

Outcome in German and South African peripartum cardiomyopathy cohorts associates with medical therapy and fibrosis markers

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Abstract

Aims This study aims to compare the clinical course of peripartum cardiomyopathy (PPCM) cohorts from Germany (G-PPCM) and South Africa (SA-PPCM) with fibrosis-related markers to get insights into novel pathomechanisms of PPCM.

Methods and results G-PPCM ($n = 79$) and SA-PPCM ($n = 72$) patients and healthy pregnancy-matched women from Germany ($n = 56$) and South Africa ($n = 40$) were enrolled. Circulating levels of procollagen type-I (PINP) and type-III (PIIINP) N-terminal propeptides, soluble ST2, galectin-3, and full-length and cleaved osteopontin (OPN) were measured at diagnosis (baseline) and 6 months of follow-up. Both cohorts received standard heart failure therapy while anticoagulation therapy was applied in 100% of G-PPCM but only in 7% of SA-PPCM patients. In G-PPCM patients, baseline left ventricular ejection fraction (LVEF) was lower, and outcome was better (baseline LVEF, $24 \pm 8\%$, full recovery: 52%, mortality: 0%) compared with SA-PPCM patients (baseline LVEF: $30 \pm 9\%$, full recovery: 32%, mortality: 11%; $P < 0.05$). At baseline, PINP/PIIINP ratio was lower in SA-PPCM and higher in G-PPCM compared with respective controls, whereas total OPN was elevated in both collectives. Cleaved OPN, which increases PIIINP levels, is generated by thrombin and was reduced in patients receiving anticoagulation therapy. High baseline galectin-3, soluble ST2, and OPN levels were associated with poor outcome in all PPCM patients.

Conclusions SA-PPCM patients displayed a more profibrotic biomarker profile, which was associated with a less favourable outcome despite better cardiac function at baseline, compared with G-PPCM patients. Use of bromocriptine and anticoagulation therapy in G-PPCM may counteract fibrosis and may in part be responsible for their better outcome.

Keywords Peripartum cardiomyopathy; Fibrosis; Biomarker; Therapy; Outcome

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Introduction

Peripartum cardiomyopathy (PPCM) is a rare disease, characterized by an onset of heart failure in women towards the end of pregnancy, during delivery, or within the first 6 months postpartum¹. The incidence of PPCM worldwide is estimated to be one in 1000 pregnancies with the highest

incidences reported in Africans and Afro-Americans.² The pathophysiology of PPCM involves increased oxidative stress, impaired angiogenesis, and activation of immune and inflammatory reactions.³ Cardiac fibrosis has been proposed to play an important role in the initiation and progression of several cardiac diseases,⁴ whereas the implication of cardiac fibrosis in the pathogenesis of PPCM is controversial.^{5,6}

Several studies have assessed the association of collagen metabolism-related biomarkers procollagen type-I N-terminal propeptide (PINP) and procollagen type-III N-terminal propeptide (PIIINP) in the diagnosis and prognosis of cardiac diseases.⁷ Moreover, increased levels of galectin-3 (Gal3) and soluble ST2 (sST2), both contemporary markers of cardiac inflammation and fibrosis, have a predictive value for adverse outcome in heart failure patients.^{8,9} Osteopontin (OPN) has been shown to enhance inflammation and fibrosis¹⁰ and is essential for collagen secretion. Thereby, OPN causes an increase of myocardial stiffness in heart failure patients.¹¹ Furthermore, there is evidence that specifically the thrombin-cleaved N-terminal OPN leads to myocardial fibrosis by enhancing the synthesis of collagen type III.¹²

While in most PPCM patients, cardiac function recovers, some patients progress into irreversible heart failure with high morbidity, the need for left ventricular assist device (LVAD), heart transplantation, and high mortality (1.4% to 28%).^{13–18} To date, it is hard to predict whether a patient will recover or remain in heart failure, as no prognostic biomarker exists for PPCM.

Comparison between PPCM collectives of different ethnic backgrounds suggests that African PPCM patients may have a different pathophysiology and a lower chance for full recovery, compared with non-African patients,^{14,15,19} but a direct comparison of different ethnic cohorts has not been performed yet. Thus, we aimed to directly compare the outcome of two large PPCM registries, one with patients from South Africa (SA-PPCM), including only patients of African ethnicity, and one from Germany (G-PPCM), with only Caucasian patients included. In these cohorts, we assessed clinical and medical history and outcome, as well as circulating factors of cardiac fibrosis, and compared them with pregnancy-matched healthy controls of each corresponding ethnic background.

Methods

Study design and patient enrolment

This study was approved by the Human Research Ethics Committee of the University of Cape Town, Cape Town, SA (R033/2013) and by the local ethics committee of Hannover Medical School, Hanover, G (3242-2016). The study complies with the Declaration of Helsinki, and all patients and controls gave written informed consent. Seventy-two PPCM patients (SA-PPCM) were enrolled at Groote Schuur Hospital (University of Cape Town, SA), and 79 patients (G-PPCM) were recruited at Hannover Medical School (Hannover Medical School, G). German patients of African origin were excluded from this study as well as SA-PPCM with Caucasian background, to allow a distinction between these two ethnicities.

Echocardiography with Doppler colour flow mapping was performed to measure left ventricular dimensions and function according to the American Society of Echocardiography guidelines.²⁰

Blood pressure and heart rate were measured non-invasively, and the New York Heart Association (NYHA) functional class of each patient at baseline and follow-up visit was evaluated by a physician who was provided with the clinical data but blinded to the laboratory results.

Analysis of outcome

The follow-up period is the duration of the pregnancy for patients who presented peripartum, plus the 6 months of follow-up visit, which was accomplished between 3 and 7 months after diagnosis. Poor outcome was defined as death, heart transplantation, LVAD implantation, or left ventricular ejection fraction (LVEF) $\leq 35\%$ at 6 months as previously described.²¹ Recovery was defined as gain of 10% in LVEF and LVEF $\geq 35\%$ at 6 months of follow-up; full left ventricular recovery was defined as LVEF $\geq 50\%$ at 6 months of follow-up.

