JACC: ADVANCES VOL. 2, NO. 2, 2023

ORIGINAL RESEARCH

ISCHEMIC HEART DISEASE

Biomarkers and Coronary Microvascular Dysfunction in Women With Angina and No Obstructive Coronary Artery Disease

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ABSTRACT

BACKGROUND Coronary microvascular dysfunction (CMD) is a major cause of ischemia with no obstructed coronary arteries.

OBJECTIVES The authors sought to assess protein biomarker signature for CMD.

METHODS We quantified 184 unique cardiovascular proteins with proximity extension assay in 1,471 women with angina and no obstructive coronary artery disease characterized for CMD by coronary flow velocity reserve (CFVR) by transthoracic echo Doppler. We performed Pearson's correlations of CFVR and each of the 184 biomarkers, and principal component analyses and weighted correlation network analysis to identify clusters linked to CMD. For prediction of CMD (CFVR $<$ 2.25), we applied logistic regression and machine learning algorithms (least absolute shrinkage and selection operator, random forest, extreme gradient boosting, and adaptive boosting) in discovery and validation cohorts.

RESULTS Sixty-one biomarkers were correlated with CFVR with strongest correlations for renin (REN), growth differentiation factor 15, brain natriuretic protein (BNP), N-terminal-proBNP (NT-proBNP), and adrenomedullin (ADM) (all P $<$ 1e-06). Two principal components with highest loading on BNP/NTproBNP and interleukin 6, respectively, were strongly associated with low CFVR. Weighted correlation network analysis identified 2 clusters associated with low CFVR reflecting involvement of hypertension/vascular function and immune modulation. The best prediction model for CFVR $<$2.25 using clinical data had area under the receiver operating characteristic curve (ROC-AUC) of 0.61 (95% CI: 0.56-0.66). ROC-AUC was 0.66 (95% CI: 0.62-0.71) with addition of biomarkers (P for model improvement = 0.01). Stringent two-layer cross-validated machine learning models had ROC-AUC ranging from 0.58 to 0.66; the most predictive biomarkers were REN, BNP, NT-proBNP, growth differentiation factor 15, and ADM.

CONCLUSIONS CMD was associated with pathways particularly involving inflammation (interleukin 6), blood pressure (REN, ADM), and ventricular remodeling (BNP/NT-proBNP) independently of clinical risk factors. Model prediction improved with biomarkers, but prediction remained moderate. (JACC Adv 2023;2:100264) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received November 4, 2022; revised manuscript received December 27, 2022, accepted January 12, 2023.

ISSN 2772-963X https://doi.org/10.1016/j.jacadv.2023.100264

ABBREVIATIONS AND ACRONYMS

ADM = adrenomedullin

AUC = area under the curve

BNP = brain natriuretic protein

CAD = coronary artery disease

CFVR = coronary flow velocity reserve

CMD = coronary microvascular dysfunction

GDF = growth differentiation factor

HFpEF = heart failure with preserved ejection fraction

IL = interleukin

LDL = low density lipoprotein

LVEF = left ventricular ejection fraction

NT-proBNP = N-terminal-proBNP

PCA = principal component analysis

REN = renin

ROC = receiver operating characteristics

WGCNA = weighted correlation network analysis

Coronary microvascular dysfunction (CMD) is a common cause of angina. Up to 50% of patients undergoing invasive angiography with no obstructive coronary artery disease (CAD) have abnormal response to adenosine stimulation.[1-3] The condition is more common in women, increases with age, hypertension and diabetes, and is associated with impaired cardiovascular prognosis.[1,4]

The underlying pathophysiological pathways are heterogenous and include intrinsic vascular dysfunction as well as perivascular structural remodeling and capillary rarefaction.[5] Small studies on selected populations have found higher levels of the inflammatory biomarkers interleukin (IL)-6, tumor necosis factor-alfa, leptin, C-reactive protein, white blood cell count, intercellular adhesion molecule-1, and soluble urokinase plasminogen activating receptor[6-10] in patients with CMD but larger, comprehensive studies of biomarkers and clustering of biomarkers in microvascular angina have not been undertaken.

We have previously shown that in a large study of women with angina and no obstructive CAD, CMD is associated with a greater risk of adverse cardiovascular outcomes,[4] and in small-scaled substudies, we found activation of pro-inflammatory pathways may be associated with CMD.[11,12]

In this study utilizing machine learning and other data-driven analyses, we aim to: 1) explore how a large panel of protein biomarkers may contribute to understanding the underlying pathophysiologic pathways in CMD; and 2) develop a risk model for the prediction of CMD using both clinical variables and protein biomarkers. To our knowledge this is the first attempt to identify a biomarker signature linked to CMD in angina.

METHODS

The iPOWER study (Improving diagnosis and treatment of women with angina pectoris and microvascular disease) was an investigator-initiated prospective cohort study. Design, rationale, and follow-up data have been published previously.[2,4,13] All women undergoing invasive angiography between March 2012 and December 2017 due to suspected angina pectoris were screened for inclusion and invited if fulfilling criteria (no epicardial stenoses (≥50%) and left ventricular ejection fraction (LVEF) >45%) (**Figure 1**). Microvascular function was assessed by transthoracic Doppler echocardiography measuring coronary flow velocity of the left anterior descending artery. Blood samples from all patients were fractionated, stored at −80 °C and processed in a randomized order. Blood plasma was analyzed by Olink Proteomics, Uppsala, Sweden, by real-time polymerase chain reaction. Please see Supplemental Appendix for details. This study was approved by the Danish Regional Committee on Biomedical Research Ethics (H-3-2012-005).

