Reversal of IFN-γ, oxLDL and prolactin serum levels correlate with clinical improvement in patients with peripartum cardiomyopathy

Olaf Forster a,1, Denise Hilfiker-Kleiner c,1, Aftab A. Ansari b, J. Bruce Sundstrom b, Elena Libhaber a, Winnie Tshani a, Anthony Becker a, Anthony Yip a, Gunnar Klein c, Karen Sliwa a,⁎

a Soweto Cardiovascular Research Unit, Department of Cardiology, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Johannesburg, South Africa
b Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, USA
c Department of Cardiology and Angiology Medical School Hannover, Germany

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Abstract

Aim: Peripartum cardiomyopathy (PPCM) is characterized by acute onset of heart failure of unknown aetiology. We aimed to identify mechanisms involved in initiation and progression of the disease.

Methods and results: Serum markers related to cardiac function, apoptosis, oxidative stress, remodelling, inflammation and the nursing hormone prolactin were analyzed in PPCM patients and healthy controls. The kinetics of these markers were compared between patients who improved cardiac function (IMP) and those patients who did not improve (NIMP), over 6 months follow-up. All patients received ACE-inhibitors, beta-blockers and diuretics. Baseline levels of TGF-beta-1 were significantly lower, MMP-9 and VEGF were not different; all other markers were significantly higher in PPCM compared with controls. Only baseline NT-proBNP levels were higher in NIMP compared with IMP. After 6 months, NT-proBNP, oxLDL and IFN-γ were significantly lower in IMP and the decrease in oxLDL, IFN-γ and prolactin was significant in IMP but not in NIMP. Significant correlations were observed between the kinetics of NT-proBNP, oxLDL, prolactin and IFN-γ in PPCM patients.

Conclusion: Baseline NT-proBNP and the failure to decrease oxLDL, IFN-γ and prolactin are associated with poor outcome in PPCM, suggesting a potential role of these factors in the pathophysiology of PPCM and for risk stratification of PPCM patients.

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Keywords: Peripartum cardiomyopathy; Africa; Predictors of outcome; Inflammatory markers; Prolactin

1. Introduction

Peripartum cardiomyopathy is characterized by the new onset of heart failure in previously healthy women between one month antepartum and five months post-delivery [1]. Clinical presentation, management of disease and outcome have been reviewed recently [2]. Studies conducted on a large cohort of PPCM patients described a pro-inflammatory response in PPCM patients with elevated plasma levels of TNF-alpha, Fas-Apo-1, IL-6 [3] and a positive correlation between C-reactive protein levels, LV end-diastolic and end-systolic diameters at the time of diagnosis [4]. We recently reported that enhanced oxidative stress in a mouse model for PPCM (mice with a cardiac specific deletion for signal transducer and activator of transcription-3, STAT3-KO) and in PPCM patients triggers the activation of Cathepsin D, an

⁎ Corresponding author. Department of Cardiology, Chris Hani Baragwanath Hospital, P.O. Bertsham 2013, Johannesburg, South Africa. Tel.: +27 11 938 1016; fax: +27 11 938 8945.
E-mail address: sliwa-hahnleke@mdh-africa.org (K. Sliwa).
1 The first two authors have contributed equally.
ubiquitous lysosomal enzyme which subsequently cleaves serum prolactin in its anti-angiogenic and pro-apoptotic 16-kDa form, which in addition seems to promote endothelial inflammation and impairs cardiomyocyte metabolism and contraction [5,6]. Full-length 23-kDa prolactin is physiologically up-regulated post-delivery and has been implicated in cardiac tissue injury and modulation of autoimmune response [7,8]. Reversible cardiac remodelling happens physiologically during the peripartum period without cardiomyocyte loss or injury and without triggering unwanted innate immune pathological responses. Thus, unbalanced oxidative stress and the subsequent generation of the 16-kDa prolactin form may represent a critical step in the initiation of endothelial damage, apoptosis, vasoconstriction and inflammation, leading to impaired cardiac function in PPCM [5,6,9,10]. In fact, pharmacological inhibition of prolactin release has been shown to prevent PPCM in STAT3-KO mice and has proved to be beneficial in a preliminary study conducted on a small group of patients with a subsequent pregnancy in PPCM [5].

