Association between proteomic biomarkers and myocardial fibrosis measured by MRI: the multi-ethnic study of atherosclerosis

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Summary

Background Cardiac magnetic resonance imaging (CMR) determines the extent of interstitial fibrosis, measured by increased extracellular volume (ECV), and replacement fibrosis with late gadolinium myocardial enhancement (LGE). Despite advances in detection, the pathophysiology of subclinical myocardial fibrosis is incompletely understood. Targeted proteomic discovery technologies enable quantification of low abundance circulating proteins to elucidate cardiac fibrosis mechanisms.

Methods Using a cross-sectional design, we selected 92 LGE+ cases and 92 LGE– demographically matched controls from the Multi-Ethnic Study of Atherosclerosis. Similarly, we selected 156 cases from the highest ECV quartile and matched with 156 cases from the lowest quartile. The plasma serum proteome was analyzed using proximity extension assays to determine differential regulation of 92 proteins previously implicated with cardiovascular disease. Results were analyzed using volcano plots of statistical significance vs. magnitude of change and Bayesian additive regression tree (BART) models to determine importance.

Findings After adjusting for false discovery, higher ECV was significantly associated with 17 proteins. Using BART, Plasminogen activator inhibitor 1, Insulin-like growth factor-binding protein 1, and N-terminal pro-B-type natriuretic peptide were associated with higher ECV after accounting for other proteins and traditional cardiovascular risk factors. In contrast, no circulating proteins were associated with replacement fibrosis.

Interpretations Our results suggest unique circulating proteomic signatures associated with interstitial fibrosis emphasizing its systemic influences. With future validation, protein panels may identify patients who may develop interstitial fibrosis with progression to heart failure.

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Keywords: Myocardial fibrosis; Interstitial fibrosis; Replacement fibrosis; Proteomic; Biomarkers; Cardiac MRI

Introduction

The presence of myocardial fibrosis is an important structural abnormality preceding symptoms of heart failure (HF) and is incorporated into the universal definition to define stage B (subclinical) HF.[1] Cardiac magnetic resonance imaging (CMR) is a non-invasive imaging modality used to detect and quantify myocardial fibrosis.[2] There are two types of myocardial fibrosis

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Research in context

Evidence before this study

Heart failure (HF) is a common cardiac disease with a high burden on the health care economy and a high mortality rate. To implement an effective preventive strategy, it is important to detect individuals at risk for developing HF. One of the most important structural findings preceding symptoms is the presence of myocardial fibrosis. There are two types of myocardial fibrosis measured by cardiac magnetic resonance (CMR). First, replacement fibrosis, that can be detected by CMR as macroscopic regions using late gadolinium myocardial enhancement (LGE). Second, interstitial myocardial fibrosis that is characterized by an overall increase in myocardial extracellular volume (ECV). Different biologic mechanisms have been proposed to explain the pathophysiology of these two types of myocardial fibrosis.

We searched PubMed from January 1990, to September 2022, for relevant studies investigated the association between novel biomarkers and myocardial fibrosis. Search terms included: "proteomics and myocardial fibrosis", "proteomics and heart failure", "proteomics and cardiovascular disease", "biomarkers and myocardial fibrosis", "biomarkers and heart failure", "biomarkers and cardiovascular disease", "cardiac MRI and myocardial fibrosis", "myocardial fibrosis and heart failure" and "types of myocardial fibrosis". Previous studies have shown an association between inflammatory biomarkers and cardiac specific biomarkers with myocardial fibrosis. These prior observations, though important, highlight the limitations of a targeted approach with a limited number of selected biomarkers as measurement of multiple biomarkers using traditional laboratory methods is expensive and can require a large blood volume.

Added value of this study

Multi-ethnic study of atherosclerosis (MESA) is a prospective, population-based cohort study consisting of 6814 women and men, aged 45–84 years who were free of overt cardiovascular disease at enrollment. We aimed to conduct two parallel, demographically matched, cross-sectional case–control pilot studies testing the hypothesis that there would be differences in the systemic pathophysiologies of CMR-defined replacement and interstitial myocardial fibrosis versus controls in MESA. We have shown interstitial myocardial fibrosis has a different proteomic signature compared to matched controls. On the other hand, no circulating proteins were associated with replacement fibrosis.

Implications of all the available evidence

Our results suggest different mechanistic pathways involved in the formation of replacement and interstitial myocardial fibrosis. Further research is recommended with larger sample size and larger number of measured proteins to confirm and add additional mechanistic insights into both forms of cardiac fibrosis.

detected by CMR. Replacement fibrosis is the result of myocyte cell death from etiologies such as necrosis and apoptosis.[3] Subsequently, inflammation triggers fibroblasts to produce collagenous scar. These changes are detectable by CMR as macroscopic focal regions of increased extracellular space quantified by late gadolinium myocardial enhancement (LGE).[4] In contrast, interstitial myocardial fibrosis is a microscopic fibrosis due to increased extracellular matrix and characterized by an overall increase in myocardial extracellular volume (ECV) without the pre-requisite macroscopic myocyte cell loss.[4]

Different biologic mechanisms have been proposed to explain the pathophysiology of myocardial fibrosis.[5] Previous studies have shown an association between elevated level of circulating inflammatory biomarkers and myocardial fibrosis.[6,7] In addition to inflammatory biomarkers, cardiac specific biomarkers including high sensitivity cardiac troponin (hs-cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) have been associated with LGE-defined replacement fibrosis and increased ECV-defined interstitial fibrosis respectively.[8,9]

While insightful, these prior observations highlight the limitations of traditional targeted biomarker research. This strategy utilizes a limited number of selected circulating biomarkers to provide mechanistic insights into differentiating the multifactorial systemic processes potentially resulting in pre-clinical cardiac fibrosis. Recent advancements in targeted discovery proteomic technologies provide the opportunity to quantify concentrations of dozens to thousands of low abundance proteins using small sample volumes.[10] Using such an approach, we aimed to conduct two parallel, matched, cross-sectional case–control pilot studies testing the hypothesis that there would be differences in the systemic pathophysiologies of CMR-defined replacement and interstitial myocardial fibrosis versus controls reflected by their associations with 92 cardiovascular-related circulating proteins in community dwelling adults free of clinical manifestation of cardiovascular disease (CVD) participating in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods

Participant population and cardiac magnetic resonance imaging

The design of MESA has been published previously.[11] Briefly, MESA is a prospective, population-based cohort study consisting of 6814 women and men, aged

45–84 years who were free of overt CVD at enrollment (July 2000 and August 2002). Participants were recruited from six US field centers (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan, NY; and St Paul, MN) and self-identified their ethnicity as White, Black, Chinese American, and Hispanic. Standard questionnaires were used to collect demographic information, medical history, medication use, gross family income, and smoking status (current, former, or never smoker). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Resting blood pressure were measured three times in a seated position and the average of the last two were collected for data analysis. Blood glucose, total, and high-density lipoprotein (HDL) cholesterol were measured in the fasting blood samples. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or the use of any hypoglycemic medication.

