PERIPARTUM CARDIOMYOPATHY – AN AUTOIMMUNE DISEASE?

Olaf Alfred Edo Manfred Forster

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand,

in fulfillment of the requirements for the degree of

Doctor of Philosophy

Johannesburg, October 2007
DECLARATION

I, Olaf Forster declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg.

Human Research Ethics Committee protocol number: 020907

Olaf Forster   (Candidate)   10th day of October 2007
DEDICATION

I thank my parents, Manfred and Margret Forster, for providing me with a good education.

I would like to dedicate this thesis to my patients and their families.
PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

PUBLICATIONS


PUBLISHED CONFERENCE PROCEEDINGS


Abstract

Introduction: Peripartum cardiomyopathy (PPCM) is defined as a disorder of unknown aetiology that occurs between one month antepartum and five months postpartum in women without pre-existing heart disease. While the incidence of PPCM has been reported between 1:2392 and 15000 live births in the USA, the disease is ubiquitous on the African continent with an incidence ranging from 1:100 to 1:1000 deliveries. The mortality rate ranges between 15 and 40.7%, while 23 to 54% of patients recover completely. Aim of this thesis was to describe the clinical profile of 100 PPCM patients, identify predictors of negative outcome, analyze differences in kinetics of cardiac function biomarkers, pro-inflammatory cytokines, markers of re-modeling and prolactin in cardiac function improvers versus non-improvers and investigate a possible autoimmune component in the pathogenesis of PPCM.

Methods: We conducted a single centre, prospective study of 100 newly diagnosed patients meeting diagnostic criteria for PPCM. All patients received standard anti-failure therapy with diuretics (furosemide, aldactone), the β-blocker carvedilol, ACE-inhibitor perindopril and digoxin if indicated. Clinical assessment, 2-dimensional echocardiographic studies and blood analysis were systematically performed at time of presentation, after six and twelve months of therapy.

Findings: Fifteen patients died within the follow-up period of six months and eight were not available for full follow-up since they moved to remote areas. Patients who completed six months of treatment showed a significant reduction of heart rate, left ventricular dimensions and significant improvement in scintigraphically and echocardiographically derived values for left ventricular ejection fraction (p<0.0001) and NYHA functional class (p<0.001). However, normalization of LVEF (>50%) was only observed in 18 (23%) of the patients. Baseline plasma levels of Fas/Apo-1 (OR = 3.56, CI 95% = 1.35–9.42) and NYHA FC (OR = 2.67, CI 95% = 1.04–6.83) were independent predictors of death.
The markers of cardiac function (NT-proBNP*, Fas/APO-1* and oxidized LDL*) and the pro-inflammatory cytokines (interleukin-1*, interleukin-6*, IFN-gamma*, TNF-alpha* and C-reactive protein) were significantly higher at baseline in 43 PPCM patients than in controls (*P<0.0001). While the marker of remodeling matrix-metalloproteinase-2* was significantly higher, transforming-growth-factor-beta1 was significantly lower in PPCM patients (P=0.002). Vascular endothelial growth factor, matrix-metalloproteinase-9 and placentatal growth factor did not differ between groups, while big endothelin-1* was significantly higher in PPCM patients.

The pregnancy related hormone prolactin was significantly higher* in PPCM patients than in peripartum controls and subsequently decreased significantly (P=0.002) in cardiac function improvers, but not in non-improvers. Analysis between cardiac function improvers and non-improvers from baseline to six months revealed significant differences for Δ IFN-gamma (P=0.0181).

Investigating a possible auto-immune component in the aetiology of PPCM, we identified β1-adrenoreceptor antibodies in serum of PPCM patients and mapped their reactivity exclusively to epitopes on the second extra-cellular loop (RAESDE and DEARRCY), while those from DCMO patients bind to epitopes on the first (30%) and second extra-cellular loop (ARRCYND and PKCCDF). The β1-adrenoreceptor agonist-like antibodies identified in PPCM patients are part of the IgG2 and IgG3 subclass, while those from DCMO patients belong to the IgG2 subclass. Furthermore we demonstrated that β1-adrenoreceptor antibodies in serum of PPCM patients prevented desensitization of the receptor. The β1-adrenoreceptor antibodies in serum of PPCM patients were not detectable in serum of non PPCM peripartum controls and are different from those found in patients with DCMO, suggesting that PPCM forms a distinct disease entity. We demonstrated a positive correlation between the activity of the β1-adrenoreceptor antibodies and serum expression levels of the marker of cardiac function NT-proBNP from baseline to twelve months (rs=0.58, 2-tailed P=0.0228), 95% CI (0.10 to 0.84).

Investigating the cause for high prolactin expression levels in serum of PPCM patients, we identified a STAT3 deficit in a PPCM mouse model. Significantly increased levels of cathepsin D cause the cleavage of
the physiological 23-kDa form of prolactin into a 16-kDa form in PPCM patients, but not in non-PPCM peripartum controls. This initiated another clinical study, investigating the effect of the prolactin-inhibitor bromocriptine in addition to standard heart failure therapy in known PPCM patients, presenting with a subsequent pregnancy. Comparison of clinical and echocardiographic data of these patients to others on standard heart failure therapy demonstrated preservation or improvement of left ventricular dimensions and systolic function as well as NYHA FC.

**Conclusion:**
While a wide range of parameters, reflecting cardiac dysfunction and pro-inflammatory immune activation, were elevated in all PPCM patients at time of first presentation, indicating their involvement in the initiation of the disease, we found significant differences over time between cardiac function improvers and non-improvers for ΔIFN-gamma (P=0.0181) serum levels, suggesting their role in disease progression.
Heightened IFN-gamma expression could indicate an ongoing T-cell mediated autoimmune response and an insult to the cardiac muscle, resulting in fibrosis and inability to improve left ventricular systolic function.
Prolactin represents a stimulatory link between the neuroendocrine and immune systems, promoting pro-inflammatory immune responses. Interestingly, we found significantly higher (P<0.0001) serum prolactin levels in PPCM patients at time of first presentation than in peripartum controls, suggesting the hormone’s role during the initial acute phase of PPCM. Several authors have described the induction of IFN-gamma by prolactin. Disease progression and the ongoing autoimmune insult by beta1-adrenergic autoantibodies appear to be driven by IFN-gamma. This pro-inflammatory cytokine remained high in PPCM non-improvers, decreased in improvers, was previously implicated in the development of autoimmune disease and its suppression leads to desensitization of β-adrenoreceptors. Together with the β1-adrenoreceptor autoantibodies that we have identified in PPCM patients and their demonstrated property to also prevent desensitization of β1-adrenoreceptors, patients experience an adrenergic overdrive, leading to cardiac myocyte insult.
While the pathogenesis of PPCM appears to be multifactorial, our task as scientists remains to find out, how the monolith was erected. Specifically, it appears promising to investigate the effects of bromocriptine in addition to standard heart failure therapy in a randomised, double-blinded clinical study. Although one could argue that prolactin regulated expression of IFN-gamma and other cytokines may explain the gender-specific differences in autoimmunity, it has been shown that elevated serum prolactin concentrations are associated with accelerated autoimmune disease in both female and male mice. Possibly, prolactin does not only play a role in the pathogenesis of PPCM, but also in other forms of cardiomyopathy, affecting males and females alike. It would be interesting to study prolactin serum expression levels in male and female patients with idiopathic DCMO. Clearly, further studies into the unfolding pathogenesis of PPCM are indicated.
ACKNOWLEDGEMENTS

My sincere gratitude is with the following people:

- Our patients at the Cardiac Clinic at Chris Hani Baragwanath Hospital for their confidence and compliance
- My supervisor, Prof. Karen Sliwa-Hähnle, for her wonderful support, guidance, patience, encouragement and for her hard work and enthusiasm
- My co-supervisor Prof. Mohammed Rafique Essop for his unconditional support and extraordinary clinical knowledge
- My co-supervisor Prof. Aftab Ahmed Ansari, Emory University, Atlanta, USA for his great support, his never ceasing optimism and encouragement, the many doors he opened and opportunities he created, but mostly for his guidance and excellent knowledge
- Winnie Tshani for her caring attitude towards our patients and ensuring compliance
- Dr. Denise Hilfiker, Medizinische Hochschule Hannover, Germany for her superb scientific work
- Walter Langhinnerich and Thomas Poese for their unconditional support
- Dr. Gerd Wallukat, Max Delbrück Centrum, Berlin, Germany, for sharing his extra-ordinary knowledge
- Dr. Bruce Sundstrom, Emory University, Atlanta, USA for his patience and guidance
- Elena Libhaber (MSC) for her support in statistical analysis
- My fellow colleagues at the Cardiac Clinic, Chris Hani Baragwanath Hospital, for their constant support and encouragement, especially Dr. Anthony Becker and Dr. Anthony Yip.
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**NOMENCLATURE**

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<th>Abbreviation</th>
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<td>AAB</td>
<td>autoantibodies</td>
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<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
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<tr>
<td>ACE</td>
<td>angiotensin-converting enzyme</td>
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<td>AIDS</td>
<td>acquired immuno-deficiency syndrome</td>
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<tr>
<td>b.p.m.</td>
<td>beats per minute</td>
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<td>β1-AR</td>
<td>β1-adrenergic receptor</td>
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<td>body mass index</td>
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<td>BNP</td>
<td>brain natriuretic peptide</td>
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<td>connective tissue growth factor</td>
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<td>dilated cardiomyopathy</td>
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<td>DC</td>
<td>dendritic cell</td>
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<td>EF</td>
<td>ejection fraction</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>endomyocardial biopsy</td>
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<td>EMBs</td>
<td>endomyocardial biopsy specimen</td>
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<td>FBC</td>
<td>full blood count</td>
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<tr>
<td>FC</td>
<td>functional class</td>
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<td>FS</td>
<td>fractional shortening</td>
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<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
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<td>HR</td>
<td>heart rate</td>
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<td>HREC</td>
<td>Human Research Ethics Committee</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>IDCM</td>
<td>idiopathic dilated cardiomyopathy</td>
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<td>IFN-gamma</td>
<td>interferon-gamma</td>
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<td>IL-1</td>
<td>Interleukin-1</td>
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<td>IL-17</td>
<td>Interleukin-17</td>
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<tr>
<td>IVSD</td>
<td>interventricular septum in diastole</td>
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<tr>
<td>IVSS</td>
<td>interventricular septum in systole</td>
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<tr>
<td>JAK</td>
<td>Janus-associated kinase</td>
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<tr>
<td>KO</td>
<td>knock-out (mouse)</td>
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<td>LFT</td>
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<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
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<td>LVEDD</td>
<td>left ventricular end diastolic diameter</td>
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<tr>
<td>LVESD</td>
<td>left ventricular end systolic diameter</td>
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<td>LVPWD</td>
<td>left ventricle posterior wall in diastole</td>
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<tr>
<td>LVPWS</td>
<td>left ventricle posterior wall in systole</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MMP-2</td>
<td>matrix metalloproteinase 2</td>
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<tr>
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<tr>
<td>NGF</td>
<td>nerve growth factor</td>
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<tr>
<td>NT-proBNP</td>
<td>N-terminal prohormone brain natriuretic peptide</td>
</tr>
<tr>
<td>NYHA FC</td>
<td>New York Heart Association functional class</td>
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<td>PDGF</td>
<td>platelet-derived growth factor</td>
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<td>PLGF</td>
<td>Placental growth factor</td>
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PPCM  peripartum cardiomyopathy
STAT3  signal transducer and activator of transcription 3
TGF-β  transforming growth factor β
TNF-alpha  tumor necrosis factor alpha
U+E  urea and electrolytes
VEGF  vascular endothelial growth factor
VSMC  vascular smooth muscle cells
1. INTRODUCTORY CHAPTERS AND LITERATURE REVIEW

1.1 Definition

Peripartum cardiomyopathy (PPCM) was first described by Virchow in 1870 and is defined as a disorder of unknown pathogenesis in which left ventricular dysfunction and symptoms of heart failure occur between the last month of pregnancy and the first five months postpartum. PPCM is an exclusion diagnosis based on the absence of an identifiable cause of heart failure or recognizable heart disease prior to the last month of pregnancy (1). It is a distinct entity of dilated cardiomyopathy (1, 2) that is incompletely characterized (3). In contrast, pregnancy related cardiomyopathy might present during the second or early third trimester. Diagnosis requires echocardiographic evidence of left ventricular systolic dysfunction (ejection fraction < 45%, fractional shortening < 30%) (4). Heart failure occurring earlier in pregnancy might be caused by previously unsuspected dilated cardiomyopathy unmasked by the haemodynamic and hormonal stress of pregnancy and possibly forms a different entity. Women developing symptoms of cardiac failure earlier than the last gestational month are diagnosed as having pregnancy-associated cardiomyopathy (5). Other possible causes of heart failure during the peripartum period, such as infectious, toxic, or metabolic disorders and ischaemic or valvular heart disease need to be considered. Complications of late pregnancy, including toxaemia and amniotic or pulmonary embolism, which may mimic heart failure, should be ruled out before the diagnosis of PPCM is made (6).

In the past, authors reporting cases of PPCM made the diagnosis solely on clinical grounds. That is, pregnant or postpartum patients with signs and symptoms of heart failure and with consistent radiographic features were given the diagnosis. With the advent of two-dimensional echocardiography techniques, it has been established that pregnant patients may at times have clinical features mimicking heart failure and yet have normal ventricular size and function. Therefore it is likely that previous studies may have erroneously included some patients with a diagnosis of PPCM. Common causes for such misdiagnoses include accelerated hypertension, diastolic ventricular dysfunction, pulmonary embolism or the high-output state of
pregnancy itself (6). The above emphasize the importance of correct diagnosis in efforts to answer important questions about incidence, prevalence, treatment, and prognosis (1).

1.2 Epidemiology of PPCM

The incidence of PPCM varies from 1:100 to 1:10000 between geographical regions (table 1.1) and tends to be associated with low socioeconomic standards (7). PPCM is most common in women of African descent (8-10). The highest incidence of pregnancy associated heart failure in the world has been reported from the Zaria province of Nigeria, but it is important to note that the incidence of PPCM might have been overestimated in some areas due to the absence of echocardiography, possibly resulting in other causes of systolic heart failure being diagnosed as PPCM. PPCM is a rare condition in Europe but a very frequent disease in Sahelian Africa (11). In Nigeria PPCM formed the most common cause of cardiac failure among the females in the north, while it is rarely reported from the south (12). While the incidence in the United States has previously been described as 1:10000 (13), recent studies reported a higher incidence of 1:2392 to 1:4000 live births (14, 15).

<table>
<thead>
<tr>
<th>Location</th>
<th>Incidence</th>
<th>Year</th>
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<td>1:1000</td>
<td>1995</td>
<td>(17)</td>
</tr>
<tr>
<td>South Africa (Johannesburg)</td>
<td>1:3000</td>
<td>1961</td>
<td>(18)</td>
</tr>
<tr>
<td>China (Taiwan)</td>
<td>1:6147</td>
<td>1992</td>
<td>(19)</td>
</tr>
</tbody>
</table>

Table 1.1: Incidence of PPCM in different geographical regions
Retrospective record review of earlier studies without echocardiography disclosed that a high percentage of these women had previous unreported underlying illness such as hypertension, mitral stenosis, thyrotoxicosis, sepsis, anaemia or pre-eclampsia that may have contributed to the onset of cardiac failure suggesting the incidence may be lower than indicated (6, 20, 21).

PPCM appears to have a more malignant course in developed countries with a proportion associated with histological evidence of myocarditis (22). There is a need for a large scale epidemiologic studies of the incidence, prevalence, determinants and outcome of cardiomyopathy in Africa to form strategies for the optimal treatment and prevention of heart muscle disease on the continent (22).
1.3 Risk factors of PPCM

Early reports suggested that PPCM was more prevalent in women above 30 years of age, but the syndrome has been reported in patients of a wide range of ages (18, 23, 24). Veille et al. reported PPCM to be more frequent in multiparous women (25). In a study from the cardiological departments of French university hospitals multiparity and twin pregnancies were identified as predisposing factors (26). Researchers from Banjul, The Gambia reported on five out of 28 patients had twin pregnancies (18%, p < 0.001) (27). Also Fillmore et al. reported an increased risk for patients with twin pregnancies (28). Although PPCM has been reported in all ethnic groups, the majority of American patients are of African descent (25). In a study from Sahelian Africa heat, hard physical exertion during pregnancy, hypertension, sodium diet, ablutions with hot water during the postpartum period, selenium deficiency and probably latent myocarditis have been named as risk factors (11). Poor nutrition was initially thought to be associated with the development of PPCM (24), but several reports have disproved this connection (29, 30).

Case reports have associated PPCM with maternal cocaine abuse (31), enterovirus infection (32) or selenium deficiency (33), but these were not confirmed epidemiologically. In a more recent report Fett et al. have ruled out a role for selenium deficiency as a risk factor for PPCM (29). On the other hand Lampert et al. reported a link between long-term oral tocolytic therapy and subsequent development of PPCM (34). The main risk factors that have been identified so far for PPCM thus include advanced maternal age, multiparity, African descent, twinning, and long-term tocolysis (6).
1.4 Aetiology of PPCM

There has been and continues to be considerable controversy with regards to the aetiology of human PPCM (8). While some studies have suggested a role for salt intolerance (35), others have shown a correlation with sexually transmitted diseases (36), Chlamydia pneumoniae (37), and strenuous aerobic exercise throughout the last trimester (38). One hypothesis that was forwarded was that PPCM was due to an interaction between prolactin-selenium resulting in selenium deficiency and/or autoimmunity (39) but data from the studies conducted by Fett et al. suggest these etiologies to be highly unlikely (29). Also in Dakar, Senegal, serum selenium and vitamin B1 levels were measured in PPCM patients and found to be normal (40).

Other studies have suggested that patients might be suffering from a quiescent heart muscle disease as a consequence of a previous viral myocarditis and that cardiac volume overload functioned as a trigger to precipitate the disease (35). A role for autoimmune effector mechanisms in the aetiology of PPCM has also been entertained. Thus, the concept that has been forwarded is that generalized immunosuppression is a hallmark of pregnancy which returns to normal shortly following delivery. However, in select cases the return to normal is accompanied by abnormal rebound resulting in autoimmunity and disease. This view was supported by a case report from Osaka, Japan, which showed that three PPCM patients developed positive anti-heart reactive antibodies as detected by an indirect immunofluorescence assay, and one had antibody to heart myosin detected by enzyme-linked immunosorbent assay shortly following delivery. The authors concluded that heart failure is induced by postpartum autoimmune myocarditis (41).

Autoantibodies that target 25 kDa, 33 kDa, or 37 kDa proteins expressed by normal cardiac tissues have been identified in PPCM patients from Haiti (42). A case report described high titers of antiactin antibodies 9 months after the delivery supporting the theory that the peripartum cardiomyopathy is of autoimmune aetiology (43), however, a study from Niamey, Niger demonstrated the absence of a humoral autoimmunity process in PPCM (44).
Sanderson et al. suggest that the primary event in PPCM reported from the Zaria province in Nigeria is fluid retention which leads to a form of high output cardiac failure. The postpartum practices in this area (taking high sodium diets and lying on heated beds) almost certainly cause the fluid to accumulate initially, but the heart may be unable to meet the demands either because of pre-existing heart muscle disease or, more likely, because of a rise of the peripheral resistance due to the volume expansion, overburdens such dilated hearts and leads to myocardial damage (45). There are few reports on familial occurrence of PPCM (46). A study from Niamey, Niger, reported an association of enterovirus infection with PPCM (32). PPCM shows a high incidence of myocarditis (47) and was identified in 29% of patients with PPCM (48). Bultmann et al. detected viral genomes in endomyocardial biopsy specimen in eight of 26 PPCM patients (30,7%) but also in 10 of 33 control subjects (30,3%) (49). The detected viruses (PVB 19, HHV 6, EBV, HCMV) have been related to inflammatory cardiomyopathy but also have a high prevalence in healthy populations (50, 51). The role of endomyocardial biopsy remains controversial and is likely to be clinically useful only if performed early in the course of the disease (52). Estrogen (53, 54), progesterone (55) and prolactin (56) have been shown to have profound effects on the cardiovascular system, but no distinct hormonal disorder has been identified in PPCM.

In summary, the literature is wealthy with studies attempting to propose aetiologies for PPCM. However, to date no study has clearly identified a distinct cause of this disease (6).
1.5 Pathophysiology of heart failure and PPCM

1.5.1 A general pathophysiological approach towards heart failure

The cardiomyopathies are a group of diseases that affect the heart muscle itself and are not the result of hypertension or congenital or acquired valvular, coronary, or pericardial abnormalities. When the cardiomyopathies are classified on an aetiological basis, two fundamental forms are recognized: A primary type, consisting of heart muscle disease of unknown cause and a secondary type, consisting of myocardial disease of known cause or associated with a disease involving other organ systems. In many cases, like PPCM however, it is not possible to arrive at a specific aetiological diagnosis, and thus it is helpful to classify the cardiomyopathies into one of three types (dilated, restrictive, hypertrophic) on the basis of differences in their pathophysiology and clinical presentation as detailed below:

- **Dilated:** Left and/or right ventricular enlargement, impaired systolic function, congestive heart failure, arrhythmias and emboli.
- **Restrictive:** Endomyocardial scarring or myocardial infiltration resulting in restriction to left and/or right ventricular filling.
- **Hypertrophic:** Disproportionate left ventricular hypertrophy, typically involving septum more than free wall, with or without an intraventricular systolic pressure gradient; usually of a non-dilated left ventricular cavity (9).
In Western populations about one in three cases of congestive heart failure (CHF) is due to dilated cardiomyopathy, with the remainder the consequence of coronary artery disease. Left and/or right ventricular systolic pump function is impaired, leading to progressive cardiac enlargement and hypertrophy, a process called remodeling. Symptoms of CHF typically appear only after remodeling has been ongoing for some time (months or even years). There is, however, no close correlation between the degree of contractile dysfunction and the severity of symptoms.

Although no cause is apparent in many cases, dilated cardiomyopathy is probably the end result of myocardial damage produced by a variety of toxic, metabolic or infectious agents. Dilated cardiomyopathy may be the late sequel of acute viral myocarditis, possibly mediated through an immunologic mechanism. Although it may occur in any patient population, it is more common in African Americans than in whites.
The prevalence of this condition is increasing. A reversible form of dilated cardiomyopathy may be found with alcohol abuse, pregnancy, thyroid disease, cocaine abuse and chronic uncontrolled tachycardia (9). PPCM is therefore a form of primary cardiomyopathy that by definition occurs exclusively during the peripartum period. 82% of women with PPCM develop cardiac symptoms in the first three postpartum months and only 7% in the last month of pregnancy (10). Echocardiography reveals features of dilated cardiomyopathy with impaired ejection fraction, global dilatation and sometimes thinned out walls. Although it is still termed idiopathic a number of mechanisms have been proposed as potential aetiological agents which include nutritional deficiencies, genetic disorders, viral or autoimmune aetiologies, hormonal problems, volume overload, alcohol, physiologic stress of pregnancy, or unmasking of latent idiopathic dilated cardiomyopathy (58).

The rare incidence of PPCM and the difficulty to set up relevant animal models have limited research and understanding of the pathogenic mechanisms involved. With the above in mind, some have suggested that the aetiology of PPCM is dependent on the interaction of pregnancy associated factors, e.g. increased haemodynamic stress, vasoactive hormones and fetal microchimerism, that co-operate in the context of essential immune and genetic environments for disease progression (59).
1.5.2 A specific pathophysiological approach towards PPCM

PPCM is defined as a disorder of unknown pathogenesis in which left ventricular dysfunction and symptoms of heart failure occur between the last month of pregnancy and the first five months postpartum. PPCM is an exclusion diagnosis based on the absence of an identifiable cause of heart failure or recognizable heart disease prior to the last month of pregnancy (1). It is a distinct entity of dilated cardiomyopathy (1, 2) and is incompletely characterized (3). Historically, PPCM has mainly been attributed to nutritional deficiencies. Recent publications focus on the role of inflammatory cytokines, cardiac myocyte apoptosis, infectious disease agents, myocarditis, auto-antibodies, hormones and genetic factors on the background of a pregnancy-specific immune-environment.

1.5.2.1 Role of immune environment

A physiologically normal immune response involves interplay of the innate and acquired immune system. Cytokines, chemokines and their natural ligands, expressed by cells of the innate and acquired immune system, regulate the quantity, quality and kinetics of such immune responses. Whereas chemokines create a biological gradient to attract haematopoietic cells into the immune environment, cytokines influence the quality of the immune response (4). Mosmann et al. described the differences between two types of helper T-cells, defined primarily by differences in the pattern of lymphokines synthesized and their different functions (table 1.5.2.1). Patterns of lymphokines synthesized are convenient and explicit markers to describe T-cell subset differences. Evidence suggesting that many of the functions of helper T-cells are predicted by the functions of the lymphokines that they synthesize after activation by cognate antigen being processed and presented by antigen presenting cells is accumulating. Depending on the infectious agent, the immune response can be classified as belonging to either Th1 or Th2, Th3 or Th0 based on the cytokine profile and is reasoned to be important to consider when inducing therapeutic immune responses (60).
Lymphokine secretion patterns | Cytokine gene expression
---|---
Th1-like (pro-inflammatory) | Synthesize **IL-2, 3, IFN-gamma, GM-CSF, lymphotoxin, TNF, TY5, met-encephalin,**
Th2-like | Synthesize **IL-3, 4, 5 and 6, GM-CSF, TNF, TY5, met-encephalin, P600**
Th0 | Synthesize Th1 and Th2-like cytokines
Th3-like (possibly identical with CD4/CD25) | Synthesize TGF-β, regulatory functions (inhibition of immune response, delivery of anti-apoptotic signals to T-cells)

Table 1.2: Cytokine gene expression profile by Th1, Th2, Th0 and Th3 helper cells (60-62)

Cerwenka et al. described the existence of the TGF-β-producing Th3 subset. TGF-β was recognized as an anti-apoptotic survival factor for T-lymphocytes and is thought to play an important regulatory role during an immune response. Th3-like cells might be identical with CD4/CD25 regulatory T-cells (62). This subset of CD4 T cells is highly potent in suppressing immune responses and its experimental depletion resulted in organ-specific autoimmunity. Changes in the frequencies of such immune cell subsets and the cytokine environment they create, can regulate the quantity and quality of the immune response (4). Ansari et al. performed a flow cytometric analysis of the frequency of CD25 among the CD4 T cells of PPCM patients and women with normal pregnancy. Frequency of CD25 increases in women with normal pregnancy during the third semester and returns to normal post delivery. In contrast all PPCM patients showed markedly lower frequency of this cell lineage. Furthermore those PPCM patients with the lowest values continued to demonstrate poor cardiac function (4).

Immunoglobulin subclass profiles in PPCM patients from Haiti, South Africa and Mozambique differ from those with IDCM from the same countries. Warraich et al. documented a selective upregulation of the IgG3 subclass, consisting of immunoglobulins with pro-inflammatory characteristics in IDCM patients. In contrast, PPCM patients had raised levels of all immunoglobulin subclasses. However, raised levels of the G3 subclass correlated with higher NYHA functional class. An increase in all the subclasses both in
frequency and in reactivity supports a relatively non-specific or a rather exaggerated humoral immune response. Whether a multitude of stimuli, differences in co-stimulatory factors or hyper-responsiveness to self-constituents following changes in host defence underlies these findings is currently not clear. Disparity in the distribution of subclass immunoglobulins in cardiomyopathies of distinct origins is suggestive of diversity in the regulatory components driving these responses in disease.

1.5.2.2 Pregnancy specific immune environment

As Peter Medawar stated in his Nobel lecture, "Immunological tolerance" may be described as a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response (63). The question how a semi-allogeneic fetus is protected from rejection by the mother is a subject of debate. Fetal cells have been shown to cross into the mother and maternal cells have been shown to cross into the fetus. 25% of adults carry long-lasting maternal haematopoietic cells. Both fetal and placental cells have also been shown to express detectable levels of major histocompatibility complex molecules. The regulatory role of such cells on the mother's immune status continues to be subject of study. Munn et al. demonstrated rapid T cell-induced rejection of all allogeneic conception when pregnant mice were treated with a pharmacologic inhibitor of indoleamine 2, 3-dioxygenase (IDO), a tryptophan-catabolising enzyme expressed by trophoblasts and macrophages. Thus, by catabolising tryptophan, the mammalian conceptus suppresses T cell activity and defends itself against rejection (64). Ansari et al. described an abnormal increase if IL-2 synthesis by peripheral blood mononuclear cells from PPCM patients as compared to other normally pregnant women, providing support to the view of an abnormal immune environment as a contributing factor in human PPCM (4). Autoimmune diseases frequently develop after delivery due to the immune rebound mechanism. In a case report from Osaka, Japan, three patients had positive anti-heart antibody detected by indirect immunofluorescence assay, and one had antibody to heart myosin detected by enzyme-linked immunosorbent assay providing suggestive evidence that heart failure is induced by postpartum autoimmune
myocarditis (41). Multiple sclerosis and rheumatoid arthritis are thought to be Th1 cytokine related diseases and usually reduce severity during pregnancy that returns postpartum. In contrast SLE which is thought to be related to Th2 type immune response often exacerbates during pregnancy, suggesting connectivity between Th1/Th2 cytokines and autoimmune diseases (4).

1.5.2.3 Microchimerism

The exchange of blood or lymphoid tissues between individuals as a result of organ transplantation, blood transfusion and pregnancy leads to microchimerism describing the persistent presence of a minor population of semi-allogeneic lymphocytes or DNA in the peripheral circulation and / or tissues of organ transplant / blood transfusion recipients and following pregnancy (4). Microchimeric cells are understood to play a vital role in tolerance-induction in humans for organ transplantation (65, 66). Male fetal CD34+ or CD34+CD38+ cells were detected in women up to 27 years after their last pregnancy. The prolonged persistence of fetal progenitor cells may have significance in development of tolerance of the fetus. Pregnancy may thus establish a long-term, low-grade chimeric state in the human female (67). In patients who have suffered recurrent spontaneous abortions of unknown cause, alloimmunization therapy using the partner's leukocytes has been reported to be effective in preventing the failure of pregnancy, proposing that alloimmunization establishes a state of microchimerism that would be the necessary allogeneic stimulus for T-cell activation, and the induction or maintenance of tolerance to the fetus during pregnancy (68). Chimerism has also been linked to autoimmune thyroid disease and systemic sclerosis (69, 70).

Fetal cells can migrate to the mothers skin during gestation, where they seem to be associated with the development of cutaneous disorders of pregnancy (71). On the other hand, microchimeric cells have been aetiologically associated with severe graft versus host reactions post bone marrow transplantation or transfusion.

Several reports have documented the occurrence of chimerism of the haematopoietic lineage cells from the fetus to the mother during pregnancy (72, 73). It is postulated that fetal cells may escape into the maternal
circulation and remain there without being rejected, due to weak immunogenicity of the paternal haplotype of the chimeric cells, or to the naturally occurring immunosuppressive state of the mother, or both. If chimeric haematopoietic cells take up residence in cardiac tissue during the immunosuppressed pregnant state and, following postpartum recovery of immune competence, are recognized as nonself by the maternal immune system, a pathologic autoimmune response may be triggered. Prior exposure to paternal major histocompatibility complex antigens expressed by spermatozoa or previous immunization from prior pregnancies may play a role in inducing local tissue inflammatory response. Cytokines and similar signaling molecules are then released, leading to non-specific bystander myocytotoxicity and myocarditis. Abnormal immunologic activity as a possible cause of PPCM is supported by high titers of autoantibodies against select cardiac tissue proteins (e.g., adenine nucleotide translocator, branched chain alpha-keto acid dehydrogenase) (74).

A large foetomaternal transfusion occurs at the time of labour and delivery in all pregnant women. This may establish fetal cell microchimerism in the mother, which may be implicated in the subsequent development of diseases such as scleroderma that are more common in women (75). A similar mechanism could play a role in the pathogenesis of PPCM.

1.5.2.4 Haemodynamic stress of pregnancy

Gave et al. performed an echocardiographic assessment of the haemodynamics in normal pregnancies and demonstrated a 10% increase in left ventricular end-diastolic volume, a 45% increase in cardiac output, and a 26% to 28% decrease in end-systolic wall stress. In addition, the left ventricle remodels in response to the haemodynamics of pregnancy, resulting in transient hypertrophy (76).

