Neurohormonal and clinical sex differences in heart failure

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Aims

Despite disparities in pathophysiology and disease manifestation between male and female patients with heart failure, studies focusing on sex differences in biomarkers are scarce. The purpose of this study was to assess sex-specific variation in clinical characteristics and biomarker levels to gain more understanding of the potential pathophysiological mechanisms underlying sex differences in heart failure.

Methods and results

Baseline demographic and clinical characteristics, multiple biomarkers, and outcomes were compared between men and women in 567 patients. The mean age of the study group was 71 ± 11 years and 38% were female. Women were older, had a higher body mass index and left ventricular ejection fraction, more hypertension, and received more diuretic and antidepressant therapy, but less ACE-inhibitor therapy compared with men. After 3 years, all-cause mortality was lower in women than men (37.0 vs. 43.9%, multivariable hazard ratio = 0.64; 95% confidence interval 0.45–0.92, P = 0.016). Levels of biomarkers related to inflammation [C-reactive protein, pentraxin 3, growth differentiation factor 15 (GDF-15), and interleukin 6] and extracellular matrix remodelling (syndecan-1 and periostin) were significantly lower in women compared with men. N-terminal pro-brain natriuretic peptide, TNF-αR1a, and GDF-15 showed the strongest interaction between sex and mortality.

Conclusion

Female heart failure patients have a distinct clinical presentation and better outcomes compared with male patients. The lower mortality was independent of differences in clinical characteristics, but differential sex associations between several biomarkers and mortality might partly explain the survival difference.

Keywords

Heart failure • Sex • Biomarkers • Aetiology • Mortality

Introduction

Heart failure (HF) is a clinical syndrome that affects both men and women. Although the total number of men and women living with heart failure is similar,1 female patients are underrepresented in clinical studies in heart failure.2,3 Therefore, evidence relating to pathophysiology, aetiology, clinical presentation, treatment, and outcome is predominantly based on data from male patients.4 A few major pharmacological and device trials in heart failure patients have performed sex-specific analyses. In contrast to patients with cardiovascular disease, these trials consistently reported an independent survival benefit for women.5–8 Sex-specific analysis of the Candesartan in Heart failure assessment of Mortality and Morbidity (CHARM)6 trial showed that differential survival is independent of age, left ventricular ejection fraction (LVEF) and the cause of heart failure. Sex-dependent differences in survival were also recently demonstrated in the Multi-center Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT),8 with women more frequently showing reverse cardiac remodelling.

However, the basic biological mechanisms related to the sex difference with regard to outcomes have not been properly addressed, despite known pathophysiological disparities involving inflammation and remodelling.9 We hypothesized that sex-specific differences in mortality are associated with disparities in biomarkers indicative of...
inflammation and remodelling. We performed sex-specific analyses on the variation in basic demographic and clinical characteristics, clinical outcomes, and levels of different biomarkers of inflammation, oxidative stress, remodelling, and cardiomyocyte stretch in a large number of heart failure patients.

**Methods**

**Study design and population**

The Coordinating study evaluating Outcomes of Advising and Counseling in Heart failure (COACH) data set was used. COACH was a multicentre, randomized, controlled, nurse-led disease management intervention trial testing whether follow-up by a cardiologist or basic or intensive additional support by a heart failure nurse improve outcomes in patients hospitalized with heart failure. No reduction of the combined endpoints of death and heart failure-related hospitalizations were seen with any intervention compared with the standard follow-up. Rationale, design, and detailed results have previously been reported elsewhere.10–14 The original COACH study included 1023 patients shortly before discharge following a heart failure hospitalization. Patients with the full continuum of LVEF were enrolled. This study refers to the subset of 567 patients from the COACH cohort, in whom samples for biomarker analysis were obtained.

**Study measures and laboratory tests**

The primary outcome measure for the present analyses was all-cause mortality within ~3 years (up to 1124 days). Secondary outcome variables were time to death or heart failure hospitalization and the number of days lost to death or hospitalization at 18 months. All other data were obtained at index hospitalization. Heart Failure with preserved Ejection Fraction was defined by LVEF $\geq$ 50%. The CES-D score15,16 was used for the assessment of depression with a score $\geq$ 16 indicating depressive symptoms; the quality of life was quantified using the Minnesota Living with Heart Failure questionnaire (MLHFQ).17 Post hoc analyses of biomarkers encompassed the markers displayed in Table 2. Biomarker analysis was performed using the following commercial assays: C-reactive protein, pentraxin 3 (PTX3), growth differentiation factor 15 (GDF-15), myeloperoxidase (MPO), galectin 3, syndecan-1, periostin, ST-2, tumour necrosis factor alpha (TNF-α), TNF-αR1a, osteopontin, RAGE, angioten- genin, endothelial cell-selective adhesion molecule (ESAM), cystatin C.

