

Sex differences in circulating proteins of patients with Rheumatoid Arthritis: a cohort study

Short Title: Sex differences in Rheumatoid Arthritis

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No authors have any financial support or other benefits from commercial sources for the work reported on in the manuscript, or any other financial interests which could create a potential conflict of interest or the appearance of a conflict of interest with regard to the work.

Abstract

Objectives: Differences in proteomic profiles between men and women may provide insights into the biological pathways that contribute to known sex differences in Rheumatoid Arthritis (RA). Studies focusing on sex differences in circulating proteins in RA patients are scarce. Our objective was to investigate the sex differences in circulating proteins of RA patients.

Methods: Cohort study enrolling 399 RA patients. 94 circulating protein-biomarkers (92CVDIIOLink®+troponin-T+c-reactive protein) were measured. Clinical, demographic, and echocardiographic characteristics were compared between men and women. The sex differences in the biomarker's expression were assessed using regression modelling.

Results: 306 (76.7%) patients were women. Compared with men, women had less visceral fat, smoked less, had diabetes and COPD less frequently and expressed more fatigue, anxiety and depression. The association with cardiovascular outcomes did not differ between sexes. After adjusting for potential confounders, women expressed higher levels of circulating proteins related to adipokine signaling and vascular function (e.g., leptin and vascular-endothelial-growth factor), whereas men expressed higher levels of circulating proteins related to extracellular matrix organization and inflammation (e.g., matrix metalloproteinase-2 and c-reactive protein). These results were not found in patients without RA.

Conclusion: Sex differences in circulating proteins reflect distinct pathways implicated in pathogenesis of RA, including inflammation, adiposity, angiogenesis, and extracellular matrix organization. These findings may help further investigations into factors underlying sex-based differences and allow

future studies focused on sex specific personalized treatment approaches in RA.

ClinicalTrials.gov ID: NCT03960515

Keywords: rheumatoid arthritis; sex differences; proteomic biomarkers; pathophysiology

Significance and Innovations

- Sex differences in circulating proteins reflect distinct pathways implicated in pathogenesis of rheumatoid arthritis (RA);
- Adipokines and endothelial/vascular inflammation may play a central role in the pathogenesis of RA in women, whereas in men the pathogenesis of RA may be more related to systemic inflammation and tissue injury;
- Sex-specific pathways may be a potential therapeutic target for the treatment of RA.

Introduction

Rheumatoid arthritis (RA) incidence is three times higher among women compared with men and has increased over the last decades(1, 2). In women, RA incidence increases linearly with age; however, men tend to have a later RA onset, with a higher risk after the sixth decade of life(3).

Genetic, hormonal, and environmental/behavioral factors may explain, at least partly, why women have higher susceptibility and prevalence of RA(4). Furthermore, RA presentation may also differ between women and men, with many studies suggesting that women have higher disease activity, lower proportion of achieved remission and poorer treatment response(5-12).

Some of the reported differences may depend on how RA disease activity is measured rather than the RA disease activity itself, because women generally report more symptoms and poorer scores on most questionnaires(13).

Given the different incidence, presentation, and prognosis of RA in men and women (including cardiovascular events), it is likely that the mechanisms involved in the pathogenesis of RA are sex-specific(14-18). Therefore, a better understanding of the sex differences in RA regarding pattern and burden of disease and cardiovascular outcomes is needed. A multimarker approach may potentially provide insight on the biological processes underlying the sex differences in patients with RA and help ascertaining if the observed sex differences are due to distinct underlying mechanisms or to different clinical expression of the disease. Another important question is whether the sex differences in the biological processes are specific of RA or also common to patients without RA.

Using a prospective cohort with detailed phenotyping and multiple circulating proteins, this study aims to investigate the sex differences in RA patients from a clinical and biological perspective, and also to assess whether the differences in protein expression (and underlying biological pathways) are specific to RA.

Methods

Study design

We conducted a prospective single-center cohort study with patients attending the Autoimmune Disease Outpatient Unit of *Centro Hospitalar do Porto*, Portugal. The study was registered in clinicaltrials.gov under the number NCT03960515(19).

This study was conducted following the principles of the Declaration of Helsinki and approved by the *Hospital de Santo António, Porto, Portugal* ethics committee under number #2016-023 (020-DEFI/020-CES). All participants provided the written informed consent before enrollment in the study.

The data used for this study are available upon reasonable request to the corresponding author.

Patient selection

We included all adult patients (18 years or older) with a diagnosis of RA and that were followed-up in our specialized RA clinic. All RA patients fulfilled the 2010 ACR/EULAR Classification Criteria(20). Patients with active neoplasm or other severe co-morbid conditions potentially associated with life expectancy shorter than six months, as well as severe dementia, inability to walk or totally dependent on a third person were excluded from our study.

The screening period for patient enrollment occurred from June 2016 to June 2018. All enrolled patients maintained their usual care under the guidance of their treating physician.

Patients were followed-up for a median of 1.5 (0.7 to 2.3) years. The cause of death and hospitalization were independently adjudicated.