STATISTICAL ANALYSIS. We considered all 184 biomarkers and the following 13 clinical risk factors: age, history of dyslipidemia or hypertension, systolic and diastolic blood pressure, body mass index, waist circumference, low density lipoprotein (LDL)-cholesterol, diabetes, HbA1c, smoking status, heart rate, and LVEF. Heart rate and diastolic blood pressure were specifically included due to potential direct hemodynamic impact on the coronary flow velocity reserve (CFVR) measure. Missing data on clinical risk factors were imputed by replacing with the adjacent observation or the mean (categorical/continuous variables, respectively). Subjects with missing data on one or more of the 184 protein biomarkers were excluded. Stata version 17 and the R-language for statistical computing version 3.6.0[14] was used for analysis.

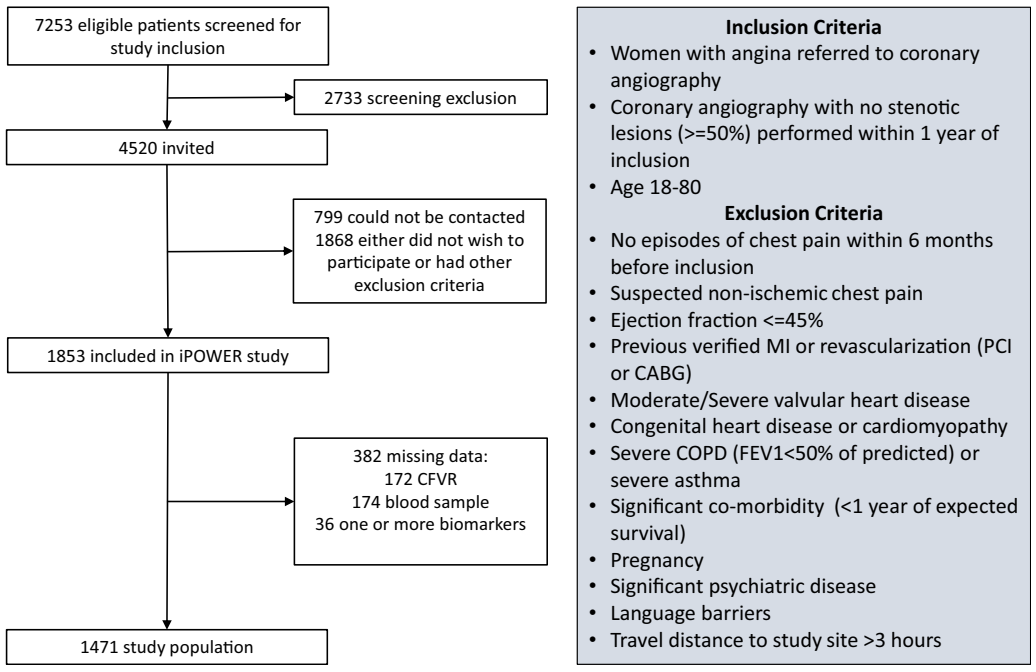
The analytical strategy comprised explorative pathway analyses and prediction modeling.

EXPLORATION OF CMD PATHWAYS. To achieve an overview of associations between protein biomarkers and CMD, we first performed Pearson's correlations of CFVR and each of the 184 biomarkers. We depicted the *P* value against the correlation coefficient in a volcano plot and adjusted for multiple testing by the Benjamini-Hochberg method. We then performed principal component analyses and weighted correlation network analyses to understand better the underlying biology reflected by the biomarkers in relation to CMD.

Principal component analysis. We included all biomarkers significantly associated with CFVR in univariate analyses in the principal component analyses (PCAs). Biomarkers were centralized to 0 and scaled to the standard deviation. We limited the number of principal components (PCs) to the number that explained 85% of the variation in the data. From these, we assigned each observation with a weighted value for each PC and tested the association with CFVR and the ability to predict CMD (CFVR <2.25).

Weighted correlation network analysis of proteomics. Weighted correlation network analysis (WGCNA) is a widely used data mining method for

FIGURE 1 Flowchart of the Study Population



CABG = coronary artery bypass graft; CFVR = coronary flow velocity reserve; COPD = chronic obstructive pulmonary disease; FEV1 = forced expiratory volume; iPOWER = Improving diagnosis and treatment of women with angina pectoris and microvascular disease; MI = myocardial infarction; PCI = percutaneous coronary intervention.

studying biological networks based on pairwise correlations between variables to identify highly correlated variables.[15,16] A co-expression network analysis of the circulating biomarkers was performed using the WGCNA R software package to identify modules (clusters) of highly co-expressed biomarkers.[17] Please see Supplemental Appendix for details.

PREDICTION MODELING. We applied conventional multiple logistic regression and machine learning approaches for prediction modeling with discovery and validation data sets. The receiver operating characteristics (ROC) area under the curve (AUC/C-statistic), sensitivity, specificity, positive predictive value, and negative predictive value for the validation set were calculated based on the observed prevalence of CFVR <2.25.