### Table 1 Baseline characteristics

| Demographics and HF characteristics | Total cohort (n = 567) | Male (n = 351) | Female (n = 216) | P-value |
|-------------------------------------|-----------------------|----------------|------------------|---------|
| Age, mean ± SD, years               | 71.0 ± 11.0           | 69.9 ± 10.6    | 72.7 ± 11.4      | 0.004   |
| Left ventricular EF, mean ± SD, %  | 32.5 ± 14.0           | 31.0 ± 13.0    | 34.9 ± 15.3      | 0.004   |
| Preserved EF, n (%)                 | 70 (15.2)             | 34 (11.8)      | 36 (20.9)        | 0.008   |
| Ischaemic aetiology, n (%)          | 232 (40.9)            | 168 (47.9)     | 64 (29.6)        | 0.000   |
| Duration of HF, median (IQR)        | 112 (22–1401)         | 123 (23–1336)  | 84 (21–1447)     | 0.770   |
| Anaemia, n (%)                      | 118 (37.8)            | 50 (26.2)      | 68 (56.2)        | 0.000   |
| NYHA class (II/III/IV), %           | 232 (40.9)            | 168 (47.9)     | 64 (29.6)        | 0.000   |
| MLHF Questionnaire, median (IQR)    | 5.0/54.7/40.3         | 6.0/53.7/40.3  | 3.3/56.3/40.4    | 0.345   |

**Clinical signs**

| Weight, mean ± SD, kg              | 77.4 ± 16.8           | 80.5 ± 16.1    | 72.2 ± 16.7      | 0.000   |
| Body mass index, mean ± SD, kg/m²  | 27.1 ± 5.5            | 26.7 ± 4.8     | 27.9 ± 6.6       | 0.020   |
| Systolic blood pressure, mean ± SD, mmHg | 118.2 ± 21.2 | 116.7 ± 20.6  | 120.6 ± 21.9    | 0.034   |
| Ankle oedema, n (%)                | 359 (64.5)            | 214 (62.2)     | 145 (68.1)       | 0.160   |

**Comorbidities [n (%)]**

| Hypertension                       | 240 (42.3)            | 135 (38.5)     | 105 (48.6)       | 0.018   |
| Diabetes                           | 173 (30.5)            | 96 (27.4)      | 77 (35.7)        | 0.037   |
| Atrial fibrillation or flutter     | 261 (46.0)            | 166 (47.3)     | 95 (44.0)        | 0.442   |
| Chronic obstructive pulmonary disease | 159 (28.0)    | 111 (31.6)     | 48 (22.2)        | 0.016   |
| Depression (CES-D score $\geq$6)  | 208 (39.3)            | 123 (37.1)     | 85 (42.9)        | 0.189   |

**Medication [n (%)]**

| ACE-inhibitor                      | 286 (50.4)            | 189 (53.9)     | 97 (44.9)        | 0.039   |
| Angiotensin receptor blocker       | 71 (12.5)             | 45 (12.8)      | 26 (12.0)        | 0.784   |
| Beta-blocker                       | 250 (44.1)            | 161 (45.9)     | 89 (41.2)        | 0.277   |
| Spironolactone                     | 166 (29.3)            | 92 (26.2)      | 74 (34.3)        | 0.041   |
| Diuretic                           | 438 (77.3)            | 258 (73.5)     | 180 (83.3)       | 0.007   |
| Digoxin                            | 146 (25.8)            | 90 (25.6)      | 56 (25.9)        | 0.940   |
| Antidepressants                    | 34 (6.0)              | 12 (3.4)       | 22 (10.2)        | 0.001   |

