

Biomarkers of Myocardial Fibrosis are Associated with Diabetes but not with Coronary Microvascular Dysfunction in Women with Angina and No Obstructive Coronary Artery Disease

Kira Bang Bove (✉ kirabove@gmail.com)

Bispebjerg Hospital

Naja Dam Mygind

Department of Cardiology, Bispebjerg University Hospital, Copenhagen, Denmark

Signe Holm Nielsen

Nordic Bioscience

Marie Mide Michelsen

Bispebjerg Hospital, Department of Cardiology

Daria Frestad Bechsgaard

Dept of Cardiology, Bispebjerg University Hospital, Copenhagen

Hannah Elena Suhrs

Dept of Cardiology, Bispebjerg Hospital, Copenhagen

Jakob Schroder

Department of Cardiology, Bispebjerg Hospital, Copenhagen

Eva Prescott

Dept of Cardiology, Bispebjerg Hospital, Copenhagen

Original investigation

Keywords: women, DM, dyslipidemia, myocardial fibrosis, biomarkers, proteoglycans, mimecan, titin, collagens

Posted Date: June 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-36838/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background Coronary microvascular dysfunction (CMD) is highly prevalent in women with no obstructive coronary artery disease and possibly related to myocardial fibrosis caused by excessive extracellular matrix (ECM) remodeling. ECM turnover can be measured in blood indicating fibrotic activity. We hypothesized that women with DM, angina and no obstructive coronary artery disease have increased ECM turnover and that this is associated with CMD.

Methods We included 344 women with angina pectoris and no obstructive coronary artery disease (187 with DM, predominantly type II) and 76 asymptomatic women without DM as controls. Biomarkers reflecting formation of type IV and VI collagen (PRO-C4 and PRO-C6) and degradation of type IV, V and VI collagen (C4M, C5M, C6M), mimecan (MIM) and titin (TIM) were measured in all participants. CMD was defined as coronary flow velocity reserve (CFVR) <2.0 assessed by transthoracic Doppler echocardiography.

Results Median age was 64.2 (IQR 57.0-70.0), slightly higher in symptomatic women with DM. Median CFVR was 2.21 (1.89-2.55) in symptomatic women with DM, 2.35 (1.96-2.77) in symptomatic women without DM and 2.63 (2.19-2.95) in controls (age-adjusted p for trend <0.001). With exception of CM5, women with DM had significantly higher levels of all ECM biomarkers than women without DM (age-adjusted p <0.01), whereas biomarkers did not differ between symptomatic women without DM and controls. High ECM biomarker levels were associated with HbA1c, high BMI, low HDL and high triglycerides (p =0.003-0.0001). There was no correlation between ECM biomarkers and CFVR.

Conclusion Women with angina pectoris and DM had increased levels of myocardial fibrosis biomarkers compared with women without DM. There was no association between CMD and biomarkers of myocardial fibrosis.

Background

Coronary microvascular dysfunction (CMD) is highly prevalent in women with angina pectoris and no obstructive coronary artery disease. CMD is a strong prognostic marker of cardiovascular morbidity and mortality,¹⁻³ is associated with cardiovascular risk factors, particularly diabetes mellitus (DM) type II and hypertension,^{4,5} and is frequent in heart failure.⁶

In CMD, transient ischemia occurs because the microvasculature cannot dilate in response to increased oxygen demand.⁷ This condition may cause chronic low-grade ischemia which promotes cardiac extracellular matrix (ECM) remodeling.^{8,9} The cardiac ECM consists primarily of collagens and proteoglycans preserving ventricular function and structure.^{10,11} Imbalanced ECM remodeling induces accumulation of collagens, expansion of the extracellular volume and myocardial fibrosis.^{12,13} Thus, CMD might be a precursor of myocardial fibrosis.

Myocardial fibrosis is associated with impaired ventricular function, remodeling and stiffness of the myocardium,^{8,14} and is a characteristic of diabetic cardiomyopathy clinically presenting as heart failure with preserved ejection fraction (HFpEF).

Collagen and proteoglycan formation and degradation fragments can be quantified in blood and may be indicative of early fibrotic disease activity.^{15,16} In a small study we have previously found that women with angina pectoris and no obstructive coronary artery disease have an imbalanced collagen turnover compared with asymptomatic controls. In this study, we investigated whether ECM turnover is associated with DM and CMD by examining seven biomarkers that have recently shown promising as markers of fibrotic activity.

Methods

Study population

The study-population was derived from the iPOWER (*ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease*) cohort conducted from May 2012 to December 2017.¹⁷ In- and exclusion criteria in iPOWER have been published previously.⁴ Briefly, inclusion criteria for the iPOWER study were angina pectoris and no obstructive coronary artery disease (defined as less than 50% stenosis assessed by coronary angiography), left ventricular ejection fraction (LVEF) > 45% and no significant valvopathy. From 1830 patients included in iPOWER we selected all women with DM (n = 187) and a random sample of women without DM (n = 157).

A control group of 76 asymptomatic women without DM or previous cardiovascular disease were recruited from the Copenhagen City Heart Study¹⁸ between June and September 2015. Blood biomarkers of collagen and proteoglycan turnover were successfully measured in all 420 participants.

Study assessments

We obtained demographic data from interviews. Clinical data included weight, abdominal circumference, blood pressure and heart rate measured at rest. Blood samples were analyzed for cholesterol levels (total, low-density lipoprotein [LDL] and high-density lipoprotein [HDL] cholesterol), and triglycerides and HbA1c. Serum was collected for biomarker analysis and immediately stored at -80 °C until analysis according to pre-defined standard operating procedures.

