkers Differentially Expressed in males and Females with Type 2 Diabetes, Recent Acute Coronary Syndrome, and With and Without Heart Failure Hospitalization.

Volcano plots visualize the differences in biomarker expression between two groups: males (**Panel A**) and females (**Panel B**) who either did or did not develop heart failure hospitalization. In these plots, each unit increase in the log<sub>2</sub> fold change represents a twofold change in biomarker levels. Additionally, higher  $-\log_{10}$  p values indicate more significant differences in biomarker expression between individuals who did and did not experience heart failure hospitalization. **Panel C** presents a Venn diagram illustrating the overlap in differential biomarker expression between males and females. In total, 25 biomarkers are identified as non-sex-specific, while 18 are sex-specific.

**Fig. 2.** Functional Enrichment of Differentially Expressed Biomarkers in Males and Females with Type 2 Diabetes, Recent Acute Coronary Syndrome, and With and Without Heart Failure Development.

Overview of the 25 non-sex-specific and 18 sex-specific biomarkers associated with the development of heart failure hospitalization in males and females. Positive Log<sub>2</sub> Fold Changes values indicate that a biomarker was more up-regulated in females with versus without heart failure hospitalization compared to males with versus without heart failure hospitalization. Likewise, negative values indicate greater up-regulation in males. K-means clustering was applied to bundle the enriched network of biomarkers into biologically relevant groups.

**Fig. 3.** Results of Network Analysis and Functional Enrichment of Cardiovascular Biomarkers Differentially Expressed in Males and Females With Versus Without Heart Failure and Diabetes.

The network illustrates the network of cardiovascular biomarkers that are differentially expressed in males and females with versus without heart failure and diabetes. With the exception of HAO1, all the illustrated biomarkers are up-regulated in males and females with versus without heart failure. Biomarkers with high opacity (i.e., high color saturation) denote sex-specific biomarkers, while biomarkers with low opacity (i.e., low color saturation) denote non-specific biomarkers.

\*Biomarkers specifically up-regulated in females with versus without heart failure

†Biomarkers specifically up-regulated in males with versus without heart failure

**Fig. 4.** Interaction Analysis of Sex-Specific Pathophysiological Biomarkers with Heart Failure Hospitalization Amongst Males Versus Females.

The figure shows hazard ratios with 95% confidence intervals of sex-specific pathophysiological biomarkers that had a significant interaction with sex for heart failure hospitalizations. The analyses were done with Fine and Gray regression models accounting for the competing risk of cardiovascular death and relevant confounders including age, HbA1c, body mass index (kg/m<sup>2</sup>), systolic blood pressure, prior heart failure hospitalization, diuretic use, history of myocardial infarction, hypertension, smoking status, and estimated glomerular filtration rate. **Abbreviations:** CA5A = Carbonic anhydrase 5A; HAOX1 = Hydroxyacid Oxidase 1; PTX3 = Pentraxin; XCL1 = Chemokine (C motif) ligand.

Figure 1.

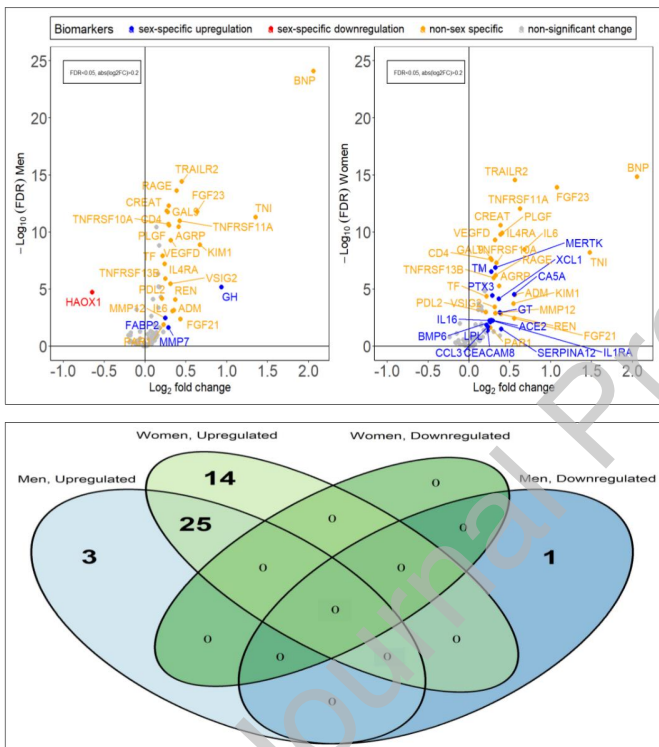


Figure 2.