Roughly 23–54% of PPCM patients recover their cardiac function within 6 months [2,11,12], others do not. The precise mechanisms leading to irreversible left ventricular dysfunction in human PPCM remain undefined.

In order to identify mechanisms involved in the onset of PPCM we compared sera and/or plasma levels of selected biomarkers in a cohort of patients presenting with acute PPCM at our clinic with age and gravida matched non-PPCM women from our local population in the peripartum period. Furthermore, we reasoned that kinetic analysis of these biomarkers over a 6-month follow-up period may contribute to the understanding of the underlying pathophysiological mechanisms of PPCM, especially why in some PPCM patients cardiac function improves, while others remain in cardiac dysfunction or even deteriorate further.

2. Methods

2.1. Study design and patient recruitment

This study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg, South Africa (PCR 990409) and complies with the Declaration of Helsinki. All patients and controls gave written informed consent before study entry. We screened a total of 54 patients to recruit 43 consecutive PPCM patients.

The study was conducted at Chris Hani Baragwanath Hospital, a tertiary institution located in Soweto, South Africa and linked to the University of the Witwatersrand, Johannesburg. It is the sole tertiary medical facility for this community. Patients were referred from local clinics, secondary hospitals, and the Department of Obstetrics at Chris Hani Baragwanath Hospital. History of pre-eclampsia and mode of delivery were obtained from the patient and confirmed by examining the obstetric card carried by each patient. The history of onset of symptoms and signs were recorded during first presentation at the cardiac clinic at Chris Hani Baragwanath Hospital (baseline) and after a follow-up period of 6 months (6-month visit). These were the two time points of the study. Clinical assessment, echocardiography, and blood analysis were performed at baseline and after 6 months of standard therapy.

Inclusion criteria: 1) age ≥ 16 and ≤ 40 years, 2) New York Heart Association functional class II–IV, 3) symptoms of congestive heart failure (CHF) that developed in the last month of pregnancy or during the first 5 months postpartum, 4) no other identifiable cause for heart failure, 5) left ventricular EF ≤ 40% by transthoracic echocardiography, and 6) sinus rhythm.

Exclusion criteria: 1) significant organic valvular heart disease, 2) systolic blood pressure > 160 mm Hg and/or diastolic blood pressure > 100 mm Hg, 3) clinical conditions other than cardiomyopathy that could increase the studied inflammatory markers, 4) treatment with anti-inflammatory drugs, 5) severe anaemia (haemoglobin concentration < 9 g/dl), and 6) metabolic disorders affecting lipoprotein metabolism i.e. thyroid disease.

Three tubes of blood (4 ml each) were collected from every patient to obtain serum and plasma for measurement of cytokine levels and complete blood count (CBC). Echocardiography was taped on video and stored within the Division of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes. Following the initial screening and baseline visits, monthly outpatient visits were scheduled for clinical assessment and evaluation of medication compliance.

All patients received treatment with diuretics and the angiotensin-converting enzyme inhibitor enalapril. Patients with an EF ≤ 25% or LV thrombus received anti-coagulation therapy. Carvedilol was added after resolution of overt heart failure, and the dose was gradually titrated up to a target of 25 mg twice daily as long as SBP was ≥ 100 mm Hg and symptoms such as dizziness did not occur.

2.2. Echocardiographic studies, assessment of New York Heart Association functional class and non-invasive blood pressure measurements

All studies were performed and interpreted by the same two operators (A.Y. and A.B.) who were blinded to the protocol. Two-dimensional targeted M-mode echocardiography with Doppler colour flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, Washington) echocardiograph attached to a 2.5- or 3.5-MHZ transducer. Systolic and diastolic left ventricular dimensions were measured according to the American Society of Echocardiography guidelines [13]. Measurements of left ventricular dimensions and function were determined on an average of ≥ 3 beats.

A physician, who was provided with the clinical data, but blinded to the study protocol and unaware of the results of the laboratory tests, evaluated the NYHA functional class of each patient during the baseline and follow-up visits.

Heart rate, systolic and diastolic blood pressure were measured non-invasively with a Critikon Dinamap vital
signs monitor 1846 and calculated as mean values from five readings. Measurements were made after a 30-minute resting period in sitting position with two-minute intervals between successive measurements.