Logistic regression. For logistic regression analyses, data were divided at random into a discovery (60%) and validation set (40%). All the cardiovascular disease (CVD) risk factors and 184 biomarkers were tested in univariate logistic regression with CFVR <2.25 as the dichotomous outcome. For an objective, data-driven technique to identify the smallest number of variables required for a practical prediction model, we applied an entry criterion of $P < 0.05$ from the univariate analysis. After modeling associations in the discovery data set, the best model was evaluated in the validation set. We further compared a model including only clinical variables with a model which included biomarkers, both developed in the discovery data set and validated in the validation set.

Machine learning. Least absolute shrinkage and selection operator (LASSO) regression, random forest (RF), adaptive boosting (AdaBoost), and extreme gradient boosting (XGBoost) models were fitted in R (version 3.6.0). A two-layer five-fold cross-validation case-control stratified by CFVR <2.25 was applied for training and ensuring independent evaluation of 5 test data sets for which the average and 95% CI performance metrics were estimated. Beyond stringent cross-validation, the machine learning methods apply regularization and pruning techniques to reduce risk of overfitting multivariate prediction models and assist feature selection for prediction. LASSO regression includes a regularization term on regression coefficients to select predictors and shrink coefficients of less important features to zero. RF, AdaBoost, and XGBoost are decision tree based with different model training techniques. All tree-based machine learning

approaches were pre-pruned to relatively short trees and minimum node size to reduce redundant features.

The machine learning models were trained using all 184 biomarkers and the 13 clinical risk factors. We conducted a rigorous stability selection procedure to ensure reliability of the biomarker signature models. We compared with feature selection based on optimization on sensitivity and on different sizes of data set by modifying k-fold split in inner and outer test yielded similar results. A robustness test of the prediction models was conducted to evaluate statistical validity of the results by comparing performance metrics for models trained on the actual prediction labels vs models trained on randomized prediction labels. We followed a standard procedure where the outcome variable (ie, CFVR <2.25) was randomly reshuffled while the corresponding protein profiles were kept intact. This was repeated in a five-fold inner and outer validation as described above. The predictive performances of the machine learning models were evaluated using area under the receiver operating characteristic curve (ROC-AUC), sensitivity, positive predictive value, specificity, and negative predictive value.

More details are given in the Supplemental Appendix.

RESULTS

BASELINE INFORMATION. The study population consisted of 1,471 women with angina with measurements on all 184 biomarkers and a valid CFVR assessment (**Figure 1**). Baseline information on study participants by level of CFVR is given in **Table 1**. Patients with CFVR <2.25 were older and more likely to have diabetes, hypertension, and dyslipidemia, whereas there were no differences in anthropometrics and LVEF. Patients with low CFVR also had higher diastolic blood pressure and heart rate.

In correlation analyses of CFVR with all 184 biomarkers, 61 biomarkers were significantly correlated with CFVR, 55 biomarkers with negative correlation, and 6 with positive correlation. After adjustment for multiple testing, 44 biomarkers remained significantly associated with CFVR (**Figure 2**) and after age-adjustment 38 (suppl volcano plots). The strongest negative correlations were seen for renin (REN), growth differentiation factor 15 (GDF15), brain natriuretic protein (BNP), N-terminal-proBNP (NT-proBNP), and adrenomedullin (ADM) (all P<1e-06). Positive correlations were relatively weak, the strongest seen for paraoxonase, matrix extracellular phosphoglycoprotein, epidermal growth factor receptor, and collagen alpha-1(I) chain (all $P < 0.01$).

TABLE 1 Baseline Characteristics of the Patients According to CFVR

	CFVR ≥2.25 (n = 825)	CFVR <2.25 (n = 646)	*P* Value
Age (y)	61.4 ± 9.4	64.5 ± 9.5	<0.001
BMI (kg/m^2)	27.0 ± 5.0	27.2 ± 5.7	0.472
Abdominal circumference (cm)	95.8 ± 13.8	97.0 ± 14.3	0.116
Hba1c	39.0 ± 7.4	40.1 ± 8.8	0.0134
Diabetes			
Yes	83 (10%)	98 (15%)	0.0039
Smoker			
Yes	123 (15%)	109 (17%)	0.367
Systolic BP (mHg)	130 ± 20.9	131 ± 21.1	0.956
Diastolic BP (mHg)	69.4 ± 12.0	67.5 ± 12.0	0.00278
Hypertension			
Yes	402 (49%)	398 (62%)	<0.001
Dyslipidemia			
Yes	494 (60%)	423 (65%)	0.0483
LDL-cholesterol (mg/dl)	2.83 ± 1.04	2.73 ± 1.02	0.0739
LVEF	56.4 ± 12.6	55.5 ± 15.3	0.275
Heart rate (beats/min)	69.8 ± 10.4	71.8 ± 11.3	<0.001

Values are mean ± SD or n (%).

BMI = body mass index; BP = blood pressure; CFVR = coronary flow velocity reserve; LDL = low density lipoprotein; LVEF = left ventricular ejection fraction.