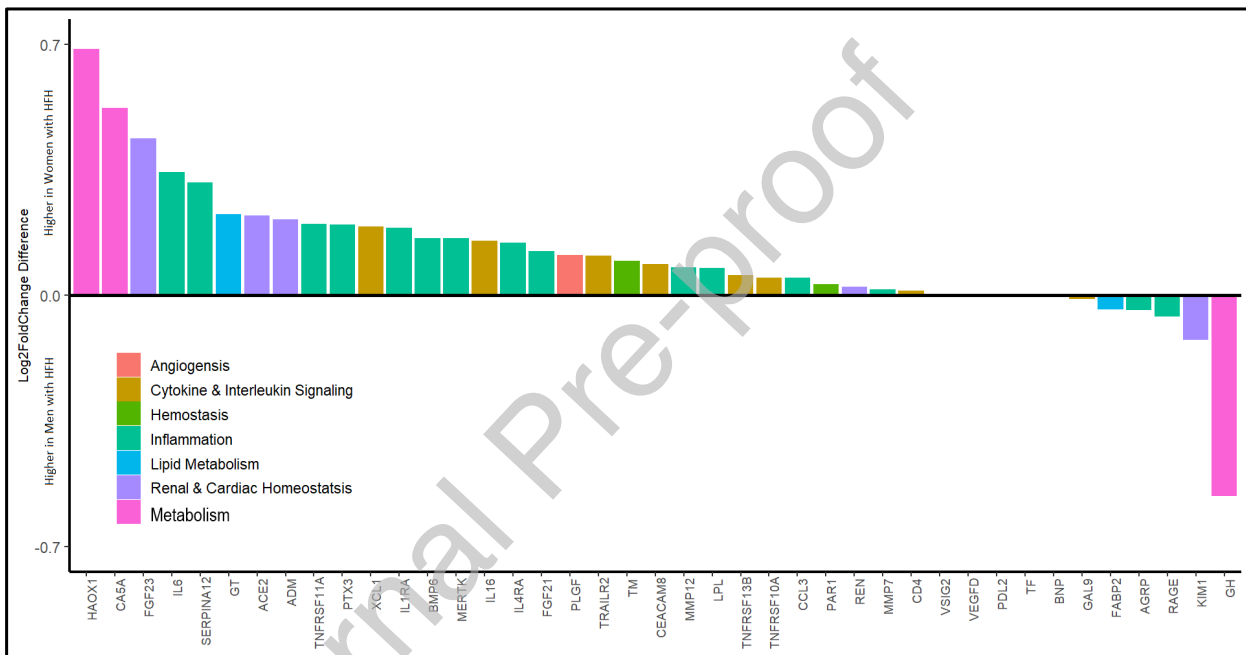




Figure 3.