Coronary microvascular function

Coronary microvascular function was assessed non-invasively by transthoracic Doppler stress echocardiography (TTDE) measuring the coronary flow velocity reserve (CFVR) of the left anterior descending artery. CFVR is the ratio of the peak diastolic flow at hyperemia to rest. We used high-dose dipyridamole (0.84 mg/kg) over 6 minutes to induce maximal hyperemia (29). All examinations were performed by the same 3 experienced echocardiographers in an unchanged setting using GE Healthcare

Vivid E9 cardiovascular ultrasound system (GE Healthcare, Horten, Norway) with a 2.7-8 MHz transducer (GE Vivid 6S probe). A standard echocardiographic examination was conducted before the CFVR examination. Measurements of myocardial function at hyperemia were obtained immediately after termination of the dipyridamole infusion. After the examination, intravenous theophylline (maximum dose 220 mg) was administered. A detailed methods description of the standard echocardiography is given in Online Appendix.

Biomarkers of fibrotic activity

Tissue turnover was determined by neo-epitope biomarkers to assess the formation of type IV and VI collagen (PRO-C4 and PRO-C6) and degradation of type IV, V, VI collagen, mimecan and titin (C4M, C5M, C6M, MIM and TIM), respectively. (Table 1).

PRO-C4 and PRO-C6 are building stones of collagen type IV and VI and have been shown to correlate with DM and to be associated with a poor outcome in patients with HFpEF.^{19–21} C4-6M are degradation fragments of collagen type IV-VI and have been linked to liver fibrosis, carotid atherosclerosis and major cardiovascular events.^{22,23} Mimecan is a small proteoglycan, upregulated and released in heart diseases.¹⁴ Titin, also known as Connectin, is a large sarcomeric protein responsible for elastic recoil and compliance.^{24,25} Decreased titin content in the sarcomere leads to deposition of fibrotic tissue.^{24,26} In the ECM remodeling process, mimecan and the cardiac isoform of titin are broken down to the neo-epitopes MIM and TIM, respectively. Both have shown promising results as blood biomarkers of fibrotic activity.^{10,12,27}

Biomarker analysis

The biomarkers were quantified by ELISA assays produced at Nordic Bioscience, Herlev, Denmark. Biomarkers were measured in serum samples from the symptomatic women with and without diabetes and from the asymptomatic controls. Briefly, ELISA assays were performed as following: Streptavidin-coated microtiter plate was incubated with a biotinylated peptide for 30 min at 20 °C. Unbound biotinylated-peptide was washed off five times with washing buffer (20 nM TRIS, 50 mM NaCl, pH 7.2). Subsequent, a selection peptide, five kit control samples and patient serum samples were added to the plate and peroxidase-labelled monoclonal antibody was added and incubated for 1 h at 20 °C (PRO-C4, C4M, C6M, MIM and TIM), 3 h at 4 °C (C5M), or 20 h at 4 °C (PRO-C6). Subsequently all ELISA assays plates, after incubated with peroxidase-labelled antibody, were washed five times with washing buffer and incubated with 3,3',5,5'-Tetramethylbenzidine (TMB) for 15 min at 20 °C in the dark. The reaction was stopped with stopping solution (1% H₂SO₄) and measured on an ELISA plate reader at 450 nm absorbance with 650 nm as reference. Standard curves were generated by the selection peptide and plotted using a 4-parametric mathematical fit model. Samples below the lower limit of measurement range (LLMR) were reported as the value of LLMR.

Statistics

Continuous variables with approximately normal distributions are expressed as mean \pm standard deviation (SD) and continuous variables with non-normal distribution as median \pm interquartile range (IQR). Pairwise comparisons of demographic, anamnestic and clinical parameters between the three groups were performed with one-way analysis of variance for continuous variables, and with χ^2 -tests for categorical variables. Age-adjusted p-value for trend across groups was calculated using linear or logistic regression analysis with the categorical variable treated as a continuous variable. Pairwise comparisons of biomarker distribution in the three groups were adjusted for age and performed by linear regression analyses with logarithmically transformed biomarker as the dependent variable. All tests were Bonferroni corrected for family-wise error rate. A two-sided p-value below 0.05 was considered statistically significant. Pairwise correlations between biomarkers and covariates of interest were calculated using Spearman's rho and reported both raw and Bonferroni corrected. Statistical analyses were performed using STATA/IC13.1 (StataCorp LP, College Station, Texas, USA).

Results

Demographics and risk factor distribution

Baseline information is presented in Table 2. As expected, symptomatic women had a significantly higher risk factor burden than asymptomatic controls. Conversely, they received more aggressive preventive medication leading to lower LDL- and total cholesterol. Women with DM were older, had higher BMI and higher prevalence of hypertension, dyslipidemia and atheromatosis on invasive coronary angiogram (Table 2). Sixteen women (8.6%) had DM Type I.

Coronary microvascular dysfunction and echocardiographic parameters

Coronary microvascular function was successfully assessed in 409 (98%) of the participants. Symptomatic women with DM had lower CFVR, indicating poorer microvascular function compared with women without DM (age-adjusted p for trend < 0.001). Using a cut-off for CFVR of ≤ 2 to define CMD, the prevalence of CMD was 33.7%, 28.7% and 17.1% in symptomatic women with DM, symptomatic women without DM and controls, respectively (age-adjusted p for trend 0.016) (Table 3).

LVEF under stress was higher in the two groups of symptomatic women than in the control group. Parameters of diastolic dysfunction (E/e' , e' and elevated filling pressure) indicated poorer function in women with diabetes than in the other groups (all $p < 0.01$). However, only few participants qualified for manifest diastolic dysfunction according to international guidelines.²⁸ (Table 3).