Patient evaluation, echocardiography and routine laboratory tests

We collected demographic data, cardiovascular history and risk factors, comorbid conditions, RA history, daily medication intake and a cardiovascular symptom questionnaire. Anxiety and depression were assessed using the Portuguese version of the Hospital Anxiety and Depression Scale(21). Physical examination included vital signs, pulmonary and cardiac auscultation, and anthropometric measures. Visceral fat was assessed using a bioelectrical impedance analysis (BIA) with Tanita® BC545 instrument. Six-minute walking test was performed according to the American Thoracic Society protocol (22).

The transthoracic echocardiogram (TTE) was performed by an experienced echocardiographer and included the M-mode, 2D and Doppler measurements acquired according to standard recommendations(23, 24). Routine laboratory tests (blood and urine samples) included a complete blood count, c-reactive protein (CRP), glucose, blood electrolytes, lipid profile, creatinine, high sensitivity troponin T (hs-TnT), and N-Terminal-pro Brain Natriuretic Peptide (NT-pro BNP).

Plasma Proteomic Biomarkers

We measured a large protein biomarker panel (Olink® CVDII panel) that comprised 92 biomarkers associated with cardiovascular and inflammatory of pathophysiological domains. Results are expressed in normalized NPX units

(<https://www.olink.com/>). The overview of the 92 circulating proteins is depicted in the **Supplement Table 1**, including their full names and Uniprot ID. In the **Supplement Table 2** the proteins are described according to their main biological functions. The full description and technical details of the analytical assay are described in the **Supplemental Addenda**.

Plasma concentrations of high-sensitivity troponin T (hsTnT) and CRP were also measured using the Elecsys Troponin T Gen 5 STAT test by Roche Diagnostics® and the enzyme-linked immunosorbent assay (Olympus CRP Latex Calibrator Normal Set®), respectively. The CVDII Olink® panel displayed a mean intra-assay and inter-assay coefficient of variation between 9.1% and 11.7%. The hs-TnT and CRP showed an intra-assay and inter-assay coefficient of variation was 10% at a concentration lower than the 99th percentile cut-off.

External assessment in patients without rheumatoid arthritis

To assess if our findings were unique to RA or whether if the sex differences in the biological processes are common to patients without RA we externally assessed our findings in two independent case-control cohorts of patients with cardiovascular risk factors, but without RA, both part of the European granted program Heart Omics and AGEing (HOMAGE). These cohorts have been previously described and the full results published(25). Briefly, a nested-matched case-control design was used with cases (patients subsequently progressing to incident HF), and controls (patients without progression to HF). Controls were matched on cohort, follow-up time, age, and sex. Two independent sample sets (Ia with 286 cases and 591 controls and Ib with 276 cases and 280 controls) were used. The analysis presented in this report are fully adjusted on the matching variables age, heart failure case, follow-up time,

smoking, diabetes mellitus, history of coronary artery disease, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate.

Statistical and bioinformatical analysis

Baseline clinical, demographic, and echocardiographic characteristics were compared between male and female patients using chi-square, t test, or Mann-Whitney tests, as appropriate. The level of significance for all hypothesis tests (*P* value) was set at 0.05. The normality of the distribution of continuous variables was evaluated by Kolmogorov–Smirnov’s test and Shapiro–Wilk’s test. Continuous variables are presented as mean and standard deviation (if normally distributed) or median and 25-75% quartiles (if non-normally distributed). We used the student’s t-test to examine the differences of the independent continuous variables between male and female groups when normally distributed; the Mann-Whitney U was used for variable without normal distribution. Categorical variables are presented as absolute (n) and relative (%) frequencies. To study whether biomarker patterns differ between male and female patients, we first identified which clinical variables better differentiated sex using stepwise (forward) logistic regression analysis including all variables with a p-value <0.1 from the Table 1 (“clinical model”). To identify the circulating proteins that better differentiated sex, we performed logistic regression analysis fully adjusted for the “clinical model” and corrected for test multiplicity using a false discovery rate (FDR) of 5% or less. As means of internal validation we have also performed a 10-fold cross-validation least absolute shrinkage and selection operator (LASSO) regression analysis. The association between sex and the composite endpoint of cardiovascular death or cardiovascular hospitalizations was performed with a Cox model. All statistical analyses were

performed with Stata® (version 16; StataCorp 2019, Stata Statistical Software: Release 16; College Station, TX: StataCorp LP).

Network analyses according to sex were performed using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) and the involved pathways were over-represented using the KEGG (Kyoto Encyclopedia of Genes and Genomes) tool.

Results

Patient selection and baseline characteristics

A total of 408 patients with RA were screened between June 2016 and June 2018. Seven patients had exclusion criteria and 2 did not perform biomarker assessment. The remaining 399 patients fulfilled the eligibility criteria and performed the biomarker study. The baseline characteristics comparing men and women are depicted in **Table 1**. Of the total 399 RA patients, 306 (76.7%) were women. Compared with men, women had less visceral fat, smoked less, and had diabetes and COPD less often. Women expressed more fatigue, anxiety and depression ($p < 0.05$ for all). Men and women displayed similar history of RA and receive similar treatment for either disease-modifying anti-rheumatic drugs (biologic or synthetic DMARDs) and non-DMARDs. In general, men expressed more features associated with a higher cardiovascular risk such as high blood pressure, lower HDL cholesterol, higher CRP levels, lower eGFR, and lower left ventricular ejection fraction. **Table 1**.

