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Published in:
Europace

DOI:
10.1093/europace/euab179

Publication date:
2022

ESC European Society of Cardiology

Europace (2022) **24**, 193–201
doi:10.1093/europace/euab179

CLINICAL RESEARCH
Atrial fibrillation

Different circulating biomarkers in women and men with paroxysmal atrial fibrillation: results from the AF-RISK and RACE V studies

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Received 9 October 2020; editorial decision 24 June 2021; accepted after revision 29 June 2021; online publish-ahead-of-print 30 July 2021

Aims The clinical risk profile of atrial fibrillation (AF) patients is different in men and women. Our aim was to identify sex differences in blood biomarkers in patients with paroxysmal AF.

Methods and results Sex differences in 92 blood biomarkers were measured in 364 patients included in our discovery cohort, the identification of a risk profile to guide atrial fibrillation therapy (AF-RISK) study, assessed by multivariable logistic regression and enrichment pathway analysis. Findings were subsequently confirmed in 213 patients included in our validation cohort, the Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) study. In the discovery cohort, mean age was 59 ± 12 years, 41% were women. CHA_2DS_2-VASc-score was 1.6 ± 1.4. A total of 46% had hypertension, 10% diabetes, and 50% had heart failure, predominantly with preserved ejection fraction (47%). In women, activated leucocyte cell adhesion molecule (ALCAM) and fatty acid binding protein-4 (FABP-4) were higher. In men, matrix metalloproteinase-3 (MMP-3), C-C motif chemokine-16 (CCL-16), and myoglobin were higher. In the validation cohort, four out of five biomarkers could be confirmed: levels of ALCAM ($P = 1.73 \times 10^{-4}$) and FABP-4 ($P = 2.46 \times 10^{-7}$) and adhesion biological pathways [false discovery rate (FDR) = 1.23×10^{-8}] were higher in women. In men, levels of MMP-3 ($P = 4.31 \times 10^{-8}$) and myoglobin ($P = 2.10 \times 10^{-4}$) and markers for extracellular matrix degradation biological pathways (FDR = 3.59×10^{-9}) were higher.

Conclusion In women with paroxysmal AF, inflammatory biomarkers were more often higher, while in men with paroxysmal AF, biomarkers for vascular remodelling were higher. Our data support the clinical notion that pathophysiological mechanisms in women and men with AF may differ.

Trial registration Clinicaltrials.gov identifier NCT01510210 for AF-RISK; Clinicaltrials.gov NCT02726698 for RACE V.

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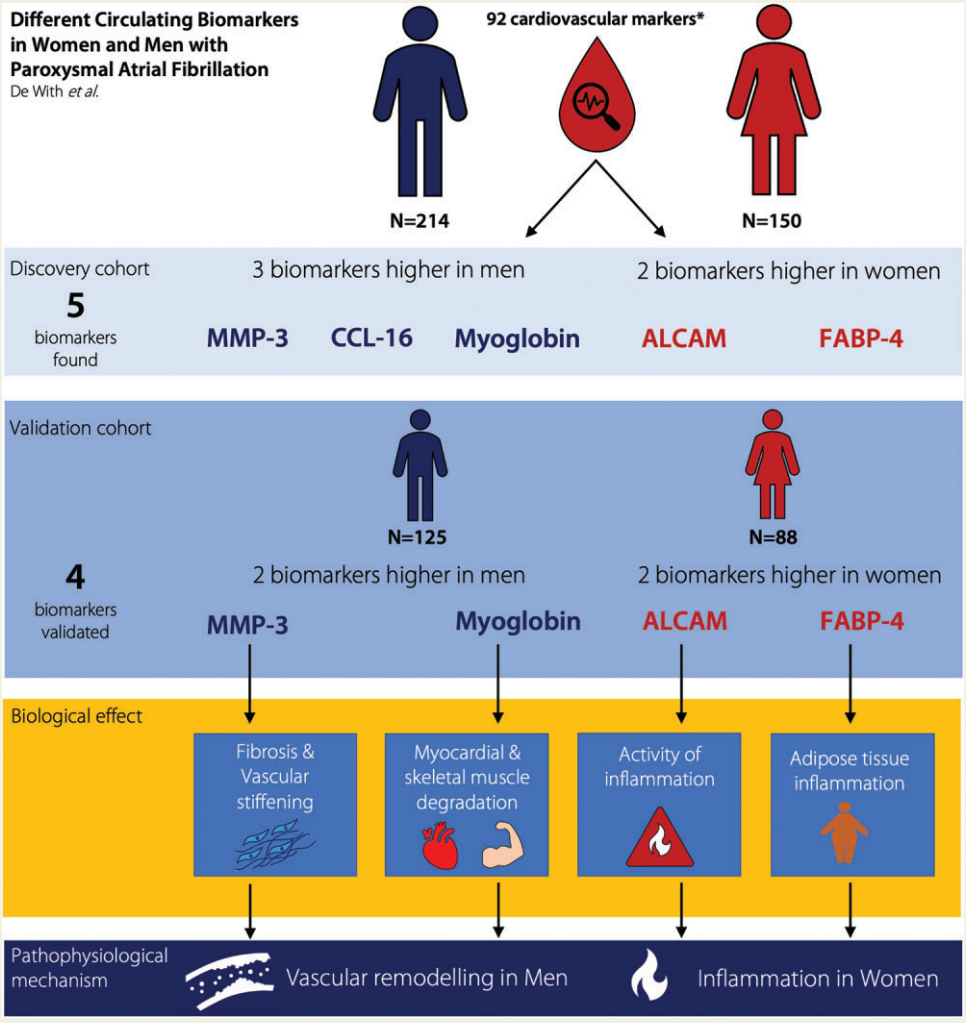
Graphical Abstract