The acquisition method of CMR images for replacement and interstitial fibrosis in MESA has previously been published.[12–14] Image acquisition for fibrosis assessment occurred at Exam 5 (April 2010–December 2011). Using 1.5 T scanner and the steady-state free precession pulse sequence, parameters of cardiac function and structure were measured. Gadolinium contrast enhanced CMR using LGE was performed among those without contraindications. Participants with an estimated glomerular filtration rate ≥45 mL/min/1.73 m^2 (≥60 mL/min/1.73 m^2 for the site at Northwestern University) and without history of allergic reaction to gadolinium were qualified to participate. LGE images were acquired 10–15 min after intravenous administration of 0.15 mmol/kg gadolinium–DTPA with breath held segmented inversion recovery sequence and acquired in the same orientations as the cine images. Inversion times were adjusted to null normal myocardium. Myocardial replacement fibrosis was defined as focal LGE either in 2 adjacent short axis slices or in one short axis and one long axis image at a corresponding location using Qmass (version 7.2, Medis).[12] For evaluation of interstitial fibrosis, 1 short axis pre-contrast modified look locker inversion recovery image at the mid slice position was acquired, repeated at 12 and 25 min after contrast injection. Interstitial myocardial fibrosis was quantified as percentage of total ECV. In MESA at exam 5 hematocrit was measured in 608 (45.5%) of the participants who underwent T1 mapping to calculate ECV. For the remainder a synthetic hematocrit was calculated that correlated closely with the measured hematocrit, and synthetic hematocrit was used to calculate myocardial ECV in those without a measured hematocrit. ECV and synthetic ECV showed high correlation.[13]

For this cross-sectional analysis, we identified participants who had undergone CMR with gadolinium at exam 5 (n = 1840) who were also free of known CVD and had stored plasma samples available for proteomic measurements (n = 1479). We applied a previously used MESA definition for interstitial fibrosis as the fourth quartile of ECV (ECVq4).[15] We randomly selected 156 cases from participants from the highest ECV quartile (ECVq4). We propensity-matched these cases to control subjects in the lowest ECV quartile (ECVq1), using 1:1 matching on a propensity score derived from age, gender, and race, with a matching caliper of 0.05. Consistent with previously published MESA studies we did not match by field centers in this study. All cases and controls were free of replacement fibrosis (LGE–). We also randomly selected 92 replacement fibrosis cases from (LGE+) and 92 matched controls (LGE–) using the same matching scheme as for ECV. Since fewer women were LGE+, we included all females with replacement fibrosis if otherwise qualified for this study.[15]

Proteomic measurements

For proteomic analysis, plasma samples drawn at the time of the MESA exam 5 encounter and stored at –80 °C. Samples (which previously underwent a single freeze-thaw) were sent to Olink (Watertown, Massachusetts) for analysis using the Olink Target 96 Cardiovascular III with 92 unique proteins. This panel was chosen based on prior work showing unique differentiation of proteins between patients with HF with reduced versus preserved left ventricular ejection fraction.[16] Reproducibility, and validation information regarding the proteins is reported by Olink (Olink Target 96 Cardiovascular panels - Olink; Accessed November 5th, 2022). A list of proteins included in the analysis are shown in Supplemental Table S1. No participants' samples were flagged for quality control issues, so all selected participants were included in the analysis. No protein level had ≥50% of samples less than the limit of detection (CHIT1 had the highest proportion with levels below the limit of detection at 5%).

Statistical analysis

Statistical analyses were performed using R statistical software version 4.2.2 (R Core Team, 2022), and R scripts are publicly available.[17] For comparing protein expression levels, demographic information, medical history, medication use, gross family income, and smoking status for ECVq1 vs. ECVq4 and LGE– vs. LGE+, Student's t-test was used to compare continuous variables and Fisher's exact test was used to compare categorical variables. Mean and standard deviations (SD) for continuous variables and proportions for categorical variables are also presented for each of the groups. For visual comparisons of protein expression levels and baseline characteristics of participants, side-by-side violin plots (Supplemental Figs. S1 and S2) and histograms (Supplemental Fig. S3) are presented to compare the distribution of protein expression levels for ECVq1 vs. ECVq4 and LGE– vs. LGE+. These Figures show a

mix of normal and near normal distribution. Given our fairly large sample size, the parametric test (t-test) provides appropriate inference. Additional sensitivity analysis statistical testing with the non-parametric Wilcoxon rank sum test was also performed for comparison.

Volcano plots are produced using the OLinkAnalyze R package (https://CRAN.R-project.org/package=OlinkAnalyze; Accessed November 5th, 2022) to visualize differences in protein expression levels across groups. The Benjamini-Hochberg procedure is used to assess the significance of differences in protein expression levels while controlling for the overall false discovery rate.[18] Differences in protein expression levels are categorized as significant after adjusting for multiple testing, significant without adjusting for multiple testing, and not significant.

To determine the significance of differences in protein expression levels while controlling for age, gender, race, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, use of lipid lowering and anti-hypertensive medications, cigarette smoking status, diabetes, estimated glomerular filtration rate (eGFR), ECV and gross family income (see Table 1), we use a Bayesian Additive Regression Tree (BART) modeling approach[19] implemented in the bartMachine R package.[20] All 92 proteins and covariates are included in the BART models used to identify group membership (ECVq1 vs. ECVq4 and LGE– vs. LGE+). BART has been shown to outperform competing models (LASSO, gradient boosting, neural nets, random forests) with respect to classification accuracy, accounts for complex interactions among proteins, and provides tools for variable selection.[19] Using BART, variable importance is measured by the proportion of splits over all regression trees including the variable, and a permutation-based test is used to assess the significance of differences in particular protein expression levels controlling for the effect of other proteins and covariates.

Pearson correlation was used to measure associations among pairs of the 92 proteins within each of the groups (ECVq1 vs. ECVq4 and LGE– vs. LGE+). To identify differences in associations among proteins between groups, the significance test of Chang et al.[21] was used to test for equality of the full 92 × 92 covariance matrices for ECVq1 vs. ECVq4 and LGE– vs. LGE+. For groups with significantly different protein expression covariances, hierarchical clustering was used for each group to identify and compare clusters of proteins that are highly positively correlated with respect to their expression levels using the dendextend[22] and WGCNA R packages.[23] Sankey plots are then presented to assess how protein clusterings vary across groups.

