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# **Cytokine pattern in pericardial tuberculosis –**

## **Correlations with disease severity**

Inauguraldissertation

zur

Erlangung der Doktorwürde

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## Abbreviations:

AAFB	Acid and Alcohol Fast Bacilli
ADA	Adenosine Deaminase
AIDS	Acquired Immunodeficiency Syndrome
BCG	Bacillus Calmette-Guérin
IL	Interleukin
CFF	Cell Free Pericardial Fluid
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
HIV	Human Immune Deficiency Virus
IFN- $\gamma$	Interferon- $\gamma$
IGRA	Interferon-Gamma Release Assay
LDH	Lactate Dehydrogenase
MTB	<i>Mycobacterium Tuberculosis</i>
NF- $\kappa$ B	Nuclear Factor Kappa B
NK cell	Natural Killer Cell
NSAID	Non-Steroidal Anti-Inflammatory Drug
PBMC	Peripheral Blood Mononuclear Cells
PPD	Purified Protein Derivate
RCF	Relative Centrifugal Force
RD 1	<i>M. tuberculosis</i> Region of Difference 1
TB	Tuberculosis

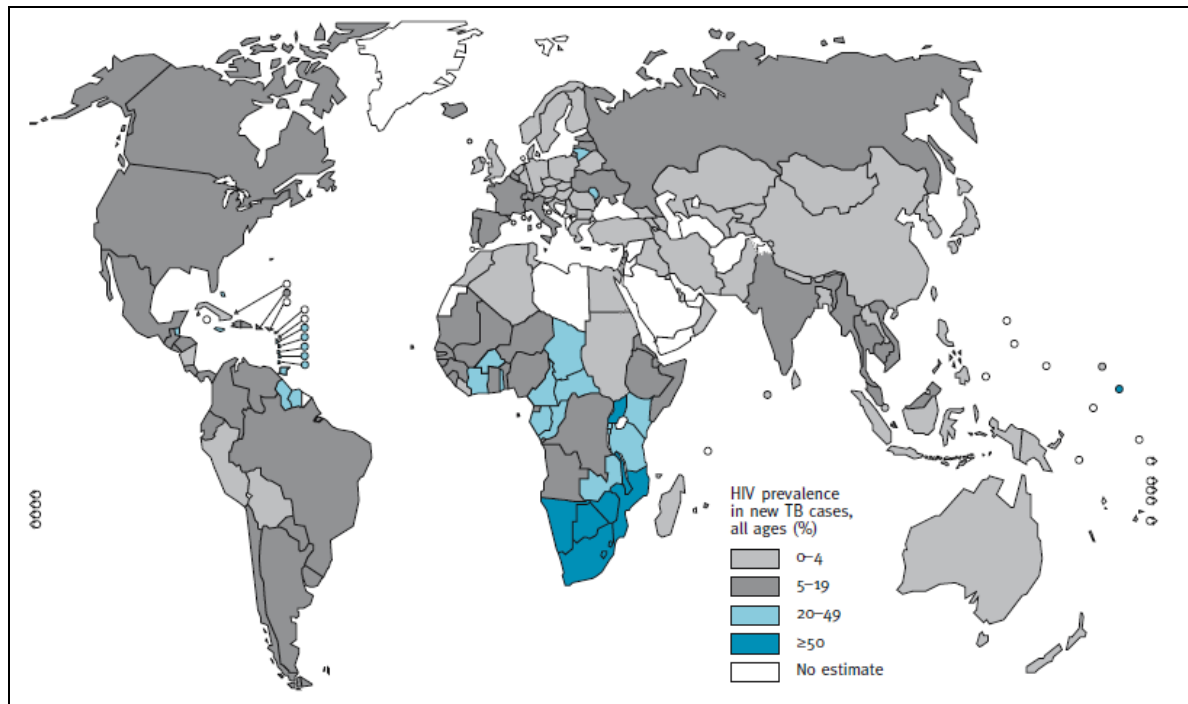
TGF- $\beta$	Transforming Growth Factor Beta
Th1	T Helper Cells Type 1
Th2	T Helper Cells Type 2
TNF	Tumor Necrosis Factor
TST	Tuberculin Skin Test
WHO	World Health Organization

# 1 Introduction

## 1.1 Global burden of HIV-infection and tuberculosis

Tuberculosis and HIV-infection are severe health problems and major causes of illness and death worldwide. The World Health Organization (WHO) estimates that over a third of the world's population is presently infected with *Mycobacterium tuberculosis*, the bacterium causing tuberculosis. According to the WHO 9.4 million new cases of tuberculosis were reported globally in 2009, of which 1.1 million were HIV-infected [1]. Approximately 80% of these HIV-infected tuberculosis cases were located in the African region. An estimated number of 1.7 million people died of tuberculosis in 2009. Four hundred thousand of these people were co-infected with HIV [1]. The African region presents the highest tuberculosis incidence rate per capita (345 per 100 000 population), which indicates that this part of the developing world is one of the most severely affected by tuberculosis.

The WHO reports an estimated number of 33.4 million people living with HIV worldwide in 2008. Two million and seven hundred thousand cases had been new infections and two million people died of the acquired immunodeficiency syndrome (AIDS) in 2008 [2]. Infections with the HI-virus are responsible for more than 5 700 deaths worldwide each day. Approximately 22.4 million HIV-infected people, 67% of the global total, are living in sub-Saharan Africa. Although the number of newly infected cases has slowly declined in the last thirteen years HIV remains a pressing health problem especially in sub-Saharan Africa [2]. The Republic of South Africa for instance is home to the world's largest population of people living with the HI-Virus (5.7 million) [2]. It is estimated that about half of all tuberculosis patients in South Africa are co-infected with HIV (Figure 1).



**Figure 1: Estimated HIV prevalence in new tuberculosis cases, 2009**

## 1.2 Pathogenesis of tuberculosis

*M. tuberculosis* (MTB) is a facultative anaerobe, slow-growing, alcoholic and acid-fast bacillus (AAFB; Ziehl-Neelsen or Kinyoun staining) which lacks an outer cell membrane. It was first described by Robert Koch in 1882 [3] who received the Nobel Prize in Physiology or Medicine for this discovery in 1905.

*M. tuberculosis* is usually transmitted by aerosol resulting in inhalation of the bacilli into the lung which is the major site of disease. Tuberculosis transmission happens through coughing and sneezing of patients suffering from active tuberculosis. Little respiratory droplets containing *M. tuberculosis* are inhaled by the next individual and reach the alveoli if the droplets are small enough. The inhaled bacilli are usually engulfed by alveolar macrophages which transport the pathogens to draining lymph nodes. The initial lesion and its inflamed lymph nodes form the so called primary complex. Cross talk between macrophages and T cells lead to the formation of well organised granulomas which contain the bacteria [4]. Usually, once macrophages engulf mycobacteria their phagosomes (the organelles formed around bacteria) merge together with lysosomes in order to mature into hydrolytic phagolysosomes. In this environment bacterial proteins can

be degraded and its peptides are then available for antigen presentation to lymphocytes. However *M. tuberculosis* is able to persist in phagosomes by arresting their maturation and inhibiting fusion with lysosomes [5]. Through this way *M. tuberculosis* is able to survive within macrophages and thus avoids the host's immune response [4, 6]. Further it prevents the induction of apoptosis of the macrophage and therefore does not get eliminated [6, 7]. Yet, not all tubercle bacilli survive, some bacteria are killed by reactive oxygen and nitrogen intermediates produced by activated macrophages [4]. Also, antimicrobial peptides such as defensins are known to play a role in the elimination of mycobacteria [8]. The immune response to *M. tuberculosis* is a mainly cellular response. Different T cell populations produce interferon- $\gamma$  (IFN- $\gamma$ ) a major cytokine of the T helper cell 1 (Th 1) response. IFN- $\gamma$  is essential in the activation of macrophages and further synergises with tumor necrosis factor (TNF) in activating macrophages [9].

Protective immunity to *M. tuberculosis* is characterised by the formation of granulomas at the site of disease [10]. These consist of *M. tuberculosis* activated macrophages and T lymphocytes. By forming a granuloma a local environment for communication between different cells of the immune system is established, moreover it prevents the dissemination of tubercle bacilli and usually keeps tuberculosis under control [10]. Within the granuloma CD4+ lymphocytes produce IFN- $\gamma$  which enables macrophages to eliminate the bacilli they are infected with. In addition to that cytotoxic T lymphocytes (CD8+) are capable of killing infected cells directly. Six to eight weeks after infection granulomas undergo caseous necrosis resulting in death of the majority of tubercle bacilli and in destruction of the surrounding host tissue [11]. This occasion coincidences with the development of the delayed type hypersensitivity response and therefore positivity of the Tuberculin Skin Test (TST) [11]. The remaining bacilli which survived caseous necrosis are believed to exist in a non-replicating hypometabolic state having adapted to the unfavourable milieu in the caseous material [11, 12].

### 1.3 Tuberculosis: Clinical features, diagnosis, treatment

Although one third of the world's population is assumed to be infected with *M. tuberculosis* only five to ten percent of this population actually develop active tuberculosis [13]. Those individuals with a persistent immune response without displaying active tuberculosis are thought to be latently infected [14]. If the infection develops into active tuberculosis clinical symptoms as cough, fever, night sweats, weight loss and loss of appetite may emerge. Certain medical conditions increase the likelihood of developing active tuberculosis such as alcoholism, diabetes mellitus, chronic renal failure, gastrectomy, immunosuppressive therapy, long-term steroid therapy and acquired T cell dependent immune defects as for instance HIV [15]. The lungs are the primary site of tuberculosis, but if tubercle bacilli gain entry to the blood stream they can affect all parts of the body, especially peripheral lymph nodes, kidneys, bones and the brain. The many foci of infection appear as tiny white tubercles in the organs. This form of tuberculosis is called miliary tuberculosis and is more common in severely immunosuppressed individuals. Mortality rates in disseminated tuberculosis are up to 21% despite treatment [16].

**Diagnosing tuberculosis** can be challenging, since there are no cardinal symptoms and clinical presentation may be multi-faceted. Laboratory parameters which may occur during active disease are: Increases in the peripheral blood leukocyte count or leukopenia, anemia (quite common in disseminated infection), increased peripheral blood monocyte counts and hyponatremia [15]. All these unspecific symptoms and blood parameters are not generally helpful in defining the disease. Abnormalities on the chest x-ray as infiltrates, ipsilateral hilar adenopathy or cavitation do not always appear, especially in HIV-infected patients normal chest x-rays may be present. Nodules and fibrotic scars on the chest film, typical for old and healed tuberculosis, may still contain slowly multiplying tubercle bacilli which present a potential for future progression to active tuberculosis.