Keywords Atrial fibrillation • Blood biomarkers • Sex differences • Inflammation • Vascular disease

What's new?

- In patients with paroxysmal atrial fibrillation (AF), inflammation biomarkers are more expressed in women in comparison to men, and vascular remodelling biomarkers are more expressed in men in comparison to women.
- In patients with paroxysmal AF, enrichment of cell adhesion pathways in women compared to men, and enrichment of extracellular matrix organization pathways in men compared to women were observed.
- Blood biomarker analysis may contribute to a personalized medicine approach in patients with paroxysmal AF.

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia around the world and the number will increase due to ageing populations and active search for early diagnosing.[1,2] The development of AF is driven by risk factors including, but not limited to, ageing, obesity, and underlying cardiovascular risk factors and diseases.[2] The cumulative prevalence of AF over the years is high and comparable in both sexes. However, women and men with AF differ regarding age and presence of comorbidities; women with AF are older, have more hypertension, valvular heart disease, and heart failure with preserved ejection fraction, and men with AF present more often coronary artery disease.[2] Whether the age difference or clustering of comorbidities are causative of the difference in clinical risk profile of AF

between women and men is yet to be determined. Other factors such as sex hormones or differentially expressed blood biomarkers representing distinct biological pathways may also play a role.[1,2]

Blood biomarkers can be seen as representation of distinct biological pathways and may differ between men and women with AF. C-reactive protein (CRP), an inflammatory biomarker, and NT-pro-B-type natriuretic peptide (NT-proBNP), a biomarker indicating cardiac stretch, have been shown to differ in women and men. CRP has been associated with AF incidence in men, whereas NT-proBNP has been associated with incident AF in women.[1] NT-proBNP and fibroblast growth factor 23, a hormone regulating biomarker associated with AF, have been suggested to help to identify those at risk for AF[3]; NT-proBNP and Cancer Antigen 125 (CA-125) have been associated with AF in patients without any concomitant disease.[4] Therefore, biomarkers may help to find guidance for a personalized approach to patients with AF.[2–4]

Our aim was to identify sex differences in blood biomarkers in patients with paroxysmal AF, to provide an insight into potential sex-specific pathophysiological mechanisms in a well-phenotyped AF population.

Methods

Study population

Patients included in the identification of a risk profile to guide atrial fibrillation therapy (AF-RISK) study were used as discovery cohort. The methods of the AF-RISK study have previously been described.[5] In short, AF-RISK was a prospective, multicentre, observational study including patients with history of AF, performed in The Netherlands between May 2011 and March 2016. Inclusion criteria were patients aged ≥18 years who presented at either the inpatient or outpatient cardiology clinic with paroxysmal AF (total AF history <2 years, or total AF history <3 years in case of ≤2 AF episodes of ≤48 h per month terminating spontaneously) or persistent AF (total AF history <2 years, and total persistent AF duration >7 days and <1 year) in whom a rhythm control strategy was preferred. Exclusion criteria were patients with history of heart failure >3 years, severe valvular disease, contra-indication for oral anticoagulation, acute coronary syndrome <1 month, or post-operative AF. In total, 386 patients had paroxysmal AF and were in sinus rhythm at the moment of blood sampling; from this amount, 364 (94%) had blood biomarker results available and were included for the current analysis.

Patients included in the Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) study were used as validation cohort.[6] In short, RACE V is an ongoing investigator-initiated, prospective, multicentre registry aiming to include 750 patients in multiple centres in The Netherlands. Inclusion criteria were patients aged ≥18 years with paroxysmal AF, a maximum AF history of 10 years since diagnosis at the moment of inclusion, a maximum CHA_2DS_2-VASc score of 5, and no other indication for anticoagulation drugs (e.g. mechanical valve prosthesis). Patients had to have at least two documented episodes of paroxysmal AF in the past year or one documented episode with at least two symptomatic episodes in the past year suspected to be AF without documentation. In patients with a Medtronic pacemaker, atrial high rate episodes (AHREs) >190 beats per minute lasting >6 min were qualified as AF episodes. Patients with other types of pacemakers, defibrillators or cardiac resynchronization therapy could not participate due to differences in AHRE algorithm and/or incompatibility with the type of home-monitoring. Further exclusion criteria were patients with a history of persistent AF, currently on amiodarone, current pregnancy or a life expectancy <2.5 years, patients with AF caused exclusively due to transient triggers (e.g. postoperative, due to infection), patients with a previous pulmonary vein isolation (PVI), or intention to undergo PVI, or diagnosed congenital heart disease. In total, 247 patients had available blood samples; from this amount, a total of 34 (14%) were excluded because of AF at the moment of sampling. Samples from the remaining 213 patients were used for the current analyses.

