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EURObservational Research Programme in Conjunction With the Heart Failure Association of the European Society of Cardiology Study Group on Peripartum Cardiomyopathy; Kodogo, Vitaris; Viljoen, Charle; Hoevelmann, Julian; Chakafana, Graham; Tromp, Jasper; Farhan, Hasan Ali; Goland, Sorel; van der Meer, Peter; Karaye, Kamilu

Published in:
JACC: Heart Failure

DOI:
10.1016/j.jchf.2023.07.028

Publication date:
2023

JACC: HEART FAILURE VOL. 11, NO. 12, 2023

PUBLISHED BY ELSEVIER

Proteomic Profiling in Patients With Peripartum Cardiomyopathy

A Biomarker Study of the ESC EORP PPCM Registry

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ABSTRACT

BACKGROUND Peripartum cardiomyopathy (PPCM) remains an important cause of maternal morbidity and mortality globally. The pathophysiology remains incompletely understood, and the diagnosis is often missed or delayed.

OBJECTIVES This study explored the serum proteome profile of patients with newly diagnosed PPCM, as compared with matched healthy postpartum mothers, to unravel novel protein biomarkers that would further an understanding of the pathogenesis of PPCM and improve diagnostic precision.

METHODS Study investigators performed untargeted serum proteome profiling using data-independent acquisition-based label-free quantitative liquid chromatography–tandem mass spectrometry on 84 patients with PPCM, as compared with 29 postpartum healthy controls (HCs). Significant changes in protein intensities were determined with nonpaired Student's *t*-tests and were further classified by using the Boruta algorithm. The proteins' diagnostic performance was evaluated by area under the curve (AUC) and validated using the 10-fold cross-validation.

RESULTS Patients with PPCM presented with a mean left ventricular ejection fraction of 33.5% ± 9.3% vs 57.0% ± 8.8% in HCs ($P < 0.001$). Study investigators identified 15 differentially up-regulated and 14 down-regulated proteins in patients with PPCM compared with HCs. Seven of these proteins were recognized as significant by the Boruta algorithm. The combination of adiponectin, quiescin sulfhydryl oxidase 1, inter-α-trypsin inhibitor heavy chain, and N-terminal pro-B-type natriuretic peptide had the best diagnostic precision (AUC: 0.90; 95% CI: 0.84-0.96) to distinguish patients with PPCM from HCs.

CONCLUSIONS Salient biologic themes related to immune response proteins, inflammation, fibrosis, angiogenesis, apoptosis, and coagulation were predominant in patients with PPCM compared with HCs. These newly identified proteins warrant further evaluation to establish their role in the pathogenesis of PPCM and potential use as diagnostic markers. (J Am Coll Cardiol HF 2023;11:1708–1725) © 2023 by the American College of Cardiology Foundation.

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ISSN 2213-1779/$36.00 https://doi.org/10.1016/j.jchf.2023.07.028

BACKGROUND

Peripartum cardiomyopathy (PPCM) is characterized by new onset left ventricular (LV) systolic dysfunction that occurs in previously healthy women toward the end of pregnancy and up to 5 months postpartum.[1,2] PPCM remains an important cause of pregnancy-related maternal morbidity and mortality globally, with a mortality of 6% in the more than 700 patients included in the EORP (EURObservational Research Programme) Registry on PPCM.[3] Potential factors contributing to the etiology of PPCM include antiangiogenic peptides, hormonal imbalances, a genetic predisposition, excessive inflammation, and autoimmune responses.[4] However, despite meaningful progress in understanding the pathophysiologic processes of PPCM, fundamental gaps in knowledge remain.

The clinical presentation of PPCM ranges from mild forms with unspecific symptoms, such as shortness of breath, fatigue, exercise intolerance, general discomfort, and peripheral edema, to severe forms with pulmonary edema and cardiogenic shock.[5] Evidence from the EORP Registry on PPCM (an ongoing prospective, international, multicenter, observational registry) reported that the time to diagnosis after initial presentation ranged from 19.4 to 38.3 days.[3] However, there is often a delay in PPCM diagnosis because patients with heart failure present with symptoms that are often attributed to the physiologic changes of pregnancy and the early postpartum period.[6]

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The absence of specific biomarkers further complicates the diagnosis of PPCM. Several molecular markers, such as natriuretic peptides, troponin, and C-reactive protein, have been up-regulated in some PPCM patients compared with healthy pregnant control subjects.[4] Unfortunately, these markers are rather nonspecific markers of heart failure, myocardial injury, and inflammation in the setting of cardiovascular disease and are not specific to PPCM. Serum levels of prolactin, microRNA-146a, plasminogen activator inhibitor-1, and placental growth factor have been used to differentiate patients with PPCM from other patients with heart failure.[7-9] However, none of these markers are specific to PPCM.

We therefore sought to explore the serum proteomic profile of patients with newly diagnosed PPCM, as compared with healthy postpartum mothers, to unravel novel protein biomarkers that would further our understanding of the pathogenesis of the disease and possibly enhance diagnosis.

MATERIAL AND METHODS

DATA SOURCE AND STUDY GROUP. The study was formally approved by the Human Research Ethics Committee of the University of Cape Town, South Africa (R033/2013), and it complied with the Declaration of Helsinki. All participants provided written informed consent before study entry.

The participants of this study represent a subset of patients who participated in the EORP registry for PPCM and a cohort of postpartum healthy controls (HCs) who had serum samples available for proteomics analysis.[3,10] Patients with PPCM had documented clinical evidence of LV systolic dysfunction (ie, left ventricular ejection fraction [LVEF] ≤45% on transthoracic echocardiography), in the absence of any other identifiable causes of heart failure, which developed within the first few months after delivery. The 29 HCs were recruited in Cape Town, South Africa, and had no clinical evidence of heart failure, as assessed by medical history, clinical examination, electrocardiogram, and echocardiogram. All participants in this study were recruited within the first 6 months after delivery; the HCs were time matched with the patients with PPCM.

Demographic and clinical data and serum samples for this study were collected from all participants (PPCM group and HCs) at the baseline visit before they took any heart failure–related medication. Serum samples were frozen immediately and were stored at −80 °C without thawing until protein analysis. The samples were shipped on dry ice from the different centers to the Cape Heart Institute in South Africa.

ABBREVIATIONS AND ACRONYMS

ADIPOQ = adiponectin

HC = healthy controls

HPLC = high-performance liquid chromatography

ITIH3 = inter-α-trypsin inhibitor heavy chain

LC-MS = liquid chromatography–mass spectrometry

LV = left ventricular

MMP = matrix metalloproteinase

NT-proBNP = N-terminal pro-B-type natriuretic peptide

PPCM = peripartum cardiomyopathy

QSOX1 = quiescin sulfhydryl oxidase 1

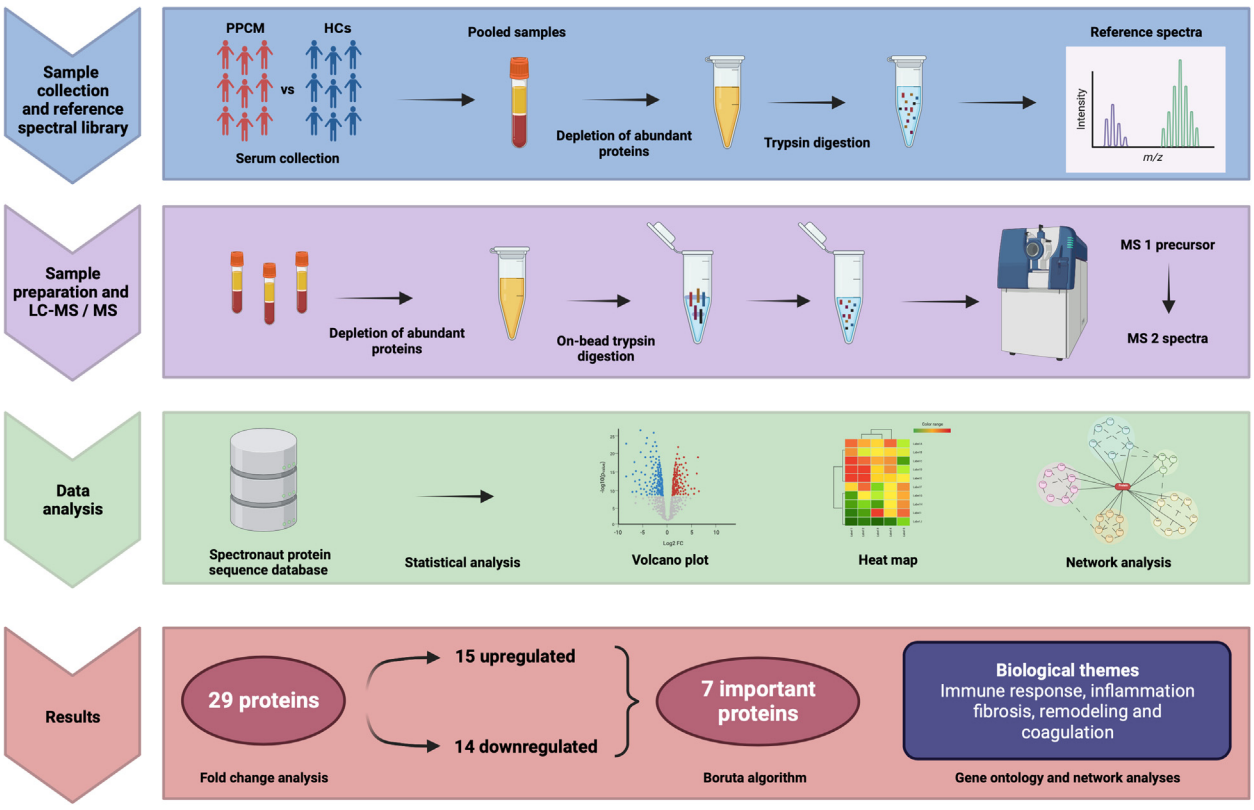
ROC = receiver-operating characteristic

ROS = reactive oxygen species

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 April 24, 2023; revised manuscript received July 14, 2023, accepted July 25, 2023.