Alterations of the extracellular matrix quantified by biomarkers

ECM turnover biomarker levels across the three groups are shown in Fig. 1. For all biomarkers, the highest values were seen among symptomatic women with DM. Five biomarkers (MIM, PRO-C4, PRO-C6, C4M

and C6M) were significantly higher in symptomatic women with DM than in women without DM (age- and Bonferroni adjusted $p = 0.001-0.03$, Fig. 1b-e) and four (TIM, MIM, Pro-C6 and C6M) were significantly higher than in controls (age- and Bonferroni adjusted $p = 0.001-0.009$, Fig. 1a-e). None of the biomarkers differed between non-diabetes patients and controls.

Association between biomarkers, clinical parameters and CFVR

HbA1c, BMI, HDL-cholesterol and serum triglyceride levels were significantly correlated with all seven biomarkers, although all correlations were weak ($r = 0.06-0.32$, $p = 0.03-0.0001$) (results not shown). In addition, the biomarkers TIM, MIM, PRO-C6, C4M and C6M were also significantly correlated with history of hypertension. After Bonferroni correction, all pairwise spearman correlations became less significant (Fig. 2). There was no correlation between any of the biomarkers and CFVR, CFV at rest or CFV under hyperemia. No systolic or diastolic measurements were correlated with biomarkers after Bonferroni correction.

Discussion

An increased turnover of ECM fragments in blood may reflect remodeling and early fibrotic disease.²⁹ In a smaller sub-sample of the iPOWER cohort we have previously demonstrated imbalanced turnover of certain collagens when compared with healthy controls³⁰ but have failed to find a relation between fibrosis on cardiac magnetic resonance imaging and CFVR assessed non-invasively, perhaps due to lack of statistical power.³¹ In this larger study we aimed to verify the increased ECM activity and determine whether DM patients, who are particularly prone to developing myocardial fibrosis, had elevated ECM turnover as a marker of myocardial fibrosis and whether this was related to impaired coronary microvascular function.

We found that women with angina pectoris and DM had significantly higher levels of ECM biomarkers although CMD did not seem to be associated with these biomarkers. Furthermore, high levels of ECM biomarkers were associated with metabolic disturbances as reflected in higher BMI, HbA1c, triglycerides and lower HDL.

Cardiovascular risk factors other than diabetes were highly prevalent in symptomatic women with DM. Ageing, hypertension and metabolic disturbances such as obesity and DM have previously been associated with myocardial fibrosis. In DM, glycation end-product deposition²⁵ and metabolic dysregulation have been described as triggers of fibroblast activation, cardiac ECM-remodeling and fibrosis. Adipositas and hypertension may also activate fibroblasts and thereby induce collagen accumulation and deposition.⁸

Biomarkers of collagen type IV and VI turnover, TIM and MIM have previously been associated with fibrotic disease or fibrotic related conditions. Collagen type IV is primarily found in the basement

membrane³⁰ and has a stabilizing function of microvessels during angiogenesis.³² Increased levels have been correlated to endocardial hypertrophy and liver fibrosis.^{33–35} Collagen type V and VI are important components of the interstitial connective tissue and contribute to the quality of the ECM by regulating the fibril size of collagen type I and III.³⁰ PRO-C6 have been associated with diabetes^{16,19} and together with PRO-C4 associated with poor prognosis in HFpEF-patients.²¹ C4M and C6M have been linked to severe liver fibrosis.²² Also, C4M has recently been found to predict major cardiovascular events and to be associated with carotid atherosclerosis.²³ Decreased titin in the sarcomere is thought to cause fibrosis,^{24,26} and circulating levels of MMP-cleaved mimecan (MIM) has previously been identified as a marker of extracellular matrix remodeling in mice.¹⁰ Although elevated in symptomatic women with DM compared with asymptomatic women, TIM was no longer correlated to DM and HbA1c after Bonferroni adjustment, whereas a strong correlation remained with BMI and blood cholesterol levels. Mimecan is a small proteoglycan with important functions in myofibril formation and angiogenesis. It is upregulated and released in heart disease such as after myocardial infarction, in conditions with pressure overload such as in hypertension, but is also released in inflammatory disease such as vasculitis.^{10,14}

In our previous study of collagen turnover in the iPOWER cohort including 71 symptomatic patients,³⁰ PRO-C6, C4M and C6M were increased when compared to asymptomatic controls, whereas no significant difference between groups was observed for PRO-C4, possibly due to lack of statistical power. C5M and C6M were found to be lower in iPOWER women than in controls. However, the previous study did not include patients with DM and are thus not directly comparable with the current results where the high values are related to the presence of DM.

To our knowledge, this is the first study to demonstrate a consistent and significant overexpression of multiple biomarkers of fibrosis in women with angina pectoris, DM and risk factors for myocardial fibrosis. Although many correlations were weak, most were highly significant even after conservative Bonferroni adjustment. Further, all biomarkers were consistently associated with DM and metabolic risk factors: BMI, HbA1c, HDL-cholesterol and triglycerides. Also, we performed Bonferroni corrected pairwise correlations and consequently, the association with DM and HbA1c disappeared for TIM and C5M.

We found no relation between CMD and ECM biomarkers. This would indicate that non-endothelial dependent CMD, as assessed in this study by dipyridamole stress, is not causally related to the development of myocardial fibrosis. Other explanations, as discussed below, is that the ECM biomarker level does not only reflect cardiac remodeling but general fibrotic activity, making direct comparisons difficult. Also, a relation between increased ECM turnover and CMD caused by endothelial inflammation may have been missed in this study as we have only assessed non-endothelial dependent CMD.^{8,12,36} Obesity, arterial hypertension and DM may induce chronic, systemic inflammation and consequently endothelial dysfunction, ECM remodeling, cardiac fibrosis and finally HFpEF.³⁷

Another explanation for the lack of relation between CMD and biomarkers is that the measured biomarker activity may reflect early stages of fibrotic disease that later may develop into manifest myocardial

fibrosis, CMD and/or HFpEF.³⁸ Risk factors for HFpEF such as female sex, ageing, hypertension, obesity, and DM³⁸ are all well presented in our population but our population of women at risk did not have HFpEF. However, they did show signs of ventricular hyper-contractibility on echocardiography (higher LVEF), and of poorer diastolic function and higher left ventricular filling pressure compared with controls.