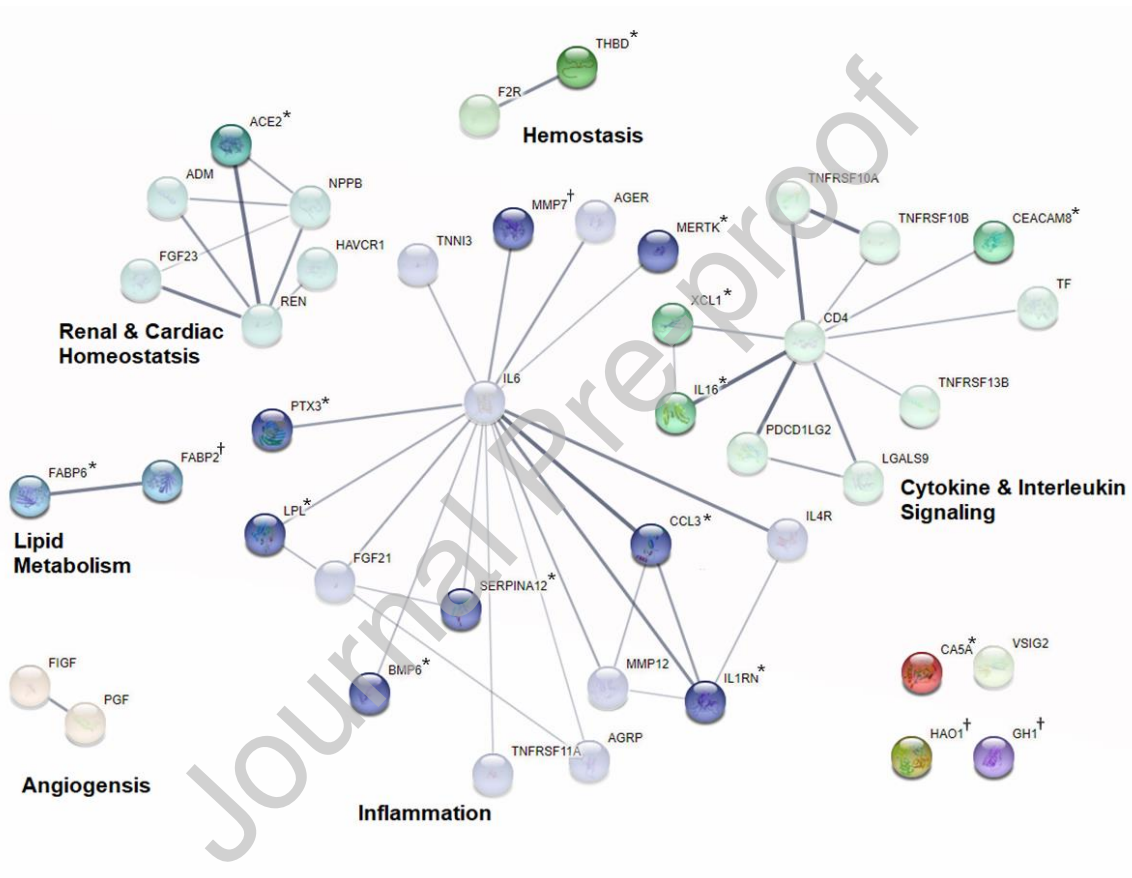
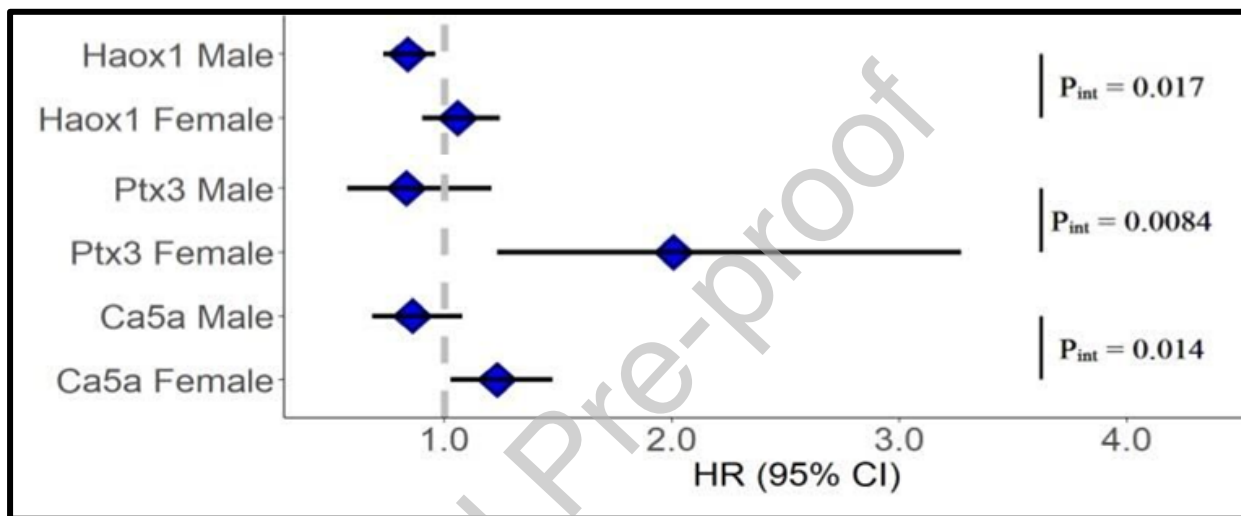


Figure 4.