Both AF-RISK and RACE V were performed in concordance with the Declaration of Helsinki. The Institutional Review Board approved both protocols. AF-RISK was registered at Clinicaltrials.gov (Clinicaltrials.gov identifier NCT01510210), as well as RACE V (Clinicaltrials.gov identifier NCT02726698) and all patients gave written informed consent.

Blood biomarkers

An electrocardiogram was performed to assess the heart rhythm prior to blood sampling. Blood sampling was performed in a similar fashion at baseline in both cohorts. EDTA anticoagulated plasma was obtained from ethylenediaminetetraacetic acid tubes and was stored at −80°C. Multiplex immunoassay by proximity extension assay (PEA) technology (Olink Bioscience, Uppsala, Sweden) was used to measure 92 biomarkers from the Olink cardiovascular panel III (full list shown in Supplementary material online, *Table S1*). The PEA technology uses a homogeneous assay that uses pairs of antibodies equipped with DNA reporter molecules. In the kits, 92 oligonucleotide-labelled antibody probe pairs are allowed to bind to their respective target if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then amplified, and subsequently detected and quantified using real-time PCR. The assay was performed in a homogeneous 96-well format without any need for washing steps. Internal controls were added to each sample and include two immunoassay controls, one extension control and one detection control. Samples for which one or more of the internal control values deviate from a pre-determined range were flagged and removed before statistical analysis. PEA results do not provide absolute concentration of the proteins; instead, proteins are expressed as normalized protein expression on a log2-transformed concentrations where a larger number represents a higher protein level in the sample, typically with the background level at around zero.

Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL for all assays for which recombinant protein antigen was available. Four biomarkers (bleomycin hydrolase, spondin-1, elafin, and cathepsin D) had ≥10% of the values below LOD and were therefore excluded. The remaining 88 biomarkers were used for the analyses.

Comorbidities

Heart failure was defined as one of the following: (i) history of heart failure admission, regardless of the left ventricular ejection fraction (LVEF); (ii) LVEF <45%; (iii) LVEF >45%, an elevated NT-proBNP (>400 ng/L) with either structural heart disease (history of left ventricular hypertrophy or wall diameter ≥ 11 mm or septum diameter ≥ 11 mm) or diastolic dysfunction (average annular e' < 8 cm/s, and deceleration time > 220 ms, and average E/e' > 8) on echocardiography.[7] Hypertension was defined by a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication. Diabetes mellitus was defined by use of antidiabetic drugs. Coronary artery disease was defined as history of myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting.

Table 1 Baseline characteristics AF-RISK discovery cohort

Characteristic	Total population (*N* = 364)	Women (*N* = 150)	Men (*N* = 214)	*P*-value
Age (years)	59 ± 12	60 ± 12	58 ± 12	0.030
History of AF (months)	6 (2–18)	5 (2–17)	6 (2–20)	0.329
Heart failure	182 (50%)	66 (44%)	116 (54%)	0.070
HFpEF	171 (47%)	65 (43%)	106 (50%)	0.289
HFrEF	11 (3%)	1 (1%)	10 (5%)	0.059
Hypertension	167 (46%)	76 (51%)	91 (43%)	0.164
Diabetes mellitus	35 (10%)	12 (8%)	23 (11%)	0.471
Coronary artery disease	21 (6%)	6 (4%)	15 (7%)	0.260
Peripheral artery disease	9 (3%)	3 (2%)	6 (3%)	0.741
Stroke or TIA	23 (6%)	10 (7%)	13 (6%)	0.830
COPD	23 (6%)	7 (5%)	16 (8%)	0.382
CHA_2DS_2-VASc score[a]	1.6 ± 1.4	2.3 ± 1.3	1.1 ± 1.2	<0.001
EHRA class[b]				0.296
I	110 (30%)	34 (23%)	76 (36%)	
II	204 (56%)	94 (63%)	110 (51%)	
III	49 (14%)	22 (15%)	27 (13%)	
Height (cm)	178 ± 10	170 ± 7	184 ± 7	<0.001
Weight (kg)	88 ± 18	81 ± 17	92 ± 17	<0.001
BMI (kg/m^2)	28 ± 5	28 ± 6	27 ± 5	0.129
Obesity (BMI > 30)	99 (27%)	43 (29%)	56 (26%)	0.633
Blood pressure (mmHg)				
Systolic	131 ± 18	134 ± 20	128 ± 15	0.004
Diastolic	78 ± 9	78 ± 11	78 ± 8	0.693
PQ time (ms)	165 ± 25	161 ± 24	168 ± 25	0.007
Left atrial volume (mL)	67 ± 21	62 ± 19	69 ± 21	0.002
Left atrial volume index (mL/m^2)	33 ± 10	33 ± 10	32 ± 10	0.696
LV ejection fraction (%)	57 ± 4	58 ± 3	57 ± 5	0.016