Biomarkers measurements

Plasma and serum were collected at baseline and at the 6 months of follow-up visit. Aliquots were stored at -80°C . N-terminal pro-brain natriuretic peptide (NT-proBNP) plasma levels were measured by routine laboratory workup as described.²² The measurement of plasma Gal3 (Human Galectin-3 Platinum Elisa, eBiosciences, San Diego, USA), sST2 (Presage[®] ST2 Assay, Critical Diagnostics, San Diego, USA), serum PINP (PINP Elisa Assay, Cloud-Clone Corp, Texas, USA), and serum PIIINP (PIIINP Elisa Assay, Cloud-Clone Corp) was conducted according to the manufacturer's instructions. OPN was measured using Bio-Plex Pro Assay (Human Cancer Biomarker Panel 1, Bio-Rad, Hercules, USA).

In addition, cleavage of OPN by thrombin was measured by western blot [Anti-Human Osteopontin (O-17) Rabbit IgG Affinity Purify, Immuno-Biological Laboratories Co. Japan].

We recruited 96 healthy pregnancy-matched women without any history of cardiac disease and with a normal physical examination. Forty controls were recruited in SA, of whom 17 women were in the prepartum period and 23 were in the early postpartum period. Fifty-six healthy women were recruited in Germany. All German controls were in the postpartum period.

Statistical analysis

Data were analysed using GraphPad Prism version 7.03 for Windows (La Jolla, California, USA) and SPSS (IBM, NYC, USA) version 24 for Windows. Continuous data are expressed

as mean \pm standard deviation or median (range) and categorical data as frequencies (%). Normal distribution was assessed using D'Agostino and Pearson omnibus normality test. χ^2 test and Fisher's exact test were used for discrete variables; unpaired *t*-test with Welch's correction or Mann-Whitney *U* test were used for continuous variables. As follow-up data were not available for all patients, some statistical analyses were only performed between the control and patients groups. For multiple-group comparison, we used one-way ANOVA with Bonferroni post-test or Kruskal-Wallis with Dunn's post-test for continuous variables and χ^2 test for discrete variables. A *P*-value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 151 patients ($n = 72$ SA-PPCM and $n = 79$ patients G-PPCM) with confirmed diagnosis for PPCM²³ were analysed (Figure 1A and B). Comparison between the two cohorts showed that the mean LVEF, heart rate, and HIV rates in

SA-PPCM at time of diagnosis were significantly higher than in G-PPCM (Table 1). G-PPCM were older, presented with higher left ventricular end-diastolic-diameter, higher rates of hypertensive disorders (including chronic hypertension and hypertensive disorders of pregnancy), hypercholesterolaemia, family history for cardiovascular disease, previous caesarean section, and more patients in NYHA class III/IV than SA-PPCM (Table 1). All other clinical baseline characteristics, including NT-proBNP levels, were comparable among G-PPCM and SA-PPCM patient cohorts (Table 1). Medical records revealed that 100% of G-PPCM and 75% of SA-PPCM received standard therapy for heart failure (beta-blocker and angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker). In addition, all G-PPCM obtained bromocriptine and anticoagulation therapy [prophylactic or therapeutic, low molecular weight heparin (LMWH), direct oral anticoagulants, and Vitamin K antagonists], while only 26% of SA-PPCM obtained bromocriptine, and only 7% received anticoagulation (Table 1). Ventricular assist device, implantable cardioverter defibrillator/cardiac resynchronization therapy defibrillator implantation, and percutaneous mechanical circulatory support were only applied in the G-PPCM cohort (Table 1).

Figure 1 Flow chart of (A) South African (SA) and (B) German (G) cohorts. Seventy-two and 79 peripartum cardiomyopathy (PPCM) patients were recruited in SA and G, respectively. Baseline clinical and echocardiography information was available for all patients. Research blood samples were collected for all SA-PPCM patients, but only 73 G-PPCM patients had blood collected at baseline. Fifty-six SA-PPCM and 73 G-PPCM patients attended their 6 months of follow-up (FU) visit, and FU research blood was taken for 22 SA-PPCM and 59 G-PPCM patients. Recovery: left ventricular ejection fraction (LVEF) $>35\%$; full recovery: LVEF $\geq 50\%$; and poor outcome: LVEF $\leq 35\%$ or death or left ventricular assist device at 6 months of FU. *FU between 3 and 7 months. NYHA-FC, New York Heart Association functional class.