EF, ejection fraction; HF, heart failure; NYHA, New York Heart Association; MLHF, Minnesota Living with Heart Failure; CES-D, Center for Epidemiologic Studies Depression; ACE, angiotensin-converting enzyme.
Table 2  Sex-specific biomarker levels

| Biomarker                  | Total cohort (n = 567) | Male (n = 351) | Female (n = 216) | P-value |
|----------------------------|------------------------|----------------|------------------|---------|
| **Inflammation**           |                        |                |                  |         |
| C-reactive protein, µg/mL  | 11.4 (4.8–33.0)        | 13.0 (5.5–33.0)| 9.0 (4.2–28.9)   | 0.018   |
| PTX 3, ng/mL               | 3.7 (2.5–5.6)          | 3.9 (2.7–5.8)  | 3.3 (2.2–5.0)    | 0.002   |
| GDF-15, ng/mL              | 2.8 (1.9–4.3)          | 3.1 (2.2–4.7)  | 2.4 (1.7–3.8)    | 0.000   |
| Osteopontin, ng/mL         | 159.2 (109.0–223.1)    | 165.7 (111.4–232.8)| 147.2 (100.9–209.3)| 0.083   |
| RAGE, ng/mL                | 2.9 (1.9–4.6)          | 3.0 (1.9–4.7)  | 2.7 (1.9–4.2)    | 0.165   |
| Interleukin 6, ng/mL       | 12.0 (6.8–24.3)        | 13.1 (7.9–28.4)| 10.9 (5.9–18.4)  | <0.001  |
| NTpro-BNP, pg/mL           | 45.8 (4.7–121.3)       | 47.3 (4.7–146.4)| 43.7 (4.8–85.0)  | 0.230   |
| TNF-α, pg/mL               | 3.1 (2.2–4.6)          | 3.1 (2.2–4.7)  | 2.9 (2.2–4.4)    | 0.500   |
| **Oxidative stress**       |                        |                |                  |         |
| MPO, ng/mL                 | 20.1 (15.6–28.1)       | 20.4 (15.7–28.4)| 19.1 (15.3–26.5)| 0.115   |
| **Remodelling**            |                        |                |                  |         |
| Syndecan-1, ng/mL          | 20.8 (15.4–28.5)       | 20.8 (15.4–28.5)| 17.7 (12.2–26.1)| 0.004   |
| Periostin, ng/mL           | 4.7 (3.4–6.6)          | 5.0 (3.5–6.6)  | 4.4 (3.1–6.3)    | 0.023   |
| Galectin 3, ng/mL          | 25.6 (21.1–32.1)       | 26.2 (21.5–32.5)| 24.9 (20.2–31.2)| 0.057   |
| TGF-β, ng/mL               | 51 (35–75)             | 48 (34–72)     | 53 (36–82)       | 0.043   |
| **Cardiomyocyte stretch**  |                        |                |                  |         |
| NTpro-BNP, pg/mL           | 2532 (1309–5721)       | 2677 (1407–6340)| 2344 (1197–5047)| 0.978   |
| ST-2, ng/mL                | 2.5 (1.4–5.4)          | 2.6 (1.5–5.4)  | 2.2 (1.2–5.5)    | 0.069   |
| **Angiogenesis**           |                        |                |                  |         |
| VEGF, ng/mL                | 63.0 (31.4–143.8)      | 58.7 (27.3–118.0)| 73.1 (36.8–189.4)| 0.003   |
| Angiogenin, µg/mL          | 5.1 (3.6–7.5)          | 5.0 (3.6–7.4)  | 5.3 (3.5–8.0)    | 0.465   |
| **Arteriosclerosis**       |                        |                |                  |         |
| ESAM, ng/mL                | 53.0 (44.5–64.3)       | 54.1 (45.5–65.1)| 51.3 (43.0–62.1)| 0.038   |
| **Renal function**         |                        |                |                  |         |
| eGFR, mL/min/1.73m²        | 53.9 +/− 20.2          | 55.8 +/− 19.9  | 50.9 +/− 20.2    | 0.006   |
| Cystatin C, µg/mL          | 11.1 (7.6–16.2)        | 11.1 (7.7–16.9)| 11.1 (7.6–15.7)  | 0.774   |
| NGAL, ng/mL                | 84.6 (60.4–123.3)      | 85.8 (61.3–135.9)| 83.8 (58.8–116.1)| 0.127   |
| **Anemia**                 |                        |                |                  |         |
| Hb, g/dL                   | 13.1 +/− 2.0           | 13.4 +/− 2.1   | 12.6 +/− 1.8     | <0.001  |
| EPOa, IU/L                 | 9.6 (5.2–16.0)         | 9.7 (5.1–16.5) | 9.5 (5.2–15.0)   | 0.569   |