Diagnosis is based on detection of *M. tuberculosis*, which cannot always be cultured successfully. Therefore diagnostics also include sputum smears of acid and alcohol fast bacilli (AAFB) and a tuberculin skin test (TST). If a patient is not producing any sputum, sputum can be induced or other specimen can be obtained

by biopsy taking, fine needle aspiration or bronchoalveolar lavage [17]. A negative AAFB sputum smear does not exclude the diagnosis of tuberculosis since approximately 50% of all culture confirmed tuberculosis cases are negative in three consecutive AAFB smears [18]. Not even a negative culture can completely exclude the diagnosis of tuberculosis. If smear is positive polymerase chain reaction (PCR) or gene probe tests can distinguish between *M. tuberculosis* and other mycobacteria.

New tests for the **immunodiagnosis of tuberculosis** called IFN- $\gamma$  release assays (IGRA) are available which are based on the detection and quantification of IFN- $\gamma$  production by peripheral blood mononuclear cells (PBMC) in response to *ex vivo* contact with two unique antigens. These two antigens called early secreted antigenic target-6 (ESAT 6) and culture filtrate protein-10 (CFP-10) are specific for *M. tuberculosis* encoded by the region of difference 1 (RD 1) of the genome of *M. tuberculosis*. A great advantage of using these antigens is their absence from bacilli Calmette-Guérin (BCG) vaccination and most nontuberculous mycobacteria. Two IGRA test systems are currently commercially available: On the one hand the enzyme-linked immunospot (ELISpot; T-Spot.TB, OxfordImmunotec, Abingdon, UK) assay enumerating single IFN- $\gamma$  producing cells after antigen contact and on the other hand the enzyme-linked immunosorbent assay (ELISA, QuantiFERON TB-Gold In-Tube, Cellestis, Carnegie, Australia) which measures IFN- $\gamma$  concentration in the supernatant of a whole blood culture. The QuantiFERON TB-Gold test additionally uses peptide p38-55 of RV2654c which is further known as TB 7.7. Both test systems have a higher specificity than the TST [19, 20] which is also true in immunosuppressed persons [21-23]. However, despite the higher specificity of the IGRAs compared to the TST, they are not capable of distinguishing between active tuberculosis and latent infection [20]. Still, recent studies have shown promising results in applying IGRAs for testing of cells obtained from other sites of infection such as from bronchoalveolar lavage [18, 24], pericardial [25] cerebro-spinal [26] and from pleural fluid [27].

**Standard treatment** of active tuberculosis consists of the so called “first-line drugs” isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampicin alone for four months [28]. If sputum tests are then negative a patient can be considered to be cured, although there still is a relapse



rate of two to three percent [29, 30]. Latent tuberculosis requires another regiment of treatment consisting of six to nine months of isoniazid alone. Fluoroquinolones, capreomycin, kanamycin and amikacin are considered to be “second-line drugs”.

The first two months of chemotherapy are supposed to kill the vast majority of bacilli and therefore eliminate the risk of transmission and the selection of drug-resistant mutants. The following four months period should eliminate the small number of viable drug-susceptible bacilli which have been able to persist despite the preceding two months chemotherapy [11].

A comprehensive diagnostic approach for a patient with possible tuberculosis includes a detailed medical history and clinical examination as well as the results of radiological, microbiological, immunological, molecular-biological and histological methods.

#### **1.4 Effect of HIV-infection on tuberculosis**

The human immunodeficiency virus (HIV) belongs to the genus *Lentivirus* being part of the family of *Retroviridae*. Being a RNA virus HIV needs to integrate itself into the host DNA by reverse transcription. Its target cells, CD4+ T helper cells, macrophages and dendritic cells all carry the CD4 molecule. Infection with HIV leads to a decrease of CD4+ T helper cells reflected by the individual becoming more and more susceptible to opportunistic infections. Disease progression leads to the acquired immunodeficiency syndrome (AIDS) which is characterised by opportunistic infections and malignancies. The most common opportunistic infections belonging to the AIDS-defining diseases include infections caused by *Toxoplasma gondii*, *Cryptococcus neoformans*, *Candida spec.*, *Herpes-simplex-virus*, *Epstein-Barr-virus*, *Varicella-zoster-virus*, *Salmonella spec.*, *Pneumocystis jirovecii* and also by *M. tuberculosis* [31]. In South Africa tuberculosis is the most common AIDS-defining condition [32]. However, tuberculosis can occur at any stage of HIV [33, 34]. Therefore it is not surprising that tuberculosis is the leading cause of death in HIV-infected individuals [35, 36]. As diagnosing tuberculosis can be difficult even in HIV-uninfected patients, it proves to be even more challenging in HIV-infected individuals. Here tuberculosis may display atypical features such as lower lobe involvement with rather diffuse infection than forming of cavitations,

hilar or mediastinal lymphadenopathies instead of parenchymal infiltrates [37-39]. Further normal chest x-rays in immunocompromised patients may occur [40]. Applying the Tuberculin Skin Test (TST) requires a functional Th1 response and is therefore not sensitive in advanced disease. Bronchoalveolar lavage, trans-bronchial biopsy and microscopic examination of expectorated sputum can however contribute to diagnosing pulmonary tuberculosis [41, 42].

Co-infection with HIV and tuberculosis is one of the greatest health problems of the world since there is pathogenic interaction between *M. tuberculosis* and HIV which leads to a progression of disease. Active tuberculosis enhances replication of HIV. This effect is mediated by the release of pro-inflammatory cytokines in response to *M. tuberculosis* such as TNF [43, 44] which induces HIV replication via a DNA binding protein called nuclear factor kappa B (NFκ-B) [45]. HIV-infected patients suffering from tuberculosis are known to have threefold increased levels of TNF compared to an only double amount in HIV-seronegative patients with tuberculosis [46]. This results in a 5- to 160-fold increase in viral load of patients with active tuberculosis which can be reduced by successful treatment [44]. Further tuberculosis often causes a reversible CD4 lymphopenia which might worsen the CD4 lymphocyte destruction caused by HIV [47, 48]. Leroy *et al.* studied survival in HIV-infected patients who did and did not develop tuberculosis and found an increased risk of death in the tuberculosis group [49]. Similar findings are presented by Whalen *et al.* who report a higher mortality rate for HIV-seropositive tuberculosis patients compared to only HIV-infected individuals [50, 51]. Still, it has to be noted that among AIDS-defining conditions (ADC) tuberculosis seems to have a better survival rate than other illnesses of that group as *P. jirovecii* pneumonia for instance [48, 52].

Another problem which might occur in co-infection with HIV and tuberculosis is the reduced ability of the immune system of immunocompromised individuals to contain the infection in form of a granuloma [53]. This allows haematogenous spread of tubercle bacilli to other sites of the body. Therefore tuberculosis may present itself as extrapulmonary disease in HIV-infected individuals [54]. All varieties of extrapulmonary tuberculosis in HIV-seropositive patients have been described: Bone marrow infiltration, hepatic, splenic, vertebral, cerebral,

meningeal, spinal and kidney involvements. Further tuberculous pericarditis can develop when the pericardium is affected by *M. tuberculosis*.

The rising HIV prevalence has resulted in an increasing incidence of tuberculosis as well. Because of that the World Health Organization (WHO) has proposed a six months Isoniazid treatment as a preventive therapy against tuberculosis in people living with HIV which resulted in a lower incidence of tuberculosis in various studies quoted by the WHO [55].

### **1.5 Pericarditis: Aetiology, diagnosis, treatment and complications**

Pericarditis is an inflammation of the pericardium, a two layered sac surrounding the heart muscle (myocardium). These two layers are usually separated by 15 to 20 ml of serous fluid (pericardial fluid), which lubricates cardiac motion. There are many causes of pericarditis such as viral, fungal or bacterial infections, trauma, surgery and myocardial infarction. Further medications, neoplasms, radiation, connective tissue diseases and uremia can also result in pericarditis, and in some cases the cause is idiopathic [56]. Common clinical features of pericarditis are chest pain and fever, dyspnoea, cough, chills and weakness may be present depending on the etiology of pericarditis [57]. The chest pain is often aggravated with inspiration or by coughing, the patient may feel relief while sitting upright and leaning forward. Although a pericardial friction rub on auscultation is highly specific for pericarditis it may not always be present [57]. Changes on electrocardiogram (ECG) such as PR-segment depression and an upwardly concave ST-segment elevation suggest acute pericarditis [56]. The most likely differential diagnosis, acute myocardial infarction, may look similar on the electrocardiogram, but unlike myocardial infarction, there is no reciprocal change, and T waves are not inverted in pericarditis [58]. On chest x-ray the cardiac silhouette may be enlarged if the fluid volume exceeds 250 ml. Echocardiography with a two-dimensional Doppler is helpful in assessing hemodynamic changes and measuring the fluid volume. Laboratory tests may clarify or exclude other causes of pericarditis. It usually includes a complete blood cell count, an erythrocyte sedimentation rate, serum chemistries and cardiac enzyme levels. Quite common are nonspecific elevations in the complete blood cell count and erythrocyte sedimentation rate. Additionally, a

purified protein derivative (PPD) skin test, HIV and tuberculosis screening, measuring the rheumatoid factor and antinuclear antibodies may be necessary laboratory tests [56, 57]. Treatment of acute pericarditis is mostly managed conservatively by relieving the pain and inhibiting the inflammatory reaction with non-steroidal anti-inflammatory drugs (NSAID). If patients do not respond to NSAID the use of corticosteroids might be more effective. Further colchicine can be used as treatment for the first episode as well as for prevention of recurrences [59].

Monitoring for complications is strongly recommended since acute pericarditis may evolve in constrictive pericarditis, which impedes cardiac filling and can result in right heart failure. Clinical features as Kussmaul's sign, a low voltage of the QRS complex and diffuse flattening of T waves in the ECG and pericardial calcifications on the chest x-ray support that diagnosis [56, 57]. Another serious complication is the occurrence of a cardiac tamponade when pericardial effusion develops rapidly or too large a volume is accumulated in the pericardial space. This life-threatening event demands for an urgent drainage by pericardiocentesis or surgical pericardiectomy [60].

## 1.6 Tuberculous pericarditis

Tuberculous pericarditis, an inflammation of the pericardium due to *Mycobacterium tuberculosis*, is a life-threatening form of extrapulmonary tuberculosis.

In developed countries tuberculosis accounts for four percent of all cases of acute pericarditis [61] compared to 69,5% in a series done in South Africa [62]. This difference is mostly due to the spread of HIV-infection in Africa, at least 50% of the patients presenting with tuberculous pericardial effusions in the Western Cape (South Africa) are co-infected with HIV [62]. Tuberculous pericarditis has varying clinical features, ranging from a purely effusive form through effusive-constrictive and constrictive pericarditis which are associated with a significant morbidity and mortality [63]. Mortality rates range from 8 to 17% in HIV-uninfected patients [64-67]. Recently, Mayosi *et al.* described an overall mortality rate of 26% for tuberculous pericarditis patients in sub-Saharan Africa, where HIV-infected

patients seemed to have a higher mortality rate (40%) than HIV-uninfected patients (17%) [68].