Data are mean (standard deviation), number of patients (%), or median (interquartile range).
AF, atrial fibrillation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; EHRA, European Heart Rhythm Association class for symptoms; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reserved ejection fraction; LV, left ventricular; TIA, transient ischaemic attack.
[a]The CHA_2DS_2-VASc score assesses thromboembolic risk. C, congestive heart failure/LV dysfunction, H, hypertension; A_2, age ≥75 years; D, diabetes mellitus; S_2, stroke/transient ischaemic attack/systemic embolism; V, vascular disease; A, age 65–74 years; Sc, sex category (female sex).
[b]In 363 patients, EHRA class data was available.

Statistical analysis

Sex differences in blood biomarkers were tested by univariable and multivariable logistic regression. In the multivariable logistic regression model additional adjustment for obesity, age, heart failure, and coronary artery disease was performed based on differences found between women and men at baseline and knowledge from previous literature.[2] The final model was tested for significant interactions. Odds ratios (ORs) per standard deviation with 95% confidence intervals (CIs) were given. Biomarkers with higher values in men were expressed as OR vs. women, biomarkers higher in women were presented as OR vs. men. Biomarkers found in the discovery cohort were subsequently tested by univariable and multivariable logistic regression in the validation cohort.

Enrichment pathway analyses were performed for blood biomarkers with higher values in women in comparison to men. The median value of each biomarker in women was divided by the median value of the same biomarker in men to produce a sex difference ratio per biomarker. This ratio was then transformed into percentage (Supplementary material online, *Figure S1*). Biomarkers found in the discovery cohort were subsequently tested in the validation cohort.

Confirmed biomarkers in the validation cohort were additionally enriched in a network analysis using STRING to identify relevant biological pathways in which the biomarkers are involved.[8] STRING is a database that provides assessment of physical and functional protein interactions which contribute to common biological processes. This knowledge derives from databases and text-mining highly calibrated, such as Gene Ontology (GO) Resource using high level groupings established by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps. Biomarkers that entered the pathway analysis were two-layer enriched.

In both multivariate logistic regression and pathway analyses, a multiple testing correction was performed using a Bonferroni correction for the 88 biomarkers that were tested. A *P*-value $<5.68 \times 10^{-4}$ ($\frac{0.05}{88}$) was considered statistically significant. Additionally, pathway enrichment underwent false discovery rate (FDR) correction for multiple testing. Pathways with higher FDR were selected as main representative processes.

Table 2 Baseline characteristics RACE V validation cohort

Characteristic	Total population (*N* = 213)	Women (*N* = 88)	Men (*N* = 125)	P-value
Age (years)	64 ± 9	66 ± 9	63 ± 10	0.011
History of AF (months)	29 (8–56)	32 (7–57)	27 (8–56)	0.973
Heart failure	62 (29%)	19 (22%)	48 (38%)	0.014
HFpEF	60 (28%)	17 (19%)	43 (34%)	0.024
HFrEF	2 (1%)	0 (0%)	2 (2%)	0.638
Hypertension	101 (47%)	40 (46%)	61 (49%)	0.732
Diabetes mellitus	21 (10%)	12 (14%)	9 (7%)	0.187
Coronary artery disease	26 (12%)	6 (7%)	20 (16%)	0.071
Peripheral artery disease	2 (1%)	0 (0%)	2 (2%)	0.638
Stroke or TIA	18 (9%)	6 (7%)	12 (10%)	0.639
COPD	16 (8%)	8 (9%)	8 (6%)	0.639
CHA_2DS_2-VASc score[a]	1.9 ± 1.3	2.6 ± 1.2	1.4 ± 1.2	<0.001
EHRA class				0.023
I	24 (11%)	6 (7%)	18 (14%)	
IIa	89 (42%)	30 (34%)	59 (48%)	
IIb	78 (37%)	40 (46%)	38 (31%)	
III	22 (10%)	12 (14%)	10 (8%)	
Height (cm)	176 ± 10	167 ± 7	183 ± 7	<0.001
Weight (kg)	87 ± 18	80 ± 17	92 ± 17	<0.001
BMI (kg/m^2)	28 ± 5	28 ± 5	28 ± 5	0.159
Obesity (BMI > 30)	60 (28%)	34 (39%)	26 (21%)	0.008
Blood pressure (mmHg)				
Systolic	136 ± 18	137 ± 19	136 ± 17	0.559
Diastolic	81 ± 10	80 ± 11	81 ± 9	0.614
PQ time (ms)	172 ± 35	169 ± 38	174 ± 34	0.349
Left atrial volume (mL)	69 ± 23	69 ± 25	69 ± 22	0.923
Left atrial volume index (mL/m^2)	35 ± 11	36 ± 12	34 ± 11	0.094
LV ejection fraction (%)	59 ± 5	60 ± 5	58 ± 5	0.031