FIGURE 1 Study Design and Methodology



A total of 113 serum samples were analyzed using data-independent acquisition (DIA). HCs = healthy controls; LC-MS/MS = liquid chromatography–tandem mass spectrometry; MS = mass spectrometry; PPCM = peripartum cardiomyopathy.

SERUM PROTEOMICS PROFILING OF HUMAN PARTICIPANTS. As illustrated in the study flowchart in Figure 1, the relative abundance profiles of proteins in 113 serum samples from PPCM patients and HCs were determined using data-independent acquisition-based label-free quantitative liquid chromatography-mass spectrometry (LC-MS). Samples were processed, digested, and analyzed at the Centre for Proteomic and Genomic Research in South Africa. A study-specific SWATH (sequential window acquisition of all theoretical fragment ion spectra) library was generated from a pooled sample made from an aliquot of each sample. All the samples underwent depletion of high-abundance proteins and subsequent high-performance liquid chromatography (HPLC)-based fractionation at the protein level and high-pH-based reverse phase fractionation at the peptide level focusing on salient biologic themes related to immune response proteins, inflammation, extracellular matrix remodeling, and blood coagulation.

To mitigate bias across the samples during sample preparation or data acquisition, samples were block randomized from preprocessing. Each country's samples were split into 6 different blocks, and each batch for the LC-MS was based on one-half of the plate. Calibrants were added after every 5 samples. Peptide medians of 2,500 ± 250 were identified in 99% of the samples.

Serum proteomics analysis with LC-MS. LC-MS was performed using an Evosep One LC system (Evosep) coupled to an AB Sciex 6600 TripleTOF mass spectrometer (AB Sciex). Peptide samples were separated on an Evosep Endurance column (150 μm × 15 cm; 1.9-μm particle size) maintained at 30 °C. Separation was achieved using a preformed linear gradient of solvents A and B (A: 0.1% formic acid; B: 100% acetonitrile/0.1% formic acid) over 21 minutes (standard 60SPD Evosep One method). For SWATH acquisition, precursor scans ranged from mass-to-charge ratio (m/z) 400 to 900 using an accumulation time of 100 ms, and fragment ions were acquired from m/z

100 to 1,800 with 15 ms accumulation time per window across 60 variable-width windows that overlapped by 0.5 Da. Collision energy spread was set to 0. The mass spectrometer was operated in positive ion mode using a NanoSpray III source (AB Sciex).

Sample preparation for library generation. Low-pH reverse phase fractionation was conducted with a Dionex Ultimate 3000 micro-HPLC system. The solvent system used was as follows: solvent A: millipore water, 0.1% formic acid; and solvent B: acetonitrile, 0.1% formic acid. For fractionation, 200 μg of F1 and 56 μg of F2 were injected onto a Waters Biosuite column (186002435; 3.5 μm × 150 mm × 2.1 mm). Ultraviolet detection of proteins was measured at 280 nm, and fractions were collected for 60 seconds.

High-pH reverse phase fractionation was conducted with a Dionex Ultimate 3000 micro-HPLC system. The solvent system used was as follows: solvent A: millipore water, 20 mM ammonium hydroxide (Sigma 338818); and solvent B: acetonitrile, 20 mM ammonium hydroxide. For fractionation, 180 μg of the peptide was injected onto a Phenomenex Gemini C-18 column (00F-4435-B0; 5 μm × 150mm × 2mm). Ultraviolet detection was measured at 214 nm, and fractions were collected for 60 seconds.

Data were analyzed by Spectronaut software version 15 (Biognosys) using a study-specific spectral library. A custom library was generated using the canonical isoform human FASTA database downloaded from the UniProt database on April 19, 2021. Specific modifications included deamidation of N and Q, oxidation of M as variable peptide modifications, and carbamidomethylation of C as a fixed modification. The Supplemental Methods contain a detailed experimental procedure.

STATISTICAL ANALYSIS. Proteomic data analysis was performed using Perseus software version 2.0.7.0 (Max Planck-Institute of Biochemistry). Statistical analysis was performed using SPSS software version 22.0 (IBM Corp) and GraphPad Prism software version 9.4.0 (GraphPad Software). Clinical data for continuous variables were expressed as mean ± SD and compared by Student's *t*-test. Categorical clinical data were compared between the PPCM and HCs groups by using the chi-square test or the Fisher exact test (when cell values were <5 or where the column marginal values were uneven).

Protein intensities were $\log_2$ transformed to stabilize the variance and reduce heteroscedasticity. Significant changes between the PPCM and HCs groups were determined by a nonpaired Student's *t*-test, and by the false discovery rate adjusted by Benjamini-Hochberg ($P < 0.05$ was considered significant).

TABLE 1 Demographics and Clinical Variables of Patients With PPCM and HCs

	PPCM (n = 84)	HCs (n = 29)	*P* Value
Age, y	30.6 ± 6.6	25.4 ± 8.0	<0.001
Parity ≥2	18 (24.3)	18 (62.1)	<0.001
Hypertension during pregnancy			0.010
No hypertension	63 (74.1)	29 (100.0)	
Hypertension without pre-eclampsia	12 (14.1)	0 (0.0)	
Pre-eclampsia	10 (11.8)	0 (0.0)	
Previous PPCM	9 (13.4)	0 (0.0)	0.038
NYHA functional class			<0.001
I/II	41 (49.0)	28 (100.0)	
III/IV	43 (51.0)	0 (0.0)	
Systolic BP, mm Hg	117.1 ± 19.4	117.1 ± 17.0	0.99
Diastolic BP, mm Hg	77.7 ± 13.4	76.9 ± 12.2	0.80
Heart rate, beats/min	95.6 ± 23.0	70.0 ± 12.0	<0.001
LVEDD, mm	58.5 ± 8.3	44.9 ± 7.0	<0.001
LVESD, mm	48.7 ± 8.7	45.0 ± 75.0	0.67
LVEF, %	33.5 ± 9.3	57.0 ± 8.8	<0.001

Values are mean ± SD or n (%).

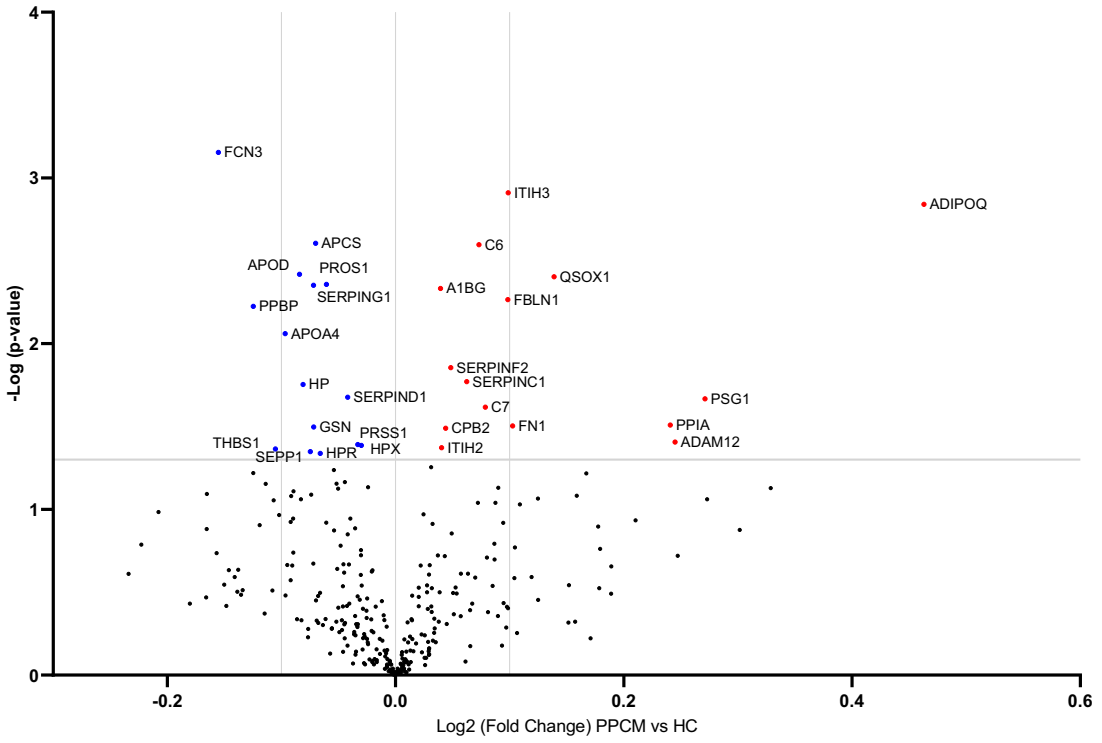
BP = blood pressure; HCs = healthy controls; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter; PPCM = peripartum cardiomyopathy.

A volcano plot was constructed from $\log_{10}$ values from the Student's *t*-test between PPCM and HCs and the $\log_2$ transformation of the fold changes. The intensities of the significant proteins were first *z*-score normalized and then normalized according to each protein (0%-100% range) before plotting the heatmap. Feature selection and classification were performed by Boruta R packages (R Foundation). Boruta performed 499 iterations (maxRuns = 500). Violin plots were used for an unadjusted bivariate comparison and distribution visualization of the significant proteins between the PPCM and HCs groups.

The diagnostic performance of each of the significantly up-regulated proteins was evaluated using the area under the curve (AUC). For this purpose, biomarkers were tested individually and in combination by using their relative intensities. Multiple biomarkers were combined by a binary logistic regression using the Hosmer-Lemeshow goodness of fit test and as guided by the findings of the Boruta algorithm. To increase the robustness of our estimates, we used 10-fold cross-validation of these AUC values. The SE of area was based on a nonparametric distribution assumption and a 95% CI. Sensitivity, specificity, and positive and negative predictive values of individual proteins, and of proteins in combination, were calculated with 95% CIs to differentiate the PPCM patients from the HCs.