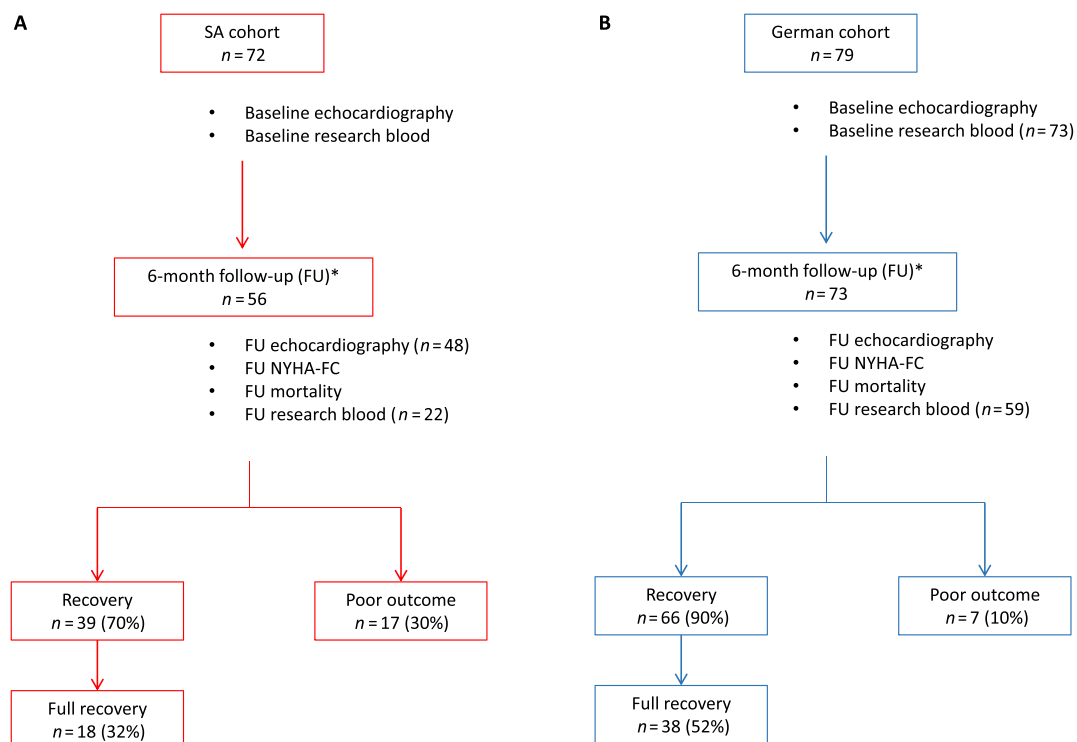


Table 1 Baseline maternal characteristics of study population (*n* = 151)

Clinical characteristic	All (<i>n</i> = 151)	SA (<i>n</i> = 72)	G (<i>n</i> = 79)	<i>P</i> -value
Age (years)	31 ± 6	29 ± 6	34 ± 5	<0.0001
Parity, median (range)	2 (0–7)	2 (0–6)	2 (1–7)	0.3216
BMI (kg/m ²)	26 (17–52)	26 (17–52)	27 (18–50)	0.2442
Systolic blood pressure (mmHg)	110 (80–168)	111 (80–168)	110 (86–145)	0.4925
Diastolic blood pressure (mmHg)	74 (50–110)	70 (50–110)	75 (50–101)	0.9976
Heart rate (bpm)	91 ± 16	96 ± 17	87 ± 15	0.0008
NYHA functional class, <i>n</i> (%)				
I/II	50 (34)	35 (49)	15 (20)	0.0002
III/IV	98 (66)	37 (51)	61 (80)	
Haemoglobin (g/dL)	11 ± 2	11 ± 2	12 ± 2	0.2357
NT-proBNP (ng/L), median (range)	3133 (8–21 290)	4440 (8–16 026)	2988 (175–21 290)	0.6572
Echocardiography				
LVEDD/BSA (mm/m ²)	33 ± 5	35 ± 5	32 ± 4	0.0009
LVESD/BSA (mm/m ²)	28 ± 5	29 ± 5	27 ± 5	0.0641
Ejection fraction (%)	27 ± 9	30 ± 9	24 ± 8	<0.0001
E velocity (cm/s)	89 ± 34	90 ± 38	87 ± 24	0.7056
A velocity (cm/s)	54 ± 26	51 ± 28	60 ± 23	0.1716
E/A (ratio)	2.1 ± 1.7	2.4 ± 2.0	1.6 ± 0.6	0.1062
General medical history, <i>n</i> (%)				
Hypertensive disorders	37 (26)	13 (18)	24 (35)	0.0229
Hypercholesterolaemia	11 (7)	1 (1)	10 (13)	0.0093
HIV	21 (14)	21 (29)	0 (0)	<0.0001
Family history of CVD	34 (24)	10 (14)	24 (35)	0.0053
Onset of clinical symptoms, <i>n</i> (%)				
Prepartum	7 (5)	5 (7)	2 (3)	0.2721
<1 week postpartum	27 (19)	8 (11)	19 (26)	0.0315
≥1 week postpartum	110 (76)	59 (82)	51 (71)	
Caesarian section, <i>n</i> (%)	50 (46)	24 (35)	26 (65)	0.0048
Medication, <i>n</i> (%)				
Beta-blocking agents	130 (86)	54 (75)	76 (96)	0.0002
ACE-inhibitors/ARB	133 (88)	54 (75)	79 (100)	<0.0001
Dobutamin	3 (2)	0 (0)	3 (4)	0.2466
Bromocriptine	97 (64)	19 (26)	78 (100)	<0.0001
Oral anticoagulation (DOACs, Vitamin K antagonists)	23 (15)	5 (7)	18 (23)	0.0114
LMWH (pat. with OAC excluded)	61 (40)	0 (0)	61 (100)	<0.0001
Devices/MCS, <i>n</i> (%)				
VAD	2 (1)	0 (0)	2 (3)	0.4977
ICD/CRT-D	5 (3)	0 (0)	5 (6)	0.0597
Other (ECMO, impella)	2 (1)	0 (0)	2 (3)	0.4977
Heart transplantation, <i>n</i> (%)	0 (0)	0 (0)	0 (0)	NA

Continuous data were expressed as mean ± standard deviation or median and range, according to normality of distribution. Comparison between the two groups was performed using Student's *t*-test for Gaussian distributed data and the Mann–Whitney *U* test where at least one column was not normally distributed. Categorical variables are presented as frequencies (percentages) and compared using Fisher's exact tests. ACE-inhibitors/ARB, angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker; BMI, body mass index; BSA, body surface area; CVD, cardiovascular disease; DOACs, direct oral anticoagulants; ECMO, extracorporeal membrane oxygenation; G, Germany; ICD/CRT-D, implantable cardioverter defibrillator/cardiac resynchronization therapy defibrillator; LMWH, low molecular weight heparin; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; MCS, mechanical circulatory support; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; OAC, oral anticoagulation; SA, South Africa; VAD, ventricular assist device.