2.3. Blood tests

For each patient at baseline and at the 6-month follow-up visit, blood samples (12 ml) were withdrawn from an antecubital vein between 10 a.m. and 12 noon. Samples were collected in pre-chilled vacutainer tubes containing ethylene-diaminetetraacetic acid or clot activator respectively and mixed rapidly. Plasma or serum was separated by centrifugation at 2500 rpm for 12 min within 15 min of collection. Aliquots were stored at −80 °C.

In order to differentiate physiologically up-regulated biomarker expression levels during the peripartum period from pathological values and to obtain reference values, we obtained serum from 20 female volunteers without history of cardiac or recent infectious disease, normal ECG and physical examination, who were in the peripartum period. Controls were comparable in terms of age, race, body mass index and parity. All serum samples used in this study were thawed only once for each of the bioassays performed.

None of the patients or volunteers received anti-inflammatory drugs during the four weeks preceding blood sampling for the determination of each of the biomarkers including the inflammatory cytokines. The measurement of serum and plasma biomarkers was conducted using commercially available enzyme linked immunosorbent assays (ELISA) according to the manufacturer’s instructions. These included NT-proBNP, prolactin (ALPCO, Wingham, NH, USA), Fas/APO-1, IFN-γ, IL-1β, IL-6, TNFα, TGF-beta-1 (Biosource, Camarillo, CA, USA), MMP-2, MMP-9, VGEF (R&D Systems, Minneapolis, MN, USA) and oxLDL (Mercodia, Uppsala, Sweden). For logistical reasons, oxLDL was only measured in 28 patients. Appropriate dilution of the plasma or serum samples were utilized for the assays as required by the manufacturer of each ELISA kit and each plasma or serum sample was assayed in duplicate. The average of the two measurements was utilized to calculate the levels of each of the biomarkers from the standard curve established with known positive standards supplied with each kit.

2.4. Statistical analysis

Data were analyzed using the SAS version 9.1 statistical program (SAS, Cary, NC, USA). Results are expressed as median [range] [14]. We used Wilcoxon Scores (Rank Sums) for comparison between all patients at baseline versus healthy controls and improvers versus non-improvers. As data were non-normally distributed, we performed log transformation of the differences of all variables between 6 months and baseline for both groups, improvers and non-improvers. Subsequently we used 1-way analysis of covariance (ANCOVA), adjusted for baseline left ventricular ejection fraction to compare between groups. Pearson’s correlation was determined for comparison between biomarkers. Significance was assumed at a two-tailed value of $p < 0.05$.

3. Results

3.1. Sera/plasma sampling and defining cardiac function in improvers and non-improvers during the acute phase of PPCM as well as in healthy controls

Thirty-eight out of 43 patients completed the follow-up period of 6 months (three patients died and two moved to remote areas and were not available for follow-up). Patients presented with a median parity of 2 [1–6] and reported onset of symptoms at a median of 11 days postpartum [−22 to 111]. The median age was 30 years [17–45] and 23.2% had undergone caesarean section. The mean haemoglobin at time of presentation was 11.3 ± 2.1 g/dl and mean BMI was 26.3 ± 7.0. Cardiac transplantation and LV assist device implantation were not available to the study population for economic reasons.

Patients presented with a mean heart rate of 99.7 ± 19 bpm, a systolic blood pressure of 113.4 ± 20.0 mm Hg and a diastolic blood pressure of 75.6 ± 13.4 mm Hg. The median LVEF among all patients was 29.5% [13–39] with a median LVESD of 4.9 mm [3.6–6.3] and a median LVEDD of 5.6 mm [4.3–7.3].

Data were obtained in parallel for plasma or serum from 20 otherwise healthy, parity and gravity matched controls from the same population. All controls were in NYHA class I, had no cardiac history, ECG abnormality or cardiac physical findings. Age, sex, body mass index and parity of controls were comparable to the PPCM patients.