PTX3, pentraxin 3; GDF-15, growth differentiation factor 15; RAGE, receptor for advanced glycation end products; TNF-α, tumour necrosis factor alpha; TNF-αR1a, tumour necrosis factor alpha receptor 1a; MPO, myeloperoxidase; TGF-β, transforming growth factor-beta; NTpro-BNP, N-terminal pro-brain natriuretic peptide; ST-2, suppression of tumourigenicity 2; VEGF, vascular endothelial growth factor; EPOa, erythropoietin alpha; ESAM, endothelial cell-selective adhesion molecule; NGAL, neutrophil gelatinase-associated lipocalin.

and neutrophil gelatinase-associated lipocalin (NGAL) were measured by Alere San Diego, Inc., San Diego, CA, USA, using competitive enzyme-linked immunosorbent assays (ELISAs) on a Luminex® platform. Transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) were analysed using a quantitative multiplexed sandwich ELISA system, SearchLight® proteome arrays, Aushon BioSystems, Billerica, MA, USA. N-terminal pro-brain natriuretic peptide (NTpro-BNP) was measured using the Elecsys proBNP ELISA by Roche Diagnostics, Mannheim, Germany. Erythropoietin alpha (EPOa) was measured using the IMMULITE® EPO ELISA by Diagnostic Products Corporation, Los Angeles, CA, USA. Estimated glomerular filtration rate (eGFR) was based on the simplified Modification of Diet in Renal Disease (MDRD) formula. Anaemia was diagnosed using the World Health Organization (WHO) definition with a haemoglobin threshold of 13.0 g/dL in men and 12.0 g/dL in women.

**Statistical analyses**

Continuous variables are presented as mean ± SD or median with interquartile range, where appropriate. Categorical variables are presented as counts and percentages. Comparisons of continuous variables were performed using either Student’s t-test or the Mann–Whitney test, as appropriate. The χ² test was used to test for categorical variables.

Hazard ratios (HRs) were calculated using univariable and multivariable Cox proportional hazards regression. The proportionality
assumption for the Cox regression analysis was evaluated on the basis of Schoenfeld residuals.

Biomarkers were used on a continuous scale for baseline sex-comparison and with log-transformation in Cox proportional hazards models.

First, univariable Cox proportional hazards regression analyses of the sex-specific outcome were performed using baseline characteristics and previously established cofounders of the COACH Risk engine.\(^\text{18}\)

Secondly, multivariable Cox proportional hazards regression was performed, adjusting for variables, which showed univariable association with 3-year mortality at \(P < 0.1\) in this cohort. The variables entered to the multivariable model comprised: age, ischaemic aetiology (i.e. previous myocardial infarction), duration of HF, MLHFQ score, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, ankle oedema, diabetes, atrial fibrillation, ACE-inhibitor therapy, beta-blocker therapy, aldosterone antagonist therapy, diuretic therapy, digoxin therapy, stroke, peripheral vascular disease, previous heart failure hospitalization, serum sodium.

Thirdly, comprehensive multivariable modelling was performed, separately adding the respective biomarkers to the model to detect the most relevant change in point estimates for relative hazard ratios. Furthermore, we studied the interaction of the individual biomarkers with the sex-effect on mortality.

Statistical analyses were performed using the STATA (version 11.0, STATA Corp, College Station, TX, USA) and R (version 2.15.1, R Foundation for Statistical Computing, Vienna, Austria) software. A two-sided \(P\)-value < 0.05 was considered statistically significant.

### Results

#### Baseline demographic and clinical characteristics

Of the 567 patients of COACH included in this analysis 216 (38\%) were female (Table 1). On average, women were 2.7 years older, showed 3.9% higher absolute LVEF and a greater proportion of preserved LVEF than men. Ischaemic aetiology of heart failure was significantly less prevalent in women. Anaemia was more than twice as common in women, while chronic obstructive pulmonary disease was more prevalent in women compared with men. The duration of heart failure, NYHA functional class and the Minnesota Living with Heart Failure Questionnaire (MLHFQ) score did not differ between sexes.