Association between proteomic biomarkers and male sex

On top of the clinical model presented in the **Supplemental Table 3**, that included age, waist circumference, smoking, diabetes, diastolic blood pressure,

hemoglobin, and eGFR as the variables with strongest association with sex (AUC =0.89), of the 94 proteomic biomarkers, 13 were retained, as differentially expressed according to sex, with an FDR <5%. **Table 2.** Of these 13 proteins, 6 had higher concentrations expressed in women: lipoprotein lipase (LPL), growth/differentiation factor 2 (GDF2), leptin (LEP), vascular endothelial growth factor D (VEGFD), interleukin-1 receptor-like 2 (IL1RL2) and a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13), and 7 had higher concentrations expressed in men: CRP, troponin-T, lactoylglutathione lyase (GLO1), placenta growth factor (PGF), C-C motif chemokine 3 (CCL3), interleukin-18 (IL18) and matrix metalloproteinase-12 (MMP12). The network analysis representation of the findings and the interplay between these proteins among men and women is displayed in the **Figure 1**. The full biomarker regression analysis is displayed in the **Supplemental Table 4**.

The LASSO model largely confirmed the same biomarkers that better differentiated sexes, indicating excellent internal validity of the data.

Supplemental Table 5.

Proteomic external assessment in patients without RA

The external assessment in patients without RA was generally not consistent with the finding of RA patients. In women, only VEGF and ADAMTS13 were directionally and consistently replicated in HOMAGE phases 1a & 1b. In men, no biomarkers were replicated in both phases (CRP and hs-TnT were not measured in HOMAGE). **Supplemental Table 6.**

Cardiovascular outcomes

There was no difference in the rates of the composite endpoint of cardiovascular death or cardiovascular hospitalization between women and

men: events 37/306 (12.1%) in women vs. 19/93 (20.4%) in men, adjusted HR 1.13, 95% CI 0.56– 2.31, p-value =0.73. **Figure 2.**

Discussion

The present study, including 399 RA patients of whom 77% women, shows that women had less visceral fat, were less frequently smokers, and had less co-morbid conditions and cardiovascular risk factors (including diabetes) than men. However, women experienced more fatigue, anxiety and depression than men. Women expressed higher levels of circulating proteins related to adipokine signaling and vascular function (e.g., LPL, LEP, GDF2, VEGFD, ADAMTS13), whereas men expressed higher levels of circulating proteins related to ischemia, extracellular matrix organization and inflammation (e.g., hs-TnT, MMP12, CCL3, CRP, IL18, GLO1), suggesting that men and women may have sex-specific biological pathways involved in RA pathogenesis. Furthermore, most of the sex differences in the expression of investigated circulating proteins were not observed in control patients with CV risk factors suggesting that these might be specific to RA.

This work aimed at substantiating potential differences in mechanistic pathways which may underly the many differences in incidence, presentation, and prognosis of RA in men and women. We examined whether a multiple biomarker approach may detect sex-specific biological pathways involved in RA, providing basis for a better understanding of the disease processes which may help conceptualizing individualized treatments.

To the best of our knowledge, this is the first report assessing a large panel of circulating proteins to study the sex differences in protein expression in

RA. For the majority of the circulating proteins found in this study, we could not find any previous study in RA populations allowing to compare our sex-specific findings. However, data from non-RA populations and animal models may serve as basis to support and reinforce our findings. Adipokines usually have higher expression in women(26). In RA, leptin has pro-inflammatory effects and correlates with measures of disease activity(27-29). Our study suggests that leptin may be involved in RA pathogenesis specifically in women, independently of abdominal obesity and diabetes. Lipoprotein lipase (LPL), also with higher expression among women in our study, has the dual function of hydrolyzing triglycerides and serve as a ligand factor for receptor-mediated lipoprotein uptake. A study with RA patients has shown that the higher level of LPL activity in this population might result from the inhibition of LPL degradation by corticosteroids(30). However, this study does not provide sex-specific associations and in our study the use of corticosteroids was similar between men and women. Therefore, our data suggest that LPL may have a sex-specific role in RA, deserving further investigation. Women also expressed higher levels of angiogenesis-related markers (GDF2, VEGFD and ADAMTS13). Growth differentiation factor 2 (or bone morphogenetic protein 9, BMP9) is a member of the TGF- β family and a potent circulating inhibitor of angiogenesis(31). Furthermore, GDF2 signaling increases vascular inflammation through direct effects on the endothelium(32). Therefore, blocking the GDF2/BMP9 pathways may be a target for reducing vascular inflammation(33). The VEGFD also plays an important role in angiogenesis by stimulating the proliferation and migration of endothelial cells and is upregulated by proinflammatory cytokines and hypoxia. Circulating VEGF concentrations are elevated in RA and correlate with

disease activity and progression(34, 35). ADAMTS13 controls the von Willebrand Factor (vWF) -mediated platelet thrombus formation by cleaving the vWF multimers into smaller forms; therefore, preventing microvascular thrombosis and inflammation(36). The IL1RL2 is a receptor for interleukin-36 mediating local (including joint) inflammatory responses(37). Compared with women, men with RA exhibit higher concentrations of systemic inflammation markers and circulating proinflammatory cytokines/chemokines (CRP, IL-18, CCL3 and GLO1)(38-40), tissue injury, fibrosis and remodeling (MMP12)(41), cardiac injury (hs-TnT)(19), and synoviocyte proliferation (PGF)(42). Together, our data suggest that adipokines and endothelial/vascular inflammation play a central role in the pathogenesis of RA in women, whereas in men the pathogenesis of RA may be more related to systemic inflammation and tissue injury(8).