CMD PATHWAY EXPLORATION. Principal component analyses. From PCA of the 61 biomarkers associated with CFVR in univariate analyses, 28 PCs explained 85% of the variance in biomarkers, and 7 of these were significantly associated with CFVR. The 10 biomarkers with highest eigenvectors for each of these 7 PCs are presented in **Table 2** (by order of strength of association with CFVR from left to right) and correlation with the 13 clinical risk factors shown in Supplemental Table 2. PC5, PC1, PC12, and PC2 were most strongly associated with CFVR. PC1 had roughly similar positive loadings across biomarkers and was associated with all clinical risk factors and thus did not point toward specific pathways. PC5 had highest positive loadings from BNP and NT-proBNP; these 2 biomarkers did not have high loading on other PCs. PC5 was positively associated with age, systolic blood pressure, and history of hypertension, diabetes and smoking and negatively with obesity and waist circumference, consistent with capturing elements of CMD associated with ventricular remodeling, as also reflected by the high loading of BNP and NT-proBNP. PC 12 had highest loading on IL-6 and showed little association with clinical risk factors, perhaps identifying a pathway to CMD involving inflammation that is independent of classical risk factors. PC 2 had highest loading from the biomarkers positively correlated with CFVR: paraoxonase, epidermal growth factor receptor, matrix extracellular

phosphoglycoprotein, and collagen alpha-1(I) chain and was inversely associated with most clinical risk factors. PC19, 9, and 10 were weakly associated with CFVR ($P > 0.01$) and had several overlapping biomarkers, most notably REN, which had high loading on both PC19 and PC9. PC9 was the PC most strongly associated with smoking.

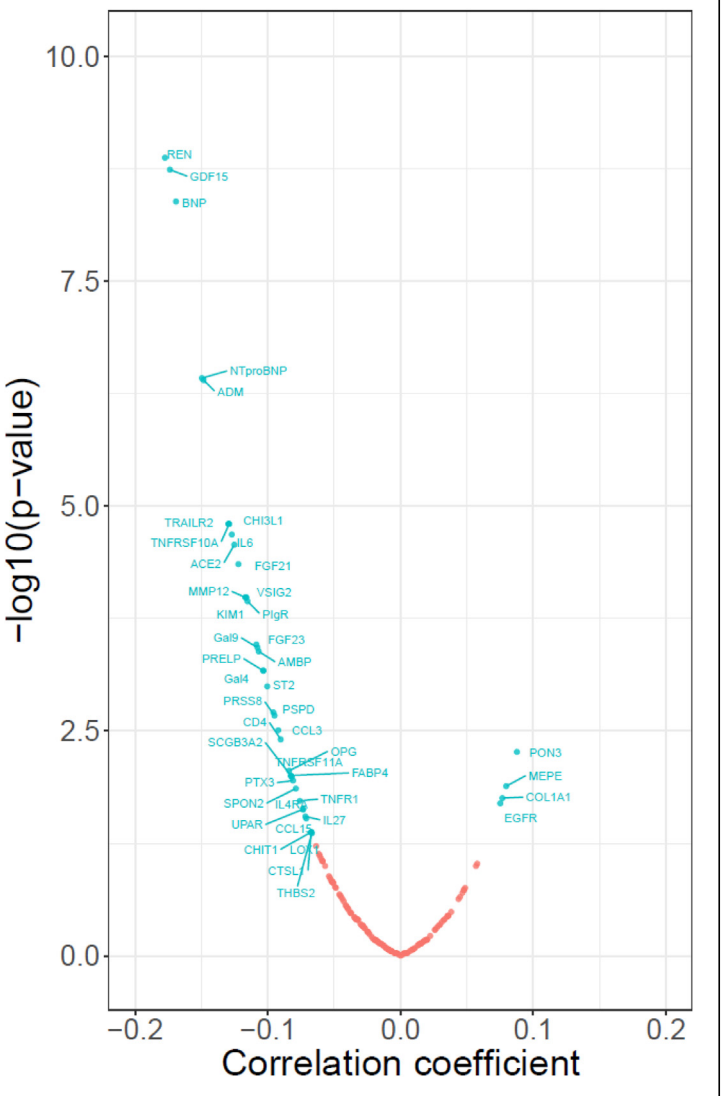
Weighted protein co-abundance network. WGCNA was used to construct protein networks and identify modules of proteins that were studied for functional enrichments based on protein-protein interactions in the clusters. The minimum module size was 5; 8 modules were constructed based on the WGCNA clustering (Supplemental Figure 1, Supplemental Table 3). Two of these were significantly negatively correlated with CFVR (blue (r = -0.12, $P = 4.2e-06$) and gray (r = −0.11, $P = 1.8e-05$) (Supplemental Figure 2). The gray and blue modules showed significant functional protein-protein interaction enrichment, $P = 2.26e-14$ and $P < 1e-16$, respectively.

The gray module included 32 biomarkers with IL-6 having the highest number of interaction partners. The biomarkers in the gray module had significant functional enrichment in hypertension and vascular disease through protein-disease associations, (Supplemental Figure 3, Supplemental Table 4, Supplemental Appendix). Of the biomarkers most strongly associated with CFVR, the module also included BNP, NTproBNP, REN, serpina12, SCGB3A2, ADAMTS3, and PSPD. Module trait relationship (Supplemental Figure 5) showed positive correlation with age, hypertension, and blood pressure and negative correlations with obesity, CFVR, and LDL. Notably, IL6, SCGB3A2, ADAMTS13, CHIT1, and REN were all among the most informative biomarkers in PC12.

The blue module included 47 biomarkers and was linked to immune modulation with CD4 having the highest clustering coefficients (Supplemental Figure 4, Supplemental Table 5, Supplemental Appendix). Of biomarkers most strongly associated with CFVR, the module included ADM, ACE2, TRAILR2, MMP12, and PRSS27. The blue module had significant positive correlations with age, waist circumference, body mass index, diabetes, systolic and diastolic blood pressure, dyslipidemia, HbA1c, heart rate, and hypertension while negatively correlated with LVEF, LDL, and CFVR (Supplemental Figure 5).

