Biomarkers and Their Relation to Cardiac Function Late After Peripartum Cardiomyopathy

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ABSTRACT

Background: Angiogenic imbalance involving the placental protein soluble Fms-like tyrosine kinase-1 (sFlt-1) and cleavage of the nursing-hormone prolactin by the enzyme cathepsin D (CD) both play a role in the pathogenesis of peripartum cardiomyopathy (PPCM). We hypothesized that angiogenic imbalance and increased activity of CD have a long-lasting impact in women with PPCM.

Methods and Results: A nationwide Danish cohort of women with PPCM (PPCM group, $n = 28$), age matched women with previous preeclampsia ($n = 28$) and uncomplicated pregnancies ($n = 28$) participated in a follow-up study including biomarker analysis, exercise testing and cardiac magnetic resonance imaging. The median time to follow-up was 91 months (range 27−137 months) for the PPCM group. Levels of sFlt-1, placental growth factor, *N*-terminal pro-natriuretic brain peptide, and copeptin were all significantly higher in the PPCM group. More women in the PPCM group had detectable CD activity (68%) compared with the preeclampsia group (29%) and uncomplicated pregnancies group (36%) ($P = .0002$). Levels of angiogenic factors and biomarkers correlated inversely with maximal exercise capacity and cardiac functional parameters assessed with cardiac magnetic resonance imaging.

Conclusions: Women with PPCM had higher biomarker levels and CD activity up to 7 years after diagnosis. Higher biomarker levels correlated inversely with maximal exercise capacity and markers of cardiac dysfunction suggesting that persistent angiogenic imbalance and increased CD activity is associated with residual cardiac dysfunction. (*J Cardiac Fail 2021;27:168−175*)

Key Words: Peripartum cardiomyopathy, heart failure, pregnancy.

Peripartum cardiomyopathy (PPCM) is a life-threatening condition presenting as acute heart failure in late pregnancy or in the months after delivery.[1] In more recent cohorts, most women recover cardiac systolic function, but complications such as need for mechanical circulatory support, heart transplantation or persistent heart failure have been reported to occur in 7%−34% with an associated mortality risk of 2%−13% within the first year after diagnosis.[2−5]

Several pathophysiologic mechanisms, including myocarditis, malnutrition, autoimmunity, and genetic predisposition, have been proposed,[6,7] but research in gestational humoral changes has provided new insights.[8] Soluble Fms-like tyrosine kinase 1 (sFlt-1) is a protein secreted from the placenta into the maternal circulation. It binds the angiogenic factors vascular endothelial growth factor and placental growth factor (PlGF), and in turn has an antiangiogenic effect on vascular homeostasis.[9] It was initially discovered that sFlt-1 levels are increased in preeclampsia, but increased levels are also observed in PPCM, and injection of sFlt-1 causes heart failure in a rodent model.[10] This angiogenic imbalance shared by PPCM and preeclampsia may explain why concomitant hypertensive disorders of pregnancy (HDP), which include gestational hypertension and preeclampsia, are present in about one-third of patients with PPCM.[3,5,11,12] Usually, the sFlt-1 serum concentration decreases to nonpregnant levels within a few weeks after delivery,[13,14] but elevated levels more than 2 years after delivery have been reported in patients with PPCM,[15] and higher baseline levels correlate with disease severity.[16]

Another pathophysiologic pathway explored involves the nursing hormone prolactin. Under certain circumstances

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Manuscript received June 13, 2020; revised manuscript received January 2, 2021; revised manuscript accepted January 2, 2021.

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1071-9164/$ - see front matter

https://doi.org/10.1016/j.cardfail.2021.01.002

such as oxidative stress and mutations in cardioprotective genes, activity of the enzyme cathepsin D (CD) increases with subsequent cleavage of prolactin into an antiangiogenic 16-kDa fragment also known as vasoinhibin.[17] Increased activity of CD and increased levels of vasoinhibin have been reported in patients with PPCM,[2,17,18] and inhibition of pituitary prolactin secretion with bromocriptine in addition to standard acute heart failure therapy seems to improve clinical outcome in these patients.[18,19]

Other biomarkers that have been investigated in PPCM include the hormone relaxin-2, which is involved in the hemodynamic adaptations to pregnancy[16,20]; the heart failure biomarkers copeptin and N-terminal pro-brain natriuretic peptide (NT-proBNP)[2,21,22]; and the marker of myocardial fibrosis/remodeling soluble suppression of tumorigenicity factor 2.[21,22]

Previously, we demonstrated impaired exercise capacity and cardiac dysfunction in a long-term follow-up study of a nationwide Danish cohort of mainly symptom-free women several years after a PPCM diagnosis.[23] The present work is based on our hypothesis that an angiogenic imbalance and increased CD activity persist after PPCM and might contribute to the persistent cardiac dysfunction observed late after PPCM.