Ethics

All participants signed written consents and all study protocols were approved by the institutional review boards of each field center.[11]

	ECVq1 (N = 156)	ECVq4 (N = 156)	p-value
Age (years)	58 (8)	58 (9)	>0.90
Gender			0.14
Female	96 (62%)	83 (53%)	
Male	60 (38%)	73 (47%)	
Race			0.80
White	76 (49%)	74 (47%)	
Chinese American	17 (11%)	21 (13%)	
Black	38 (24%)	40 (26%)	
Hispanic	25 (16%)	21 (13%)	
BMI (kg/m^2)	29.6 (5.6)	27.3 (5.5)	<0.001
Systolic BP (mmHg)	124 (21)	118 (19)	0.022
Diastolic BP (mmHg)	68 (9)	67 (10)	0.70
Use of anti-hypertension Medication	77 (49%)	71 (46%)	0.50
Total Cholesterol (mg/dl)	179 (33)	181 (37)	0.70
HDL Cholesterol (mg/dl)	53 (14)	57 (18)	0.032
Use of lipid lowering medication	72 (46%)	53 (34%)	0.028
Cigarette smoking status			<0.001
Never	76 (49%)	50 (32%)	
Former	78 (50%)	86 (55%)	
Current	2 (1%)	20 (13%)	
Diabetes			0.03
Normal	89 (57%)	112 (72%)	
Impaired fasting glucose	35 (22%)	20 (13%)	
Diabetes	32 (21%)	24 (16%)	
eGFR (mL/min/1.73 m^2)	85 (14)	86 (15)	0.60
ECV (%)	23.5 (1.0)	30.3 (1.5)	<0.001
Gross family income			0.11
$0–$19,999	22 (15%)	20 (13%)	
$20,000–$49,999	63 (42%)	48 (31%)	
$50,000 or more	66 (44%)	85 (56%)	

Figures are numbers (%) and mean (standard deviation). BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; ECV, extracellular volume.

Table 1: **Baseline characteristics of participants without and with interstitial fibrosis [ECV quartile 1 (q1) vs. ECV quartile 4 (q4)].**

Role of funders

Funders had no role in study design, data collection, data analysis, interpretation or writing of the manuscript.

Results

Tables 1 and 2 show baseline characteristics of participants with ECVq1 vs. ECVq4 (low versus high interstitial fibrosis) and LGE– vs. LGE+ (without and with replacement fibrosis). By design, demographics were matched based on the type of fibrosis being assessed. Compared with participants with ECVq1, participants with ECVq4 had lower systolic blood pressure (SBP), lower body mass index (BMI), higher high-density lipoprotein (HDL) cholesterol, a lower prevalence of diabetes, but were more likely current or former smokers.

	LGE– (N = 92)	LGE+ (N = 92)	p-value
Age (years)	62 (10)	62 (10)	0.90
Sex			0.80
Female	17 (18%)	16 (17%)	
Male	75 (82%)	76 (83%)	
Race			>0.90
White	47 (51%)	47 (51%)	
Chinese American	1 (1%)	2 (2%)	
Black	29 (32%)	28 (30%)	
Hispanic	15 (16%)	15 (16%)	
BMI (kg/m^2)	27.9 (4.2)	28.7 (5.1)	0.20
Systolic BP (mmHg)	122 (18)	127 (18)	0.078
Diastolic BP (mmHg)	70 (10)	72 (10)	0.08
Use of anti-hypertension Medication	51 (55%)	54 (59%)	0.70
Total Cholesterol (mg/dl)	172 (33)	179 (34)	0.20
HDL Cholesterol (mg/dl)	49 (13)	52 (13)	0.20
Use of lipid lowering medication	36 (39%)	32 (35%)	0.50
Cigarette smoking status			0.12
Never	26 (28%)	34 (37%)	
Former	58 (63%)	44 (48%)	
Current	8 (9%)	13 (14%)	
Diabetes			0.50
Normal	59 (64%)	49 (53%)	
Impaired fasting glucose	19 (21%)	27 (29%)	
Diabetes	14 (15%)	16 (17%)	
eGFR (mL/min/1.73m^2)	82 (12)	81 (15)	0.4
Gross family income			>0.90
$0–$19,999	12 (13%)	12 (13%)	
$20,000–$49,999	30 (33%)	32 (36%)	
$50,000 or more	48 (53%)	46 (51%)	

Figures are numbers (%) and mean (standard deviation). BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; ECV, extracellular volume.

Table 2: **Baseline characteristics without and with replacement fibrosis (LGE– and LGE+).**

Compared to LGE– participants, LGE+ were not different with respect to CVD risk factors. In a sensitivity analysis using nonparametric Wilcoxon rank sum test for all continuous variables (Supplemental Tables S4 and S5) p-values varied slightly, but significant findings remain unchanged with one exception (p-value for variable HDL cholesterol: 0.032 vs. 0.09 for t-test vs. rank sum test respectively).

There were 13 circulating proteins with levels that were lower and 4 proteins that were higher in participants with high versus low interstitial fibrosis controlling the overall false discovery rate at 5% as shown in the volcano plot in Fig. 1a. Proteins with lower levels included plasminogen activator inhibitor 1 (PAI), Cathepsin D (CTSD), Low-density lipoprotein (LDL) receptor, Fatty acid-binding protein 4 (FABP4), Bleomycin (BLM) hydrolase, Proprotein convertase subtilisin/kexin type 9 (PCSK9), Tissue-type plasminogen activator (t-PA), E-selectin (SELE), Retinoic acid receptor responder protein 2 (RARRES2), Cathepsin Z (CTSZ), Interleukin-1 receptor type 2 (IL-1RT2), Integrin b2 (ITGB2), and Scavenger receptor cysteine-rich type 1 protein M130 (CD163). Proteins with higher levels included Insulin-like growth factor-binding protein (IGFBP)-1, IGFBP-2, NT-proBNP, and Serum paraoxonase/lactonase 3 (PON3). In Supplemental Figs. S1 and S2, violin plots are shown for all 92 proteins to visually compare the distributions in expression levels for ECVq1 vs. ECVq4 and LGE– vs. LGE+ respectively.

The importance plot (Fig. 2a) shows the relative importance of the top 20 proteins for identifying participants with a lowest versus a highest quartile volume of interstitial fibrosis. The top three proteins, PAI, IGFBP-1 and NT-proBNP, are found to be significantly associated with a low versus high ECV while controlling for other proteins and CVD risk factors using the Bayesian additive regression tree (BART) model ($p = 0.010$).

We next performed a similar analysis with the cases and controls for replacement fibrosis. No protein levels were found to be different between participants who were LGE– vs. LGE+ controlling the overall false discovery rate at 5% (volcano plot, Fig. 1b). An importance plot analysis shows the relative importance of top 20 proteins for identifying participants with replacement fibrosis (Fig. 2b). However, even the two most important proteins, NT-proBNP and IGFBP-1, are not significantly associated with replacement fibrosis while controlling for other proteins and CVD risk factors using BART ($p = 0.053$), consistent with the individual protein analysis results. In a sensitivity analysis using the nonparametric Wilcoxon rank sum test to create the volcano plots (Supplemental Figs. S4 and S5) 16 of the 17 proteins remained statistically different between ECV groups with only the protein CTSZ falling just below the threshold for significance. The top three proteins (PAI, IGFBP-1, and NT-proBNP) evaluated further using the Bayesian Additive Regression Tree approach remain the most significant for both parametric and non-parametric statistical testing.