The pathogenesis of tuberculous pericarditis can be variable, since there are many ways in which the pericardium may be involved in tuberculosis. The most common form is thought to arise by the breakdown of infection in mediastinal lymph nodes which spread directly into the pericardium, in rare cases the pericardium can be infected by direct spread from tuberculous pneumonia, infected visceral pleura or from the rib. Further, the pericardium can be seeded in miliary tuberculosis, but in those cases other organ manifestations might dominate the clinical presentation [69]. From the pathological point of view four stages of tuberculous pericarditis can be distinguished: (1) Fibrinous exudation with many tubercle bacilli, early granuloma formation with loose organisation of T cells and macrophages; (2) serosanguineous effusion with lymphocytes, monocytes and foam cells dominating the exudate; (3) absorption of the effusion, organisation of granulomatous caseation and pericardial thickening which is caused by fibrin and collagenosis finally leading to fibrosis and (4) constrictive scarring which can impact diastolic filling and display symptoms of constrictive pericarditis [70].

Constrictive pericarditis, recurring effusions and tamponade are common complications of tuberculous pericardial disease. In a North Indian emergency ward 60% of all tamponade cases were caused by *M. tuberculosis* [71]. Unfortunately, constriction cannot be prevented by antituberculous treatment and may still develop throughout the course of disease [72]. Here surgical intervention such as pericardiectomy may be required.

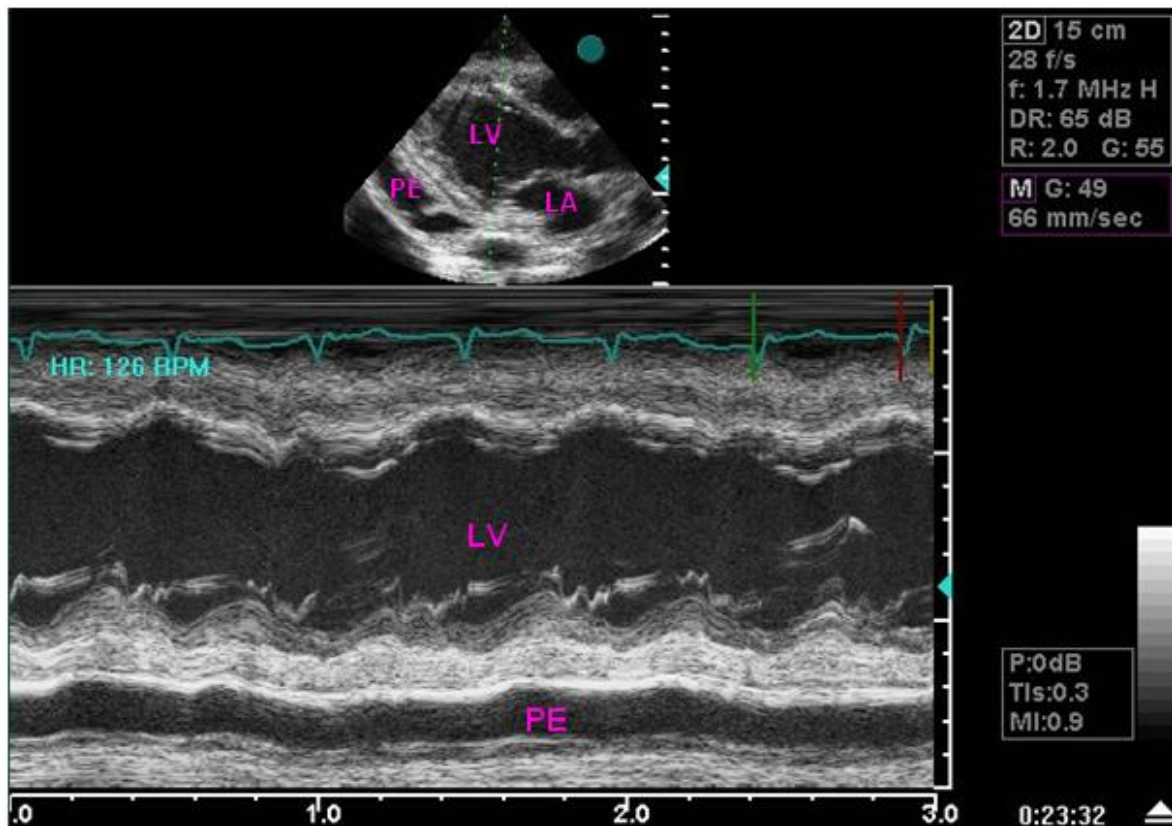


Figure 2: Echocardiographic picture in M-mode of a patient presenting with effusive-constrictive pericarditis due to tuberculous pericarditis. (LV denotes left ventricle, LA denotes left atrium, PE denotes pericardial effusion.) (The image has been kindly provided by Professor Bongani Mayosi, Head of the Department of Medicine, University of Cape Town, Rep. South Africa.)

The **diagnosis of tuberculous aetiology** in pericardial effusion involves additional tests and tools as those described in pericarditis in general. This diagnosis is further based on histopathology of the pericardial tissue or culture of *M. tuberculosis* from pericardial tissue or fluid, the presence of tuberculosis elsewhere in the body with otherwise unknown cause of pericarditis or response to specific treatment [61]. Chest radiography can demonstrate pericardial effusion or give evidence of pulmonary tuberculosis. Mediastinal node enlargements however can only be detected by magnetic resonance imaging or computer tomography and not by chest x-ray [73]. Also, pathological features of the pericardium such as thickening or calcifications can be detected on chest computed tomography. Echocardiography can contribute to diagnosis since features as pericardial

thickening, fibrin strands and exudative coating are more common in tuberculous pericarditis than in effusions of other origin [74]. Biochemical tests contribute to diagnosis, because tuberculous pericardial effusions are typically exudative in contrast to transudative effusions due to renal failure [75]. Light's criteria are very reliable in identifying an exudate when at least one of the following statements applies to the effusion: (1) Pleural fluid protein divided by serum protein  $> 0.5$ ; (2) pleural fluid lactate dehydrogenase (LDH) divided by serum LDH  $> 0.6$  and (3) pleural fluid LDH is greater than two thirds of the upper limit of normal serum LDH [76]. These criteria can also be applied on pericardial fluid. Cegielski *et al.* tested the utility of polymerase chain reaction (PCR) which is not yet as suitable as conventional methods due to less sensitivity and false-positive results [77]. Elevated levels of adenosine deaminase (ADA) with a cut-off level of 30 U/l in pericardial fluid strongly suggest a tuberculous aetiology, also very high levels of IFN- $\gamma$  in pericardial effusions with a cut off value of 200 pg/l resulted in a sensitivity and specificity of 100% for the diagnosis of tuberculosis [78]. Very high ADA levels in pericardial fluid are also believed to have a predictive value in the development of constrictive pericarditis [79]. Another indirect marker in pericardial fluid for tuberculous pericarditis is the amount of lysozyme in pericardial fluid which strongly correlates with the ADA activity [80]. A recent study has also shown a promising result in applying the ELISpot assay on pericardial fluid [25].

**Treatment** of tuberculous pericardial disease basically consists of effective drainage of the pericardial effusion if necessary and anti-tuberculous drugs according to the WHO guideline [28]. This regimen includes therapy of rifampicin, isoniazid, pyrazinamide and ethambutol for at least two months followed by isoniazid and rifampicin for four months. The benefit of adjunctive corticosteroids remains still controversial. They seem to reduce mortality and morbidity in tuberculous pericarditis, however this needs to be assessed in larger clinical trials which also differentiate between HIV-infected and -uninfected individuals [63, 81-83].

## 1.7 Key cytokines in immune response to *M. tuberculosis* and immunity at the site of disease

### 1.7.1 Pro-inflammatory cytokines

**IFN- $\gamma$** , a product of natural killer (NK) cells, macrophages and T cells, is a major cytokine involved in the control of *M. tuberculosis*. It enables macrophages to effectively killing of intracellular bacteria by activating them and inducing antimicrobial mechanisms such as hydrogen peroxide [84]. Mice with an interruption in the IFN- $\gamma$  gene were unable to control a usually sublethal dose of *M. tuberculosis* and displayed a progressive and disseminated tissue destruction and necrosis with very high numbers of tubercle bacilli [85]. Further those mice failed to produce reactive nitrogen intermediates necessary for killing intracellular bacilli and therefore could not constrain the growth of acid-fast bacilli [86]. Additionally IFN- $\gamma$  synergises with tumor necrosis factor (TNF) in activating macrophages [9]. **TNF**, mainly a macrophage product, is also essential in the immune response against tuberculosis, not only because of its synergism with IFN- $\gamma$ , it is also crucial for the development and maintenance of the granuloma. TNF knockout mice have an impaired granuloma formation and fail to prevent disease progression [87, 88]. **IL-1 $\beta$**  being produced by monocytes, macrophages and dendritic cells is a pro-inflammatory cytokine highly expressed at the site of disease in human tuberculosis [89]. It also plays an important role in the immune response to intracellular pathogens. IL-1 type I receptor deficient mice are more susceptible to pulmonary tuberculosis which is demonstrated in an impaired granuloma formation, bacterial outgrowth in the lung and increased mortality [90].

**IL-6** having both pro- and anti-inflammatory properties [91] is elevated at the site of infection in pleural tuberculosis [92]. Its role in immunity to *M. tuberculosis* is controversial. On the one hand it is known to suppress the T cell response [91] and to restrain the production of TNF and IL-1 $\beta$  [93] but on the other hand IL-6 deficient mice develop a lethal form of tuberculosis that was still controlled by IL-6 competent mice which suggests an also supportive role of IL-6 in the immune response to *M. tuberculosis* [94]. **IL-17** and **IL-22** two relatively novel cytokines have both pro-inflammatory properties. Th17 cells, a distinct lineage of effector CD4<sup>+</sup> T cells, produce IL-17 and are believed to coexpress IL-22 by some authors



[95], others reported a CD4<sup>+</sup> T cell population producing IL-22 which is distinctive from IL-17 producing T cells [96]. Further IL-17 seems to play an important role in neutrophil recruitment to the airways [97]. Th17 memory cells induced by a novel tuberculosis vaccine were able to recruit IFN- $\gamma$  producing Th1 cells to the lung by up-regulating chemokines [98]. Th1 cytokines however are believed to suppress IL-17 expression [96]. Concerning IL-22, elevated levels have been reported in bronchoalveolar lavage of patients with pulmonary tuberculosis [96]. As previously mentioned IL-22 is believed to be produced by Th17 cells by some authors [95], others hold a distinctive CD4<sup>+</sup> T cell population responsible for IL-22 production [96]. Further, NK cells produce IL-22 in response to *Mycobacterium tuberculosis* [99]. Dhiman *et al.* determined an important contribution of IL-22 to immune defense against *M. tuberculosis* by enhancing phagolysosomal fusion which results in an inhibition of intracellular mycobacterial growth [99]. The role of IL-17 and IL-22 in immunity to *M. tuberculosis* is however still incompletely understood and remains to be evaluated in future studies.