Methods

Study Population

Survivors from a 10-year (2005−2014) nationwide Danish cohort of women with a diagnosis of PPCM were invited to participate in a clinical follow-up study (PPCM group). The identification of this cohort using nationwide registries and subsequent validation of the diagnosis through chart review has been described in detail elsewhere.[5] We also invited age-matched women with a history of severe preeclampsia (PE group) or uncomplicated pregnancies (UCP group) to participate. Severe preeclampsia was defined according to the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy.[24] The PE group and the UCP group were recruited from a population-based obstetric database that covers the Capital Region and accounts for approximately one third of all deliveries in Denmark.[25] A total of 84 women with 28 in each of the 3 groups were finally included in this study.[23]

Study Protocol and Procedures

The basic design and methods of the study have been described previously, but are summarized here.[23] The study protocol included nonfasting venous blood sampling, maximal cardiopulmonary exercise testing and cardiac magnetic resonance imaging (CMR). All measurements were performed during one visit in the same sequential order.

Biomarker Analyses

The following analyses were performed at Department of Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet. Standard laboratory analyses, including NT-proBNP and plasma prolactin were performed on the day of sample collection. Prolactin levels were measured using immunofluorometric assay on B.R.AH.M.S. Kryptor Compact PLUS from Thermo Fisher Scientific (Waltham, MA), and NT-proBNP levels were assessed using Sandwich Elektrochemiluminescens-immunoassay on Cobas 8000 from Roche Diagnostics International Ltd. (Rotkreuz, Switzerland). For other analyses, serum and plasma were stored at −80°C until the time of analysis.

For measurement of circulating relaxin-2, we used a commercial assay from R&D Systems (Minneapolis, MN). CD activity was determined with a fluorometric assay from AnaSpec Inc. (Fremont, CA). We modified the assay set up to measure plasma concentrations by increasing the enzymatic reaction time to 90 minutes. Also, the sample volume was downscaled to 10 μL. Soluble suppression of tumorigenicity factor 2 was measured by an enzyme-linked immunosorbent assay from SopaChem (Eke, Belgium). MR-proANP and copeptin was quantitated on a Kryptor Plus platform using reagents from Thermo Fisher Scientific.

At the Department of Clinical Biochemistry, Aarhus University Hospital, plasma concentrations of vascular endothelial growth factor, sFlt-1, and PlGF were assessed by a V-PLEX Custom Human Biomarkers assay from Meso Scale Diagnostics (Rockville, MD) according to the manufacturer's instructions.

Maximal Cardiopulmonary Exercise Testing

A total of 79 women completed a maximal cardiopulmonary exercise test using an upright exercise bicycle (Ergoselect, Ergoline, Germany). A ramp exercise protocol with an initial workload of 50 W followed by 50 W increments every 2 minutes until complete exhaustion was used. Maximal exercise capacity was defined as peak oxygen consumption (peak VO_2) and was calculated as milliliters per kilogram per minute (standard pressure temperature dry).

CMR

CMR was performed using a 1.5 T magnetic resonance scanner (GE Optima MR450, GE Healthcare). Two-, 3-, and 4-chamber views as well as a transaxial and a short axis cine stack covering the whole heart were obtained allowing analysis of atrial and ventricular volumes as well as systolic and diastolic functional parameters. A total of 79 women completed CMR.