Lastly, we utilized hierarchical clustering to determine if protein clusters were different between those with low versus elevated myocardial ECV and those without and with replacement fibrosis. Consistent with our findings based on the individual protein analysis, we identified significantly different covariances among the 92 proteins in those with a low versus a high volume of interstitial fibrosis ($p = 0.04$) and not between those without versus with replacement fibrosis ($p = 0.67$). Fig. 3a and b show visualization by hierarchical clustering of participants with a low and high myocardial ECV respectively. The individual proteins that were significantly different in the volcano plot (Fig. 1a) after correction for false discovery are shown in red asterisk.

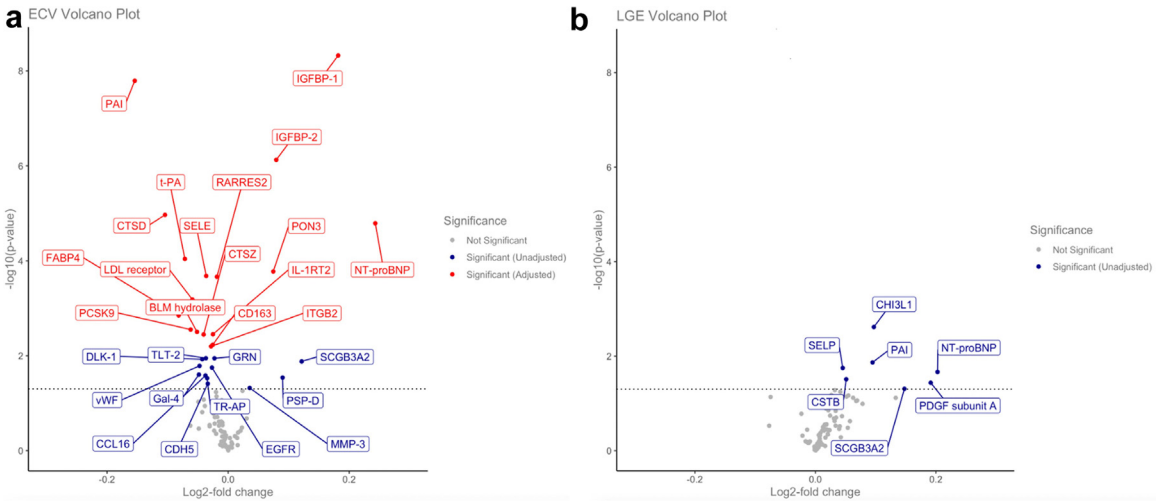

Fig. 1: Volcano plots for the proteins of interest in interstitial (a) and replacement (b) fibrosis respectively. In the analysis of proteins of interstitial fibrosis, 17 proteins of interest were identified, including plasminogen activator inhibitor 1 (PAI), Cathepsin D (CTSD), Low-density lipoprotein (LDL) receptor, Fatty acid-binding protein 4 (FABP4), Bleomycin (BLM) hydrolase, Proprotein convertase subtilisin/kexin type 9 (PCSK9), Tissue-type plasminogen activator (t-PA), E-selectin (SELE), Retinoic acid receptor responder protein 2 (RARRES2), Cathepsin Z (CTSZ), Interleukin-1 receptor type 2 (IL-1RT2), Integrin b2 (ITGB2), and Scavenger receptor cysteine-rich type 1 protein M130 (CD163). Proteins with higher levels included Insulin-like growth factor-binding protein (IGFBP)-1, IGFBP-2, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and Serum paraoxonase/lactonase 3 (PON3). The full names of proteins that were statistically significant only prior to adjustment for false discovery rate can be found in Supplemental Table S1. Conversely, no proteins were identified as significantly different in levels based on the presence or absence of replacement fibrosis as shown in the volcano plot after adjusting for multiple testing.

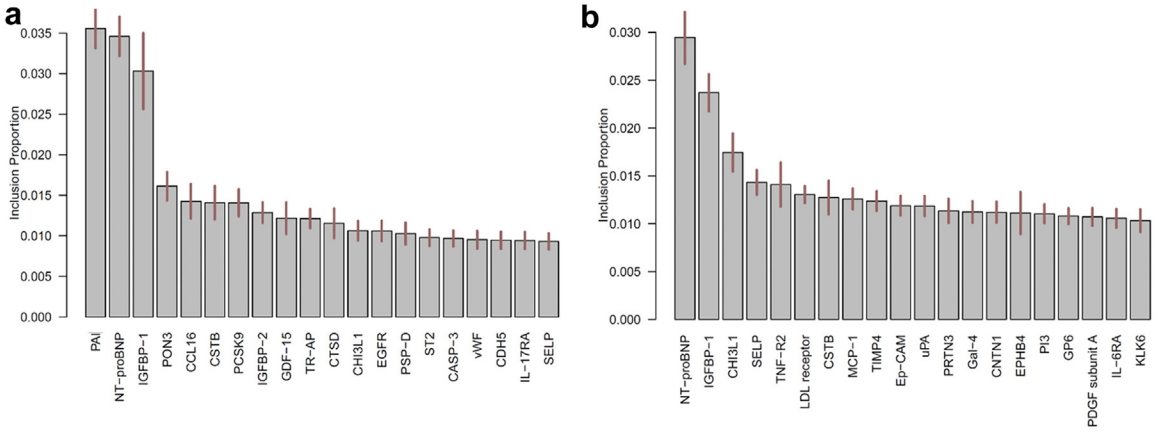

Fig. 2: Importance plots of the top 20 proteins associated with interstitial (a) and replacement (b) fibrosis. In the analysis of interstitial fibrosis, only three of the 20 proteins were found to be statistically significant predictors of disease after accounting for demographics and traditional cardiovascular disease risk factors (see statistical methods for variables). These proteins include plasminogen activator inhibitor (PAI), insulin-like growth factor binding protein 1 (IGFBP-1), and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Similar to the volcano plot analysis, no proteins were determined to be of statistical importance when accounting for demographics and cardiovascular disease risk factors with replacement fibrosis.

To provide a qualitative visual assessment of the differences in clustering between ECVq1 and ECVq4, we divided the 92 proteins into 5 major clusters separately for ECVq1 and ECVq4. The Sankey plot (Fig. 3c) illustrates that the majority of proteins remain clustered together for ECVq1 and ECVq4 participants, but there are some proteins that shift into different clusters. Supplemental Fig. S6 provides additional visualizations to compare the hierarchical clustering of participants with ECVq1 vs. ECVq4.