Data are mean (standard deviation), number of patients (%), or median (interquartile range).
AF, atrial fibrillation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; EHRA, European Heart Rhythm Association class for symptoms; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reserved ejection fraction; LV, left ventricular; TIA, transient ischaemic attack.
[a]The CHA_2DS_2-VASc score assesses thromboembolic risk. C, congestive heart failure/LV dysfunction; H, hypertension; A_2, age ≥75 years; D, diabetes mellitus; S_2, stroke/transient ischaemic attack/systemic embolism; V, vascular disease; A, age 65–74 years; Sc, sex category (female sex).

Results

Patient characteristics

Clinical characteristics of patients from the discovery cohort are shown in *Table 1*. Patient characteristics were comparable to the entire AF-RISK cohort (data not shown).[5] In short, mean age was 59 ± 12 years, 150 (41%) were women. CHA_2DS_2-VASc-score was 1.6 ± 1.4. A total of 182 (50%) had heart failure [171 (47%) with preserved and 11 (3%) with reduced ejection fraction] and 167 (46%) hypertension. Women compared to men were slightly older (60 ± 12 vs. 58 ± 12 years, $P = 0.03$) and had slightly higher LVEF (58 ± 3 vs. 57 ± 5, $P = 0.01$).

Baseline characteristics from the validation cohort are shown in *Table 2*. Compared to the discovery cohort, the proportion of women was comparable in the validation cohort (41%). CHA_2DS_2-VASc-score was 1.9 ± 1.3. A total of 62 (29%) had heart failure [60 (28%) with preserved and 2 (1%) with reduced ejection fraction] and 101 (47%) hypertension. Within the validation cohort, women compared to men were older (66 ± 9 vs. 63 ± 10 years, $P = 0.01$), had less often heart failure (22 vs. 38%, $P = 0.01$) and had more often obesity (39 vs. 21%, $P = 0.008$). Patients in the validation were older in comparison to the discovery cohort (64 ± 9 vs. 59 ± 12 years, $P < 0.01$) and had longer history of AF (29 vs. 6 months, $P < 0.01$).

Biomarker analysis

The multivariable logistic regression in the discovery cohort showed that levels of activated leucocyte cell adhesion molecule (ALCAM, $P = 4.03 \times 10^{-4}$) and fatty acid binding protein-4 (FABP-4, $P = 4.48 \times 10^{-12}$) were higher in women. While levels of matrix metalloproteinase-3 (MMP-3 $P = 6.46 \times 10^{-13}$), C-C motif chemokine-16 (CCL-16 $P = 4.17 \times 10^{-5}$), and myoglobin ($P = 2.34 \times 10^{-4}$) were higher in men (*Figure 1*).