Furthermore, an internal validation was performed to confirm the significance of the proteins identified

FIGURE 2 Volcano Plot Showing the Differential Level of Proteins Between PPCM and HCs Groups (n = 84 vs n = 29)



Each point represents a protein. The false discovery rate-corrected *P* values were calculated by permutation (250 times). Red represents proteins up-regulated in peripartum cardiomyopathy (PPCM) patients, whereas blue represents down-regulated proteins. Labeled proteins had significant changes ($P < 0.05$). ADAM12 = disintegrin and metalloproteinase domain-containing protein 12; ADIPOQ = adiponectin; APCS = serum amyloid P-component; APOA4 = apolipoprotein A-IV; APOD = apolipoprotein D; A1BG = α_{1B}-glycoprotein; C6 = complement component C6; C7 = complement component C7; CPB2 = carboxypeptidase B2; FBLN1 = fibulin-1; FCN3 = ficolin-3; FDR = false discovery rate; FN1 = fibronectin 1; HCs = healthy controls; HP = haptoglobin; HPR = haptoglobin-related protein; HPX = hemopexin; ITIH2 = inter-α-trypsin inhibitor heavy chain H2; ITIH3 = inter-α-trypsin inhibitor heavy chain H3; PPBP = platelet basic protein; PPIA = peptidyl-prolyl cis-trans-isomerase A; PROS1 = vitamin K–dependent protein S; PRSS1 = serine protease 1; PSG1 = pregnancy-specific β_1-glycoprotein 1, QSOX1 = quiescin sulfhydryl oxidase 1; SEPP1 = selenoprotein P; SERPINC1 = antithrombin-III; SERPIND1 = heparin cofactor 2; SERPINF2 = α_2-antiplasmin; SERPING1 = plasma protease C1 inhibitor; THBS1 = thrombospondin-1.

by the Boruta feature selection algorithm for the diagnosis of PPCM. The proteins were considered as input features for the machine learning classification model. The objective of this test was to predict PPCM through a K-Neighbors Classifier. We used the caret software package (Classification and Regression Training) in R (R Foundation) for preprocessing, model training, model prediction, and model evaluation. The data set was split into training and testing sets. We used 0.8 as the percentage of splitting. We then set the method to repeated cross-validation (10 folds and 10 repetitions). The model was set to knn (K nearest neighbors) for model training, the optimization metric was set to Accuracy. Once the model was trained, we evaluated its performance using the *predict()*-function to predict the output for the test set. Finally, we used the pROC library to plot sensitivity vs specificity of the model.

The Gene Ontology (GO) and pathway enrichment analysis were used to group differentially expressed proteins according to molecular functions, biologic processes, and protein class. A functional pathway annotation network to visualize functionally grouped terms comprehensively was plotted using ClueGO, a Cytoscape plug-in.[11] The relationship between the selected terms is defined on the basis of their shared genes. ClueGO initially creates a binary gene-term matrix and then calculates a term-term matrix by using chance-corrected κ statistics to determine the association strength between the terms. Terms are linked on the basis of a κ score (>0.4). Pseudo-heat maps that delineate the salient biologic themes that

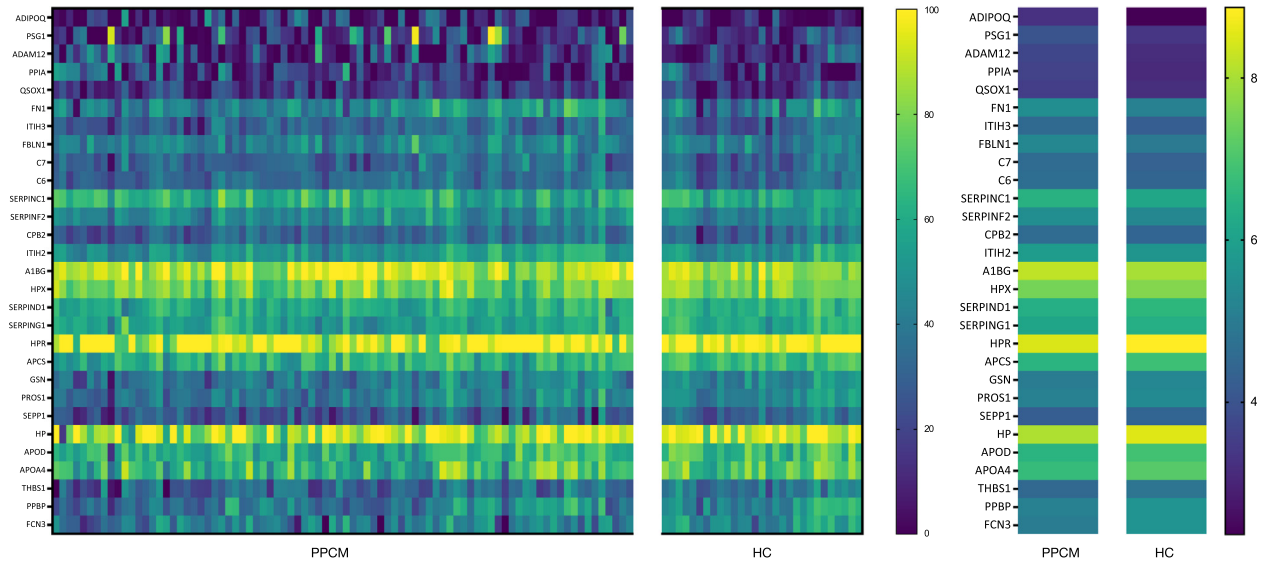
TABLE 2 Differentially Expressed Proteins Between Patients With Peripartum Cardiomyopathy and Healthy Controls

UniProtId	Gene Name	Protein Description	Mean Expression in Cases (log_2)	Mean Expression in Control Subjects (log_2)	Fold-Change (log_2)	FDR Corrected *P* Value
Up-regulated[a]						
Q15848	ADIPOQ	Important adipokine involved in the control of fat metabolism and insulin sensitivity, with direct antidiabetic, antiatherogenic, and anti-inflammatory activities	1.706	1.243	0.463	<0.001
P11464	PSG1	Belongs to the immunoglobulin superfamily	2.013	1.742	0.271	0.022
O43184	ADAM12	Involved in skeletal muscle regeneration, specifically at the onset of cell fusion	1.9	1.654	0.245	0.039
P62937	PPIA	PPIAs accelerate the folding of proteins	1.860	1.62	0.241	0.031
O00391	QSOX1	Catalyzes the oxidation of sulfhydryl groups in peptide and protein thiols to disulfides with the reduction of oxygen to hydrogen peroxide	1.821	1.682	0.139	0.004
P02751	FN1	Catalyzes the post-translational oxidative deamination of peptidyl lysine residues	2.459	2.356	0.103	0.031
Q06033	ITIH3	May act as a carrier of hyaluronan in serum or as a binding protein between hyaluronan and other matrix protein	2.178	2.079	0.099	0.001
P23142	FBLN1	May play a role in cell adhesion along protein fibers within the extracellular matrix	2.407	2.309	0.098	0.005
P10643	C7	Constituent of the membrane attack complex (that plays a key role in the innate and adaptive immune response	2.195	2.116	0.079	0.024
P13671	C6	Constituent of the membrane attack complex that plays a key role in the innate and adaptive immune response by forming pores in the plasma membrane of target cells	2.226	2.153	0.073	0.003
P01008	SERPINC1	Serine protease inhibitor in plasma that regulates the blood coagulation cascade	2.69	2.628	0.062	0.017
P08697	SERPINF2	Serine protease inhibitor	2.455	2.407	0.048	0.014
Q96IY4	CPB2	Down-regulates fibrinolysis by removing C-terminal lysine residues from fibrin	2.189	2.145	0.044	0.032
P19823	ITIH2	May act as a carrier of hyaluronan in serum or as a binding protein between hyaluronan and other matrix protein	2.547	2.507	0.040	0.042
P04217	A1BG	Response to elevated platelet cytosolic calcium	3.039	3	0.039	0.005
Down-regulated[b]						
O75636	FCN3	May function in innate immunity through activation of the lectin complement pathway	2.329	2.484	−0.155	0.001
P02775	PPBP	Stimulates DNA synthesis, mitosis, glycolysis, intracellular cAMP accumulation	2.357	2.482	−0.125	0.006
P07996	THBS1	Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions	2.168	2.273	−0.105	0.043
P06727	APOA4	May have a role in chylomicrons and VLDL secretion and catabolism	2.741	2.838	−0.097	0.009
P05090	APOD	Occurs in the macromolecular complex with lecithin-cholesterol acyltransferase	2.701	2.785	−0.084	0.004
P00738	HP	Induces morphologic changes and detachment through cytoskeletal reorganization	3.011	3.092	−0.081	0.018
P49908	SEPP1	Responsible for some of the extracellular antioxidant defense properties of selenium	2.073	2.148	−0.075	0.045
P07225	PROS1	Anticoagulant plasma protein	2.359	2.431	−0.072	0.004
P06396	GSN	Calcium-regulated, actin-modulating protein	2.333	2.405	−0.072	0.032
P02743	APCS	Interacts with DNA and histones and may scavenge nuclear material released from damaged circulating cells	2.701	2.771	−0.070	0.002
P00739	HPR	Primate-specific plasma protein associated with apolipoprotein L-I (-containing HDL)	3.083	3.149	−0.066	0.046
P05155	SERPING1	Plays a potentially crucial role in regulating important physiologic pathways	2.616	2.677	−0.061	0.004
P05546	SERPIND1	Thrombin inhibitor activated by the glycosaminoglycans	2.674	2.716	−0.042	0.021
P02790	HPX	Binds heme and transports it to the liver for breakdown and iron recovery	2.904	2.934	−0.030	0.041

[a]Differentially up-regulated proteins between patients with peripartum cardiomyopathy and healthy controls are arranged according to their fold changes. [b]Differentially down-regulated proteins between patients with peripartum cardiomyopathy and healthy controls are arranged according to their fold changes. Protein descriptions were obtained from String database (https://string-db.org/).