Strengths and limitations

In the iPOWER study, participants were consecutively included and systematically examined. All participants except the asymptomatic controls had a clinical invasive coronary angiography performed ruling out obstructive coronary artery disease (defined by > 50% stenosis of coronary arteries). The prevalence of cardiovascular risk factors was high. However, if we had been able to include participants with more impaired ventricular function or more pronounced CMD, the population might have had more myocardial fibrosis which we might have been able to measure by increased levels of circulating biomarkers. We did not measure the endothelial dependent component of coronary microvascular function and have therefore not examined the relationship between endothelial-dependent microvascular function and biomarker turnover

Further research is needed with the aim of detecting cardio-specific biomarkers of fibrosis. Until then, it is possible to misinterpret fibrosis as myocardial when biomarkers may be increased due to fibrosis in other organs than the heart.

Conclusion

Women with angina pectoris, DM and a high cardiovascular risk factor burden have increased turnover of biomarkers reflecting early fibrotic disease compared with women without DM and few risk factors. Biomarkers were associated with BMI, HbA1c and cholesterol levels but not with non-endothelial dependent CMD. To better evaluate the relation between CMD and myocardial fibrosis future studies would benefit from longitudinal follow-up and include measurements of endothelial dependent CMD.

Abbreviations

CFV(R) = coronary flow velocity (reserve)

CMD = coronary microvascular dysfunction

ECM = extracellular matrix

ELISA = enzyme-linked immunosorbent assay

GLS = global longitudinal strain

HDL = high-density lipoprotein

HFpEF = heart failure with preserved ejection fraction

iPOWER = ImProve diagnOsis and treatment of Women with angina pEctoris and micRovessel disease

LDL = low-density lipoprotein

LLMR = lower limit of measurement range

LVEF = left ventricular ejection fraction

MMP = matrix metalloproteinase

TTDE = transthoracic Doppler echocardiography

Abbreviations of biomarkers are explained in Table 1.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki³⁹ and was approved by the Danish Regional Committee on Biomedical Research Ethics (H-3-2012-005). All participants have given written informed consent upon oral and written information.

Consent for publication

Not applicable.

Availability of data and materials

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests

SHN is employed at Nordic Bioscience. The other authors of this manuscript have no conflicts of interest.

Funding

This work was supported by The Danish Heart Foundation (grant number: 11-10-R87-B-A3628-22678).

Authors' contributions

KBB participated in data collection and echocardiographic analyses, performed the analysis and interpretation of data and drafted this manuscript.

NDM, MMM, AP, DF, HES, JS participated in the data collection and the echocardiographic analyses and revised this manuscript critically. SHN conducted the biomarker analysis and revised this manuscript critically. EP designed the iPOWER study, participated in drafting this manuscript and revised it critically.

Acknowledgements

The authors would like to thank the Danish Heart Foundation, the University of Copenhagen and the Danish Research Foundation for financial support. We also thank the Department of Cardiology at Bispebjerg Hospital and all collaborators in the iPOWER group. Finally, we thank all the participating women in iPOWER for their time and willingness to contribute to the research.

References

1. Pepine CJ, Anderson RD, Sharaf BL, et al. Coronary Microvascular Reactivity to Adenosine Predicts Adverse Outcome in Women Evaluated for Suspected Ischemia. *J Am Coll Cardiol*. 2010;55(25):2825-2832. doi:10.1016/j.jacc.2010.01.054.
2. Sicari R, Rigo F, Cortigiani L, Gherardi S, Galderisi M, Picano E. Additive Prognostic Value of Coronary Flow Reserve in Patients With Chest Pain Syndrome and Normal or Near-Normal Coronary Arteries. *Am J Cardiol*. 2009;103:626-631. doi:10.1016/j.amjcard.2008.10.033.
3. Murthy VL, Naya M, Taqueti VR, et al. Effects of sex on coronary microvascular dysfunction and cardiac outcomes. *Circulation*. 2014;129(24):2518-2527. doi:10.1161/CIRCULATIONAHA.113.008507.
4. Mygind ND, Michelsen MM, Pena A, et al. Coronary Microvascular Function and Cardiovascular Risk Factors in Women With Angina Pectoris and No Obstructive Coronary Artery Disease: The iPOWER Study. *J Am Heart Assoc*. 2016;5(3):e003064. doi:10.1161/JAHA.115.003064.
5. Borbély A, Van Heerebeek L, Paulus WJ. Transcriptional and Posttranslational Modifications of Titin Implications for Diastole. 2009. doi:10.1161/CIRCRESAHA.108.191130.
6. Shah SJ, Lam CSP, Svedlund S, et al. Prevalence and correlates of coronary microvascular dysfunction in heart failure with preserved ejection fraction: PROMIS-HFpEF. *Eur Heart J*. 2018; (August):1-12. doi:10.1093/eurheartj/ehy531.
7. Duncker DJ, Koller A, Merkus D, Canty JM. Regulation of Coronary Blood Flow in Health and Ischemic Heart Disease. *Prog Cardiovasc Dis*. 2015;57(5):409-422. doi:10.1016/j.pcad.2014.12.002.
8. Frangiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. *J Clin Invest*. 2017;127(5):1600-1612. doi:10.1172/JCI87491.