Despite lower cardiac function at baseline in the German- compared to the South African PPCM cohort, the 6 months recovery rate is better in the German cohort

Of the 151 recruited patients, 6 months of follow-up data was available for 129 patients (56 SA-PPCM and 73 G-PPCM) (Figure 1A and B). G-PPCM displayed a higher rate of recovery at follow-up compared with SA-PPCM (G-PPCM: 90%, *n* = 66/73; SA-PPCM: 70%, *n* = 39/56; *P* = 0.0053) (Figure 1). In addition, there were more G-PPCM with full

cardiac recovery compared with SA-PPCM (G-PPCM: 52%, *n* = 38/73; SA-PPCM: 32%, *n* = 18/56; *P* = 0.0314) (Figure 1). In turn, almost three times more SA-PPCM were in terminal heart failure or had died compared with G-PPCM (SA-PPCM: 30%, *n* = 17/56, mortality rate: 14%, *n* = 8/56 died; G-PPCM: 10%, *n* = 7/73, mortality rate: 0%, *n* = 0/73) (Figure 1). Most clinical characteristics were similar between recovered and non-recovered patients of both cohorts, with only LVEF being lower and left ventricular end-systolic diameter being larger in patients who did not recover (Table 2).

Table 2 Baseline characteristics for patients with recovery of left ventricular ejection fraction vs. patients with non-recovery of left ventricular ejection fraction ($n = 129$)

Characteristic	Recovered PPCM ($n = 105$)			Non-recovered PPCM ($n = 24$)		
	All ($n = 105$)	SA ($n = 39$)	G ($n = 66$)	All ($n = 24$)	SA ($n = 17$)	G ($n = 7$)
Clinical						
Age (years)	32 ± 6	29 ± 6 ^{§§§}	34 ± 5 ^{§§}	30 ± 5	29 ± 5	32 ± 6
Parity, median (range)	2 (1–7)	2 (1–6)	2 (1–7)	2 (0–4)	2 (0–4)	3 (1–4)
BMI (kg/m ²)	26 (17–50)	26 (17–43)	26 (19–50)	26 (18–38)	24 (18–33)	27 (18–38)
Systolic blood pressure (mmHg)	110 (82–168)	110 (82–168)	111 (86–145)	11 (80–160)	110 (80–160)	110 (90–140)
Diastolic blood pressure (mmHg)	75 (50–110)	75 (50–110)	75 (50–100)	70 (60–101)	70 (60–100)	74 (60–101)
Heart rate (bpm)	89 ± 16	95 ± 18	86 ± 14 ^{§§}	97 ± 17	101 ± 15	92 ± 18
NYHA functional class, n (%)						
I/II	35 (34)	23 (59) ^{§§§, #}	12 (19)	8 (34)	5 (29)	3 (43)
III/IV	68 (66)	16 (41)	52 (81)	16 (66)	12 (71)	4 (57)
Haemoglobin (g/dL)	12 ± 2	12 ± 2	12 ± 2	12 ± 2	11 ± 2	12 ± 2
Echocardiography						
LVEDD/BSA (mm/m ²)	33 ± 5	35 ± 5 ^{§§§}	31 ± 4	35 ± 6	35 ± 6	36 ± 7
LVEDS/BSA (mm/m ²)	27 ± 4 ^{**}	29 ± 5	26 ± 4	31 ± 6	31 ± 6	33 ± 8
Ejection fraction (%)	28 ± 9 ^{**}	32 ± 9 ^{§§§, §§, #}	25 ± 8	22 ± 10	25 ± 9	15 ± 7
E velocity (cm/s)	87 ± 34	87 ± 42	86 ± 25	94 ± 30	96 ± 32	86 ± 12
A velocity (cm/s)	52 ± 26	44 ± 26	61 ± 24	58 ± 23	59 ± 25	50 ± 5
E/A (ratio)	2.2 ± 2.0	2.8 ± 2.5	1.6 ± 0.76	1.9 ± 1.1	1.9 ± 1.2	2.8 ± 0.0
Co-morbidities, n (%)						
Chronic hypertension	29 (30)	9 (23)	20 (35)	4 (17)	2 (11)	2 (33)
Hypercholesterolaemia	9 (9)	1 (3)	8 (12)	0 (0)	0 (0)	0 (0)
HIV	12 (12)	12 (31) ^{§§§}	0 (0) ^{§§§}	6 (25)	6 (35)	0 (0)
Family history of CVD	24 (25)	2 (5) ^{§§§}	22 (38)	4 (18)	3 (17)	1 (20)
Onset of the clinical symptoms, n (%)						
Prepartum	3 (3)	2 (5)	1 (2)	2 (9)	1 (6)	1 (20)
<1 week postpartum	19 (19)	2 (5) ^{§§}	17 (26)	2 (9)	1 (6)	1 (20)
≥1 week postpartum	80 (78)	35 (90) [§]	45 (71)	18 (82)	15 (88)	3 (60)
Caesarian section, n (%)	34 (44)	12 (30) ^{§§}	22 (63) ^{§§}	5 (31)	3 (21) [€]	2 (100)

Continuous data were expressed as mean ± standard deviation or median and range, according to normality of distribution; categorical variables were expressed as frequencies (percentages). BMI, body mass index; BSA, body surface area; CVD, cardiovascular disease; G, Germany; LVEDD, left ventricular end-diastolic diameter; LVEDS, left ventricular end-systolic diameter; NYHA, New York Heart Association; PPCM, peripartum cardiomyopathy; SA, South Africa.

* $P < 0.05$.

** $P < 0.01$ 'All recovered' vs. 'All non-recovered'.

$P < 0.01$, using Student's t -test for Gaussian distributed data and the Mann-Whitney U test where at least one column was not normally distributed for comparison of 'All recovered' vs. 'All non-recovered'.

§ $P < 0.05$.

§§ $P < 0.01$.