Women had 8.3 kg lower average body weight, but 1.1 kg/m\(^2\) higher BMI. Systolic blood pressure was higher by 3.9 mmHg in women compared with men. Hypertension and diabetes were more common in women, while chronic obstructive pulmonary disease was more prevalent in men. No difference between male and female patients was found regarding atrial fibrillation or flutter; neither for signs of depression. However, women used more antidepressants compared with men. Women also received less ACE-inhibitor therapy, but more diuretics and spironolactone compared with men.

#### Biomarker levels

Table 2 provides an overview of the biomarker levels in male and female patients. Women had consistently lower values than men for inflammatory markers C-reactive protein, PTX3, GDF-15, and Interleukin 6, while no statistical difference was detectable for osteopontin, TNF-\(\alpha\) and TNF-\(\alpha\)R1a, and MPO, a marker of oxidative stress. In addition, lower levels of the remodelling markers syndecan-1 and periostin were found in women, while lower galectin-3 levels were not significant. Transforming growth factor-beta was significantly higher in women compared with men. Levels of the myocardial stretch markers NTpro-BNP and ST-2 were not significantly different between sexes. The angiogenesis marker VEGF was significantly higher in women, while there was no sex difference for Angiogenin. Endothelial cell-selective adhesion molecule, a marker of arteriosclerosis, was significantly lower in female compared with male patients. No sex differences in the levels of the biomarkers of renal function cystatin C and NGAL were found, whereas eGFR was lower by 4.9 mL/min/1.73 m\(^2\) in women compared with men. Haemoglobin levels were 0.8 g/dL lower in women, whereas no different levels of the erythropoiesis marker EPO could be detected between both sexes.

#### Outcomes

##### Mortality

The estimated 3-year event rates are 44\% (39–49\%) for males and 37\% (31–44\%) for females, respectively. Table 3 shows the age-adjusted and multivariable association of sex with mortality. Female heart failure patients had lower age-adjusted 3-year all-cause mortality compared with male patients (HR \(=\) 0.71; 95% CI: 0.54–0.93, \(P = 0.014\)). Figure 1 shows the sex-specific Kaplan–Meier survival curves.

### Table 3: Sex-specific outcome analyses

| Variable | Sex | Adjusted HR (95% CI) | Z (P) |
|----------|-----|----------------------|-------|
| Age adjusted | Sex | 0.71 (0.54–0.93) | −2.45 (0.014) |
| Multivariable | Sex | 0.64 (0.45–0.92) | −2.41 (0.016) |

The univariate and age-adjusted point estimates for the adjusted HR (95% CI) and the \(Z\) and \(P\) values for sex.

### Figure 1: Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality.
curves for 3-year all-cause mortality. In a multivariable model, we adjusted for the clinical risk markers for mortality in this patient cohort. Even after full adjustment, female sex was associated with a 36% lower mortality risk (HR = 0.64; 95% CI 0.45–0.92, P = 0.016). The proportionality assumption held ($\chi^2 = 16.92; P = 0.716$).

### Biomarkers

Table 4 shows the association between the individual biomarkers, sex, and mortality and also the P-values for interaction of individual biomarkers with sex. Concerning the association between female sex and mortality, the change in point estimate for the hazard ratio was most pronounced when adding NTpro-BNP [from 0.64