1.5.2.5 Hormones

The profound effects of estrogen (53, 54), progesterone (55) and prolactin (56) on the cardiovascular system have been demonstrated in general, but no distinct hormonal disorder has been identified in PPCM. Coulson
et al. reported on abnormalities of relaxin, primarily an ovarian hormone produced during pregnancy, recently found in cardiac atria, shown to have positive inotropic and chronotropic properties and potentially involved in excessive relaxation of the cardiac skeleton (77). Sex steroid regulation of the production of the lymphokine interferon-gamma as well as other cytokines may help explain the gender-specific differences in the immune system, including autoimmunity (78). The prevalence of autoimmune diseases in women may be the consequence of a bidirectional signaling network between hormones and the immune system that regulates female reproductive life. Certain estrogens have been thought to improve rheumatoid arthritis which is Th1-mediated and exacerbate Th2-mediated SLE (79).

Estrogen and prolactin have a reciprocal endocrinologic relationship and both hormones have pleiotropic effects on the immune system. Despite the presence of a number of confounding variables, these hormones modulate autoimmunity. Estrogen appears to suppress cell-mediated and augment humoral-based immunity. Prolactin appears to stimulate both, cell and humoral-based immunity. Both hormones have been shown to modulate IFN-gamma secretion. These similar sets of data, derived from experimental models, human autoimmune disease and pregnant patients with autoimmune diseases suggests disparate effects of estrogen and prolactin on autoimmune responses and disease pathogenesis. The endocrinologic and immune effects of estrogen may directly or indirectly stimulate or inhibit immune responses. These dichotomous effects have limited its successful pharmacologic manipulation in human autoimmune disease with estrogen compounds, tamoxifen, oral contraceptives, anti-gonadotropic agents, or ovulation induction regimens. In contrast, reduction of immuno-stimulatory concentrations of prolactin with bromocriptine has successfully suppressed development or expression of murine and human autoimmune disease (80). Prolactin has been implicated in cardiac tissue injury, modulation of the autoimmune response and maintenance of pregnancy (81, 82).

Progesterone appears to promote Th2 cells and suppress the generation of Th1-like immune responses. Testosterone has been shown to regulate T cell cytokine secretion and possess anti-inflammatory properties associated with immunosuppression (83).
1.5.2.6 Inflammatory cytokines

Stress-activated pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-alpha) or interleukin-1 have been implicated in the pathophysiology of idiopathic dilated cardiomyopathy (84). TNF-alpha is a pleiotropic inflammatory cytokine and serves a variety of functions, many of which are not yet fully understood. The cytokine possesses both growth stimulating and growth inhibitory properties and appears to have self-regulatory properties as well. For instance, TNF-alpha induces neutrophil proliferation during inflammation, but it also induces neutrophil apoptosis upon binding to the TNF-R55 receptor (85). TNF-alpha is a 26 kDa protein and is produced by many different cell types. The main sources in vivo are stimulated monocytes, fibroblasts, and endothelial cells. Macrophages, T-cells and B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells and keratinocytes also stimulate production of TNF-alpha. Glioblastoma cells constitutively produce TNF-alpha and the factor can be detected also in the cerebrospinal fluid. Human milk also contains TNF-alpha. Physiological stimuli for the synthesis of TNF-alpha are IL-1, bacterial endotoxins, PDGF and oncostatin M. In fibroblasts the synthesis of TNF-alpha is stimulated by IFN-β, TNF-alpha, PDGF and viral infections. In thymic stromal cells the synthesis of TNF-alpha can be induced by NGF. TNF-alpha can also stimulate or inhibits its own synthesis, depending upon the cell type. In epithelial, endothelial, and fibroblastic cells secretion of TNF-alpha is induced by IL-17.

Beneficial functions of TNF-alpha include its role in the immune response to bacterial, and certain fungal, viral, and parasitic invasions as well as its role in the necrosis of specific tumors. TNF-alpha acts as a key mediary during local inflammatory immune response. It is an acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNF-alpha secreted by the macrophage causes blood clotting which serves to contain the infection. Without TNF-alpha, mice infected with gram-negative bacteria experience septic shock (86). Tracey and Cerami suggest that low levels of TNF-alpha may aid in maintaining homeostasis by regulating
the body's circadian rhythm and that low levels of TNF-alpha promote the remodeling or replacement of injured and senescent tissue by stimulating fibroblast growth (87). The pathological activities of TNF-alpha include the promotion of growth of certain tumor cells, although it causes necrosis of other types of tumors. High levels of TNF-alpha correlate with increased risk of mortality (88). TNF-alpha participates in disorders of inflammatory and non-inflammatory origin (89). Originally, sepsis was believed to result directly from the invading bacteria itself, but it was later recognized that host system proteins, such as TNF-alpha induce sepsis in response. Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of TNF-alpha and other cytokines. Lipopolysaccharide from bacteria cell walls are a potent stimulus for TNF-alpha synthesis (87). TNF-alpha exhibits acute and chronic effects. If TNF-alpha remains in the body for a long time, it loses its anti-tumor activity. This can occur due to cytokine polymerization, shedding of TNF-alpha receptors by tumor cells, excessive production of anti-TNF antibodies, found in patients with carcinomas or chronic infection, and disruptions in the alpha-2 macroglobulin proteinase system which may deregulate cytokines. Prolonged overproduction of TNF-alpha results in cachexia. Cachectin and TNF-alpha were once considered different proteins, but in 1985 researchers discovered that the two proteins were homologous (90).
Picture 1.1: A resolution structure of mouse tumor necrosis factor, towards modulation of its selectivity and trimerisation (91).

All known members of the TNF-alpha cytokine family induce hepatic expression of acute phase proteins. Acute, high dose exposure to TNF-alpha causes shock and tissue damage, catabolic hormone release, vascular leakage syndrome, adult respiratory distress disorder, gastrointestinal necrosis, acute renal tube necrosis, adrenal hemorrhage, decreased muscle membrane potentials, disseminated intravascular coagulation and fever. Chronic, low dose exposure to TNF-alpha has been associated with subendocardial inflammation, endothelial activation, protein catabolism, lipid depletion, insulin resistance and enhanced tumor cell reproduction. Sliwa et al. reported significantly higher plasma levels of TNF-alpha and interleukin-6 in PPCM patients as compared to age-matched healthy controls (92).

One hypothetical advantage of treatment with anti-TNF-alpha antibodies results from its role in multiple types of inflammation. It is often difficult to determine that inflammation in burn and trauma victims are of
infectious aetiology and warrant treatment with antibiotics; therefore another treatment strategy might involve anti-TNF-alpha therapy (89). Strategies for preventing TNF-alpha activity include neutralization of the cytokine via either anti-TNF-alpha antibodies, soluble receptors, or receptor fusion proteins; suppression of TNF-alpha synthesis via drugs such as cyclosporine A, glucocorticoids, or cytokine IL-10 and by inhibition of secondary mediators such as IL-1, IL-6 or nitric oxide (87).

The rationale for using immuno-modulating agents to treat patients with heart failure is based on the fact that excessive enhancement of pro-inflammatory cytokines appears to mimic many aspects of the heart failure phenotype (93). Inflammatory cytokines have been shown to play a key role in the pathogenesis of atherosclerosis and coronary artery disease (94) and it has been suggested that sustained TNF-alpha expression after myocardial infarction and in persistent ischemia may have detrimental effects on the remodeling process (95). However, recent trials with anticytokine agents such as etanercept and infliximab showed time- and dose-dependent worsening of heart failure (93, 96, 97). These rather discouraging results may be explained by the mechanisms of action of these agents. Infliximab exerts its effects by fixing complement in cells (93), which in the heart is reported to lead to cardiac myocyte lysis (98). Etanercept stabilizes TNF-alpha and hence leads to an accumulation of TNF-alpha in the peripheral circulation (93). In comparison, the effects of pentoxifylline are to reduce the synthesis of TNF-alpha by blocking transcriptional activation (99, 100). Furthermore, pentoxifylline has been shown to inhibit apoptosis in different human cell types in vitro (101, 102) and in vivo (102). Hence, pentoxifylline is likely to be a more promising anticytokine agent. Indeed, Sliwa et al. were able to report on improvements in cardiac function with pentoxifylline (103), which were associated with reductions of inflammatory marker levels. These results were in line with their previous work performed in patients with idiopathic dilated cardiomyopathy (104-106). Plasma levels of NT-pro BNP have been used in several clinical trials to assess the efficacy of medical therapy (107). Sliwa et al. also observed reductions in NT-pro BNP in patients treated with pentoxifylline in a randomized study of patients with ischaemic cardiomyopathy (103) and were able to
confirm the efficacy of pentoxifylline in ischaemic heart failure. While pentoxifylline did not abolish increments in circulating TNF-alpha concentrations in patients, experimental studies have suggested that physiological levels of TNF-alpha have cytoprotective effects on the heart during ischaemic events (108, 109). Although we are not aware of any large-scale study that has evaluated the safety of pentoxifylline in patients with heart failure, this pharmacological agent has been in clinical use for more than 25 years for conditions such as peripheral and cerebrovascular disease (110). Furthermore, because patients with peripheral vascular disease frequently also have coronary artery disease and heart failure, it is important to note that large trials with more than 10000 such patients have not reported increases in mortality in patients treated with pentoxifylline (110).

Furthermore, beneficial effects of pentoxifylline have been reported in HIV-positive patients. Apoptosis is a significant cause of CD4 T cell death. Wanchu et al. demonstrated a significant decline of caspase 1 and caspase 8 that are involved in Fas/Apo-1 and TNF-alpha facilitated apoptosis in HIV-positive patients and concluded that this might result in reduced apoptosis and improved CD4 lymphocyte survival (111). The same group described a reduction of nitric oxide in HIV-1 positive patients after administration of pentoxiphylline (112). Swords et al. described an association between enhanced HIV replication and increased production of TNF-alpha. They observed partial inhibition of HIV-1 induction using pentoxifylline and matrix metalloproteinase (MMP) inhibitor I (113).

Data on the effects of pentoxifylline in patients with PPCM is only available from small, non-randomised studies. Furthermore the impact of HIV-1 infection in PPCM and the potential beneficial effects of pentoxifylline have not been studied yet.

1.5.2.7 Cardiac myocyte apoptosis

Loss of myocytes due to apoptosis occurs in patients with end-stage cardiomyopathy and may contribute to progressive myocardial dysfunction (114), but its importance in pathogenesis is unknown. Transgenic mice with cardiac-restricted overexpression of G alpha(q) exhibit a lethal PPCM accompanied by apoptosis.
Hayakawa et al. demonstrated a reduction in cardiac myocyte apoptosis by caspase inhibition through administration of the polycaspase inhibitor IDN-1965 and improved left ventricular function and survival in pregnant G alpha(q) mice, suggesting that cardiac myocyte apoptosis plays a causal role in the pathogenesis of cardiomyopathy (115). Fas/APO-1 is an apoptosis signaling surface receptor, known to trigger cell death in a variety of cell types (116). Sliwa et al. observed significantly higher plasma levels of Fas/APO-1 in PPCM patients compared with healthy volunteers (92).

1.5.2.8 Myocarditis

Several lines of evidence suggest that PPCM may be the result of myocarditis due to a viral illness or an autoimmune aetiology (52, 117, 118). On the background of a relatively immunosuppressed state during pregnancy, susceptibility to cardiotrophic viruses is relatively higher than normal (119). The role of endomyocardial biopsy remains controversial and is likely to be clinically useful only if performed early in the course of the disease (52).

Bultmann et al. detected viral genomes in endomyocardial biopsy specimen in eight of 26 PPCM patients (30.7%) but also in 10 of 33 control subjects (30.3%) (49). The detected viruses (PVB 19, HHV 6, EBV, HCMV) have been related to inflammatory cardiomyopathy but also have a high prevalence in healthy populations (50, 51). In this regard, it is important to note that myocarditis was identified in 29% of patients with peripartum cardiomyopathy (48) and in a separate study cardiac biopsy analysis of patients with PPCM showed a high incidence of myocarditis (47). Kuhl et al. amplified viral genomes in endomyocardial biopsies of 165 (67.4%) among 245 patients with idiopathic dilated cardiomyopathy and found a similar spectrum of viruses (EV=9.4%, ADV=1.6%, PVB19=51.4%, HHV-6=21.6%, EBV=52.0%, HCMV=0.8%), including 45 cases (27.3%) with multiple infections (120).
1.5.2.9 Autoantibodies

A possible association with antimyocardial antibodies has been suggested, although their aetiologic role for PPCM has not been demonstrated (121, 122). It is difficult to conclude from current literature if the presence of autoantibodies (AAB) in sera of patients with cardiomyopathy is result of the disease process or a cause. In more than half of 30 Haitian PPCM mothers, plasma autoantibodies were found. The targets of such autoantibodies include human cardiac myosin heavy chain (200 kDa), putative cardiac transaldolase (37 kDa), putative cardiac myosin light chain (27 kDa) and yet unidentified 25 and 33 kDa cardiac proteins. It is uncertain if these AAB are merely secondary epiphenomena or if they could be directly contributing to cardiomyocyte injury. These autoantibodies were unique to PPCM patients and could not be detected in sera from controls or patients with other forms of cardiomyopathies (4, 29, 59, 123). In contrast, a study from Niamey, Niger demonstrates the absence of a humoral autoimmunity process in PPCM (44).

Case report The presence of a high titer of antiactin antibodies 9 months after the delivery supports the theory that the peripartum cardiomyopathy was of autoimmune aetiology (43).

Additional evidence for an immune hypothesis comes from the presence of antimyosin antibodies, adenine nucleotide translocator (ANT), and branched-chain [alpha]-ketoacid dehydrogenase in patients with peripartum cardiomyopathy, in comparison with control subjects (1).

All immunoglobulins of the G subclass are up-regulated in peripartum cardiomyopathy. Immunoglobulin G3–positive patients have a higher New York Heart Association (NYHA) class and more advanced symptoms at initial presentation (124).

Pregnancy leads to structural, physiological, hormonal and immunological changes in healthy mothers to accommodate a healthy fetus and the mechanisms involved in the pathogenesis of PPCM have to be viewed in this context. Ansari et al. proposed that the dynamic balance between pregnancy, immune environment, fetal microchimerism and genetics is critical in determining the progression toward PPCM. Increased haemodynamic stress during late stage pregnancy leads to pregnancy induced hypertension (PIH) that
triggers myocyte hypertrophy and cardiac remodeling. Increasing levels of hormones such as progesterone, relaxin and estradiol during normal pregnancy promote vasodilatation and have been reported to buffer such pregnancy induced hypertension. Preliminary data suggest that reduced plasma levels of these three hormones in PPCM patients and thus a decreased buffering capacity against PIH, could result in the cardiac tissue pathology of PPCM patients. As a response to reparative maternal cardiac remodeling, fetal cells in the maternal circulation may be selectively recruited because of their undifferentiated characteristics and their potential to integrate into the host cardiac tissue. Ansari et al demonstrated significant increase in the levels of male genomic chromosomal DNA in PPCM patients relative to normal pregnancies, which further supports the opportunity for fetal microchimerism to play a role in the development of PPCM. During the cardiac remodeling process, fetal microchimeric stem cells under the influence of local cytokines and growth factors may differentiate into fetal dendritic cells (DCs). These would process maternal cardiac antigens and then migrate to regional lymph nodes where they present these self-antigens to maternal B- and T-helper cells in a different semi-allogeneic MHC context. As outlined above, plasma of PPCM patients contains autoantibodies that recognize unique cardiac antigens. The role of an abnormal immune environment in the development of autoimmune mediated PPCM is supported by significant differences in the cytokine potential and decrease in frequency of CD4/CD25 regulatory cells in the peripheral blood of PPCM patients (4).

Wallukat et al. described the presence of agonist-like autoantibodies directed against the β1-adrenoceptor and/or the muscarinic M2-receptor in sera of patients with idiopathic dilated cardiomyopathy and Chagas' disease. In patients with dilated cardiomyopathy the first as well as the second extracellular loop was identified as an antibody epitope. In Chagas' disease the anti-β1-adrenoceptor antibody recognizes only one epitope on the second extracellular loop. The anti-β1-adrenoceptor antibodies acting like the β-adrenergic agonist isoprenaline and exert a positive chronotropic effect in cultured rat cardiomyocytes. In contrast to isoprenaline the antibody caused no downregulation of the β-adrenergic signal transduction cascade within six hours. The anti-M2 receptor antibodies recognize in both diseases an epitope on the second extracellular
loop. The anti-M2-receptor antibody exerts a negative chronotropic response in cultured cardiomyocytes. This antibody induced no downregulation of the muscarinic M2-receptor. The negative chronotropic effect was unabated for 6 hours. Based on these findings it is believed that the agonist-like autoantibodies acting against the $\beta_1$-adrenoceptor and the muscarinic M2-receptor may play a role in the pathogenesis of dilated cardiomyopathy and Chagas' disease (125).

We therefore analysed serum of PPCM patients for agonist-like autoantibodies directed against the $\beta_1$-adrenoceptor and correlated these with the kinetics of clinical improvement or lack thereof in chapter six of this thesis.
1.5.2.10 Infectious disease agents

A number of infectious disease agents have long been suspected to be involved in the pathogenesis of PPCM. This includes a study from Niamey, Niger that reported a relationship between enterovirus infection and PPCM (32). It has been suggested that such patients might be suffering from a quiescent heart muscle disease from a previous viral myocarditis and that stress induced volume overload was sufficient to trigger the disease process (35). Others have reported a correlation of PPCM with sexually transmitted diseases (36) including Chlamydia pneumoniae (37). Latent viral myocarditis has been reported as a risk factor for PPCM in a study from Sahelian Africa (11). In light of such a view, Sainani et al. reported a virus aetiology of heart disease that was supported by the successful isolation of one of the subtypes of Coxsackie B virus in 19 out of 55 patients (126). However, only one of these patients had PPCM.

Ansari et al. performed extensive polymerase chain reaction and in situ hybridization studies of cardiac tissues explanted from PPCM, IDC M and non-PPCM, non-IDCM patients trauma cases and found similar transcripts in about the same frequency in all patients. In another effort by the same investigators no significant evidence of mycobacteria, helicobacter, archaeobacter or eubacteria was detected in cardiac tissues of PPCM patients.

Ansari et al. hypothesized that immune responses against virus infected cardiomyocytes prompt lysis of such cells, leading to exposure of the immune system to intracellular cardiac tissue proteins. These are normally sequestered from the immune system against which self-tolerance was not achieved and hence such proteins now serve as neo-antigens and the autoimmune process becomes initiated, explaining the presence of antibodies against intracellular cardiac tissue proteins in sera of patients with PPCM (4).
1.5.2.11 Genetic factors

Genetic factors have been implicated to play a role in a number of autoimmune diseases. This was underlined by the documentation of a significantly higher incidence of diseases such as primary biliary cirrhosis in monozygotic twins than expected in the population in which the study was performed. Individual familial cases of PPCM have been reported (8), but altogether there are few reports on familial occurrence of PPCM (46). It has been suggested that PPCM is due to familial DCM which becomes unmasked by pregnancy (4). Fett et al. have reported a case of PPCM in a mother and her daughter in Haiti (29). Most of the attention has been paid to a role for MHC genes and susceptibility to autoimmune diseases, including experimental autoimmune encephalomyelitis, IDDM and rheumatoid arthritis. It appears that not only MHC but also sets of other non-MHC genes likely play a role. This includes genes encoded by 6p21, genes encoded by chromosomes 5 and 19 and polymorphisms for genes encoding for a number of cytokines, chemokines and/or promoters of such cytokines and chemokines (4).

STAT3 (one of a family of signal transducer and activators of transcription) was initially identified as APRF (acute-phase response factor), an intracellular DNA-binding protein that binds to interleukin 6 (IL-6)-responsive elements. Subsequently, it has been shown that STAT3 is activated by the entire family of IL-6–related cytokines, peptide growth factors, and hormones (127-129). Although initially considered to be a mere target gene of the IL-6–mediated inflammatory response, STAT3 is by now known to direct a wide variety of biologic processes, such as cell survival and apoptosis, inflammation, angiogenesis, and cardiac hypertrophy (129-131).

Experimental studies indicate that IL-6-related cytokines, signaling via the shared receptor gp130, and the Janus kinase (JAKs) - STAT pathway, provide a critical cardiomyocyte survival pathway in vivo in normal healthy conditions. Podewski et al. found that signaling via gp130 and JAK-STAT is profoundly altered in DCM. Importantly, tyrosine-phosphorylation of JAK2 is reduced in the face of increased gp130 phosphorylation, indicating impaired downstream activation of this critical pathway in DCM (132).
STAT-3 participates in a wide variety of physiological processes and directs seemingly contradictory responses such as proliferation and apoptosis (133). The constitutive activation of STAT3 promotes tumor growth and angiogenesis and is associated with drug resistance in cancer therapy. In contrast, in the heart, the down-regulation of STAT3 has been associated with end-stage heart failure in patients (132).

Osugi et al. provided the first evidence that activation of STAT3 controls vessel growth in vivo and suggests that STAT3 contributes to cardiac adaptation by regulating vascular function under the conditions of stress (134). Giordano et al. established the critical importance of cardiac myocyte-derived vascular endothelial growth factor in cardiac morphogenesis and determination of heart function (135), suggesting that VEGF may be an important target gene mediating pro-angiogenic effects of STAT3. To elucidate the role of STAT3 in cardiac muscle and in particular for cardiac protection against physiological and pathophysiological stresses, Hilfiker-Kleiner et al. generated mice harbouring a cardiomyocyte-restricted knockout of STAT3 using the standard Cre/loxP-regulated gene knock out (KO) system. STAT3-deficient mice developed reduced myocardial capillary density and increased interstitial fibrosis within the first four postnatal months, followed by dilated cardiomyopathy with impaired cardiac function and premature death. Conditioned medium from STAT3-deficient cardiomyocytes generated in vitro inhibited endothelial cell proliferation and increased fibroblast proliferation, suggesting the presence of paracrine factors attenuating angiogenesis and promoting fibrosis in vitro. Hilfiker-Kleiner et al. thus established a novel role for STAT3 in controlling paracrine circuits in the heart, essential for postnatal capillary vasculature maintenance, interstitial matrix deposition balance, and protection from ischaemic injury and heart failure (133, 136).

STAT3 KO mice displayed an upregulation of CTGF, an endogenous inhibitor of VEGF activity (137) and increased expression levels of TSP1, which suppresses capillary formation by inhibiting VEGF release and inducing endothelial cell apoptosis (133, 138, 139). Moreover expression of the potent anti-angiogenic factor TIMP1 was augmented in STAT3 KO hearts (133, 140). In short, one could say that STAT3 suppresses an anti-angiogenic gene program in the adult heart (141).
While studying the STAT-3 KO mice, Hilfiker et al. not only observed the upregulation of CTGF, TSP1 and TIMP1, but also OPN, TNC and PAI-1. These antiangiogenic factors are also involved in the formation of interstitial matrix (142, 143), which is consistent with the profibrotic state in these animals. Vice versa there is evidence that enhanced interstitial matrix formation inhibits angiogenesis (144). This supports the concept that the anti-angiogenic and profibrotic cardiac phenotypes of KO mice are mediated, at least in part, by overlapping paracrine mechanisms. Alternative explanations for the enhanced fibrosis in the STAT-3 KO myocardium, such as replacement fibrosis caused by myocyte loss as a result of decreased myocardial blood supply or oxidative stress secondary to tissue hypoxia / ischaemia driving fibrosis have also been forwarded (133). In this regard, it is important to note that the development of severe cardiac fibrosis in aging STAT-3 KO mice was shown to be associated with impaired cardiac function, increased apoptosis, ventricular remodeling, and heart failure with generalized oedema and enhanced mortality (i.e. typical features noted in patients with dilated cardiomyopathy and end-stage heart failure). Hilfiker et al. have shown that the expression of both, total and phosphorylated STAT3 protein, are markedly reduced in failing human hearts. Therefore it is conceivable that reduced STAT3 expression and activation in patients with heart failure adversely affects fundamental cardioprotective mechanisms (in analogy to what is observed in KO mice), thereby contributing to the progression of heart failure (133). It is therefore not an unexpected finding that cardiomyocyte specific deletion of STAT3 impairs cardiac response to neurohormonal activation, ischaemia, doxorubicin treatment and bacterial toxins, functioning to interfere directly with pathways involved with cytoprotective effects (133).

In summary, the extent of angiogenesis in vivo depends on the local balance between proangiogenic and antiangiogenic molecules, whereby paracrine and autocrine circuits in cardiomyocytes and endothelial cells play a crucial role. In this setting, STAT3, in its function as a signaling molecule and an enhancer or repressor of gene transcription, appears to play a central role in regulating angiogenesis in the postnatal heart under physiologic and pathophysiological conditions (141).
1.5.2.12 Nutritional deficiencies
Walsh described the nutritional background of his patients as being extremely deficient, particularly in conjunction with multiparity and in most cases sequential pregnancies (8).

A role of nutritional deficiency in PPCM was supported by a study conducted in Niamey (Republic of Niger): Plasma albumin and pre-albumin levels were lower in patients with PPCM than they were in controls (P < 0.001). For retinol binding protein, the difference was not statistically significant. The plasma concentrations of selenium and zinc were lower in patients than they were in controls (48±25 versus 77±16 ng/ml and 0.90±0.21 versus 1.17±0.25 micrograms/ml, respectively, P < 0.001) whereas that of copper was higher (2.03±0.37 versus 1.23±0.20 micrograms/ml, P < 0.001). The mean plasma zinc: copper ratio was lower in patients than controls (0.44 versus 0.95). It was concluded from these studies that such differences might be aetiological factors or biological consequences of the peripartum cardiac failure due to cardiomyopathy. Nutritional abnormalities were thus reasoned to play a role in the pathophysiology of the disease (145).

In a more recent study from Haiti on the other hand, Fett et al. report that neither a macronutrient deficiency (protein and iron) nor a micro-nutrient deficiency (vitamin A, vitamin B-12, vitamin C, vitamin E, β-carotene or selenium) played a significant role in the high incidence and prevalence of PPCM in the Haitian population, concluding that future studies of PPCM in this population should focus on other potential aetiologic and risk factors (29, 146).

Thus while poor nutrition was initially thought to be associated with the development of PPCM (24), several more recent reports suggest caution in the interpretation of these data (29, 30). Differences in the nutritional status based on the geographical location of the population needs to be considered within this context.
1.6 Clinical Presentation and Diagnosis

1.6.1 Physiologic features

Normal pregnancy is associated with an expansion of blood volume, an increase in metabolic demands, relative anaemia and changes in vascular resistance that are associated with ventricular dilatation and increase in cardiac output. These physiologic changes are due to an increase in preload and heart rate accompanied by a decrease in afterload, peaking during the second trimester of pregnancy. Decompensation of patients with subclinical valvular, ischaemic or myopathic heart disease usually occurs during this time. The early stage of PPCM can easily be missed, because many symptoms and signs of pregnancy are similar to those of early congestive heart failure (e.g. dyspnoea, abdominal discomfort, fatigue) (6, 147). Elkayam et al. report that 7% of their US patients were diagnosed within one month before delivery and 75% of patients were diagnosed during the first month postpartum (5) while Sliwa et al. observed onset of symptoms in South African patients primarily during the postpartum period (92, 148), which is in accordance with findings by Fett et al. in Haitian patients (14, 149). The symptoms and signs are similar to those in patients with idiopathic dilated cardiomyopathy (9) and can be complicated by thromboembolic events and arrhythmia. Echocardiography usually demonstrates features of dilated cardiomyopathy (DCM) with impaired ejection fraction, global dilatation and sometimes thinned out walls.

1.6.2 Symptoms and signs

The symptoms and signs are similar to those in patients with idiopathic dilated cardiomyopathy (9). Patients uniformly present with signs and symptoms of left heart failure (8). On physical examination one usually finds a young woman in moderate to severe respiratory distress and elevated jugular venous pressure. The heart is enlarged and there is an active left ventricular impulse. A left parasternal impulse due to an enlarged right ventricle may also be present. On auscultation a holosystolic murmur representing mitral and sometimes also tricuspid incompetence may be present. The murmur usually disappears as cardiac function
improves. In some patients mitral regurgitation persists and may be due to cardiomyopathic involvement of the papillary muscles (150).

Peripheral oedema and upper abdominal discomfort (congested liver) are present in 50% of the cases. Chest pain occurs in half of the women and might in some instances be due to pulmonary embolism (151). As a rule, the presence of ascites has not been noted during the earlier phases of the initial episode of cardiac insufficiency (8). Patients are usually in regular sinus rhythm although some complain of palpitations. Haemoptysis may be the presenting feature of pulmonary embolus, to which these patients are particularly predisposed (23).

Walsh et al. observed a high incidence of colicky, severe, persistent abdominal pain. With return of cardiac compensation these symptoms generally subsided, although they tended to persist to a mild degree for many months without demonstrable congestive heart failure or intrinsic gastrointestinal disease (8). Non-specific palpitations, chest pain and abdominal pain are common and tend to contribute to some confusion during the initial clinical evaluation. The use of the New York Heart Association functional classification is confounded by simultaneously occurring signs and symptoms of normal pregnancy and may not reflect the severity of underlying cardiac dysfunction (152). Table 1.6.2 provides a summary of the frequency by which some of these symptoms are noted in PPCM patients.
<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Signs</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnoea</td>
<td>Increased jugular venous pressure</td>
<td>60%</td>
</tr>
<tr>
<td>Cough</td>
<td>Cardiomegaly</td>
<td>100%</td>
</tr>
<tr>
<td>Orthopnea</td>
<td>Third heart sound</td>
<td>100%</td>
</tr>
<tr>
<td>Oedema</td>
<td>Pulmonary rales</td>
<td>60%</td>
</tr>
<tr>
<td>Paroxysmal nocturnal dyspnoea</td>
<td>Loud pulmonic valve component of second heart sound</td>
<td>93%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Mitral and/or tricuspid regurgitation, peripheral oedema</td>
<td>60%</td>
</tr>
<tr>
<td>Palpitations</td>
<td>Arrhythmias</td>
<td>7%</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>Embolic phenomena</td>
<td>26%</td>
</tr>
<tr>
<td>Chest pain</td>
<td>Embolic phenomena</td>
<td>50%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>Hepatomegaly, Ascites</td>
<td>67% 47%</td>
</tr>
</tbody>
</table>

**Table 1.3:** Symptoms and signs in patients with PPCM (6, 8, 10, 23)

The chest X-ray usually shows cardiomegaly (23). The left ventricle is consistently enlarged and there may be left atrial enlargement as well (10). Pulmonary venous congestion and bibasilar infiltrates are commonly seen (8, 18, 23, 151). Serial thoracic teleroentgenograms with barium in the oesophagus in all patients initially show generalized cardiomegaly involving all four chambers. In 1/3 of the patients a double shadow ascribable to left atrial enlargement has been noted on postero-anterior views of the chest (8).

The electrocardiogram (ECG) usually does not demonstrate any major abnormalities (30). The ECG often shows non-specific findings such as tachycardia and non-specific ST-T wave changes.
1.6.3 Echocardiographic studies

Geva et al. performed systematic echocardiographic assessment of the haemodynamics in normal pregnancies and demonstrated a 10% increase in left ventricular end-diastolic volume, a 45% increase in cardiac output, and a 26% to 28% decrease in end-systolic wall stress. In addition, the left ventricle remolds in response to the haemodynamics of pregnancy, resulting in transient hypertrophy (76).

The echocardiogram usually shows a dilated left ventricle with marked impairment of overall systolic performance (30, 45). Sliwa et al. observed a diastolic restrictive pattern in 21 of 29 patients at presentation (92). In addition, regional heterogeneities in systolic wall thickening, a B notch on M-mode tracing, mitral regurgitation, atrial enlargement and a small haemodynamically insignificant pericardial effusion may be noted. In approx. 25% of patients left ventricular thrombi were diagnosed by echocardiography (92). A fractional shortening value less than 20% and a left ventricular end diastolic dimension 6 cm or greater at the time of diagnosis was associated with a more than 3-fold higher risk for persistent left ventricular dysfunction. Along with being an important diagnostic tool in PPCM, echocardiography may provide significant prognostic information with regards to recovery of cardiac function (153).

In patients presenting with PPCM, inotropic contractile reserve during dobutamine stress echocardiography accurately correlates with subsequent recovery of LV function and confers a benign prognosis (154).

1.6.4 Tissue studies

During autopsy the heart of PPCM patients is usually soft and grossly enlarged (350-650g) with dilatation of all four chambers (8, 10). Specimens demonstrate paleness of the myocardium. Although ventricular mural thrombi are often seen, there is no evidence of detectable coronary artery, valvular or pericardial disease. Endocardial thickening and pericardial fluid have been noted occasionally (8, 10, 23, 24, 151, 152, 155). No vascular changes of hypertension have so far been seen (23).

In 1971 Demakis et al. performed thoracotomy for myocardial and pericardial biopsies. Results of these studies showed that the pericardium appeared normal. The most prominent findings in these myocardial
biopsies were hypertrophy of myocardial fibers and varying degrees of fibrosis (23). Other authors demonstrated non-specific myofiber hypertrophy, myofiber degeneration, fibrosis, interstitial oedema and occasionally lymphocytic infiltration in antemortem biopsy specimens (18, 156, 157).