9. Jarvelainen, Sainio A, Koulu M, Wight TN PR. Extracellular Matrix Molecules: Potential Targets in Pharmacotherapy. *Pharmacol Rev.* 2009;61(2):198-223. doi:10.1124/pr.109.001289.provided.
10. Barascuk N, Vassiliadis E, Zheng Q, et al. Levels of Circulating MMCN-151, a Degradation Product of Mimecan, Reflect Pathological Extracellular Matrix Remodeling in Apolipoprotein E Knockout Mice. *Biomark Insights.* 2011;6:97-106. doi:10.4137/BMI.S7777.
11. Schwartzkopff B, Motz W, Frenzel H, Vogt M, Knauer S, Strauer BE. Structural and functional alterations of the intramyocardial coronary arterioles in patients with arterial hypertension. *Circulation.* 1993;88(3):993-1003. doi:10.1161/01.CIR.88.3.993.
12. Crea F, Noel Bairey Merz C, Beltrame JF, et al. The parallel tales of microvascular angina and heart failure with preserved ejection fraction: a paradigm shift. *Eur Heart J.* 2017;38:473-477. doi:10.1093/eurheartj/ehw461.
13. Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenes Tissue Repair.* 2012;5(1):1-13.
14. de Boer RA, De Keulenaer G, Bauersachs J, et al. Towards better definition, quantification and treatment of fibrosis in heart failure. A scientific roadmap by the Committee of Translational Research of the Heart Failure Association (HFA) of the European Society of Cardiology. *Eur J Heart Fail.* 2019;21(3):272-285. doi:10.1002/ejhf.1406.
15. Jenkins G, Saini G, Rgn B, et al. Longitudinal change in collagen degradation biomarkers in idiopathic pulmonary fibrosis: an analysis from the prospective, multicentre PROFILE study. *Lancet Respir Med.* 2015;3:462-472. doi:10.1016/S2213-2600(15)00048-X.
16. Genovese F, Karsdal MA, Leeming DJ, Scholze A, Tepel M. Association of Versican Turnover with All-Cause Mortality in Patients on Haemodialysis. *PLoS One.* 2014;9(10):111134. doi:10.1371/journal.pone.0111134.
17. Prescott E, Abildstrøm SZ, Aziz A, et al. Improving diagnosis and treatment of women with angina pectoris and microvascular disease: The iPOWER study design and rationale. *Am Heart J.* 2014;167(4):452-458. doi:10.1016/j.ahj.2014.01.003.
18. Schnohr P, Jensen G, Lange P, Scharling H, Appleyard M. The Copenhagen City Heart Study. Purpose of the Copenhagen City Heart Study. *Eur Hear J Suppl.* 2001;3(Suppl H):1-83.
19. Pilemann-Lyberg S, Rasmussen DGK, Hansen TW, et al. Markers of collagen formation and degradation reflect renal function and predict adverse outcomes in patients with type 1 diabetes. *Diabetes Care.* 2019;42(9):1760-1768. doi:10.2337/dc18-2599.
20. Rasmussen DGK, Hansen TW, Von Scholten BJ, et al. Higher collagen VI formation is associated with all-cause mortality in patients with type 2 diabetes and microalbuminuria. *Diabetes Care.* 2018;41(7):1493-1500. doi:10.2337/dc17-2392.
21. Julio A Chirinos , Lei Zhao , Federica Genovese , Mary Ellen Cvijic , Michael Basso , Alexander Reese-Petersen , Melissa Yarde , Zhaoqing Wang , Dietmar SJulio A Chirinos , Lei Zhao , Federica Genovese , Mary Ellen Cvijic , Michael Basso , Alexander Reese TPC. Abstract 14508: High Collagen Type VI

- Formation is a Strong Independent Predictor of Poor Outcomes in Heart Failure With Preserved Ejection Fraction: The TOPCAT Trial. In: *Circulation*. ; 2019:Vol 140, suppl 1.
22. Nielsen MJ, Kazankov K, Leeming DJ, et al. Markers of collagen remodeling detect clinically significant fibrosis in chronic hepatitis C patients. *PLoS One*. 2015;10(9):1-12. doi:10.1371/journal.pone.0137302.
 23. Nielsen SH, Tengryd C, Edsfeldt A, et al. Markers of basement membrane remodeling are associated with higher mortality in patients with known atherosclerosis. *J Am Hear Assoc*. 2018;7(21):1-10. doi:10.1161/JAHA.118.009193.
 24. Hein S, Scholz D, Fujitani N. Altered expression of titin and contractile proteins in failing human myocardium. *J Mol Cell Cardiol*. 1994;26:1291-1306.
 25. Franssen C, González Miqueo A. The role of titin and extracellular matrix remodelling in heart failure with preserved ejection fraction. *Netherlands Hear J*. 2016;24(4):259-267. doi:10.1007/s12471-016-0812-z.
 26. Kostin S, Hein S, Arnon E, Scholz D, Schaper J. *The Cytoskeleton and Related Proteins in the Human Failing Heart*. Vol 5.; 2000.
 27. Vassiliadis E, Rasmussen LM, Byrjalsen I, et al. Clinical evaluation of a matrix metalloproteinase-12 cleaved fragment of titin as a cardiovascular serological biomarker. *J Transl Med*. 2012;10(1). doi:10.1186/1479-5876-10-140.
 28. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. doi:10.1093/ehjci/jew082.
 29. Nielsen SH, Mygind ND, Michelsen MM, et al. Accelerated collagen turnover in women with angina pectoris without obstructive coronary artery disease: An iPOWER substudy. *Eur J Prev Cardiol*. 2018;25(7):719-727. doi:10.1177/2047487318758750.
 30. Nielsen SH, Mygind ND, Michelsen MM, et al. Accelerated collagen turnover in women with angina pectoris without obstructive coronary artery disease: An iPOWER substudy. *Eur J Prev Cardiol*. 2018:204748731875875. doi:10.1177/2047487318758750.
 31. Mygind, Naja Dam; Michelsen, Marie Mide; Pena, Adam; Wayyum, Abbas Ali; Frestad, Daria; Christensen, Thomas Emil, Ghotbi Adam Ali; Dose, Nynne; Faber, Rebekka; Vejlstrup, Niels; Hasbak, Philip; Kjaer, Andreas; Prescott E. Coronary microvascular function and myocardial fibrosis in women with angina pectoris and no obstructive coronary artery disease: the iPOWER study. *J Cardiovasc Magn Reson*. 2016;18(76):1-12. doi:10.1186/s12968-016-0295-5.
 32. Lindsey ML, Iyer RP, Zamilpa R, et al. A Novel Collagen Matricryptin Reduces Left Ventricular Dilation Post-Myocardial Infarction by Promoting Scar Formation and Angiogenesis. *J Am Coll Cardiol*. 2015;66(12):1364-1374. doi:10.1016/j.jacc.2015.07.035.
 33. Asbun J, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *J Am Coll Cardiol*. 2006;47(4):693-700. doi:10.1016/j.jacc.2005.09.050.