CMR Image Analysis

We used semiautomated CMR software (cvi42, Circle Cardiovascular Imaging, Canada) for image analysis. Left ventricular (LV) epicardial and endocardial borders were traced on the short axis stack to calculate LV volumes, myocardial mass and ejection fraction. From the short axis stack, a LV volume−time curve was constructed and LV end-systolic volume, LV end-diastolic volume, LV peak filling rate (LVPFR), LV stroke volume, and LV ejection

fraction (LVEF) were obtained. To assess LV diastolic function, left atrial (LA) endocardial borders were traced on the transaxial cine stack and a LA volume−time curve was constructed, which enabled determination of the 3 LA emptying volumes that contributed to total LV filling: LA passive emptying volume (LAPEV), LA active emptying volume, and LA conduit volume,[26] which further allowed calculation of the LA passive emptying fraction and LA active emptying fraction.[23] All volumes and LV mass were indexed to body surface area according to the Mosteller method. We defined diastolic dysfunction as LVPFR/LV end-diastolic volume ratio of less than 2.5.[27]

Statistical Analyses

Continuous data are presented as means and standard deviations (SD) in case of normally distributed data and as medians and ranges in case of non-normally distributed data. Global differences between the 3 groups were analyzed using analysis of variance in case of normal distribution and Kruskal−Wallis test in case of non-normal distribution. Graphical methods were used to test for normality of the data. Categorical data are presented as numbers and percentages and differences were analyzed using χ^2 test or Fisher's exact test as appropriate. A 2-sided P value of less than .05 was considered statistically significant.

For variables that were significant in the global test, post hoc analyses were performed using Student t test or Mann−Whitney U test as appropriate to compare the PPCM group with the PE group and the UCP group, respectively, as well as to compare the PE group with the UCP group. To adjust for multiple comparisons, the Bonferroni method was applied, and P values were multiplied by 3 accordingly.

Post hoc analyses of covariance with adjustment for body mass index (BMI) were done for biomarkers that differed between the 3 groups in the global tests. We further compared biomarker levels in the PPCM group by concomitant HDP and in all women by diastolic dysfunction using the Mann−Whitney U test. Spearman correlation analyses were used to explore associations between the levels of angiogenic factors, cardiac biomarkers and cardiac functional parameters. Statistical analyses were performed using SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC) and R for Windows (R: A language and environment for statistical computing, version 4.0.3).

The study protocol was approved by the Danish Data Protection Agency (RH-2016-174, I-Suite 04729) and the Capital Region's Committee on Biomedical Research Ethics (H-1-2014-131). All participants provided written informed consent.

Results

Demographic and clinical characteristics of the 84 participants in relation to the index pregnancy and at follow-up are presented in Table 1. The mean LVEF at PPCM diagnosis was 27% and 4 of the participating women (14%) had suffered a major adverse event (persistent heart failure with an LVEF of 35% or less at 12 months after PPCM diagnosis or mechanical circulatory support requirement).

The PPCM group had a median time to follow-up of 91 months (range 27−137 months) after the index delivery. Most women in the PPCM group (86%) reported to be without symptoms of heart failure at follow-up, but they had significantly lower exercise capacity and LVEF compared with the PE group and the UCP group. At follow-up mean LVEF in the PPCM group was 62% (SD 6%, range 53%−73%), 69% (SD 4%, range 66%−76%) in the PE group, and 67% (SD 5%, range 62%−76%). Furthermore, they exhibited CMR signs of diastolic dysfunction with significantly lower LVPFR, LAPEV, and LA passive emptying fraction compared with the 2 other groups (Table 1). A total of 4 women in the PPCM group did not undergo exercise testing for the following reasons: extreme obesity (BMI > 60 kg/m^2) (1 woman), physical inability caused by multiple sclerosis developed after PPCM onset (1 woman), white coat hypertension with an in-hospital resting systolic blood pressure of more than 200 mm Hg (1 woman), and technical problems with the equipment and inability to reschedule the test (1 woman). Also, 1 woman from the UCP group did not undergo exercise testing technical problems with the equipment and an inability to reschedule the test. In addition, 3 women in the PPCM group did not undergo CMR for the following reasons: implantable cardioverter defibrillators incompatible with CMR (2 women) and claustrophobia (1 woman). Both in the PE group and in the UCP group, 1 woman could not complete CMR owing to claustrophobia.[23]

Women in the PPCM group had significantly higher median levels of NT-proBNP, copeptin, and PlGF compared with women in both the PE group and in the UCP group (Table 2). The median level of sFlt-1 was higher among women in the PPCM group compared with women in the UCP group, but no differences were found in sFlt-1 levels between the PPCM group and the PE group or between the PE group and the UCP group. We were not able to detect CD enzyme activity in all blood samples, but it was detected in significantly more women in the PPCM group than in the other 2 groups of participants. Adjustment for BMI was done for the 5 biomarkers that differed between groups in the global tests (sFlt-1, PlGF, copeptin, NT-proBNP, and detectable CD enzyme activity); PlGF and copeptin levels were still significantly higher in the PPCM group in these analyses (Supplementary Table S1). No other differences in the median levels of biomarkers were found between the groups.