PREDICTION MODELS. Logistic regression in discovery and validation data set. Data were split at random 60:40 into a discovery and validation data set. After considering the a priori selected 13 clinical risk factors, the following 3 risk factors were significantly associated with impaired CFVR in multivariable adjusted model in the discovery data set: Age, diastolic blood pressure, and heart rate. The ROC-AUC in the validation cohort for this model based on clinical risk factors was 0.61 (95% CI 0.56-0.66). When adding biomarkers, the best model retained 5 biomarkers in the model: REN, BNP, chitinase-3-like protein (CHI3L1, also known as

FIGURE 2 Volcano Plot Showing Correlations Between 184 Biomarkers and Coronary Flow Velocity Reserve After Adjustment for Multiple Testing (Benjamini-Hochberg)



Forty-four biomarkers significantly associated with CFVR ($P < 0.05$) are depicted in **blue**: on the **left** are negative correlations, on the **right** positive correlations. Explanation for abbreviations for protein biomarkers is given in the Supplemental Table 1.

TABLE 2 Principal Components Associated With Coronary Flow Velocity Reserve

	PC5		PC1		PC12		PC2	
	Biomarker	Loading	Biomarker	Loading	Biomarker	Loading	Biomarker	Loading
1	BNP	0.371400	TRAILR2	0.199113	IL-6	0.374660	PON3	0.311759
2	NTproBNP	0.36120	TNFRSF11A	0.191228	SCGB3A2	0.313553	EGFR	0.292205
3	ADAMTS13	−0.274076	PGF	0.185412	MMP3	0.262252	MEPE	0.272181
4	EGFR	−0.273627	TNFRSF10A	0.180614	ADAMTS13	−0.244763	COL1A1	0.257342
5	GDF15	0.216225	SPON2	0.179553	CHIT1	−0.240334	IGFBP2	0.255146
6	BOC	−0.214201	ADM	0.176305	FABP2	−0.237533	PLC	0.202806
7	MEPE	−0.208370	TNFR1	0.174049	ST2	0.219723	IL18BP	0.201117
8	IGFBP2	0.199133	GDF15	0.169820	AMBP	−0.210039	OPG	0.198633
9	PSPD	0.19758	IL18BP	0.166418	REN	0.201904	MMP3	0.198401
10	MMP12	0.191672	PLC	0.164218	CTSL1	0.185104	IL1ra	−0.167283
Eigenvalue proportion	3.42%		30.26%		1.59%		8.13%	
P value for correlation with CFVR	<0.0001		<0.0001		0.0027		0.006	

The table gives the 10 highest loading biomarkers for each of the 7 principal components (PCs) significantly associated with coronary flow velocity reserve (CFVR). Biomarkers are given by order of their loading from top to bottom and principal components by order of strength of association with CFVR from left to right. For abbreviations of biomarkers please see Supplemental Table 1.

PC = principal component.

Continued on the next page

YKL 40), protein delta homolog 1(DLK1), and aminopeptidase N. Higher age, heart rate, REN, BNP, and CHI3L1 were associated with higher odds of impaired CFVR while higher diastolic blood pressure, DLK1, and aminopeptidase N were associated with lower odds. ROC-AUC in the validation cohort for this biomarker model was 0.66 (95% CI: 0.62-0.71) (P = 0.01 for model improvement). However, while the same clinical risk factors were selected, the biomarkers selected in the model varied when discovery-validation sets were resampled. This reflects the strong correlation between biomarkers and underlines that the importance of the 5 specific biomarkers listed above should not be overemphasized.

Cross-validated machine learning algorithms. To get an unbiased estimate of how well the clinical features and biomarkers can be applied for diagnosis of CMD, 4 different machine learning models were trained with similar cross-validation. This included a logistic regression model regularized by L1 (LASSO), RF, XGBoost, and AdaBoost. LASSO regression had the least predictive power for CMD where the mean ROC-AUC in the cross-fold validation cohort was 0.58 (95% CI: 0.53-0.64), while RF was 0.66 (95% CI: 0.61-0.71), AdaBoost 0.63 (95% CI: 0.56-0.71), and XGBoost was 0.63 (95% CI: 0.58-0.67).

The most important features in the models are listed in **Table 3** and corresponding importance plots in Supplemental Figures 6 to 9. Overall, the biomarkers most strongly associated with CFVR were represented in RF, LASSO, and XGBoost (REN, GDF15, BNP/NTproBNP, and ADM). RF and XGBoost showed overlapping biomarkers of importance, including REN, ADM, BNP/NTproBNP, PRSS27, Serpina12, and MMP12. Age had high importance in RF and XGBoost but not in LASSO and AdaBoost. The AdaBoost algorithm identified a different set of biomarkers that interestingly showed considerable overlap with PC1, perhaps identifying a protein signature for a different pathway to CMD.

The XGBoost algorithm also identified age, REN, and BNP among the 10 most important features. Overlapping with RF or LASSO were PRSS27, serpina 12, MMP12, and heart rate, while the 2 features diastolic blood pressure and LVEF were only represented in this model.