| Covariate | Point estimate for sex (multivariable model + individual biomarker) | Interaction (multivariable model + interaction term) |
|-----------|------------------------------------------------------------------|---------------------------------------------------|
|           | Adjusted HR (95% CI) | Z (P) | P-value |
| Inflammation |                                     |                  |               |
| C-reactive protein (n = 567) | 0.66 (0.46–0.94) | −2.30 (0.022) | 0.217 |
| PTX (n = 567) | 0.68 (0.47–0.97) | −2.10 (0.035) | 0.105 |
| GDF-15 (n = 567) | 0.73 (0.50–1.05) | −1.72 (0.086) | 0.072 |
| Osteopontin (n = 567) | 0.65 (0.46–0.94) | −2.30 (0.021) | 0.548 |
| RAGE (n = 567) | 0.64 (0.44–0.91) | −2.45 (0.014) | 0.214 |
| Interleukin 6 (n = 526) | 0.71 (0.48–1.05) | −1.71 (0.087) | 0.272 |
| TNF-α (n = 464) | 0.58 (0.38–0.87) | −2.65 (0.008) | 0.567 |
| TNF-α R1α (n = 567) | 0.66 (0.46–0.95) | −2.24 (0.025) | 0.057 |
| Oxidative stress |                                     |                  |               |
| MPO (n = 567) | 0.64 (0.47–0.92) | −2.43 (0.015) | 0.078 |
| Remodelling |                                     |                  |               |
| Syndecan (n = 567) | 0.66 (0.46–0.95) | −2.23 (0.026) | 0.093 |
| Periostin (n = 567) | 0.65 (0.45–0.93) | −2.38 (0.017) | 0.333 |
| Galectin 3 (n = 567) | 0.63 (0.44–0.91) | −2.47 (0.014) | 0.084 |
| TGF-β (n = 547) | 0.62 (0.43–0.90) | −2.52 (0.012) | 0.277 |
| Cardiomyocyte stretch |                                     |                  |               |
| NTpro-BNP (n = 538) | 0.79 (0.54–1.14) | −1.25 (0.212) | 0.039 |
| ST-2 (n = 567) | 0.65 (0.45–0.93) | −2.36 (0.018) | 0.624 |
| Angiogenesis |                                     |                  |               |
| VEGF (n = 515) | 0.65 (0.45–0.92) | −2.39 (0.017) | 0.283 |
| Angiogenin (n = 567) | 0.65 (0.45–0.94) | −2.31 (0.021) | 0.318 |
| Arteriosclerosis |                                     |                  |               |
| ESAM (n = 567) | 0.65 (0.45–0.93) | −2.37 (0.018) | 0.082 |
| Renal function |                                     |                  |               |
| eGFR (n = 557) | 0.60 (0.41–0.85) | −2.81 (0.005) | 0.135 |
| Cystatin C (n = 567) | 0.65 (0.45–0.93) | −2.36 (0.019) | 0.723 |
| NGAL (n = 562) | 0.69 (0.48–0.99) | −2.01 (0.044) | 0.141 |
| Anaemia |                                     |                  |               |
| Hb (n = 312) | 0.52 (0.32–0.85) | −2.61 (0.009) | 0.588 |
| EPOα (n = 565) | 0.64 (0.45–0.92) | −2.41 (0.016) | 0.239 |

The change in point estimates for relative hazard ratios of the sex- and biomarker-variables if the respective biomarkers are added separately to a model adjusting for variables which showed univariate association with 3-year mortality at $P < 0.1$: age, ischaemic aetiology (i.e., previous myocardial infarction), duration of HF, MLwHF score, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, ankle oedema, diabetes, atrial fibrillation, ACE-inhibitor therapy, beta-blocker therapy, aldosterone antagonist therapy, diuretic therapy, digoxin therapy, stroke, peripheral vascular disease, previous heart failure hospitalization, and serum sodium. PTX3, pentraxin 3; GDF-15, growth differentiation factor 15; RAGE, receptor for advanced glycation end products; TNF-α, tumour necrosis factor alpha; TNF-α R1α, tumour necrosis factor alpha receptor 1α; MPO, myeloperoxidase; TGF-β, transforming growth factor-beta; NTpro-BNP, N-terminal pro-brain natriuretic peptide; ST-2, suppression of tumorigenicity 2; VEGF, vascular endothelial growth factor; ESAM, endothelial cell-selective adhesion molecule; eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; Hb, haemoglobin; EPOα, erythropoietin alpha.
Figure 2 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on growth differentiation factor-15 tertiles for men (A) and women (B).

Figure 3 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on tumour necrosis factor-αR1a tertiles for men (A) and women (B).

Figure 4 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on myeloperoxidase tertiles for men (A) and women (B).
Figure 5 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on syndecan tertiles for men (A) and women (B).

Figure 6 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on galectin 3 tertiles for men (A) and women (B).

Figure 7 Sex-stratified Kaplan–Meier curves for 3-year all-cause mortality based on N-terminal pro-brain natriuretic peptide tertiles for men (A) and women (B).
In addition to NT-pro-BNP and GDF-15, we found that TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had a different prognostic value in male vs. female patients. Kaplan–Meier survival curves for all biomarkers with significant interaction between sex and outcome are shown in Figures 2–8.

**Discussion**

This is the first study to report on biomarker-related differences between male and female heart failure patients. We confirmed that female heart failure patients have a better prognosis compared with male patients, which could not be explained by the difference in clinical characteristics. Interestingly, several biomarkers were lower in women, and in addition to NT-pro-BNP, GDF-15, TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had a sex-dependent prognostic value.