34. Leeming DJ, Nielsen MJ, Dai Y, et al. Enzyme-linked immunosorbent serum assay specific for the 7S domain of Collagen Type IV (P4NP 7S): A marker related to the extracellular matrix remodeling during liver fibrogenesis. *Hepatol Res.* 2012;42(5):482-493.
35. Veidal SS, Karsdal MA, Vassiliadis E, et al. MMP mediated degradation of type VI collagen is highly associated with liver Fibrosis - Identification and validation of a novel biochemical marker assay. *PLoS One.* 2011;6(9):1-9. doi:10.1371/journal.pone.0024753.
36. Shah SJ, Kitzman DW, Borlaug BA, et al. Phenotype-specific treatment of heart failure with preserved ejection fraction. *Circulation.* 2016;134(1):73-90. doi:10.1161/CIRCULATIONAHA.116.021884.
37. Paulus WJ, Tschöpe C. A Novel Paradigm for Heart Failure With Preserved Ejection Fraction Comorbidities Drive Myocardial Dysfunction and Remodeling Through Coronary Microvascular Endothelial Inflammation. *J Am Coll Cardiol.* 2013;62(4):263-271. doi:10.1016/j.jacc.2013.02.092.
38. Michalska-Kasiczak M, Bielecka-Dabrowa A, Von Haehling S, Anker SD, Rysz J, Banach M. Biomarkers, myocardial fibrosis and co-morbidities in heart failure with preserved ejection fraction: An overview. *Arch Med Sci.* 2018;14(4):890-909. doi:10.5114/aoms.2018.76279.
39. Carlson R V, Boyd KM, Webb DJ. The revision of the Declaration of Helsinki: past, present and future. *Br J Clin Pharmacol.* 2004;57(6):695-713. doi:10.1111/j.1365-2125.2004.02103.x.

Tables

Table 1. Overview of measured biomarkers representing bone and tissue turnover

Biomarker	Measures	Reference
Formation Biomarkers		
PRO-C4	Internal epitope in the 7S domain of type IV collagen	36
PRO-C6	C-terminal of released C5 domain of type VI collagen α 3 chain	42
Degradation Biomarkers		
C4M	MMP-2,9,12 mediated degradation of type IV collagen alpha 1 chain	43
C5M	MMP-2,9 mediated degradation of type V collagen	44
C6M	MMP-2 mediated degradation of type VI collagen	45
MIM	MMP-9,12 mediated degradation of mimecan	12
TIM	MMP-12 mediated degradation of titin	30
MMP: Matrix-metalloproteinase.		

Table 2. Baseline variables across groups.

Variable	iPOWER+DM n=187	iPOWER-DM n=157	Controls n=76	P- value*
Self-reported data				
Hypertension, n (%)	141 (75.8)	85 (54.5)a	14 (20.6)b, c	<0.001
Hyperlipidemia, n (%)	153 (82.7)	98 (63.2) a	16 (23.5)b, c	<0.001
Family history of IHD, n (%)	100 (56.8)	108 (69.7)a	18 (26.5) b, c	0.002
Smoking (current), n (%)	20 (10.9)	33 (21.0)	7 (10.1)	0.695
Peripheral vascular disease, n (%)	24 (13.0)	26 (16.8)	5 (7.2)	0.611
Clinical assessment				
Age (years)	64.4 (9.1)	61.7 (9.9)a	62.1 (8.8)	0.023
BMI (kg/m ²), median (IQR)	29.8 (26.4; 34.6)	25.1 (22.8; 28.9) a	24.4 (22.3; 26.4) b	<0.001
Systolic blood pressure (mmHg)	135.8 (21.8)	128.6 (19.3) a	115.7 (16.8) b, c	<0.001
Diastolic blood pressure (mmHg)	72.3 (19.8)	68.0 (12.3) a	60.1 (8.2) b, c	<0.001
Heart rate (bpm)	74.3 (12.1)	68.5 (9.8) a	69.6 (12.2) b	<0.001
Atheromatosis at CAG, n (%)	90 (50.8)	56 (35.9)	N/A	0.037
Laboratory tests				
Total cholesterol (mmol/L)	4.4 (1.0)	5.0 (1.1) a	5.4 (0.8) b, c	<0.001
HDL cholesterol (mmol/L)	1.4 (0.5)	1.7 (0.6) a	1.9 (0.5) b	<0.001
LDL cholesterol (mmol/L)	2.2 (0.9)	2.8 (1.0) a	3.0 (0.8) b	<0.001
Triglycerides (mmol/L)	1.7 (0.9)	1.2 (0.7) a	1.0 (0.5) b	<0.001
HbA1c (mmol/mol)	52.6 (12.6)	37.1 (4.2) a	36.7 (3.2) b	<0.001
Medical treatment				
Statin, n (%)	141 (75.4)	73 (46.8) a	9 (11.8) b, c	<0.001
Beta blockers, n (%)	65 (34.8)	49 (31.4)	2 (2.6) b, c	<0.012
Calcium antagonist, n (%)	58 (31.0)	40 (25.6)	6 (7.9) b, c	<0.068
ACE-inhibitor / ARB, n (%)	108 (58.7)	48 (31.0) a	10 (13.3) b, c	<0.001