In a post hoc sensitivity analysis, we excluded outlying values of NT-proBNP (>50 pmol/L) and sFlt-1 (>100 pg/mL). In this analysis, the observed differences between groups remained significant (Supplementary Table S2 and Supplementary Figs. 1 and 2).

No significant differences were found in the PPCM group by concomitant HDP or not (Table 3).

In an analysis of biomarker levels in all 84 women by diastolic dysfunction, defined as LVPFR/LV end-diastolic

Table 1. Comparison of Demographic and Clinical Characteristics Between Women With Peripartum Cardiomyopathy, Preeclampsia, and Uncomplicated Pregnancies in the Index Pregnancy and at Follow-up

	PPCM (n = 28)	Preeclampsia (n = 28)	Uncomplicated pregnancy (n = 28)	*P* value
Index pregnancy characteristics				
Age at delivery, years	30.7 (6.0)	30.5 (5.0)	31.0 (5.2)	.73
Race, n (%), Caucasian Black	28 (100) 0	28 (100) 0	27 (96) 1 (4)	.364
Prepregnancy BMI, kg/m^2	28.3 (6.4)	22.8 (3.2)	21.3 (1.8)	<.0001
Parity, n (%)				
0	12 (43)	24 (86)	12 (43)	
1	11 (39)	3 (11)	10 (36)	.007
≥2	5 (18)	1 (3)	4 (21)	
Concomitant HDP, n (%)				
Gestational hypertension	2 (7)	0	0	
Preeclampsia	11 (39)	28 (100)	0	<.0001
HELLP	2 (7)	0	0	
Other comorbidities, n (%)	8 (29)†	4 (14)	8 (29)	.350
Follow-up characteristics				
Median time from index delivery to follow-up (range), months	91 (27-137)	95 (26-143)	101 (25-146)	.603
Age, years	38.0 (6.9)	39.1 (5.3)	38.8 (5.6)	.754
BMI, kg/m^2	30.0 (8.4)	23.3 (4.1)	22.6 (3.0)	<.001
NYHA functional class, n (%)				
I	24 (86)	28 (100)	28 (100)	
II	3 (11)	0	0	.078
III	1 (3)	0	0	
On daily antihypertensive/heart failure medication‖, n (%)	13 (46)*,†	3 (11)	0	<.0001
Systolic BP, mm Hg	129 (16)†	129 (16)§	119 (11)	.019
Diastolic BP at rest, mm Hg	83 (14)†	82 (10)§	73 (9)	.007
Peak VO_2, mL/kg/min	29.6 (7.2)*,†	43.2 (11.1)	45.4 (10.2)	<.0001
Left ventricular ejection fraction, %	62 (6)*,†	69 (4)	67 (5)	<.0001
Left ventricular peak filling rate, mL/s/m^2	229 (49)*,†	276 (57)	265 (45)	.005
Left ventricular end-diastolic volume, mL/m^2	84 (14)	78 (10)	80 (10)	.233
Left ventricular end-systolic volume, mL/m^2	31 (7)*	25 (8)	27 (6)	.008
Left atrial passive emptying volume, mL/m^2	13 (5)*,†	19 (4)	20 (3)	<.0001
Left atrial active emptying volume, mL/m^2	11 (4)	9 (2)	9 (2)	.129
Left atrial active emptying fraction, %	38 (9)	35 (9)	35 (8)	.359

BMI, body mass index; BP, blood pressure; HELLP, hemolysis elevated liver enzymes low platelets; HDP, hypertensive disorders of pregnancy; NYHA, New York Heart Association; PPCM, peripartum cardiomyopathy.

Global analyses of difference between means, medians and proportions across the 3 groups were performed by analysis of variance, Kruskal-Wallis, or χ^2 test, respectively.

*The PPCM group was significantly different compared with preeclampsia group (post hoc analyses were performed by Student *t* test, Mann−Whitney *U* test, or χ^2 test; *P* values were multiplied by 3 to correct for multiple comparisons, a.m. Bonferroni).