During re-assessment after 6 months of standard cardiac failure therapy, 25 patients were classified as cardiac function improvers (IMP) and 13 as non-improvers (NIMP). Patients were classified as cardiac function improvers if their LVEF determined by echocardiography improved by at least 10 units (i.e. 25 to 35%) and their NYHA class improved by at least one grade [3]. Data were then analyzed between cardiac function improvers and non-improvers (Table 1). PPCM patients who were identified as cardiac function improvers had a significantly lower median LVEF

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>IMP median baseline</th>
<th>Range</th>
<th>n</th>
<th>NIMP median baseline</th>
<th>Range</th>
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<td>25</td>
<td>32</td>
<td>18–40</td>
<td>13</td>
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<td>LVEF (%) 6 months</td>
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<td>25–63</td>
<td>25</td>
<td>34</td>
<td>21–46</td>
<td>13</td>
<td>0.0064</td>
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<tr>
<td>NYHA baseline</td>
<td>3</td>
<td>2.0–4.0</td>
<td>25</td>
<td>3</td>
<td>2.0–4.0</td>
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<td>NS</td>
</tr>
<tr>
<td>NYHA 6 months</td>
<td>1</td>
<td>1–3</td>
<td>25</td>
<td>2</td>
<td>1–3</td>
<td>13</td>
<td>0.03</td>
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</table>
at baseline than non-improvers (Table 1). While LVEF increased in the IMP group to a median of 50.0% after 6 months of treatment, the median LVEF of non-improvers increased only to 34% (Table 1). The median NYHA class was 3.0 at baseline among improvers and non-improvers (Table 1). The median NYHA class after 6 months of treatment was 1 (range 1–3) among improvers and 2 (range 1–3) among non-improvers (p = 0.03).

3.2. Serum levels of biomarkers for cardiac function, oxidative stress, apoptosis, inflammation and nursing in patients with acute PPCM versus healthy controls

3.2.1. Cardiac function

Measurements of a long list of proteins associated with cardiac function have been utilized in the past to assess their role in patients with various forms of cardiomyopathies. Based on the published results, we chose to measure serum levels of NT-proBNP, a biochemical marker of heart failure [15]. As shown in Table 2, NT-proBNP levels were markedly increased in patients with acute PPCM compared to controls.

3.2.2. Oxidative stress and apoptosis

oxLDL, an indicator of oxidative stress [16], and Fas/Apo-1 as a marker for apoptosis [2], were as previously shown [5] both significantly elevated in all PPCM patients at baseline as compared with controls (Table 2).

3.2.3. Remodelling

Among the proteins involved in cardiac tissue remodelling, we reasoned that matrix-metallo-proteinase-2 (MMP-2), matrix-metallo-proteinase-9 (MMP-9), the anti-apoptotic survival factor for T-lymphocytes termed transforming-growth factor β-1 (TGF-β-1) and vascular endothelial growth factor (VEGF) may provide the most informative clues as to the role of remodelling in PPCM. The median baseline serum levels of MMP-2 were significantly higher in samples from PPCM patients than in controls (Table 2). In contrast, serum levels of MMP-9 and VEGF were not different between PPCM patients and controls (Table 2). Interestingly, TGF-β-1 was significantly lower in samples from PPCM patients than in controls (Table 2), which is in keeping with findings from Haiti [17].

3.2.4. Inflammation

None of the patients included in this study had a readily identifiable concomitant inflammatory disease. Mean white cell count at time of presentation was 6.9 ± 3.6 × 10⁹/l and well within normal limits. We measured levels of the prototype Th1-like pro-inflammatory cytokines interferon-γ (IFN-γ), interleukin-1β (IL-1β), IL-6, tumour necrosis factor alpha (TNF-alpha) and C-reactive protein (CRP). Median baseline levels of all these pro-inflammatory markers were significantly higher among PPCM patients as compared to peripartum controls (Table 2).

3.2.5. Prolactin

There was a striking difference in prolactin serum levels between PPCM patients and controls. As shown in Table 2, PPCM patients (NIMPs and IMPs) displayed 3-fold higher median prolactin serum levels at baseline compared with age and gravida matched peripartum controls (Table 2).