**Sex-related clinical characteristics**

Female patients in COACH showed the typical female clinical presentation pattern of heart failure, which has been characterized in many other studies and registries. Female heart failure patients are generally older, have more preserved LVEF, suffer less frequently from ischaemic cardiomyopathy, and show more hypertension and signs of congestion compared with male patients. These classic sex-specific manifestations could be interpreted as reflections of the underlying sex disparities in pathophysiology and natural development of heart failure over time. However, these differences alone do not explain the survival benefit in women.

**Survival differences**

The survival benefit for women in the present study is consistent with the results of other studies: O’Meara et al. reported an independent survival benefit for women in the CHARM trial, accounting for LVEF and the cause of heart failure [adjusted hazard ratio (HR), 0.77; 95% CI: 0.69 to 0.86; P < 0.001]. Notably, this large cohort comprised patients with both reduced and preserved EF. Alla et al. published the sex-specific findings of the Digitalis Investigation Group (DIG) trial showing comparable results, with independent survival benefit for women irrespective of LVEF, cause of heart failure or duration of heart failure. Similarly, population-based studies consistently report lower mortality for female heart failure patients, precluding trial-specific selection bias as an explanation for the survival benefit. A recent large individual patient data meta-analysis powerfully supports this finding. However, none of the studies performed to date has adequately clarified a biological background for the survival benefit for women with heart failure.

**Biomarkers**

Overall, lower baseline levels of biomarkers indicative of inflammation and remodelling suggest less biological activity in the respective pathophysiological pathways in women compared with men. This may imply a different biological disease expression, but could also reflect natural biological variation between sexes. However, in healthy populations, women show higher basic levels of C-reactive protein, PTX3, RAGE, galectin 3, and NT-pro-BNP. Other studies report that GDF-15, VEGF, NGAL, and EPOa were similar in men and women. Lower normal levels of TNF-α, TGF-β, ESAM, GFR, cystatin C, and haemoglobin have been reported in women. Sex-specific population-based data are scarce for the remaining markers. Reference levels from cohorts of healthy volunteers of each sex can be found in Supplementary material online, Table S1. This suggests that women hospitalized for heart failure have a distinct biological disease expression compared with men.

**Experimental differences in pathophysiological pathways between sexes**

Our observation, that biomarkers related to inflammation and remodelling were significantly lower in women, might reflect the sex-dependent different aetiology of heart failure, sex-characteristic remodelling pattern, and the influence of comorbidities, all of which are associated with a distinctive increase in biomarkers of...
inflammation and remodelling. Biologically, the sex differences are most likely attributable to the effects of oestrogen on the corresponding pathophysiological pathways, as shown by experimental data in animals and humans in cardiovascular disease and heart failure.³⁹

The main demographic and aetiological sex difference in heart failure is a predominance of myocardial infarctions and the presence of ischaemic heart disease in men over women. Inflammation is one of the key processes in myocardial damage and the post-myocardial infarction remodelling process, which might explain higher inflammatory activation in male heart failure patients. However, there is a well-known profound interaction of female sex and oestrogen with the specific remodelling pattern and the progression to heart failure.⁴⁰ Female sex is reliably associated with a slowed and attenuated development of adverse cardiac remodelling and heart failure in various animal models and human studies on myocardial injury,⁴¹,⁴² pressure,⁴³–⁴⁶ and volume overload.⁴⁷–⁴⁹ Therefore, it can be speculated that the lower concentrations of inflammatory and remodelling biomarkers in women are to be regarded as a surrogate of less scar or adverse remodelling burden.

Notably, most of the comorbidities, which might potentially confound the levels of inflammation makers by being associated with an increase of respective values, are preferentially seen in women with heart failure. Thus, diabetes,⁵⁰ BMI,⁵¹,⁵² and depression⁵³ have previously been shown to increase inflammatory biomarkers.

Additionally, age is known to influence the expression of inflammatory markers. With increasing age, the level of expression of inflammatory markers increases in the general population.⁵⁴ Notably, with regard to the fact that women were 2.7 years older on average in our study population than men, the lower level of inflammatory markers among women in our study population of heart failure patients compared with men appears remarkable.