Electron microscopic studies of sections of the myocardium in congestive cardiomyopathy show an increase in the number and size of mitochondria, presence of dense intra-mitochondrial inclusions, fragmentation of the cristae, varying degrees of myofibrillar destruction, fragmentation of sarcoplasmatic reticulum, increased number of lipofuscin granules and increased glycogen deposition. Electron microscopic studies of myocardial sections from a patient with PPCM showed essentially similar results (158, 159). Histochemical studies have shown an increased deposition of neutral lipid deposits in the myofibrils and a decrease in myocardial oxidative enzymes, especially succinic dehydrogenase activity. Variable areas of interstitial fibrosis and areas of muscle degeneration were seen dispersed throughout the myocardium (8).

All patients exhibited severe chronic passive congestion of the lungs and intraabdominal organs. Microscopically all hearts demonstrated significant variation in fiber size and oedematous and partially hyalinised fibers which were interspersed irregularly. Variable areas of fibrosis were seen throughout. The presence of inflammatory cells was highly variable. The amount of lipofuscin granules was within normal limits. The number of mast cells per unit area of PPCM tissue examined appeared to be similar to that seen in similar tissues from otherwise normal donors using cresyl violet stain. No detectable levels of metachromasia or accumulation of PAS-positive material was found. There was little fatty acid change except in areas with heavy deposition of relatively large fat droplets. Such areas correspond to those with diminished or absent succinic dehydrogenase and cytochrome oxidase activity. Walsh et al. interpreted these changes as evidence of mitochondrial damage with resulting impairment of oxidation of lipids which therefore accumulate. There were individual fibers with diminished succinic dehydrogenase reaction and no lipid droplets as well as others with normal enzymatic reactions and small lipid droplets. All fibers containing fat droplets demonstrated a very reduced response for both oxidative enzymes. These changes were strikingly different from those seen in alcoholic cardiomyopathy (8).
Other authors found that pathologic studies of cardiac tissues have so far failed to identify any significant difference between PPCM and other forms of primary congestive cardiomyopathy. However, it should be stressed that specimens of myocardium that have been studied were obtained several months to several years after the onset of the disease (10). It is important to note, however, that a case for the presence of inflammation within cardiac tissues of PPCM patients has been made by a number of studies. Thus, a study of cardiac tissues obtained at autopsy from three PPCM patients from Haiti, demonstrated an inflammatory infiltrate consisting of mononuclear cells including T-lymphocytes (CD 8) and monocyte/macrophage lineage cells (CD 68) in one patient. Cardiac tissues from the second patient showed non-specific changes in the myocardium similar to those that may be seen in dilated cardiomyopathy of various aetiologies, including PPCM. The third patient’s cardiac tissue additionally showed areas of interstitial fibrosis, highlighting the difficulty of finding focal myocarditis in limited autopsy tissue (123).

Similarly several additional studies have reported an inflammation of the myocardium which was shown to be sometimes associated with pericarditis and demonstrated histological evidence of myocarditis on endomyocardial biopsy samples (52, 118, 160).

One of these studies reported from the Stanford University Medical Centre which involved a retrospective review of endomyocardial biopsy specimens from 34 patients with PPCM reported an incidence comparable to that found in age- and sex-matched controls (118).

Bultmann et al. detected viral genomes in endomyocardial biopsy specimen in eight of 26 PPCM patients (30.7%) but also in 10 of 33 control subjects (30.3%) (49). The detected viruses (PVB 19, HHV 6, EBV, HCMV) have been related to inflammatory cardiomyopathy but also have a high prevalence in healthy populations (50, 51). A study by Sanderson et al. of EMB in 11 African women with PPCM in Nairobi were consistent with healing myocarditis in 5 patients (161).

In a study from Johns Hopkins Hospital myocarditis was demonstrated in EMB specimens in 14 of 18 patients with newly diagnosed PPCM. Of these, 10 were treated with immunosuppressive therapy. Nine of the 10 treated patients with myocarditis had subjective and objective improvement. Follow-up
endomyocardial biopsies in these patients showed resolution or substantial improvement in myocarditis. Four patients with myocarditis not treated with immunosuppressives also improved. The role of endomyocardial biopsy remains controversial and is likely to be clinically useful only if performed early in the course of the disease (52).

**Picture 1.2:** Exterior aspect of the globally dilated heart of a patient with PPCM (162)

**Picture 1.3:** Interior aspect of the globally dilated heart of a patient with PPCM (163)
1.7 Current therapeutic approaches towards heart failure in PPCM

1.7.1 Treatment of acute heart failure in PPCM

Treatment is directed toward symptomatic relief and improvement of cardiac function and similar to other forms of congestive heart failure. The maintenance of a SaO2 within the normal range (95-98%) is important to maximize oxygen delivery to the tissues and tissue oxygenation, thus helping to prevent end-organ dysfunction and multiple organ failure. This is best achieved by first ensuring that there is a patent airway and then by administration of an increased FiO2. Endotracheal intubation is indicated if these measures fail to improve tissue oxygenation. The use of CPAP and NIPPV in acute cardiogenic pulmonary oedema is associated with a significant reduction in the need for tracheal intubation and mechanical ventilation. Respiratory muscle fatigue is the most frequent reason for endotracheal intubation and mechanical ventilation in AHF. It may be diagnosed by decreased respiratory rate associated with hypercapnia and a confused state of mind. Invasive mechanical ventilation should only be used if acute respiratory failure does not respond to vasodilators, oxygen therapy and/or CPAP or NIPPV (164).

Morphine is indicated in the early stage of treatment of patients with severe AHF, especially if associated with restlessness and dyspnoea. Morphine induces venodilation and mild arterial dilatation and reduces heart rate. Anticoagulation should be initiated unless contraindicated to avoid both, venous and arterial thromboembolic events. Careful monitoring of INR and PTT is advised since autoanticoagulation due to hepatic congestion may be present.

Vasodilators are indicated as first line therapy if hypoperfusion is associated with an adequate blood pressure and signs of congestion with low diuresis, to open the peripheral circulation and to lower pre-load. Angiotensin converting enzyme (87)-inhibitors are not indicated in the early stabilization of patients with HF. Administration of diuretics is indicated in the presence of symptoms secondary to fluid retention. There has been no study with β-blocker therapy in AHF targeted to acutely improve the condition. On the contrary AHF has been considered a contraindication for this treatment.
It is important to note that management will differ in women who are still pregnant since the threshold to perform X-rays or a CT scan will be much higher. Before the administration of drugs, contra-indications during pregnancy need to be observed.

In patients with chronic heart failure, β-blockers should be initiated when the patient has stabilized after the acute episode (usually after 4 days). Inotropic agents are indicated in the presence of peripheral hypoperfusion (hypotension, decreased renal function) with or without congestion or pulmonary oedema refractory to diuretics and vasodilators.

Temporary mechanical circulatory assistance may be indicated in patients with AHF who are not responding to conventional therapy and where there is a potential for myocardial recovery or as a bridge to heart transplantation or interventions that may result in significant recovery of the heart function (intra-aortic balloon pump, left ventricular assist device) (164). In clinical experience, PPCM often shows remarkable spontaneous improvement. The decision for heart transplantation should therefore only be made very carefully, after all other options have been exhausted and sufficient time for recovery has been allowed.

Cardiac transplantation has been performed successfully in PPCM patients. Favourable outcomes have been attributed to the young age of the recipients and to the recent onset of heart failure, resulting in minimal end-organ damage. In view of the success that has been achieved by transplantation in these young and otherwise healthy mothers, aggressive measures such as temporary life support in form of cardiopulmonary bypass or a left ventricular assist device until availability of transplant (165) have been advocated.

1.7.2 Treatment of chronic heart failure in PPCM

Angiotensin-converting enzyme-inhibitors are recommended as first-line therapy in patients with a reduced left ventricular systolic function less than 40-45% with or without symptoms (166), but are contraindicated during pregnancy because of teratogenicity (1). Vasodilator therapy reduces afterload and improves cardiac output, resulting in a reduction in left ventricular end-diastolic pressure and a decrease in pulmonary and systemic vascular resistances. Godsel et al. view an ACE-inhibitor as the most valuable medication, not only
because of its direct beneficial effects on the heart but also because of its potential benefit to interrupt the chain of events in the pathobiology of PPCM (167). ACE-inhibitors should be uptitrated to dosages shown to be effective in the large controlled trials in heart failure and not on symptomatic improvement alone. Diuretics are essential for symptomatic treatment when fluid overload is present and manifest as pulmonary congestion or peripheral oedema, but their use should be carefully considered during pregnancy. In patients with chronic heart failure diuretics should be administered in combination with ACE-inhibitors and β-blockers if tolerated.

B-blockers should be considered for treatment of all patients with stable, mild, moderate and severe heart failure, unless there is a contraindication. B-blocker therapy reduces hospitalizations, improves the NYHA functional class and leads to less worsening of heart failure. The initial dose should be low and increased slowly and progressively to the target dose used in the large clinical trials (166). Carvedilol reduces the risk of death as well as the risk of hospitalization for cardiovascular causes in patients with heart failure. Vasodilating β-blockers such as carvedilol also reduce afterload through alpha-1 adrenergic blockade. Lowes et al. reported functional improvement in IDCM patients related to treatment with β-blockers and an association with changes in myocardial gene expression. B-blocker treated patients who improved left ventricular ejection fraction had an increase in sarcoplasmatic reticulum calcium ATPase mRNA and alpha-myosin heavy chain mRNA and a decrease in β-myosin heavy chain mRNA (168). Up-titration should be adapted to individual responses. Aldosterone receptor antagonists are recommended in addition to ACE-inhibitors, β-blockers and diuretics in advanced heart failure (NYHA III-VI) with systolic dysfunction to improve survival and morbidity. In the RALES study, low doses of aldactone, added to standard of care for severe heart failure, improved survival by 30% and lowered hospitalization by 35% (169). Angiotensin II receptor blockers (ARB) can be used as an alternative to ACE-inhibition in symptomatic patients intolerant to ACE-inhibitors to improve morbidity and mortality, but are also contra-indicated during pregnancy. Digoxin therapy is associated with an increased risk of death from any cause among women with heart failure and depressed left ventricular systolic function (170). Retrospective analysis of data from the DIG
trial indicates a beneficial effect of digoxin on morbidity and no excess mortality in women at serum concentrations from 0.5 to 0.9 ng/ml, whereas serum concentrations > or =1.2 ng/ml seem harmful (171). Ahmed et al. found lower hospitalisation rates without increasing odds for death among women with heart failure and left ventricular ejection fraction below 35% with serum digoxin concentrations between 0.5 – 1.1 ng/ml. Negative outcome was documented in women with higher serum digoxin concentrations or higher LVEF (172).

Digoxin is a class C drug and should be avoided during pregnancy. While ACE-inhibitors and ARB's are contra-indicated during pregnancy, hydralazine might be the vasodilator of choice although controversy exists (173). Among the vasodilators, nitrates are another alternative during pregnancy. It is important to note that venous therapeutic modalities mentioned in the article have never been studied specifically in PPCM patients but in DCM patients.

Thromboembolic phenomena have been reported in PPCM patients. Pregnant patients are at increased risk of thromboembolic complications due to the hypercoagulable state of late pregnancy that may persist up to six weeks postpartum. Left ventricular systolic dysfunction resulting in blood stasis, additionally predisposes patients to develop left ventricular, pulmonary and cerebral thrombemboli. The decision for anticoagulation should be made after careful consideration, that should include dilated left ventricular dimensions and low ejection fraction. It is important to stress that not all PPCM patients need to be anticoagulated. During the last weeks of pregnancy low-molecular heparin is the agent of choice while warfarin is preferred postpartum. The need for early delivery and the mode of delivery should be assessed through collaboration between obstetricians, cardiologists and anesthesiologists (1). If possible, pregnancy should be permitted to continue to term in PPCM patients diagnosed during the last month of gestation. Urgent delivery of the fetus may be considered for patients who present with advanced heart failure with haemodynamic instability. Patients with adequate cardiac output may tolerate induction and vaginal delivery. Critically ill patients who require inotropic therapy or mechanical support should undergo caesarean delivery
1.7.3 Immunomodulatory therapy

Stress-activated pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) or interleukin 1 (IL-1) have been implicated in the pathophysiology of idiopathic dilated cardiomyopathy (84). TNF-alpha is a pleiotropic inflammatory cytokine and serves a variety of functions, many of which are not yet fully understood. The cytokine possesses both growth stimulating properties and growth inhibitory processes, and it appears to have self regulatory properties as well. For instance, TNF-alpha induces neutrophil proliferation during inflammation, but it also induces neutrophil apoptosis upon binding to the TNF-R55 receptor (85). TNF-alpha is a 26 kDa protein and is produced by many different cell types. The main sources in vivo are stimulated monocytes, fibroblasts, and endothelial cells. Macrophages, T-cells and B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells and keratinocytes also stimulate production of TNF-alpha. Glioblastoma cells constitutively produce TNF-alpha and the factor can be detected also in the cerebrospinal fluid. Human milk also contains TNF-alpha.

Physiological stimuli for the synthesis of TNF-alpha are IL-1, bacterial endotoxins, PDGF and Oncostatin M. In fibroblasts the synthesis of TNF-alpha is stimulated by IFN-β, TNF-alpha, PDGF and viral infections. In thymic stromal cells the synthesis of TNF-alpha can be induced by NGF. TNF-alpha can also stimulate or inhibits its own synthesis, depending upon the cell type. In epithelial, endothelial, and fibroblastic cells secretion of TNF-alpha is induced by IL-17.

Beneficial functions of TNF-alpha include its role in the immune response to bacterial, and certain fungal, viral, and parasitic invasions as well as its role in the necrosis of specific tumors. TNF-alpha acts as a key mediary during local inflammatory immune response. It is an acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and neutrophils to a site of infection. TNF-alpha secreted by the macrophage causes blood clotting which serves to contain the infection. Without TNF-alpha, mice infected with gram-negative bacteria experience septic shock (86). Tracey and Cerami suggest that low levels of TNF-alpha may aid in maintaining homeostasis by regulating
the body's circadian rhythm and that low levels of TNF-alpha promote the remodeling or replacement of injured and senescent tissue by stimulating fibroblast growth (87). The pathological activities of TNF-alpha include the promotion of growth of certain tumor cells, although it causes necrosis of other types of tumors. High levels of TNF-alpha correlate with increased risk of mortality (88). TNF-alpha participates in disorders of inflammatory and non-inflammatory origin (89). Originally sepsis was believed to result directly from the invading bacteria itself, but it was later recognized that host system proteins, such as TNF-alpha induce sepsis in response. Exogenous and endogenous factors from bacteria, viruses, and parasites stimulate production of TNF-alpha and other cytokines. Lipopolysaccharide from bacteria cell walls are a potent stimulus for TNF-alpha synthesis (87). TNF-alpha exhibits acute and chronic effects. If TNF-alpha remains in the body for a long time, it loses its anti tumor activity. This can occur due to cytokine polymerization, shedding of TNF-alpha receptors by tumor cells, excessive production of anti-TNF antibodies, found in patients with carcinomas or chronic infection, and disruptions in the alpha-2 macroglobulin proteinase system which may deregulate cytokines. Prolonged overproduction of TNF-alpha results in cachexia. Cachectin and TNF-alpha were once considered different proteins, but in 1985 researchers discovered that the two proteins were homologous (90).

All known members of the TNF-alpha cytokine family induce hepatic expression of acute phase proteins. Acute, high dose exposure to TNF-alpha causes shock and tissue damage, catabolic hormone release, vascular leakage syndrome, adult respiratory distress disorder, gastrointestinal necrosis, acute renal tube necrosis, adrenal hemorrhage, decreased muscle membrane potentials, disseminated intravascular coagulation, fever. Chronic, low dose exposure to TNF-alpha has been associated with subendocardial inflammation, endothelial activation, protein catabolism, lipid depletion, insulin resistance and enhanced tumor cell reproduction.

Sliwa et al. reported significantly higher plasma levels of TNF-alpha and interleukin-6 in PPCM patients as compared to age matched healthy controls (92). One hypothetical advantage of treatment with anti-TNF-alpha antibodies results from its role in multiple types of inflammation. It is often difficult to determine that
inflammation in burn and trauma victims are of infectious aetiology and warrant treatment with antibiotics. Therefore another treatment strategy might involve anti-TNF-alpha therapy (89). Strategies for preventing TNF-alpha activity include neutralization of the cytokine via either anti-TNF antibodies, soluble receptors, or receptor fusion proteins, suppression of TNF-alpha synthesis via drugs such as cyclosporine A, glucocorticoids, or cytokine IL-10 and by inhibition of secondary mediators such as IL-1, IL-6 or nitric oxide (87).

The rationale for using immunomodulating agents to treat patients with heart failure is based on the fact that excessive enhancement of pro-inflammatory cytokines appears to mimic many aspects of the heart failure phenotype (93). In addition, inflammatory cytokines have been shown to play a key role in the pathogenesis of atherosclerosis and coronary artery disease (94) and it has been suggested that sustained TNF-alpha expression after myocardial infarction and in persistent ischemia may have detrimental effects on the remodeling process (95). However, recent trials with anticytokine agents such as etanercept and infliximab showed time- and dose-dependent worsening of heart failure (93, 96, 97). These rather discouraging results may be explained by the mechanisms of action of these agents. Infliximab exerts its effects by fixing complement in cells (93), which in the heart is reported to lead to cardiac myocyte lysis (98). Etanercept stabilizes TNF-alpha and hence leads to an accumulation of TNF-alpha in the peripheral circulation (93). In comparison, the effects of pentoxifylline are to reduce the synthesis of TNF-alpha by blocking transcriptional activation (99, 100). Furthermore, pentoxifylline has been shown to inhibit apoptosis in different human cell types in vitro (101, 102) and in vivo (102). Hence, pentoxifylline is likely to be a more promising anticytokine agent. Indeed, Sliwa et al. were able to demonstrate improved outcome in PPCM patients who received pentoxifylline in addition to standard heart failure therapy. In a non-randomised, single-centre study with 59 prospectively enrolled PPCM patients the composite end-point of poor outcome (either death, NYHA FC III or IV at latest follow-up and/or failure to improve LVEF by 10 absolute units) occurred in 52% of patients on standard heart failure therapy alone and in 27% of patients who received
additional pentoxifylline. From all baseline characteristics analysed between the two groups, treatment with pentoxifylline was the only independent predictor of outcome (p=0.04) using logistic regression analysis (148). These results are in line with Sliwa et al.'s previous work on patients with idiopathic dilated cardiomyopathy (104-106). However, the effects of additional pentoxifylline in the treatment of PPCM need to be interpreted carefully since PPCM is a rare disorder, limiting studies to small numbers of patients and because PPCM has a spontaneous recovery rate of approximately 30%, making statistical interpretation difficult.

The beneficial effects of pentoxifylline observed in PPCM patients are likely to have been mediated by several mechanisms. Patients treated with pentoxifylline showed a marginal decrease in plasma TNF-alpha concentrations but significant reductions in plasma Fas/Apo-1 concentrations. Because programmed cell death has been recognized as a contributing cause of myocyte loss in myocardial infarction (174) and TNF-alpha augments this process through the stimulation of apoptosis (175), the combined reduction of TNF-alpha and Fas/Apo-1 concentrations in PPCM patients may explain the clinical benefits observed with pentoxifylline therapy. In addition, the acute-phase protein CRP was reduced in those patients who were treated with pentoxifylline. CRP has direct pro-inflammatory effects on endothelial cells, including the expression of adhesion molecules and monocyte chemotactic protein-1 (176). Furthermore, CRP is implicated in the synthesis of TNF-alpha (177). Hence, a reduction in serum CRP concentrations could have beneficial effects on the progression of cardiac dysfunction. Plasma levels of NT-pro BNP have been used in several clinical trials to assess the efficacy of medical therapy (107). Sliwa et al. also observed reductions in NT-pro BNP in patients treated with pentoxifylline in a randomized study of patients with ischaemic cardiomyopathy (103) and were able to confirm the efficacy of pentoxifylline in ischaemic heart failure. While pentoxifylline did not abolish increments in circulating TNF-alpha concentrations in patients, experimental studies have suggested that physiological levels of TNF-alpha have cytoprotective effects on the heart during ischaemic events (108, 109). Although we are not aware of any large-scale study that has evaluated the safety of pentoxifylline in patients with heart failure, this pharmacological agent has been in
clinical use for more than 25 years for conditions such as peripheral and cerebrovascular disease (110).

Patients with peripheral vascular disease frequently also have coronary artery disease and heart failure. It is therefore important to note that large trials with more than 10000 such patients have not reported increases in mortality in patients treated with pentoxifylline (110).

Furthermore beneficial effects of pentoxifylline have been reported in HIV-positive patients. Apoptosis is a significant cause of CD4 T cell death. Wanchu et al. demonstrated a significant decline of caspase 1 and caspase 8 that are involved in Fas/Apo-1 and TNF-alpha facilitated apoptosis in HIV-positive patients and concluded that this might result in reduced apoptosis and improved CD4 lymphocyte survival (111). The same group described a reduction of nitric oxide in HIV-1 positive patients after administration of pentoxiphylline (112). Swords et al. described an association between enhanced HIV replication and increased production of TNF-alpha. They observed partial inhibition of HIV-1 induction using pentoxifylline and matrix metalloproteinase (MMP) inhibitor I (113).
1.7.4 Anticoagulatory drugs

Thromboembolic phenomena have been reported in up to 53% of PPCM patients (8, 178). Because of the high incidence, Demakis et al. recommend anticoagulants for the duration of cardiomegaly (10). In general, pregnant patients are at an increased risk of thromboembolic complications due to a hypercoagulable state of late pregnancy, which is associated with increased concentrations of coagulation factors II, VII, VIII, X and of plasma fibrinogen as well as augmented platelet adhesiveness (179). These changes may persist up to six weeks postpartum (180, 181). Left ventricular dysfunction results in partial blood stasis, which predisposes additionally to the formation of left ventricular, pulmonary and cerebral thrombemboli. During the last weeks of pregnancy low-molecular weight heparin is the agent of choice while warfarin is preferred postpartum.

1.7.5 Immunosuppressive therapy

A case report by Rached et al. from Argentina described good results of immunosuppressive therapy in one patient with PPCM (182). Immunosuppression has been attempted in PPCM patients with biopsy-proven myocarditis (117). However, most EMB studies in PPCM patients did not reveal conclusive results. Although Maisch et al. recommend heart catheterization with endomyocardial biopsy to allow for the exact diagnosis of the underlying cardiac process (inflammatory and/or viral vs. autoreactive myocarditis or non-inflammatory or nonviral forms) (160) this is not an established routine procedure. Until a link between immunosuppressive therapy and resolution of myocarditis can be established in PPCM patients, the use of immunosuppressive agents is not recommended (6). In fact the benefit of immunosuppressive and antiviral therapy is discussed controversially (183).

In a small retrospective study Bozkurt et al. treated women with PPCM with intravenous immunoglobulin and reported a greater improvement in ejection fraction during early follow-up than patients treated conventionally (184).
1.7.6 Cardiac transplantation

Cardiac transplantation has been performed successfully in PPCM patients (165, 185). Favourable outcomes have been attributed to the young age of the recipients and to the recent onset of heart failure, resulting in minimal end-organ damage. In view of the successful transplantation in these young and otherwise healthy mothers, aggressive measures such as temporary life support in form of cardiopulmonary bypass or a left ventricular assist device until availability of transplant (165) have been advocated. Tubal ligation has been recommended for women who have received heart transplants due to the mother's reduced life expectancy and teratogenicity of immunosuppressive drugs (186). But Carvalho et al. even reported on a successful subsequent pregnancy in a PPCM patient post-transplant (187). Data on the outcome of pregnancies among heart transplant recipients demonstrates that despite frequent complications these pregnancies can be managed as high-risk cases (188).

1.7.7 Other forms of treatment

1.7.7.1 Salt and water restriction

Salt and water restriction are important in patient management, particularly in women with symptoms and signs of heart failure. Once heart failure symptoms have been controlled, modest exercise may improve symptoms as well as peripheral muscular and arterial tone (1).

1.7.7.2 Prolonged bed rest

Burch et al. stressed the value of prolonged bed rest in altering the course of PPCM and advocate bed rest for three months after the heart size has returned to normal (20). Also Walsh et al. advocated prolonged, complete bed rest for periods in excess of one year in some instances or until the heart had returned to normal size (8).
1.7.7.3 Herbal medicine

Bark extracts prepared from Terminalia Arjuna, an Indian medicinal plant, when administered at a dose of 500 mg qid for a 2 week period, patients with idiopathic dilated cardiomyopathy and PPCM showed continued improvement in symptoms, signs, effort tolerance and NYHA Class as compared with patients administered placebo using the same dosage scheme (189). The significance of this finding remains to be established as is the case with studies utilizing unconventional forms of alternative medicines from natural sources.

1.7.8 Recommended contraceptive methods for PPCM patients

Appropriate birth-control measures are recommended for patients with enlarged hearts. Oral contraceptives should be avoided due to the increased incidence of thromboembolism. However, the use of quarterly injections of depot hormone or other methods of preventive family planning need to be encouraged.
1.8 Prognosis

1.8.1 Maternal outcome

Before the routine application of ACE-inhibitors and β-blockers in patients with heart failure, Demakis et al. reported in 1971 that the clinical course of PPCM is related to the return of heart size to normal within six months which occurred in approximately half of their patients. The mortality of patients whose heart size returned to normal was 14%. Of those who maintained cardiomegaly beyond six months, 85% died, all as a result of myocardial failure (23). The most common cause of death was congestive heart failure, which was frequently exacerbated by pulmonary emboli, subsequent pregnancies or supraventricular arrhythmias such as atrial fibrillation and atrial flutter (10). Patients in whom left ventricular function recovers have significantly improved survival (19, 190, 191).

More recent studies confirm the importance of the recovery of cardiac dimensions. Echocardiography is an important diagnostic tool in PPCM and may provide significant prognostic information with regards to recovery of cardiac function. End diastolic dimensions of 6 cm or greater at the time of diagnosis were associated with a more than 3-fold higher risk for persistent left ventricular dysfunction (153). Dorbala et al. studied the left ventricular contractile reserve in seven PPCM patients during dobutamine stress echocardiography and documented a correlation (r=0.79) with subsequent recovery of LV function (154).

In a cohort of 100 patients from South Africa, Sliwa et al. reported a mortality of 15% within a six months period. Baseline plasma levels of Fas/Apo-1 (OR=1.30, CI 95%=1.11-1.54) and NYHA FC (OR=2.88, CI 95%=1.10-7.53) were identified as independent predictors of death (192). Sliwa et al. did not confirm variables previously reported by others, such as age above 30 years, higher parity, later onset of symptoms after delivery and twin deliveries as predictors of outcome (92).

In a study from the US, Elkayam et al. report a maternal mortality of 9% within a period of 2.2 years. Death was described as sudden in four patients and as a result of complications from heart transplantation in two patients. Heart transplantation was performed in 4% of the patients. Three percent of the patients required
implantation of an automatic implantable cardioverter-defibrillator, and 2% required implantation of a permanent pacemaker during the follow-up period. LVEF at the time of diagnosis was 29±11% and improved to 46±14% (5) at follow-up. Normalization of LVEF occurred in 54% and was more likely in patients with LVEF >30% at diagnosis. (5).

Felker et al. found better survival rates in 51 PPCM patients than in patients with other causes of cardiomyopathy (n=1230) (193). In a study from Haiti, the ratio of PPCM deaths for the 5-year period was 47.1 per 100,000 births compared with the US ratio of 0.62 per 100,000 births. The mortality rate was 15.3% during a mean follow-up period of 2.2 years. 28% of patients who were observed for at least six months, regained normal left ventricular function. The difference in left ventricular echocardiographic features at diagnosis between deceased patients and survivors was not statistically significant, but a statistically significant difference occurred at diagnosis between the recovered and the non-recovered group for mean ejection fraction (28% vs. 23%; P<0.001) and fractional shortening (17% vs. 14%; P=0.004) (14).

**1.8.2 Neonatal outcome**

There is little systematic evidence that infants born to women with PPCM are adversely affected, although one study did report a premature delivery rate of 21% in 14 women. Results of a more recent study from the USA that described neonatal outcome in PPCM mothers is also of interest. However, this study requires careful interpretation since pregnancy related hypertension was reported in 43% of these patients who would otherwise be excluded from the analysis of these data since the diagnosis utilized for the inclusion of these patients did not follow a strict and generally acceptable diagnosis of PPCM as appreciated at our institution. The mode of delivery was a by cesarean section in 40 patients, which was performed for obstetrical reasons in 70% of the patients, cardiac reasons in 10% and unknown reasons in 20% of the patients. The duration of pregnancy (56 patients) ranged from 24 to 42 weeks, with an average of 37.7±3.5 weeks. Premature delivery (<37 weeks) was reported in 25% of these patients. Birth weight (51 patients) ranged between 1350 and 5000 g, with a mean of 3092±745 g. The incidence of small-for-date infants was 5.9%. There were two
stillbirths and one neonatal death. Congenital anomalies in the newborn were reported in four cases and included hypospadias, coarctation of the aorta, dysmorphogenesis, and macrosomia. Neonatal complications were reported in six cases and included one case each of hypothermia, poor suckling, apnea with seizure requiring intubation, hypoglycemia and death and two cases of pulmonary oedema (5). As stated above, while these are interesting data, their interpretation with reference to a strict diagnosis of PPCM remains a problem.
1.9 Subsequent pregnancy in PPCM

One of the most common issues for women surviving an episode of PPCM is whether it is safe to become pregnant again (13). If cardiac failure recurs in subsequent pregnancies, it once again manifests clinically in the peripartum period (155). The mechanism of recurrent symptomatic heart failure in patients with a history of PPCM and recovered left ventricular function has been attributed to a significant physiological increase in blood volume, stroke volume and heart rate during pregnancy (194).

Women with a history of peripartum cardiomyopathy who have regained normal resting left ventricular size and performance have decreased contractile reserve revealed by the use of a dobutamine challenge test. Ventricles of these women may respond suboptimal to haemodynamic stress in spite of evidence of recovery by routine echocardiographic evaluation (195). These haemodynamic changes are expected to cause symptomatic deterioration in patients with persistent unmasked subclinical myocardial dysfunction, which may exist even in patients who seem to recover their left ventricular function (195). At the same time the findings of a significant depression in left ventricular function associated with subsequent pregnancy suggest that worsening of symptoms are also due to reactivation of the underlying idiopathic process responsible for the development of the cardiomyopathy during a previous pregnancy (13).

If a subsequent pregnancy occurs, it should be managed in close collaboration between an obstetrician and a cardiologist (1). The prognosis of a subsequent pregnancy in known PPCM appears to be related to left ventricular dimensions at onset of subsequent pregnancy.

Elkayam et al. conducted a record review among members of the American College of Cardiology in the United States and Chris Hani Baragwanath Hospital and described the outcome of 60 subsequent pregnancies in 44 women with a history of PPCM. Among the first subsequent pregnancies in the 44 women, 28 occurred among women, in whom left ventricular function had returned to normal (group 1) and 16 occurred in women with persistent left ventricular dysfunction (group 2). The pregnancies were associated with a reduction in mean left ventricular ejection fraction in each group (from 56 ±7 % to 49 ±10 % in group 1, p=0.002 and from 36±9 % to 32±11 % in group 2, p=0.08). During these pregnancies, a
decrease of more than 20% in left ventricular ejection fraction occurred in 21% of women in group 1 and 25% of those in group 2, and symptoms of heart failure occurred in 21% of women in group 1 and 44% of those in group 2. The mortality rate was 0% in group 1 and 19% in group 2 (p=0.06) (196). Although the likelihood of maternal death seems to be very low in women who recover their left ventricular function before a subsequent pregnancy, the fact that a reduction in left ventricular ejection fraction and symptomatic heart failure may occur during subsequent pregnancy needs to be considered (13).

Results from other studies of subsequent pregnancies are important to summarize. Thus, a study conducted by Walsh et al. concerned observations on six subsequent full-term pregnancies in six patients with previously documented PPCM. At least two were complicated by the appearance of congestive heart failure in spite of digitalis. Subsequent to each of the six pregnancies cardiac insufficiency became worse postpartum. Moreover these six patients experienced four additional pregnancies that did not go to term. Existing congestive heart failure may have become more severe following abortion of a three month fetus. The remaining three pregnancies terminating at six, six and seven months respectively were neither complicated by congestive heart failure nor followed by recurrence of PPCM (8).