†The PPCM group was significantly different compared with uncomplicated pregnancy group (post hoc analyses were performed by Student *t* test, Mann−Whitney *U*, or χ^2 test; *P* values were multiplied by 3 to correct for multiple comparisons, a.m. Bonferroni).

§Preeclampsia group significantly different compared with uncomplicated pregnancy group (post hoc analyses were performed by Student *t* test, Mann−Whitney *U*, or χ^2 test; *P* values were multiplied by 3 to correct for multiple comparisons, a.m. Bonferroni).

‖Daily antihypertensive/heart failure medications: angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, beta-blockers, calcium antagonists, and diuretics.

volume ratio of less than 2.5, NT-proBNP was the only biomarker that differed significantly between groups, with a higher median NT-proBNP in the subgroup with diastolic dysfunction (Table 4).

Among the biomarkers that differed between the 3 groups, sFlt-1 correlated inversely with a marker of diastolic function, LAPEV (Spearman r = −0.32, *P* = .003). PlGF level correlated inversely with LAPEV (Spearman r = −0.29, *P* = .010) and peak VO_2 (Spearman r = −0.47, *P* < .0001). Copeptin level correlated inversely with another marker of diastolic function, LA passive emptying fraction (Spearman r = −0.26, *P* = .020) and peak VO_2 (Spearman r = −0.38, *P* = .0006). Also, NT-proBNP correlated with LV end-systolic volume (Spearman r = 0.25, *P* = .024). Finally, BMI correlated with levels of sFlt-1 (Spearman r = 0.24, *P* = .03), PlGF (Spearman r = 0.33, *P* = .002), and copeptin (Spearman r = 0.37, *P* = .0006) (Supplementary Table S3).

Discussion

In this nationwide follow-up study of asymptomatic women late after PPCM, we found that levels of NT-proBNP, copeptin, sFlt-1, and PlGF, despite overlaps, were significantly higher compared with age-matched control groups of women with previous severe preeclampsia and previous uncomplicated pregnancies, respectively. Exploratory analyses revealed correlations between levels of biomarkers and angiogenic factors and markers of diastolic dysfunction as well as maximal exercise capacity.

No other studies have, to the best of our knowledge, reported on biomarker analyses this late after PPCM

Table 2. Levels of Biomarkers in the 3 Groups of Women at Follow-up

	PPCM ($n = 28$)	Preeclampsia ($n = 28$)	Uncomplicated pregnancy ($n = 28$)	P value‡
sFlt-1, pg/mL	74.9 (46.8−142.5)†	67.6 (42.3−99.1)	63.4 (46.5−94.4)	.0065
PlGF, pg/mL	7.0 (5.6−9.5)*,†	6.0 (1.6−10.2)	5.8 (3.6−8.8)	.0004
sFlt-1/PlGF ratio	10.3 (5.0−25.4)	11.3 (6.2−38.5)	11.1 (6.4−19.4)	.372
VEGF, pg/mL	82.1 (31.1−413.0)	58.1 (28.9−601.8)	57.1 (28.0−166.0)	.075
VEGF/sFlt-1 ratio	1.00 (0.48−4.71)	0.94 (0.37−7.05)	0.92 (0.44−2.83)	.672
NT-proBNP, pmol/L	13.8 (5.0−188.0)*,†	6.8 (5.0−19.9)	6.9 (50.0−0.8)	.0154
Copeptin, pmol/L	5.6 (2.3−45.5)*,†	3.2 (1.3−8.8)	3.7 (2.2−8.9)	.0001
MR-proANP, pmol/L	48.0 (17.9−303.5)	49.7 (28.0−113.3)	66.0 (32.0−129.9)	.127
ST2, ng/mL	25.0 (8.1−51.3)	23.9 (11.4−61.5)	27.2 (13.1−66.7)	.893
Prolactin, 10^{-3} IU/L	149.0 (62−267)	143.5 (76−507)	145.0 (59−580)	.485
Detectable CD activity (yes/no)	19 (68%)*,†	8 (29%)	10 (36%)	.0073
Relaxin-2, pg/mL	2.7 (1.7−20.6)	3.0 (1.7−27.0)	2.5 (1.7−18.0)	.527
Creatinine, μmol/L	71.6 (16.1)	69.0 (10.0)	68.7 (9.3)	.616

All data are presented as medians and ranges except detectable CD enzyme activity, which is presented as numbers and percentages.