The 4 different ML models trained provide different decision boundaries when classifying patients to low or high CFVR which is reflected in the difference of the feature importance. Boosting models, such as XGBoost and AdaBoost, create classifiers that have specialized strategies to try correctly to classify the 'difficult-to-classify' observations. AdaBoost works by weighting the predictions from stumps (short decision trees) higher for observations that are difficult to classify. This nature of AdaBoost may explain the notably different feature importance ranking.

The robustness test of prediction models trained on a data set with randomized outcome yielded ROC-AUC ranging from 0.49 to 0.52. Performance metrics are given in Supplemental Table 6.

TABLE 2 Continued

PC19		PC9		PC10	
Biomarker	Loading	Biomarker	Loading	Biomarker	Loading
XCL1	0.392075	IL27	−0.310998	ADAMTS13	0.304134
REN	0.389946	REN	−0.291131	PIgR	0.252646
CTSL1	−0.352725	FABP2	−0.265492	IL4RA	−0.248399
PSPD	0.270867	PSPD	0.262868	COL1A1	−0.232109
CCL3	−0.243718	FGF21	0.254257	XCL1	−0.231514
KIM1	−0.240667	COL1A1	0.245283	AMBP	0.226923
PSGL1	−0.225694	SCGB3A2	0.203058	NTproBNP	0.220057
SCGB3A2	−0.204858	PRSS8	0.201850	MMP3	0.211671
PRSS8	0.184928	Gal4	−0.198421	FABP2	−0.207976
CHI3L1	0.168220	MMP3	−0.191168	PSGL1	0.204125
1.17%		1.94%		1.75%	
0.0297		0.0385		0.043	

DISCUSSION

This paper applied data-driven analyses to identify activated pathways and develop prediction models for CMD in patients with angina and no obstructive CAD. Approximately one-third of the 184 cardiovascular biomarkers assessed were significantly associated with impaired CFVR. The individual biomarkers and pathway patterns identified indicate links to inflammation, hypertension, and cardiac remodeling. The prediction models developed with different statistical approaches were superior to models with only clinical data but had only moderate ability to identify CMD in these patients.

The individual biomarkers most strongly associated with CFVR that also emerged as biomarkers of importance in pathways and prediction models were REN, GDF15, BNP (and its prohormone, NT-proBNP), ADM, CHI3L1, TRAILR2, and IL-6. Additionally, IL-6 was a core protein in the gray module of protein-protein interaction and loaded highest on PC12. TRAILR2 scored highest on PC1 and was the most important biomarker in the AdaBoost derived prediction model. These biomarkers and their possible role in CMD are briefly described below.

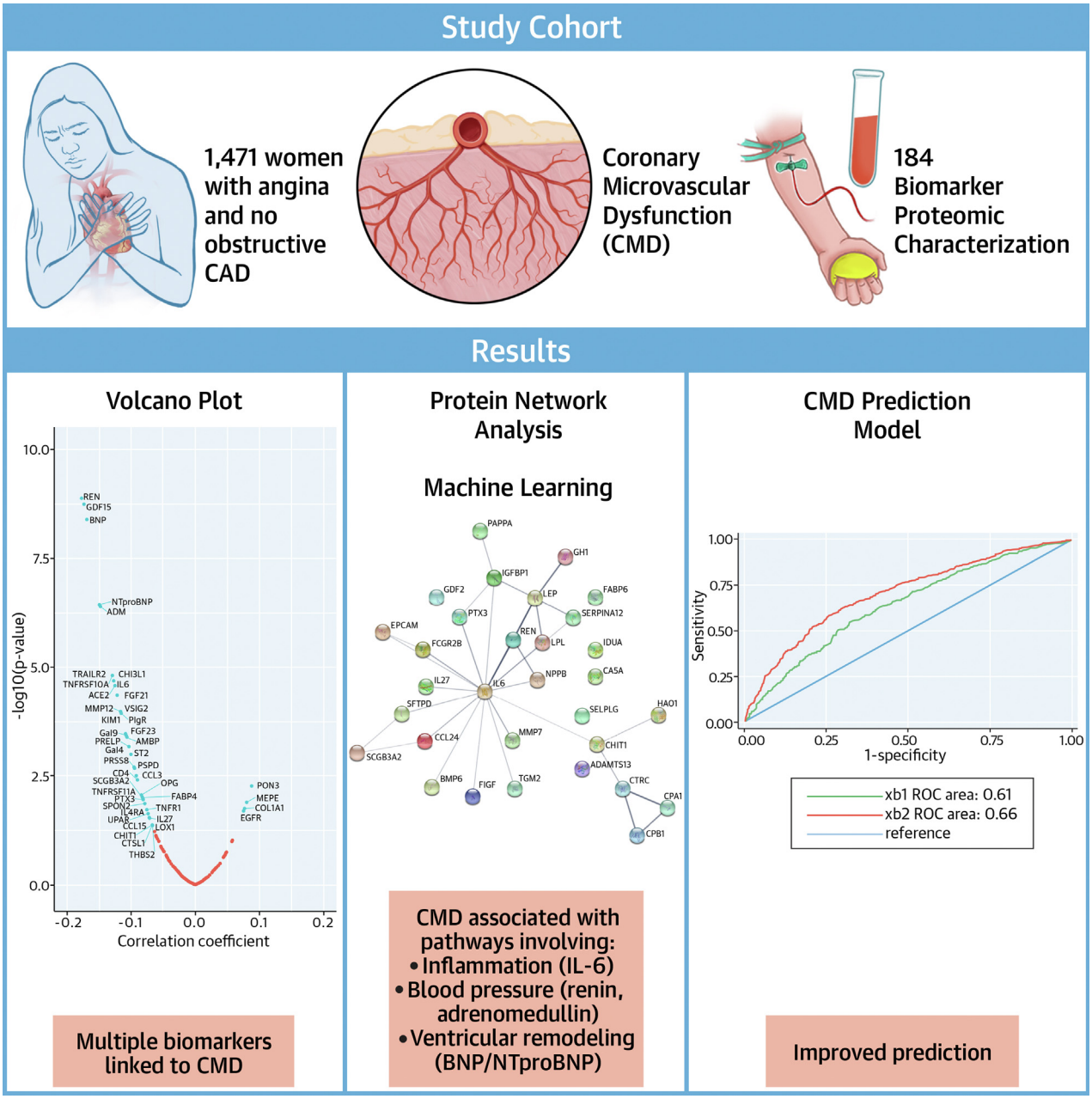
RENIN, BNP, AND NT-proBNP. REN, BNP, and NTproBNP emerged as 3 of the biomarkers most strongly linked to CMD. Together with IL-6, these were core proteins in the gray module identified by WGCNA and prominent biomarkers in PCs associated with CFVR. REN is well-known for its role in the REN-angiotensin-aldosterone system as a protein secreted by the kidneys resulting in downstream vasocon-