3.3. Kinetics of biomarker profile in the course of the disease in patients that improved versus patients that did not improve from PPCM

3.3.1. Cardiac function

The baseline median serum levels of NT-proBNP were significantly higher in non-improvers than in improvers (NIMP: 2203.1 fmol/ml [1733–3078]) versus IMP: 1632 fmol/ml [886–2885]) p = 0.0013). After 6 months, NT-proBNP levels were still significantly (p = 0.0018) lower in IMP compared with NIMPs (IMPs: 935 fmol/ml, range: 331–2059 fmol/ml, NIMP: 1713 fmol/ml, range: 883–2895 fmol/ml). NT-proBNP decreased in both groups with no significant difference in the kinetics of the decrease (Table 3).
3.3.2. Oxidative stress
oxLDL was increased to a similar degree in IMP and NIPM at baseline (IMP: 16.4 I.U./ml, range: 14.6–19.4 versus NIMP: 16.7 I.U./ml, range: 15.8–18.4, \( p = \) NS), the values after 6 months revealed a significant reduction (\( p < 0.001 \)) of median oxLDL levels among IMP (IMP 6 months: 12.2 I.U./ml, range: 9.2–14.6; median \( \Delta \) IMP: −4.3 I.U./ml) while values in NIMP did not decrease (NIMP 6 months: 17.2 I.U./ml, range: 16.6–19.2; median \( \Delta \) NIMP: +0.6 I.U./ml).

3.3.3. Apoptosis
Fas/Apo-1 was increased to a similar degree in IMP and NIPM and decreased markedly in both groups and 6-month levels were not significantly different between IMP and NIMP (Table 3).

3.3.4. Remodelling
Levels of MMP-9, VEGF and TGF-\( \beta \)-1 were similar between IMP and NIPM at baseline and after 6 months and MMP-2 decreased to similar extent in IMP and NIMP at baseline versus 6 months (Table 3).

3.3.5. Inflammation
No significant differences were noted in the levels of IL-1\( \beta \), IL-6, TNF-alpha and CRP between IMP and NIPM at baseline. After 6 months follow-up, no significant change in IL-1\( \beta \) and IL-6 levels was observed, while TNF-alpha decreased in both NIMP and IMP to a similar degree (Table 3).

In contrast, while the levels of IFN-\( \gamma \) were similar between IMP and NIPM at baseline (IMP: 2.95 I.U./ml, range: 1.87–5.59 versus NIPM: 2.09 I.U./ml, range: 1.82–3.79, \( p = \) NS), the values after 6 months revealed a significant reduction (\( p = 0.0181 \), Fig. 1) of median IFN-\( \gamma \) levels among IMP (IMP 6 months: 1.3 I.U./ml, range: 0.1–5.5 I.U./ml; median \( \Delta \) IMP baseline versus 6 months: −1.87) while values in NIMP did not decrease (NIMP 6 months: 3.0 I.U./ml, range: 1.0–5.2 I.U./ml; median \( \Delta \) NIMP baseline versus 6 months: +0.86).

Interestingly, 4 out of 25 patients in the IMP group displayed an increase in IFN-\( \gamma \) serum levels (range: +0.6 to +2.1 I.U./ml). Two of these patients with increasing IFN-\( \gamma \) (+1.0, respectively +0.6 I.U./ml) died after the 6-month follow-up. No patient with decreasing IFN-\( \gamma \) died after the 6-month observation period, even if they belong to the NIMP group. In the NIMP group, 4 out of 13 patients experienced a decrease of IFN-\( \gamma \) (range: −1.0 to −2.2 I.U./ml). Three of

![Fig. 1. IFN-\( \gamma \) (I.U./ml) serum levels at baseline and after 6 months follow-up. (A) In IMP IFN-\( \gamma \) levels decrease in the majority of patients. (B) In contrast, in most patients classified as non-improvers (NIMP) IFN-\( \gamma \) fails to decrease within the 6-month follow-up. Median \( \Delta \) baseline to 6 months in IMP versus median \( \Delta \) baseline to 6 months in NIMP, \( p = 0.0181 \).](image-url)