CD, cathepsin D; MR-proANP, mid-region pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-brain natriuretic peptide; PlGF, placenta growth factor; PPCM, peripartum cardiomyopathy; sFlt-1, soluble Fms-like tyrosine kinase-1; ST2, suppression of tumorigenicity-2; VEGF, vascular endothelial growth factor.

Global test of difference between groups using nonparametric (Kruskal-Wallis) test.

*The PPCM group was significantly different compared with preeclampsia group (post hoc analyses were performed by the Mann−Whitney U test).

†The PPCM group was significantly different compared with uncomplicated pregnancy group (post hoc analyses were performed by the Mann−Whitney U test).

diagnosis. Goland et al.[15] combined biomarker analyses with echocardiography in 29 women with recovered LVEF at a mean of 32 months after PPCM diagnosis and compared the results with a control group. It was found that women with PPCM had significantly higher sFlt-1 levels and persistent echocardiographic signs of systolic and diastolic dysfunction.[15]

In other studies, the sFlt-1 level was measured at the time of PPCM diagnosis. Higher baseline levels of sFlt-1 were associated with subsequent higher New York Heart Association functional class and adverse clinical events in the prospective IPAC cohort.[16] Mebazaa et al[21] obtained blood samples for analysis from 83 women at the time of PPCM diagnosis (median of 4 weeks postpartum) and compared results with those of healthy women within 24 hours of delivery and with patients with acute heart failure of other etiologies. It was found that sFlt-1 levels in women with PPCM were like those of acute heart failure patients, but lower than those of healthy delivering women.[21] This finding may, at least in part, be explained by the rapid physiologic decrease in sFlt-1 level seen postpartum in normal healthy women, where sFlt-1 levels have been reported to decrease from approximately 6000 to 8000 pg/mL in term gestations (37−40 weeks) to around 100 pg/mL 5−6 weeks postpartum.[13] Whether postpartum sFlt-1 levels differ between women with PPCM and healthy delivering women matched on time since delivery remains unknown.

Table 3. Biomarker Levels at Follow-up in Women With Peripartum Cardiomyopathy by Hypertensive Disorder of Pregnancy (HDP) in the Index Pregnancy

	HDP ($n = 15$)	No HDP ($n = 13$)	P value
NT-proBNP, pmol/L	7.5 (5.0−129.0)	20.4 (5.0−188.0)	.109
sFlt-1, pg/mL	74.9 (46.8−102.5)	74.0 (52.1−142.5)	.908
ST2, ng/mL	24.3 (11.2−32.2)	27.2 (8.1−51.3)	.475
PlGF, pg/mL	7.2 (6.0−9.5)	6.9 (5.6−9.0)	.147
Copeptin, pmol/L	5.0 (3.4−45.5)	5.6 (2.3−16.8)	.908
MR-proANP, pmol/L	47.4 (17.9−303.5)	56.0 (30.9−195.0)	.102
VEGF, pg/mL	84.9 (36.2−413.0)	82.0 (31.1−133.3)	.322
VEGF/sFlt-1 ratio	1.1 (0.6−4.7)	0.9 (0.5−1.7)	.147
Prolactin, 10^{-3} IU/L	160.0 (75.0−267.0)	138.0 (62.0−248.0)	.782
Detectable CD activity (yes/no)	12 (80%)	7 (54%)	.139
Relaxin-2	2.8 (1.8−18.3)	2.6 (1.7−20.6)	.712

All data are presented as medians and ranges except detectable cathepsin D enzyme activity, which is presented as numbers and percentages.

Abbreviations as I Tables 1 and 2.

Table 4. Biomarker Levels in All Women by Diastolic Dysfunction Defined as an LVPFR/LVEDV Ratio of Less Than 2.5 (Indexed to Body Surface Area).