### 1.7.2 Anti-inflammatory cytokines

**IL-10** is produced by macrophages and T cells at the site of disease in pleural tuberculosis [100]. It is known to inhibit the T cell response to *M. tuberculosis* by repressing the production of major cytokines such as IFN- $\gamma$ , TNF and IL-12 [101-103]. However, IL-10 also has a tissue protective role in tuberculous pleurisy and pericarditis, since a high level of IL-10 was associated with less tissue damage [104, 105]. IL-10 production can be enhanced by transforming growth factor beta (TGF- $\beta$ ). These two cytokines seem to act synergistically in the suppression of IFN- $\gamma$  production by T cells [103]. Mycobacterial products induce the production of **TGF- $\beta$**  by monocytes and dendritic cells at the site of disease. TGF- $\beta$  is a suppressor of cell mediated immunity as it antagonizes the production of pro-inflammatory cytokines, antigen presentation and cellular activation in macrophages and also inhibits the IFN- $\gamma$  production by T cells [106]. Further TGF- $\beta$  has pro-fibrotic properties, it induces fibroblasts to produce and also remodel extracellular matrix which can result in pleural or cardiac fibrosis [107, 108].

Various studies have addressed the immunology at the site of disease, especially in pleural tuberculosis. Two major categories of T cells have been described: Th1 and Th2 cells. This classification is based on the pattern of cytokines which they

secrete: Th1 cells mainly secrete IFN- $\gamma$  and IL-2 whereas Th2 cells produce IL-4, IL-5 and IL-13 [109]. Th2 cells protect against helminthes infections while Th1 cells are crucial for protection against intracellular bacteria.

## **1.8 Cytokines at the site of disease in pericardial tuberculosis compared to other pericardial diseases**

Cytokine levels in pericardial effusion either due to tuberculosis ( $n = 19$ ), malignancy ( $n = 6$ ) or infections other than tuberculosis ( $n = 5$ ) have been evaluated by Burgess *et al.*. Cytokines of interest in that study were IFN- $\gamma$ , interleukin-1 (IL-1), IL-2, IL-6, TNF, IL-10 and IL-4. The IFN- $\gamma$  level was significantly higher in tuberculous pericardial effusions than in the other two diagnostic groups. TNF was elevated in infective and tuberculous effusions compared to the malignant ones. Concentrations of IL-1 and IL-2 were higher in infective than in tuberculous effusions, but undetectable in the malignant group. IL-6 however, being elevated in all diagnostic groups, had its highest concentrations in the malignant effusions. Further, IL-10 was elevated in all cases whereas IL-4 was undetectable in any specimen.

Because of this cytokine pattern Burgess *et al.* suggest that tuberculous pericardial effusions develop due to a hypersensitivity reaction mediated by Th1 lymphocytes [110]. Corresponding findings are reported in a second study by Reuter *et al.*, however they carried out a further division of the group with tuberculous effusions by comparing cytokine levels between HIV-infected and -uninfected individuals of that group. The measured cytokines IL-4, TNF, IL-10 and IFN- $\gamma$  did not differ between HIV-seropositive and -seronegative patients. Additionally, histopathological features such as tissue necrosis were correlated to cytokine levels. IL-10 concentrations were lower in effusions associated with necrosis whereas the sample with the highest measurement of IL-10 was free of tissue necrosis which suggests a tissue protective role for IL-10 [105].

## **1.9 Rationale for the study**

Pericardial tuberculosis is a life-threatening form of extrapulmonary tuberculosis which can result in serious complications such as constrictive pericarditis or tamponade. Its immunopathology however is poorly understood. The compartmentalisation of Th1 cytokines to the site of disease, namely pericardial fluid, has only been evaluated by Burgess and Reuter [105, 110]. This study also assessed the cytokine profile in pericardial fluid of tuberculous pericarditis patients, but further cytokines were taken into consideration such as IL-17, IL-22 and TGF- $\beta$ . Additionally, alterations of cytokine levels of HIV-infected and -uninfected individuals were investigated.

Further no research has been done analyzing the relationship between cytokine profiles and disease severity in pericardial tuberculosis. Therefore the aim of this study is to assess the cytokine pattern in pericardial tuberculosis and to investigate whether pericardial disease severity in tuberculous pericarditis has distinct immunological correlates.

Apart from better understanding the immunopathology of pericardial tuberculosis the findings of this study may ultimately lead to a better treatment and improved diagnosis of tuberculous pericarditis.

## 2 Materials and Methods

From January 2006 to October 2007 patients with a diagnosis of tuberculous pericarditis were enrolled in this study at four community hospitals in Cape Town, Republic of South Africa (Somerset Hospital, GF Jooste Hospital, Karl Bremer Hospital and Victoria Hospital) and then referred to the central study centre (Groote Schuur Hospital). Most of the patients underwent voluntary counselling and testing for HIV-1 infection.

Each patient underwent a full clinical examination, chest X-ray, electrocardiogram and echocardiography. Patients with a pericardial effusion larger than 10 mm underwent pericardiocentesis. Ten to 20 ml of pericardial fluid was sampled for routine biochemistry (including adenosine deaminase (ADA)) and microbiological examinations. Ten to 20 ml of venous blood for a serum specimen was obtained by a peripheral venipuncture.

Additionally, 26 control patients were recruited from the Department of Cardiac Surgery at Groote Schuur Hospital, Cape Town, South Africa. These patients underwent open cardiac surgery for valve replacements or bypass operations and agreed to have 10 ml of pericardial fluid and 10 ml of blood taken for this study. These patients were HIV-seronegative and did not have active tuberculosis at the time of the study or did develop tuberculosis within the following three months. The study was approved by the research ethical committee of the University of Cape Town (REC 402/2005, REC 289/2007).

### 2.1 Diagnostic criteria

Tuberculous pericarditis was diagnosed if *M. tuberculosis* could be found in stained smear or culture of pericardial fluid or when acid-fast bacilli or caseating granuloma were seen on histological examination of the pericardium. In patients with microscopical or histopathological evidence of extrapericardial tuberculosis, lymphocytic pericarditis with an elevated ADA pericardial fluid titre and a good response to anti-tuberculous chemotherapy a diagnosis of tuberculous pericarditis was also assumed.

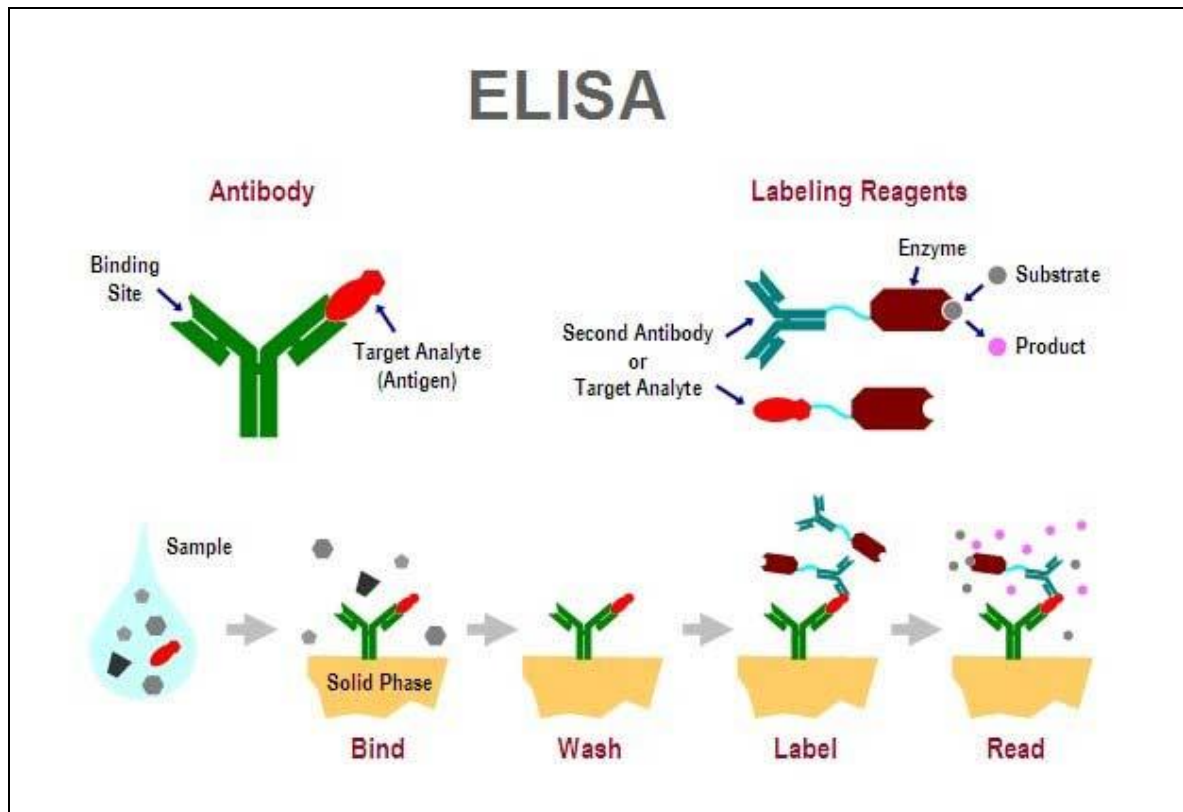
## **2.2 Separation of serum from blood samples**

Blood samples (BD Vacutainer serum vial) were centrifuged at 3000 RCF for 10 minutes. The separated serum was removed and stored with 0.1% of Sodium Azide in a cryotube vial (Nunc) at -80°C until enzyme-linked immunosorbent assays were performed.

## **2.3 Separation of pericardial fluid cells**

The pericardial fluid was first transferred into 50 ml Falcon universal tubes (Sterilin) and then centrifuged at 700 RCF for 20 minutes with the brake off. One ml of cell free fluid was removed and stored in a cryotube vial (Nunc) at -80°C for future enzyme-linked immunosorbent assays. The remaining cell free fluid in the tube was poured off leaving a pellet consisting of pericardial fluid cells (PFC) behind. These cells were washed and counted so as to set up ELISpot assays for future work. The remaining pericardial fluid cells were frozen in a 1:1 dilution of RPMI/10%FCS and cell freeze medium.