§§§ $P < 0.001$ 'SA recovered' vs. 'G recovered'.

§ $P < 0.05$.

§§ $P < 0.01$.

§§§ $P < 0.001$ 'G recovered' vs. 'SA non-recovered'.

§§§§ $P < 0.001$ 'SA recovered' vs. 'G non-recovered'.

$P < 0.05$ 'SA recovered' vs. 'SA non-recovered'.

€ $P < 0.05$.

€€€ $P < 0.001$ 'SA-non-recovered' vs. 'G non-recovered' using Kruskal-Wallis test and Dunn's multiple comparison test for continuous data and χ^2 test for comparison of categorical variables.

Baseline cardiac function, heart dimensions, and heart rate predict outcome in German and South African peripartum cardiomyopathy

Comparing clinical and medical history between survivors and PPCM patients who either died or underwent LVAD implantation revealed that an increased heart rate, a higher NYHA functional class, and treatment with dobutamine were

associated with an adverse outcome in both collectives, whereas treatment with bromocriptine and treatment with beta-blocker were associated with a better outcome (Table 3). However, these results could not be confirmed by logistic regression analyses, due to the low number of patients with poor outcome. Interestingly, HIV seems not to be associated with poor outcome in the SA cohort (Supporting Information, Table S2).

Table 3 Baseline characteristics for survivors and non-survivors/LVAD patients ($n = 151$)

Clinical characteristic	Survivors ($n = 141$)	Non-survivors and LVAD ($n = 10$)	P-value
Age (years)	31 ± 6	31 ± 6	0.6186
Parity, median (range)	2 (1–7)	2 (0–4)	0.6918
BMI (kg/m^2)	26 (17–52)	27 (23–33)	0.6570
Systolic blood pressure (mmHg)	112 (82–168)	103 (80–136)	0.2141
Diastolic blood pressure (mmHg)	75 (50–110)	70 (60–100)	0.8601
Heart rate (bpm)	90 ± 16	106 ± 10	0.0110
NYHA functional class, n (%)			
I/II	50 (36)	0 (0)	0.0165
III/IV	88 (64)	10 (100)	
Haemoglobin (g/dL)	11 ± 2	12 ± 2	0.5454
Echocardiography			
LVEDD/BSA (mm/m^2)	33 ± 5	35 ± 7	0.8705
LVESD/BSA (mm/m^2)	28 ± 5	30 ± 7	0.4092
Ejection fraction (%)	27 ± 9	28 ± 17	0.9065
E velocity (cm/s)	88 ± 34	96 ± 29	0.6173
A velocity (cm/s)	54 ± 27	57 ± 21	0.8055
E/A (ratio)	2.2 ± 1.8	1.8 ± 0.3	0.9693
Deceleration time (ms)	104 ± 93	162 ± 36	0.2961
Co-morbidities, n (%)			
Chronic hypertension	37 (28)	0 (0)	0.1120
Hypercholesterolaemia	11 (8)	0 (0)	1.0000
HIV	18 (13)	3 (30)	0.1504
Family history of CVD	31 (24)	3 (33)	0.4503
Onset of the clinical symptoms, n (%)			
Prepartum	6 (4)	1 (10)	0.3937
<1 week postpartum	25 (19)	2 (20)	1.0000
≥1 week postpartum	103 (77)	7 (70)	
Caesarian section, n (%)	47 (47)	3 (33)	0.5004
Medical therapy, n (%)			
Beta-blocker	125 (89)	5 (50)	0.0052
ACE-inhibitors/ARB	126 (89)	7 (70)	0.1002
Bromocriptine	94 (67)	3 (30)	0.0341
Dobutamine	1 (1)	2 (20)	0.0115
Biomarkers, median (range)			
Galectin-3 (ng/mL)	7.97 (2.11–42.5)	14.70 (10.1–37.1)	0.0002
sST2 (ng/mL)	27.11 (2.79–297.6)	55.39 (33.6–191.6)	0.0037
PINP (ng/mL)	14.78 (2.40–57.0)	8.39 (2.40–17.3)	0.0117
PIIINP (ng/mL)	117.2 (8.49–305.1)	161.6 (78.9–277.2)	0.0965
PINP/PIIINP	0.126 (0.01–1.36)	0.054 (0.01–0.122)	0.0035
OPN (pg/mL)	41 271 (355.3–201 216)	84 285 (20 267–329 213)	0.0257

Continuous data were expressed as mean ± standard deviation or median and range, according to normality of distribution. Comparison between the two groups was performed using Student's *t*-test for Gaussian distributed data and the Mann–Whitney *U* test where at least one column was not normally distributed. Categorical variables are presented as frequencies (percentages) and compared using Fisher's exact tests. ACE-inhibitors/ARB, angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker; BMI, body mass index; BSA, body surface area; CVD, cardiovascular disease; LVAD, left ventricular assist device; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; NYHA, New York Heart Association; OPN, osteopontin; PIIINP, procollagen type-III N-terminal propeptide; PINP, procollagen type-I N-terminal propeptide; sST2, soluble ST2.