	Diastolic Dysfunction ($n = 12$)	No Diastolic Dysfunction ($n = 67$)	P value
NT-proBNP, pmol/L	12.5 (5.0−129.0)	6.8 (5.0−90.8)	.031
sFlt-1, pg/mL	69.9 (46.8−108.9)	67.4 (42.3−102.5)	.517
ST2, ng/mL	21.1 (8.1−38.9)	25.6 (11.4−66.7)	.123
PlGF, pg/mL	6.4 (5.1−9.5)	6.4 (1.6−10.2)	.235
Copeptin, pmol/L	3.8 (2.5−45.5)	3.7 (1.3−11.2)	.405
MR-proANP, pmol/L	57.1 (32.0−303.5)	50.3 (17.9−129.9)	.312
VEGF, pg/mL	79.0 (43.2−214.5)	59.3 (28.0−601.8)	.104
VEGF/sFlt-1 ratio	1.1 (0.7−2.8)	0.9 (0.4−7.0)	.246
Prolactin, 10^{-3} IU/L	133.0 (75.0−369.0)	144.0 (59.0−580.0)	.290
Detectable CD activity (yes/no)	8 (67%)	29 (43%)	.135
Relaxin-2	2.7 (1.7−15.5)	2.6 (1.7−27.0)	.886

All data are presented as medians and ranges except detectable CD enzyme activity, which is presented as numbers and percentages.

LVEDV, left ventricular end-diastolic diameter; LVPFR, left ventricular peak filling rate. Other abbreviations as in Tables 1, 2, and 3.

The median PlGF levels were higher in the PPCM group both at the time of follow-up in our study and at the time of PPCM diagnosis in Mebazaa's study. This finding contrasts with preeclampsia, where sFlt-1 levels are elevated but PlGF levels are low resulting in a high sFlt-1/PlGF ratio.[28] This factor is being used as a first trimester screening tool and in the second and third trimesters of pregnancy as a diagnostic tool to predict the imminent development of preeclampsia.[29] The sFlt-1/PlGF ratio and sFlt-1 levels have further been reported to remain high up to 1 year after preeclampsia and to be associated with arterial aging.[30] Both sFlt-1 and PlGF may be elevated in patients with heart failure, and in a cohort of almost 800 nonpregnant patients with stable heart failure higher levels of both sFlt-1 and PlGF were associated with adverse outcomes.[31] This discrepancy between PlGF levels observed in/after preeclampsia (low) and in heart failure (high) may, in theory, be useful in PPCM diagnostics. However, further prospective studies, including comparisons between PlGF levels in matched women with PPCM and normal controls, are required to take the normal physiologic decrease in PlGF levels postpartum into account.

The mean BMI was higher in the PPCM group in our cohort and correlated positively with PlGF and sFlt-1 levels in the combined 3 groups in our study. It has previously been shown that sFlt-1 is expressed in adipose tissue,[32] but in contrast with our findings levels have been found to be inversely correlated with BMI in both pregnant and nonpregnant populations.[32,33] PlGF is also expressed in adipose tissue,[34] and a higher PlGF level is associated with higher BMI and the metabolic syndrome.[35] It may reflect that overweight women in our PPCM group have increased adipose tissue expression of PlGF, which somehow triggers increased sFlt-1 release that subsequently causes cardiac dysfunction in susceptible women, but this putative mechanism remains hypothetical. Other sources of extraplacental sFlt-1 and PlGF secretion may be platelet−monocyte aggregates, endothelial cells and bone marrow.[31,36,37]

Hilfiker-Kleiner et al. elegantly demonstrated how increased expression and activity of the enzyme CD and consequently cleavage of prolactin to the antiangiogenic and cardiomyocyte-damaging 16 kDa vasoinhibin plays a pivotal role in the pathophysiology of PPCM.[2,17] We found that significantly more women with PPCM had detectable CD activity supporting the concept of this pathway, which further seems to persist for as long as 7 years (median) after PPCM. This finding could suggest that interventions specifically targeting the mechanisms underlying PPCM probably should not be restricted the acute and subacute phase of the disease in case of persistent heart failure.

During pregnancy, the level of the hormone relaxin-2 increases as relaxin-2 participates in the hemodynamic adaptations to pregnancy such as vasodilation, increasing cardiac output, heart rate and plasma volume.[20] In the IPAC cohort, higher relaxin-2 levels at baseline were associated with higher LVEF 2 months after PPCM diagnosis.[16] Lower relaxin-2 levels were found in a German cohort of women diagnosed with PPCM within the first week postpartum compared with normal postpartum women, whereas no differences were found when PPCM was diagnosed later.[38] Also, we observed no differences at follow-up. Studies suggest that a role of relative relaxin deficiency in heart failure or PPCM may not be prominent as human recombinant relaxin, serelaxin, has failed to improve outcome both clinically in acute heart failure,[20,39] and in an experimental mouse model of PPCM.[38]