## 2.4 Enzyme-linked immunosorbent assays



**Figure 3: General procedure of an enzyme-linked immunosorbent assay:** The sample containing the protein target is added to the plate which is pre-coated with an antibody to the protein. The protein binds to that antibody, washing removes contents of the sample which are not of interest. A secondary capture antibody is added which also binds to the protein. The secondary antibody is either already attached to an enzyme or the enzyme can be added in a following step. The plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. This absorbance can be read with an ELISA reader.

Enzyme-linked immunosorbent assays were carried out on serum and cell free pericardial fluid samples for the following cytokines: IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6, IL-10, IL-17, IL-22 and TGF- $\beta$ . The procedure will be elucidated in detail on the basis of the IFN- $\gamma$  specific ELISA, which also applies for the assays of the other cytokines.

### 2.4.1 IFN- $\gamma$ specific ELISA

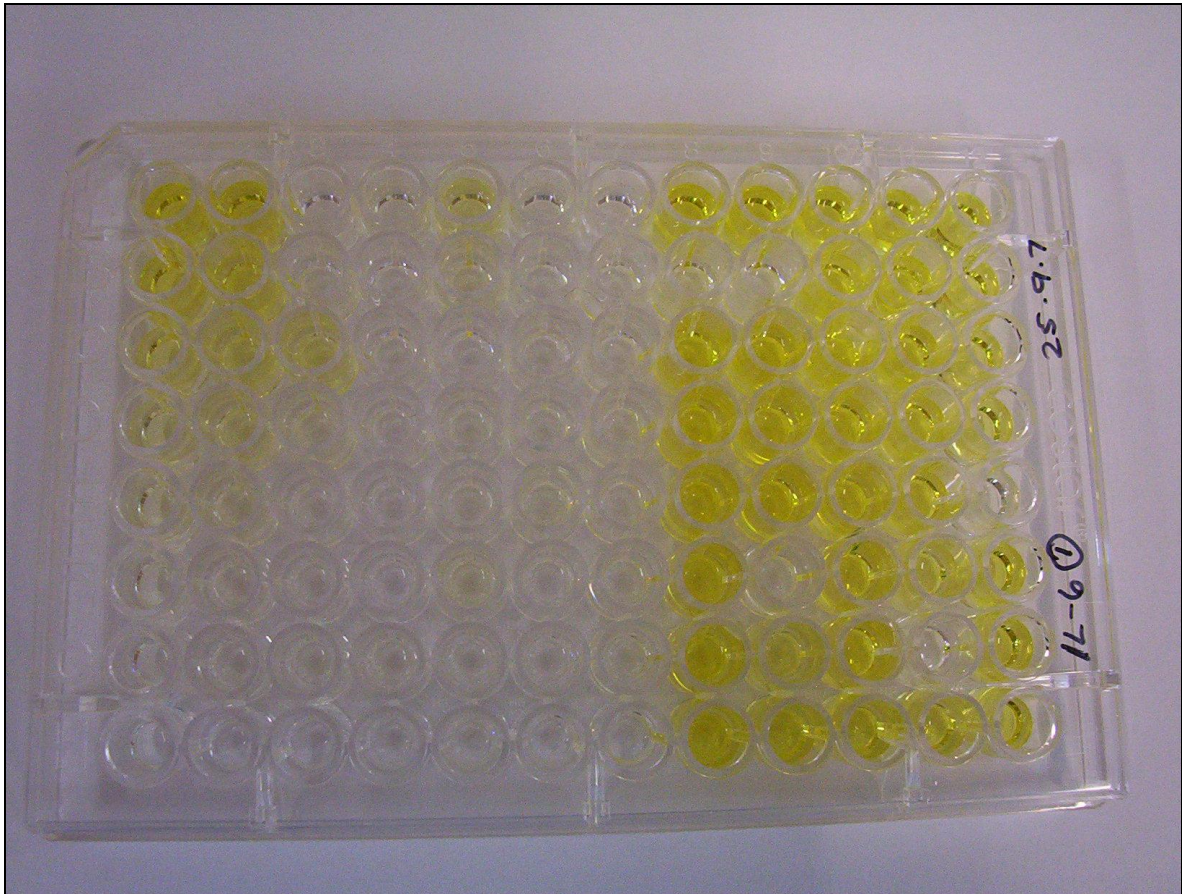
An IFN- $\gamma$  specific ELISA was carried out on the cell free pericardial fluid and the matching serum. The plate was coated with 50  $\mu$ l per well of the mouse anti-human IFN- $\gamma$  (BD catalogue number 551221) with a final concentration of 2  $\mu$ g/ml in bicarbonate coating buffer (0.1 M carbonate buffer, 8.4 g NaHCO<sub>3</sub> were added to 1 l of deionized water). The plate was sealed (sealing films Greiner catalogue number 67001) and incubated overnight at 4°C. Afterwards the plate was washed twice with PBS-Tween (wash buffer was made by adding 500 ml of 10 x PBS (Sigma-D1408) to 4.5 l of deionized water, to this solution 2.5 ml of Tween 20 (Sigma P-1379) was added). The plate was blocked with 200  $\mu$ l per well of PBS/10%FCS before it was incubated for two hours at room temperature. Again, the plate was washed twice with PBS-Tween.

Standards and samples were both diluted beforehand. The standard, a recombinant human IFN- $\gamma$  (BD catalogue number 554617) had an initial concentration of 20000 pg/ml and was then serially diluted in PBS-Tween/10%FCS to give concentrations of 6666, 2222, 740, 246, 82 and 0 pg/ml. The standards were added at 100  $\mu$ l per well in duplicate wells. For making up PBS-Tween/10%FCS 5 ml of 10% FCS were added to 45 ml of PBS, to this solution 25  $\mu$ l of Tween was added. The samples were diluted 1:3 in PBS-Tween/10%FCS (33  $\mu$ l of sample + 66  $\mu$ l of PBS-Tween/10%FCS) and added at 100  $\mu$ l per well. Subsequently, the plate was incubated overnight at 4°C.

The next morning the plate was washed four times with PBS-Tween before 100  $\mu$ l of the biotinylated mouse anti-human IFN- $\gamma$  detection antibody at 1  $\mu$ g/ml in PBS-Tween/10%FCS was added to each well. This procedure was followed by an incubation time of 45 minutes at room temperature. Afterwards, the plate was washed six times with PBS-Tween and 100  $\mu$ l of Avidin-Peroxidase (Sigma A3151) at 1 mg/ml in PBS/3%BSA diluted 1:1000 in PBS-Tween/10%FCS was added to every well. The plate was then incubated for 30 minutes at room temperature in the dark.

After that the plate was washed eight times with PBS-Tween and 100  $\mu$ l of the OPD solution was added to each well (SigmaFast OPD tablet set (P9187), both tablets were added consecutively to 20 ml of distilled water). The reaction was

stopped by adding 50  $\mu\text{l}$  of 2 M  $\text{H}_2\text{SO}_4$  to each well. Finally, the plate was read at an absorbance of 490 nm. The results were recorded and the concentrations were calculated from a standard curve, which had been constructed from the standard concentrations. Please see the supplements for the standard curves and detection limits of each ELISA.



**Figure 4: IL-6 ELISA plate after development**



### 2.4.2 ELISAs for TNF, IL-1 $\beta$ , IL-6, IL-17, IL-22, IL-10 and TGF- $\beta$

The following ELISAs were used for the other cytokines:

**Table 1: Enzyme-linked immunosorbent assays, manufacturers and catalogue numbers**

TNF specific ELISA	BD Biosciences, Human TNF ELISA Set, catalogue number 555212
IL-1 $\beta$ specific ELISA	R&D Systems, DuoSet ELISA Development System catalogue number DY201
IL-6 specific ELISA	eBioscience, catalogue number 88-7066
IL-17 specific ELISA	eBioscience, catalogue number 88-7371
IL-22 specific ELISA	Komabiotech, catalogue number K0131234
IL-10 specific ELISA	MABTECH, catalogue number 3430-1H
TGF- $\beta$ specific ELISA	R&D Systems, catalogue number DY 240

## 2.5 Statistical analysis

Statistical analysis of the data was performed using the program GraphPad Prism (GraphPad Version 4.03, January 21, 2005, USA) and SPSS 8.0 (May 1998). D'Agostino and Pearson omnibus normality test was applied to ascertain whether the data sets were normally distributed. A two-way ANOVA was applied for analysis of the data. For further analysis paired t-tests, unpaired t-tests, Wilcoxon matched pairs test and Mann-Whitney test were used. The measured cytokine concentrations were correlated with various clinical data indicative of ventricular dysfunction and severity of pericardial disease. Spearman's rank correlation was

performed. So as to avoid a significant correlation due to chance a Bonferoni correction was performed on p-values, which means, that all p-values of correlations were multiplied by the number of the comparisons made minus one ( $p\text{-value} * (n-1)$ ). A p-value of 0.05 or less was considered to be statistically significant.

### 3 Results

#### 3.1 Patients' details

##### 3.1.1 Tuberculosis patients

Twenty nine male patients (72.5%) and eleven female patients (27.5%) with tuberculous pericarditis were enrolled. The mean patient age was 37 years (standard derivation 14.5). The majority of patients was HIV-seropositive (65%) while 22.5% of patients were HIV-seronegative. A group of five patients (12.5%) could not be tested. Details of the patients are summarized in the table below. Thirteen patients had a definite diagnosis of tuberculosis, 22 patients had a probable diagnosis of tuberculosis whereas five patients were not classified into these groups, but still enrolled in the study because of clinical features likely to be due to tuberculous pericarditis.

**Table 2: Tuberculous pericarditis patients' details**

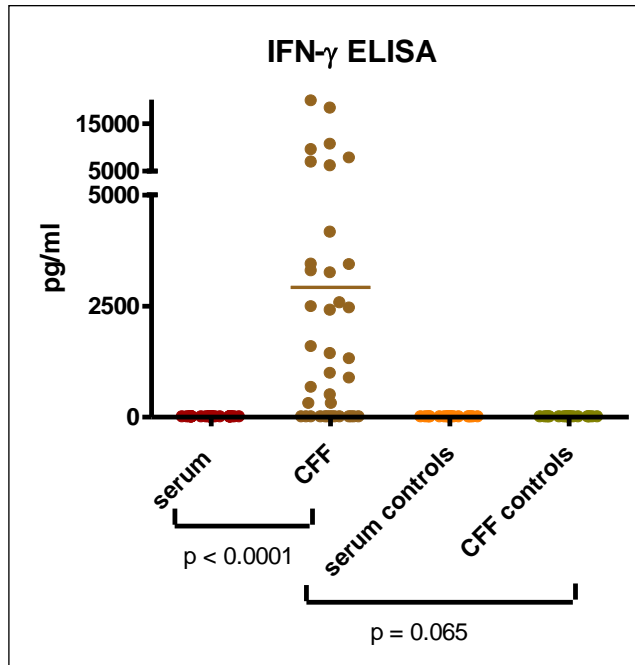
	total	HIV-infected	HIV-uninfected	unknown HIV status
total number of patients recruited	40 (100%)	26 (65%)	9 (22.5%)	5 (12.5%)
gender (male = M, female = F)	29M/11F	19M/7F	6M/3F	4M/1F
mean age in years (SD)	37.0 (14.5)	33.85 (9.2)	38.67 (17.6)	50.40 (21.3)

##### 3.1.2 Control patients

Twenty-six control patients undergoing open heart surgery for valve replacements or bypass operations were recruited. Of these 18 (69.2%) were female and 8 (30.8%) male, the mean age was 51 years.