Pathophysiological rationale

There is a strong pathophysiological rationale that the female cardiovascular response to damage is different from that in men. Men are prone to remodelling with LV-dilatation and fibrosis while women more frequently remodel with marked concentric hypertrophy and smaller LV cavity volumes.⁵⁵ These different mechanistic adaptions imply that heart failure does not necessarily depend on reduction of LVEF, but includes heart failure with preserved EF,⁵⁶ which is more common in women.²³ However, arbitrary dichotomization of heart failure into preserved or reduced LVEF, as used in many clinical trials, does not appear to adequately explain sex differences in heart failure presentation and outcome. As Adams et al.⁵⁷ demonstrated, female gender is significantly associated with better survival (P < 0.001), depending on the primary aetiology of heart failure instead of baseline ventricular function. Women had better survival than men when heart failure aetiology was non-ischaemic. This relationship has also been proven by the results from the BEST study, where the prognostic benefit of non-ischaemic heart failure aetiology was stressed.⁵⁸ Our own results in the total COACH cohort match these findings, by showing a pronounced survival benefit for women with non-ischaemic heart disease (31.6 vs. 39.9%; age-adjusted hazard ratio = 0.65; 95% confidence interval 0.45–0.94, P = 0.022). While differences in age obviously do not explain the sex difference in survival, a sex difference in symptom and disease burden may. At time of heart failure hospitalization women may present at earlier biological stage of heart failure, while men often present at a pathophysiologically more advanced stage of (mostly ischaemic) cardiomyopathy with already reduced LVEF, translating to a survival disadvantage during the follow-up.

This hypothesis integrates gender (psychosocial) and sex (biological) aspects, and COACH uniquely allows the analysis of both features simultaneously. In our study cohort women did not differ from men regarding NYHA class, and Minnesota Living with Heart failure questionnaire scores at index admission. Although also not significantly differing in terms of current suffering from depression, as defined by a CES-D score ≥ 16, the rate of depression in women was higher and they showed more concomitant antidepressant use, suggestive for a higher depression prevalence in women. Depression is a common co-morbidity in heart failure, especially in women,⁵⁹,⁶⁰ a finding confirmed in our population. Although it has previously been linked to worse mortality in heart failure with reduced⁶¹ and preserved LVEF,⁶² we found no association with all-cause mortality in our cohort, which may be explained by treatment effects related to specific antidepressants or study participation. Although baseline elevations of inflammatory biomarkers such as IL-6 and C-reactive protein have previously been associated with depressive symptoms in the COACH cohort,⁶³ there was no sex-specific correlation, and in the present sex-specific analysis women had even lower baseline levels of inflammatory markers.

Study limitations

This study is affected by the typical limitations of post hoc analyses, necessitating cautious interpretation. COACH had no specific design to warrant sufficient power for analyses of the sex subgroups. No a priori hypotheses on the sex subgroups were stated in advance. Furthermore, post hoc biomarker analysis in a subset of patients introduces potential selection bias. Assignment of biomarkers to individual pathophysiological process categories is somewhat arbitrary and cannot account for the diverse biological activity of individual markers. The lack of data on oestrogen levels or menopause does not allow respective differentiation. No data regarding previous pregnancies of female patients are available in COACH, which precludes investigation of a link between previous pregnancies and biomarkers. Study inclusion and biomarker sampling in COACH were done just before discharge, in a stable clinical condition. Therefore in COACH patients cannot be considered to have acute heart failure, but they are also not completely comparable with chronic heart failure patients. This study is based on biological subgroup classification, is exploratory in nature, and aims to generate new hypotheses. A causal relationship cannot be concluded from the present data and the hypothesis generating results should be confirmed in separate analyses.

Conclusion

Female heart failure patients have a different clinical presentation and better outcomes compared with male patients. Several biomarkers related to inflammation and remodelling were significantly lower in women and NTpro-BNP, GDF-15, TNF-αR1a, MPO, syndecan, galectin 3, and ESAM had sex-dependent prognostic value.

Our findings indicate that the biological state of heart failure at admission is less advanced in women compared with men and suggest...
the sex-specific natural history and course of remodelling may be of particular relevance. There is an unmet need to clarify the pathophysiological processes involved in sex differences in heart failure. Especially in women, current study data are scarce and that requires preferential inclusion of women in clinical trials and related preliminary planning of studies to bridge the gap in current knowledge between men and women.

Supplementary material
Supplementary material is available at European Heart Journal online.

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Ethical approval
The study was approved by the local Ethics Committee and conducted in accordance with Declaration of Helsinki guidelines. All patients provided written informed consent. Additional consent was obtained for 36-month follow-up.

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