The association with cardiovascular outcomes did not differ between sexes, but the number of events was overall low. Nonetheless, men experienced nominally more cardiovascular events than women, which may reflect a greater cardiac involvement in men with RA (also supported by the higher concentration of hs-TnT in men).

Limitations

Several limitations should be acknowledged in our study. This is an observational study and thus causality cannot be ascertained. Our cohort came from a single center and some of the findings may reflect specific socio-demographic characteristics and local practice patterns which may limit the generalization of the findings. The lower representativeness of men (with a ratio of 1:3 vs. women, compatible with the epidemiology of the disease) and a small

number of events, decreased the statistical power for comparing outcomes between sexes. .We have studied the Olink® CVDII panel, hs-TnT and CRP. Whether a larger protein screening could provide more information might worth exploring. Our study included ambulatory patients only, and our findings cannot be generalized to hospitalized RA patients. Finally, studies in larger populations to validate our results are needed.

Conclusion

Sex differences in circulating proteins reflect distinct pathways implicated in pathogenesis of RA, adipokines and endothelial/vascular inflammation play a central role in the pathogenesis of RA in women, whereas in men the pathogenesis of RA may be more related to systemic inflammation and tissue injury. Our study provides novel hypotheses and highlights the need for a better understanding of the sex differences in RA.

Acknowledgements

JPF, PR, and FZ are supported by the RHU Fight-HF, a public grant overseen by the French National Research Agency (ANR) as part of the second “Investissements d’Avenir” program (reference: ANR-15-RHUS-0004), by the French PIA project “Lorraine Université d’Excellence” (reference: ANR-15-IDEX-04-LUE), Contrat de Plan Etat Lorraine IT2MP and FEDER Lorraine.

Disclosures

None.

Authors’ Contributions

M.B.F., drafted the manuscript; J.P.F., performed the statistical analysis and drafted the manuscript; A.S.B., performed the bioinformatical analysis; all authors read, provided critical input and approved the final version of the manuscript.

Dr. João Pedro Ferreira is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

References

1. Wolfe AM, Kellgren JH, Masi AT. The epidemiology of rheumatoid arthritis: a review. II. Incidence and diagnostic criteria. *Bull Rheum Dis*. 1968;19:524-9.
2. Myasoedova E, Crowson CS, Kremers HM, Therneau TM, Gabriel SE. Is the incidence of rheumatoid arthritis rising?: results from Olmsted County, Minnesota, 1955-2007. *Arthritis Rheum*. 2010;62:1576-82.
3. Symmons DP, Barrett EM, Bankhead CR, Scott DG, Silman AJ. The incidence of rheumatoid arthritis in the United Kingdom: results from the Norfolk Arthritis Register. *Br J Rheumatol*. 1994;33:735-9.
4. Favalli EG, Biggoggero M, Crotti C, Becciolini A, Raimondo MG, Meroni PL. Sex and Management of Rheumatoid Arthritis. *Clin Rev Allergy Immunol*. 2019;56:333-45.
5. Iikuni N, Sato E, Hoshi M, Inoue E, Taniguchi A, Hara M, et al. The influence of sex on patients with rheumatoid arthritis in a large observational cohort. *J Rheumatol*. 2009;36:508-11.
6. Sokka T, Toloza S, Cutolo M, Kautiainen H, Makinen H, Gogus F, et al. Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA study. *Arthritis Res Ther*. 2009;11:R7.
7. Kvien TK, Heiberg, Lie E, Kaufmann C, Mikkelsen K, Nordvag BY, et al. A Norwegian DMARD register: prescriptions of DMARDs and biological agents to patients with inflammatory rheumatic diseases. *Clin Exp Rheumatol*. 2005;23(5 Suppl 39):S188-94.
8. Forslind K, Hafstrom I, Ahlmen M, Svensson B, Group BS. Sex: a major predictor of remission in early rheumatoid arthritis? *Ann Rheum Dis*. 2007;66:46-52.
9. Symmons DP. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. *Best Pract Res Clin Rheumatol*. 2002;16:707-22.
10. Flouri I, Markatseli TE, Voulgari PV, Boki KA, Papadopoulos I, Settas L, et al. Comparative effectiveness and survival of infliximab, adalimumab, and etanercept for rheumatoid arthritis patients in the Hellenic Registry of Biologics: Low rates of remission and 5-year drug survival. *Semin Arthritis Rheum*. 2014;43:447-57.
11. Tengstrand B, Ahlmen M, Hafstrom I. The influence of sex on rheumatoid arthritis: a prospective study of onset and outcome after 2 years. *J Rheumatol*. 2004;31:214-22.
12. Kuiper S, van Gestel AM, Swinkels HL, de Boo TM, da Silva JA, van Riel PL. Influence of sex, age, and menopausal state on the course of early rheumatoid arthritis. *J Rheumatol*. 2001;28:1809-16.
13. van Vollenhoven RF. Sex differences in rheumatoid arthritis: more than meets the eye. *BMC Med*. 2009;7:12.
14. Elemam NM, Hannawi S, Maghazachi AA. Role of Chemokines and Chemokine Receptors in Rheumatoid Arthritis. *Immunotargets Ther*. 2020;9:43-56.
15. Burska A, Boissinot M, Ponchel F. Cytokines as biomarkers in rheumatoid arthritis. *Mediators Inflamm*. 2014;2014:545493.
16. Crowson CS, Rollefstad S, Ikdahl E, Kitas GD, van Riel P, Gabriel SE, et al. Impact of risk factors associated with cardiovascular outcomes in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2018;77:48-54.
17. Lew J, Sanghavi M, Ayers CR, McGuire DK, Omland T, Atzler D, et al. Sex-Based Differences in Cardiometabolic Biomarkers. *Circulation*. 2017;135:544-55.
18. Despres JP. Body fat distribution and risk of cardiovascular disease: an update. *Circulation*. 2012;126:1301-13.
19. Ferreira MB, Fonseca T, Costa R, Marinhoc A, Carvalho HC, Oliveira JC, et al. Prevalence, risk factors and proteomic bioprofiles associated with heart failure in rheumatoid arthritis: The RA-HF study. *Eur J Intern Med*. 2020.
20. Villeneuve E, Nam J, Emery P. 2010 ACR-EULAR classification criteria for rheumatoid arthritis. *Rev Bras Reumatol*. 2010;50:481-3.

21. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M. Validation study of a Portuguese version of the Hospital Anxiety and Depression Scale. *Psychol Health Med*. 2007;12:225-35; quiz 35-7.
22. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166:111-7.
23. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2015;16:233-70.
24. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2015;28:1-39 e14.
25. Ferreira JP, Verdonschot J, Collier T, Wang P, Pizard A, Bar C, et al. Proteomic Bioprofiles and Mechanistic Pathways of Progression to Heart Failure. *Circ Heart Fail*. 2019;12:e005897.
26. Lau ES, Paniagua SM, Guseh JS, Bhambhani V, Zanni MV, Courchesne P, et al. Sex Differences in Circulating Biomarkers of Cardiovascular Disease. *J Am Coll Cardiol*. 2019;74:1543-53.
27. Toussirot É, Michel F, Binda D, Dumoulin G. The role of leptin in the pathophysiology of rheumatoid arthritis. *Life Sci*. 2015;140:29-36.
28. Fatel ECS, Rosa FT, Simao ANC, Dichi I. Adipokines in rheumatoid arthritis. *Adv Rheumatol*. 2018;58:25.
29. Skalska U, Kontny E. Adiponectin Isoforms and Leptin Impact on Rheumatoid Adipose Mesenchymal Stem Cells Function. *Stem Cells Int*. 2016;2016:6532860.
30. Kidani T, Sakayama K, Masuno H, Takubo N, Matsuda Y, Okuda H, et al. Active-dimeric form of lipoprotein lipase increases in the adipose tissue of patients with rheumatoid arthritis treated with prednisolone. *Biochim Biophys Acta*. 2002;1584:31-6.
31. Morrell NW, Bloch DB, ten Dijke P, Goumans MJ, Hata A, Smith J, et al. Targeting BMP signalling in cardiovascular disease and anaemia. *Nat Rev Cardiol*. 2016;13:106-20.
32. Helbing T, Wiltgen G, Hornstein A, Brauers EZ, Arnold L, Bauer A, et al. Bone Morphogenetic Protein-Modulator BMPER Regulates Endothelial Barrier Function. *Inflammation*. 2017;40:442-53.
33. Yao Y, Bennett BJ, Wang X, Rosenfeld ME, Giachelli C, Lusis AJ, et al. Inhibition of bone morphogenetic proteins protects against atherosclerosis and vascular calcification. *Circ Res*. 2010;107:485-94.
34. Ramirez J, Ruiz-Esquide V, Pomes I, Celis R, Cuervo A, Hernandez MV, et al. Patients with rheumatoid arthritis in clinical remission and ultrasound-defined active synovitis exhibit higher disease activity and increased serum levels of angiogenic biomarkers. *Arthritis Res Ther*. 2014;16:R5.
35. Taylor PC. VEGF and imaging of vessels in rheumatoid arthritis. *Arthritis Res*. 2002;4 Suppl 3(Suppl 3):S99-107.
36. Lu K, Liu L, Xu X, Zhao F, Deng J, Tang X, et al. ADAMTS13 ameliorates inflammatory responses in experimental autoimmune encephalomyelitis. *J Neuroinflammation*. 2020;17:67.
37. Boutet MA, Nerviani A, Lliso-Ribera G, Lucchesi D, Prediletto E, Ghirardi GM, et al. Interleukin-36 family dysregulation drives joint inflammation and therapy response in psoriatic arthritis. *Rheumatology (Oxford)*. 2020;59:828-38.
38. Zhang G, Liu HB, Zhou L, Cui XQ, Fan XH. CCL3 participates in the development of rheumatoid arthritis by activating AKT. *Eur Rev Med Pharmacol Sci*. 2018;22:6625-32.
39. Joosten LA, Radstake TR, Lubberts E, van den Bersselaar LA, van Riel PL, van Lent PL, et al. Association of interleukin-18 expression with enhanced levels of both interleukin-1beta and tumor necrosis factor alpha in knee synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum*. 2003;48:339-47.