## 3.2 Cytokine measurements by ELISA

### 3.2.1 IFN- $\gamma$

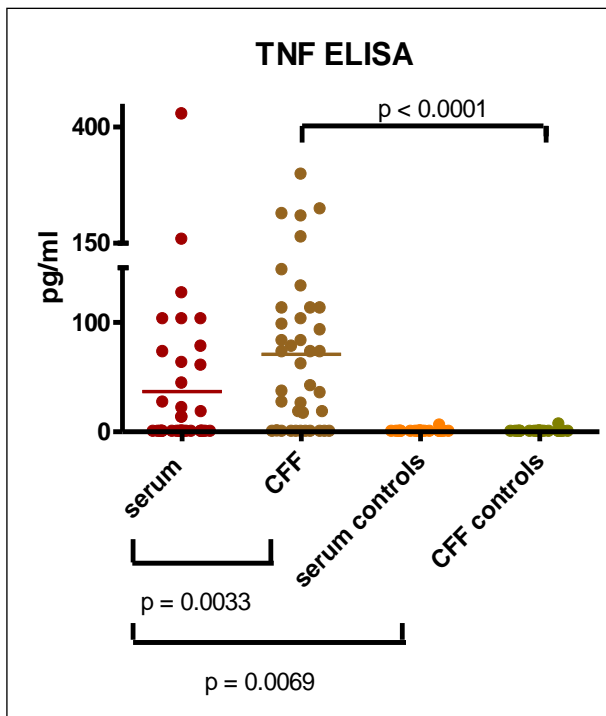


**Figure 5: IFN- $\gamma$  levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

IFN- $\gamma$  production in serum and cell free pericardial fluid was measured by ELISA. Elevated IFN- $\gamma$  levels were found in cell free pericardial fluid of the tuberculous pericarditis patients whereas IFN- $\gamma$  concentrations of all other specimen were below the minimum range of detection. The IFN- $\gamma$  level was higher in cell free pericardial fluid of tuberculous pericarditis patients compared to serum of the same patients ( $p < 0.0001$ ). Still, in cell free pericardial fluid of 14 tuberculous pericarditis patients no IFN- $\gamma$  could be detected although

two of them had a positive *M. tuberculosis* culture and three of them a positive PCR. Regarding a positive culture or PCR as the gold standard for tuberculous pericarditis the diagnostic sensitivity of an IFN- $\gamma$  level  $> 143.5$  pg/ml (lower detection limit of the ELISA) for tuberculous pericarditis would be 67% (95% CL 42 – 85%).

### 3.2.2 TNF

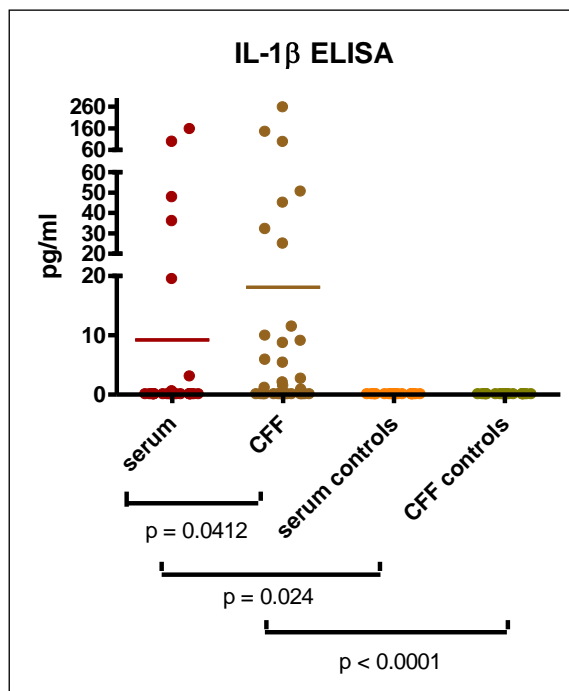


**Figure 6: TNF levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

A tumor necrosis factor specific ELISA was carried out on serum extracted from blood and on cell free pericardial fluid. Elevated levels of TNF were measured in specimen of tuberculosis patients compared to control specimen with a significant difference between serum of the two groups ( $p = 0.0069$ ) and between pericardial fluid of both groups ( $p < 0.0001$ ). TNF concentrations in pericardial fluid were higher than in serum of tuberculosis patients ( $p = 0.0033$ ). Ten of the tuberculous pericarditis patients had undetectable TNF levels in their cell free pericardial fluid (lower detection limit 10 pg/ml)

although two of them had a positive PCR. If a positive culture or PCR is regarded as the gold standard for the diagnosis of tuberculous pericarditis the diagnostic sensitivity of a TNF level  $> 10$  pg/ml would be 80% (95% CL 55 – 93%).

### 3.2.3 IL-1 $\beta$



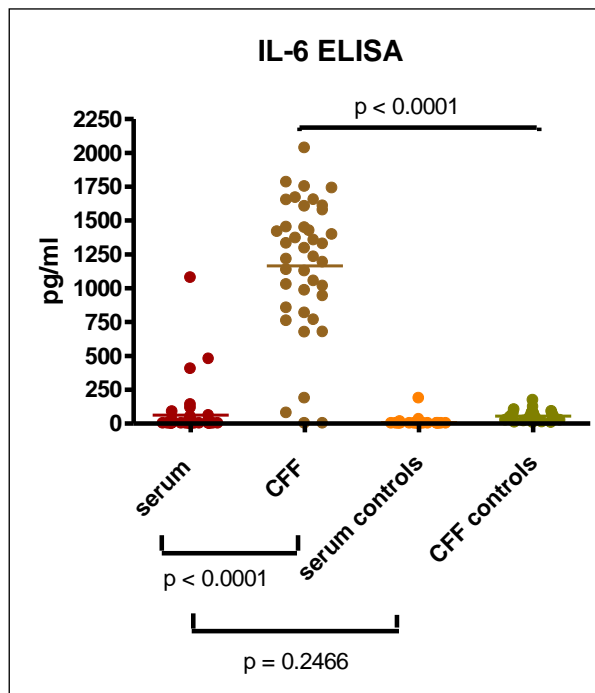
**Figure 7: IL-1 $\beta$  levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

IL-1 $\beta$  level > 2.28 pg/ml for tuberculous pericarditis would then be 67 % (95% CL 42 – 85%).

IL-1 $\beta$  concentration was higher in serum of tuberculous pericarditis patients than in serum of the control patients ( $p = 0.024$ ), the same was true for cell free pericardial fluid ( $p < 0.0001$ ), respectively. In tuberculosis patients a higher level of IL-1 $\beta$  could be detected in cell free pericardial fluid when compared to serum ( $p = 0.0412$ ). Still 21 tuberculous pericarditis patients had no detectable IL-1 $\beta$  concentrations in their cell free pericardial fluid (lower detection

limit 2.28 pg/ml), although three of them had a positive culture, one a positive PCR and one a positive culture and PCR. The diagnostic sensitivity of an IL-

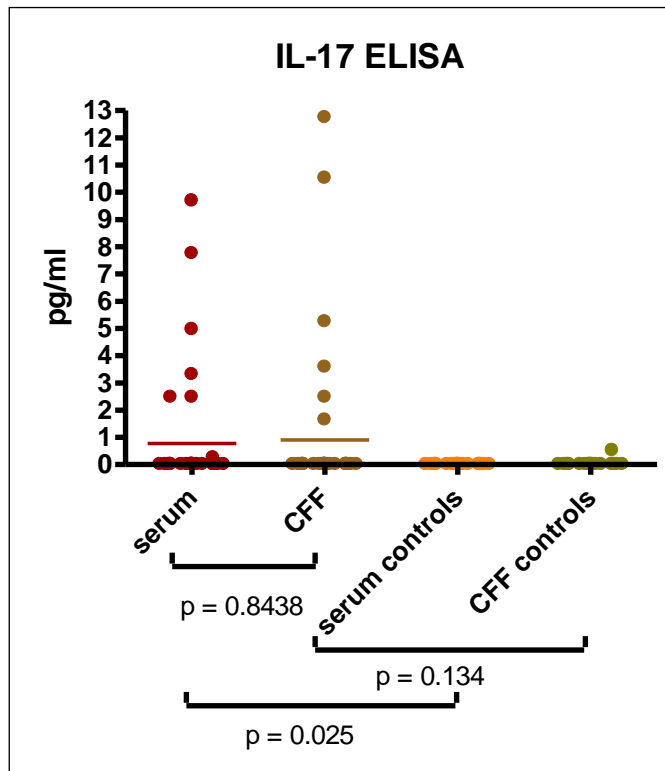
### 3.2.4 IL-6



**Figure 8: IL-6 levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

IL-6 was elevated in pericardial fluid ( $p < 0.0001$ ) but not in serum ( $p = 0.2466$ ) of tuberculous pericarditis patients compared to the controls. Comparing cell free pericardial fluid of the tuberculosis patients to serum of the same patients a higher IL-6 level was detected in the pericardial fluid ( $p < 0.0001$ ).

### 3.2.5 IL-17

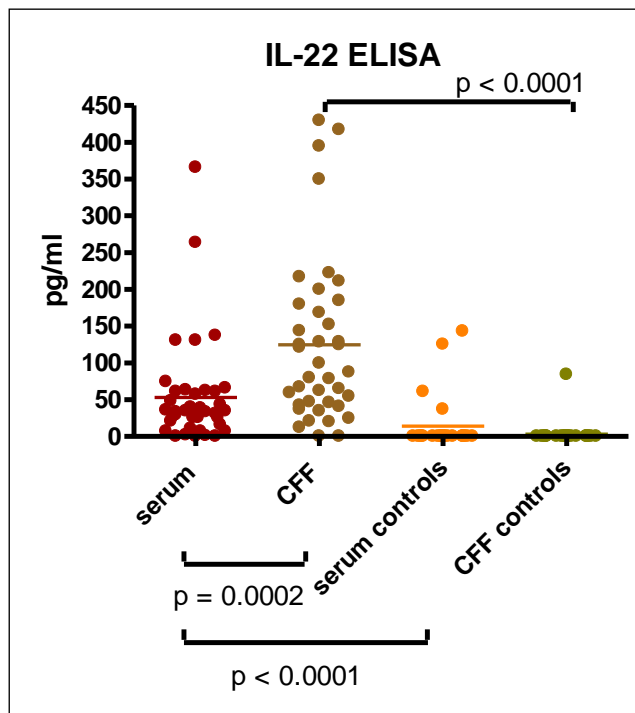


IL-17 concentrations were slightly elevated in tuberculous pericarditis patients compared to control subjects with a p-value of 0.025 for serum and 0.134 for pericardial fluid. Levels of IL-17 did not actually differ in serum and cell free pericardial fluid of tuberculous pericarditis patients ( $p = 0.8438$ ).