Comparison of circulating biomarkers for fibrosis in healthy pregnancy-matched South African and German controls

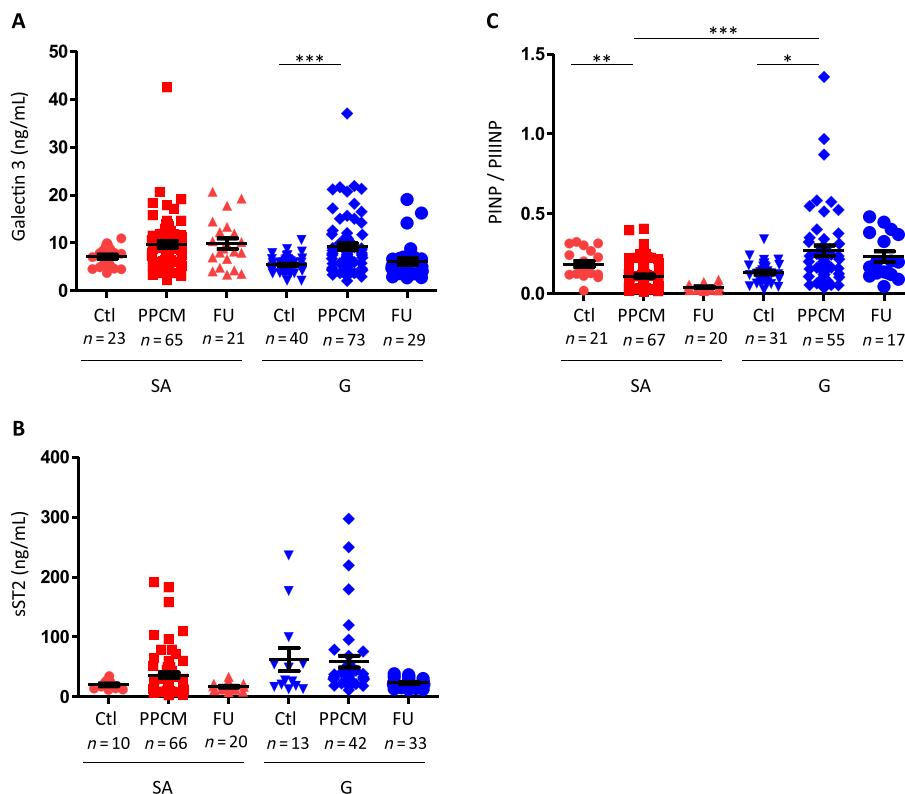
Because circulating biomarker levels may vary between subjects with different ethnic backgrounds, blood samples from pregnancy-matched and postpartum-matched healthy controls with comparable ethnic background were recruited for each cohort (SA controls and G controls). Levels of Gal3 and PINP, as well as the ratio between PINP and PIIINP, were higher, levels of sST2 and PIIINP were similar, and total OPN levels were lower in pregnancy-matched SA controls compared with pregnancy-matched G controls (Supporting

Information, *Table S1*). Due to these differences in circulating biomarker profiles between pregnancy-matched healthy controls of SA and G ethnicity, all biomarker measurements in PPCM were always compared with their respective pregnancy-matched control collectives.

Galectin-3 and soluble ST2 in South African and German peripartum cardiomyopathy patients

Plasma Gal3 levels were not different between SA controls and SA-PPCM at baseline and follow-up (*Figure 2A*). In G-PPCM, Gal3 levels were increased at baseline compared with

Figure 2 Galectin-3, soluble ST2 (sST2), osteopontin levels, and ratio of procollagen type-I N-terminal propeptide (PINP) to procollagen type-III N-terminal propeptide (PIIINP) levels in German (G) and South African (SA) controls and peripartum cardiomyopathy (PPCM) patients at baseline and follow-up (FU). Bar graphs display (A) galectin-3 plasma and (B) sST2 serum levels in G-PPCM and SA-PPCM patients at baseline and after 6 months of FU as well as in their respective controls. (C) Ratio of PINP to PIIINP levels in G and SA controls and PPCM patients at baseline and FU. The FU data are only represented but were not compared with the control and PPCM groups, as described in the Methods section. A number of subjects are indicated, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ using Kruskal–Wallis test and Dunn’s multiple comparison test.



G controls (Figure 2A). Serum sST2 levels were not statistically different in SA-PPCM and in G-PPCM at baseline and follow-up compared with respective controls (Figure 2B).

PINP, PIIINP, and PINP/PIIINP ratio in South African and German peripartum cardiomyopathy patients

Procollagen type-I N-terminal propeptide did not differ between PPCM patients and controls in both collectives nor between G-PPCM and SA-PPCM (Supporting Information, Table S3).

Procollagen type-III N-terminal propeptide levels were increased at baseline in SA-PPCM patients compared with their controls (Supporting Information, Table S3). In G-PPCM, PIIINP levels were lower compared with G controls (Supporting Information, Table S3). Accordingly, the ratio of PINP to PIIINP (PINP/PIIINP) was significantly lower at baseline in SA-PPCM compared with SA-controls, while it was higher at baseline in G-PPCM, compared with G controls (Figure 2C).

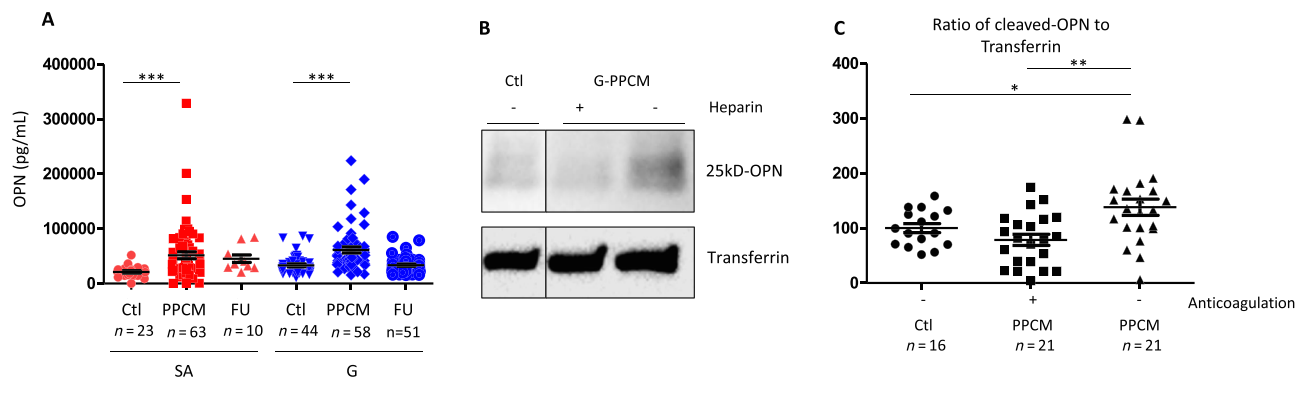
Total osteopontin and cleaved osteopontin in South African and German peripartum cardiomyopathy patients

Circulating levels of total OPN were elevated in both cohorts at baseline compared with their healthy controls (Figure 3A). Western blot analyses showed that the thrombin-cleaved N-terminal OPN fragment was elevated in PPCM patients not treated with anticoagulation therapy, whereas it was comparable with healthy postpartum controls in PPCM patients treated with anticoagulation therapy (Figure 3B and C).