40. Imas JJ, Ruiz Zamarreno C, Zubiarte P, Sanchez-Martin L, Campion J, Matias IR. Optical Biosensors for the Detection of Rheumatoid Arthritis (RA) Biomarkers: A Comprehensive Review. *Sensors (Basel)*. 2020;20.
41. Wang X, Liang J, Koike T, Sun H, Ichikawa T, Kitajima S, et al. Overexpression of human matrix metalloproteinase-12 enhances the development of inflammatory arthritis in transgenic rabbits. *Am J Pathol*. 2004;165:1375-83.
42. Kumkumian GK, Lafyatis R, Remmers EF, Case JP, Kim SJ, Wilder RL. Platelet-derived growth factor and IL-1 interactions in rheumatoid arthritis. Regulation of synoviocyte proliferation, prostaglandin production, and collagenase transcription. *J Immunol*. 1989;143:833-7.

Table 1. Baseline characteristics of men and women.

Baseline characteristics	Women (N=306)	Men (N=93)	P value
Biometric and demographic			
Age (years)	58.0 ± 13.4	61.0 ± 12.3	0.056
BMI (Kg/m ²)	26.5 ± 4.5	26.6 ± 4.3	0.93
Waist circumference category*	157 (51.3%)	28 (30.1%)	<0.001
Visceral fat	7.8 ± 3.0	11.9 ± 4.1	<0.001
Smoking status			
Never	235 (76.8%)	24 (25.8%)	<0.001
Past	39 (12.7%)	45 (48.4%)	
Current	32 (10.5%)	24 (25.8%)	
Comorbidities			
Diabetes mellitus	33 (10.8%)	20 (21.5%)	0.008
Dyslipidemia	152 (49.7%)	38 (40.9%)	0.14
Myocardial infarction	3 (1.0%)	3 (3.2%)	0.12
Angina pectoris	22 (7.2%)	3 (3.2%)	0.17
Ischemic stroke or transient ischemic attack	10 (3.3%)	4 (4.3%)	0.64
Valvular heart disease	9 (2.9%)	1 (1.1%)	0.31
Atrial fibrillation	11 (3.6%)	3 (3.2%)	0.87
COPD	9 (2.9%)	9 (9.7%)	0.006
Prior heart failure	18 (5.9%)	6 (6.5%)	0.84
Hypertension	133 (43.5%)	51 (54.8%)	0.054
Anemia	59 (19.3%)	11 (11.8%)	0.098
Chronic kidney disease	12 (3.9%)	1 (1.1%)	0.18
RA history			
Time since RA diagnosis (years)	12.5 ± 10.8	10.8 ± 9.0	0.18
RF or anti-CCP positive	241 (78.8%)	75 (80.6%)	0.69
Articular erosions	105 (37.6%)	34 (41.5%)	0.53
DAS28 value with ESR	2.9 ± 1.2	2.6 ± 1.3	0.13
Symptoms and physical exam			
Dyspnea	91 (29.7%)	22 (23.7%)	0.25
Fatigue	196 (64.1%)	46 (49.5%)	0.012
Systolic blood pressure (mmHg)	132.5 ± 19.6	137.2 ± 18.4	0.042
Diastolic blood pressure (mmHg)	75.2 ± 10.0	79.7 ± 10.4	<0.001
Heart rate (bpm)	80.2 ± 13.2	82.2 ± 18.0	0.24
Echocardiogram			
LVEF (biplane)	61.7 ± 6.9	58.7 ± 7.3	<0.001
LVEF <50%	12 (4.4%)	8 (10.0%)	0.059
Left ventricular hypertrophy	28 (10.3%)	8 (9.5%)	0.85
Left atrium volume (biplane indexed)	32.6 ± 11.2	31.8 ± 9.7	0.57

Laboratorial data			
Hb (g/dl)	12.9 ± 1.2	14.1 ± 1.7	<0.001
eGFR (ml/min/1.73m ²)	89.8 ± 20.3	83.5 ± 20.4	0.010
Na ⁺ (mmol/L)	142.0 ± 2.6	142.1 ± 2.0	0.87
K ⁺ (mmol/L)	4.4 ± 0.4	4.6 ± 0.4	0.006
Total cholesterol (mg/dl)	187.3 ± 37.3	183.1 ± 32.8	0.33
LDL cholesterol (mg/dl)	99.7 ± 31.1	103.4 ± 28.6	0.32
HDL cholesterol (mg/dl)	61.7 ± 16.3	53.1 ± 16.1	<0.001
Triglycerides (mg/dl)	125.5 ± 65.0	125.6 ± 54.8	0.98
NT-pro BNP (pg/ml)	88.0 (47.1, 174.1)	70.0 (31.4, 145.0)	0.041
CRP (mg/dl)	2.9 (1.1, 6.5)	5.8 (2.2, 12.3)	<0.001
Medication			
ACEi/ARB	108 (35.3%)	44 (47.3%)	0.037
B-blocker	32 (10.5%)	11 (11.8%)	0.71
MRA	1 (0.3%)	1 (1.1%)	0.37
Loop diuretic	8 (2.6%)	2 (2.2%)	0.80
Statin	117 (38.2%)	33 (35.5%)	0.63
Corticosteroids	138 (45.1%)	43 (46.2%)	0.85
Methotrexate	189 (62.0%)	59 (64.1%)	0.71
NSAIDs	82 (26.8%)	19 (20.4%)	0.22
Biological DMARDs	63 (20.6%)	12 (12.9%)	0.097
Six-minute walk test (m)	375(330-420)	390 (330-450)	0.34
Anxiety and Depression status			
HADS A (points)	6.0 (3.0, 10.0)	4.0 (2.0, 7.0)	<0.001
HADS D (points)	5.0 (2.0, 9.0)	3.0 (1.0, 5.0)	<0.001