ADAM12 = disintegrin and metalloproteinase domain-containing protein 12; ADIPOQ = adiponectin; APCS = serum amyloid P-component; APOA4 = apolipoprotein A-IV; APOD = apolipoprotein D; A1BG = α_{1B}-glycoprotein; cAMP = cyclic adenosine monophosphate; CPB2 = carboxypeptidase B2; C6 = complement component C6; C7 = complement component C7; FBLN1 = fibulin-1; FCN3 =ficolin-3; FDR = false discovery rate; FN1 = fibronectin 1; GSN = gelsolin; HDL = high-density lipoprotein; HP = haptoglobin; HPR = haptoglobin-related protein; HPX = hemopexin; ITIH2 = inter-α-trypsin inhibitor heavy chain H2; ITIH3 = inter-α-trypsin inhibitor heavy chain H3; PPBP =platelet basic protein; PPIA = peptidyl-prolyl cis-trans-isomerase A; PROS1 = vitamin K-dependent protein S; PSG1 = pregnancy-specific β_1-glycoprotein 1; QSOX1 = quiescin sulfhydryl oxidase 1; SEPP1 = selenoprotein P; SERPINC1 = antithrombin-III; SERPIND1 = heparin cofactor 2; SERPINF2 = α_2-antiplasmin; THBS1 =thrombospondin-1; SERPING1 = plasma protease C1 inhibitor; VLDL = very low-density lipoprotein.

FIGURE 3 Heat Map Visualization for the Differentially Expressed Proteins Between Patients With PPCM and Healthy Controls



The color scale illustrates the relative expression level of each protein across all the patients and controls; yellow and green indicate higher and lower expression compared with the median expression value (black), respectively. The color intensity indicates the degree of protein up-regulation or down-regulation. Abbreviations as in Figure 2.

are mapped by the differentially expressed proteins between PPCM patients and HCs were created on the basis of the ClueGO network.

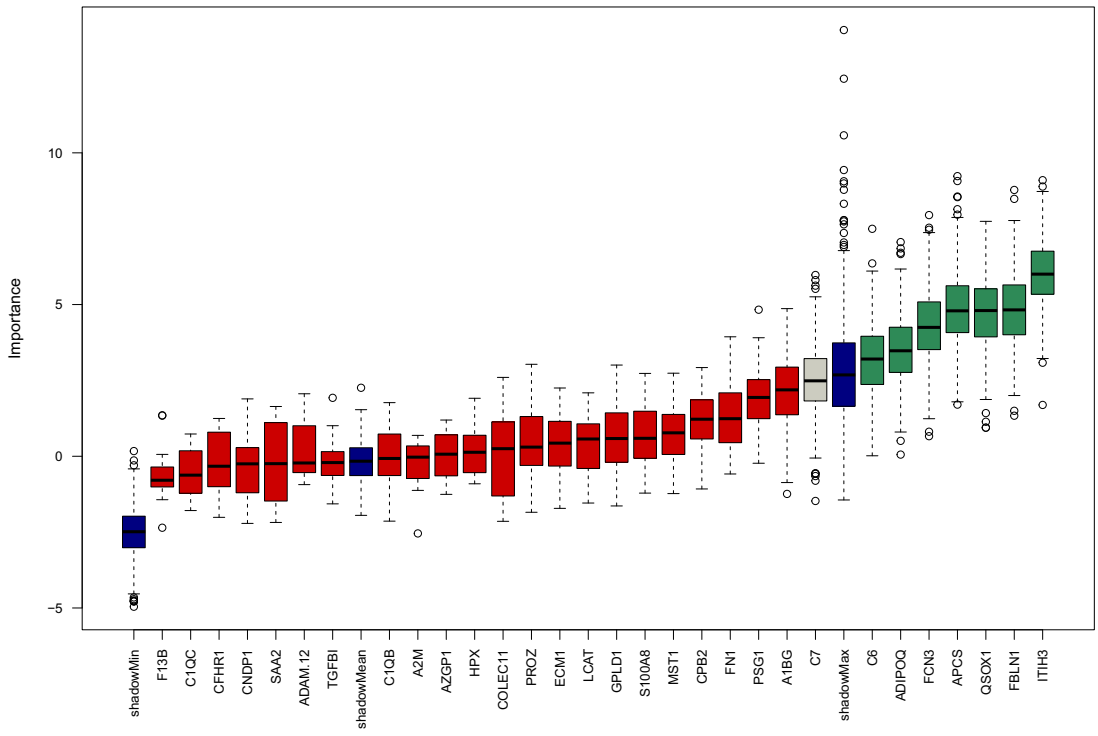
RESULTS

BASELINE CLINICAL CHARACTERISTICS OF PPCM AND HC GROUPS. In this study, 84 patients with PPCM were recruited across 7 countries (South Africa [47.6%], Nigeria [21.4%], Iraq [13.0%]; Israel [7.1%], Germany [5.9%], Poland [2.3%], and the Netherlands [2.3%]), and 29 healthy postpartum women were recruited from South Africa. The clinical characteristics of patients with PPCM and the HCs are summarized in Table 1. The PPCM cohort was older than the postpartum HCs (30.6 ± 6.6 years vs 25.3 ± 6.9 years; $P < 0.001$) and more frequently had a singleton pregnancy (75.7% vs 41.5%; $P < 0.001$).

Among the patients with PPCM, 13.4% had a previous diagnosis of PPCM, 14.1% had hypertension during pregnancy, and 11.8% had pre-eclampsia. Forty-three (51%) of the patients with PPCM were moderately to severely symptomatic of heart failure (NYHA functional class III or IV) at the time of diagnosis. Although there were no differences between the groups for systolic or diastolic blood pressure, patients with PPCM had significantly higher heart rates (95.6 ± 23.0 beats/min vs 72.6 ± 13.6 beats/min; $P < 0.001$). Similarly, on echocardiography, there were significant differences in LV end-diastolic diameter (58.5 ± 8.3 mm vs 45.6 ± 6.8 mm; $P < 0.001$) and LVEF (33.5% ± 9.3% vs 57.2% ± 8.9%; $P < 0.001$) between the patients with PPCM and the HCs.

DIFFERENTIAL PROTEIN EXPRESSION BETWEEN PPCM PATIENTS AND HCs. Proteomic profiling revealed a total of 329 proteins from the depleted serum samples. Fold change analysis identified 29 differentially regulated proteins between PPCM patients and HCs (Figure 2). Of these, 15 proteins were significantly up-regulated, whereas 14 were significantly down-regulated in the patients with PPCM, as compared with the HCs (Figure 2). Adiponectin (ADIPOQ) ($\log_2$ fold change 1.378; $P = 0.001$), quiescin sulfhydryl oxidase 1 (QSOX1) (1.101; $P = 0.004$), inter-α-trypsin inhibitor heavy chain H3 (ITIH3) (1.071; $P = 0.001$), pregnancy-specific β_1-glycoprotein 1 (1.207; $P = 0.022$), disintegrin metalloproteinase domain-containing protein 12 (1.185; $P = 0.039$), peptidyl-prolyl cis-trans-isomerase (1.182; $P = 0.031$), and fibronectin (1.074; $P = 0.031$) were among the top 7 up-regulated proteins, whereas ficolin-3 (FCN3) (0.898; $P = 0.001$), platelet basic protein (0.917; $P = 0.006$), thrombospondin-1 (0.930; $P = 0.043$), apolipoprotein A-IV (0.935; $P = 0.009$), apolipoprotein D (0.943;

FIGURE 4 Boruta Feature Selection and Classification



Boruta automatically performed feature selection on the significantly changed proteins. Seven proteins were classified as important, represented in green. Twenty-two proteins were classified as unimportant, represented in red. C7 was classified as tentative, represented in ivory. AZGP1 = alpha-2-glycoprotein 1; A2M = alpha-2-macroglobulin; CFHR1 = complement factor H related 1; CNDP1 = carnosine dipeptidase 1; COLEC11 = collectin subfamily member 11; C1QB = complement C1q B chain; C1QC = complement C1q C chain; ECM1 = extracellular matrix protein 1; F13B = coagulation factor XIII B chain; GPLD1 = glycosylphosphatidylinositol specific phospholipase D1; LCAT = lecithin-cholesterol acyltransferase; MST1 = macrophage-stimulating protein; PROZ = protein Z, vitamin K-dependent plasma glycoprotein; SAA2 = serum amyloid A2; S100AB = calcium binding protein A2; TGFBI = transforming growth factor-β inhibitor; other abbreviations as in Figure 2.

P = 0.004), haptoglobin (0.945; P = 0.018), and selenoprotein P (0.95; P = 0.045) were among the top down-regulated. Table 2 provides the details of all significantly up-regulated or down-regulated proteins.

A heat map visualization of the changes in expression also demonstrated the varied expression levels of each significantly regulated protein (Figure 3). The distribution of the differentially expressed proteins between PPCM patients and HCs is further illustrated in violin plots in Supplemental Figure 1.

FEATURE SELECTION OF BIOMARKERS USING BORUTA. We incorporated a Boruta automated feature selection and classification for selection of important protein markers from the differently changed proteins. The Boruta algorithm confirmed 7 proteins as significant in the differentiation between PPCM and HC groups (ie, ADIPOQ, QSOX1, ITIH3, complement component 6 (C6), fibulin-1 (FBLN1), FCN3, and serum amyloid P-component (APCS), whereas 22 proteins were confirmed unimportant (Figure 4). Complement component 7 was regarded as a tentative protein because it could not be classified as either important or unimportant.