In the experience of Demakis et al. the influence of a subsequent pregnancy was related to whether the heart size had returned to normal. 21 subsequent pregnancies occurred in 8 patients whose heart size had returned to normal (group A). There was a temporary recurrence of postpartum cardiac failure following three pregnancies in two of these patients. In each case the patient responded promptly to therapy. Six patients whose heart size did not return to normal (group B) had subsequent pregnancies. Although these six patients maintained cardiomegaly, they were not in clinical cardiac failure before onset of the subsequent pregnancy. There was no change in cardiac function in 3 of these patients. The other three patients experienced marked increase in cardiac symptoms in the last trimester or in the early postpartum period. In each case this initiated a permanent deterioration in cardiac function that resulted in death. This experience would strongly suggest that patients whose heart size has not returned to normal should avoid becoming pregnant (23).
Sutton et al. conclude that PPCM patients whose left ventricular function returns to normal may undertake further pregnancy with a normal fetal outcome and a low risk of recurrent left ventricular dysfunction (197).
2. METHODS

The low incidence of PPCM in the northern hemisphere is one of the reasons why previous studies of this disease have relied to a great extent on case reports, studies with small numbers of patients or a review of medical records from many hospitals for defining the potential actio-pathogenesis of human PPCM. The high incidence of PPCM in a single centre in South Africa provides therefore a unique opportunity to initiate studies of the mechanism of pathogenesis, clinical features and prognostic markers in this disease.

2.1 Study objectives

- To describe the clinical profile of 100 patients diagnosed with PPCM
- To document the kinetics of cardiac function biomarkers, pro-inflammatory cytokines, markers of remodeling and prolactin in 40 PPCM patients over a six months period
- To study the presence of autoantibodies in PPCM
- To document the outcome of subsequent pregnancy in PPCM

2.2 Informed consent

This study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, Johannesburg, South Africa (PRC 990409) and complies with the Declaration of Helsinki. All patients and controls gave written informed consent before study entry. As data developed during the course of this study and new aspects of the pathogenesis of PPCM emerged, several amendments to the original protocol became necessary and were also approved by the HREC. The nature of this study was explained to all patients in their native language by an interpreter and each patient was provided sufficient time to consider entry into the study and ask questions. Informed consent was signed before entry into the study and performance of any study related procedures. Please find the relevant documents attached in the appendix.
2.3 Methods

The general methods are outlined in this chapter, while the methods specific to each section of the results are described in the methods section of the respective chapters.

2.3.2 Recruitment of patients

We enrolled 100 African women with PPCM attending the Cardiac Clinic at Chris Hani Baragwanath Hospital. All patients received standard therapy for PPCM (β-blocker, angiotensin-converting enzyme inhibitors, diuretics and if indicated digoxin). Patients with an ejection fraction ≤ 25% or LV thrombus additionally received anticoagulation therapy. All patients were followed up at the Cardiac Clinic, Chris Hani Baragwanath Hospital every month. Reassessments of the initial examinations were performed after six and 12 months or at anytime in between when clinically indicated.

2.3.3.1 Inclusion criteria

At the time of entry into the study each patient had to meet the following criteria:

- Symptoms of congestive heart failure that developed in the last month of pregnancy or in the first 5 months postpartum
- No other identifiable cause for heart failure and no demonstrable cardiac disease in the last three months of pregnancy
- Left ventricular ejection fraction less than 40% by transthoracic echocardiography
- New York Heart Association functional class II - IV
- Sinus rhythm documented on ECG
- Eligible patients in whom high-quality echocardiographic images could be obtained

2.3.3.2 Exclusion criteria

- Systolic blood pressure > 160 mmHg and/or diastolic blood pressure > 100 mmHg
• Significant organic valvular heart disease

• Clinical conditions other than cardiomyopathy that could increase inflammatory markers i.e. active tuberculosis, sepsis, rheumatoid arthritis, allergy

• Significant liver disease (defined as enzymes > 2 times the upper limit of normal) and severe anaemia (haemoglobin concentration < 9.0 g/dl)

• Any clinical condition that according to the investigators preclude inclusion in the study or are a contraindication for the study medication pentoxifylline (malignancy, cachexia, COPD, recent retinal haemorrhage, recent cerebral bleed)

2.3.4 Study visits

Following the initial screening and baseline visits, monthly outpatient visits were scheduled for clinical assessment and evaluation of medication compliance. All baseline examinations were repeated after six months. After completing six months of therapy, patients were given a choice to extend their participation in the study to twelve months and continue monthly outpatient visits with another assessment of all baseline examinations after twelve months.

At the time of enrolment, and at six and twelve months following enrolment, physical and echocardiographic examinations were performed. In addition an ECG was done and blood pressure and heart rate were measured as described in the methods section. Echocardiography was taped on video and stored at the Division of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

• Follow up visit 2 (after 1 month): Check up
• Follow up visit 3 (after 2 months): Check up
• Follow up visit 4 (after 3 months): Check up
• Follow up visit 5 (after 4 months): Check up
• Follow up visit 6 (after 5 months): Check up
**Follow up visit 7 (after 6 months):** A physical examination was performed, blood pressure and heart rate measured, blood samples taken. Echocardiography was done, taped on video and stored at the Division of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

After completing six months the patients were informed that they could decide to either continue participating in this study for an additional 6 months to complete 12 months of study or no longer participate in the study without any change in the clinical care they would receive if they did decide to terminate their participation. The ones who elected to continue participating in the study were subjected to the protocol below:

- Follow up visit 8 (after 7 months): Check up
- Follow up visit 9 (after 8 months): Check up
- Follow up visit 10 (after 9 months): Check up
- Follow up visit 11 (after 10 months): Check up
- Follow up visit 12 (after 11 months): Check up

**Follow up visit 13 (after 12 months):** A physical examination was performed, blood pressure and heart rate measured, blood samples taken. Echocardiography was done, taped on video and stored at the Division of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

**2.3.5 Echocardiographic studies**

All studies were performed and interpreted by the same operator who was blinded to the protocol. Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, Washington) echocardiograph attached to a 2.5 or 3.5 MHz transducer. Left ventricular dimensions were measured according to the American Society of Echocardiography guidelines (198). Measurements of LV dimensions and function were determined on an
average of ≥ 3 beats. Diastolic mitral flow was assessed by pulsed-wave Doppler echocardiography from the apical four-chamber view. E-wave deceleration time was measured as the interval between the early peak diastolic velocity and the point at which the steepest deceleration slope was extrapolated to the zero line. All studies were recorded on videotape.

2.3.6 Assessment New York Heart Association functional class
A physician who was provided with the clinical data, but blinded to the study protocol and unaware of the results of the laboratory tests assigned each patient to the NYHA FC during baseline and follow-up visits.

2.3.7 Blood pressure measurements
Non-invasive blood pressure measurements were performed by use of a Critikon Dinamap vital signs monitor 1846. Resting heart rate, systolic and diastolic blood pressures were calculated as mean values from 5 readings. Measurements were made after a 30 minute rest period in sitting position with 2 minute intervals between successive measurements.

2.3.8 Research specific blood tests
A small research laboratory has been established at the Division of Cardiology at Chris Hani Baragwanath Hospital adjacent to the clinic. A volume of 21 ml of blood was withdrawn from an antecubital vein and collected in prechilled vacutainer tubes containing ethylenediaminetetraacetic acid and mixed rapidly. Plasma was separated by centrifugation at 2500 rpm for 12 minutes within 15 minutes of collection. Aliquots were stored at minus 70 degree Celsius. All plasma or serum samples used in this study were thawed only once for the measurement of biomarkers by commercially available enzyme linked immunosorbent assays (ELISA). The average of 2 measurements in undiluted plasma or serum was calculated. In order to obtain reference values for the population studied, plasma and serum was obtained from 20 female, age and HIV-
status matched volunteers who were in the peripartum period. All volunteers were physically examined and clinically had no concomitant infections that would influence biomarker levels. None of the patients or volunteers received anti-inflammatory drugs during the four weeks preceding the sample they donated for the determination of inflammatory cytokines.

All blood specimens were collected at Chris Hani Baragwanath Hospital and the plasma and serum obtained stored on site at minus 70 degrees Celsius. The aliquotted samples were then transferred on dry ice to the three different institutions as stated below, where the respective laboratory measurements were performed. Detailed protocols for each test are included in the appendix:

Emory University School of Medicine
Department of Pathology & Laboratory Medicine
Room 2309 WMB
1639 Pierce Drive, NE
Atlanta, GA  30322

- Pro-inflammatory biomarkers: CRP, TNF-alpha, IL-6 and IFN-gamma (measured at baseline, after six and twelve months)
- Markers of remodeling: MMP-2, MMP-9, TGF-β and VEGF (measured at baseline, after six and twelve months)
- Markers of cardiac function: ACE, NT-proBNP and Fas/APO-1 (measured at baseline, after six and twelve months)
Identification and quantification of agonistic autoantibodies against $\beta_1$-adrenergic receptor
(measured at baseline and after six months)

16-kDa Prolactin (post-delivery)
23-kDa Prolactin (post-delivery)
Cathepsin D

In contrast to the original protocol, selenium levels were not measured since Fett et al ruled out a role for the effect of selenium levels and described the presence of normal selenium levels in a cohort of PPCM patients from Haiti (29).

2.4 Statistical analysis

The applied statistical methods are outlined in the individual chapters.
FIRST SECTION OF RESULTS

3.0 CLINICAL PROFILE OF 100 PATIENTS DIAGNOSED WITH PPCM

3.1 Introduction

As outlined in the introductory chapter one, a low incidence of PPCM in the northern hemisphere (1:15000) as compared to countries like Nigeria, Haiti and South Africa with an incidence between 1:100 to 1:1000 respectively, is one of the reasons why numerous previous studies on PPCM relied on case reports, small numbers of patients, a review of medical records or even a survey among 15000 members of the American College of Cardiology (199) by mail in an effort to obtain basic information that totaled 123 patients (5). Only patients in Haiti were studied prospectively by the same group of doctors, but given the political situation, researchers were faced with practical and logistical difficulties.

Many reports on PPCM were written before the advent of echocardiography that is essential in the diagnosis of PPCM. As a result previous reports and studies most likely included a significant percentage of patients with a non-PPCM cause of cardiac malfunction that clinically mimic heart failure. The criteria for PPCM were re-defined in 1997 (1) emphasizing the absence of other underlying cardiac conditions to help refine the diagnosis of human PPCM. This problem of inaccurate diagnosis is reflected by the fact that select studies of human PPCM included a high percentage of patients with other cardiac conditions, e.g. a history of hypertension during pregnancy in 43% of patients (5). Furthermore, ACE-inhibitors or β-blockers were not available during many of the early studies, impacting negatively on outcome.

In view of the above, systematic data collection to study the aetiology and the potential pathogenic mechanisms of PPCM was deemed difficult. The high incidence of PPCM in a single tertiary centre in South Africa provides therefore an unique opportunity to conduct a prospective study of the mechanism of the pathogenesis and clinical features of this disease in a significant number of these clinically well characterized patients that have a high probability to provide data that can withstand objective statistical analysis which have been heretofore not possible.
3.2 Patients and methods

3.2.1 Study design and patient enrolment

The protocol was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg, South Africa and complies with the Declaration of Helsinki. All patients and controls gave written informed consent before study entry. The objective of the study was to recruit a total of 100 consecutive patients (who happened to be black and who represent the population being seen at that clinic) with PPCM which required screening of a total of 144 patients, 44 of whom did not fit the inclusion criteria of the study. The study was conducted at the Chris Hani Baragwanath Hospital, a tertiary hospital linked to the University of the Witwatersrand, located in Soweto, South Africa, which 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 at the first presentation of the patients at the Chris Hani Baragwanath Hospital cardiac clinic (baseline) and after a follow-up period of 6 months (6 months visit). These were the two time points of the study.

Clinical assessment, echocardiography, and blood analysis were done at baseline and after 6 months of standard therapy. Inflammatory markers were measured at baseline only. All patients received treatment with diuretics and the angiotensin-converting enzyme inhibitor accupril. 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 slowly titrated up to a target of 25 mg twice daily as long as SBP was ≥ 100 mmHg or symptoms such as dizziness did not occur. Patients attended the cardiac clinic at least once a month for routine follow-up.

Inclusion and exclusion criteria are outlined in chapter 2. In addition 20 healthy, age, sex, body mass index and parity matched females were recruited from the local population for purposes of control.
Plasma levels of CRP were measured as part of routine investigation by the hospital laboratory utilizing a commercially available enzyme-linked immunosorbent assay (Roche Diagnostics GmbH, Mannheim, Germany). The assay utilized had a sensitivity of 0.1-10 mg/l and included standards which were run in parallel. Obtained values were used to calculate plasma levels in patient and control samples.

### 3.2.2 TNF-alpha and Fas/APO-1 levels

Fifteen milliliters of blood were drawn from each patient and controls during day time (10.00 to 12.00 a.m.) from the antecubital vein and collected into pre-chilled vacutainer tubes containing ethylenediaminetetraacetic acid. Plasma was separated by centrifugation at 2500 r.p.m. for 12 min within 15 min of collection. Aliquots were stored at minus 80 degrees Celsius. Plasma levels of TNF-alpha and Fas/Apo-1 were measured using commercially available enzyme-linked immunoassays (Amersham, Maidstone, USA and Calbiochem, San Diego, CA, USA, respectively) and performed according to manufacturers’ instructions. The manufacturer supplied a reference range for normal values. However, as those values are obtained in samples from western population groups, we collected blood from 20 otherwise healthy, age, race, sex, body mass index, and parity comparable controls recruited from the local population.

### 3.2.3 Functional class, echocardiography and cardiac scintigraphy

A physician who was provided the clinical data, but was blinded to the protocol and unaware of the results of the laboratory tests, performed the assignments of each patient to the NYHA FC during baseline and follow-up visits. The same physician evaluated all patients. A multiple-gated equilibrium cardiac blood pool scintigraphic technique (MUGA) was used to measure LVEF (Elscint Apex 409, Chicago, IL, USA), and calculations of LV performance were made as previously described (200). Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, WA, USA) echocardiograph attached to a 2.5 or 3.5 MHz transducer. All studies were performed and interpreted by the same operator who was unaware of the other parameters investigated.
All studies were recorded on videotape. LV dimensions were measured according to the American Society of Echocardiography Guidelines (198). Measurements of LV dimensions and function were determined on an average of ≥ 3 beats.

3.2.4 Statistical analysis

Data were analysed using the SAS version 9.1 statistical program (SAS, Cary, NC, USA). Results are expressed as mean±SD or median (201). The paired t-test was used for the comparison of baseline data with the 6 months data, whereas the Wilcoxon matched pair test was used for variables measured in a continuous scale and with a non-normal distribution. The McNemar test was used for calculating the differences on the basis of the NYHA FC by grouping the patients into two classes (I + II and III + IV). Comparison of numerical data between groups of patients was carried out using non-parametric Mann–Whitney–Wilcoxon test. Significance was assumed at a two-tailed value of P< 0.05. Spearman correlation coefficients were calculated for continuous data. Univariate logistic regression was used to select possible predictors of mortality. Continuous variables were tested for linearity generating partial residual plots. As non-linear effects were detected for all the variables, they were transformed into an ordinal scale by tertiles. The multiple logistic regression was performed including predictor variables [NYHA FC, end-diastolic diameter (EDD), end-systolic diameter (ESD), EF, Fas/Apo1, aspartate amino transferase (AST) and SBP] that had a P-value less than 0.15 from the univariate analysis. A good fitting model was indicated in stepwise, forward, and backward logistic regression. The resulting model for mortality selected Fas/APO-1 and NYHA FC as explanatory variables with a P-value less than 0.05 being considered significant.
3.3 Results

3.3.1 Characteristics of study patients

The characteristics of the study population at time of first presentation to the cardiac clinic (baseline) are shown in Table 3.1. Although 91% of the study patients were diagnosed as PPCM patients for the first time, the remaining 9 (9%) PPCM patients had been diagnosed at a previous pregnancy. These nine patients had recovered their LV function and experienced a subsequent episode of PPCM. None of the patients had identifiable causes for heart failure. There was no association between history of hypertension and eclampsia during pregnancy or use of tocolytic agents (9%). At the day of first presentation at the clinic (baseline), 26 patients were in NYHA FC II, 49 were in FC III, and 25 were in FC IV. LV thrombi were detected on echocardiography in 16% of the patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.6±6.6</td>
</tr>
<tr>
<td>Body Mass Index (kg/cm²)</td>
<td>25.6±5.1</td>
</tr>
<tr>
<td>Blood pressure (mmHg) systolic</td>
<td>111.1±17.4</td>
</tr>
<tr>
<td>Blood pressure (mmHg) diastolic</td>
<td>70.4±13.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>93.5±18.5</td>
</tr>
<tr>
<td>Left ventricular EDD (mm)</td>
<td>61.6±7.1</td>
</tr>
<tr>
<td>Left ventricular ESD (mm)</td>
<td>53.1±7</td>
</tr>
<tr>
<td>Ejection fraction echocardiography (%)</td>
<td>25.9±8.2</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>10.8±13.2</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8±1.1</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.2±0.8</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.5±1.7</td>
</tr>
<tr>
<td>Fas/Apo-1 (U/l)</td>
<td>6.3±4.1</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>4.9±4.2</td>
</tr>
</tbody>
</table>
Table 3.1 Baseline characteristics of study population (n=100) EDD= end-diastolic diameter; ESD= end-systolic diameter; TNF= tumor necrosis factor

3.3.2 Follow up

Fifteen patients died within the follow-up period of 6 months, and eight patients moved to remote areas and were not available for full follow-up assessments. Patients lost to follow-up were observed for a median of 3 months (range 1–5 months). They were contactable through phone and were alive. Eleven patients died despite optimal medical therapy because of progression of heart failure in hospital and the other four patients experienced sudden death. All the patients died during the first 3 months after enrolment in the longitudinal study. Women who had a prior history of PPCM had no difference in mortality when compared with the women who had no previous history of PPCM. However, they had an intensive monitoring by the cardiologist and obstetrician throughout their pregnancy and post-partum. Baseline characteristics of patients that were not available for full follow-up assessment did not differ from the others. None of these patients died. Cardiac transplantation or LV assist device for the population studied was unavailable for the duration of the trial due to economic reasons.

3.3.3 Medication

During the first months after enrolment, patients received standard therapy for heart failure, which included furosemide [n = 96, median daily dose 160 mg (80–250)], accupril [n = 96, median daily dose 10 mg (10–20)], and carvedilol [n = 95, median daily dose 25 mg (6.25–50)]. Carvedilol was uptitrated as long as SBP was > 100 mmHg or symptoms such as dizziness occurred. Clinical data and NYHA FC were compared at first presentation at the cardiac clinic at baseline (n = 100) and after 6 months of standard care (n = 77) as detailed in table 3.2.
3.3.4 Left ventricular function, dimensions and heart rate

Patients who completed 6 months of treatment showed a significant reduction of heart rate, left ventricular dimensions and significant improvement in scintigraphic and echocardiographic derived values for left ventricular ejection fraction (p< 0.0001, Table 3.2) and NYHA functional class (p< 0.001). However, normalization of LVEF (>50%) was only observed in 18 (23%) of the patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>6 months</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>111.6±16.8</td>
<td>116.1±17.6</td>
<td>0.018</td>
</tr>
<tr>
<td>Diastolic BP(mmHg)</td>
<td>71.0±12.6</td>
<td>72.6±11.3</td>
<td>0.37</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>92.8±18.4</td>
<td>74.2±12.9</td>
<td>0.001</td>
</tr>
<tr>
<td>NYHA FC (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC I = 0</td>
<td>FC I = 55</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>FC II = 23</td>
<td>FC II = 17</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>FC III = 38</td>
<td>FC III = 5</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>FC IV = 16</td>
<td>FC IV = 0</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>EDD ( mm)</td>
<td>61.2±7.1</td>
<td>55.6±8.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>53.4±7.7</td>
<td>43.7±10.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECHO EF (%)</td>
<td>26.2±8.2</td>
<td>42.9±13.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MUGA EF (%)</td>
<td>23.9±8.1</td>
<td>43.1±15.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3.2 Clinical variables and left-ventricular function at baseline and after 6 months follow-up (n=77); BP=blood pressure; HR=heart rate; EDD= end-diastolic diameter; ESD= end-systolic diameter

3.3.5 C-reactive protein, TNF-alpha, FasApo-1 and other blood result

The median plasma level of C-reactive protein for the 100 PPCM patients was 10.0 mg/L (range 1–90) with 45% of patients having values of .10 mg/L (table 1). Only ten patients had a C-reactive protein level of 3 mg/L. Baseline plasma levels of C-reactive protein correlated positively with LV end-diastolic (rs = 0.33, P = 0.0026) and endsystolic dimensions (rs = 0.35, P = 0.0012), whereas the correlation with LVEF (rs = 0.20.27, P = 0.015) was inverse (figure 3.1). Plasma C-reactive protein levels also correlated inversely with levels of total cholesterol (rs = 0.29, P = 0.01). Baseline plasma levels of C-reactive protein, TNF-alpha,
and Fas/Apo-1 were elevated in patients with PPCM when compared with 20 age, sex, body mass index, and parity comparable healthy volunteers (TNF-alpha 4.9±4.2 vs. 1.4±1.3 pg/mL, Fas/Apo-1 6.3±4.1 vs. 0.84±0.2 U/L, C-reactive protein 10.8±13.2 vs. 3.1±0.9 mg/L, P = 0.01). There was no correlation between baseline plasma levels of C-reactive protein, TNF-alpha, and Fas/Apo-1 among the PPCM patients.

Figure 3.1 Correlation co-efficients of total cholesterol and C-reactive protein vs. parameters of LV dimensions and function.

3.3.6 Predictors of outcome:

In the population studied, mortality remained high (15%). Significant differences in the baseline data between deceased patients and survivors were seen in NYHA FC, and values of SBP, end-diastolic and end-
systolic dimensions, LVEF, plasma AST, a marker of hepatic congestion and liver cell death (table 3.3), and plasma levels of Fas/Apo-1 (table 3.3 and Figure 3.2). Logistic regression analysis of NYHA FC, SBP, EDD, ESD, EF, AST, and Fas/Apo-1 revealed that only the baseline plasma levels of Fas/Apo-1 (OR = 3.56, CI 95% = 1.35–9.42) and NYHA FC (OR = 2.67, CI 95% = 1.04–6.83) were independent predictors of death (tables 3.4 and 3.5).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Deceased (n=15)</th>
<th>Survivors (n=77)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.9±1.2</td>
<td>32±6.4</td>
<td>0.30</td>
</tr>
<tr>
<td>No of children (n)</td>
<td>2.3±1.2</td>
<td>2.9±1.4</td>
<td>0.14</td>
</tr>
<tr>
<td>Onset of symptoms post delivery (months)</td>
<td>2.1±1.4</td>
<td>2.2±1.3</td>
<td>0.68</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>98.4±21.0</td>
<td>92.2±18.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>102.1±22.1</td>
<td>111.6±16.8</td>
<td>0.03</td>
</tr>
<tr>
<td>NYHA FC</td>
<td>3.3±0.7</td>
<td>2.0±0.8</td>
<td>0.04</td>
</tr>
<tr>
<td>EDD (mm)</td>
<td>64.9±5.8</td>
<td>61.2±7.2</td>
<td>0.03</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>57.7±7.3</td>
<td>53.4±7.7</td>
<td>0.03</td>
</tr>
<tr>
<td>ECHO EF (%)</td>
<td>22.2±6.0</td>
<td>26.2±8.2</td>
<td>0.04</td>
</tr>
<tr>
<td>AST (iU/l)</td>
<td>50.4±34.4</td>
<td>34.8±24.0</td>
<td>0.04</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>12.4±7</td>
<td>10.4±14.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>4.3±1.1</td>
<td>4.3±0.9</td>
<td>0.87</td>
</tr>
<tr>
<td>Fas/Apo-1 (U/ml)</td>
<td>9.8±4.2</td>
<td>5.9±3.8</td>
<td>0.002</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>4.8±5.1</td>
<td>5.1±4.2</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Table 3.3 Baseline characteristics of deceased versus surviving patients
BP=blood pressure; HR=heart rate; EDD=end-diastolic diameter; ESD=end-systolic diameter; TNF=tumor necrosis factor and AST=Aspartate amino transferase
Figure 3.2: Baseline plasma inflammatory markers of deceased patients vs. survivors. Only differences in Fas/Apo-1 were significant (p=0.002)
### Table 3.4 Univariate logistic regression analysis of survivors vs. deceased patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.96</td>
<td>0.89–1.04</td>
<td>0.3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.96</td>
<td>0.93–1.00</td>
<td>0.06</td>
</tr>
<tr>
<td>NYHA FC</td>
<td>2.35</td>
<td>1.03–5.34</td>
<td>0.04</td>
</tr>
<tr>
<td>EDD (mm)</td>
<td>1.07</td>
<td>0.99–1.16</td>
<td>0.07</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>1.07</td>
<td>0.99–1.15</td>
<td>0.06</td>
</tr>
<tr>
<td>Echo EF (%)</td>
<td>0.95</td>
<td>0.89–1.02</td>
<td>0.14</td>
</tr>
<tr>
<td>Fas/Apo-1(U/ml)</td>
<td>1.25</td>
<td>1.08–1.45</td>
<td>0.003</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>1.02</td>
<td>0.99–1.04</td>
<td>0.07</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>0.99</td>
<td>0.86–1.14</td>
<td>0.83</td>
</tr>
</tbody>
</table>

### Table 3.5: Backward logistic regression analysis (all variables from univariate analysis were included (P < 0.15), but were not part of the final model, as they were not significant.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas/APO-1 (U/L) (tertiles)</td>
<td>3.56</td>
<td>1.35-9.42</td>
<td>0.01</td>
</tr>
<tr>
<td>NYHA FC</td>
<td>2.67</td>
<td>1.04-6.83</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### 3.4 Summary

Plasma markers of inflammation were significantly elevated in PPCM patients and correlated with increased left ventricular dimensions and lower ejection fraction at presentation. Baseline Fas/Apo-1 and higher NYHA functional class were the only predictors of mortality. These results contribute to previous findings by our group and others that apoptosis and chronic inflammation may contribute to the pathogenesis of PPCM (4, 115, 148) and deserve studies aimed at defining the mechanisms for such chronic insults. Despite standard medical therapy normalization of LVEF was only observed in 23% of this cohort of African PPCM patients.
SECOND SECTION OF RESULTS

4. KINETICS OF CARDIAC FUNCTION BIOMARKERS, PRO-INFLAMMATORY CYTOKINES, MARKERS OF RE-MODELING AND PROLACTIN IN 43 PATIENTS WITH PERIPARTUM CARDIOMYOPATHY OVER A SIX MONTHS PERIOD

4.1 Introduction

Peripartum cardiomyopathy is a disabling condition characterized by new onset of heart failure in previously healthy women between one month antepartum and five months post-delivery (1). While established treatment options do not differ from those of other patients with cardiomyopathy, the aetiology of PPCM has been subject of numerous studies suggesting a wide range of contributing factors, such as multiparity (25), twin pregnancy (26, 27), advanced maternal age (6), tocolytic therapy (34), entero- (32) and cardiotropic viruses (49-51), heat (11), poor nutrition and selenium deficiency (24), maternal cocaine abuse (31), sexually transmitted diseases (36), chlamydia pneumoniae (37) and strenuous aerobic exercise (38). However, due to the rare incidence of PPCM, these data were either limited to small numbers of patients, obtained before the advent of echocardiography or not acquired in a systematic fashion.

Recent studies conducted on a large cohort of PPCM patients by Sliwa et al. in South Africa described a pro-inflammatory response in PPCM patients with elevated levels of TNF-alpha, IL-6 (148) and a positive correlation between C-reactive protein levels and LV end-diastolic and end-systolic diameters (192).

T cell-mediated autoimmune responses have been discussed in the pathogenesis of dilated cardiomyopathy and the role of IFN-gamma in the progression from early (viral) to late (autoimmune) phases of myocarditis has been documented. Treatment with antibody to IFN-gamma reduced early disease, but had little effect on the severity of cardiac lesions at later times (202). CD4(+) Th1 cells promote activation of the autoimmune CD8(+) alpha beta TCR(+) effectors via an IFN-gamma mediated mechanism (203). The inhibition of T cell responses and suppression of Th1-type and inflammatory cytokines via inactivation of nuclear factor-kappaB by administration of a statin in autoimmune myocarditis have also been reported. The lipid lowering agent fluvastatin reduced production of Th1-type cytokines including IFN-gamma and inhibited expression of inflammatory cytokine mRNAs in the myocardium. Infiltration of CD4-
positive T cells into the myocardium and T cell proliferative responses were suppressed by fluvastatin. (204). Li et al. documented improved cardiac function in mice after reduction of IFN-gamma levels with atorvastatin (205). We reasoned that measurement of IFN-gamma as an indicator of an ongoing autoimmune process would provide some insights as to the potential role of this cytokine in perpetuating autoimmune insult in the heart of PPCM patients.

Fett and colleagues prospectively followed a large cohort of patients in Haiti and reported an association between high levels of C-reactive protein and PPCM. They hypothesized that Fas-mediated apoptosis contributes to cardiomyocyte loss, left ventricular dilatation and eventually clinically detectable left ventricular systolic failure (147). The role of programmed cell death (apoptosis) is an area of intense investigation (206) and has been identified as an essential process in the progression to heart failure (207). Indeed, Sliwa et al. have identified Fas/APO-1 as a predictor of death in PPCM patients (148). Cenac et al. found that low values of NT-ProBNP and hsCRP indicate complete remission of cardiac failure and normal heart volume in PPCM. In their view the synchronous variation of NT-ProBNP/hsCRP and their positive correlation supported the hypothesis of inflammatory process (myocarditis) in PPCM (208).

Plasma oxLDL is a marker of oxidative stress in patients with dilated cardiomyopathy (209). Tsutamoto and colleagues reported that left ventricular dysfunction in dilated cardiomyopathy may be partly due to the oxidative stress measured by plasma oxLDL. They also found a significant positive correlation between oxLDL and TNF-alpha, concluding that TNF-alpha may stimulate oxidative stress in the failing heart (210). In a different study Tsutsui et al. found a significant negative correlation between the plasma level of oxLDL and left ventricular ejection fraction and a significant positive correlation between the plasma level of oxLDL and plasma norepinephrine level (209).

Ansari et al. have focused on the specific immune environment of pregnancy (4). An echocardiographic assessment of the haemodynamics in normal pregnancies demonstrated a 10% increase in left ventricular end-diastolic volume, a 45% increase in cardiac output and a 26% to 28% decrease in end-systolic wall stress. In order to accommodate higher pre-load during pregnancy, the left ventricle undergoes re-modeling
and reversible left ventricular hypertrophy (76, 211). Cardiac remodeling is the restructuring and reshaping of the heart that underlies heart failure progression and is a major determinant of the clinical course of chronic heart failure, irrespective of its etiology (206). Matrix metalloproteinases can alter myocardial extracellular matrix and thereby contribute to adverse ventricular remodeling in progressive heart failure (212). Cardiac-specific, constitutively active MMP-2 expression leads to impaired contraction and diminished responses to inotropic stimulation (213) and has been identified as an independent predictor of mortality in patients with chronic heart failure (214). The selective inhibition of MMP-2, MMP-9 and others has been shown to reduce replacement fibrosis and interstitial fibrosis by 29% in dogs (215). Vellaichamy et al. report that reduced NPRA signaling activates MMP-2 and 9, TGF-β1 and TNF-alpha expression in Npr1-/- mouse hearts, promoting hypertrophic growth and extracellular matrix remodeling, leading to the development of cardiac hypertrophy, myocardial fibrosis, and congestive heart failure (216). TNF-alpha may stimulate the expression of MMP’s, contribute to myocardial remodeling and lead to the development and progression of congestive heart failure (217).
The importance of fibrosis in different cardiac pathologies appears to be increasingly relevant and is thought to be partially mediated by transforming growth factor-β1 (TGF-β1), a potent stimulator of collagen-producing cardiac fibroblasts. Watanabe et al. demonstrated a reduction in the area of myocardial fibrosis and expression of TGF-β-1 levels in a model of rats with dilated cardiomyopathy through inhibition of the RAAS with ACE-inhibitors (218, 219). Previously, TGF-β1 had been implicated solely as a modulator of the myocardial remodeling seen after infarction. However, recent studies indicate that dilated, ischaemic and hypertrophic cardiomyopathies are all associated with raised levels of TGF-β1 (220).

Hilfiker-Kleiner and colleagues demonstrated an association between enhanced cardiomyocyte apoptosis and fibrosis with increased levels of VEGF in the pressure-overloaded mouse heart (221). Other others
studied the influence of VEGF on capillary density (222). Heart size and cardiac function are angiogenesis dependent, and disruption of coordinated tissue growth and angiogenesis in the heart contributes to the progression from adaptive cardiac hypertrophy to heart failure. Shiojima et al. showed that inhibition of angiogenesis via a decoy VEGF receptor in mice with dilated cardiomyopathy led to decreased capillary density, contractile dysfunction, and impaired cardiac growth. (223).