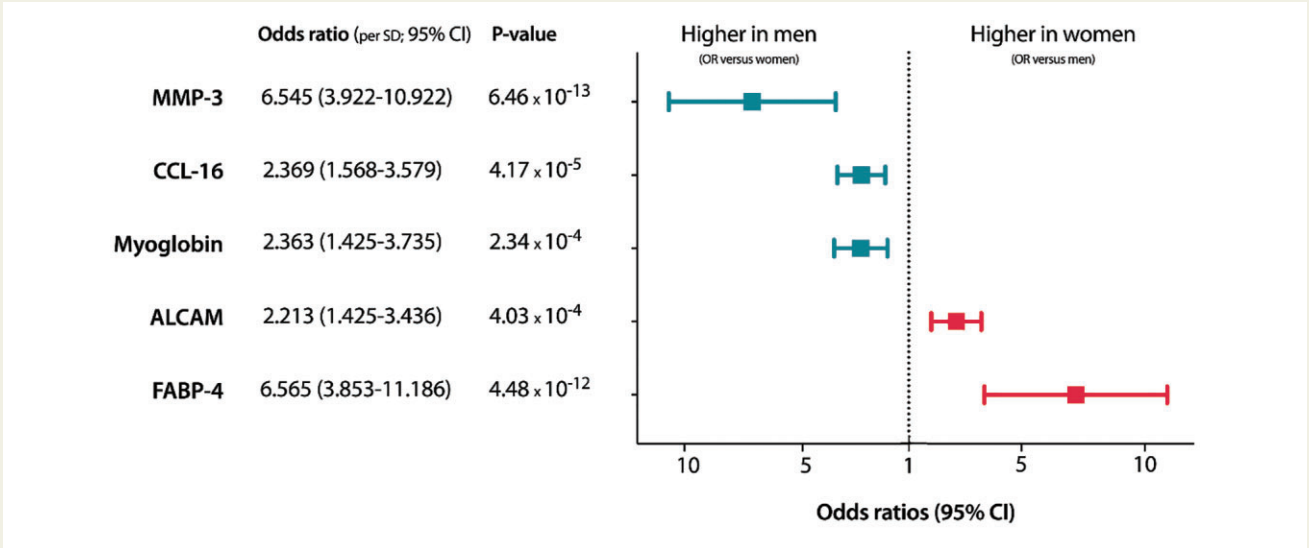


Figure 1 Blood biomarker sex differences in discovery cohort. ALCAM, activated leucocyte cell adhesion molecule; CCL-16, C-C motif chemokine-16; CI, confidence interval; FABP-4, fatty acid binding protein-4; MMP-3, matrix metalloproteinase-3; OR, odds ratio; SD, standard deviation.

Table 3 Multivariate logistic regression results of biomarkers in validation cohort

	OR	95% CI	*P*-value
MMP-3	6.289[a]	3.257–12.195	4.31×10^{-8}
CCL-16			NS
Myoglobin	3.135[a]	1.712–5.747	2.10×10^{-4}
ALCAM	3.165	1.735–5.774	1.73×10^{-4}
FABP-4	5.975	3.030–11.78	2.46×10^{-7}

[a]Odds ratios are expressed vs. women.
ALCAM, activated leucocyte cell adhesion molecule; CCL-16, C-C motif chemokine-16; CI, confidence interval; FABP-4, fatty acid binding protein-4; MMP-3, matrix metalloproteinase-3; NS, not statistically significant; OR, odds ratio; SD, standard deviation.

The five biomarkers found in the discovery cohort were univariably tested in the validation cohort and all but CCL-16 (univariably OR 1.090, 95% CI 0.829–1.432, $P = 0.537$) were confirmed to be differently expressed between sexes (*Table 3*). Based on differences on baseline characteristics and knowledge from previous literature,[2] it was adjusted for obesity, age, and heart failure; after this adjustment, only FABP-4 remained higher in women (OR 7.442, 95% CI 3.680–15.051, $P = 2.32 \times 10^{-8}$). MMP-3 (OR 8.403, 95% CI 4.329–16.393, $P = 3.12 \times 10^{-10}$) remained higher in men.

In the pathway analysis, six biomarkers in women and eight in men were statistically significant (Supplementary material online, *Figure S2*), which included the biomarkers from the multivariate logistic regression analysis in the discovery cohort. These biomarkers were subsequently tested in the validation cohort; six remained statistically significant in women, FABP-4, ALCAM, NT-proBNP, contactin-1 (CNTN1), metalloproteinase inhibitor 4 (TIMP4), and integrin beta-2 (ITGB2); three remained statistically significant in men matrix, extracellular phosphoglycoprotein (MEPE), myoglobin, and MMP3 (Supplementary material online, *Table S2* and *Figure S3*).

After a two-layer protein enrichment, in women compared to men, pathways with higher FDR under GO analysis showed cell-cell adhesion (FDR = 1.23×10^{-8}), integrin-mediated signalling pathway (FDR = 3.83×10^{-8}), and cell adhesion (6.13×10^{-8}); moreover, cell adhesion molecules (FDR = 5.19×10^{-12}) pathways resulted under KEGG analysis. In men, extracellular matrix organization (FDR 3.59×10^{-9}) pathway resulted from GO analysis without any results under KEGG analysis (*Figure 2*).

Discussion

The aim of this study was to identify sex differences in blood biomarkers in patients with AF. We identified five biomarkers that were differently expressed between sexes with paroxysmal AF. In a validation cohort, four out of five markers were confirmed to be differently expressed between sexes.

Blood biomarkers in women

In women, ALCAM and FABP-4 were higher. Cell adhesion molecules, like ALCAM, are involved in leucocyte recruitment in case of tissue damage. In patients with stroke, ALCAM has been associated with long-term mortality.[9] Also, Lim *et al.*[10] previously showed that higher levels of ALCAM were associated with early recurrence after AF ablation. Moreover, cell adhesion mechanisms increase the adhesiveness of platelets and leucocytes incrementing the risk of thrombogenesis even when in sinus rhythm.[11] FABP-4 is mainly expressed in adipose tissue and represents around 6% of the total protein adipocytes. It has been associated a systemic pro-inflammatory state, development of atherosclerosis and metabolic syndrome. In the presence of coronary artery