RECEIVER-OPERATING CHARACTERISTIC CURVES AND OPTIMAL BIOMARKER COMBINATIONS. The area under the receiver-operating characteristic (ROC) analyses (Table 3, Figure 5) demonstrate the discriminative value of the proteins and protein combinations in differentiating patients with PPCM from HCs. N-terminal pro-B-type natriuretic peptide (NT-proBNP) had the best sensitivity to differentiate patients with PPCM from HCs (AUC: 0.808; 95% CI: 0.722-0.894). However, as demonstrated in Table 3, the sensitivity of NT-proBNP could be further

TABLE 3 Diagnostic Accuracy of Proteins, and Their Combinations, in Differentiating Patients With Peripartum Cardiomyopathy From Healthy Controls

Protein/Protein Combinations	AUC	95% CI
NT-proBNP/ITIH3/QSOX1/ADIPOQ	0.898	0.835-0.96
NT-proBNP/QSOX1	0.862	0.79-0.935
NT-proBNP/ITIH3	0.854	0.78-0.927
NT-proBNP/ADIPOQ	0.847	0.77-0.923
NT-proBNP/FBLN1	0.844	0.767-0.92
NT-proBNP/FN1	0.833	0.753-0.914
NT-proBNP (ng/L)	0.808	0.722-0.894
QSOX1/ADIPOQ/ITIH3	0.748	0.682-0.869
ADIPOQ/ITIH3	0.741	0.662-0.853
QSOX1/ITIH3	0.734	0.627-0.836
FN1/ITIH3	0.73	0.627-0.832
ADIPOQ/FN1	0.73	0.613-0.832
ADIPOQ/FBLN1	0.73	0.618-0.824
ADIPOQ/QSOX1	0.721	0.619-0.815
FN1/FBLN1	0.719	0.608-0.818
FBLN1	0.705	0.594-0.828
ADIPOQ	0.704	0.599-0.814
QSOX1/FBLN1	0.70	0.578-0.81
QSOX1/FN1	0.69	0.583-0.80
FN1	0.653	0.57-0.799
ITIH3	0.647	0.566-0.798
C6	0.645	0.55-0.799
A1BG	0.645	0.554-0.771
ECM1	0.643	0.542-0.766
QSOX1	0.642	0.538-0.766
C7	0.632	0.528-0.772
TGFBI	0.621	0.514-0.739
ADAM12	0.587	0.488-0.726
PSG1	0.587	0.48-0.713

The area under the curve (AUC) of the identified proteins estimates the predictive accuracy of each protein and protein combinations.

ECM1 = extracellular matrix protein 1; NT-proBNP = N-terminal pro-B-type natriuretic peptide; other abbreviations as in Table 2.

improved when combined with other biomarkers. Notably, the combination of NT-proBNP, QSOX1, ADIPOQ, and ITIH3 had the best discriminative performance (AUC: 0.898; 95% CI: 0.835-0.960) in differentiating the patients with PPCM from the healthy postpartum mothers.

CLASSIFICATION MODEL, TRAINING, AND EVALUATION. ITIH3, FBLN1, QSOX1, APCS, FCN3, ADIPOQ, and C6 identified as important proteins by the Boruta feature selection algorithm were used to develop a machine learning classification model to predict PPCM. The trained machine learning model achieved artificial intelligence accuracy of 76.2% for correct predictions of PPCM, thus indicating that the model is realistic and maybe good enough for clinical use. Supplemental Figure 2 shows the ROC curve (specificity vs sensitivity) of the model.

FUNCTIONAL ANALYSIS OF THE IDENTIFIED PROTEINS. We then conducted GO analysis of the 29 significant proteins on the basis of molecular function, biologic process, and cellular component (Figure 6). The proteins were grouped into 4 molecular function classes, including extracellular matrix structural constituent (GO:00005201), binding (GO:1901681), and enzyme regulator (GO:0030234). Annotation according to biologic process revealed that 18.5% (5 proteins) were biologic regulation enzymes (GO:0065007), 22.2% (6 proteins) were metabolic process modulators (GO:008152), and 14.8% were defense/immunity proteins. Supplemental Table 1 lists the proteins from the differentially expressed proteins that contributed to each GO.

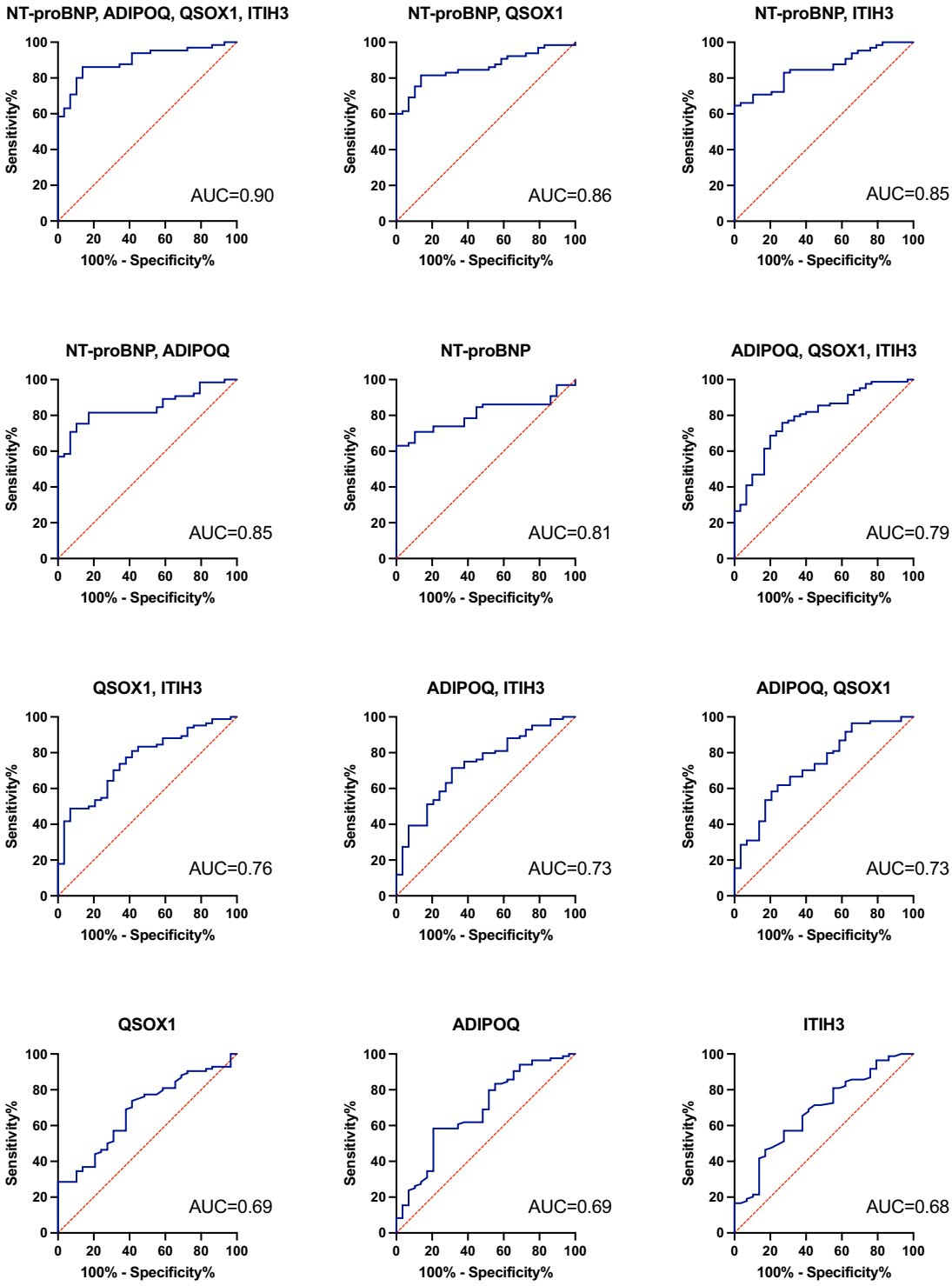
Different protein classes were involved in several differentially regulated processes between PPCM patients and HCs, a finding possibly suggesting differences in pathways likely enriched during PPCM signaling. Figure 7 provides a dynamic network structure that is based on pathways, as well as protein interaction data from the 29 significantly changed proteins between PPCM patients and HCs. Overall, the differentially regulated proteins observed were largely linked to salient biologic themes that may contribute to the pathophysiology of PPCM, including blood coagulation, inflammatory response, humoral immune response, response to oxidative stress, and leukocyte migration. The pseudo-heat maps in Table 4 delineate the main themes and the involved proteins.

DISCUSSION

To the best of our knowledge, this is the first multinational proteomic profile of patients with PPCM. In this multicenter proteomics study, we used an ethnically diverse group of PPCM patients to identify protein biomarker patterns in the serum that discriminate between PPCM patients and HCs. We found significant changes in the expression of 29 serum proteins in patients with PPCM. The identified proteomic signature delineates the complexity of the pathophysiology of PPCM. Moreover, we identified ADIPOQ, QSOX1, and ITIH3 as potential markers for a PPCM diagnosis, and a multiple-marker approach (combination of NT-proBNP, QSOX1, ADIPOQ, and ITIH3) further improved their diagnostic value (Central Illustration).