**Figure 9: IL-17 levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**



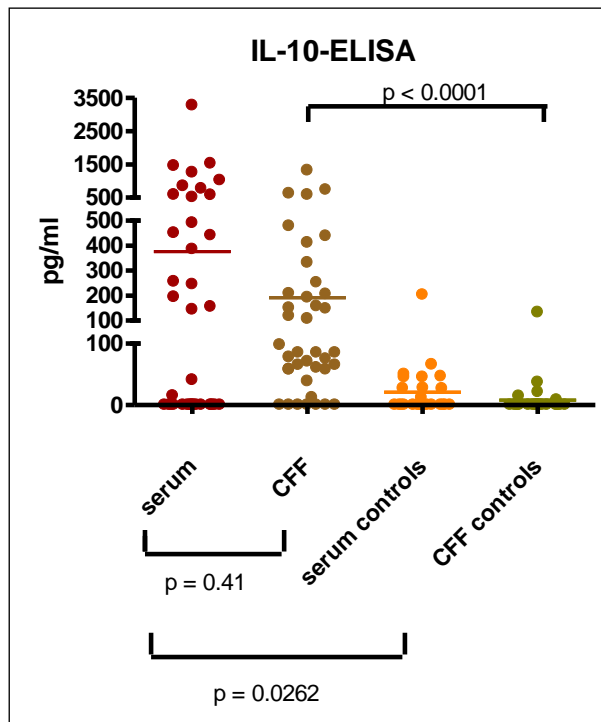
### 3.2.6 IL-22



**Figure 10: IL-22 levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

Levels of IL-22 in serum and cell free pericardial fluid of tuberculous pericarditis patients were higher compared to the control group ( $p < 0.0001$ ,  $p < 0.0001$  respectively). Also IL-22 concentration in pericardial fluid of the tuberculosis patients was higher than in their serum ( $p = 0.0002$ ).

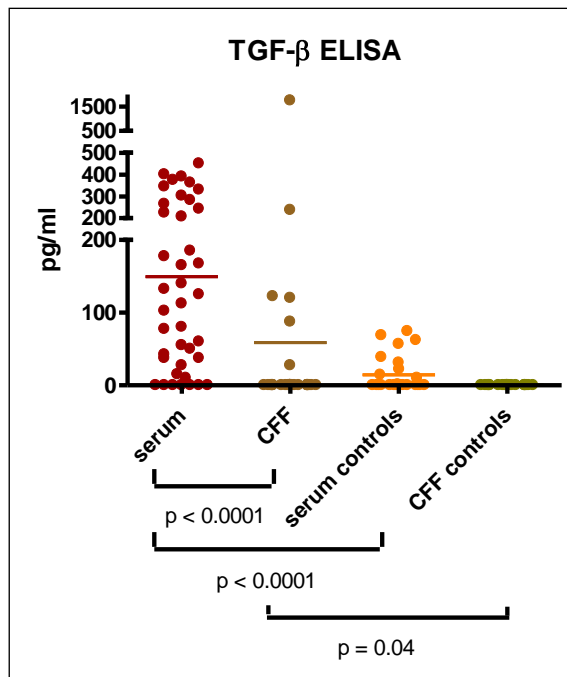
### 3.2.7 IL-10



**Figure 11: IL-10 levels measured by ELISA in serum and cell free pericardial fluid (CFF) of tuberculosis patients and controls**

Serum IL-10 level ( $p = 0.0262$ ) and cell free pericardial fluid IL-10 ( $p < 0.0001$ ) of tuberculous pericarditis patients were elevated in comparison to the control patients. In samples from patients with tuberculous pericarditis was a moderately higher production of IL-10 in serum compared to pericardial fluid, however this difference was not of statistical significance ( $p = 0.41$ ).

### 3.2.8 TGF- $\beta$



**Figure 12: TGF- $\beta$  levels measured by ELISA in serum and cell free pericardial fluid of tuberculosis patients and controls**

Higher TGF- $\beta$  levels were measured in serum and cell free pericardial fluid of tuberculous pericarditis patients compared to the controls ( $p < 0.0001$  for serum,  $p = 0.04$  for pericardial fluid). Concentration of TGF- $\beta$  was higher in serum than in pericardial fluid of the tuberculosis patients ( $p < 0.0001$ ).



**Table 5: Results of ANOVA test comparing tuberculosis patients and controls; patients and controls include cell free pericardial fluid (CFF) and serum; df denotes degrees of freedom, SS denotes sum of squares, MS denotes mean square**

cytokines	patients vs. controls	df	SS	MS	F	significance	$\eta^2$
IFN- $\gamma$	patients	1	58284700.34	58284700.3	8.19	0.01	0.07
	controls						
TNF	patients	1	89573.76	89573.76	25.81	0.00	0.17
	controls						
IL-1 $\beta$	patients	1	5816.54	5816.54	5.94	0.02	0.05
	controls						
IL-6	patients	1	10397824.0	10397824.0	117.73	0.00	0.48
	controls						
IL-17	patients	1	21.66	21.66	5.92	0.02	0.04
	controls						
IL-22	patients	1	205742.54	205742.54	35.04	0.00	0.22
	controls						
IL-10	patients	1	2217920.24	2217920.24	15.27	0.00	0.11
	controls						
TGF- $\beta$	patients	1	295411.32	295411.32	9.95	0.00	0.07
	controls						

**Table 6: Summary table of median and mean cytokine concentrations in pg/ml (interquartile range) in HIV-infected and HIV-uninfected tuberculous pericarditis patients; CFF denotes cell free pericardial fluid, HIV denotes Human Immune Deficiency Virus**

**Table 7: Results of ANOVA test comparing HIV-infected and HIV-uninfected tuberculosis patients; the groups of HIV-infected and –uninfected patients include cell free pericardial fluid (CFF) and serum; df denotes degrees of freedom, SS denotes sum of squares, MS denotes mean square**

cytokines	HIV-infected vs. -uninfected	df	SS	MS	F	significance	$\eta^2$
IFN- $\gamma$	HIV-infected	1	18453060.05	18453060.00	2.08	0.16	0.03
	HIV-uninfected						
TNF	HIV-infected	1	4517.70	4517.70	0.70	0.41	0.01
	HIV-uninfected						
IL-1 $\beta$	HIV-infected	1	7500.21	7500.21	4.03	0.05	0.06
	HIV-uninfected						
IL-6	HIV-infected	1	501414.11	501414.11	4.05	0.05	0.06
	HIV-uninfected						
IL-17	HIV-infected	1	4.31	4.31	1.17	0.28	0.02
	HIV-uninfected						
IL-22	HIV-infected	1	8363.85	8363.85	0.91	0.34	0.01
	HIV-uninfected						
IL-10	HIV-infected	1	224463.05	224463.05	0.87	0.35	0.01
	HIV-uninfected						
TGF- $\beta$	HIV-infected	1	96921.00	96921.00	1.76	0.19	0.03
	HIV-uninfected						

Patients with tuberculous pericarditis were evaluated further by their HIV-serostatus: HIV-seropositive patients, HIV-seronegative patients and patients with unknown HIV status. Cytokine levels in serum and pericardial fluid of HIV-infected and HIV-uninfected individuals were compared. Findings are summarized in Table 6 and Table 7 above.

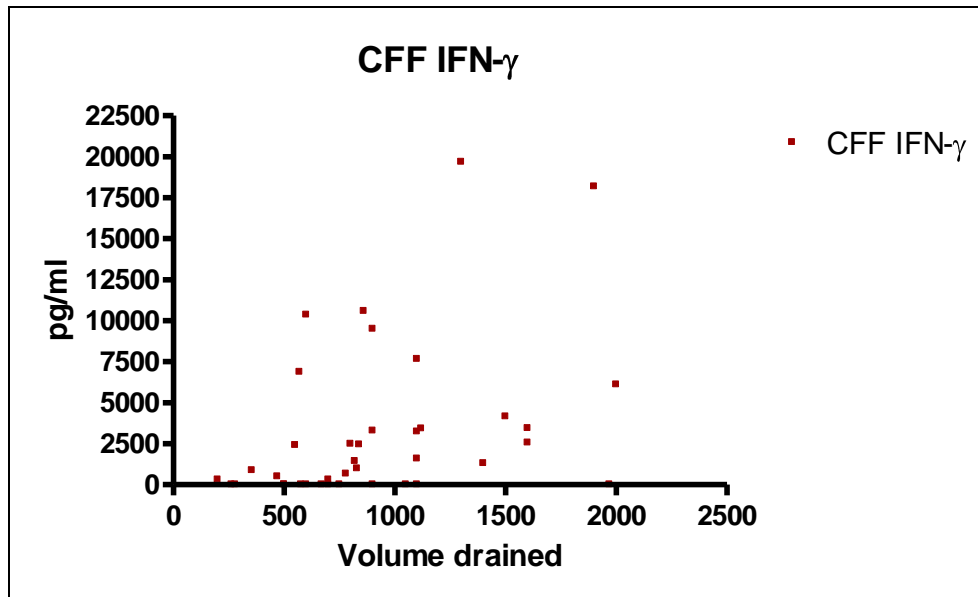
As displayed in Table 6 and Table 7 there were significant differences in levels of IL-1 $\beta$  and IL-6 among HIV-infected and HIV-uninfected patients. IL-1 $\beta$  levels were elevated in serum and cell free pericardial fluid of HIV-uninfected patients compared to HIV-infected patients. IL-6 however seemed to be more concentrated in cell free pericardial fluid of HIV-infected patients.

### **3.5 Correlation with clinical data**

After having measured cytokine concentrations in serum and pericardial fluid of tuberculous pericarditis patients these concentrations were correlated with clinical data indicating the severity of pericardial tuberculosis such as size of effusion, right atrial pressure, pulsus paradoxus and opening pressure at pericardiocentesis which indicates the degree of cardiac compression. Left ventricular ejection fraction (LVEF), left ventricular shortening fraction (LVSF) and E/A ratio have been chosen as indicators of ventricular dysfunction. Also, electrocardiogram (ECG) data indicative of a severe pericarditis such as a high heart beat rate per minute, a large R wave in the limb leads, the existence of ST-elevations and many leads with a negative T wave were taken into consideration.