Discussion

In this cross-sectional study of demographically matched cases and controls in individuals without

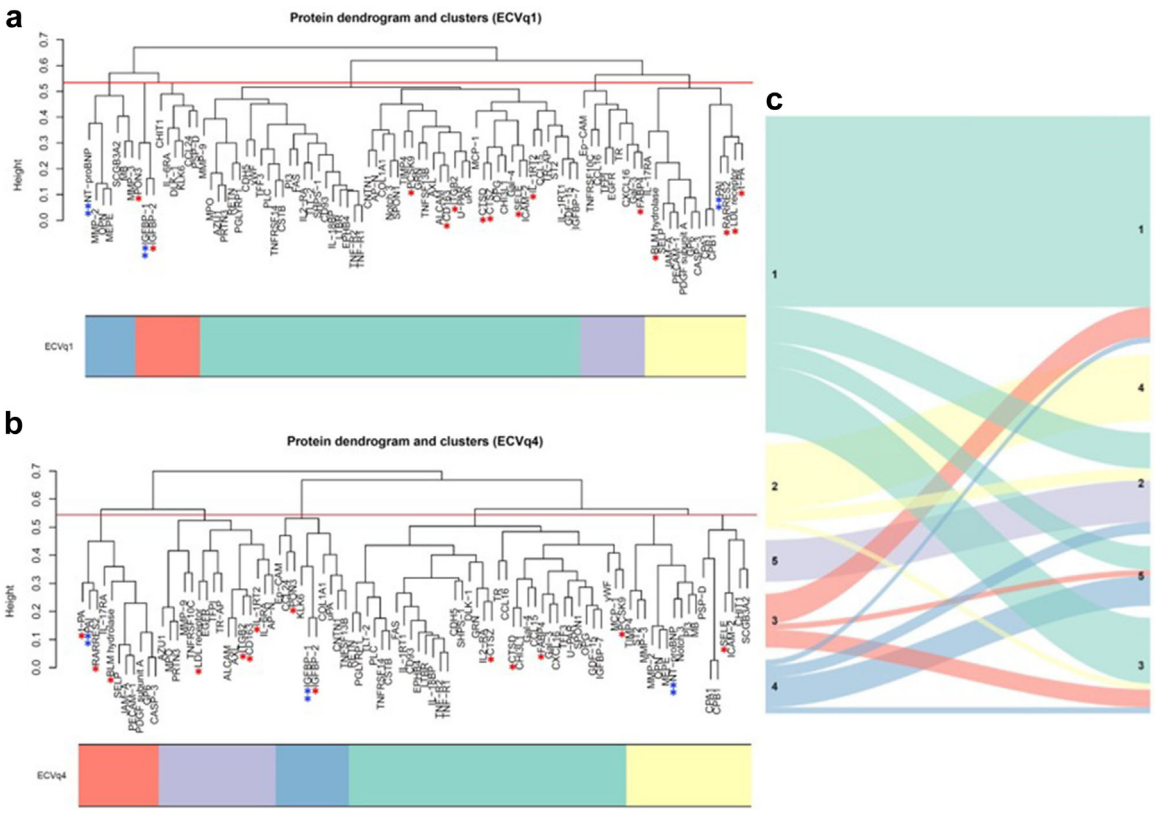

Fig. 3: Hierarchical clustering results of protein groupings for interstitial fibrosis for those without (a, ECVq1) and with (b, ECVq4) interstitial fibrosis. The clusters for interstitial fibrosis included proteins that were also found to be statistically significant in the volcano plot analysis noted with a red asterisk and 3 proteins that remained statistically significant using Bayesian additive regression tree noted with a blue asterisk. A total of 5 clusters were created for ECVq1 and shown as the color bar at the bottom of Fig. 3a. Redistribution of the 5 clusters for ECVq4 are shown in the color bar at the bottom of Fig. 3b. In the Sankey plot (c), the same cluster color scheme is used and a shift or absence of a shift in proteins to different clusters is qualitatively shown between those with and without interstitial fibrosis.

known CVD, we investigated the association of 92 circulating proteins associated with CVD with both interstitial and replacement fibrosis assessed by CMR. Although these proteomics methods have previously been implemented to study the pathophysiology of HF, coronary artery disease and preclinical phenotypes of HF,[24–26] our study is among the first to show differentially regulated proteins in CMR imaged myocardial fibrosis using targeted PEA proteomics in a population-based study. Previous studies have focused on identifying the individual proteins associated with the myocardial fibrosis, but not a comprehensive analysis of the plasma proteome in a community-based study sample or have focused on characterizing the proteome for only one form of myocardial fibrosis.[27–29]

We demonstrate that there are multiple unique circulating proteins associated with a greater myocardial extracellular volume related to interstitial fibrosis, with a variety of potential mechanisms, but are in large part focused on inflammatory mechanisms of action supporting pre-clinical research on the importance of a systemic inflammatory milieu for the development of cardiac fibrosis.[5,6] Potential mechanisms of the 17 differentiated proteins between ECVq1 and ECVq4 are shown in Supplemental Table S2. There has been substantial interest in identifying clinical phenotypes of populations at risk for cardiac fibrosis and subsequent symptomatic HF using proteomics.[26] Our cross-sectional data linking preclinical interstitial fibrosis imaging findings with a potential proteomic signature lends support to such an approach. In contrast, the absence of an association of the same proteins with replacement fibrosis compared to matched controls identifies that myocyte cell loss with subsequent macroscopic "scar" in the absence of known myocardial infarction is mediated through different mechanisms than its interstitial fibrosis counterpart. While our 92 proteins only represent a portion of the total circulating proteome associated with CVD and an inflammatory response, our findings suggest that a systemic inflammatory response plays a lesser or different role in replacement fibrosis versus interstitial fibrosis.

Interstitial fibrosis is seen with aging and in response to stressors such as hypertension.[30] In this type of fibrosis, the extracellular volume is increased because of deposition of more extracellular matrix (ECM). One key finding of our study was the presence of differentially regulated circulating proteins representing subclinical inflammation, and other additional CVD mechanisms, supporting the concept that interstitial fibrosis is driven by a host of heterogeneous systemic processes. This is reflected by the diversity of unique proteins with multiple roles in cardiac physiology (see Supplemental Table S2) and provides support for this form of fibrosis often being associated with heart failure with preserved ejection fraction, a disorder typically associated with multiple systemic pathophysiologies.[31,32] Proteins were found to be differentially regulated despite controlling for demographics such as advanced age and female gender known to be associated with interstitial fibrosis in the same cohort.[13] Importantly, after accounting for traditional cardiovascular risk factors and other circulating proteins in our BART analysis, several of the circulating proteins including NT-proBNP, IGFBP-1, PAI remained the most important factors significantly associated with interstitial fibrosis.