TABLE 3 Ranking of 10 Most Important Variables and Model Performance Metrics for Each of the Algorithms

Ranking	Random Forest (Gini)	Random Forest (Accuracy)	LASSO	XGBoost	AdaBoost
1	REN	REN	REN	REN	TRAILR2
2	Age	Age	BNP	Age	ALCAM
3	ADM	ADM	DLK1	PRSS27	PGF
4	PRSS27	GDF15	IL-6	ADM	EPHB4
5	GDF15	NTproBNP	PRSS27	SERPINA12	TNFRSF11A
6	MMP12	BNP	IL17RA	Dia BP	IGFBP7
7	PON3	Heart rate	vWF	LVEF	MMP2
8	SERPINA12	PRSS27	PSPD	MMP12	MERTK
9	vWF	MMP12	SELE	BNP	TNFR1
10	NTproBNP	MEPE	CHI3L	Heart rate	SPON2
AUC	0.66 (0.61-0.71)		0.58 (0.53-0.64)	0.63 (0.58-0.67)	0.63 (0.56-0.71)
Sensitivity	0.47(0.40-0.55)		0.41 (0.31-0.51)	0.45 (0.35-0.54)	0.39 (0.26-0.52)
Specificity	0.73 (0.64-0.81)		0.76 (0.65-0.87)	0.72 (0.65-0.79)	0.80 (0.75-0.85)
PPV	0.57 (0.51-0.63)		0.58 (0.47-0.68)	0.56 (0.51-0.60)	0.60 (0.51-0.69)
NPV	0.64 (0.61-0.67)		0.62 (0.58-0.66)	0.62 (0.59-0.65)	0.63 (0.58-0.67)

AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value. For abbreviations of biomarkers please see Supplemental Table 1.

CENTRAL ILLUSTRATION **184 Protein Biomarkers and Coronary Microvascular Dysfunction in Women With Angina and No Obstructive CAD**



Prescott E, et al. JACC Adv. 2023;2(2):100264.

striction and increase in blood pressure and sodium retention (via angiotensin I and II activation). The natriuretic peptide BNP may be elevated in response to REN. REN is also a paracrine antifibrotic factor and REN, BNP, and NT-proBNP are all raised in patients with heart failure. Together with the well-established strong association between hypertension and microvascular dysfunction, the increased levels of REN and natriuretic peptides may thus reflect an ongoing adaptive cardiac remodeling in microvascular angina. An important novel paradigm describes a possible causal pathway from the mainly metabolic risk factors obesity, diabetes and hypertension through low-grade systemic inflammation to CMD,

cardiac remodeling, diastolic dysfunction and ultimately development of heart failure with preserved ejection fraction (HFpEF).[18] There is some evidence supporting this paradigm from cross-sectional studies[6,9-12,19-26] and CMD has been linked to increased future risk of HFpEF development.[27] Moreover, studies in mice have found that exposure to hypertensive and metabolic stress ('two-hit-model') leads to inflammation, impaired CFVR, and HFpEF.[28] The biomarker analyses presented here confirm associations between CMD and biomarkers reflecting primarily inflammation, hypertension, and remodeling. However, the cross-sectional nature of the current analyses impedes causal inference and prospective studies that intervene in the causal pathway are needed to establish this link.

GDF15, ADM, CHI3L1, TRAILR2, AND IL-6. GDF15 is a well-established cardiovascular risk marker that is also upregulated with age, in obesity, diabetes, inflammation, cancer, pulmonary and renal disease. Biological actions are poorly defined and understood but GDF15 was increased in patients with atrial fibrillation,[29] heart failure[30] and CAD[31] and associated with impaired prognosis.[31,32] In previous iPOWER substudies, GDF15 was associated with impaired CFVR.[11,12] No other study has reported a link between GDF15 and CMD.