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Median ( \Delta ) IMP baseline versus 6 months</th>
<th>Range</th>
<th>( n )</th>
<th>Median ( \Delta ) NIMP baseline versus 6 months</th>
<th>Range</th>
<th>( n )</th>
<th>( p )-value</th>
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<tr>
<td>NT-proBNP (fmol/ml)</td>
<td>−696</td>
<td>−1604 to 50</td>
<td>25</td>
<td>−491</td>
<td>−1542 to 257</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Fas/APO-1 (ng/ml)</td>
<td>−0.08</td>
<td>−0.56 to 0.59</td>
<td>27</td>
<td>−0.11</td>
<td>−0.49 to 0.13</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>oxLDL (I.U./ml)</td>
<td>−4.3</td>
<td>−6.8 to (−0.5)</td>
<td>21</td>
<td>0.56</td>
<td>−2.9 to 2.4</td>
<td>5</td>
<td>0.003</td>
</tr>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>−64.13</td>
<td>−174.9 to 51.4</td>
<td>24</td>
<td>96.08</td>
<td>−132.2 to −48.9</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-( \beta )-1 (ng/ml)</td>
<td>−4.15</td>
<td>−19.7 to 23.2</td>
<td>24</td>
<td>−1.2</td>
<td>−12.4 to 2.00</td>
<td>10</td>
<td>NS</td>
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<tr>
<td>IL-1( \beta ) (pg/ml)</td>
<td>9.3</td>
<td>−113.55 to 99.6</td>
<td>24</td>
<td>−6.05</td>
<td>−89.25 to 44.20</td>
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<td>NS</td>
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<tr>
<td>IL-6 (pg/ml)</td>
<td>−48.2</td>
<td>−259.8 to 78.6</td>
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<td>0.25</td>
<td>−89.25 to 52.7</td>
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<tr>
<td>TNF-alpha (pg/ml)</td>
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<td>−169.35 to 100.95</td>
<td>24</td>
<td>−18.18</td>
<td>−98.95 to 27.65</td>
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<td>NS</td>
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<td>CRP (mg/l)</td>
<td>−4.4</td>
<td>−146.3 to 55.2</td>
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<td>−8.6</td>
<td>−79.4 to 3.5</td>
<td>11</td>
<td>NS</td>
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<tr>
<td>Prolactin (ng/ml)</td>
<td>−9.23</td>
<td>−47.7 to 10.9</td>
<td>25</td>
<td>−6.11</td>
<td>−28.9 to 6.9</td>
<td>13</td>
<td>NS</td>
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Fig. 1. IFN-\( \gamma \) (I.U./ml) serum levels at baseline and after 6 months follow-up. (A) In IMP IFN-\( \gamma \) levels decrease in the majority of patients. (B) In contrast, in most patients classified as non-improvers (NIMP) IFN-\( \gamma \) fails to decrease within the 6-month follow-up. Median \( \Delta \) baseline to 6 months in IMP versus median \( \Delta \) baseline to 6 months in NIMP, \( p = 0.0181 \).
these patients had only moderate left ventricular dysfunction at baseline (basal EF 43%, 36% and 39%) and two patients in this group displayed, despite only moderate increases in EF to almost normal function after the 6-month observation period (EF: 48% and 58%).

3.3.6. Prolactin

There were no significant differences in the levels of prolactin in samples from IMP versus NIMP at baseline (IMP: 28.8 ng/ml; range: 9.6–66.6 versus NIMP: 27.65 ng/ml, range: 10.8–54.0 ng/ml p=NS). After 6 months, there was a significant decrease in the median prolactin serum levels among IMPs compared with baseline (IMP 6 months: 19.6 ng/ml, range: 7.8–43.5 ng/ml; median Δ −9.2 ng/ml, p=0.0068). The level of decrease in NIMP, however, did not reach statistical significance (NIMP 6 months: 21.5 ng/ml, range: 21.1–46.6 ng/ml; median Δ −6.1 ng/ml, p=0.45) (Table 3).

3.4. Correlation of the kinetics of NT-proBNP and IFN-γ with the kinetics of other biomarkers and cardiac function in the course of the disease in patients that improved versus patients that did not improve from PPCM

The relatively small sample of patients and large number of studied parameters do not allow the use of multivariate analysis. Instead, we performed correlation analysis for the 2 markers, NT-proBNP and IFN-γ, for which a potential prognostic value was established.

Correlation analyses of the kinetics of NT-proBNP with Δ IFN-γ (p<0.0001), Δ oxLDL (p=0.049), Δ prolactin (p=0.02) (Fig. 2A, B, C) and Δ NYHA (p=0.015) were significant. In contrast, correlations of Δ NT-proBNP with Δs of other serum markers and with Δ %EF were not significant.