Analyses of the role of ethnicity among the South African collective

Patients from the SA cohort were from two different ethnic groups, with 45 Black African patients (62%) and 27 coloured patients (37%). The coloured patient population is from a mixed ancestry (a population group of admixed ethnicity including Indigenous African European and Asian origins), and

Figure 3 Total and cleaved N-terminal osteopontin (OPN) levels in German (G) and South African (SA) peripartum cardiomyopathy (PPCM) patients and respective controls and in relation to anticoagulation therapy. Bar graphs display (A) total OPN plasma levels in G-PPCM and SA-PPCM patients at baseline and after 6 months of follow-up (FU) as well as in their respective controls. The follow-up data are only represented but were not compared with the control and PPCM groups, as described in the Methods section. Western blots from (B) serum samples and (C) quantitative protein levels of 25 kDa of cleaved N-terminal OPN normalized for transferrin content. A number of subjects are indicated, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. Ctrl using one-way ANOVA with Bonferroni post hoc test.



the Black African patients are non-admixed Indigenous African women (a population group of native or Indigenous Africans with various ethnicities ranging from Zulu to Xhosa). PINP levels were lower in SA Black African patients when compared with other SA-PPCM from mixed ancestry ($P = 0.0416$). Interestingly, both groups presented with no difference in baseline and follow-up cardiac function (Supporting Information, *Table S4*).

Serum markers for fibrosis may predict adverse outcome in South African peripartum cardiomyopathy patients

During the 6 months of follow-up period, eight patients from the SA cohort had died, and two patients from the G cohort underwent LVAD implantation. All non-surviving SA-PPCM and LVAD G-PPCM presented with heart failure NYHA class III/IV ($P = 0.0156$) at time of diagnosis, but baseline LVEF was not different compared with mean LVEF of the whole PPCM cohort (*Table 3*). Significant differences in the baseline biomarker levels between deceased/LVAD patients and all other PPCM patients were seen in plasma Gal3 ($P = 0.0002$), serum sST2 ($P = 0.0037$), and plasma OPN ($P = 0.0257$) levels, which were all elevated in deceased/LVAD patients (*Table 3*). Serum PINP levels and PINP/PIIINP ratio were significantly decreased in the non-survivors/LVAD vs. survivors ($P = 0.0117$ and $P = 0.0035$), whereas PIIINP levels were not different between the two groups. Interestingly, significant differences in the baseline biomarker levels (Gal3, sST2, PINP, and OPN) between recovered patients and non-recovered PPCM patients were only seen in SA cohort (Supporting Information, *Table S5*). In addition, higher NT-proBNP levels seem to be associated with mortality in SA-

PPCM (1654 ± 1444 ng/L in survivors vs. 4332 ± 3308 ng/L in non-survivors, $P = 0.0173$). Because no G-PPCM in this cohort died, this association could not be performed in the G cohort.

Discussion

The present study is the first to directly compare PPCM patients of African or Caucasian origin in a prospective manner with regard to baseline clinical presentation, treatment, outcome, and biomarker profiles. The study confirmed previous observations¹⁵ that African descent PPCM patients seem to have a lower recovery rate, despite an initial better cardiac function and lower NYHA class. It also showed that clinical presentation differs with regard to heart rate (higher in SA-PPCM patients) and co-morbidities, that is, markedly higher HIV infection rate but lower rate of hypertension associated with pregnancy and hypercholesterolaemia, compared with G-PPCM.

Beside potential influences by the genetic background, the higher infection rate of HIV, which is a well-described difference between these two populations (2016. Global Aids Update 2016. <http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016>), might be another explanation for worse outcome in SA-PPCM. However, previous analyses between African HIV-positive and HIV-negative PPCM patients¹⁷ revealed no influence of HIV infection on the outcome of PPCM patients, which was confirmed in the present study.

Another explanation might derive from differences in diagnosis and treatment. In fact, G-PPCM appear to be diagnosed earlier after delivery, a feature known to increase the chance

for early treatment and for recovery.²⁴ In addition, G-PPCM had access to device therapy, which are lifesaving tools and which may also explain better recovery and lower adverse events and mortality in the G cohort. Finally, the vast majority of G-PPCM (96%) were treated according to the BOARD system,²⁵ that is, with beta-blocker, angiotensin-converting enzyme inhibitors/angiotensin II receptor blocker, bromocriptine, and anticoagulation (LMWH), while substantially fewer patients in the SA cohort (26%) obtained this treatment. In SA, bromocriptine is often not prescribed if formula milk cannot be provided by the local clinic. Mixed feeding is not encouraged in HIV-positive mothers as it facilitates HIV transmission.

Nevertheless, we cannot exclude a different disease pathogenesis between the two populations. We thus analysed circulating biomarkers associated with cardiac remodelling, inflammation, and fibrosis, that is, Gal3, sST2, PINP, PIIINP, and OPN, in comparison with respective healthy postpartum controls from each ethnic background.