In vivo and in vitro studies suggest that the effector hormones, angiotensin II and aldosterone, of the RAAS are primarily involved in regulating the structural remodeling of the myocardial collagen matrix (224). Inflammation is a key mechanism in the initiation and progression of cardiomyopathy. Angiotensin II, the major effector peptide of the RAAS, plays a significant role in the advent and perpetuation of inflammatory cardiovascular disease. Among others, TNF-alpha, and C-reactive protein have diagnostic and prognostic values in cardiovascular disease and are modified by angiotensin-converting enzyme inhibitors (225). Hyperactivation of the RAAS, heightened sympathetic drive and uncontrolled synthesis of inflammatory cytokines exacerbates ventricular contractile dysfunction in heart failure patients (226). Several studies suggest a link between the pathogenesis of congestive heart failure, circulating levels of TNF alpha IL-1, IL-6 (227) and clinical status (228).

Besides the RAAS, most endocrinal glands are affected by chronic heart failure and elevated serum prolactin levels were observed as the most frequent hormonal disturbance in a group of male patients with chronic heart failure of different aetiologies (229) as well as in males and females with ischaemic or dilated cardiomyopathy (230). Prolactin represents a stimulatory link between the neuroendocrine and immune systems, but its involvement in the neurohumoral adaptations to heart failure has not been explored (230). It is physiologically upregulated postdelivery and has been implicated in cardiac tissue injury and modulation of the autoimmune response (81, 82).

While this process of remodeling happens physiologically during the peripartum period without myocyte loss or injury and thus without triggering unwanted innate immune pathological responses (231), some women develop PPCM during this period.
We therefore stratified our approach to study the kinetics of the above described markers of cardiac function, re-modeling and inflammation as well as levels of the pregnancy associated hormone prolactin in patients presenting with PPCM to our clinic for diagnosis and anti-failure therapy. While the incidence of PPCM has been reported between 1: 2392 and 4000 live births in the USA (13, 14) and 1:300 in Haiti (14), PPCM is ubiquitous on the African continent with an incidence ranging from 1: 100 to 1: 1000 deliveries (22). In an attempt to improve the understanding of the mechanisms and pathways involved in the pathogenesis of PPCM, we enrolled 41 patients with new onset of the disease and systematically obtained clinical and echocardiographic data as well as serum/plasma samples from all patients at baseline and after six month of treatment. In order to identify mechanisms involved in the onset of PPCM, we measured the biomarkers outlined above and compared these to non-PPCM women from our local population in the peripartum period. This was necessary to distinguish such markers that would be physiologically upregulated in all women during the peripartum period from those that are pathologically elevated. Since PPCM has a high rate of recovery ranging between and 23 and 54% (5, 192), we were also interested in the kinetics of biomarkers that differed between such patients who subsequently improved their cardiac function and those who did not. We therefore obtained another set of serum/plasma samples after six months of therapy and repeated the clinical and echocardiographic examinations at the same time. We grouped the results into patients who improved their cardiac function versus those who did not and describe the kinetics of these biomarkers over time.

4.2 Patients and Methods

4.2.1 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 (PRC 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 six months (6 months visit). These were the two time points of the study. Clinical assessment, echocardiography, and blood analysis were done at baseline and after 6 months of standard therapy. Three tubes of blood (4 ml each) were collected from every patient to obtain serum and plasma for measurement of cytokine levels and FBC. Echocardiography was taped on video and stored at 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 \( \leq 25\% \) or LV thrombus received anti-coagulation therapy. Carvedilol was added after resolution of overt heart failure, and the dose was slowly titrated up to a target of 25 mg twice daily as long as SBP was \( \geq 100 \text{ mmHg} \) or symptoms such as dizziness did not occur. Patients attended the cardiac clinic at least once a month for routine follow-up.

4.2.3 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 operator who was blinded to the protocol.

Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, Washington) echocardiograph attached to a 2.5 or 3.5 MHz transducer. Left ventricular dimensions were measured according to the
American Society of Echocardiography guidelines (198). Measurements of LV dimensions and function were determined on an average of \( \geq 3 \) beats. All echocardiographic studies were recorded on videotape and stored at Chris Hani Baragwanath Hospital, Division of Cardiology for future reference and audit purposes.

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 FC of each patient during 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.

### 4.2.4 Research specific blood tests

A volume of 8 ml of blood was withdrawn from an antecubital vein and collected in prechilled vacutainer tubes containing ethylenediaminetetraacetic acid or clot activator respectively and mixed rapidly. Plasma or serum were separated by centrifugation at 2500 rpm for 12 minutes within 15 minutes of collection. Aliquots were stored at minus 70 degree Celsius.

In order to differentiate physiologically upregulated 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, time after delivery and parity. All serum samples used in this study were thawed only once.

None of the patients or volunteers received anti-inflammatory drugs during the four weeks preceding the sample they donated for the determination of inflammatory cytokines. All blood specimens were collected at Chris Hani Baragwanath Hospital and the plasma and serum obtained stored on site at minus 70 degrees.
Celsius. The aliquotted samples were then transferred on dry ice to Emory University School of Medicine, Department of Pathology & Laboratory Medicine, Atlanta, USA.

All plasma and serum samples used in this study were thawed only once for the measurement of biomarkers by commercially available enzyme linked immunosorbent assays (ELISA) according to the manufacturer’s instructions: Big endothelin-1 (Assay Designs, Ann Arbor, MI, USA), NT-proBNP, prolactin (ALPCO, Wingham, NH, USA), 17-beta estradiol, Fas/APO-1, IFN-gamma, IL-1beta, IL-6, TNF-alpha, TGF-beta1 (Biosource, Camarillo, CA, USA), ACE, MMP-2, MMP-9, PLGF, VEGF (R&D Systems, Minneapolis, MN, USA) and oxLDL (Mercodia, Uppsala, Sweden). Detailed protocols for each test are included in the appendix. Due to logistical reasons, oxLDL was measured only in 28 patients. The average of two measurements in undiluted plasma or serum was calculated.

4.2.5 Statistical analysis

Data were analysed using the SAS version 9.1 statistical program (SAS, Cary, NC, USA). Results are expressed as median (range) (201). We used Wilcoxon Scores (Rank Sums) for comparison between all patients at baseline versus healthy controls and improvers vs. non-improvers as data were non-normally distributed data.

As data were non-normally distributed, we performed log transformation to the differences of all variables between six 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. Significance was assumed at a two-tailed value of P< 0.05.
4.3 Results

Thirty-eight out of 43 patients completed the follow-up period of six months - three patients had died and two had 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 - 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 or LV assist device for the population studied was unavailable due to economic reasons.

In order to identify abnormal values in PPCM patients, we compared data to 20 controls from the same population who were also in the peripartum period. All were in NYHA FC 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 six months of standard cardiac failure therapy 25 patients were classified as cardiac function improvers and 13 as non-improvers. Patients were considered to be cardiac function improvers if their LVEF as determined by echocardiography improved by at least 10 units and their NYHA FC improved by at least one grade. Data were then analysed between cardiac function improvers and non-improvers.

4.3.1 Markers of cardiac function in improvers and non-improvers of PPCM

Patients presented with a heart rate of 99.7±19 beats per minute, a mean systolic blood pressure of 113.4±20.0 mmHg and a diastolic blood pressure of 75.6±13.4 mmHg. 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]. Representing markers of cardiac function, we measured levels of angiotensin-converting enzyme (87), the marker of apoptosis Fas/APO-1, oxidized low density lipoprotein (oxLDL) and the indicator of heart failure NT-proBNP in PPCM patients at baseline and after 6 months. We compared values to samples
obtained from controls from the same population, who were in the peripartum period, from the same age group and parity without cardiac dysfunction or history thereof.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Median baseline</th>
<th>Range</th>
<th>n</th>
<th>Controls</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas/APO-1 (ng/ml)</td>
<td>0.34</td>
<td>0.13-5.29</td>
<td>38</td>
<td>0.13</td>
<td>0.09-0.30</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>oxLDL (IU/ml)</td>
<td>16.1</td>
<td>15.3-19.4</td>
<td>28</td>
<td>8.6</td>
<td>7.5-9.3</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NT-proBNP (fmol/ml)</td>
<td>1727.2</td>
<td>988.7-3077</td>
<td>36</td>
<td>339.5</td>
<td>184.6-715.6</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4.1: Markers of cardiac function between PPCM patients and controls at baseline

NT-proBNP is a marker of cardiac failure and PPCM patients had significantly elevated median serum levels at baseline as compared with healthy peripartum controls (P<0.0001) and median serum levels of NT-proBNP were significantly higher in non-improvers than in improvers (P=0.0013) at baseline (tables).

While median plasma levels of the marker of apoptosis Fas/APO-1 and the marker of oxidative stress oxLDL were significantly (p<0.0001) elevated among PPCM patients as compared to healthy controls (P<0.0001), differences between improvers and non-improvers at baseline and after six months were non-significant.
PPCM patients who were later identified as cardiac function improvers had a median LVEF at baseline of 23.0 [13-39] which is significantly lower (p=0.0084) than in non-improvers. While LVEF increased in this group to a median of 50.0% [25-63] after six months of treatment, the median LVEF of non-improvers was 32.0 [18-40] at baseline and increased only to 34% [21-46] after six months of treatment.

The median NYHA FC was 3.0 [2.0-4.0] at baseline among improvers as well as among non-improvers (p=NS). The median NYHA FC after six months of treatment was 1 [1-3] among improvers and 2 [1-3] among non-improvers.

Table 4.2: Markers of cardiac function between cardiac function improvers and non-improvers at baseline

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Improvers median baseline</th>
<th>Range</th>
<th>N</th>
<th>Non-improvers median</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas/APO-1 (ng/ml)</td>
<td>0.33</td>
<td>0.12-5.29</td>
<td>27</td>
<td>0.34</td>
<td>0.12-0.82</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>NT-proBNP (fmol/ml)</td>
<td>1635.1</td>
<td>885.9-2884.7</td>
<td>25</td>
<td>1818.1</td>
<td>1427.8-3077.7</td>
<td>11</td>
<td>0.0013</td>
</tr>
</tbody>
</table>
Table 4.3: Median Δ of markers of cardiac function from baseline to six months between improvers and non-improvers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median Δ Improvers baseline vs. 6 months</th>
<th>Range</th>
<th>n</th>
<th>Median Δ Non-improvers baseline vs. 6 months</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas/APO-1 (ng/ml)</td>
<td>-0.08</td>
<td>-0.56-0.59</td>
<td>27</td>
<td>-0.11</td>
<td>-0.49-0.13</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>oxLDL (IU/ml)</td>
<td>-4.12</td>
<td>-6.81-(-0.46)</td>
<td>21</td>
<td>0.85</td>
<td>-2.88-2.44</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>NT-proBNP (fmol/ml)</td>
<td>-616.28</td>
<td>-2419.1-255.55</td>
<td>24</td>
<td>-287.23</td>
<td>-1008.5-257.05</td>
<td>10</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4.4: Markers of re-modeling between PPCM patients and controls at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients median baseline</th>
<th>Range</th>
<th>n</th>
<th>Controls median</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>368.5</td>
<td>308.2-474.9</td>
<td>36</td>
<td>142.2</td>
<td>111.8-184.9</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>873.2</td>
<td>465.3-1400.7</td>
<td>36</td>
<td>751.3</td>
<td>465.1-1338.8</td>
<td>21</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-β-1 (ng/ml)</td>
<td>8.5</td>
<td>3.7-22.7</td>
<td>36</td>
<td>16.4</td>
<td>9.0-30.9</td>
<td>21</td>
<td>0.002</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>83.8</td>
<td>1.1-1903.6</td>
<td>38</td>
<td>103.5</td>
<td>32.3-602.9</td>
<td>21</td>
<td>NS</td>
</tr>
</tbody>
</table>

We reasoned that matrix-metallo-proteinase-2 (MMP-2), matrix-metallo-proteinase-9 (MMP-9), the anti-apoptotic survival factor for T-lymphocytes transforming-growth factor β-1 (TGF-β-1) and vascular endothelial growth factor (VEGF) could serve as markers of re-modeling. Median baseline serum levels of MMP-2 were significantly higher among PPCM patients as compared to healthy controls (P<0.0001), while
levels of MMP-9 and VEGF were not. Interestingly, TGF-β-1 was significantly (P=0.002) lower in PPCM patients than among controls. None of these markers of re-modeling differed significantly between improvers and non-improvers at baseline or over time.

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Improvers median</th>
<th>Range</th>
<th>n</th>
<th>Non-improvers median</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>356.7</td>
<td>307.2-461.0</td>
<td>25</td>
<td>391.1</td>
<td>288.3-474.9</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>780.4</td>
<td>1400.7-334.1</td>
<td>25</td>
<td>961.9</td>
<td>465.3-1384.8</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-β-1 (ng/ml)</td>
<td>8.55</td>
<td>3.05-22.65</td>
<td>25</td>
<td>7.30</td>
<td>2.4-16.45</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>79.0</td>
<td>0.0-1742.1</td>
<td>27</td>
<td>131.0</td>
<td>0.0-1903.6</td>
<td>11</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4.5: Medians of markers of re-modeling between improvers and non-improvers at baseline
### Table 4.6: Median Δ of markers of re-modeling from baseline to six months between improvers and non-improvers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Improvers baseline vs. 6 months</th>
<th>Range</th>
<th>n</th>
<th>Non-improvers baseline vs. 6 months</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Δ MMP-2 (ng/ml)</td>
<td>-64.13</td>
<td>-174.9-51.4</td>
<td>24</td>
<td>96.08</td>
<td>-132.2-(-489)</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ MMP-9 (ng/ml)</td>
<td>-125.33</td>
<td>-560.7-226.3</td>
<td>24</td>
<td>-149.75</td>
<td>-207.7-298.15</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ TGF-β-1 (ng/ml)</td>
<td>-4.15</td>
<td>-19.7-23.2</td>
<td>24</td>
<td>-1.2</td>
<td>-12.4-2.00</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ VEGF (pg/ml)</td>
<td>5.04</td>
<td>-821.38-851.64</td>
<td>27</td>
<td>-11.59</td>
<td>-679.12-263.30</td>
<td>11</td>
<td>NS</td>
</tr>
</tbody>
</table>

#### 4.3.3 Kinetics of pro-inflammatory cytokines

None of the patients included in this study had a concomitant inflammatory disease. Mean white cell count at time of presentation was 6.9±3.6 x10⁹/L and well within normal limits. We measured levels of the Th1-like proinflammatory cytokines interferon-gamma (IFN-gamma), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha) and C-reactive protein in this group of PPCM patients. Median baseline levels of all these pro-inflammatory markers were significantly higher among PPCM patients as compared to peripartum controls. While we were unable to detect significant differences of these markers between improvers and non-improvers at baseline, the kinetics over time revealed a significant reduction of median IFN-gamma levels among improvers (P=0.0181).
Table 4.7: Pro-inflammatory cytokine levels between PPCM patients and controls at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients median baseline</th>
<th>Range</th>
<th>N</th>
<th>Controls median</th>
<th>Range</th>
<th>n</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-gamma (IU/ml)</td>
<td>2.9</td>
<td>1.9-5.6</td>
<td>36</td>
<td>0.16</td>
<td>0.05-0.31</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-1 β (pg/ml)</td>
<td>91.3</td>
<td>44.1-197.2</td>
<td>36</td>
<td>32.0</td>
<td>17.7-69.8</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>111.0</td>
<td>52.7-333.8</td>
<td>36</td>
<td>30.1</td>
<td>18.3-54.3</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>123.8</td>
<td>70.0-412.5</td>
<td>36</td>
<td>16.5</td>
<td>9.3-39.3</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>12.2</td>
<td>1.0-250.7</td>
<td>39</td>
<td>2.6</td>
<td>1.0-42.8</td>
<td>21</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

We measured levels of the Th1-like proinflammatory cytokines interferon-gamma (IFN-gamma), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha) and C-reactive protein in this group of PPCM patients. Median baseline levels of all these pro-inflammatory markers were significantly higher among PPCM patients as compared to peripartum controls. While we were unable to detect significant differences of these markers between improvers and non-improvers at baseline, the kinetics over time revealed a significant reduction of median IFN-gamma levels among improvers (P=0.0181).
<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Improvers median</th>
<th>Range</th>
<th>N</th>
<th>Non-improvers median</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-gamma (IU/ml)</td>
<td>2.95</td>
<td>1.87-5.59</td>
<td>25</td>
<td>2.09</td>
<td>1.82-3.79</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>IL-1 β (pg/ml)</td>
<td>86.8</td>
<td>37.9-140.4</td>
<td>25</td>
<td>96.8</td>
<td>36.3-197.2</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>111.4</td>
<td>40.5-333.8</td>
<td>25</td>
<td>104.7</td>
<td>36.3-197.2</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>135.1</td>
<td>58.5-330.0</td>
<td>25</td>
<td>111.3</td>
<td>51.2-412.5</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>CRP</td>
<td>12.1</td>
<td>1.0-250.7</td>
<td>27</td>
<td>12.2</td>
<td>1.0-88.4</td>
<td>12</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 4.8:** Medians of pro-inflammatory cytokines between improvers and non-improvers at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Improvers baseline vs. 6 months</th>
<th>Range</th>
<th>N</th>
<th>Non-improvers baseline vs. 6 months</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Δ IFN-gamma (IU/ml)</td>
<td>-2.13</td>
<td>-5.43-2.06</td>
<td>24</td>
<td>1.21</td>
<td>-1.03-1.91</td>
<td>10</td>
<td>0.0181</td>
</tr>
<tr>
<td>Median Δ IL-1 β (pg/ml)</td>
<td>9.3</td>
<td>-113.55-99.6</td>
<td>24</td>
<td>-6.05</td>
<td>-89.25-44.20</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ IL-6 (pg/ml)</td>
<td>-48.2</td>
<td>-259.8-78.6</td>
<td>24</td>
<td>0.25</td>
<td>-89.25-52.7</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ TNF-alpha (pg/ml)</td>
<td>-38.05</td>
<td>-169.35-100.95</td>
<td>24</td>
<td>-18.18</td>
<td>-98.95-27.65</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ CRP</td>
<td>-4.4</td>
<td>-146.3-55.2</td>
<td>27</td>
<td>-8.6</td>
<td>-79.4-3.5</td>
<td>11</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 4.9:** Median Δ of pro-inflammatory cytokines from baseline to six months between improvers and non-improvers
As in other forms of heart failure, patients usually present with tachycardia and fluid retention. Big endothelin-1 (Big E) is the precursor of endothelin-1. Activation is through endothelin converting enzyme. Endothelin-1 has a positive inotropic and chronotropic effect. Chronic inotropic and chronotropic stimulation leads to cardiac myocyte damage, apoptosis and progression of heart failure. Endothelin-1 also increases aldosterone activity which again leads to more fluid retention. Median big E plasma levels were significantly higher in PPCM patients than in controls (P<0.0001), but levels of placental growth factor (PLGF) did not show any significant differences between patients and controls, improvers vs. non-improvers or over time.

### Table 4.10: Other markers between PPCM patients and controls at baseline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients median baseline</th>
<th>Range</th>
<th>n</th>
<th>Controls median</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big E (pg/ml)</td>
<td>10.1</td>
<td>0.89-43.1</td>
<td>37</td>
<td>1.72</td>
<td>0.08-8.54</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PLGF (pg/ml)</td>
<td>6.17</td>
<td>0.00-376.6</td>
<td>38</td>
<td>4.57</td>
<td>2.48</td>
<td>21</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 4.11: Other markers between improvers and non-improvers at baseline

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Improvers</th>
<th>Range</th>
<th>n</th>
<th>Non-improvers</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Big E (pg/ml)</td>
<td>7.75</td>
<td>0.0-43.1</td>
<td>26</td>
<td>12.89</td>
<td>0.0-32.1</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Median PLGF (pg/ml)</td>
<td>6.32</td>
<td>0.0-376.6</td>
<td>27</td>
<td>6.01</td>
<td>1.28-10.94</td>
<td>11</td>
<td>NS</td>
</tr>
</tbody>
</table>
## 4.3.5 Hormones

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients baseline</th>
<th>Range</th>
<th>n</th>
<th>Controls</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median estradiol (pg/ml)</td>
<td>48.2</td>
<td>0.00-1547.0</td>
<td>38</td>
<td>0.00</td>
<td>0.00-309.6</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Median prolactin (ng/ml)</td>
<td>24.7</td>
<td>9.6-66.6</td>
<td>36</td>
<td>7.40</td>
<td>2.85-18.95</td>
<td>21</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4.13: Hormone levels between PPCM patients and controls at baseline

Kinetics of median estradiol plasma levels were difficult to interpret as the range of values is wide. In contrast, median prolactin serum levels were significantly higher in PPCM patients as compared with peripartum controls (P<0.0001), although there were no significant differences between improvers and non-improvers at baseline or after six months. It is interesting to note though, that median prolactin serum levels among improvers decreased significantly (P=0.002) from 26.6 ng/ml [9.6-66.6] at baseline to 18.9 ng/ml [3.35-43.5] after six months. During the same time interval the decrease among non-improvers from 20.9 ng/ml [10.8-54.0] to 17.8 ng/ml [2.1-46.6] was non-significant (P=0.069)
### Table 4.14: Median of hormones between improvers and non-improvers at baseline

<table>
<thead>
<tr>
<th>Parameters at baseline</th>
<th>Improvers</th>
<th>Range</th>
<th>n</th>
<th>Non-improvers</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median estradiol (pg/ml)</td>
<td>62.1</td>
<td>0.0-1547.0</td>
<td>27</td>
<td>0.0</td>
<td>0.0-358.9</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Median prolactin (ng/ml)</td>
<td>26.6</td>
<td>9.6-66.6</td>
<td>25</td>
<td>20.85</td>
<td>10.8-53.95</td>
<td>11</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 4.15: Median Δ of hormones from baseline to six months between improvers and non-improvers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Improvers baseline vs. 6 months</th>
<th>Range</th>
<th>N</th>
<th>Non-improvers baseline vs. 6 months</th>
<th>Range</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Δ estradiol (pg/ml)</td>
<td>0</td>
<td>-1392.69-912.31</td>
<td>27</td>
<td>0</td>
<td>-108.05-330.86</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Median Δ prolactin (ng/ml)</td>
<td>-5.03</td>
<td>-47.65-10.85</td>
<td>24</td>
<td>-4.08</td>
<td>-28.85-6.9</td>
<td>10</td>
<td>NS</td>
</tr>
</tbody>
</table>
5. IDENTIFICATION OF AGONISTIC AUTOANTIBODIES AGAINST THE BETA 1-ADRENERGIC RECEPTOR AND THEIR POSITIVE CORRELATION WITH NT-PROBNP SERUM EXPRESSION LEVELS IN PERIPARTUM CARDIOMYOPATHY

5.1 Introduction

A general introduction to PPCM has been given in chapter one. We analysed the kinetics in serum and plasma expression levels of biomarkers in our previous studies (chapter 4 of this thesis), reflecting cardiac function, processes of remodeling, levels of pro-inflammatory cytokines and prolactin in PPCM patients over a period of six months. Several authors have described recovery of LV systolic function in up to 54% of PPCM patients (5, 192), while others develop chronic heart failure. In order to identify molecular pathways that determine whether cardiac function recovers or becomes chronically dysfunctional, we analyzed our data for differences between cardiac function improvers and non-improvers. While a wide range of parameters was elevated in all PPCM patients at time of first presentation, indicating their involvement in the initiation of the disease, we found significant differences over time between the two groups for IFN-gamma (P=0.0181) and ACE serum levels (P=0.0493), indicating their role in disease progression. While higher ACE expression levels in cardiac function non-improvers reflect ongoing adrenergic stimulation, heightened IFN-gamma expression could indicate ongoing T-cell mediated autoimmune response and insult to the cardiac muscle, resulting in failure to improve left ventricular systolic function. Interestingly, Abel et al. observed that IFN-gamma response may play a role in the susceptibility of patients to develop chronic Chagas’ disease cardiomyopathy (232). Autoimmune mediated myocardial damage is likely to be a pathogenic mechanism for acquired dilated cardiomyopathies. Several authors described the possible role of agonist-like AAB that act against the β1-AR and the muscarinic M2-receptor in the pathogenesis of DCMO and Chagas' disease (125, 233). Beta-adrenergic signaling plays an important role in the natural history of dilated cardiomyopathies. Chronic activation of β-adrenergic receptors during periods of cardiac stress ultimately harms the failing heart by mechanisms that include alterations in gene expression (234). The myocardial β-AR are part of the family of G-protein coupled receptors. Three subtypes have been distinguished (β1-, β2- and β3-adrenoceptors), consisting of seven membrane-spanning domains, three intracellular...
and three extracellular loops, one extracellular N-terminal domain and one intracellular C-terminal tail (235). The β1-ARs mediate chronotropic and inotropic effects of catecholamines via the stimulatory G-protein (Gs). The β-adrenergic myocardial responsiveness and expression of the β1-AR receptor on the mRNA and protein level are reduced in DCMO patients, while expression of the inhibitory G-protein G(i) and the G-protein receptor kinase are increased. This kinase induces uncoupling of β-AR and may either be induced through high catecholamine release or by agonist-like autoantibodies directed against the β1-AR found in patients with DCMO (125, 235, 236). The exposure to high levels of circulating catecholamines has been reported as toxic to cardiac myocytes, leading to myofibrillar degradation and increased cardiac collagen volume fraction mediated by β-AR stimulation (237, 238). Beta1-AAB enhance the beating frequency of cultured neonatal rat cardiomyocytes, increase L-Type Ca2+ current, APD and contractility in freshly isolated cardiomyocytes mediated via β1-AR, possibly contributing to β1-AR mediated cardiotoxicity in heart failure (239). The postpartum exacerbation of autoimmune diseases has been described previously and is secondary to immune system changes during a normal pregnancy (240).

The development of end-stage heart failure often involves an initial insult to the myocardium that reduces cardiac output and leads to a compensatory increase in sympathetic nervous system activity. Acutely, the sympathetic hyperactivity through the activation of β-adrenergic receptors increases heart rate and cardiac contractility, which compensate for decreased cardiac output. However, chronic exposure of the heart to elevated catecholamine levels may lead to further pathologic changes, resulting in continued elevation of sympathetic tone and a progressive deterioration in cardiac function. On a molecular level, altered β-AR signalling plays a pivotal role in the genesis and progression of heart failure (241).

As described in chapter 4 of this thesis, we found significantly higher ACE serum levels over time in cardiac function non-improvers than in improvers (P=0.0493). In vivo and in vitro studies suggest that the effector hormones of the RAAS, angiotensin II and aldosterone, are primarily involved in regulating the structural remodeling of the myocardial collagen matrix (224). Henrich et al. found that renin release elicited by a circulating β-agonist functions independently of prostaglandin synthesis and that the pathway operates
via an extrarenal mechanism (242). Inflammation is a key mechanism in the initiation and progression of cardiomyopathy. Angiotensin II plays a significant role in the advent and perpetuation of inflammatory cardiovascular disease (225). Hyperactivation of the RAAS, heightened sympathetic drive and uncontrolled synthesis of inflammatory cytokines exacerbate ventricular contractile dysfunction in heart failure patients (226).

In the present study we investigated the incidence of β1-AR AAB in serum of patients with PPCM and their activity at time of presentation, after six and twelve months. We related the time-course pattern of their activity with changes in NYHA FC, LVEF, LVESD, heart rate and NT-proBNP expression levels. NT-proBNP is suitable for heart failure monitoring and correlates well with clinical course in heart failure patients as described by others (243).

5.2 Patients and Methods

5.2.1 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 (PRC 990409) and complies with the Declaration of Helsinki. All patients and controls gave written informed consent before entry into the study. We screened a total of 39 patients to recruit 22 consecutive patients who met the inclusion criteria which are: (i) age ≥ 16 and ≤ 40, (ii) New York Heart Association functional class (NYHA FC) II-IV, (iii) symptoms of congestive heart failure that developed in the last month of pregnancy or during the first five months post-delivery, (iv) no other identifiable cause for heart failure, (v) left ventricular ejection fraction (LVEF) ≤ 40% by transthoracic echocardiography and (i) sinus rhythm.

Exclusion criteria: (i) significant organic valvular heart disease, (ii) systolic blood pressure (SBP) > 160 mmHg and/or diastolic blood pressure (DBP) > 100 mmHg, (iii) clinical conditions other than cardiomyopathy that could increase inflammatory markers, (iv) treatment with anti-inflammatory drugs, (v)
severe anaemia (haemoglobin < 9 g/dL), (vi) metabolic disorders affecting lipoprotein metabolism, i.e. thyroid disease.

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. The hospital is the sole tertiary medical facility for this community. Patients were referred from the department of obstetrics of our hospital, secondary hospitals and local clinics. Obstetric history, incl. pre-eclampsia and mode of delivery, was obtained from all patients and supplemented with data from the patients’ obstetric file. Clinical status, symptoms and signs, echocardiography and blood analysis were assessed at time of presentation (baseline), after six and twelve months of standard heart failure treatment. These were the three time points of the study. Besides the clinically indicated routine blood tests, we collected one additional tube of blood (4 ml) from every patient to obtain serum for measurement of research-specific β1-AR AAB and NT-proBNP expression levels.

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 \( \leq 25\% \) or LV thrombus received anticoagulatory therapy. The β-blocker carvedilol was added after resolution of overt heart failure and the dose was slowly titrated up to a target of 25 mg twice daily as long as SBP was \( \geq 100 \text{ mmHg} \) or symptoms such as dizziness did not occur.

Primary endpoints of this study were the activity of β1-AR AAB in serum (measured as the number of additional beats per minute), New York Heart Association functional class (NYHA FC), left-ventricular ejection fraction (LVEF) and NT-proBNP serum expression levels at time of presentation, after six and twelve months.

5.2.2 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 operator who was blinded to the protocol.

Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, Washington) echocardiograph attached to a 2.5 or 3.5 MHz transducer. Left ventricular dimensions were measured according to the American Society of Echocardiography guidelines (198). Measurements of LV dimensions and function were determined on an average of ≥ 3 beats. All echocardiographic studies were recorded on videotape and stored at Chris Hani Baragwanath Hospital, Division of Cardiology for future reference and audit purposes.

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 FC of each patient during 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.

5.2.3 Research specific blood tests

In order to measure the activity of β1-AR AAB and NT-proBNP serum expression levels, a volume of 4 ml of blood was withdrawn from an antecubital vein, collected in a prechilled vacutainer tube containing clot activator and mixed rapidly. Serum was separated by centrifugation at 2500 rpm for 12 minutes within 15 minutes of collection. All serum samples from patients and controls were obtained at Chris Hani Baragwanath Hospital and stored on site at minus 70 degrees Celsius. To measure the activity of β1-AR AAB the aliquotted samples were transferred on wet ice to Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany. The immunoglobulin fraction was isolated from 1ml serum samples by ammonium sulfate precipitation at a saturation of 40% overnight. After centrifugation, the precipitate was washed and dissolved in buffer containing 154 mmol/l NaCl and 10 mmol/l sodium phosphate, pH 7.2. We repeated two cycles of precipitating, washing, centrifugation and dissolving. Finally, the precipitated
immunoglobulins were taken up in 0.7 ml buffer and dialyzed at 4°C for 60 hours against 1 L of this buffer. The buffer was exchanged five times during dialysis.

Serum expression levels of Fas/Apo-1 were measured according to the manufacturers’ instructions, using commercially available enzyme immunoassays (ALPCO, Windham, NH, USA) and performed after shipment on dry ice at Emory University School of Medicine, Department of Pathology & Laboratory Medicine, Atlanta, USA.

In order to differentiate physiologically upregulated NT-proBNP expression levels during the peripartum period from pathological values and to obtain reference values for the activity of β1-AR AAB, we obtained serum from ten 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.