Legend: BMI, body mass index; COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis; RF, rheumatoid factor; Anti-CCP, anti-citrulline antibody; DAS28 VS (ESR), Disease Activity Score for Rheumatoid Arthritis with Erythrocyte Sedimentation Rate; LVEF, left ventricular ejection fraction; Hb, hemoglobin; eGFR, estimated Glomerular Filtration Rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NT-pro BNP, N-terminal pro B-type Natriuretic Peptide; CRP, c-reactive protein; ACEi/ARB, angiotensin converting enzyme inhibitors or angiotensin-receptor blockers; MRA, mineralocorticoid receptor antagonists; MTX, methotrexate; NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, disease-modifying anti-rheumatic drugs; HADS, Hospital Anxiety and Depression Scale.

*Waist circumference category defined as >88 cm for women and >102 cm for men.

Data are presented as mean ± standard deviation, median (25% and 75% quartiles) or frequency (percentage) as appropriate. Bold face values indicate statistical significance.

Table 2. Circulating proteins that better differentiate between sexes.

Biomarker	OR (95%CI)	P value*
Higher expression in women		
LPL	8.33 (3.70-20.0)	0.0001
GDF2	4.76 (2.50-9.09)	0.0001
LEP	4.00 (2.63-5.88)	0.0001
VEGFD	7.14 (2.43-20.0)	0.0003
IL1RL2	3.84 (1.81-8.33)	0.0004
ADAMTS13	33.3 (3.03-99.0)	0.0037
Higher expression in men		
CRP	1.68 (1.32-2.14)	0.0001
hs-TnT	3.14 (1.76-5.61)	0.0001
GLO1	2.64 (1.55-4.50)	0.0003
PGF	5.27 (2.05-13.6)	0.0006
CCL3	1.82 (1.28-2.58)	0.0008
IL18	2.26 (1.29-3.98)	0.0044
MMP12	1.88 (1.20-2.95)	0.0055

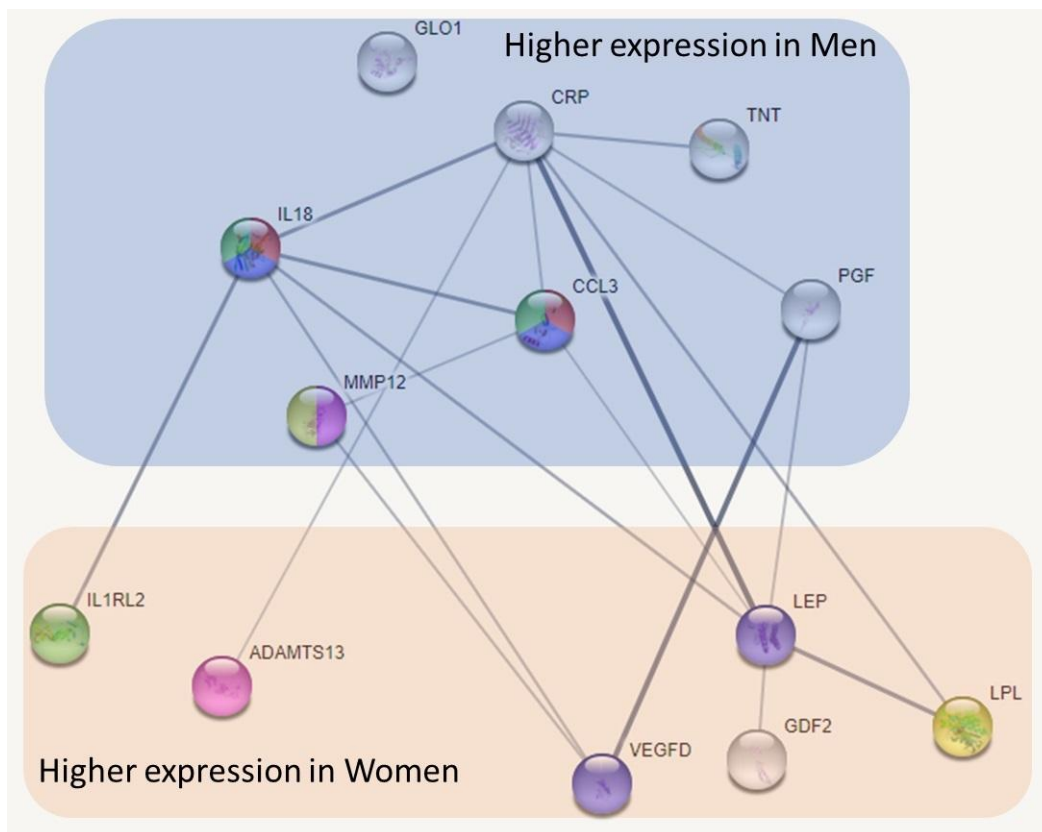
Legend: OR, Odds ratio; CI, confidence interval. ADAMTS13, Adisintegrin and metalloproteinase with thrombospondin motifs 13; LPL, Lipoprotein lipase; VEGFD, Vascular endothelial growth factor D; GDF2, Growth/differentiation factor 2; LEP, Leptin; IL1RL2, Interleukin-1 receptor-like 2; CRP, c-reactive protein; CCL3, C-C motif chemokine 3; MMP12, Matrix metalloproteinase-12; IL18, Interleukin-18; GLO1, Lactoylglutathione lyase; hs-TnT, high sensitivity troponin T; PGF, Placenta growth factor.