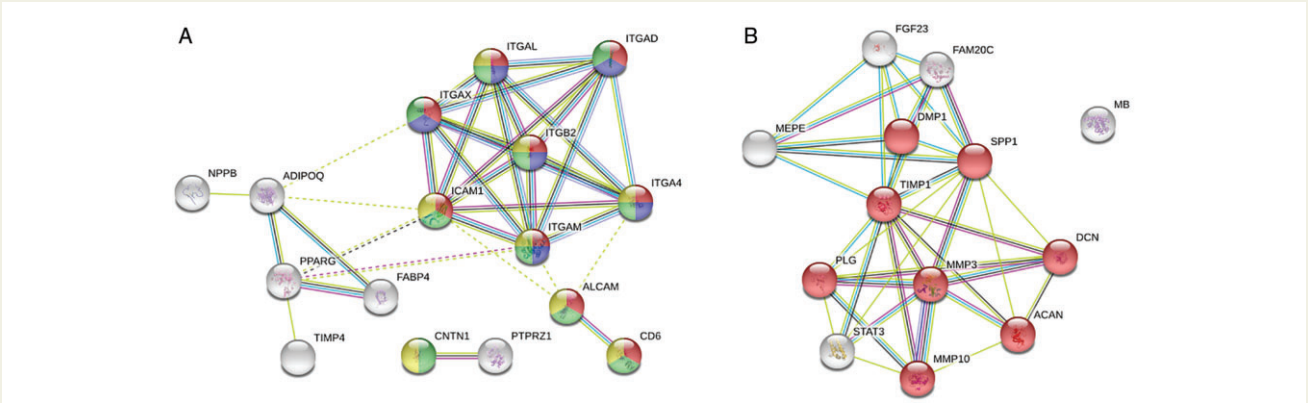


Figure 2 Pathway enrichment analysis of biomarkers confirmed in validation cohort. (*A*) Pathway enrichment analysis with layers of proteins on validated biomarkers in women in comparison to men from both cohorts. (*B*) Pathway enrichment analysis with layers of proteins on validated biomarkers in men in comparison to women from both cohorts. (*C*) Biological processes with higher FDR in women in comparison to men. Colours represent biological processes in which the proteins (nodes) are involved as depicted in *A*. (*D*) Biological processes with higher FDR in women in comparison to men. Colours represent biological processes in which the proteins (nodes) are involved as depicted in *B*. ACAN, aggrecan; ALCAM, activated leucocyte cell adhesion molecule; CD6, T-cell differentiation antigen CD6; CNTN1, contactin-1; DCN, decorin; DMP1, dentin matrix acidic phosphoprotein 1; FDR, false discovery rate; GO, Gene Ontology; ICAM1, intercellular adhesion molecule 1; ITGA4, integrin subunit alpha 4; ITGAD, integrin subunit alpha D; ITGAL, integrin subunit alpha L; ITGAM, integrin subunit alpha M; ITGAX, integrin subunit alpha X; ITGB2, integrin subunit beta 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; MMP10, integrin subunit beta 2; MMP3, matrix metalloproteinase-3; PLG, plasminogen; SPP1, secreted phosphoprotein 1; TIMP1, metallopeptidase inhibitor 1.

disease, FABP-4 has been associated with left ventricular hypertrophy, systolic dysfunction, clinical heart failure. Increasing levels of FABP-4 have been associated with increased cardiovascular mortality in both women and men, even after adjusting for potential cofounders.[12] Higher FABP-4 levels have also been linked to postoperative AF.[13] López-Canoa *et al.*[14] have shown an association between higher levels of FABP-4 in women with AF in comparison to men, as well as recurrence of AF after ablation in both sexes. In contrast to what we found, Magnussen *et al.*[1] previously found that CRP was associated with incident AF in men but not in women, but with very low hazards ratio; this may be explained by the lack of specificity of CRP, or use of a relatively healthy population; the latter is supported by the low values that were found. CRP levels were not routinely performed in our population and a direct comparison cannot be made. Despite the fact that our biomarker panel consists of 32 inflammatory markers, only two were found to be higher in women. This can be explained by the fact that most inflammatory markers are derived from similar biological pathways and are therefore not all included in the final multivariable model. Therefore, these data suggest that the contribution of inflammation seems to be more critical in AF substrate formation in women (*Graphical abstract*). Of interest, in our study, women had less heart failure with preserved ejection fraction (HFpEF) in both cohorts as compared to men, reaching statistical significance in the validation cohort. This is in contrast to previous data showing that women more often suffer from HFpEF.[2] This might be due to the fact that we only included paroxysmal self-terminating AF, implying less severe remodelling. Also, the fact that hypertension occurred in comparable percentage in men and women may have contributed to this observation. This clearly highlights that there still is a gap of knowledge in sex differences in patients with all types of AF warranting further research.