Of the 3 proteins found to be important in our BART analysis of high interstitial fibrosis volume (ECVq4), NT-proBNP and IGFBP-1 were upregulated, and PAI was downregulated. NT-proBNP has been extensively studied as a marker of myocardial stretch and wall tension.[33] However, NT-proBNP also has been implicated in myocardial fibrosis. Liu et al. in a cross-sectional study of 1334 MESA participants have shown the association between NT-proBNP and interstitial fibrosis detected by CMR T1 mapping indices including ECV.[9] Increased NT-proBNP, which represents the biologically inert amino terminal portion of BNP with longer half-life, likely reflects a counter-reactive response to pro-fibrotic stimulation by cardiac production of BNP which has antifibrotic effects.[34,35]

While not widely studied in myocardial fibrosis, IGFBP-1 and IGFBP-2 have been studied in idiopathic pulmonary fibrosis (IPF). In IPF, compared to healthy controls, IGFBP-2 and to a lesser extent IGFBP-1 have been found to be increased.[36,37] In patients treated with antifibrotic therapy, IGFBP-2 decreased from baseline but still remained significantly elevated relative to healthy controls.[36] IGFBP-1 and -2 may be increased in the interstitial fibrosis cases as IGFBPs in general bind strongly to insulin-like growth factor (IGF) to either increase half-life or alter function.[36] Although it was not measured as part of the PEA panel used in this analysis, IGF-1 and -2 have previously been found to attenuate cardiac fibrosis in animal models.[38,39] These findings suggest elevated IGFBP-1 and -2 may prevent the antifibrotic activities of IGF in interstitial fibrosis, whereas this same association is not observed in replacement fibrosis. Of note, a different insulin-like growth factor binding protein, IGFBP-7, has been previously indicated in activating fibrotic mechanisms and plays a role in fibrosis and diastolic dysfunction.[40]

Plasminogen activator inhibitor 1, a serine protease inhibitor, is also implicated in the pathophysiology of fibrosis formation in different tissues.[41] It has been found that PAI-1 inhibits urokinase plasminogen activator and tissue plasminogen activator 1. This inhibition affects downstream ECM remodeling preventing fibrosis.[41] While there are conflicting views on the role of PAI-1 in pro- and anti-fibrotic functions, it is believed in myocardial fibrosis elevated PAI-1 prevents progression to fibrosis.[41,42] In animal models of reactive fibrosis associated with aging, hypertension, and dilated cardiomyopathy, elevated PAI-1 was found to reduce cardiac fibrosis.[43,44] Lower levels of PAI-1 have been suggested to alter signaling which would otherwise prevent collagen deposition in the ECM.[41] This is in alignment with our findings as higher myocardial ECV reflecting increased interstitial fibrosis was associated with lowered PAI-1.

Interestingly, in our case–control where we matched for demographic factors known to be strongly associated with increased ECV in the MESA cohort, other risk factors such as higher blood pressure, higher BMI and impaired fasting glucose/diabetes were significantly greater in the ECVq1 controls than the ECVq4 cases. This suggests that differences in proteins associated with interstitial fibrosis were not just driven by a greater prevalence of traditional risk factor phenotypes associated with systemic inflammation and interstitial fibrosis but may point to unique mechanisms. While caution is required for null findings in this modestly sized pilot study, it is of interest that key circulating proteins, including galectin-3, soluble ST2, and growth differentiation factor-15 in which elevated levels have been associated with fibrosis in animal models and long-term prognosis in participants with and without known CVD where not among the proteins that were significantly different after correction for false discovery between those with a low versus a high volume of interstitial cardiac fibrosis.[45–50] Whether this may be related to the analytical properties of the assays, the population studied, or study design will need to be determined. Another interesting aspect of identifying a unique proteomic pattern for interstitial fibrosis is the recognition of the reversible nature of interstitial fibrosis whether it be with relief of pressure overload with aortic valve replacement or in symptomatic patients with HF as demonstrated that treatment with a sodium-glucose transport protein 2 (SGLT2) inhibitor results in a decreased ECV.[51,52] These findings suggest that proteomic expression patterns may provide suitable targets for primary prevention strategies.

Conversely, replacement fibrosis represents irreversible replacement of cardiomyocytes following cell death with scar tissue, such as after myocardial

infarction or prolonged exposure to stressors.[53] In our analysis, replacement fibrosis was not associated with significant differences in circulating protein levels even though unrecognized replacement fibrosis has been previously associated with left ventricular remodeling, incident HF and death.[54,55] Our findings support the contention that replacement fibrosis, compared to interstitial fibrosis, may be less driven by ongoing systemic processes reflected by this diverse group of 92 circulating proteins with known associations with CVD. Understanding the pathophysiology of myocardial fibrosis is a major step towards finding novel therapeutic interventions to stop or potentially reverse the pathologic process.

While our study has several strengths, it has limitations as well. This was a pilot-level study rather than a definitive analysis. Despite the size of the MESA cohort, cases (i.e. LGE+ without prior CVD) are limited, as was demographic matching of subjects with ECVq1 to those with ECVq4. As a result, baseline characteristics of the interstitial fibrosis groups showed differences in terms of some CVD risk factors including SBP, BMI, HDL cholesterol, diabetes, and smoking status, which may have impacted differential protein levels unrelated to the pathophysiology of interstitial fibrosis. Our BART analysis should have in-part accounted for the traditional risk factors as well as a social determinant of health (income) as confounders, but not other uncaptured potential clinical factors. That said, it is of interest that the control group had a greater burden of several of the traditional cardiovascular risk factors. Our proteomics findings are in contrast to comorbidity associated inflammation that has been identified from prior proteomics analysis of patients with symptomatic HFpEF.[6,56] While we were able to measure 92 proteins, several other markers of fibrosis (i.e., Transforming growth factor-β, and IGF-1 and -2) were not included. Furthermore, a prior study using MESA measured vascular cell adhesion molecule 1 (VCAM-1) and found higher levels were associated with incident HFpEF.[57] Interestingly we didn't see an association of intercellular adhesion molecule (ICAM) with cardiac fibrosis despite a prior association in younger adults with longitudinal development of decreased left ventricular global longitudinal strain.[58] Additionally, proteins which are involved in early stages of fibrosis formation might not be detectable in later stages. Future analysis can include a wider array of PEA panels to capture proteins implicated in the inflammatory and immune functions of myocardial fibrosis and determine whether a cross sectional association with interstitial fibrosis translates to longitudinal prediction of incident HFpEF. Interstitial fibrosis is just one of multiple multi-system mechanisms implicated in the development of symptomatic HFpEF.[6,31] Despite this, the presence of rigorously obtained CMR imaging for both interstitial and replacement fibrosis in a racially and ethnically diverse group of participants free of CVD is unique and allows us to provide important mechanistic insights into these imaging correlates with targeted discovery proteomics. Finally, we might have missed some non-linear complex correlations between proteins by using Pearson correlations for clustering which identify linear correlations only. While we did not produce scatter plots for all pairwise combinations of the 92 proteins to assess linearity, we provide scatter plots for ECVq1 and ECVq4 for 3 highly correlated, 3 moderately correlated, and 3 uncorrelated pairs of proteins using Pearson correlation (Supplemental Figs. S7 and S8). For these pairs of proteins, there does not appear to be any complex, non-linear associations.