Aspirin, n (%)	111 (59.4)	68 (43.6) a	1 (1.3) b, c	<0.001
----------------	------------	-------------	--------------	--------

*P value from age-adjusted trend test (linear and logistic regression). ACE-inhibitor / ARB: angiotensin converting enzyme-inhibitor/angiotensin II receptor blocker. CAG: coronary angiography. IHD: ischaemic heart disease. a p<0.05 for comparison with +DM, b p<0.05 for comparison with +DM, c p<0.05 for comparison with -DM. Pairwise comparisons are Bonferroni corrected.

Table 3. Echocardiographic variables across groups

Variable	iPOWER+DM	iPOWER-DM	Controls	P-value*
mean (SD) or otherwise indicated	n=187	n=157	n=76	
CFV at rest (m/s), median (IQR)	0.24 (0.20; 0.31)	0.23 (0.19; 0.29)	0.21 (0.19; 0.25) ^{b,c}	0.001
CFV at stress (m/s), median (IQR)	0.54 (0.44; 0.63)	0.56 (0.47; 0.68)	0.53 (0.47; 0.62)	0.764
CFVR, median (IQR)	2.21 (1.89; 2.55)	2.35 (1.96; 2.77) ^a	2.63 (2.19; 2.95) ^b	<0.001
Low CFVR (<=2.0), n (%)	63 (33.7)	45 (28.7)	13 (17.1) ^b	0.016
LVEF at rest (%)	57.4 (6.3)	58.9 (6.0)	56.0 (4.4) ^c	0.506
LVEF at stress (%)	62.7 (5.6)	63.6 (6.3)	59.8 (4.6) ^{b, c}	0.010
GLS end-systolic at rest (%)	20.2 (2.7)	21.3 (3.2) ^a	21.2 (2.8) ^c	0.007
GLS end-systolic at stress (%)	22.5 (2.8)	23.6 (3.1) ^a	23.7 (3.6) ^c	0.005
Left atrium volume index (mL/m ²)	28.1 (8.4)	28.2 (8.6)	23.4 (6.3) ^{b, c}	<0.001
E/e'	9.7 (3.8)	7.8 (2.4) ^a	7.9 (2.7) ^b	<0.001
e' lateral wall (cm/sec)	8.7 (2.5)	10.0 (2.8) ^a	10.3 (2.6) ^b	<0.001
Elevated filling pressure, n (%)	47 (29.2)	26 (17.7)	7 (10.8) ^b	<0.005

*P value from age-adjusted trend test (linear and logistic regression). a p<0.05 for comparison with +DM, b p<0.05 for comparison with +DM, c p<0.05 for comparison with -DM. Pairwise comparisons are Bonferroni corrected. CFV(R): coronary flow velocity (reserve). LVEF: left ventricular ejection fraction. GLS: global longitudinal strain. Filling pressure was considered as normal if E/e'<8 or E/e' was 8-12 and LAVI<34 mL/m² and high if E/e' was 8-12 and LAVI>34 or E/e' was >12