Δ IFN-γ correlated negatively, as expected, with Δ %EF (p=0.009). In addition, significant positive correlations of Δ IFN-γ with Δ oxLDL (p<0.0001) and with Δ prolactin (p=0.027) were observed, while correlations of Δ IFN-γ with Δs of all other serum markers were not significant.

4. Discussion

The present study was conducted to compare values of a set of biomarkers involved in cardiac function, oxidative stress, apoptosis, inflammation, remodelling and pregnancy in a cohort of patients diagnosed with acute PPCM in comparison with healthy age and pregnancy matched controls from the same population. Furthermore, the kinetics of the same set of biomarkers was monitored over 6 months follow-up, in PPCM patients who improved (IMP) versus those who did not improve (NIMP). The aim was to identify potential pathways and/or mechanisms involved in the initiation and progression of the disease in an effort to stratify possible future treatment options.

Among the factors assessed in the present study, biomarkers for heart failure, oxidative stress, inflammation and apoptosis (NT-proBNP, oxLDL, IFN-γ, IL-1β, IL-6, TNFα and CRP) were substantially up-regulated in PPCM patients at baseline.

The observation, that all PPCM patients displayed significantly higher baseline levels of oxLDL (indicative for increased oxidative stress) and prolactin compared with pregnancy matched healthy women, supports the notion of our previously published concept that oxidative stress and increased prolactin are crucial factors for the initiation of PPCM [5].

In our previous work, we demonstrated that blockade of prolactin by bromocriptine, a drug used to stop lactation in women for many years, prevented the onset of PPCM in a mouse model of the disease [5]. Patients with one episode of PPCM display an extremely high risk for a relapse in a
subsequent pregnancy [2]. In line with a central role for prolactin to trigger PPCM in humans, it is of interest to note that in a pilot study, inhibition of prolactin secretion by bromocriptine in patients with a subsequent pregnancy prevented the expected onset of heart failure [5]. Furthermore, in a recent publication we presented two cases with acute PPCM where bromocriptine, in addition to standard therapy for heart failure, was associated with recovery from the disease [18]. These data support the notion that prolactin is a crucial factor for the initiation of PPCM. While elevated prolactin levels are present in all women in the early postpartum phase, the pathophysiological effect of prolactin derives from its cleaved subform, 16-kDa prolactin, of which substantial levels are detected in the serum of patients with acute onset of PPCM but not in healthy nursing women [5]. The cleavage of prolactin is achieved by the proteases such as Cathepsin D, which can be activated by unbalanced oxidative stress [5]. Increased oxidative stress as indicated by oxLDL was present in all PPCM patients and enhanced activation of Cathepsin D has been observed in a subset of this patient collective [5]. Moreover, this study revealed a substantial up-regulation of a second prolactin cleaving protease, MMP-2 [19], in PPCM patients at baseline. Levels of 16-kDa prolactin have not been quantified separately in the majority of patients analyzed in this study, but it was detected in 3 patients who were included in this and a previous study [5].

The 16-kDa prolactin is responsible for endothelial damage, i.e. apoptosis in endothelial cells and vasoconstriction and it impairs cardiomyocyte metabolism and contractility [5,20]. Recent findings also imply a pro-inflammatory role of 16-kDa prolactin [5,6].

Endothelial damage and apoptosis of vascular cells, appear to be a crucial event in PPCM [5,21]. In line with this notion, we observed a significant elevation of the pro-apoptotic Fas/APO-1 in PPCM patients at baseline compared with controls which is in line with previous observations [2]. Additionally, oxLDL sensitizes human vascular smooth muscle cells (VSMC) to Fas/APO-1 (CD95) mediated apoptosis [22] and may thereby, together with 16-kDa prolactin, contribute to apoptosis in the vasculature during the acute phase of PPCM. Moreover, IFN-γ, which is also up-regulated in PPCM patients at baseline, suppresses vessel formation by directly interfering with VEGF signalling [23]. Further in line with a systemic pro-apoptotic and impaired regenerative situation, serum levels of the anti-apoptotic survival factor TGF-β-1 were decreased in PPCM patients compared with controls which is in keeping with reports from Haiti [17]. TGF-β-1 and its signalling mediator (MCP-1) play a crucial role in vessel formation by enhancing migration of mural cells toward the endothelium [12], and may thereby contribute to a lower regeneration capacity of the vasculature in PPCM patients.