In conclusion interstitial myocardial fibrosis has a different proteomic signature compared to matched controls, a similar finding was not present for replacement fibrosis in community dwelling participants without known CVD. These findings highlight the chronic systemic influences on interstitial, but not replacement fibrosis and suggest opportunities for early intervention and prevention of interstitial fibrosis. Further research is recommended with larger sample size and larger number of measured proteins to confirm and add additional mechanistic insights into both forms of cardiac fibrosis.

Contributors

HB, SAM, CD and SAB wrote the first draft of the manuscript. SLS performed randomization for cases and controls selection. SAB performed the statistical analysis. HB, SAB and CD have verified the underlying data. All authors contributed to the study design, data interpretation and final revision of the manuscript. All authors reviewed and approved the final version of the manuscript.

Data sharing statement

The MESA data including data that were used in this analysis are available upon request through the following website: BioLINCC: Multi-Ethnic Study of Atherosclerosis (MESA) (nih.gov).

Declaration of interests

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Drs. Seliger and deFilippi are co-owners on a patent awarded to the University of Maryland (US Patent Application Number: 15/309,754) entitled: "Methods for Assessing Differential Risk for Developing Heart Failure."

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104490.

References

1 Bozkurt B, Coats AJS, Tsutsui H, et al. Universal definition and classification of heart failure: a report of the heart failure society of America, heart failure association of the European society of cardiology, Japanese heart failure society and writing committee of the universal definition of heart failure: endorsed by the Canadian heart failure society, heart failure association of India, cardiac society of Australia and New Zealand, and Chinese heart failure association. *Eur J Heart Fail*. 2021;23(3):352–380.
2 Mewton N, Liu CY, Croisille P, Bluemke D, Lima JA. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. *J Am Coll Cardiol*. 2011;57(8):891–903.
3 Ambale Venkatesh B, Volpe GJ, Donekal S, et al. Association of longitudinal changes in left ventricular structure and function with myocardial fibrosis: the Multi-Ethnic Study of Atherosclerosis study. *Hypertension*. 2014;64(3):508–515.
4 Ambale-Venkatesh B, Liu CY, Liu YC, et al. Association of myocardial fibrosis and cardiovascular events: the multi-ethnic study of atherosclerosis. *Eur Heart J Cardiovasc Imaging*. 2019;20(2):168–176.
5 Frangogiannis NG. Cardiac fibrosis. *Cardiovasc Res*. 2021;117(6):1450–1488.
6 Paulus WJ, Zile MR. From systemic inflammation to myocardial fibrosis: the heart failure with preserved ejection fraction paradigm revisited. *Circ Res*. 2021;128(10):1451–1467.
7 Suthahar N, Meijers WC, Silljé HHW, de Boer RA. From inflammation to fibrosis-molecular and cellular mechanisms of myocardial tissue remodelling and perspectives on differential treatment opportunities. *Curr Heart Fail Rep*. 2017;14(4):235–250.
8 Seliger SL, Hong SN, Christenson RH, et al. High-sensitive cardiac troponin T as an early biochemical signature for clinical and subclinical heart failure: MESA (Multi-Ethnic study of atherosclerosis). *Circulation*. 2017;135(16):1494–1505.
9 Liu CY, Heckbert SR, Lai S, et al. Association of elevated NT-proBNP with myocardial fibrosis in the multi-ethnic study of atherosclerosis (MESA). *J Am Coll Cardiol*. 2017;70(25):3102–3109.
10 Lam MP, Ping P, Murphy E. Proteomics research in cardiovascular medicine and biomarker discovery. *J Am Coll Cardiol*. 2016;68(25):2819–2830.
11 Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156(9):871–881.
12 Turkbey EB, Nacif MS, Guo M, et al. Prevalence and correlates of myocardial scar in a US cohort. *JAMA*. 2015;314(18):1945–1954.
13 Liu CY, Liu YC, Wu C, et al. Evaluation of age-related interstitial myocardial fibrosis with cardiac magnetic resonance contrast-enhanced T1 mapping: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2013;62(14):1280–1287.
14 Volpe GJ, Rizzi P, Nacif MS, et al. Lessons on quality control in large scale imaging trials: the multi-ethnic study of atherosclerosis (MESA). *Curr Cardiovasc Imaging Rep*. 2015;8(5):13.
15 Shabani M, Dutta D, Ambale-Venkatesh B, et al. Rare genetic variants associated with myocardial fibrosis: multi-ethnic study of atherosclerosis. *Front Cardiovasc Med*. 2022;9:804788.
16 Tromp J, Westenbrink BD, Ouwerkerk W, et al. Identifying pathophysiological mechanisms in heart failure with reduced versus preserved ejection fraction. *J Am Coll Cardiol*. 2018;72(10):1081–1090.
17 Bruce S. *MESAproteomics (version 1.0.0)*. Source code. 2023. https://doi.org/10.5281/zenodo.7558173. Available from:.
18 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57:289–300.
19 Chipman HA, George EI, McCulloch RE. BART: bayesian additive regression trees. *Ann Appl Stat*. 2010;4:266–298.
20 Kapelner A, Bleich J. bartMachine: Machine learning with bayesian additive regression trees. *J Stat Softw*. 2016;70(4):1–40.
21 Chang J, Zhou W, Zhou WX, Wang L. Comparing large covariance matrices under weak conditions on the dependence structure and its application to gene clustering. *Biometrics*. 2017;73(1):31–41.
22 Galili T. dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering. *Bioinformatics*. 2015;31(22):3718–3720.
23 Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008;9:559.
24 Michelhaugh SA, Januzzi JL Jr. Finding a needle in a haystack: proteomics in heart failure. *JACC Basic Transl Sci*. 2020;5(10):1043–1053.
25 Bom MJ, Levin E, Driessen RS, et al. Predictive value of targeted proteomics for coronary plaque morphology in patients with suspected coronary artery disease. *eBioMedicine*. 2019;39:109–117.
26 Ferreira JP, Pizard A, Machu JL, et al. Plasma protein biomarkers and their association with mutually exclusive cardiovascular phenotypes: the FIBRO-TARGETS case-control analyses. *Clin Res Cardiol*. 2020;109(1):22–33.
27 Ho JE, Shi L, Day SM, et al. Biomarkers of cardiovascular stress and fibrosis in preclinical hypertrophic cardiomyopathy. *Open Heart*. 2017;4(2):e000615.
28 Lander BS, Zhao Y, Hasegawa K, et al. Comprehensive proteomics profiling identifies patients with late gadolinium enhancement on cardiac magnetic resonance imaging in the hypertrophic cardiomyopathy population. *Front Cardiovasc Med*. 2022;9:839409.
29 Mohammad MA, Koul S, Egerstedt A, et al. Using proximity extension proteomics assay to identify biomarkers associated with infarct size and ejection fraction after ST-elevation myocardial infarction. *Sci Rep*. 2020;10(1):18663.
30 Hinderer S, Schenke-Layland K. Cardiac fibrosis–a short review of causes and therapeutic strategies. *Adv Drug Deliv Rev*. 2019;146:77–82.
31 Shah SJ, Borlaug BA, Kitzman DW, et al. Research priorities for heart failure with preserved ejection fraction: national heart, lung, and blood institute working group summary. *Circulation*. 2020;141(12):1001–1026.
32 Mohammed SF, Hussain S, Mirzoyev SA, Edwards WD, Maleszewski JJ, Redfield MM. Coronary microvascular rarefaction and myocardial fibrosis in heart failure with preserved ejection fraction. *Circulation*. 2015;131(6):550–559.
33 Cao Z, Jia Y, Zhu B. BNP and NT-proBNP as diagnostic biomarkers for cardiac dysfunction in both clinical and forensic medicine. *Int J Mol Sci*. 2019;20(8):1820.
34 Li Y, Liu J, Cao Y, et al. Predictive values of multiple non-invasive markers for myocardial fibrosis in hypertrophic cardiomyopathy patients with preserved ejection fraction. *Sci Rep*. 2021;11(1):4297.
35 Tamura N, Ogawa Y, Chusho H, et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci U S A*. 2000;97(8):4239–4244.
36 Guiot J, Bondue B, Henket M, Corhay JL, Louis R. Raised serum levels of IGFBP-1 and IGFBP-2 in idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2016;16(1):86.
37 Hirota N, Ito T, Miyazaki S, Ebina M, Homma S. Gene expression profiling of lung myofibroblasts reveals the anti-fibrotic effects of cyclosporine. *Tohoku J Exp Med*. 2014;233(4):283–293.
38 Troncoso R, Ibarra C, Vicencio JM, Jaimovich E, Lavandero S. New insights into IGF-1 signaling in the heart. *Trends Endocrinol Metab*. 2014;25(3):128–137.
39 González-Guerra JL, Castilla-Cortazar I, Aguirre GA, et al. Partial IGF-1 deficiency is sufficient to reduce heart contractibility, angiotensin II sensibility, and alter gene expression of structural and functional cardiac proteins. *PLoS One*. 2017;12(8):e0181760.
40 Januzzi JL Jr, Packer M, Claggett B, et al. IGFBP7 (Insulin-like growth factor-binding protein-7) and neprilysin inhibition in patients with heart failure. *Circ Heart Fail*. 2018;11(10):e005133.
41 Ghosh AK, Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol*. 2012;227(2):493–507.