Figures

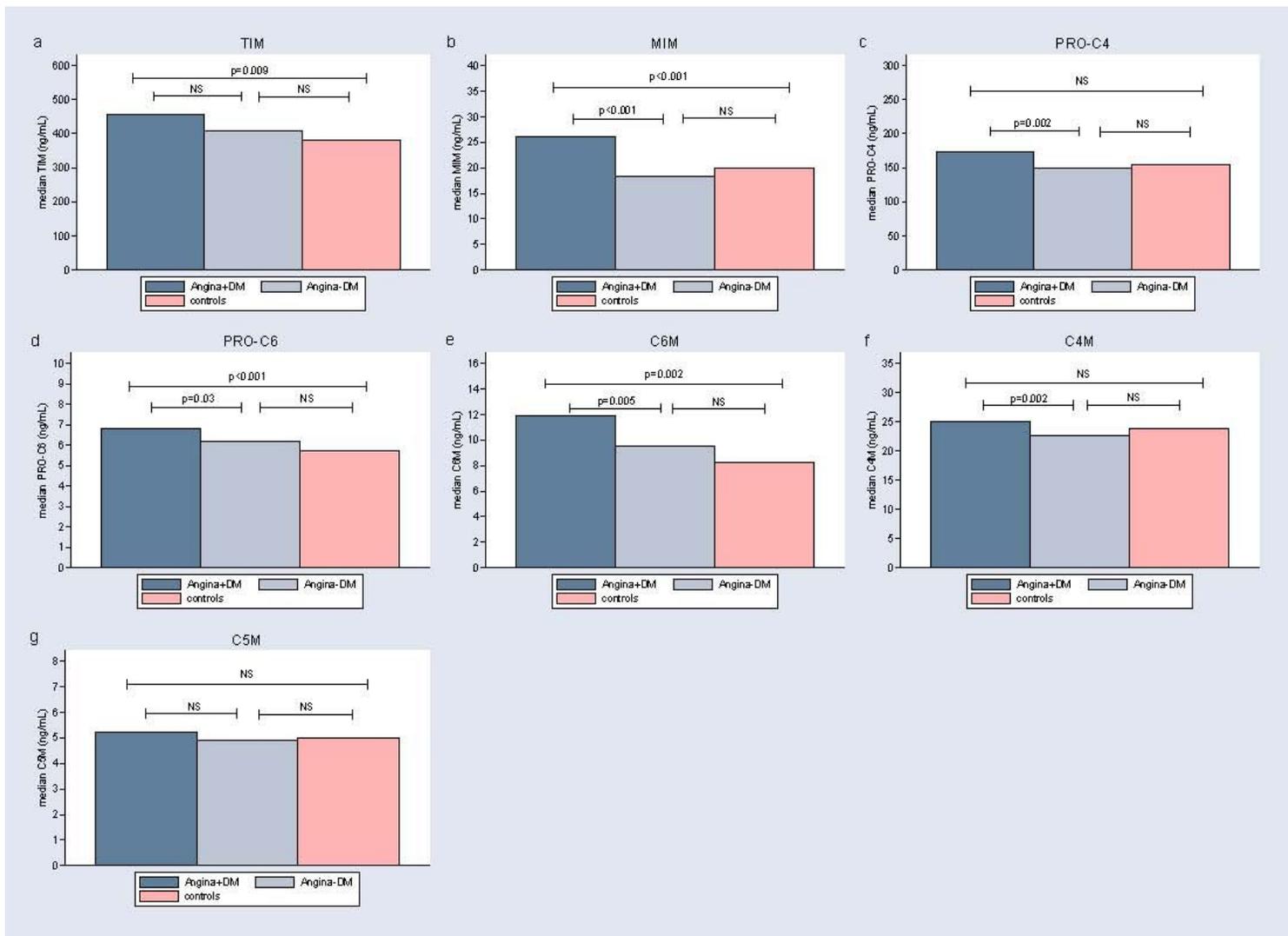


Figure 1

Figure 1

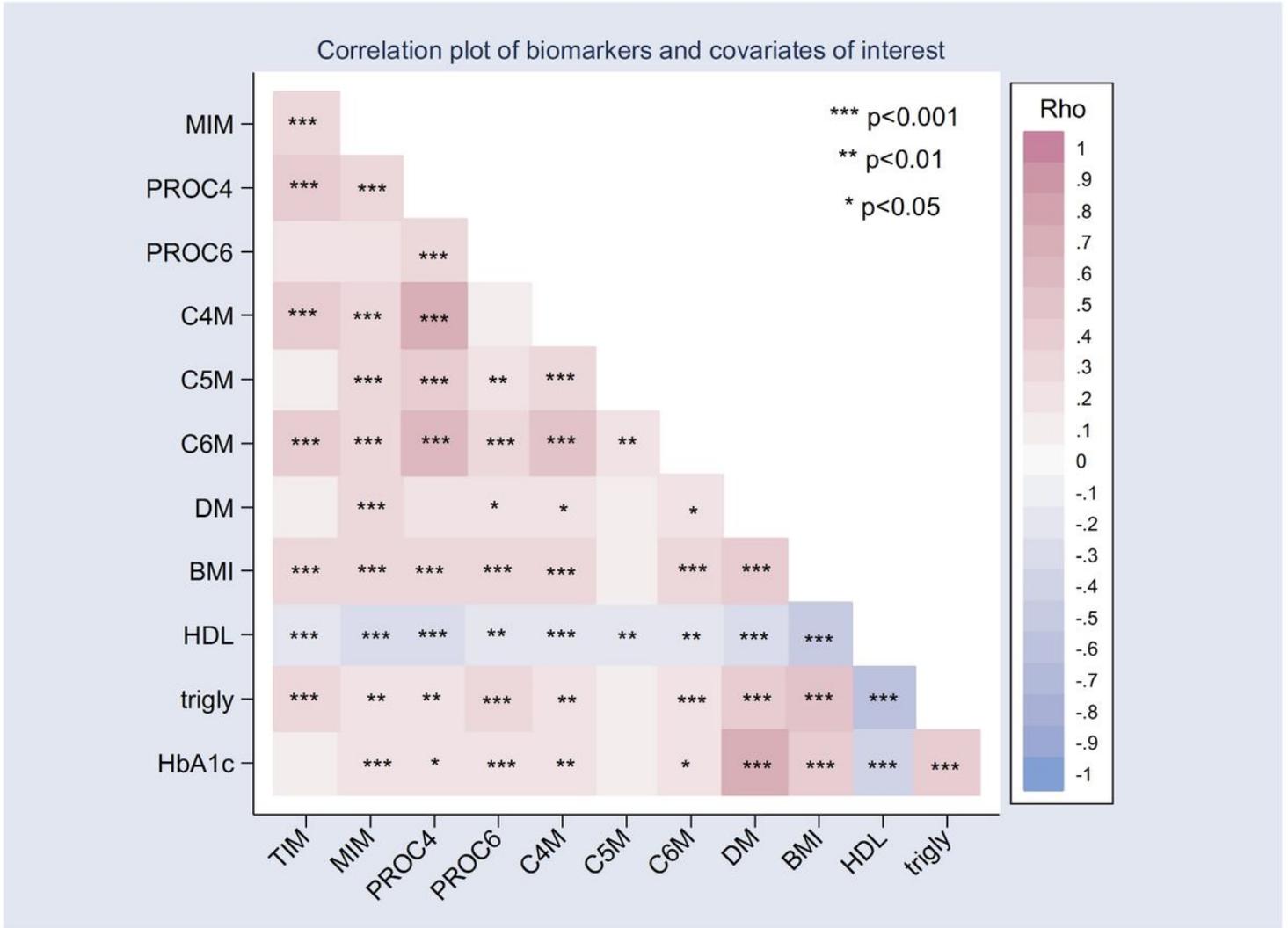


Figure 2

Figure 2

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [OnlineAppendix.pdf](#)
- [lettertoeditor.pdf](#)