ADM is a circulating vasodilator peptide hormone with natriuretic effects that also stimulates angiogenesis and was one of the biomarkers with greatest importance for CFVR in the RF and XGBoost models. ADM is increased in acute decompensation, was recently suggested as a biomarker of prognosis and risk stratification in heart failure and may prove a target for pharmacological intervention.[30] Together with GDF15 and YKL40, ADM was associated with impaired prognosis in a small study of patients with HFpEF.[32] The only previous study to link CMD to ADM found that in 32 patients undergoing invasive angiography, coronary flow reserve was associated with ADM.[33]

CHI3L1, also known as YKL-40, is a glycoprotein regarded as a non-disease-specific biomarker of inflammation and tissue remodeling. CHI3L1 increases with age and is elevated in CAD as well as in diseases characterized by inflammation, including heart failure, stroke, and diabetes. CHI3L1 is expressed in macrophages and smooth muscle cells in atherosclerotic plaques formation, particularly in early stages of atherosclerosis. Moreover, higher levels of CHI3L1 have been associated with thromboembolic events.[34] No other study has reported on associations between CMD and CHI3L1.

TRAILR2 is a receptor for the pro-apoptotic protein tumor necrosis factor-related apoptosis inducing ligand (TRAIL). Studies have indicated TRAILR2 is associated with adverse CVD outcomes, but more studies are needed to understand its precise role.[35] No previous studies have linked TRAILR2 to CMD.

CFVR was strongly associated with IL-6 ($P = 1.0e\text{-}06$) and from a treatment perspective this may be the most important finding of this study. IL-6 is acknowledged as a driver of atherosclerosis [36] and specifically targeting IL-1β upstream from IL-6 lead to improved CVD outcomes in the seminal, proof-of-concept CANTOS trial (Canakinumab anti-inflammatory thrombosis outcome study). This is the first study to link CMD to IL-6 in patients with angina and no obstructive CAD. Future studies may test the effect of IL-6 inhibition in this patient group.

PREDICTION OF CMD. A model to predict CMD likelihood could be useful as a point-of-care test prior to referral to invasive or noninvasive diagnostic tests for CMD. A simple model with clinical risk factors, including age, hypertension and diabetes, factors all known to be risk factors for CMD, better-than-random in predicting CMD with an ROC-AUC of 0.63. Adding biomarkers to the model significantly improved prediction but the model only performed moderately well, with ROC-AUC below 0.70. The clinical utility of a prediction model to rule-in and rule-out CMD from a panel of biomarkers could thus not be established. Such a model would need to be refined and further validated before apt for clinical use.

The aim of studying proteomics in microvascular angina was twofold and partly overlapping: We wished to study patterns of protein biomarkers that might help understand and separate the underlying mechanisms for microvascular dysfunction. With the limited availability of noninvasive and invasive methods to diagnose microvascular angina, we also wished to develop a predictive model to rule-in or rule-out CMD in patients with angina and no obstructive CAD. Although proteomics plays a crucial role in biomarker discovery, the modest discriminative ability of this technique indicates that there are still hidden mechanisms in protein regulatory networks that may not be sufficiently elucidated by biomarkers on the plasma levels. There may be multiple underlying mechanisms of CMD—structural and functional and pertaining to vascular function or cardiac remodeling—that have different protein signatures.

STUDY LIMITATIONS. Small studies have shown associations between CMD and selected inflammatory biomarkers. The present study is appreciably the

largest to assess biomarkers in CMD, and multiple protein biomarkers were assessed simultaneously. Other strengths include an unselected and well-characterized study population in which CMD has been shown to be associated with CVD outcomes.[4] An important strength is that we apply multiple methods to data analyses and we base conclusions on the combined results.

The main limitation of the study is the cross-sectional nature of data hindering causal inference. Some individual biomarker associations will be subject to type I error, but no conclusions were drawn from individual associations that were not very strong. In the more advanced analyses (PCA, WGCNA, and ML), this limitation because of multiple testing does not apply and, further, care was taken to draw conclusions on patterns of results.

We did not perform external validation. To our knowledge, no similar data population for external validation exists. We did not include symptom characteristics in the phenotype characterization as we have previously reported no association between CFVR and symptom characteristics in the iPower cohort.[2]

By inducing maximum hyperemia for CFVR assessment by dipyridamole infusion, we evaluated primarily non-endothelium dependent vasodilatation and results are not comparable to coronary vascular function assessed by eg, acetylcholine provocation. We only included women and we do not know whether results apply also to male peers.

CONCLUSIONS

In this large study of women with angina and no obstructive CAD, we identified multiple biomarkers that were associated with CMD. We further identified potential pathways involving inflammation, hypertension, and cardiac remodeling linking these biomarkers to microvascular angina (**Central Illustration**). Results should be confirmed in other study populations and prospective studies are needed to determine whether biomarkers are causally related to future CMD development and whether modification of the underlying pathways may lead to improved coronary microvascular function. Prediction models were significantly improved but predictive abilities remained moderate.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This study received funding from the Danish Heart Foundation, the Danish Research Council, and the Kaj and Lise Jensen Foundation. Dr Nielsen is a full-time employee at Novo Nordisk Research Centre Oxford, United Kingdom since June 2021. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A comprehensive characterization of biomarker profile in patients with angina and no obstructive CAD is consistent with inflammation, hypertension, and ventricular remodeling being intrinsically linked to CMD.

TRANSLATIONAL OUTLOOK: If validated in other cohorts of patients with microvascular angina, our findings support considering inflammation as a therapeutic target in patients with angina and CMD.

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KEY WORDS angina, ANOCA, INOCA, microvascular

APPENDIX For supplemental information, tables, and figures, please see the online version of this paper.