5.2.4 Cell culture and antibody characterization

Autoantibodies against the β1-adrenoreceptor from patients with dilated cardiomyopathy (DCM) increase the beating frequency of cultured neonatal rat cardiomyocytes (239). Neonatal rat cardiomyocytes were isolated and cultured as previously described (244). Briefly, single cells were dissociated from minced ventricles of one to three days old Sprague Dawley rats with 0.2% solution of crude trypsin and cultured with a density of 160,000 cells/cm² in a SM 20-I medium, containing 10% neonatal calf serum and 2 µmol/l fluorodeoxyuridine to prevent proliferation and overgrowth of any non-myocytes (245). The cells were incubated with 2 ml fresh serum-containing medium on the fourth or eight day respectively. The contracting rate of eight to ten spontaneously beating single or clustered cardiomyocytes was counted for 15 seconds on the heated desk (37°C) of an inverted microscope. In order to yield results, we repeated this procedure in different cultures. The isolated autoantibodies (immunoglobulins), agonistic and antagonistic drugs, peptides, etc. were added either separately or cumulatively, as indicated. The immunoglobulin preparations were pretreated with monoclonal anti-human IgG1, IgG2, IgG3 and IgG4 antibodies for one hour at room
temperature in order to determine the IgG subclass. The samples were treated with a mouse anti-human IgG antibody to enlarge the immunoglobulin complex. After further 30 min. the samples were centrifuged for 15 minutes at 13,000 rpm (Biofuge Fresco, Heraeus Instruments, Ostenrode, Germany) to obtain the supernatant used in the experiments. The antibodies were neutralized with peptides corresponding to the first, second, and third extra-cellular loop of the β1-AR in order to define the extra-cellular receptor structures that are recognized by the antibodies. The epitopes were identified using short overlapping peptides of the extra-cellular loops. In this experiment 50 µl of the human IgG preparation were incubated for one hour at room temperature with 50 µl of the short overlapping peptides (10µg/ml). The samples were centrifuged at 13,000 r.p.m. (as described above) to use the supernatant with the neutralized antibodies in the experiments.

5.2.5 Statistical analysis

Analyse-it version 1.71 statistical program (Analyse-it for Microsoft Excel, Leeds, UK) was used to describe the means ± standard deviation or medians and their range in [ ] as indicated. P-values for parametric data between baseline, six and twelve months were calculated by use of ANOVA 1-way repeated measures. P-values for non-parametric data between baseline, six and twelve months were calculated by use of the Friedman test. Significance was assumed at a two-tailed value of P<0.05 and all significant results were calculated by both, ANOVA 1-way repeated measures and the Friedman test. In cases where results differed between the tests, we displayed the less significant P-value. We used Spearman rank correlation to analyze a possible correlation between differences of β-1 AR AAB activity and differences in NT-proBNP expression levels at the three time points of the study.

5.3 Results

After a follow-up period of twelve months, data from 20 out of 22 enrolled patients were available. Two patients had moved to remote areas and were not available for follow-up. Patients presented with a median
parity of 2 [1-5] and reported onset of symptoms at a median of 17.5 days postpartum [8-30]. The mean age was 28.9±6.3 years and 9.1% had undergone caesarean section. The mean haemoglobin at time of presentation was 12.6±1.4 g/dl and median BMI was 23.0 [21.3-27.5]. Cardiac transplantation or LV assist device were unavailable for the population studied for economic reasons. All patients in this cohort were HIV-negative.

5.3.1 Functional characterization of β1-adrenoreceptor antibodies in PPCM patients

The basal contracting rate of the spontaneously beating neonatal rat cardiomyocytes in the described model was 162 ± 8 beats/ min. Addition of the antibody-containing IgG preparation from PPCM patients to the spontaneously beating rat cardiomyocytes exerted a positive chronotropic response in these cells. The effect was dose dependent with a maximal response at an antibody dilution of 1:50 (Fig.5.1).

![Figure 5.1: Dose response curve of AAB against the β1-adrenoreceptor in PPCM patients](image-url)
The positive chronotropic effect was antagonized by the selective β1-adrenergic antagonist bisoprolol (1µM). Addition of the angiotensin II blocker losartan (1µM) or the α1-adrenergic receptor antagonist prazosin (1µM) did not influence the agonistic effect induced by the β1-adrenoreceptor antibodies. Moreover, the peptide YFLL (1µg/ml) that was described as an inhibitory peptide of the human platelet thrombin receptor PAR-1 (proteinase activated receptor), was also without inhibitory effect (figure 5.2).

![Figure 5.2: AT1-, α1- and β1- adrenergic receptor antagonist effect](image)
The agonistic effect of the diluted IgG preparation (1:20) from patients diagnosed with PPCM and DCMO in comparison to healthy controls is demonstrated in figure 5.3. While the addition of the selective β1-adrenergic antagonist bisoprolol inhibited the positive chronotropic effect of the synthetic β1- and β2-sympathomimetic isoprenaline on cardiomyocytes only partially, the effect induced by antibodies isolated from patients with PPCM was blocked completely, identifying them as β1-adrenoreceptor antibodies. Importantly, the addition of immunoglobulins obtained from non-PPCM controls, did not alter the cardiomyocyte beating rate.

![Figure 5.3: Influence of the β1-adrenergic antagonist bisoprolol](image)

In order to further define the β1-adrenoreceptor antibodies, we treated them with peptides corresponding to the first, second and third extra-cellular loop of the β1-adrenoreceptor. Beta-1 adrenoreceptor antibodies obtained from serum of PPCM patients exclusively recognized epitopes on the second extra-cellular loop, while those from DCMO patients bind to epitopes of the first (30%) and second extra-cellular loop (70%). In another set of experiments we characterized the epitopes recognized by the β1-adrenoreceptor antibodies of PPCM and DCMO patients. The β1-adrenoreceptor antibodies of PPCM patients were neutralized by the second extra-cellular loop peptides RAESDE and DEARRCY (figure 5.4), while the other peptides of this
loop did not influence the agonist-like activity of the antibodies. In contrast, the β1-adrenoreceptor antibodies of DCMO patients were neutralized by the peptides ARRCYND and PKCCDF (figure 5.5), while the epitopes DEARRCY and ARRCYND partially overlap.

Figure 5.4: Epitope analysis in PPCM patients

Figure 5.5: Epitope analysis in DCMO patients
Subsequently, we identified the IgG subclasses of agonistic β1-adrenoreceptor antibodies. From previous investigations we know that functional antibodies represent immunoglobulins of the IgG class (data not shown). Therefore, we used monoclonal anti-human IgG1, IgG2, IgG3 and IgG4 antibodies to precipitate the functional antibodies of the immunoglobulin fraction. Figure 5.6 shows agonist-like antibodies of the IgG2 and IgG3 subclass prepared from PPCM patient serum samples. The second extra-cellular loop β1-adrenoreceptor antibodies of DCM patients are exclusively antibodies of the IgG2 subclass (Figure5.7).

Figure 5.6: IgG subclasses of β1-adrenergic AAB in PPCM patients (II. loop)

Figure 5.7: IgG subclasses of β1-adrenergic AAB in DCMO patients (II. loop)
The addition of the $\beta_1/2$ adrenoreceptor agonist isoprenaline (10 µM) caused a positive chronotropic effect in the spontaneously beating rat cardiomyocytes and induced desensitization of the $\beta$-adrenergic response. After two hours of incubation with isoprenaline the chronotropic response was moderately reduced. However, after a washing procedure and subsequent re-stimulation with isoprenaline, cell response reached only one third of the agonist’s maximal response. In contrast, the $\beta_1$-adrenoreceptor antibodies of PPCM patients exerted a long-lasting stimulatory effect on the cultured rat cardiomyocytes that was not reversed by washing with fresh, warm complete cell culture medium, but was interrupted by the $\beta_1$-adrenergic antagonist bisoprolol. Apparently the addition of bisoprolol removed the $\beta_1$-adrenergic receptor antibodies from their binding sites, because removal of $\beta_1$-adrenergic antagonist did not induce an increase in beating rate. Under these conditions renewed stimulation with isoprenaline exerted a maximal response in beating rate, while long-lasting stimulation of $\beta_1$-adrenoreceptors usually desensitizes the $\beta_1$-adrenoreceptor signaling cascade. These results suggest that the PPCM patient’s $\beta_1$-adrenoreceptor antibodies prevented desensitization of the $\beta_1$-adrenoreceptor (figures 5.8 and 5.9).

**Figure 5.8:** The $\beta_1$-adrenoreceptor autoantibody in a dilution of 1:40 exerts a long-lasting positive chronotropic effect without desensitization of the $\beta_1$-adrenoreceptor signal cascade
Figure 5.9: The β1-adrenoreceptor autoantibody prevents receptor desensitization induced by the β1-adrenoreceptor agonist isoprenaline.

5.3.2 Clinical status of PPCM patients and positive correlation of β1-adrenoreceptor antibodies with NT-proBNP serum expression levels

Patients presented with a mean heart rate of 93.4±16.7 b.p.m., systolic blood pressure of 105.9±17.0 mmHg and a diastolic blood pressure of 69.8±13.9 mmHg. The mean LVEF among all patients at time of presentation was 29.3±8.5% with a mean LVEDD of 5.5±0.6 mm. and LVESD of 4.8±0.7 mm. Median levels of NT-proBNP were 1673.7 fmol/ml [1076.5-2593.3] and significantly higher (P<0.0001) than in controls 339.5 fmol/ml [252.8-440.6]. Addition of baseline serum samples from human PPCM patients as described above revealed the presence of agonist-like AAB directed against the β1-adrenoreceptor in all PPCM patients, but not in peripartum non-PPCM controls (figure 5.1 and 5.3) and was quantified as 23.2 [19.2-27.2] additional beats per minute.
Patients were followed-up once a month or more frequently, if clinically indicated. At time of re-assessment after six months, mean haemoglobin (13.6±1.3 g/dl, P=0.15) and median BMI were stable 23.3 [20.8-25.6] (P=0.27) as compared to baseline. The mean heart rate of the initially tachycardiac patients had dropped significantly (P=0.0006) since time of first presentation to 76.0±11.8 beats per minute and within normal range, while systolic (105.1±14.5 mmHg, P=0.91) and diastolic blood pressure were unchanged (67.5±12.7 mmHg, P=0.42). The mean LVEF had improved significantly (P<0.0001) since baseline assessment to 43.7±11.6%, mainly due to a significant reduction of LVESD (4.0±0.8 mm, P=0.0006), while the reduction of mean LVEDD (5.2±0.8 mm, P=0.09) was non-significant. During the same time interval NYHA FC (1.5±0.7, P<0.0001) and NT-proBNP levels improved significantly (P=0.0075) to 936.2 fmol/ml [713.7-
Simultaneously the activity of agonist-like AAB directed against the β1-adrenoreceptor decreased significantly (P=0.0006) to 15.2 [10.4-19.2] as shown in figure 5.10.

![Figure 5.11](image)

**Figure 5.11:** Decreasing serum expression levels of NT-proBNP (fmol/ml) on the y-axis over time in months (x-axis)

After twelve months of anti-failure treatment, mean haemoglobin (13.0±1.6 g/dl, P=0.98) and median BMI were unchanged 23.1 [20.5-26.1] (P=0.07) as compared to assessment at six months. The mean heart rate (82.0±14.1 b.p.m., P=0.1), systolic (110.0±15.5 mmHg, P=0.08) and diastolic blood pressure (69.5±12.5 mmHg, P=0.50) were stable as well. Also the mean LVEF (43±13.0 %, P=0.88), LVEDD (5.1±0.8 mm, P=0.65), LVESD (4.0±0.9 mm, P=0.51) and NYHA FC (1.5±0.6 (P=0.71) remained unchanged. However, the activity of the agonist-like AAB directed against β1-AR continued to decrease significantly (P=0.0076) to 1.9 [0.00-14.5] in parallel with serum expression levels of NT-proBNP to 722.3 fmol/ml [480.3-1628.1] (P=0.0076). While changes in β-1 adrenoreceptor AAB activity and LVEF or NYHA FC did not correlate,
we found a significant positive correlation with NT-proBNP from baseline to twelve months (rs=0.58, 2-tailed P=0.0228), 95% CI (0.10 to 0.84).

**Figure 5.12:** Positive correlation between kinetics of β-1 adrenoreceptor AAB and NT-proBNP expression levels
6. THE ADDITION OF BROMOCRIPTINE TO STANDARD HEART FAILURE THERAPY PREVENTS DETERIORATION OF LEFT VENTRICULAR DIMENSIONS AND SYSTOLIC FUNCTION IN PPCM PATIENTS WITH A SUBSEQUENT PREGNANCY

6.1 Introduction

A general introduction to PPCM has been given in chapter one and readers are referred to the more specific introduction to subsequent pregnancy in chapter 1.9. Recurrence of heart failure in a subsequent pregnancy in PPCM patients has been well described and women should be counseled to avoid a subsequent pregnancy after diagnosis of PPCM (9). If ventricular function does not return to normal after pregnancy, subsequent pregnancies have been associated with maternal mortality rates of 19 to 50 percent (196). Even in those whose LV function returns to normal, deaths have been reported with subsequent pregnancies (58).

Fett et al. described the outcome of 16 subsequent pregnancies in 15 women with PPCM after the index pregnancies. Eight of these patients experienced worsening heart failure; of these, one died and one regained normal left ventricular systolic function. Seven patients tolerated pregnancy without worsening heart failure, and ventricular function recovered in these patients within 30 months after the subsequent pregnancy. However, all but one patient became pregnant before full recovery of LV systolic function and against medical advice. Eight of 15 patients had worsening heart failure during subsequent pregnancy and only one of these patients regained normal LV systolic function after subsequent pregnancy. Seven patients showed no worsening of heart failure during subsequent pregnancy and recovered normal LV systolic function during or after the subsequent pregnancy. Except for recovery of LV systolic function, the authors could not find a distinguishing feature between the group that fully recovered and the group that continued to have abnormal heart function (246).

As much as doctors can advise patients to avoid subsequent pregnancies in known PPCM, some patients do present with a subsequent pregnancy, creating an ethically challenging dilemma. On the one hand the life of the mother should be preserved, especially if there are other children depending on her. On the other hand every effort should be made to safe the life of the unborn child.
In 2004 Hilfiker-Kleiner et al. described the occurrence of PPCM in cardiac tissue specific STAT3 knock-out mice. As detailed in figure 6.1, the physiological postpartum activation of the prolactin receptor in healthy women results in secretion of a 24 kDa prolactin. Postpartum activation of this prolactin receptor in STAT3 knock out mice resulted in cathepsin D facilitated cleavage of the 24 kDa prolactin into 16 and 8 kDa proteins. In vitro the 16 kDa prolactin inhibited endothelial cell proliferation and increased fibroblast proliferation. STAT3-deficient female mice showed increased cardiac apoptosis and reduced cardiac function and survival postpartum. Bromocriptine, a dopamine- D2 receptor antagonist that inhibits prolactin secretion, prevented PPCM in these mice, restored cardiac function and structure and prevented apoptosis (133, 136, 141).

**Figure 6.1:** Mechanism of STAT3 knock-out in the pathogenesis of PPCM, adapted from Hilfiker-Kleiner et al. (133)

In light of the above findings, it seems appropriate to discuss the role of sex hormones and their effect on PPCM. It has long been established that both estrogen and prolactin have a reciprocal endocrinologic relationship and both hormones have pleiotropic effects on the immune system. Despite the presence of a
number of confounding variables, these hormones modulate immune response and have been implicated in
the development of autoimmunity. However, mechanisms by which this modulation occurs remain obscure.
Estrogen appears to suppress cell-mediated and augment humoral-based immunity. Prolactin appears to
stimulate both cell and humoral-based immunity. Both hormones have been shown to modulate IFN gamma
secretion. Experimental models of human autoimmune disease and autoimmune disease during pregnancy
suggest disparate effects of estrogen and prolactin on autoimmune responses and disease pathogenesis. In the
NZBXNZW (B/W) F1 mouse model of lupus, prolactin accelerates disease expression, whereas estrogen,
devoid of its prolactin stimulating properties, is immunosuppressive and inhibits IL-2 production. Estrogen
may directly or indirectly stimulate or inhibit immune responses due to its endocrinologic and immune
effects. These dichotomous effects have limited its successful pharmacologic manipulation in human
autoimmune disease with estrogen compounds, tamoxifen, oral contraceptives, antigonadotropic agents, or
ovulation induction regimens. In contrast, reduction of immunostimulatory concentrations of prolactin with
bromocriptine has successfully suppressed development, induction or expression of murine and human
autoimmune disease (80).
The occurrence of a subsequent pregnancy in PPCM patients is rare and therefore the number of patients
studied for this specific recurrent disease is small. Nonetheless, notwithstanding the small numbers, it's
occurrence prompted a closer examination of the clinical and laboratory parameters and hence the rationale.
Since the use of bromocriptine in addition to standard heart failure therapy was being evaluated in this
population at the initiation of this study, the role of bromocriptine therapy on subsequent pregnancy can be
viewed as the assessment of it's potential use as a preventive medication. Further studies are nonetheless
needed to draw statistically meaningful conclusions and to determine a potential preventive/prophylactic and
possibly curative effect of bromocriptine. However, since our data on PPCM patients with a subsequent
pregnancy is very encouraging and the condition carries a high mortality rate, we decided to present it at an
early stage.
6.2. Methods

6.2.1 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 (PRC 990409) and complies with the Declaration of Helsinki. All patients and controls gave written informed consent before entry into the study. Since PPCM occurs in previously healthy women who cannot be identified beforehand, we enrolled patients who had recovered from a previous episode of PPCM and presented with a subsequent pregnancy. All PPCM patients had been routinely advised to avoid subsequent pregnancies and referred to the gynecologist for choice of contraceptive method. However, some patients did present with a subsequent pregnancy and decided to carry the pregnancy to term. The inclusion criteria were: (i) documented previous episode of PPCM and (ii) decision to carry on with the current pregnancy.

Exclusion criteria: (i) significant organic valvular heart disease, (ii) systolic blood pressure (SBP) > 160 mmHg and/or diastolic blood pressure (DBP) > 100 mmHg, (iii) clinical conditions other than cardiomyopathy that could increase inflammatory markers, (iv) treatment with anti-inflammatory drugs, (v) severe anaemia (haemoglobin < 9 g/dL), (vi) metabolic disorders affecting lipoprotein metabolism, i.e. thyroid disease.

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. Obstetric history, incl. pre-eclampsia and mode of delivery, was obtained in close collaboration with the Department of Obstetrics at our hospital. Clinical status, symptoms and signs, echocardiography and blood analysis were assessed pre- and post-delivery and three months postpartum. These were the three time points of the study. Besides the clinically indicated routine blood tests, we collected one additional tube of blood (4 ml) from every patient to obtain plasma for measurement of research-specific cathepsin-D, 16- and 23-kDa prolactin expression levels. Following the initial screening and baseline visits, monthly outpatient visits were scheduled for clinical assessment and evaluation of medication compliance.
6.2.2 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 operator who was blinded to the protocol. Two-dimensional targeted M-mode echocardiography with Doppler color flow mapping was performed using a Hewlett Packard Sonos 5500 (Philips, Bothell, Washington) echocardiograph attached to a 2.5 or 3.5 MHz transducer. Left ventricular dimensions were measured according to the American Society of Echocardiography guidelines (198). Measurements of LV dimensions and function were determined on an average of ≥3 beats. All echocardiographic studies were recorded on videotape and stored at Chris Hani Baragwanath Hospital, Division of Cardiology for future reference and audit purposes.

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 FC of each patient during 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.

6.2.3 Research specific blood tests

In order to measure cathepsin-D, 16- and 23-kDa prolactin expression levels, 4 ml of blood were withdrawn from an antecubital vein, collected in a prechilled vacutainer tube containing clot activator and mixed rapidly. Serum was separated by centrifugation at 2500 rpm for 12 minutes within 15 minutes of collection. All serum samples from patients and controls were obtained at Chris Hani Baragwanath Hospital and stored on site at minus 70 degrees Celsius. In order to differentiate physiologically upregulated cathepsin-D, 16- and 23-kDa prolactin expression levels during the peripartum period from pathological values, we obtained serum from 5 female volunteers in the peripartum period without history of cardiac or recent infectious
Western blot for 16kDa prolactin: For immuno-precipitation 100 μl human serum was incubated with the PRL antibody (10 μl, 3 h) and subsequently with protein A-agarose (Roche) (50 μl over night) in 1xPBS and complete mini protein inhibitor cocktail (Roche). Then A-agarose protein complex was precipitated and washed three times with RIPA buffer. Finally the pellet was re-suspended in 1xLaemmli buffer loaded on 12% SDS page gel. Proteins were transferred to nitrocellulose membrane (Amersham) and incubated with the PRL antibody. Bands were visualized with ECL (Amersham) on x-ray film (Kodak).

Cathepsin D Activity Assay: CD activity was determined in patient serum using the InnoZyme CD Immunocapture Activity Assay Kit (Calbiochem) and a FLUOstar Galaxy.

6.2.4 Treatment

During pregnancy all patients received treatment with the diuretic furosemide and the β-blocker carvedilol at clinically indicated doses. Post-delivery we added the angiotensin-converting-enzyme inhibitor enalapril as long as SBP was ≥ 100 mmHg or symptoms such as dizziness did not occur. While the first four consecutive patients received standard heart failure therapy alone as described above, the next four consecutive patients additionally received bromocriptine 2.5mg twice daily, starting four hours post-delivery for two months.

6.2.5 Statistical analysis

Analyse-it version 1.71 statistical program (Analyse-it for Microsoft Excel, Leeds, UK) was used to describe the means ± standard deviation or medians (range) as indicated. P-values for parametric data between groups at the three time points of the study were calculated by using an independent samples t-test. P-values for non-parametric data were calculated with a Median test. Significance was assumed at a two-tailed value of P<0.05. Single parameters were calculated by continuous summary descriptives as means ± standard
deviation or medians with range [ ] as appropriate. Due to the small sample size we did not calculate P-values between the three time points of the study.

6.3. Results

6.3.1 Clinical and echocardiographic data of PPCM patients presenting with subsequent pregnancy either on standard heart failure therapy alone or receiving additional bromocriptine

All eleven patients were indigenous black women (age range 26 to 39 years) and presented with a median gravida of 4 [2-5]. The subsequent pregnancy occurred one to 6 years after the PPCM index pregnancy. None were twin or multiple pregnancies and none of the patients had pregnancy associated hypertension or eclampsia. All patients had a normal vaginal delivery at term. All except one patient were in NYHA FC I at onset of subsequent pregnancy and remained asymptomatic until delivery. Two patients on standard treatment alone died from severe refractory heart failure within eight weeks postdelivery despite optimal medical therapy. Changes in left ventricular ejection fraction are shown in table 6.1.

<table>
<thead>
<tr>
<th>Predelivery parameters</th>
<th>Group 1 (n=5)</th>
<th>Group 2 (n=6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ejection fraction (%)</td>
<td>48.2±12.2</td>
<td>47.4±9.0</td>
<td>0.9068**</td>
</tr>
<tr>
<td>Mean LVEDD (cm)</td>
<td>5.29±0.09</td>
<td>5.94±0.76</td>
<td>0.0951**</td>
</tr>
<tr>
<td>Mean LVESD (cm)</td>
<td>3.99±0.35</td>
<td>4.42±0.95</td>
<td>0.3655**</td>
</tr>
<tr>
<td>Mean NYHA FC</td>
<td>1.4±0.89</td>
<td>1.0±0</td>
<td>0.3466**</td>
</tr>
</tbody>
</table>

Table 6.1: Pre-delivery parameters of PPCM patients with subsequent pregnancy.
Group 1: Standard heart failure therapy plus bromocriptine
Group 2: Standard heart failure therapy only
** 2-tailed p-value

Four of the six patients had persistent cardiomegaly and impaired ejection fraction (40%) at onset of the subsequent pregnancy. At eight months of pregnancy, left ventricular ejection fraction remained unchanged. However, one month postpartum a significant deterioration (10% decrease in ejection fraction) was observed in all but one patient. The single patient whose ejection fraction remained unchanged had normal systolic
function to start with. At three months postpartum, two of the six patients on standard therapy died due to heart failure, with no improvement in ejection fraction in the remaining patients. Both deaths occurred in patients who had persistent cardiomegaly and impaired ejection fraction at onset of subsequent pregnancy.

Figure 6.2: Time course pattern of left ventricular ejection fraction in PPCM patients with subsequent pregnancy on standard heart failure therapy and with addition of bromocriptine

In the bromocriptine group heart failure symptoms occurred in one patient during the last month of pregnancy and coincided with defaulting treatment for three weeks. All patients in the bromocriptine group maintained left ventricular ejection fraction and the patient who had defaulted recovered well. None of the patients in the bromocriptine group died. As shown in table 6.1, echocardiographic parameters did not differ significantly between groups pre-delivery.
<table>
<thead>
<tr>
<th>Post-delivery parameters</th>
<th>Group 1 (n=5)</th>
<th>Group 2 (n=4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median LVEF (%)</td>
<td>37.6 (37.0)</td>
<td>30.3 (39.0)</td>
<td>0.7143*</td>
</tr>
<tr>
<td>Mean LVEDD (cm)</td>
<td>5.72±0.29</td>
<td>6.25±0.39</td>
<td>0.0507**</td>
</tr>
<tr>
<td>Median LVESD (cm)</td>
<td>4.55 (1.2)</td>
<td>5.35 (1.8)</td>
<td>0.3333*</td>
</tr>
</tbody>
</table>

**Table 6.2:** Post-delivery parameters of PPCM patients with subsequent pregnancy.  
Group 1: Standard heart failure therapy plus bromocriptine  
Group 2: Standard heart failure therapy only  
* Median test exact, double 1-tailed p-value  
** 2-tailed p-value

<table>
<thead>
<tr>
<th>Three months post-delivery parameters</th>
<th>Group 1 (n=5)</th>
<th>Group 2 (n=4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean LVEF (%)</td>
<td>48.8±11.0</td>
<td>25.5±5.5</td>
<td>0.0069**</td>
</tr>
<tr>
<td>Median LVEDD (cm)</td>
<td>5.8 (0.7)</td>
<td>6.6 (1.1)</td>
<td>0.3333*</td>
</tr>
<tr>
<td>Median LVESD (cm)</td>
<td>4.1 (1.2)</td>
<td>5.8 (1.3)</td>
<td>0.3333*</td>
</tr>
</tbody>
</table>

**Table 6.3:** Three months post-delivery parameters of PPCM patients with subsequent pregnancy.  
Group 1: Standard heart failure therapy plus bromocriptine  
Group 2: Standard heart failure therapy only  
* Median test exact, double 1-tailed p-value  
** 2-tailed p-value
6.3.2 Presence of 16-kDa prolactin isoform in human PPCM patients and increased cathepsin D activity

![Figure 6.3: Increased cathepsin D activity and presence of 16-kDa prolactin in PPCM patients in comparison to controls.](image)

![Figure 6.4: Presence of 23-kDa prolactin during the peripartum period in all samples, but absence of 16-kDa prolactin in bromocriptine-treated PPCM patients and healthy peripartum controls.](image)

We observed significantly increased levels of cathepsin D activity in serum of lactating PPCM patients as compared to lactating non-PPCM women (figure 6.3) from the same population. In addition, we readily detected the cleaved 16kDa prolactin by Western blot (figure 6.4) in serum of three out of the five lactating PPCM patients with documented cardiac dysfunction at the time of serum collection (mean EF: 24±7%).

Sera from five non-PPCM patients failed to demonstrate detectable levels of the 16-kDa prolactin. Interestingly, we were unable to demonstrate the presence of the 16-kDa prolactin in serum of patients treated with bromocriptine 2.5 mg twice daily in addition to standard heart failure therapy.
7. DISCUSSION AND CONCLUSIONS

7.1 Discussion: Clinical profile of 100 patients diagnosed with PPCM

This study documented the clinical profile of 100 PPCM patients at a tertiary level hospital in South Africa and examined the role of plasma/serum pro-inflammatory markers at the time of diagnosis and clinical outcome after 6 months of treatment. Despite appropriate and optimal clinical care including ACE-inhibitors and carvedilol, 15% of patients died and only 23% of the studied population normalized their LVEF after 6 months of therapy. Prognosis of patients with PPCM seems to vary according to topographical region. Whereas Felker et al. (193) reported a 94% survival rate in 52 patients diagnosed at John Hopkins Hospital in the USA, 14% died in a prospectively studied population in Haiti and only 20% regained normal left ventricular function (149). Poor socioeconomic status, subtle yet undefined nutritional deficiencies, genetic factors and inadequate pre- and postnatal care could contribute to these geographical differences. The only options for patients who do not regain normal left ventricular function are the use of left ventricular assist devices and cardiac transplantation. However, such options are not available in resource poor settings, such as in Haiti and South Africa. Patients that died had lower NYHA FC, LVEF and larger left ventricular dimensions at diagnosis compared to those who survived, whereas age, parity or onset of symptoms did not appear to play a role. Demakis et al. found that pathological findings in the myocardium of patients whose hearts had returned to normal size within six months of treatment and those whose hearts had not returned to normal size were indistinguishable (23).

CRP is an acute-phase protein which recognizes a range of pathogenic targets including membranes of apoptotic and reactive cells (200). As this inflammatory marker is associated with adverse prognosis in patients with idiopathic dilated cardiomyopathy (247, 248), we investigated if levels of plasma CRP at baseline could predict outcome in patients with PPCM. Almost half of the population investigated had raised levels of CRP reflecting possibly the presence of a low-grade chronic inflammatory process due to the release of endotoxin or endotoxin like substances and subsequent release of proinflammatory cytokines (249). However, we did not find a correlation with NYHA FC or death. None of the patients with PPCM
presented with symptoms during the antepartum period. This is in contrast to studies performed by others (6) and more in keeping with a study from Haiti (149) documenting that 96% of patients with PPCM developed heart failure in the post partum period. Our failure to include PPCM patients during the prepartum period in the present study was not due to a lack of identifying such patients since the cardiologists at Chris Hani Baragwanath Hospital are routinely involved in the care of pregnant patients presenting with symptoms and signs of congestive cardiac failure. The majority of cases developed symptoms in the first 4 weeks post partum. Twenty percent of patients studied were primiparous. We could not confirm factors mentioned by others (6) as multiparity, older age, or long-term use of tocolytic agents to be associated with the development of PPCM. At presentation this group of patients had acute onset heart failure of short duration. There was no evidence of chronic disease or cardiac cachexia that could account for a low lipid profile being a marker of severe, chronic disease. Mean plasma levels of total cholesterol of the patient population studied was $4.2\pm0.8$ mmol/L and low compared to that reported in other studies (250-252). In a study by Rauchhaus and colleagues with an established plasma cholesterol cut-off level of $< 5.2$ mmol/L, low total cholesterol level was found to be predictive for impaired 1-year survival (251). In line with findings by others (250, 251) demonstrating an increase in the rate of mortality with low serum total cholesterol levels, we found an association of low total cholesterol levels with larger left ventricular dimensions and lower EF. There was a trend, but no statistically significant association between the rate of mortality which could possibly be explained by the short duration of the trial, the limited number of patients studied and the spontaneous recovery rate typical for patients with PPCM. Levels of low plasma cholesterol correlated positively with the levels of the inflammatory marker CRP. These findings are in support of the endotoxin-lipoprotein hypothesis (249) suggesting that lower plasma levels of total cholesterol provide lesser protection against endotoxins making a susceptible group of patients more prone to severe heart failure. A recent trial by Albert and colleagues (253) showed a significant variation in the distribution of plasma CRP levels among various ethnic groups living in the United States. Median plasma CRP levels were significantly higher among black women compared to their white, Hispanic or Asian counterparts. Since 40% of the variance of plasma CRP
levels is genetically determined and PPCM is much more frequent in black patients one could hypothesize that an increase in the intensity of an inflammatory response could be one of many factors contributing towards the development of PPCM. This is supported by our previous research in PPCM patients presenting with subsequent pregnancy where we observed an exaggerated postpartum pro-inflammatory cytokine surge possibly playing a role in the development of PPCM (254).

Plasma levels of Fas/Apo-1 in the PPCM patients were significantly higher compared to healthy controls and a predictor of mortality, indicating that cardiac myocyte apoptosis may play a causal role in the pathogenesis of PPCM. This view is further supported by a non-randomised study in patients with PPCM where we have demonstrated improved clinical outcome in patients receiving pentoxifylline, an immuno-modulating agent, when used as a supplement to conventional therapy (148) as discussed below.
7.2 Discussion: Kinetics of cardiac function biomarkers, pro-inflammatory cytokines, markers of re-modeling and prolactin in 43 PPCM patients over a six months period

**Prolactin cytokine feedback loop:** We assessed the kinetics of biomarkers reflecting cardiac function, processes of inflammation and re-modeling as well as the hormone prolactin in a cohort of patients diagnosed with PPCM. The aim was to identify pathways and/or mechanisms involved in the initiation and progression of the disease in an effort to stratify possible future treatment options. While we found a whole range of biomarkers significantly elevated in patients vs. controls at baseline, reflecting their role during initiation of the disease, the significantly different kinetics between baseline and six months in improvers vs. non-improvers were narrowed down to the pro-inflammatory cytokine IFN-gamma that is involved in fibrosis and autoimmune activation and the regulatory angiotensin-converting enzyme, suggesting their involvement in progression of the disease.