42 Samarakoon R, Higgins SP, Higgins CE, Higgins PJ. The TGF-β1/p53/PAI-1 signaling axis in vascular senescence: role of caveolin-1. *Biomolecules*. 2019;9(8):341.

43 Gupta KK, Donahue DL, Sandoval-Cooper MJ, Castellino FJ, Ploplis VA. Plasminogen activator inhibitor-1 protects mice against cardiac fibrosis by inhibiting urokinase-type plasminogen activator-mediated plasminogen activation. *Sci Rep*. 2017;7(1):365.

44 Baumeier C, Escher F, Aleshcheva G, Pietsch H, Schultheiss HP. Plasminogen activator inhibitor-1 reduces cardiac fibrosis and promotes M2 macrophage polarization in inflammatory cardiomyopathy. *Basic Res Cardiol*. 2021;116(1):1.

45 Weinberg EO, Shimpo M, De Keulenaer GW, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation*. 2002;106(23):2961–2966.

46 Wang TJ, Wollert KC, Larson MG, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation*. 2012;126(13):1596–1604.

47 Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Galectin-3 is independently associated with cardiovascular mortality in community-dwelling older adults without known cardiovascular disease: the Rancho Bernardo Study. *Am Heart J*. 2014;167(5):674–682.e1.

48 Sharma UC, Pokharel S, van Brakel TJ, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110(19):3121–3128.

49 Lok SI, Winkens B, Goldschmeding R, et al. Circulating growth differentiation factor-15 correlates with myocardial fibrosis in patients with non-ischaemic dilated cardiomyopathy and decreases rapidly after left ventricular assist device support. *Eur J Heart Fail*. 2012;14(11):1249–1256.

50 Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem*. 2017;63(1):140–151.

51 Krayenbuehl HP, Hess OM, Monrad ES, Schneider J, Mall G, Turina M. Left ventricular myocardial structure in aortic valve disease before, intermediate, and late after aortic valve replacement. *Circulation*. 1989;79(4):744–755.

52 Requena-Ibáñez JA, Santos-Gallego CG, Rodriguez-Cordero A, et al. Mechanistic insights of empagliflozin in nondiabetic patients with HFrEF: from the EMPA-TROPISM study. *JACC Heart Fail*. 2021;9(8):578–589.

53 Ambale-Venkatesh B, Lima JA. Cardiac MRI: a central prognostic tool in myocardial fibrosis. *Nat Rev Cardiol*. 2015;12(1):18–29.

54 Cheong BY, Muthupillai R, Wilson JM, et al. Prognostic significance of delayed-enhancement magnetic resonance imaging: survival of 857 patients with and without left ventricular dysfunction. *Circulation*. 2009;120(21):2069–2076.

55 Kwong RY, Chan AK, Brown KA, et al. Impact of unrecognized myocardial scar detected by cardiac magnetic resonance imaging on event-free survival in patients presenting with signs or symptoms of coronary artery disease. *Circulation*. 2006;113(23):2733–2743.

56 Sanders-van Wijk S, Tromp J, Beussink-Nelson L, et al. Proteomic evaluation of the comorbidity-inflammation paradigm in heart failure with preserved ejection fraction: results from the PROMIS-HFpEF study. *Circulation*. 2020;142(21):2029–2044.

57 Patel RB, Colangelo LA, Bielinski SJ, et al. Circulating vascular cell adhesion molecule-1 and incident heart failure: the multi-ethnic study of atherosclerosis (MESA). *J Am Heart Assoc*. 2020;9(22):e019390.

58 Patel RB, Colangelo LA, Reiner AP, et al. Cellular adhesion molecules in young adulthood and cardiac function in later life. *J Am Coll Cardiol*. 2020;75(17):2156–2165.