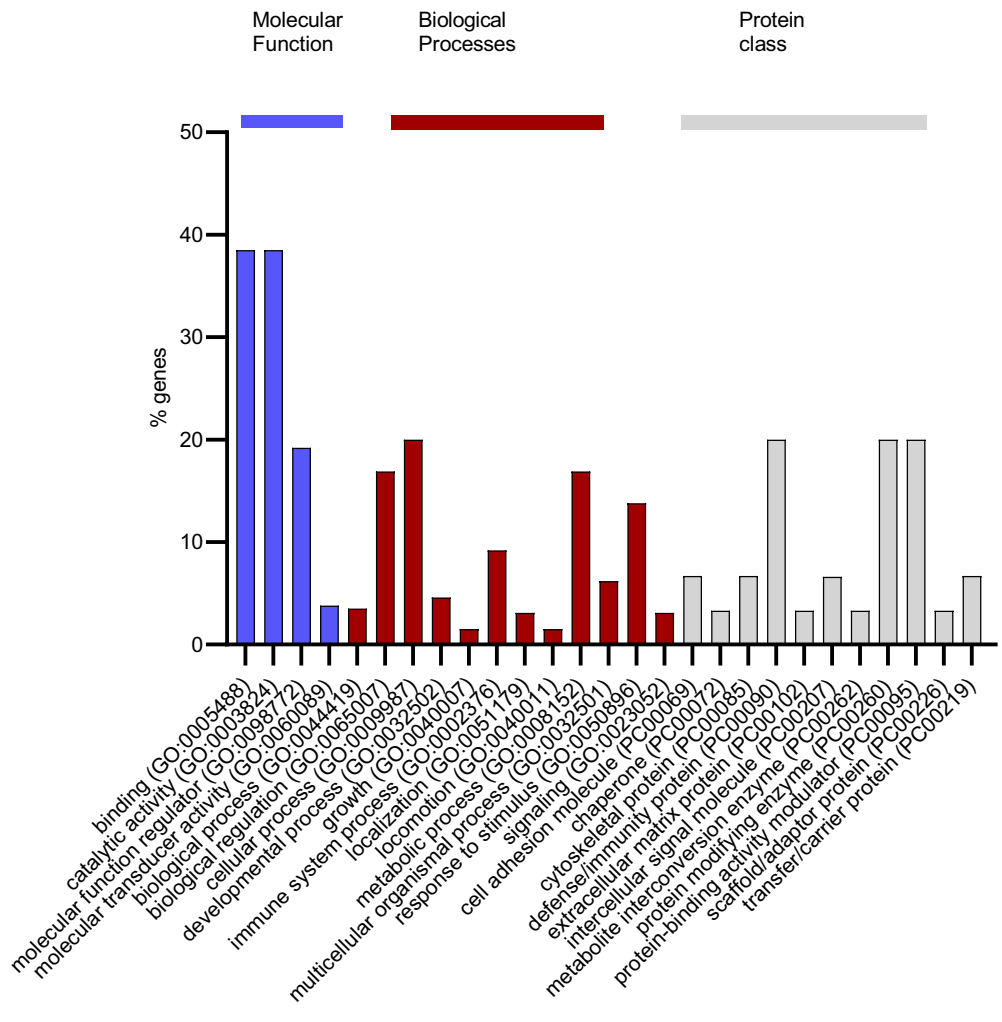
DIAGNOSTIC STRENGTH OF THE SIGNIFICANT PROTEINS. B-type natriuretic peptide is mainly synthesized, processed, and secreted by myocytes in the left ventricle as a response to myocytes stretched by

FIGURE 5 ROC Curves Estimating the Predictive Accuracy of Identified Proteins and Protein Combinations in Patients With Peripartum Cardiomyopathy and Healthy Controls



AUC = area under the curve; NT-proBNP = N-terminal pro-B-type natriuretic peptide; ROC = receiver-operating characteristic; other abbreviations as in Figure 2.

FIGURE 6 Gene Ontology Classification of Differentially Expressed Proteins in Patients With Peripartum Cardiomyopathy and Healthy Controls



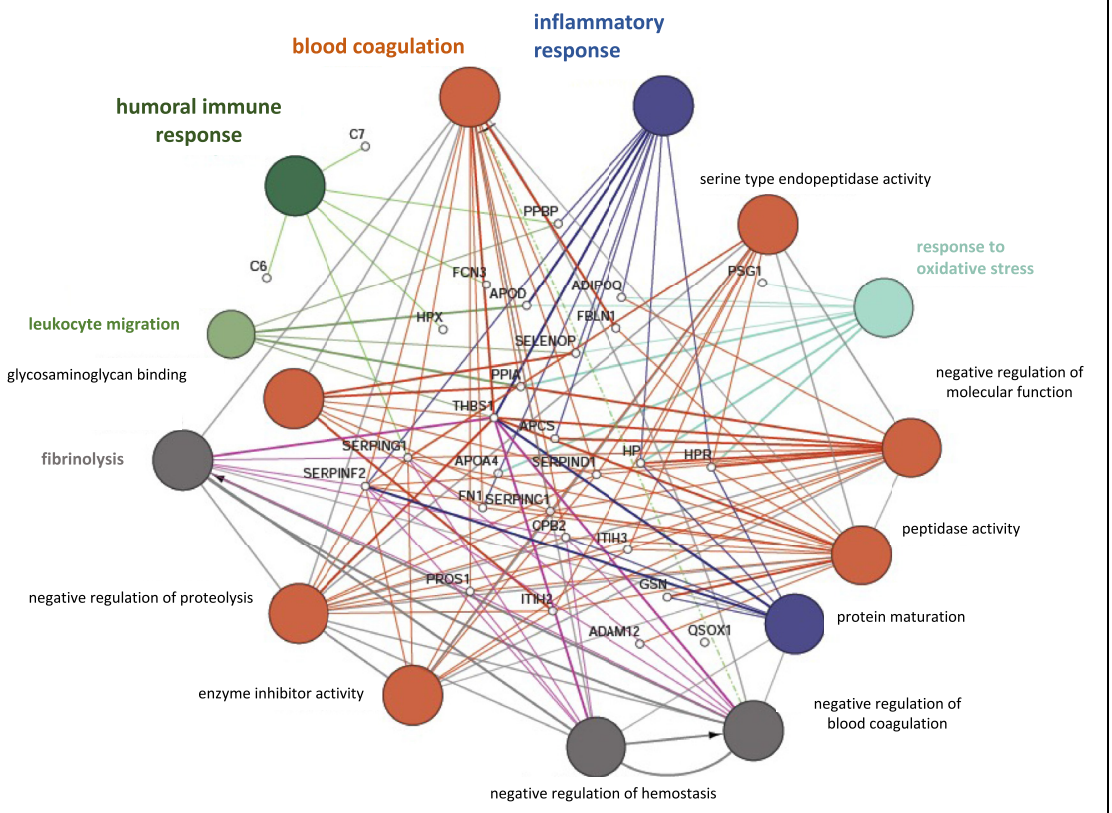
The proteins were grouped according to molecular function, biologic processes, and protein classes.

pressure overload or volume expansion of the ventricle.[12] In clinical practice, NT-proBNP is used to screen for heart failure.[13] Additionally, it has also been shown to be a valuable prognostic marker for pregnant women with heart disease.[14] Indeed, NT-proBNP has been shown to have prognostic value in predicting LV recovery in PPCM but remains nonspecific for PPCM.[14]

An important implication of this study is that the combination of NT-proBNP with ADIPOQ, QSOX1, and ITIH3 added to the predictive ability of NT-proBNP alone to identify women with PPCM. Studies on coronary artery disease and other cardiovascular diseases such as acute myocardial infarction have shown that a multimarker approach improved the diagnostic accuracy of biomarkers and their value in risk stratification.[15,16] Considering the complex pathophysiology of PPCM, detecting a single elevated biomarker, such as NT-proBNP, may underestimate total risk. A multibiomarker approach to diagnosis and risk assessment in PPCM is therefore warranted.

ROLE OF DIFFERENTIALLY EXPRESSED PROTEINS IN THE PATHOPHYSIOLOGY OF PPCM. ADIPOQ is an adipocyte-derived cytokine (adipokine), which is also synthesized in cardiac muscle cells and connective tissue cells within the heart.[17] ADIPOQ was shown to exert antiapoptotic, antihypertrophic, antifibrotic, and antioxidative properties at the myocardial level.[18] It has also been shown to have a

FIGURE 7 **ClueGO Functional Analysis Network of the Up-Regulated and Down-Regulated Proteins Between Patients With Peripartum Cardiomyopathy and Healthy Controls**



Functionally grouped network with terms as nodes linked on the basis of their κ score level (≥0.4), where only the label of the most significant term per group is shown. The node size represents the term enrichment significance. Functionally related groups partially overlap. SELENOP = selenoprotein P; other abbreviations as in Figure 2.

cardioprotective role in dilated cardiomyopathy.[19] Moreover, hyperadiponectinemia has previously been reported to be associated with cardiac (renal and pulmonary) diseases.[20] Controversially, ADIPOQ has been associated with high mortality in patients with advanced heart failure.[21]

Interestingly, the bloodstream ADIPOQ appears in different molecular subfractions that may exert different biologic functions.[22] Further research is needed to elucidate the mechanisms of ADIPOQ secretion and subfractionation and the roles ADIPOQ plays in different cardiovascular disease pathophysiology. The exact molecular signaling pathway of systemic and cardiac-derived ADIPOQ has been largely elucidated in experimental studies. All forms of ADIPOQ complexes have been found to mediate their cellular effects by binding to ADIPOQ receptors, AdipoR1 and AdipoR2. AdipoR forms complexes with effectors such as APPL1 that are highly expressed in the heart. Such a complex leads to activation of adenosine monophosphate-activated protein kinase, which mediates many cardioprotective effects. It has been reported that ADIPOQ increases *Akt* serine/threonine kinase 1 phosphorylation in cardiomyocytes.[22] The Akt signaling pathway is a signal transduction pathway that promotes survival and growth in response to extracellular signals. The Akt signaling pathway is activated by estrogens during pregnancy and plays an important role in cardioprotection.[23] The Akt level decreases in response to decreased estrogen level in the postpartum period. In turn, the signal transducer and activator of transcription-3 (STAT3) level increases in the postpartum period to provide the needed cardioprotection.[4] However, the activation of Akt in the postpartum phase lowers the antioxidative defense, and if combined with low STAT3 conditions, it accelerates inflammation and fibrosis in the peripartum heart.[24] It is therefore plausible that ADIPOQ-induced Akt phosphorylation

TABLE 4 Pseudo-Heat Map Showing Biologic Themes Contributed by the Differential Expressed Proteins Between the Peripartum Cardiomyopathy and Healthy Control Groups