**Table 1.** Characteristics of Male and Females Patients with Type 2 Diabetes, Acute Coronary Syndrome, and Heart Failure

Characteristic	Men				Females			
	All Male (N=3651)	No HFH (N=3534)	HFH (N=117)	<i>p</i> <i>value</i> *	All Females (N=1729)	No HFH (N=1651)	HFH (N=78)	<i>p</i> <i>value</i> *
<b>Demographics</b>								
Age (years)	59.76±9.74	59.63±9.71	63.45±9.84	<0.001	63.26±9.90	63.24±9.88	63.67±10.33	0.725
Body weight (kg)	85.26±18.94	85.32±18.87	83.53±21.00	0.366	75.73±17.97	75.57±17.50	79.23±25.91	0.221
Body-mass index (kg/m <sup>2</sup> )	29.12±5.29	29.12±5.24	29.24±6.56	0.839	30.23±6.11	30.14±5.96	31.96±8.61	0.069
Time between ACS and randomization (days)	47.39±21.87	47.46±21.85	45.07±22.50	0.259	48.74±22.24	49.00±22.32	43.29±19.80	0.016
<b>Medical History</b>								
Duration of diabetes (years)	8.40±7.75	8.25±7.58	13.11±10.81	<0.001	10.74±8.76	10.60±8.60	13.79±11.16	0.015
Current smoker	592 (16.21)	576 (16.30)	16 (13.68)	0.529	142 (8.21)	136 (8.24)	6 (7.69)	1.000
Hypertension	2910 (79.70)	2810 (79.51)	100 (85.47)	0.145	1559 (90.17)	1486 (90.01)	73 (93.59)	0.399
Myocardial infarction	3283 (89.92)	3170 (89.70)	113 (96.58)	0.023	1451 (83.92)	1377 (83.40)	74 (94.87)	0.011
Percutaneous coronary interventions	2394 (65.57)	2319 (65.62)	75 (64.10)	0.810	978 (56.56)	933 (56.51)	45 (57.69)	0.929
Coronary-artery bypass graftings	514 (14.08)	480 (13.58)	34 (29.06)	<0.001	174 (10.06)	155 (9.39)	19 (24.36)	<0.001
Congestive heart failure	931 (25.50)	858 (24.28)	73 (62.39)	<0.001	602 (34.82)	547 (33.13)	55 (70.51)	<0.001
Stroke	256 (7.01)	242 (6.85)	14 (11.97)	0.051	132 (7.63)	126 (7.63)	6 (7.69)	1.000
Peripheral arterial disease	337 (9.23)	311 (8.80)	26 (22.22)	<0.001	177 (10.24)	159 (9.63)	18 (23.08)	<0.001
<b>Index ACS</b>								
Myocardial infarction	2928 (80.20)	2822 (79.85)	106 (90.60)	0.007	1224 (70.79)	1159 (70.20)	65 (83.33)	0.011
Unstable angina requiring hospitalization	712 (19.50)	701 (19.84)	11 (9.40)		502 (29.03)	490 (29.68)	12 (15.38)	
Missing data	11 (0.30)	11 (0.31)	0 (0)		3 (0.17)	2 (0.12)	1 (1.28)	
<b>Laboratory</b>								
Glycated hemoglobin (%)	8.00±1.08	7.99±1.08	8.14±1.04	0.131	8.12±1.12	8.12±1.13	8.10±1.01	0.883