Thus, data from this study are consistent with the notion from previous studies [5,21] that oxidative stress mediated prolactin cleavage and subsequent endothelial damage plays a crucial role in the onset and early phase of PPCM [5,21]. Among the factors analyzed in this study, baseline NT-proBNP levels correlated directly with outcome in PPCM patients, that is, NIMPs had higher baseline NT-proBNP levels than IMPs, while a low baseline left ventricular function (% EF) was not predictive for an adverse outcome. These findings are consistent with studies performed after myocardial infarction suggesting that elevated plasma levels of NT-proBNP are a better predictor of poor outcome than the ejection fraction measured by echocardiography [24]. Further investigation of NT-proBNP in the course of PPCM showed that NT-proBNP serum levels remained significantly higher in NIMPs compared with IMPs and the kinetics of NT-proBNP correlated positively with NYHA class over 6 months confirming the value of NT-proBNP as a marker for ventricular wall stress and heart failure also in PPCM. In addition, the kinetics of NT-proBNP correlated significantly with oxLDL, IFN-γ and prolactin, a marker profile more specifically associated with PPCM. While these observations support a causal relation of these factors with the pathophysiology of the disease, they may serve for risk stratification of PPCM patients. In fact, one unique feature of PPCM is that some patients recover and some do not. Recovery sometimes progresses over 6 to 12 months. Over the same period of time other patients will not or only slightly improve and may need additional medical treatments such as assist device, defibrillator or heart transplantation. Therefore, the kinetics of NT-proBNP in correlation with the kinetics of other more disease specific markers (oxLDL, IFN-γ, prolactin) may serve to distinguish patients with poor prognosis from those who may recover. However, the patient numbers are too low to be conclusive and further studies with larger collectives are required.

Our study showed for the first time that the kinetics of IFN-γ was significantly associated with the outcome of PPCM: Most IMPs displayed a significant decrease in IFN-γ over 6 months of follow-up, while most NIMPs did not. The observation, that 2 out of 4 IMPs with increasing IFN-γ serum levels died after 6 months despite increased cardiac function suggests that even in patients with the ability to improve cardiac function substantially, positive IFN-γ kinetics may indicate a higher risk for morbidity and mortality. In contrast, the fact that decreasing IFN-γ in NIMPs was mainly associated with mild forms of PPCM suggests that decreasing IFN-γ serum levels seem to be associated with less severe forms of the disease. A limiting factor of this study, however, is, that we were not able to evaluate the cause for increased IFN-γ in PPCM patients and therefore cannot exclude that increases in IFN-γ may result from additional PPCM independent disease factors such as unrecognized infections.

The observation, that the kinetics of IFN-γ differed significantly from baseline to 6 months between IMP and NIMP, together with its correlation with NT-proBNP suggests a potential role of this factor for disease progression in PPCM patients. In this regard, it has been reported that IFN-γ reduced papillary muscle contraction and decreased responsiveness to beta-adrenoreceptor [25], a notion which fits well with the observed failure to improve left ventricular function in NIMP.
Furthermore, the kinetics of IFN-γ correlated positively with prolactin, the factor thought to play a major role for initiating PPCM [5]. 16-kDa prolactin strongly enhances adhesion of inflammatory cells to the endothelium and stimulates the expression of IFN-γ responsive genes such as interferon-stimulated protein (28 kDa and 15 kDa) and interferon responsive factor 1 [6,26]. Therefore, prolactin (mainly the 16-kDa form) together with IFN-γ may induce a strong inflammatory reaction, which seems to revert among IMP while it progresses into chronic inflammation with characteristics of an autoimmune process in NIMP. However, data presented here are descriptive and it is beyond the scope of this study to investigate their role in the progression of PPCM.

5. Conclusion
With regard to novel insights into the pathophysiology of PPCM, observations from this study support our previously published work [5] suggesting that oxidative stress, inflammation and prolactin may be interconnected in a vicious circle which is responsible for initiating PPCM. In addition, the tight correlation of NT-proBNP, as a marker for ventricular wall stress and heart failure, with a more disease specific marker set, i.e. IFN-γ, oxLDL and prolactin, may serve for risk stratification of PPCM patients and should be further explored in larger patient collectives.

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