Themes	Proteins	PPCM	HC
External encapsulating structure organization	QSOX1; CPB2; FBLN1; ADAM12; SERPINF2	Yes	No
	GSN	No	Yes
Leukocyte migration	PPIA; APOD	Yes	No
	SELENOP; PPBP	No	Yes
Humoral immune response	C6; C7; FCN3	Yes	No
	PPBP; HPX; SERPING1	No	Yes
Response to oxidative stress	PSG 1; PPIA; ADIPOQ	Yes	No
	SELENOP; APOA4; HP; HPR	No	Yes
Inflammatory response	SERPINF2; ADIPOQ; FN1; ADAM12	Yes	No
	HP; HPR; THBS1; SELENOP; APOD; APCS; PPBP	No	Yes
Blood coagulation	CPB2; FBLN1; SERPINF2; THBS1; FN1; PPIA	Yes	No
	PROS1; SERPIND1; SERPING1; SELENOP	No	Yes
Fibrinolysis	CPB2; SERPINF2	Yes	No
	PROS1; THBS1; SERPING1	No	Yes
Zymogen activation	CPB2; SERPINF2	Yes	No
	THBS1; HP; HPR	No	Yes
Glycosaminoglycan binding	FN1; PPIA; ITIH2; THBS1; SERPINC1	Yes	No
	SELENOP; SERPIND1	No	Yes
Regulation of peptidase activity	SERPINF2; SERPINC1; FN1; ITIH2; ITIH3	Yes	No
	GSN; SERPING1; SERPIND1; THBS1; PROS1	No	Yes
Hemostasis	CPB2; SERPINC1; SERPINF2; PPIA; FN1	Yes	No
	PROS1; THBS1; SERPIND1; SERPING1	No	Yes
Negative regulation of protein metabolic process	CPB2; ITIH2; SERPINF2; SERPINC1; ITIH3; PPIA; ADIPOQ; FBLN1	Yes	No
	PROS1; SERPIND1; THBS1; APOD; APCS	No	Yes
Negative regulation of catalytic activity	ITIH2; ITIH3; SERPINC1; ADIPOQ; SERPINF2	Yes	No
	PROS1; APCS; THBS1; HPR; HP; SERPIND1; SERPING1	No	Yes
Regulation of wound healing	SERPINC1; CPB2; SERPINF2	Yes	No
	APCS; SERPING1; PROS1; THBS1	No	Yes
Negative regulation of response to external stimulus	APCS; PROS1; THBS1; SERPING1; ADIPOQ; CPB2; SERPINF2	Yes	No
Peptidase activity	ADAM 12; FBLN1; SERPINF2; CPB2; ITIH2; ITIH3; FN1; SERPIND1; SERPINC1	Yes	No
	GSN; PROS1; THBS1; HPR; HP; SERPING1	No	Yes
Positive regulation of response to external stimulus	HPX; FCN3; THBS1	No	Yes
	CPB2; SERPINF2	Yes	No

SELENOP = selenoprotein P; other abbreviations as in Tables 1 and 2.

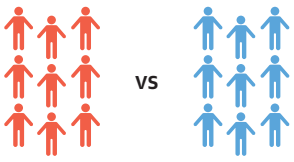
may modulate PPCM pathophysiology through a signaling cascade in which prolactin cleavage proteins such as matrix metalloproteinases (MMPs) and cathepsin D are further activated (Figure 8).

QSOX1, a sulfhydryl oxidase, is a catalyst for forming disulfide bonds in peptides and proteins. QSOX1 has been implicated in protein folding, extracellular matrix production, redox regulation, protection from apoptosis, and angiogenesis.[25] An experimental study revealed the potential cardioprotective role of QSOX1 upon acute stress in mice.[26] However, QSOX1 has been identified as a potential biomarker and independent predictor of LV dysfunction after myocardial infarction and decompensated heart failure.[27,28] Deceased level of STAT3 in the postpartum phase increases the reactive oxygen species (ROS), which in turn will increase cathepsin D and MMP activity. MMPs play a role in cleaving 23-kDa prolactin into the antiangiogenic, and apoptotic, and proinflammatory 16-kDa prolactin. Interestingly, QSOX1 has also been shown to activate MMPs post-translationally.[29] Given that MMPs facilitate proteolytic cleavage of prolactin in its antiangiogenic N-terminal 16-kDa fragment, QSOX1 could potentially have a regulatory role on MMP cleavage activity during PPCM signaling. Previous studies also indicated that QSOX1 is involved in several processes, including ROS activation, angiogenesis, apoptosis, and proliferation, that are also linked with pathophysiologic processes associated with PPCM (Figure 8).[25]

ITIH3, one of the constituents of plasma serine protease inhibitors, is related to the proinflammatory process and myocardial infarction.[30] Numerous studies identified up-regulation of inflammatory

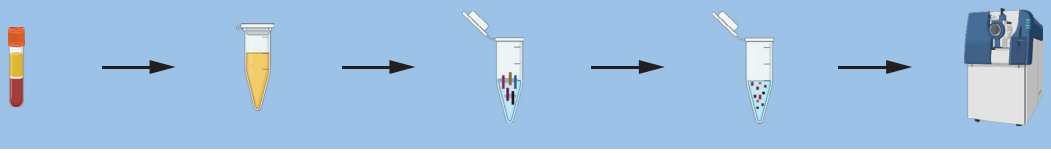
CENTRAL ILLUSTRATION **Serum Proteome Profile of Patients With Newly Diagnosed Peripartum Cardiomyopathy (n = 84) Was Compared With Matched Healthy Postpartum Mothers (n = 29)**

84 Patients With PPCM
- NYHA III/IV in 51% of cohort
- Mean LVEDD 58.5 ± 8.3
- Mean LVEF 33.5% ± 9.3%

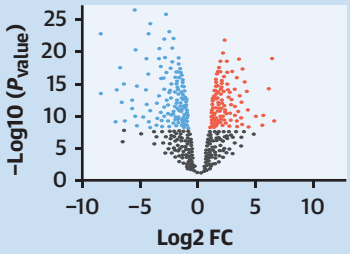


29 Healthy Postpartal Controls
- Asymptomatic
- Mean LVEDD 44.9 ± 7.0
- Mean LVEF 57.0% ± 8.8%

Proteomic Profiling Performed Using LC-MS/MS, Focusing on Salient Biological Themes Related to Immune Response Proteins, Inflammation, Extracellular Matrix Remodeling, and Coagulation



Volcano Plot

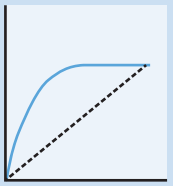


In patients with PPCM
- 15 proteins were significantly up-regulated
- 14 proteins were significantly down-regulated

Boruta Algorithm

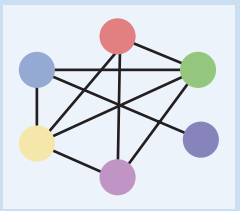
7 important proteins were identified to differentiate patients with PPCM from HCs: ADIPOQ, QSOX1, ITIH3, C6, FBLN1, FCN3, and APCS

AUC / ROC Curves



Combination of NT-proBNP, ADIPOQ, QSOX1 and ITIH3 had the best discriminative performance in differentiating PPCM from HC (AUC: 0.90)

Network Analyses



Network analyses confirmed the interaction of proteins regulating immune and inflammatory processes, fibrosis, and coagulation

Implications and Relevance of These Research Findings

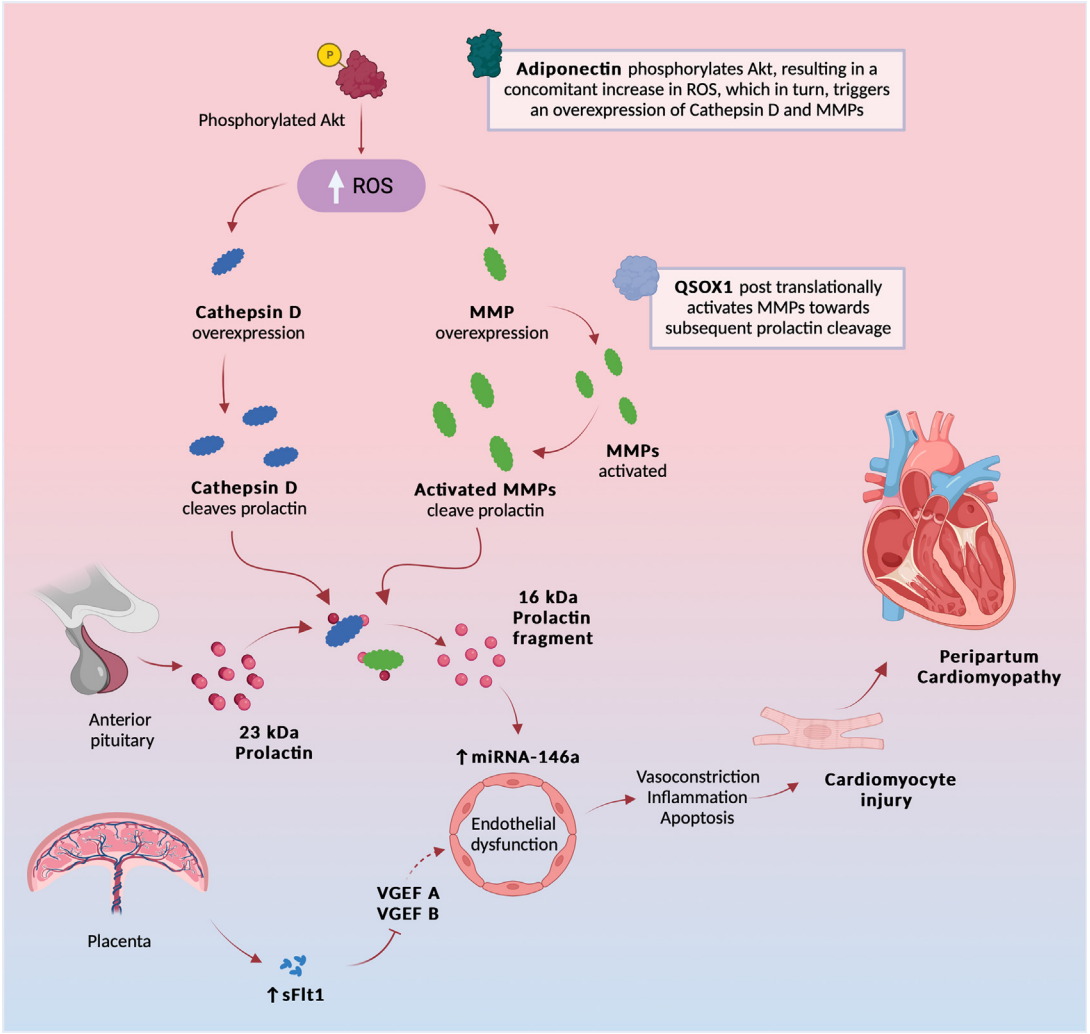
This study confirms the **complexity and multifactorial nature** of the **pathophysiology** of PPCM. Future research should explore the salient biological themes related to immune response proteins, inflammation, extracellular matrix remodeling, and coagulation, to further our understanding of the disease.