Even though the median NT-proBNP level was within the normal range and there were overlaps between groups, the NT-proBNP level was significantly higher in the PPCM group compared with the other 2 groups of study participants. NT-proBNP is the gold standard biomarker of heart failure of various etiologies and elevated levels have repeatedly been noted in PPCM. Baseline levels have in some but not all studies proven useful as a prognostic marker.[2,21,40,41]

Unlike Mebazaa et al,[21] we found a higher median level of copeptin in women with PPCM compared with both control groups. Copeptin is derived from the vasopressin pre−pro-hormone proarginine vasopressin and is a prognostic marker in heart failure, but its use in PPCM remains uncertain. The close relation of vasopressin to prolactin given the pituitary origin raises the question whether more general pituitary dysfunction could be an important feature of PPCM, but this remains speculative.

It has been questioned whether women with concomitant HDP should be included under the PPCM diagnosis as outcome seem to differ with some reports of a more favorable disease course in women with concomitant HDP.[42] In this study, no differences in biomarker levels between women with PPCM by concomitant HDP were observed at long-term follow-up.

Our study has strengths and limitations that must be considered. First, there is a risk of selection bias because the PPCM participants, in comparison with nonparticipants, may represent a less severe subgroup of patients with PPCM. However, an analysis of characteristics of participants and nonparticipants in our study has previously been reported[23] and no differences in baseline characteristics, major adverse events or other outcome variables 12 months after diagnosis could be identified. Owing to the retrospective identification of our PPCM cohort, we did not have data on baseline biomarker or angiogenic factor levels and, therefore, our study does not provide any new knowledge with regards to the prognostic significance of these biomarkers. But we report the longest time to clinical follow-up in a nationwide setting and show how different pathophysiologic pathways could be affected late after PPCM; both the affected biomarkers and the reduced maximal exercise capacity could be related to residual deconditioning in patients with prior PPCM but alterations in biomarkers may also reflect reverse causality.

Biomarkers and angiogenic factor may be influenced by other factors such as age, renal function, and BMI, but owing to the relatively small sample size, we chose to only perform minimal adjustments for these possible mediators, which is a limitation of the results. We have previously shown that the difference in peak VO_2 between groups was attenuated with adjustment for age and BMI at follow-up.[23]

Numbers are relatively small, and comparisons, for example, between women with and without concomitant HDP, should be perceived as exploratory. Also, we performed many exploratory correlation analyses and found correlations between biomarker levels and cardiac functional parameters as well as BMI. Correlations do not imply causality, but it could be hypothesized that persistently increased levels of sFlt-1 and PlGF as well as CD activity contribute to or reflect chronic cardiac impairment late after PPCM diagnosis. Future studies should investigate this and address whether interventions that reduce sFlt-1, PlGF and/or CD can improve clinical outcome.

Conclusion

Women with PPCM had significantly higher levels of sFlt-1, PlGF, copeptin, and NT-proBNP and more often detectable CD activity 7 years after PPCM diagnosis compared with 2 control groups of women with previous severe preeclampsia and previous uncomplicated pregnancy, respectively, matched on age and year of index delivery. Elevated biomarker levels further correlated inversely with maximal exercise capacity and markers of diastolic dysfunction suggesting that both a persistent angiogenic imbalance and increased CD activity are associated with residual cardiac dysfunction.

Declaration of Competing Interest

All authors have reviewed and approved the final manuscript. All authors have read the journal authorship agreement and policy on disclosure of potential conflicts of interest. After the preparation of this article, ASE has been employed by Novo Nordisk A/S, Denmark. JPG, MJ, MGH, KS, NV, FG, and PD have no conflicts of interest.

Acknowledgments

We thank Jesper Kromann, radiographer, CMR technologist, of the Department of Diagnostic Radiology, Copenhagen University Hospital, Rigshospitalet, Annemie S. Bojer, MD, and Marie Bayer Elming, MD, PhD, both of the Department of Cardiology, Copenhagen University Hospital, Rigshospitalet for assistance with CMR image recording and analysis.

Funding

The work was funded by The Danish Heart Foundation, Rigshospitalet's Research Foundation, Arvid Nilsson's Foundation, and Aase & Ejnar Danielsen's Foundation. The funding sources had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2021.01.002.

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