*All the biomarkers had a FDR lower than 5% after adjustment (see the methods section for details).

The biomarkers are ordered by strength of the association (p-value).

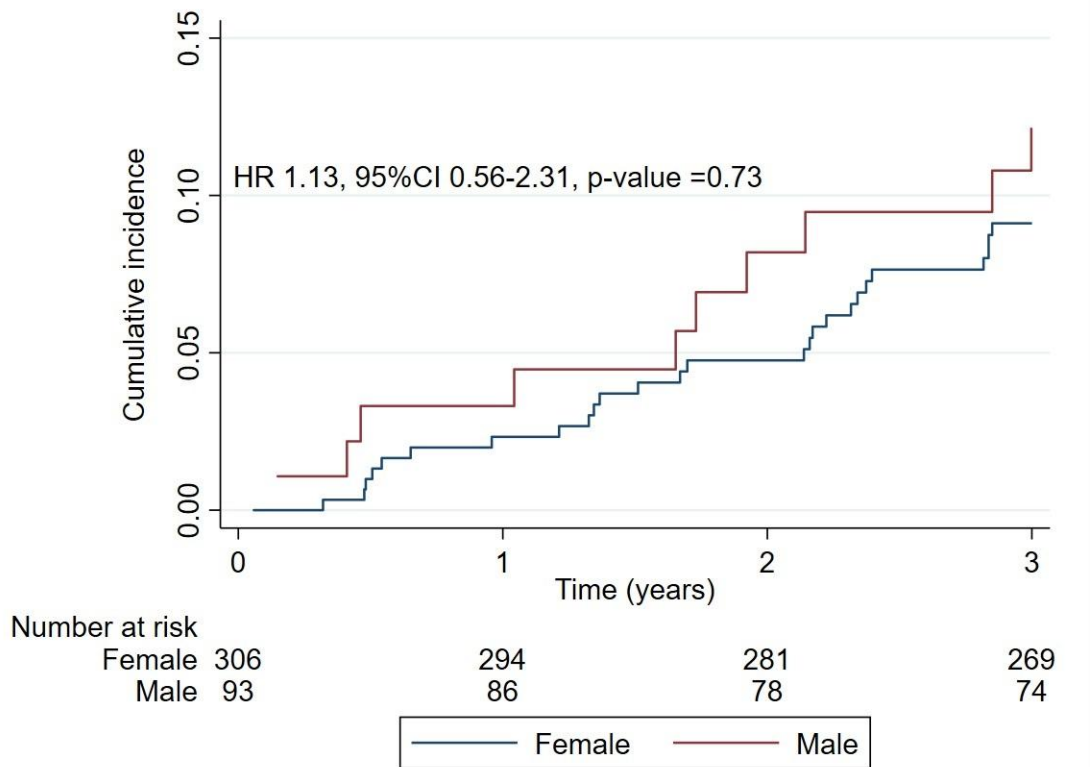
To facilitate the interpretation of the data, we present all the biomarkers in terms of “higher association” with women and men i.e., the inverse odds ratio (1/OR) is presented for women by changing the indicator variable.

Figure 1. Network analysis of protein expression in men and women



Legend: Most relevant proteins network built utilizing the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins). The edges (lines) indicate both functional and physical protein associations) and their thickness signifies the strength of data support as defined by STRING. Color of the nodes indicates: KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways significantly over-represented (red: rheumatoid arthritis; blue: cytokine-cytokine receptor interactions) and annotated Uniprot keywords (green: inflammatory response, magenta: metalloproteases, yellow: Extra-cellular matrix). **GLO1** denotes Lactoylglutathione lyase; **CRP**, C-reactive protein; **TNT**, high-sensitivity troponin; **IL18**, Interleukin-18; **PGF**, Placenta growth factor; **CCL3**, C-C motif chemokine 3; **MMP12**, Matrix metalloproteinase-12; **LEP**, Leptin; **IL1RL2**, Interleukin-1 receptor-like 2; **ADAMTS13**, A disintegrin and metalloproteinase with thrombospondin motifs 13; **LPL**, Lipoprotein lipase; **GDF-2**, Growth/differentiation factor 2; **VEGFD**, Vascular endothelial growth factor D.

Figure 2. Composite endpoint of CV death or CV hospitalization between women and men



Legend: CV, cardiovascular; HR, hazard ratio; CI, confidence interval.

Model adjusted on age, waist circumference, smoking, diabetes, diastolic blood pressure, hemoglobin, and estimated glomerular filtration rate.