This process could be further enhanced through pro-inflammatory cytokines like TNF-alpha, IL-1 β and IL6 (all of these were higher in our PPCM patients than in controls, \( P<0.0001 \)) that again increase ACTH release by stimulating secretion of corticotrophin-releasing hormone (255). Inflammatory cytokines promote a "prolactin–cytokine positive feedback loop" by stimulating the release of pituitary prolactin (80). Vice versa, elevated serum prolactin concentrations have been correlated with increased levels of inflammatory cytokines. Brand et al. demonstrated that prolactin causes an increase in the binding activity of the intracellular transcription factors nuclear factor-kappaB (NFkappaB) and interferon regulatory factor-1 (IRF-1), which are known to promote TNF-alpha and IL-12 secretion, suggesting that prolactin promotes a pro-inflammatory immune responses (256). Di Rosa et al. observed that prolactin, which is structurally related to several cytokines and is involved in regulating monocyte/macrophage functions, upregulates chitotriosidase, a chitinolytic enzyme that is mainly produced by activated macrophages. The group exposed human monocytes/macrophages to pro-inflammatory stimuli such as IFN-gamma, TNF-alpha and lipopolysaccharide, resulting in increased levels of chitotriosidase mRNA, as well as chitotriosidase activity.
(257). Mavoungou et al. documented a strong positive correlation between prolactin and the pro-inflamatory cytokines TNF-alpha, IFN-gamma, IL-1 and IL-6 in microfilaraemic women (258).

**Autoimmune disease:** Interestingly, serum prolactin levels were significantly higher (P<0.0001) among PPCM patients than in peripartum controls in our study and serum levels decreased significantly (P=0.002) among improvers, but not in non-improvers. As thoroughly reviewed by others, autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome, rheumatoid arthritis, Hashimoto's thyroiditis and multiple sclerosis affect women predominantly (81, 259-261). Cyclic estrogen concentrations differ not only between female and male, but also appear to differ between races for unknown cause. African and Asian women have higher serum 17-β-estradiol concentrations than Caucasians (262), an epidemiological coincidence with the increased frequency of SLE in populations of African and Asian ancestry (263). Amelioration of autoimmune diseases like SLE, RA and MS during pregnancy followed by post-partum exacerbation has been previously described by others (264, 265). Interestingly, prolactin and the immunosuppressive drug cyclosporine appear to be antagonistic through a receptor-based mechanism (266). Investigations of prolactin in the lupus NZB/W mouse have shown that elevated serum prolactin concentrations are associated with accelerated autoimmune disease in both female and male mice (267-269). Schwarz et al. demonstrated induction of IFN-gamma gene expression in a Nb2 rat T lymphoma cell line induced by prolactin (270). Breidthardt et al. reported that human recombinant prolactin significantly amplified IFN-gamma yields after stimulation with either PHA or lipopolysaccharide in peripheral human whole blood cultures (271).

Notably, in our present study the median Δ of IFN-gamma differed significantly (P=0.0181) from baseline to six months between cardiac function-improvers and non-improvers, suggesting a role for this cytokine not only during the initiation of PPCM, but also as an important factor in disease progression. This might reflect ongoing autoimmune activation and fibrosis, leading to irreversible cardiomyopathy among cardiac function non-improvers with PPCM. It is interesting to note that Abel et al. observed that IFN-
gamma response may play a role in the susceptibility of patients to develop chronic Chagas’ disease cardiomyopathy (232).

**Lack of protection from apoptosis:** Fas/APO-1 (CD95) is a type I membrane protein belonging to the tumor necrosis factor receptor family, is ubiquitously expressed and induces a death signal when bound to its ligands that is typically confined to inflammatory cells (272, 273). Elevated baseline levels of Fas/APO-1 have previously been described as a predictor of mortality in PPCM patients (192). In the present study we were able to demonstrate their significant (P<0.0001) elevation vs. controls at baseline and were able to identify dysregulated molecular pathways involved in the expression levels of Fas/APO-1 in PPCM patients. On the one hand, a cellular lack of protection from increased Fas/APO-1 activity could be secondary to significantly (P=0.002) decreased serum levels of the antiapoptotic survival factor for T-lymphocytes TGF-β1 in PPCM patients as compared to healthy controls. Cerwenka et al. described the existence of a TGF-β-producing Th3 subset that might be identical with CD4/CD25 regulatory T-cells (62) and play an important inhibitory role during immune response and deliver anti-apoptotic signals to T-cells. This subset of CD4 T cells is highly potent in suppressing immune responses and its experimental depletion resulted in organ-specific autoimmunity (4). On the other hand we found significantly increased levels of oxidized low density lipoprotein (oxLDL) among PPCM patients (P<0.0001). Takarada et al. investigated the contribution of oxLDL to Fas/APO-1 mediated apoptosis in human vascular smooth muscle cells (VSMC) and provided evidence that oxLDL sensitizes human vascular smooth muscle cells to Fas/APO-1 (CD95) mediated apoptosis and that oxLDL is involved in Fas/APO-1 signal transduction, proposing a mechanism by which oxLDL upregulates cell surface Fas/APO-1 by inhibition of Fas/APO-1 degradation through the ubiquitin–proteasome pathway (274). We therefore postulate that apoptosis is a crucial mechanism in the pathogenesis of PPCM and increased activity in PPCM patients results from a lack of the protective, antiapoptotic survival factor for T-lymphocytes TGF-β1 in combination with the inhibition of Fas/APO-1 degradation mediated by high levels of oxLDL.
Differential effects between the pro-inflammatory cytokine IL-1β and the anti-inflammatory cytokine TGF-β1 have been described by Ng et al. (199) and could help to understand the high median serum levels of IL-1β in PPCM patients (P<0.0001) in the presence of decreased TGF-β1 serum levels among PPCM patients (P=0.002).

B-Type natriuretic peptide (BNP) is considered an important component of the adaptive mechanism that helps reduce the load on the myocardium through systemic vasodilatation, reduction in venous return, and reduction in vascular volume (275). NT-proBNP concentrations have been shown to provide information similar to BNP, and the validity of the assay as a clinical tool is well documented (276). Intravascular volume is known to expand during pregnancy by 40-50% (277) and return to nearly its non-pregnancy value within a week post-delivery (278). The mean weight loss of 2–3 kg during this week is attributed to diuresis. Lev-Sagie et al. (279) studied the mean maternal blood NT-proBNP concentration in 62 women and documented a mean plasma concentration before delivery of 81±32 ng/L and 165±102 ng/L after delivery (n = 62, P <0.001). The 2-fold increase in NT-proBNP after delivery suggests that BNP may be involved in postpartum diuresis. Recently, it was found that BNP mRNA of the left ventricle was increased in postpartum rats. The authors of that study concluded that natriuretic peptides may be involved in the adaptation to volume alterations associated with pregnancy (280). Kale et colleagues reported significantly elevated levels of serum Nt-proBNP in pre-eclamptic patients (430+/−28.91 pg/mL) as compared to normotensive pregnant women (74+/−16.82 pg/mL, P < 0.001) (281). In our study, we documented significantly elevated serum levels of NT-proBNP in PPCM patients at baseline vs. non-PPCM controls in the peripartum period. Cardiac function non-improvers had significantly higher levels (P=0.0013) than improvers at baseline. While Cenac et al. reported that low NT-proBNP values indicate complete remission of cardiac failure and normal heart volume (208) in PPCM patients, we observed a reduction of median serum NT-proBNP levels, but not a normalization of these values in our patients after six months.

**Fibrosis:** The importance of fibrosis in organ pathology and dysfunction appears to be increasingly relevant to a variety of distinct diseases. In particular, a number of different cardiac pathologies seem to be caused by
a common fibrotic process (220). Examining markers of re-modeling in our cohort of PPCM patients, we did not record significant changes in plasma levels of VEGF or MMP-9. However, MMP-2 serum levels were significantly elevated at baseline (P<0.0001). Martin-Chouly et al. described an overexpression of MMP-2 in lung fibroblasts leading to tissue destruction associated with airway inflammation and were able to demonstrate selective inhibition of pro-MMP-2 secretion induced by TNF-alpha with phosphodiesterase type 4 inhibitors (282). Therefore increased baseline levels of TNF-alpha could explain initial elevation of MMP-2 serum levels. Fibrosis is thought to be partially mediated by TGF-β1, a potent stimulator of collagen-producing cardiac fibroblasts. Previously, TGF-β1 had been implicated solely as a modulator of the myocardial remodeling seen after infarction. However, recent studies indicate that dilated, ischaemic and hypertrophic cardiomyopathies are all associated with raised levels of TGF-β1 (220). In contrast, median baseline TGF-β1 serum levels of PPCM patients were lower than in controls (P=0.002), suggesting that remodeling does not play a key role in the aetiology of PPCM.

As expected, our results reflect cardiac failure in this cohort of PPCM patients. Interestingly, we were able to demonstrate oxidative stress that might contribute to left ventricular dysfunction and tachycardia. Other than in dilated, ischaemic and hypertrophic cardiomyopathy, decreased levels of TGF-β1 suggest a lack of antiapoptotic protection rather than myocardial re-modeling as an aetiologic factor in the pathogenesis of PPCM, giving a possible explanation for the high rate of spontaneous recovery in this disease entity. Our results might reflect a prolactin induced pro-inflammatory immune response accompanied by apoptosis at onset of disease, although it is difficult to say whether elevated postpartum prolactin levels induce the pro-inflammatory response, or if a pro-inflammatory immune environment induces prolactin secretion. However, the expression of IFN-gamma, that differed significantly between cardiac function improvers and non-improvers over time, appears to be interrelated with the kinetics of the pituitary gland hormone prolactin. Double-blind, placebo-controlled studies on the use of the prolactin inhibitor bromocriptine are limited, but clinical observations and trials support the use of bromocriptine as a nonstandard primary or adjunctive therapy in the treatment of recalcitrant rheumatoid arthritis, SLE, Reiter's
syndrome and psoriatic arthritis unresponsive to traditional approaches (283). While the role of prolactin in the pathogenesis of PPCM deserves further investigation, clinicians should consider the possible negative effect of drugs in these patients that potentially increase prolactin levels, such as the calcium-channel blocker verapamil, alpha-methylldopa, selective serotonin-uptake inhibitors such as fluoxetine and H2-receptor antagonists like cimetidine.

The significant kinetics of IFN-gamma levels between improvers and non-improvers are likely to reflect an ongoing T-cell mediated autoimmune response and insult to the cardiac muscle of these PPCM patients, resulting in failure to improve left ventricular function. It would be interesting to study, whether agonistic autoantibodies against the β 1-adrenergic receptor that were found in patients with other forms of dilated cardiomyopathy (125, 235, 236) also exist in PPCM patients and if their presence or absence would be related to kinetics of left ventricular function.
7.3 Discussion: Identification of agonistic autoantibodies against the beta1-adrenergic receptor and their positive correlation with NT-proBNP serum expression levels in PPCM

We have demonstrated the presence of agonist-like AAB directed against the β1-adrenoceptor in serum of all 22 PPCM patients in this cohort and mapped their reactivity to the second extracellular loop (RAESDE and DEARRCY). These AAB were not present in any of the non-PPCM peripartum controls, differ from those found in serum of local DCMO patients in recognized epitopes and IgG subclass, suggesting that PPCM is a distinct form of cardiomyopathy.

The activity of the agonist-like β1-adrenoceptor AAB correlated with expression levels of NT-proBNP, but not with left ventricular ejection fraction and NYHA FC. The correlation of NT-proBNP changes with the clinical course in heart failure patients has been described before (243, 284) and increased circulating concentrations of NT-proBNP have been observed in asymptomatic left ventricular dysfunction (285-287). While clinical and echocardiographic assessment between six and twelve months indicate stabilization of left ventricular systolic function in our PPCM patients, the continued and significant decrease of NT-proBNP levels is likely to reflect ongoing resolution of asymptomatic heart failure and immune reconstitution mechanisms on the molecular level, accompanied by decreasing β1-adrenoceptor AAB activity. Possibly, the expression of NT-proBNP serum levels is a more subtle marker of myocardial dysfunction than clinical and echocardiographic assessment in this cohort of PPCM patients.

Obviously it would be interesting to know, whether the decrease in β1-adrenoceptor AAB activity induced clinical improvement in PPCM patients or vice versa. Immunization of rats with a synthetic peptide corresponding to the second extracellular loop of the β1-adrenoceptor induced cardiac dysfunction and desensitization of the β1-adrenoceptor, suggesting a pathogenetic role of the autoantibodies (236, 288). Gimenez et al. showed progressive decrease in left ventricular wall thickness, LV mass and fractional shortening in β1-adrenoceptor immunized mice accompanied by a decrease in β1-adrenoceptor density, myofibril disarray and fibrosis, pointing towards remodeling as a consequence of the long-term presence of
anti-receptor antibodies (233). Stork et al. identified β1-adrenoreceptor autoantibodies as independent predictors of increased all-cause and cardiovascular mortality in a model with 65 DCM patients (289). Abnormalities in β-adrenergic receptor signal transduction are not only involved in functional cardiac impairment, but also in structural changes in the transition from compensated cardiac hypertrophy to decompensated heart failure (168, 290). Veliotes et al. suggest that aldosterone receptor blockade, through load-independent effects, may be useful in preventing the transition from compensated LVH to dilatation and pump dysfunction mediated by chronic β-adrenoreceptor activation (291). Immunoabsorption and plasmapheresis assisted removal of immunoglobulins in anti-β1-adrenoreceptor positive DCMO patients resulted in improved cardiac function and quality of life (236, 292). Dorffel et al. found increased antibody titers accompanied by deterioration of cardiovascular function in patients with severe idiopathic dilated cardiomyopathy and documented improved short- and long-term haemodynamics after immunoabsorption (293). Jahns et al. described the β1-adrenoreceptor-directed autoimmune attack as a possible cause of cardiomyopathy, which is now referred to as anti-β1-AR-induced dilated immune-cardiomyopathy (294). However, autoantibodies might not be directly responsible for many of the manifestations of autoimmune disease (295). While these data indicate a clinical relevance of β1-adrenoreceptor AAB in DCM and encourage further research into antibody-directed strategies as a therapeutic principle (289), Tabak'ian et al. found that detection of antibodies did not depend on the aetiology of systolic cardiac failure (IHD, DCM) or the severity of haemodynamic impairment (296). Mobini et al. showed similar acute and prolonged improvement of haemodynamics and left ventricular ejection fraction during immunoabsorption therapy in both, antibody-positive and -negative DCMO patients, suggesting that beneficial haemodynamic effects induced by immunoabsorption are not directly associated with the removal of β1-adrenoreceptor autoantibodies. However, the group only measured antibodies against the second extracellular loop (297). Similarly, Larsson et al. found that improvement of cardiac function is not due to neutralization of β1-adrenoreceptor autoantibodies (298).
However, a possible explanation for improvement of left ventricular systolic function in the presence of β1-adrenoreceptor AAB would be the recognition of an epitope by the AAB that does not correlate with disease, e.g. an upregulation of uterine β1-adrenoreceptors may absorb all the agonist-like AAB directed against the β1-adrenoreceptor. One could also imagine patients to improve cardiac function in the presence of agonist-like AAB directed against β1-adrenoreceptor, if the intracellular pathway desensitized in vivo while the β1-adrenoreceptors are expressed.

The diminished beta-adrenergic myocardial responsiveness in DCMO patients (235) might serve a protective purpose since chronic beta-adrenoreceptor stimulation induces myocardial apoptosis (299) and heart failure in transgenic animals (235). Interestingly, Ishikawa et al. described the suppression of heterologous desensitization of β-adrenoreceptor by IFN-gamma (300), suggesting that high IFN-gamma levels, as we found in non-improving PPCM patients (chapter 4 of this thesis), lead to β-adrenoreceptor being highly susceptible towards stimulation. In this light our results help to understand the progression of PPCM from acute heart failure in a setting of pro-inflammatory activation to chronic heart failure, accompanied by high serum expression levels of IFN-gamma and angiotensin converting enzyme in the presence of PPCM-specific agonist-like β1-adrenoreceptor autoantibodies.
7.4 Discussion The addition of bromocriptine to standard heart failure therapy prevents deterioration of left ventricular dimensions and systolic function in PPCM patients with a subsequent pregnancy

Subsequent pregnancy in PPCM is associated with a risk of reoccurrence or deterioration of heart failure or even death (13). The exact mechanism of recurrent depression of cardiac function associated with subsequent pregnancy is not clear but might be related to reactivation of the same underlying idiopathic process responsible for the initial cardiomyopathy (196) and may exist even in patients who seem to recover their left ventricular function (195). While Reimold et al. suggest that the haemodynamic stress of pregnancy might be responsible for deterioration of left ventricular function (301), Sliwa et al. documented deterioration of LVEF uniformly postpartum (254).

Hilfiker et al derived a STAT3 cardiac tissue specific knock out mouse model that presented with symptoms much alike human PPCM. Studies conducted by her suggest that cathepsin D facilitates cleavage of the 24 kDa prolactin into 16 and 8 kDa proteins postdelivery causing in vitro inhibition of endothelial cell proliferation, increased fibroblast proliferation and increased cardiac apoptosis and reduced cardiac function and survival postpartum. Bromocriptine, a dopamine- D2 receptor antagonist that inhibits prolactin secretion, prevented PPCM in these mice, restored cardiac function and structure and prevented apoptosis (133, 136, 141). In the present study we were able to demonstrate the presence of the cleaved 16 kDa prolactin in three of five PPCM patients, but not in non-PPCM controls during the postpartum period, leading us to the conclusion that bromocriptine would also have beneficial effects in human PPCM patients.

The present prospective study provides for the first time a comparative description of the effect of bromocriptine as an add on therapy in subsequent pregnancy in human PPCM patients. We compared data from a cohort of four patients with subsequent pregnancy on standard heart failure therapy alone with another cohort of four patients with subsequent pregnancy in PPCM who was treated with bromocriptine 2.5 mg twice daily for two months in addition to standard heart failure therapy. All patients who received additional bromocriptine survived and even improved their left ventricular ejection fraction from 49.0±8.1%
at onset of subsequent pregnancy to 52.5±5.5% at three months postdelivery. This is in contrast to our findings in patients on standard heart failure therapy alone, in which one patient died and left ventricular ejection fraction deteriorated from 47.6±11.9% at baseline to 38.7±20.6%. The data in our bromocriptine group is also in contrast to findings by Elkayam et al. who described significant deterioration of LVEF from 36±9% to 32±11% (p=0.08) in women with left ventricular dysfunction prior to subsequent pregnancy (mortality 19%) and in women who had normal LVEF prior to subsequent pregnancy and experienced a deterioration of LVEF from 56±7% to 49±10% (p=0.002). It is important though to point out that mortality was 0% in 40 patients who had recovered left ventricular function prior to subsequent pregnancy (196).

One could argue that patients in our cohort who did not receive bromocriptine also had greater left ventricular dimensions (5.9±0.7 as compared to 5.0±0.6cm, p=NS) and therefore a greater chance to deteriorate their LVEF. The report by Elkayam failed to include measurements of left ventricular dimensions of the subsequent pregnancy patients, but we described the outcome of six subsequent pregnancies out of whom two patients with persistent cardiomegaly died within three months postdelivery (254). During risk assessment of patients with a subsequent pregnancy in PPCM it appears advisable to consider left ventricular dimensions as well as left ventricular ejection fraction. We documented significantly higher cathepsin D serum expression levels in PPCM patients than in healthy controls. A link between cathepsin D, autophagic degeneration and cell death has been suggested in several models of heart failure (302, 303) and might play a causal role in PPCM.

While mortality appears to be very low in patients with normal left ventricular function and dimensions at onset of subsequent pregnancy, the risk is higher when these parameters are impaired. The results obtained by the addition of bromocriptine to standard heart failure treatment in this study are encouraging. Bromocriptine may represent a novel therapeutic approach in the treatment of PPCM, but the data need to be considered as preliminary and need to be confirmed in a larger cohort of patients.
7.5 Conclusions

We described the clinical profile of 100 patients presenting with PPCM to our clinic and found a positive correlation between baseline C-reactive protein plasma levels and LV end-diastolic (rs = 0.33, P = 0.0026) and end-systolic dimensions (rs = 0.35, P = 0.0012). The mortality rate was 15% within six months and logistic regression analysis revealed baseline plasma levels of Fas/Apo-1 (OR = 3.56, CI 95% = 1.35–9.42) and NYHA FC (OR = 2.67, CI 95% = 1.04–6.83) as independent predictors of death.

While several authors have described recovery of LV systolic function in up to 54% of PPCM patients (5, 192), other patients develop irreversible heart failure. In order to identify molecular pathways that determine whether cardiac function recovers or becomes chronically dysfunctional, we studied the kinetics of biomarkers reflecting cardiac function, processes of inflammation and re-modeling as well as the hormone prolactin over a six months period. While a wide range of parameters, reflecting cardiac dysfunction and pro-inflammatory immune activation, were elevated in all PPCM patients at time of first presentation, indicating their involvement in the initiation of the disease, we found significant differences over time between cardiac function improvers and non-improvers for ΔIFN-gamma (P=0.0181), indicating its role in disease progression. Heightened IFN-gamma expression could indicate an ongoing T-cell mediated autoimmune response and an insult to the cardiac muscle, resulting in fibrosis and inability to improve left ventricular systolic function.

In a next step we analysed serum of PPCM patients, identified beta1-adrenergic autoantibodies in all PPCM patients and mapped their reactivity to the second extracellular loop (RAESDE and DEARRCY). These autoantibodies were not present in any of the non-PPCM peripartum controls and are different from those in serum of DCMO patients, suggesting that PPCM is a distinct form of cardiomyopathy.

NT-proBNP correlates well with clinical course in heart failure patients (243) and statistical analysis of the activity of the beta1-adrenergic autoantibodies found in our cohort of PPCM patients revealed a positive correlation with NT-proBNP serum expression levels (rs=0.58, 2-tailed P=0.0228), 95% CI (0.10 to 0.84).
Prolactin represents a stimulatory link between the neuroendocrine and immune systems (230). Several authors have suggested that prolactin promotes pro-inflammatory immune responses (256, 258). Interestingly, we found significantly higher (P<0.0001) serum prolactin levels in PPCM patients at time of first presentation than in peripartum controls, suggesting the hormone’s role during the initial acute phase of PPCM. Interestingly, several authors have described the induction of IFN-gamma by prolactin (270), (271). Disease progression and the ongoing autoimmune insult by beta1-adrenergic autoantibodies appear to be driven by IFN-gamma. This pro-inflammatory cytokine remained high in PPCM non-improvers, decreased in improvers, has previously been implicated in the development of autoimmune disease (202) and its suppression leads to desensitization of β-adrenoreceptors (300).

Hyperactivation of inflammatory cytokines exacerbate ventricular contractile dysfunction in heart failure patients (226).

We measured significantly higher cathepsin D serum expression levels in PPCM patients than controls at time of first presentation. Circulating cathepsin D is a ubiquitous lysosomal enzyme with high renin sequence homology. Cathepsin D release from damaged myocardial tissue could contribute to angiotensin formation by acting as an enzymatic alternate to renin (304). Furthermore, cathepsin D has been shown to efficiently cleave PRL into its 16kDa form as found in our PPCM patients (305, 306).

In view of the above it appeared reasonable to interrupt the expression of the pituitary gland hormone prolactin during the time when onset or exacerbation of disease would normally be expected. Therefore we administered bromocriptine in addition to standard heart failure therapy in patients with known PPCM who presented with a subsequent pregnancy. Instead of the expected deterioration, left ventricular dimensions and systolic function remained stable or even improved. Although the number of patients in this interventional analysis is too small to draw statistically meaningful conclusions, the results are very encouraging.
While the pathogenesis of PPCM appears to be multifactorial, our task as scientists remains to find out, how the monolith was erected. Specifically, it appears promising to investigate the effects of bromocriptine in addition to standard heart failure therapy in a randomised, double-blinded clinical study. Although some authors argue that prolactin regulated expression of IFN-gamma and other cytokines may explain the gender-specific differences in autoimmunity (78), others have shown that elevated serum prolactin concentrations are associated with accelerated autoimmune disease in both female and male mice (267-269). Possibly, prolactin does not only play a role in the pathogenesis of PPCM, but also in other forms of cardiomyopathy, affecting males and females alike. It would be interesting to study prolactin serum
expression levels in male and female patients with idiopathic DCMO. Clearly, further studies into the unfolding pathogenesis of PPCM are indicated.
STUDY LIMITATIONS

The results of this thesis should be assessed in a larger study population.
REFERENCES


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245. Albert MA, Glynn RJ, Buring J, Ridker PM. C-reactive protein levels among women of various ethnic groups living in the United States (from the Women's Health Study). Am J Cardiol. 2004 May 15;93(10):1238-42.


APPENDIX

Informed Consent and Human Research Ethics Committee Clearance Documents
UNIVERSITY OF THE WITWATERSRAND
HUMAN RESEARCH ETHICS COMMITTEE
INFORMED CONSENT

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT

Each patient must receive, read and understand this document before any study-related procedure!

STUDY NUMBER: 020907

STUDY TITLE: Peripartum Cardiomyopathy – an autoimmune disease?

SPONSOR: Department of Cardiology, Chris Hani Baragwanath Hospital

INVESTIGATOR: Prof. Karen Sliwa-Hähnle, Dr. Olaf Förster

INSTITUTION: Department of Cardiology, Chris Hani Baragwanath Hospital

Time and date of first informed consent discussion:

Date (dd/mm/yyyy):

Time:
INTRODUCTION:
Good day, my name is Dr. Olaf Förster / Prof. Karen Sliwa-Hähnle. I am a Medical Doctor at Chris Hani Baragwanath Hospital, Department of Cardiology. I would like you to consider participating in a research study, entitled "Peripartum Cardiomyopathy – an autoimmune disease?"

- Before agreeing to participate, it is important that you read and understand the following explanation of the purpose of the study, the study procedures, benefits, risks, discomforts, and precautions as well as the alternative procedures that are available to you, and your right to withdraw from the study at any time. This information leaflet is to help you to decide if you would like to participate. You should fully understand what is involved before you agree to take part in this study.
- If you have any questions, do not hesitate to ask me.
- You should not agree to take part unless you are satisfied about all the procedures involved.
- You may not participate in another medical research study, nor take any other investigational medicine during your participation in this study. You should not have participated in an investigational medicine research study within the past 30 days.
- Please be completely truthful with me regarding your health history, since you may otherwise harm yourself by participating in this study.
- If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy to keep.
- If you have a personal doctor. please discuss with or inform him/her of your possible participation in this study. If you wish, I can also notify your personal doctor in this regard.

PURPOSE OF THE STUDY:
- You have been diagnosed as suffering from "Peripartum Cardiomyopathy" and I would like you to consider taking part in the research of a new medicine called "Pentoxifylline".
- The purpose of this study is to determine the improvement of your cardiac function

LENGTH OF THE STUDY AND NUMBER OF PARTICIPANTS:
- The study will be performed at Chris Hani Baragwanath Hospital
- Approximately 100 patients will participate in this study
- The patients will be between the ages of 18 and 50 years
You will be required to come for follow up to Cardiac Clinic at Chris Hani Baragwanath Hospital once a month for a duration of 6 months.
- Once you have completed 6 months of treatment you can decide to continue with this study for another 6 months to complete a total of 12 months. You will experience no disadvantage if you decide not to continue with this study after 6 months. In that case you will be treated as a regular patient in the Cardiac Clinic at Chris Hani Baragwanath Hospital.

PROCEDURES:
If you agree to take part in this study, you will first be asked questions and examined to see if you qualify for this study. Before receiving your first dose of study medicine, I will examine you and draw several blood samples from you.
At each following visit you will undergo:

- Enrolment visit: Examination plus ECG plus bloods (15 tubes for FBC, U+E, CRP, LFT, TLR3, immunoglobulins, 4 x serum, 3 x plasma for cytokines, selenium and autoantibodies, 4 x for PBMC, HIV, CD4) plus Echocardiography. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.
- Follow up visit 2 (after 1 month): Check up
- Follow up visit 3 (after 2 months): Check up
Follow up visit 4  (after 3 months):  Check up
Follow up visit 5  (after 4 months):  Check up
Follow up visit 6  (after 5 months):  Check up
Follow up visit 7  (after 6 months):  Check up plus bloods (15 tubes for FBC, U+E, CRP, LFT, TLR3, immunoglobulins, 4 x serum, 3 x plasma for cytokines, selenium and autoantibodies, 4 x for PBMC, HIV, CD4) plus Echocardiography. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

Follow up visit 8  (after 7 months):  Check up
Follow up visit 9  (after 8 months):  Check up
Follow up visit 10  (after 9 months):  Check up
Follow up visit 11  (after 10 months):  Check up
Follow up visit 12  (after 11 months):  Check up
Follow up visit 13  (after 12 months):  Check up plus bloods (15 tubes for FBC, U+E, CRP, LFT, TLR3, immunoglobulins, 4 x serum, 3 x plasma for cytokines, selenium and autoantibodies, 4 x for PBMC, HIV, CD4) plus Echocardiography. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

After completing 6 months you can decide if you prefer to continue participating in this study for an additional 6 months to complete 12 months of study or if you do not wish to extend your participation in the study.

The blood samples will be analysed to determine the effect of the treatment you received on your "Peripartum Cardiomyopathy" and will help to find out the cause of "Peripartum Cardiomyopathy". The echocardiography will be done to determine the effect of the treatment you received on the performance of your heart muscle.

WILL ANY OF THESE STUDY PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

- Venipunctures (i.e. drawing of blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions. A total of 75 ml of blood (i.e. 15 teaspoons) will be collected every 6 months.
- Echocardiography is a commonly used diagnostic procedure in Cardiology that allows the examiner to assess the function of your heart muscle. It does not pose any harm to the patient
- ECG (Electrocardiogram): This examination allows the doctor to determine the rhythm of your heart. It is a commonly used diagnostic procedure and does not pose any harm to the patient.
- As part of the study your HIV status will be checked. HIV pre-test and post-result counseling will be provided.
  (  ) I would like to know the HIV test result and wish to participate in appropriate counseling
  (  ) I would not like to know the HIV test result
**BENEFITS:**
The potential benefit from your participation in this study may be control of your "Peripartum Cardiomyopathy". However, you may not benefit from this study. Your participation in this study will contribute to medical knowledge that may help other patients that, that like you, have "Peripartum Cardiomyopathy"

**ALTERNATIVE TREATMENT:**
If you decide not to take part in this study you will still receive the best current care, from your usual doctor; this may or may not include the study medicine.

**BENEFITS AND RISKS OF STANDARD ALTERNATIVE TREATMENT:**
Participating in the study you will always receive standard treatment.

**ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS STUDY?**
Due to your diagnosis "Peripartum Cardiomyopathy" you should not fall pregnant again. Therefore effective contraception is strongly recommended.

**INTERACTIONS:**
It is important that you let me know of any medicines (both prescriptions and over-the-counter medicines), alcohol or other substances that you are currently taking.

**RIGHTS AS A PARTICIPANT IN THIS STUDY:**
- Voluntary: Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.
- Discontinuation of study treatment: You must inform me if you wish to stop taking your study medicine. I will supervise any discontinuation with your health as the first priority.
- New findings: I will provide you with any additional information that becomes available during the study, which may affect your willingness to continue on the study

**WITHDRAWAL FROM THIS STUDY:**
- Your withdrawal will not affect your access to other medical care.
- I retain the right to withdraw you from the study if it is considered to be in your best interest. If your participation is ended early, you may be asked to return for study-ending tests and procedures for your safety.
- If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.
- Pregnancy: Peripartum Cardiomyopathy is defined as a cardiomyopathy without any other attributable cause in the period of one month antepartum to 5 months postpartum. Pregnancy is therefore no defined reason for withdrawal from the study.

**EMERGENCY CARE AND HOSPITALISATION:**
If you seek emergency care or if hospitalisation is required at any time during the study, please inform the treating doctor that you are/were enrolled in this research study and that you are diagnosed with "Peripartum Cardiomyopathy". Please ask the treating doctor to inform me about your condition.
FINANCIAL ARRANGEMENTS:
- The Department of Cardiology, Chris Hani Baragwanath Hospital will provide payment for all study procedures and reasonable medical expenses that you may incur as a direct result of this study as determined by the Department of Cardiology, Chris Hani Baragwanath Hospital and me.
- Neither you nor your medical scheme will be expected to pay for any study medication, study related visit or study procedures.

REIMBURSEMENT FOR STUDY PARTICIPATION:
You will not be paid to participate in this study but your transport costs will be reimbursed adequately.

ABPI STATEMENT ON COMPENSATION:
All patients enrolled in this study are public hospital patients at Chris Hani Baragwanath Hospital. No specific sponsor exists for this study. It is an investigator driven study.