Creatinine at baseline (mg/dL)	1.12±0.42	1.11±0.42	1.39±0.55	<0.001	0.99±0.46	0.98±0.44	1.32±0.64	<0.001
HsTnI (ng/L)	47.74±240.89	46.19±232.47	95.47±424.80	0.226	79.79±1066.79	77.18±1082.55	135.78±643.10	0.460
eGFR								
Mean±SD (ml/min/1.73 m <sup>2</sup> )	73.81±20.76	74.26±20.55	45.07±22.50	<0.001	64.85±21.52	65.54±21.32	50.27±20.69	<0.001
<60 ml/min/1.73 m <sup>2</sup> - no. (%)	884 (24.21)	828 (23.43)	56 (47.86)	<0.001	681 (39.39)	629 (38.10)	52 (66.67)	<0.001

Numbers are mean ± SD for numeric variables and counts (%) for categorical variables. Clinical characteristics were compared between male and females, and between HFH and no HFH, using independent sample t-tests for continuous variables and  $\chi^2$  tests for categorical variables.

Abbreviations: SD = Standard Deviation, HsTnI = High Sensitivity Troponin I, eGFR = estimated Glomerular Filtration Rate

\* Difference between individuals who experienced heart failure hospitalization and those who did not

## Lay summary

This study studies the sex-specific variations in heart failure development and progression in individuals with type 2 diabetes following acute coronary syndrome. Interleukin-6 emerged a central driver of heart failure, offering potential therapeutic avenues applicable to both genders. Additionally, the observed elevation of specific circulating proteins associated with immunological pathways in female heart failure patients contributes to our understanding of the unique pathophysiological landscape in this clinical context.

## Key Point

- This research investigates sex-specific differences in the development and progression of heart failure in individuals with type 2 diabetes (T2D) following acute coronary syndrome (ACS).
- Interleukin-6 emerges as a central factor in the pathogenesis of heart failure, representing a potential therapeutic target applicable to both sexes.
- Elevated levels of specific circulating proteins related to immunological pathways in female heart failure patients provide valuable insights into the distinctive pathophysiological mechanisms underlying heart failure progression.

## Pathophysiological Sex-Differences in Heart Failure Progression after Acute Coronary Syndrome: Insights from the EXAMINE Trial

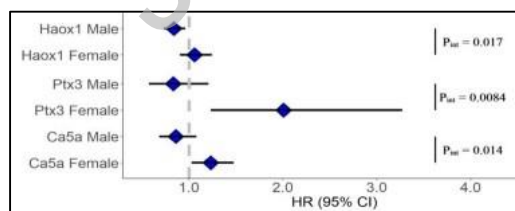


**POPULATION** 5,380 people with type II diabetes and recent acute coronary syndrome (ACS) enrolled in EXAMINE

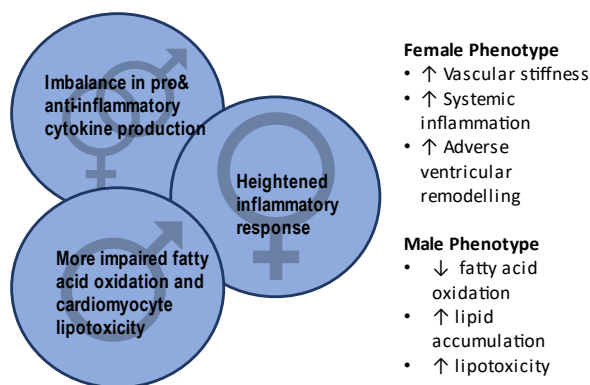
**AIM:** Identify sex-specific pathophysiological variations and circulating biomarkers in heart failure hospitalization (HFH).

Out of 43 biomarkers associated with HFH following ACS, 14 biomarkers were uniquely up- or down-regulated in females and 4 in males, suggesting sex-specific pathophysiological variations. Interleukin-6 emerged as a central pathological node in network analyses.

Three biomarkers were associated with increased risk of heart failure hospitalization after ACS in females compared to males.



## Sex-Specific Pathophysiological Variations



Higher levels of immunological in females developing heart failure suggest sex-specific variations leading to heart failure hospitalizations.