Procollagen type-III N-terminal propeptide appeared significantly up-regulated at diagnosis in SA-PPCM but not in G-PPCM; Gal3 and sST2 showed high overlaps between PPCM patients and respective controls. Thus, Gal3 and sST2 seem not to be suited as diagnostic markers, and for PIIINP, further data are needed to explore its value as a potential diagnostic marker for PPCM specifically of African ethnics. All measured biomarkers except PIIINP appeared to differentiate between the survivors and non-survivors groups or LVAD implantation. However, it is important to note that information on prognosis derive mainly from SA-PPCM patients, as the number of patients with adverse outcome in the G-PPCM was very low and further analyses in larger cohorts also of different ethnic background are needed to confirm a potential value of Gal3, sST2, PINP, and OPN as prognostic biomarkers.

The differential and completely opposite expression of collagen I and III in both cohorts is interesting and pinpoints possible ethnicity-specific pathomechanisms between African and non-African patients.

Because of PPCM incidence being the highest in Nigeria, Haiti, and SA, we thought for decades that PPCM was a 'Black' ethnicity linked disease. This assumption is wrong as PPCM is associated with different ethnic groups.² However, the role of ethnicity in the pathogenesis of PPCM has never been studied. In the ARIC study, the authors showed that Black patients will preferentially develop eccentric hypertrophy rather than concentric hypertrophy, which is mainly presented by White patients.²⁶ It has been suggested that eccentric remodelling in Black patients involves cardiomyocyte death and replacement fibrosis, which is characterized by an increase of collagen III.²⁷ On the other hand, concentric left ventricular remodelling is consecutive to inflammation, cardiomyocytes hypertrophy, and reactive fibrosis,²⁸ which preferentially promote collagen I to collagen III expression.²⁹

Thus, the collagen profiling might explain different recovery rate in the two collectives as the consequence of different remodelling in both populations suffering from the same disease. According to this hypothesis, lower recovery rates in the SA collective might be due to more cardiac tissue loss and replacement fibrosis, while better recovery and reversibility of the disease in the G-PPCM collective are associated with transient reactive fibrosis. However, more sophisticated imaging data would be needed to confirm the nature of cardiac fibrosis in PPCM patients.

Osteopontin has been shown to promote myocardial stiffness in heart failure patients.¹¹ Furthermore, OPN promotes cardiac fibrosis via up-regulation of microRNA-21 in fibroblasts.³⁰ Here, we report that OPN is up-regulated in PPCM patients from both collectives at baseline. More recently, especially the thrombin-cleaved N-terminal OPN was shown to induce myocardial fibrosis by enhancing the synthesis of PIIINP.¹² In the present study, we observed that plasma levels of thrombin-cleaved N-terminal OPN were reduced in PPCM patients treated with anticoagulation (please note that anticoagulation included only LMWH, direct oral anticoagulants, and Vitamin K antagonists but not *platelet aggregation inhibitors*). Better outcomes in G-PPCM may be explained at least in part by consistent application of anticoagulation, leading to lower levels of the profibrotic PIIINP, pointing to lower fibrotic activity. In turn, as thrombin-cleaved OPN induces PIIINP and anticoagulation was not used in most SA-PPCM patients, this may explain the higher levels of PIIINP observed in SA-PPCM patients, which might subsequently contribute to their lower recovery rate. Therefore, different anticoagulation treatment regimens between G-PPCM and SA-PPCM could also explain differences in outcome between the two collectives. A recent study showed no benefit of an anticoagulation therapy in heart failure patients.³¹ However, the condition in PPCM patients cannot be compared with general heart failure patients because (i) the physiology of the peripartum phase per se increases coagulation activity (as a protection from extensive bleeding at delivery) and (ii) the pathophysiology of PPCM further increases the thrombotic risk due to heart failure and impairment in the vasculature.³ In this regard, with the observed increased thrombin-dependent cleavage of OPN, our data identify a novel prothrombotic pathomechanism in PPCM patients that is specifically targeted by LMWH therapy. This potential novel pathomechanism and the potential benefit of LMWH therapy need confirmation in larger cohorts and eventually in randomized multicentre trial.

In conclusion, at diagnosis, SA-PPCM and G-PPCM patients present different clinical and biomarker profiles. These can be attributed to ethnical differences and to differences regarding health care access (earlier diagnosis in G-PPCM). Differences in baseline biomarkers profiles between the two cohorts support the notion of ethnical differences. Outcome also differs markedly with substantially better outcome in

G-PPCM compared with SA-PPCM patients despite initial more severe disease presentation in G-PPCM. Besides genetic and ethnic aspects, earlier diagnosis, different medical treatment regimens, and access to device therapy in G-PPCM could contribute to the better clinical outcome in G-PPCM. The present data also suggest a novel pathomechanism of PPCM, which may have an impact on the outcome of PPCM patients by regulating fibrosis. This pathomechanism includes elevated circulating OPN levels, its processing in a profibrotic fragment by thrombin, and a subsequent up-regulation of PIIINP. Anticoagulation therapy may be able to brake this cycle and thereby not only prevent thrombosis but also contribute to better recovery of PPCM patients, a feature supported by the high use of anticoagulation therapy and the better outcome of G-PPCM patients compared with SA-PPCM patients.

Limitations

Because of the low incidence of PPCM, we included patients from the G-PPCM registry who were treated in different centres from all over Germany. Therefore, especially in the G cohort, clinical data were partially missing in some patients. Also, due to the limited amount of blood samples available, especially during the follow-up, not all biomarkers could be measured in all patients. Furthermore, the C-terminal PICP, closely related to collagen I formation, was not assessed in our study.

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Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Circulating levels of Galectin 3, PINP, PINP/PIIINP and OPN but not sST2 and PIIINP differ between German and South African postpartum controls

Table S2. HIV is not involved in SA-PPCM patient's baseline cardiac function and outcome and doesn't influence fibrosis-related biomarkers circulating levels

Table S3. PINP and PIIINP in German and South African controls and PPCM patients presenting postpartum

Table S4. Role of ethnicity in patient's characteristics within SA-cohort

Table S5. Biomarkers levels in German and South African PPCM patients according to their LVEF recovery

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