ETHICAL APPROVAL:
- This clinical study protocol has been submitted to the University of the Witwatersrand, Human Research Ethics Committee (HREC) and written approval has been granted by that committee.
- The study has been structured in accordance with the Declaration of Helsinki (last updated: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human subjects. A copy may be obtained from me should you wish to review it.
- This study is investigator driven and I do not have any financial or personal interests that may bias my actions.

SOURCE OF ADDITIONAL INFORMATION:
- For the duration of the study, you will be under the care of Dr. Olaf Förster. If at any time between your visits, you feel that any of your symptoms are causing you any problems, or you have any questions during the study, please do not hesitate to contact me.
  Doctors from the Department of Cardiology who are working on this study are:
  Professor Karen Sliwa-Hähnle 083-457-4823
  Dr. Olaf Förster 082-555-9859
  They can be contacted at the above 24 hour telephone numbers.
- If you want any information regarding your rights as a research participant, or complaints regarding this research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at the following number: 011-717-2229
- For research information you can contact Prof Huddle, Head of Department of Medicine, Chris Hani Baragwanath Hospital on 011-933-8940
- South African Medicines Control Council: If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:
  The Registrar
  SA Medicines Control Council
  Department of Health
  Private Bag X828
  Pretoria 0001
  Fax: 012-323-4474
  E-mail: labusa@health.gov.za
CONFIDENTIALITY:

- All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.
- This information will be reviewed by authorized representatives of the Department of Cardiology, Chris Hani Baragwanath Hospital. The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC), the South African Medicines Control Council (MCC) and/or the United States Food and Medicine Administration (FDA), as well as your personal doctor. Therefore, you hereby authorize me to release your medical records to the Department of Cardiology, Chris Hani Baragwanath Hospital, its employees or agents, domestic and foreign regulatory health authorities, the South African Medicines Control Council and the University of the Witwatersrand, Human Research Ethics Committee (HREC). These records will be utilized by them only in connection with carrying out their obligations relating to this clinical study.

Any information uncovered regarding your test results or state of health as a result of your participation in this study will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this study but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases of communicable diseases where a legal duty of notification of the Department of Health exists. In this case, you will be informed of my intent to disclose such information to the authorized state agency.

PERSONAL DOCTOR / SPECIALIST NOTIFICATION OPTION:
Please indicate below, whether you want me to notify your personal doctor or your specialist of your participation in this study:

(   ) Yes, I want you to inform my personal doctor / specialist of my participation in this study.
(   ) No, I do not want you to inform my personal doctor / specialist of my participation in this study
(   ) I do not have a personal doctor / specialist
INFORMED CONSENT:

- I hereby confirm that I have been informed by the study doctor, Dr. Olaf Förster / Prof Karen Sliwa-Hähnle, about the nature, conduct, benefits and risks of the clinical study "Peripartum Cardiomyopathy – an autoimmune disease?", protocol number 020907.
- I have also received, read and understood the above written information (Patient Information leaflet and Informed Consent) regarding this clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Patient: ____________________________________________________________
Printed Name  Signature/Mark/Thumbprint  Date and Time

I, Prof. Karen Sliwa-Hähnle / Dr. Olaf Förster, herewith confirm that the above patient has been fully informed about the nature, conduct and risks of the above study.

Study Doctor: ____________________________________________________________
Printed Name  Signature  Date and Time

Study nurse/translator/other person explaining Informed Consent (Designation):

Printed Name  Signature  Date and Time

Witness (if applicable):

Printed Name  Signature  Date and Time
INFORMED CONSENT CONCERNING EXTENSION OF MY PARTICIPATION IN THE STUDY “PERIPARTUM CARDIOMYOPATHY-AN AUTOIMMUNE DISEASE?” FROM 6 MONTHS TO 12 MONTHS

• I hereby confirm that I have been informed by the study doctor, Dr. Olaf Förster / Prof Karen Sliwa-Hähnle, about the nature, conduct, benefits and risks of the clinical study "Peripartum Cardiomyopathy – an autoimmune disease?", protocol number 020907
• I have also received, read and understood the above written information (Patient Information leaflet and Informed Consent) regarding this clinical study.
• I am aware that the results of the study, including personal details regarding my sex, age, date of birth, and diagnosis will be anonymously processed into a study report.
• In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
• I may, at any stage, without prejudice, withdraw my consent and participation in the study.
• I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

(   ) Yes, I would like to extend my participation in this study from 6 months to 12 months
(   ) No, I would not like to extend my participation in this study from 6 months to 12 months.

I am aware that I may attend the Cardiac Clinic at Baragwanath Hospital as a regular patient for further follow up independent from my decision concerning the extension of my participation in this study.

Patient: ___________________________________________________________________
Printed Name                     Signature/Mark/Thumbprint   Date and Time

I, Prof. Karen Sliwa-Hähnle / Dr. Olaf Förster, herewith confirm that the above patient has been fully informed about the nature, conduct and risks of the above study.

Study Doctor: ___________________________________________________________________
Printed Name                     Signature                     Date and Time

Study nurse/translator/other person explaining Informed Consent (Designation):
________________________________________________________________________
Printed Name                     Signature                     Date and Time

Witness (if applicable):
________________________________________________________________________
Printed Name                     Signature                     Date and Time
INFORMED CONSENT FOR PARENTS/LEGAL GUARDIANS:
(On behalf of minors under 18 years old)

Prof. Karen Sliwa-Hähnle / Dr. Olaf Förster has provided me with a copy of the Patient Information Leaflet and Consent regarding the clinical study about "Peripartum Cardiomyopathy – an autoimmune disease?" 020907 and has fully explained to me the nature, risks, benefits and purpose of the study. The study doctor has given me the opportunity to ask any questions concerning both the medicine and the study. It has been explained to me that I will be free to withdraw my child from the study at any time without any disadvantage to future care. I have understood everything that has been explained to me and I consent for my child to participate in this clinical study.

Parent / Legal Guardian:

Printed Name   Signature/Mark/Thumbprint  Date and Time

Patient Assent: *(Seven years old and above)

Printed Name   Signature/Mark/Thumbprint  Date and Time

(* Minors competent to understand must participate as fully as possible in the entire procedure)

Study Doctor:

Printed Name   Signature  Date and Time

Research nurse/translator/other person explaining Informed Consent (Designation):

Printed Name   Signature  Date and Time

Witness (if applicable):

Printed Name   Signature  Date and Time
VERBAL PATIENT INFORMED Consent:
(Applicable when patients cannot read or write or are incapable of giving consent)

• I, the undersigned, Prof. Karen Sliwa-Hählen / Dr. Olaf Förster have read and have explained fully to the patient, named __________________________ and/or her relative/friend/legal representative, __________________________ the patient information leaflet.

• The account I have given has explained both the possible risks and benefits of the study as well as the alternative treatments available for his/her illness. The patient and/or her relative/friend/legal representative understands these.

• The patient and/or her relative/friend/legal representative indicated that he/she understands that the patient will be free to withdraw from the study at any time for any reason and without jeopardizing his/her subsequent treatment.

• I have also informed the patient and/or his/her relative/friend/legal representative of the existence of relevant compensation arrangements in case of an injury attributable to the medicine(s) used in the clinical study, to which he/she agrees.

I hereby certify that, the patient and/or his/her relative/friend/legal representative acting on her behalf, have agreed to participate in this study.

Patient:

Printed Name    Signature/Mark/Thumbprint    Date and Time

Study Doctor:

Printed Name    Signature    Date and Time

Research nurse/translator/other person explaining Informed Consent (Designation):

Printed Name    Signature    Date and Time

Parent/Legal Guardian/Legal Representative/Friend:

Printed Name    Signature/Mark/Thumbprint    Date and Time

Witness (if applicable):

Printed Name    Signature    Date and Time
INFORMED CONSENT ON DNA SAMPLING:

As part of the study 2 tubes of blood (10 ml approx. 2 teaspoons) will be drawn for genetic testing / RNA sampling. These samples will be sent to Emory University in Atlanta, USA and to Medizinische Hochschule Hanover, Germany for testing. Testing may also be done at the University of the Witwatersrand / Chris Hani Baragwanath Hospital, South Africa. Any of these results will not be available to any unauthorized person.

- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
- I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Patient: ________________________________________________________________
Printed Name Signature/Mark/Thumbprint Date and Time

Study Doctor: __________________________________________________________
Printed Name Signature Date and Time

Study nurse/translator/other person explaining Informed Consent:

Printed Name Signature Date and Time

Witness (if applicable):

Printed Name Signature Date and Time
INFORMED CONSENT FOR NON-PPCM CONTROLS ON DNA SAMPLING:

As part of the study on Peripartum cardiomyopathy (PPCM) blood from people who are not affected by PPCM needs to be analysed. The purpose is to compare results from PPCM patients with results of people who are not affected by PPCM. 2 tubes of blood (10 ml approx. 2 teaspoons) will be drawn for genetic testing / RNA sampling. These samples will be sent to Emory University in Atlanta, USA and to Medizinische Hochschule Hanover, Germany for testing. Testing may also be done at the University of the Witwatersrand / Chris Hani Baragwanath Hospital, South Africa. Any of these results will not be available to any unauthorized person. Blood samples may be stored on the premises of the University of the Witwatersrand for future analysis related to PPCM.

- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, number of children and diagnosis will be anonymously processed into a study report.
- As part of the study your blood will be analysed for HIV. This result will not be available to you or any unauthorized person. In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
- I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Non-PPCM Control Volunteer:

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INFORMED CONSENT FOR NON-PPCM CONTROLS ON BLOOD TESTS FOR CYTOKINES, AUTOANTIBODIES AND OTHER BLOOD TESTS RELATED TO RESEARCH ON PERIPARTUM CARDIOMYOPATHY:

As part of the study on Peripartum cardiomyopathy (PPCM) blood from people who are not affected by PPCM needs to be analysed. The purpose is to compare results from PPCM patients with results of people who are not affected by PPCM. 8 tubes of blood (40 ml approx. 8 teaspoons) will be drawn for analysis of cytokines, autoantibodies and other tests related to research on PPCM. These samples will be sent to Emory University in Atlanta, USA and to Medizinische Hochschule Hanover, Germany for testing. Testing may also be done at the University of the Witwatersrand / Chris Hani Baragwanath Hospital, South Africa. Any of these results will not be available to any unauthorized person. Blood samples may be stored on the premises of the University of the Witwatersrand for future analysis related to PPCM.

• I am aware that the results of the study, including personal details regarding my sex, age, date of birth, number of children and diagnosis will be anonymously processed into a study report.
• As part of the study your blood will be analysed for HIV. This result will not be available to you or any unauthorized person.
• In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
• I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Non-PPCM Control Volunteer:

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Study nurse/translator/other person explaining Informed Consent:

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EXTENSION OF THE PROTOCOL "PERIPARTUM CARDIOMYOPATHY – AN AUTOIMMUNE DISEASE?" TO ASSESS THE EFFECT OF BROMOCRIPTINE IN WOMEN PRESENTING WITH A SUBSEQUENT PREGNANCY AND A PREVIOUS EPISODE OF PERIPARTUM CARDIOMYOPATHY

PURPOSE OF THE STUDY:
• You have previously been diagnosed with "Peripartum Cardiomyopathy" (PPCM). You are now presenting with a subsequent pregnancy. As you may remember we explained to you initially that this may not be good for your heart. Recent research has suggested that a drug called Bromocriptine may reduce the risk to your heart. I would like you to consider taking part in the research of a new medicine called "Bromocriptine".
• The purpose of this study is to determine the improvement of your cardiac function
• This study will compare your standard treatment for "Peripartum Cardiomyopathy" with Bromocriptine added. The results will be compared to data that was collected from patients before this new treatment was considered for this condition.

LENGTH OF THE STUDY AND NUMBER OF PARTICIPANTS:
• The study will be performed at Chris Hani Baragwanath Hospital
• Approximately 6 patients will participate in this study
• The patients will be between the ages of 18 and 50 years
• You will be required to come for follow up to Cardiac Clinic at Chris Hani Baragwanath Hospital once a month for a duration of 8 months before giving birth up to 6 months after giving birth.
• Once you have completed 6 months after giving birth, you can decide to continue with the study on "PPCM – an autoimmune disease?". You will experience no disadvantage if you decide to discontinue your participation. In that case you will be treated as a regular patient in the Cardiac Clinic at Chris Hani Baragwanath Hospital.

PROCEDURES:
If you agree to take part in this study, you will first be asked questions and examined to see if you qualify for this study. Before receiving your first dose of study medicine, I will examine you and draw several blood samples from you. If you are already in an advanced stage of pregnancy there will be less follow up visits before delivery.
At following visits you will undergo:
• Enrolment visit: Examination plus ECG plus bloods (14 tubes for FBC, U+E, LFT, 4 x serum, 3 x plasma for cytokines, autoantibodies, 4 x for PBMC, HIV, CD4) plus Echocardiography. Echocardiography will be taped on video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.
• Follow up visit 2 (after 1 month): Check up
• Follow up visit 3 (after 2 months): Check up
• Follow up visit 4 (after 3 months): Check up
• Follow up visit 5 (after 4 months): Check up
• Follow up visit 6 (after 5 months): Check up
• Follow up visit 7 (after 6 months): Check up
• Follow up visit 8 (after 6 months / before delivery): Check up plus bloods (14 tubes for FBC, U+E, LFT, 4 x serum, 3 x plasma for cytokines, autoantibodies, 4 x for PBMC, HIV, CD4) plus Echocardiography. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.
Follow up visit 9  (after delivery): **Check up plus bloods** (14 tubes for FBC, U+E, LFT, 4 x serum, 3 x plasma for cytokines, autoantibodies, 4 x for PBMC, HIV, CD4) plus **Echocardiography**. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

Follow up visit 10  (1 month post partum):  Check up

Follow up visit 11  (2 months post partum):  Check up

Follow up visit 12  (3 months post partum):  **Check up plus bloods** (14 tubes for FBC, U+E, LFT, 4 x serum, 3 x plasma for cytokines, autoantibodies, 4 x for PBMC, HIV, CD4) plus **Echocardiography**. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

Follow up visit 13  (4 months post partum):  Check up

Follow up visit 14  (5 months post partum):  Check up

Follow up visit 15  (6 months post partum):  **Check up plus bloods** (14 tubes for FBC, U+E, LFT, 4 x serum, 3 x plasma for cytokines, autoantibodies, 4 x for PBMC, HIV, CD4) plus **Echocardiography**. Echocardiography will be taped on Video and stored in the Department of Cardiology at Chris Hani Baragwanath Hospital for further reference and audit purposes.

Any analysis of serum, plasma, PBMC will be strictly in direct connection with our research on Peripartum Cardiomyopathy.

The blood samples will be analysed to determine the effect of the treatment you received on your "Peripartum Cardiomyopathy" and will help to find out the cause of "Peripartum Cardiomyopathy". The Echocardiography will be done to determine the effect of the treatment you received on the performance of your heart muscle.

WILL ANY OF THESE STUDY PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

- Venipunctures (i.e. drawing of blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions. A total of 70 ml of blood (i.e. 14 teaspoons) will be collected every 6 months.

- Echocardiography is a commonly used diagnostic procedure in Cardiology that allows the examiner to assess the function of your heart muscle. It does not pose any harm to the patient

- ECG (Electrocardiogram): This examination allows the doctor to determine the rhythm of your heart. It is a commonly used diagnostic procedure and does not pose any harm to the patient.

- As part of the study your HIV status will be checked. HIV pre-test and post-result counseling will be provided.

  ( ) I would like to know the HIV test result and wish to participate in appropriate counseling

  ( ) I would not like to know the HIV test result

RISKS OF THE STUDY MEDICINE:
Bromocriptine is a registered drug in South Africa. It has been used for many years to treat diseases other than Peripartum Cardiomyopathy, such as Parkinson's disease. If you participate in this study you will be among the first people to use Bromocriptine for prevention of Peripartum Cardiomyopathy.

UNFORSEEN RISKS:
The study medicine is investigational and there may be other risks or side effects which are unforeseen or unknown. You should immediately contact me if any side effects occur throughout your participation in this study.

**BENEFITS:**
The potential benefit from your participation in this study may be control of your "Peripartum Cardiomyopathy". However, you may not benefit from this study. Your participation in this study will contribute to medical knowledge that may help other patients that, like you, have "Peripartum Cardiomyopathy"

**ALTERNATIVE TREATMENT:**
- Alternative treatment in the form of standard treatment is used to treat "Peripartum Cardiomyopathy"
- If you decide not to take part in this study you will still receive the best current care, from your usual doctor; this may or may not include the study medicine.

**BENEFITS AND RISKS OF STANDARD ALTERNATIVE TREATMENT:**
Participating in the study you will always receive standard treatment. In addition you will receive Bromocriptine

**ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS STUDY?**
You should not participate in the study if you have one of the following:
- Hypersensitivity to Bromocriptine
- Systolic blood pressure > 170 mmHg/<100 mmHg or diastolic > 105 mmHg
- Clinical conditions other than cardiomyopathy that could increase plasma levels of inflammatory markers (sepsis, rheumatoid arthritis etc.)
- Significant liver disease (defined as enzymes > 2 times the upper limit of normal) or impaired renal function (defined as urea / creatinine more than 1.5 upper limit of normal)
- Contraindications to bromocriptine (history of psychotic disorders, parkinsonism with dementia, compromised cerebral circulation, ischaemic heart disease, liver disease, history of peptic ulcers)

Due to your diagnosis "Peripartum Cardiomyopathy" you should not fall pregnant again. Therefore effective contraception is strongly recommended.

**INTERACTIONS:**
It is important that you let me know of any medicines (both prescriptions and over-the-counter medicines), alcohol or other substances that you are currently taking.

**RIGHTS AS A PARTICIPANT IN THIS STUDY:**
- Voluntary: Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your access to other medical care.
- Discontinuation of study treatment: You must inform me if you wish to stop taking your study medicine. I will supervise any discontinuation with your health as the first priority.
- New findings: I will provide you with any additional information that becomes available during the study, which may affect your willingness to continue on the study

**WITHDRAWAL FROM THIS STUDY:**
- Your withdrawal will not affect your access to other medical care.
• I retain the right to withdraw you from the study if it is considered to be in your best interest. If your participation is ended early, you may be asked to return for study-ending tests and procedures for your safety.
• If you did not give an accurate history or did not follow the guidelines of the study and the regulations of the study facility, you may be withdrawn from the study at any time.
• Pregnancy: Peripartum Cardiomyopathy is defined as a cardiomyopathy without any other attributable cause in the period of one month antepartum to 5 months postpartum. Pregnancy is therefore no defined reason for withdrawal from the study.

EMERGENCY CARE AND HOSPITALISATION:
If you seek emergency care or if hospitalisation is required at any time during the study, please inform the treating doctor that you are/were enrolled in this research study and that you are diagnosed with "Peripartum Cardiomyopathy". Please ask the treating doctor to inform me about your condition.

FINANCIAL ARRANGEMENTS:
• The Department of Cardiology, Chris Hani Baragwanath Hospital will provide payment for all study procedures and reasonable medical expenses that you may incur as a direct result of this study as determined by the Department of Cardiology, Chris Hani Baragwanath Hospital and me.
• Neither you nor your medical scheme will be expected to pay for any study medication, study related visit or study procedures.

REIMBURSEMENT FOR STUDY PARTICIPATION:
You will not be paid to participate in this study but your transport costs will be reimbursed adequately.

ABPI STATEMENT ON COMPENSATION:
All patients enrolled in this study are public hospital patients at Chris Hani Baragwanath Hospital. No specific sponsor exists for this study. It is an investigator driven study.

ETHICAL APPROVAL:
• This clinical study protocol has been submitted to the University of the Witwatersrand, Human Research Ethics Committee (HREC) and written approval has been granted by that committee.
• The study has been structured in accordance with the Declaration of Helsinki (last updated: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human subjects. A copy may be obtained from me should you wish to review it.

SOURCE OF ADDITIONAL INFORMATION:
• For the duration of the study, you will be under the care of Dr. Olaf Förster. If at any time between your visits, you feel that any of your symptoms are causing you any problems, or you have any questions during the study, please do not hesitate to contact me.
• Doctors from the Department of Cardiology who are working on this study are:
  Professor Karen Sliwa-Hähnle 083-457-4823
  Dr. Olaf Förster 082-555-9859
  They can be contacted at the above 24 hour telephone numbers.

• If you want any information regarding your rights as a research participant, or complaints regarding this research study, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at the following number: 011-717-2229
• For research information you can contact Prof Huddle, Head of Department of Medicine, Chris Hani Baragwanath Hospital on 011-933-8940
• South African Medicines Control Council: If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Medicines Control Council (MCC) at:

The Registrar  
SA Medicines Control Council  
Department of Health  
Private Bag X828  
Pretoria 0001  
Fax: 012-323-4474  
E-mail: labusa@health.gov.za

CONFIDENTIALITY:
• All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.
• This information will be reviewed by authorized representatives of the Department of Cardiology, Chris Hani Baragwanath Hospital.

The information might also be inspected by the University of the Witwatersrand. Human Research Ethics Committee (HREC), the South African Medicines Control Council (MCC) and/or the United States Food and Medicine Administration (FDA), as well as your personal doctor. Therefore, you hereby authorize me to release your medical records to the Department of Cardiology, Chris Hani Baragwanath Hospital, its employees or agents, domestic and foreign regulatory health authorities, the South African Medicines Control Council and the University of the Witwatersrand, Human Research Ethics Committee (HREC). These records will be utilized by them only in connection with carrying out their obligations relating to this clinical study.

Any information uncovered regarding your test results or state of health as a result of your participation in this study will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this study but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases of communicable diseases where a legal duty of notification of the Department of Health exists. In this case, you will be informed of my intent to disclose such information to the authorized state agency.
PERSONAL DOCTOR / SPECIALIST NOTIFICATION OPTION:
Please indicate below, whether you want me to notify your personal doctor or your specialist of your participation in this study:
( ) Yes, I want you to inform my personal doctor / specialist of my participation in this study.
( ) No, I do not want you to inform my personal doctor / specialist of my participation in this study
( ) I do not have a personal doctor / specialist
INFORMED CONSENT ON BROMOCRIPTIN FOR PATIENTS PRESENTING WITH A SUBSEQUENT PREGNANCY AND A PREVIOUS EPISODE OF PERIPARTUM CARDIOMYOPATHY:

- I hereby confirm that I have been informed by the study doctor, Dr. Olaf Förster / Prof Karen Sliwa-Hähnle, about the nature, conduct, benefits and risks of the clinical study "Peripartum Cardiomyopathy – an autoimmune disease?", protocol number 020907. I had sufficient time and opportunity to seek advice before deciding to participate in this study. I received sufficient information on the option to terminate my pregnancy. I have decided out of my own free will not to terminate my pregnancy. All my question in this regard have been answered.
- I have also received, read and understood the above written information (Patient Information leaflet and Informed Consent) regarding this clinical study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the Department of Cardiology, Chris Hani Baragwanath Hospital or on its behalf.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

Patient: __________________________
Printed Name  Signature/Mark/Thumbprint  Date and Time

I, Prof. Karen Sliwa-Hähnle / Dr. Olaf Förster, herewith confirm that the above patient has been fully informed about the nature, conduct and risks of the above study.

Study Doctor: __________________________
Printed Name  Signature  Date and Time

Study nurse/translator/other person explaining Informed Consent (Designation):

Printed Name  Signature  Date and Time

Witness (if applicable):

Printed Name  Signature  Date and Time
02 September 2003

Dr O Förster,
Investigator
Department of Cardiology and Paediatric Cardiology
Chris Hani Baragwanath Hospital
P.O. Box Bertram
Soweto
2013
Fax: 011 938 8945

Dear Dr Förster,

PROTOCOL: PENTOXIFYLLINE - PERIPARTUM CARDIOMYOPATHY-AN AUTOIMMUNE DISEASE? A RANDOMISED TRIAL TO ASSESS THE EFFECTS OF HIGH DOSE PENTOXIFYLLINE IN PATIENTS WITH PERIPARTUM CARDIOMYOPATHY: AMENDMENT TO ETHICS REF: 890409

ETHICS REFERENCE NO: 020807

RE: ACKNOWLEDGEMENT OF THE FOLLOWING:

* Protocol Amendment dated July 2003
* Patient Information Leaflet and Informed Consent Version 01.09.2003 (Prof Karen Sliwa-Hähnle / Dr Olaf Förster)
* Informed Consent on DNA Sampling Version 01.09.2003 (Prof Karen Sliwa-Hähnle / Dr Olaf Förster)
* Prof K Sliwa-Hähnle updated CV and signed Wits Declaration
* Dr O Förster CV and signed Wits Declaration
* MCC Notification Letter - MCC Reference N2/10/8/2 (1029)
* Medical Faculty Research Endowment Fund from Iris Ellen Hodges CV
* Noted:
  Prof Karen Sliwa-Hähnle and Dr Olaf Förster will be attending the Introduction Good Clinical Practice Course in October 2003.
  Rene Wills will clarify the MCC Approval with the HPCSA.

Ethics Approval will follow shortly. Many thanks for your assistance.

The above has been noted for the Ethics Committee information and records.

KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / SPONSOR / STUDY CO-ORDINATORS - WHERE APPLICABLE

Regards,

MISS MERLEESA NAIDOO
For and on behalf of the Human Research Ethics Committee: (Medical)
FAXED AND MAIL

07 October 2004

Dr OA Förster,

Chris Hani Baragwanath Hospital
Department of Cardiology
P.O. Bertsham
Soweto
2013

Fax: 011 938 8945

Dear Dr Förster,

PROTOCOL: PENTOXIFYLLINE - PERIPARTUM CARDIOMYOPATHY-AN AUTOIMMUNE DISEASE? A RANDOMISED TRIAL TO ASSESS THE EFFECTS OF HIGH DOSE PENTOXIFYLLINE IN PATIENTS WITH PERIPARTUM CARDIOMYOPATHY: AMENDMENTS TO ETHICS REFERENCE 990409

ETHICS REFERENCE NO: 020907

RE : APPROVAL FOR PROTOCOL AMENDMENT AND PATIENT INFORMATION LEAFLET AND INFORMED CONSENT

We acknowledge receipt of your letter dated 05 October 2004 with the following documentation pertaining to the above-captioned trial.

Amendment Date: 10-Mar-04 Amendment Version: Protocol Amendment & Patient Information Leaflet and Informed Consent, Version 2

Amendment Number: No. 2 Received Date: 05-Oct-04

The following has been approved by the Wits Human Research Ethics Committee: (Medical)

* Research Protocol Amendment dated 10 March 2004, Version 2 (Title: Peripartum Cardiomyopathy - An Autoimmune Disease?)

* Patient Information Leaflet and Informed Consent, Version 2 dated 10 March 2004 - (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent for Parent/Legal Guardians (On behalf of minors under 18 year old) - Version 2 dated 10 March 2004 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Verbal Patient Informed Consent (Applicable when patients cannot read or write or are incapable of giving consent) - Version 2 dated 10 March 2004 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent of DNA Sampling - Version 2 dated 10 March 2004 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy - An Autoimmune Disease?" From 6 Months to 12 Months, Version 2 dated 10 March 2004 (Prof Karen
Sliwa-Hähnle and Dr Olaf Förster

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy - An Autoimmune Disease?" From 12 Months to 18 Months, Version 2 dated 10 March 2004 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

The following correspondence has been acknowledged:
* Letter to Dr Olaf Förster from the University of the Witwatersrand dated 6 May 2004
* Letter to Dr Olaf Förster from Emory University dated 25 January 2004
* Letter to Dr Olaf Förster from South African Heart Association dated 5 April 2004
* Letter to Dr Olaf Förster from MRC South Africa dated 19 March 2004
* Article by Dr Denise Hitcher-Kleiner
* Abstract: Autoantibodies against 61-adrenergic receptor in sera of patients with peripartum cardiomyopathy by Dr G Wallukat
* Abstract from the recent ISHR meeting in Brisbane by Prof Kris dos Remedios
* STAT3 and post partum cardiomyopathy

1. THIS APPROVAL IS SUBJECT TO THE FOLLOWING PROVISOS:
* A copy of the MCC Approval and/or MCC Notification letter must be submitted to the Ethics Regulatory Office Secretariat before the study commences.

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of:
  - Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor’s opinion are suspected to be related to the study drug. (International and Local Reports).
  - Any data received during the trial which may cast doubt on the validity of the continuation of the study.

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:
* The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:
* The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year (March and September) to the Office for Pharmaceutical Trials and a report of the final results, at the conclusion of the study.

4. TRANSPORT AND STORAGE OF BLOOD AND TISSUE SAMPLES IN SOUTH AFRICA:
* If blood specimens are to be stored for future analysis and is planned that such analysis will be done outside Wits then the blood must be stored at Wits with release of sub-samples only once projects have been approved by the local Research Ethics Committee applicable to where the research will be done as well as by the Wits Human Research Ethics Committee (Medical).

5. REIMBURSEMENT TO PATIENTS FOR TRANSPORT:
* The Human Research Ethics Committee: Medical does not agree with the views as stipulated by the Medicines Control Council of SA and that reimbursement will be appropriate according to the situation and to the discretion of the Principal Investigator.

6. GENETIC TESTING

* Please note that in the future the Human Research Ethics Committee: Medical will unlikely approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. WE AWAIT YOUR RESPONSES AS REQUESTED:

* MCC Approval and/or Notification before the above study may commence.

* Kindly forward the above to the undersigned at fax: 011 274 9281 at your earliest convenience.

The above has been noted for the Ethics Committee information and records.

**KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / STUDY CO-ORDINATORS**

Regards,

PROF PETER CLEATON-JONES
For and on behalf of the Human Research Ethics Committee: Medical
FAXED AND MAIL

04 February 2005

Dr OA Förster,

Chris Hani Baragwanath Hospital
Department of Cardiology
P.O. Bertsham
Soweto
2013

Fax: 011 938 8945

Dear Dr Förster,

PROTOCOL: PENTOXIFYLLINE - PERIPARTUM CARDIOMYOPATHY-AN AUTOIMMUNE DISEASE?
A RANDOMISED TRIAL TO ASSESS THE EFFECTS OF HIGH DOSE PENTOXIFYLLINE IN PATIENTS
WITH PERIPARTUM CARDIOMYOPATHY: AMENDMENTS TO ETHICS REFERENCE 990409

ETHICS REFERENCE NO: 020907

RE : APPROVAL FOR PROTOCOL AMENDMENT AND PATIENT INFORMATION LEAFLET AND
INFORMED CONSENT

We acknowledge receipt of your letter dated 03 February 2005 with the following documentation pertaining
to the above-captioned trial.

Amendment Date: 25-Jan-05
Amendment Version: Research Protocol
Informed Consent
Forms Version 3

Amendment Number: No. 3
Received Date: 03-Feb-05

The following has been approved by the Wits Human Research Ethics Committee: (Medical)

* Research Protocol Amendment dated 25 January 2005, Version 3 (Title: Peripartum Cardiomyopathy -
An Autoimmune Disease?)

* Patient Information Leaflet and Informed Consent, Version 3 dated 25 January 2005 - (Prof Karen Sliwa-
Hähnle and Dr Olaf Förster)

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy -
An Autoimmune Disease?" From 6 Months to 12 Months, Version 3 dated 25 January 2005  (Prof Karen
Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy -
An Autoimmune Disease?" From 12 Months to 18 Months, Version 3 dated 25 January 2005  (Prof Karen
Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy -
An Autoimmune Disease?" From 18 Months to 24 Months, Version 3 dated 25 January 2005  (Prof Karen
Sliwa-Hähnle and Dr Olaf Förster)
* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy - An Autoimmune Disease?" From 24 Months to 30 Months, Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent Concerning Extension of My Participation in the Study "Peripartum Cardiomyopathy - An Autoimmune Disease?" From 30 Months to 36 Months, Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent for Parents/Legal Guardians (On behalf of minors under 18 year old) - Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Verbal Patient Informed Consent (Applicable when patients cannot read or write or are incapable of giving consent) - Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent of DNA Sampling - Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent for Non-PPCM Controls on DNA Sampling, Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)


* Extension of the Protocol "Peripartum Cardiomyopathy - An Autoimmune Disease?" to assess the effect of Bromocriptine in women presenting with a subsequent pregnancy and a previous episode of Peripartum Cardiomyopathy, Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

* Informed Consent on Bromocriptin for Patients presenting with a subsequent Pregnancy and a previous episode of Peripartum Cardiomyopathy, Version 3 dated 25 January 2005 (Prof Karen Sliwa-Hähnle and Dr Olaf Förster)

The above has been noted for the Ethics Committee information and records.

KINDLY FORWARD TO THE RELEVANT INVESTIGATORS / CRA / STUDY CO-ORDINATORS

Regards,

PROF PETER CLEATON-JONES

For and on behalf of the Human Research Ethics Committee: (Medical)