Blood biomarkers in men

In men, levels of MMP-3 and myoglobin were higher. MMP-3 is part of the family of matrix metalloproteinases that are involved in extracellular matrix degradation and deposition. MMP-3 has been associated with vascular remodelling, including atrial stiffening and coronary artery disease[15] and has also been suggested as potential therapeutic target in atherosclerosis.[16] Moreover, Yue *et al.*[17] concluded that the excess of proteins involved in extracellular matrix biological pathways may lead to tissue fibrosis, contributing to vascular remodelling; this affects mechanical and electrical function, and therefore can promote AF. Different studies have reported contrasting results between the association of MMP-3 and LAVI (left atrial volume indexed).[18] LAVI is comparable between men and women in the current analysis; we could speculate that the association between MMP-3 and LAVI is not present in this population. However, a conclusive statement of this association cannot be drawn since levels of biomarkers are relative measures from the population, making them not comparable to absolute measures. Myoglobin can be detected in case of muscle degradation. Recurrent episodes of silent ischaemia, also in patients with subclinical coronary artery disease may be the underlying substrate for myocardial myoglobin release.[19] In addition, higher muscle mass in men could contribute to the observed outcome. The combination of MMP-3 and myoglobin may indicate that in men, vascular remodelling plays an important role in AF substrate formation. Prevalence of clinical coronary artery disease was, however, not different between sexes in our discovery nor validation

cohorts. When corrected for differences in underlying disease, MMP-3 remained associated with higher values in men. This could indicate that subclinical vascular disease is more prominent in men (*Graphical abstract*). This in accordance with findings from the Rotterdam study which described subclinical atherosclerosis as an independent risk factor for new-onset AF but not only in men.[20] Subclinical atherosclerosis, which may be present in many patients with AF, was, however, not routinely assessed in our discovery cohort. Since the biomarker panel used in this analysis did not include CA-125, our results cannot be compared to previous findings of this biomarker.[4]

Strengths and limitations

Limitations of the current analysis include the use of a biomarker panel with relative values, which impairs comparison with absolute values of other cohorts. Also, this was a cross-sectional study which precludes definite conclusions regarding cause–effect relations. In addition, the AF duration of the validation cohort is longer than in the discovery cohort, implying greater atrial remodelling substrate and differentiated expression of blood biomarkers. Furthermore, information regarding frequency of menstrual cycles in women was not collected, which could have provided an insight on the association of hormones and biomarker expression in women in pre- and post-menopausal periods. Lastly, residual confounding may have affected results, despite adjustment-analysis. Strengths of the current analysis are the well-phenotyped cohorts and the availability of a large number of biomarkers representative of multiple biological pathways. Furthermore, the use of two analytical approaches and two independent cohorts yielded synergic results.

Conclusion

In conclusion, in this exploratory analysis, we identified biomarkers differentially expressed in women and men with paroxysmal AF. In a validation cohort, four out of five biomarkers were confirmed. In women with paroxysmal AF, inflammatory biomarkers were higher, while in men with paroxysmal AF biomarkers for vascular remodelling were higher. Our data suggest that pathophysiological mechanisms in women and men with AF may differ. This advocates more research on sex differences in AF and endorses a personalized medicine approach, taking sex differences into account.

Supplementary material

Supplementary material is available at *Europace* online.

Acknowledgements

The authors acknowledge the support from the Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON 2014-9): Reappraisal of Atrial Fibrillation: interaction between hyperCoagulability, Electrical remodelling, and Vascular destabilisation in the progression of AF (RACE V); grant support to the institution from Medtronic; and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie (agreement 754425).

Funding

This work was supported by a grant from the Dutch Heart Foundation (NHS2010B233).

Conflict of interest: H.T.C. has received research support from Bayer and Pfizer and is consultant for Alveron and shareholder with Coagulation profile. J.G.L.M.L. has a consultancy agreement with Medtronic. All remaining authors have declared no conflicts of interest.

Data availability

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study.

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EP CASE EXPRESS

doi:10.1093/europace/euab089
Online publish-ahead-of-print 2 June 2021

The pulmonary vein that stumped us

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A 76-year-old man presented for catheter-based atrial fibrillation (AF) ablation. Sixteen years earlier, the patient had left-sided small cell lung cancer treated with chemotherapy and radiation. Left atrial mapping (*Panels A* **and** *B*) as well as contrast injections (*Panel C*) revealed complete occlusion of the proximal left superior pulmonary vein (PV). Following isolation of the right PVs, a spline-based multi-electrode catheter was positioned into the left superior PV stump. With infusion of isoproterenol up to 4 mcg/min, runs of triggered firing were appreciated from the left superior PV stump (*Panel D*). Following isolation of the left PVs, no arrhythmias remained inducible on isoproterenol up to 20 mcg/min. It is well appreciated that the PV muscle sleeves play a prominent role in the initiation of AF. Disruption of these sleeves might be predicted to reduce their triggering role but a prior report of patients post pneumonectomy found that PV stumps continue to be active triggering sites for AF. Our findings are somewhat unique in that our patient had no history of pneumonectomy but are in agreement with data that fully intact PV muscle sleeves are not necessary for the PV anatomy to trigger AF.

The full-length version of this report can be viewed at: https://www.escardio.org/Education/E-Learning/Clinical-cases/Electrophysiology.