A multibiomarker approach had better diagnostic value in differentiating patients with PPCM from healthy postpartum women. A combination of **ADIPOQ, QSOX1 and ITIH3 together with NT-proBNP** correctly identified 90% of patients with PPCM and should be explored as a **new diagnostic testing modality**.

Kodogo V, et al. J Am Coll Cardiol HF. 2023;11(12):1708–1725.

Proteomic profiling performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). A volcano plot demonstrated that 15 proteins were significantly up-regulated, whereas 14 proteins were significantly down-regulated in patients with peripartum cardiomyopathy (PPCM). This was refined, by using the Boruta algorithm, which identified 7 important proteins that could differentiate patients with peripartum cardiomyopathy from healthy controls (HCs). Among these, combination of N-terminal pro-B-type natriuretic peptide (NT-proBNP), adiponectin (ADIPOQ), quiescin sulfhydryl oxidase 1 (QSOX1), and inter-α-trypsin inhibitor heavy chain H3 (ITIH3) had the best discriminative performance in differentiating peripartum cardiomyopathy from HCs. Network analyses confirmed the interaction of proteins regulating immune and inflammatory processes, fibrosis, and coagulation. This study confirms the complexity and multifactorial nature of the pathophysiology of peripartum cardiomyopathy. In this regard, a multibiomarker approach (combination of ADIPOQ, QSOX1, and ITIH3 together with NT-proBNP) had better diagnostic value in differentiating patients with peripartum cardiomyopathy from healthy postpartum women and should be explored as a new diagnostic testing modality. APCS = serum amyloid P component; AUC = area under the curve; C6 = complement component C6; FBLN1 = fibulin-1; FC = fold change; FCN3 = ficolin-3; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; ROC = receiver-operating characteristic.

FIGURE 8 Proposed Model on the Roles of ADIPOQ and QSOX1 in Peripartum Cardiomyopathy Signaling



Adiponectin (ADIPOQ) facilitates Akt phosphorylation, which in turn results in the generation of enhanced reactive oxygen species (ROS). This causes an up-regulation of cathepsin D and matrix metalloproteinases (MMPs). Quiescin sulfhydryl oxidase 1 (QSOX1) then post-translationally modifies matrix metalloproteinases, which can then cleave prolactin into the 16-kDa fragment that plays a role in the development of peripartum cardiomyopathy. sFLT = soluble fms-like tyrosine kinase-1; VEGF = vascular endothelial growth factor.

pathways in PPCM.[31,32] Nonetheless, to date, no study has specifically proposed ITIH3 as a potential biomarker of cardiovascular disease. Although the detailed biologic role of ITIH3 protein in cardiac disease is still to be clarified, our findings suggest that this protein may be involved in the pathophysiology of PPCM and warrants further mechanistic investigations.

CLINICAL RELEVANCE OF THE IDENTIFIED PROTEINS. The significant proteins contribute to several functional themes, including autoimmune system, blood coagulation, inflammation, and response to oxidative stress. Several compelling pieces of evidence support the view that PPCM is an autoimmune disease with multiple contributing factors and effector mechanisms.[33] High titers of autoantibodies against selected cardiac tissue proteins, such as the adenine nucleotide translocator, the branched-chain ketoacid dehydrogenase, cardiac myosin, and the β_2-adrenergic receptor proteins, have been found in the majority of women with PPCM.[33,34]

Approximately 7% of the more than 700 patients included in the PPCM EORP study had a thrombotic

event.[3] Several proteins involved in blood coagulation such as carboxypeptidase B2, FBLN1, fibronection-1, α_2-antiplasmin, and peptidyl-prolyl cis-trans-isomerase A were up-regulated in PPCM patients. The hypercoagulable state has been suggested to represent a protective adaptation to prevent hemorrhaging after delivery.[35] However, other events such as cardiac dilatation and endothelial injury may exacerbate the clotting in the postpartum period and lead to pathologic conditions.[36]

Interestingly, proteins that were observed to be down-regulated in patients with PPCM are implicated in mitochondrial adaptation to increased energy demands, vital processes needed to prevent heme-driven oxidative stress in the postpartum phase and lipid metabolism.

Mitochondria have a crucial role in producing and regulating ROS within most mammalian cells, including cardiomyocytes.[37] The ROS scavenging network coordinately works to maintain proper basal ROS levels and redox signaling in cells to control mitochondrial oxidative stress.[37] Haptoglobin and hemopexin, reported to counteract heme-driven oxidative stress in cardiovascular and non-cardiovascular conditions, were down-regulated in patients with PPCM.[38,39] Mitochondrial dysfunction has been implicated as a possible cause of heart diseases, including dilated cardiomyopathy.[40] Taken together, down-regulated proteins in PPCM provide insight into the importance of an integrated mitochondrial ROS production and scavenging system in the postpartum heart. However, the link between mitochondrial dysfunction and PPCM needs further investigation.

STUDY STRENGTHS AND LIMITATIONS. The present study has some limitations but also the strength of a relative larger sample size of patients presenting with PPCM (all postpartum) and postpartum HCs. The samples analyzed for this study were collected from a multiethnicity PPCM group. Differences may arise within subgroups. Several PPCM risk factors, such as ethnicity, account for the varied occurrence and phenotypes of PPCM in different regions. The identified proteins warrant further validation and quantification in a larger cohort. Moreover, involvement of the identified proteins in the pathogenesis of PPCM needs to be investigated in known murine models of PPCM. An important limitation is that we did not have another cohort for external validation of our model; nevertheless, we validated our model internally. This study also did not have a control arm of female patients with heart failure of other causes.

Because our measurements are based on relative protein expression and not on absolute values, our results do not indicate the precise protein cutoff levels that could be used for diagnostic purposes in PPCM. This would require future research using measurements (eg, enzyme-linked immunosorbent assay testing) to determine the actual protein concentration that would differentiate patients with PPCM from healthy women.

Future studies should aim to investigate proteins that could help to differentiate PPCM from other forms of cardiomyopathy. However, PPCM is a common diagnosis in many regions such as Africa. Finding an age-matched (non-PPCM) cardiomyopathy would be challenging. Several countries involved in the project unfortunately do not allow for the export of genetic material to centers where DNA analysis could be performed. This restriction limits elaboration on the genetics of the identified proteins.

CONCLUSIONS

We explored the serum proteome profiling of patients with PPCM and identified salient biologic themes related to immune response proteins, inflammation, extracellular matrix remodeling, and blood coagulation. This study confirms the complexity and multifactorial nature of the pathophysiology of PPCM. Moreover, a multibiomarker approach had better diagnostic value in differentiating patients with PPCM from healthy postpartum women. A combination of ADIPOQ, QSOX1, and ITHI3 together with NT-proBNP correctly identified 90% of patients with PPCM and should be explored as a diagnostic testing modality. Further research to elucidate the involvement of mitochondrial dysfunction in the pathophysiology of PPCM is needed.

ACKNOWLEDGMENTS The authors thank all participants for being a part of this study, as well as the members of the EORP for the study coordination, and they thank Ms Olivia Briton for her tremendous efforts with import and export logistics related to the shipment of the samples on dry ice and Ms Elani Muller for statistical advice.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Since the start of EORP, the following companies have supported the whole research program: Abbott Vascular International (2011-2021), Amgen Cardiovascular (2009-2018), AstraZeneca (2014-2021), Bayer AG (2009-2018), Boehringer Ingelheim (2009-2019), Boston Scientific (2009-2012), Bristol-Myers Squibb and Pfizer Alliance (2011-2019), Daiichi Sankyo Europe GmbH (2011-2020), Daiichi Sankyo Europe GmbH and Eli Lilly and Company Alliance (2014-2017), Edwards

Lifesciences (2016-2019), Gedeon Richter Plc (2014-2016), Menarini Int Op (2009-2012), Merck Sharp & Dohme-Merck and Co (2011-2014), Novartis Pharma AG (2014-2020), ResMed (2014-2016), Sanofi (2009-2011), Servier (2009-2021), and Vifor (2019-2022). Dr Hoevelmann has received speaker honoraria from Boehringer Ingelheim. Dr Tromp has received support from the National University of Singapore Start-up Grant, the Tier 1 Grant from the Ministry of Education, and the CS-IRG New Investigator Grant from the National Medical Research Council; has received consulting or speaker fees from Daiichi Sankyo, Boehringer Ingelheim, Roche Diagnostics, and Us2.ai; and owns patent US-10702247-B2 unrelated to the present work. Dr Sliwa has funded the proteomic analysis using her unconditional research grants; and has received support from Servier: Institut La Conference Hippocrate for funding of a statistical analysis program. 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 multibiomarker approach had better diagnostic value in differentiating patients with PPCM from healthy postpartum women. A combination of ADIPOQ, QSOX1, and ITIH3 together with NT-proBNP correctly identified 90% of patients with PPCM and should be explored as a new diagnostic testing modality.

TRANSLATIONAL OUTLOOK: This study confirms the complexity and multifactorial nature of the pathophysiology of PPCM. Future research should explore the salient biologic themes related to immune response proteins, inflammation, extracellular matrix remodeling, and coagulation, to further our understanding of the disease.

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KEY WORDS heart failure, peripartum cardiomyopathy, pregnancy, serum proteomics

APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.