Furthermore the group of patients was split into two groups with chronic heart failure according to the New York Heart Association (NYHA) stage II/III and NYHA stage IV so as to determine whether the cytokine concentrations between these groups differed. The same was done according to heart rhythm and patients were divided into a sinus rhythm and atrial fibrillation group so as to ascertain whether cytokine patterns differed between those groups.

A positive correlation was established between pericardial effusion size in millilitre and the concentration of IFN- $\gamma$  in cell free pericardial fluid (Spearman  $r$  of 0.47;  $p$  = 0.0168) which is shown in Figure 13.



**Figure 13: Correlation of IFN- $\gamma$  in pericardial fluid with the size of effusion**

There were no other significant correlations of cytokine levels and clinical data indicative of left ventricular dysfunction or pericardial disease severity found (supplements Table 10).

Additionally, patients were grouped according to their NYHA stage. Cytokine levels of patients with NYHA stage II/III and NYHA stage IV were compared. Findings are listed in Table 8. Cytokine levels of patients with NYHA stage II/III and NYHA stage IV did not differ significantly.



**Table 8: Cytokine levels according to New York Heart Association (NYHA) stage in median pg/ml (interquartile range)**

summary table NYHA-stage				
cytokines	NYHA II/III	NYHA IV	p-value	corrected p-value
<b>CFF IFN-<math>\gamma</math></b>	1428.0 (0.0 – 3423.0)	978.0 (0.0 – 3798.0)	0.77	5.36
<b>serum TNF</b>	0.0 (0.0 – 43.0)	13.0 (0.0 – 60.63)	0.26	1.82
<b>CFF TNF</b>	83.0 (27.0 – 148.0)	18.0 (0.0 – 83.0)	0.12	0.84
<b>serum IL-1<math>\beta</math></b>	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.59	4.10
<b>CFF IL-1<math>\beta</math></b>	0.0 (0.0 – 10.0)	1.1 (0.0 – 9.9)	0.50	3.51
<b>serum IL-6</b>	0.0 (0.0 – 12.0)	0.0 (0.0 – 73.0)	0.54	3.78
<b>CFF IL-6</b>	1317.0 (745.0 – 1660.0)	1223.0 (984.0 – 1435.0)	0.67	4.70
<b>serum IL-17</b>	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.98	6.86
<b>CFF IL-17</b>	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.78	5.49
<b>serum IL-22</b>	28.25 (18.25 - 58.88)	34.5 (8.88 – 63.88)	0.47	3.30
<b>CFF IL-22</b>	87.0 (44.5 - 205.1)	120.8 (40.13 – 173.9)	0.69	4.84
<b>serum IL-10</b>	0.0 (0.0 - 471.0)	245.0 (0.0 – 588.0)	0.33	2.32
<b>CFF IL-10</b>	98.0 (62.0 – 270.0)	85.0 (58.0 – 253.0)	0.89	6.21
<b>serum TGF-<math>\beta</math></b>	113.0 (43.0 – 243.0)	176.0 (33.0 – 346.0)	0.68	4.78
<b>CFF TGF-<math>\beta</math></b>	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.81	5.70

ECG-data indicating a severe pericarditis such as a high heart beat rate per minute, a large R wave in the limb leads, the existence of ST-elevations and many leads with a negative T wave were analyzed and correlations were sought between these data sets and cytokine levels in serum and pericardial fluid. However there were no significant correlations found. Findings are listed in Table 11 (supplements). Additionally cytokine levels of patients with sinus rhythm and atrial fibrillation were compared. A higher level of TGF- $\beta$  in serum of patients with atrial fibrillation compared to patients with sinus rhythm could be measured ( $p = 0.04$ ). Findings are summarized in Table 12 (supplements).

### 3.6 Cytokines in effusive and effusive-constrictive pericarditis

Patients with tuberculous pericarditis were also divided into two groups, depending whether they were presenting with effusive or effusive-constrictive pericarditis. Thirty-three patients could be definitely assigned into these groups due to their clinical presentation on echocardiography and pericardiocentesis. Cytokine concentrations in cell free pericardial fluid were compared between both groups. Findings are listed in Table 9. Cytokine levels were not significantly different in

effusive and effusive-constrictive pericarditis except for a higher level of IL-17 in CFF of patients with a pure effusive form of pericarditis ( $p = 0.01$ ).

**Table 9: Cytokine levels in cell free pericardial fluid (CFF) according to the clinical form of pericarditis, concentrations are given in median pg/ml (interquartile range)**

CFF	effusive pericarditis (n = 20)	effusive-constrictive pericarditis (n = 13)	p-value
IFN- $\gamma$	483.0 (0.0 - 2463.0)	2568.0 (400.5 – 6491.0)	$p = 0.113$
TNF	78.0 (18.0 - 148.0)	62.0 (0.32 - 108.0)	$p = 0.423$
IL-1 $\beta$	0.0 (0.0 - 9.9)	0.0 (0.0 - 19.0)	$p = 0.862$
IL-6	1310.0 (901.0 - 1592.0)	1331.0 (954.0 - 1434.0)	$p = 0.941$
IL-17	0.0 (0.0 - 0.82)	0.0 (0.0 - 0.0)	$p = 0.010$
IL-22	93.25 (50.75 - 189.5)	124.5 (41.38 - 168.3)	$p = 0.897$
IL-10	97.0 (60.0 - 207.0)	85.0 (29.0 - 336.0)	$p = 0.985$
TGF- $\beta$	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	$p = 0.579$

## 4 Discussion

The immunopathogenesis of pericardial tuberculosis is not well defined. Therefore the aim of this study was to gain a better knowledge of tuberculous pericarditis by evaluating anti- and pro-inflammatory cytokine profiles in serum and at the site of disease and by correlating these profiles with clinical data indicative of disease severity.

Tuberculosis is the most common cause of pericarditis in Africa which includes the Republic of South Africa where the prevalence of tuberculosis is 808 per 100 000 [111]. By comparison Germany has a prevalence of 5.9 [111]. The Groote Schuur Hospital being located in this high prevalence area in Cape Town, Republic of South Africa, is called on by many patients presenting with HIV-infection and/or tuberculosis. Further patients are often referred to this hospital if complicated forms of infections develop, tuberculous pericarditis being one of them, which can be treated more appropriately in the Cardiology Department. These conditions allow the Groote Schuur Hospital to be the tertiary referral centre and to work as an ideal place of enrolment of patients for this study.

Forty patients from Cape Town in the Republic of South Africa with a definite or assumed diagnosis of tuberculous pericarditis have been enrolled in this study. Serum and pericardial fluid samples were taken in order to measure concentrations of cytokines by enzyme-linked immunosorbent assay (ELISA). Further, 26 patients undergoing open-heart surgery for bypass-operations, valve reconstruction or valve replacements were recruited to serve as a control group. None of these patients had active tuberculosis nor were they HIV-seropositive.

Pro-inflammatory cytokines IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6 and IL-22 were elevated in pericardial fluid of the tuberculosis patients compared to pericardial fluid of the control group. Further, concentrations of these cytokines were higher in pericardial fluid of the tuberculosis patients compared to their serum. In contrast, anti-inflammatory cytokines IL-10 and TGF- $\beta$  were higher in serum compared to pericardial fluid of the tuberculous pericarditis patients. Measurements of these cytokines in the tuberculous pericarditis group were still significantly elevated compared to levels in the control group.

Cytokine concentrations in serum and pericardial fluid were correlated with clinical data indicating the disease severity of tuberculous pericarditis. The main finding of this study is a positive correlation between the level of IFN- $\gamma$  in cell free pericardial fluid and the size of the pericardial effusion which is shown in Figure 13.

#### **4.1 Pathogenesis of tuberculous pericarditis**

In the era before antituberculous treatment was available tuberculous pericarditis had been rapidly fatal with an early mortality rate over 80%. However since the introduction of chemotherapy much lower mortality rates have been reported such as 3 to 17% in one study [66] and 26% in another [68]. Still there is considerable urgency in establishing the correct diagnosis which should result in initiating the proper treatment. Various causes of pericarditis have to be taken into consideration while establishing the diagnosis such as infections (bacterial, viral, fungal), rheumatic diseases, neoplastic origins, immunologic causes, radiation, uremia, chest trauma or post-myocardial infarction [58]. Appropriate diagnostic tools for tuberculous pericarditis have been mentioned in chapter 1.6, for now however the focus will remain on the immunopathogenesis of tuberculosis and especially pericardial tuberculosis.

Involvement of the pericardium in tuberculosis infection most often occurs by breakdown of infection in mediastinal lymphnodes especially those at the tracheobronchial bifurcation into the pericardium [69]. Lymphocytes are the dominant cell type in tuberculous pericardial effusions [105]. An efficient protective immune response to *M. tuberculosis* is dependent on the activity of macrophages and on the production of pro-inflammatory cytokines by CD4+ and CD8+ lymphocytes [112, 113]. Various studies have assessed the cytokine profile at the site of disease in tuberculosis and agreed on the necessity of a Th1 cytokine profile for the elimination of tubercle bacilli [100, 110, 114]. However it is yet controversial to what a degree the Th2 response is involved in immunity to tuberculosis.

## 4.2 Cytokine levels at the site of disease

Evaluating the cytokine profile at the site of disease is one approach to gain a better understanding of the pathogenesis of tuberculous pericarditis. Several studies have assessed cytokine levels at the site of disease particularly in pleural tuberculosis. Shimokata *et al.* studied levels of IL-1, IL-2 and IFN- $\gamma$  in pleural fluid of tuberculous pleurisy patients and malignant pleurisy patients and found them to be highly elevated in the tuberculosis group thus suggesting a presence of local cellular immunity mediated by those lymphokines [115]. Barnes *et al.* also provide strong evidence for the compartmentalization of Th1 cytokines to the site of disease in pleural tuberculosis since they measured highly elevated levels of IFN- $\gamma$  at the site of disease and could not detect IL-4 or IL-5, both being main cytokines of the Th2 response, in pleural fluid [100]. High levels of IL-12 in pleural fluid after stimulation of pleural fluid cells with *M. tuberculosis* have been reported which also supports the presence of a predominant Th1 response in pleural tuberculosis, as IL-12 is known to induce a Th1 response [116]. Lin *et al.* evaluated cytokine mRNA expression in lymph nodes of tuberculosis patients and found high IFN- $\gamma$  levels whereas IL-4 expression was low [114]. Elevated levels of IFN- $\gamma$ , IL-2 and IL-1 $\beta$  give evidence of a compartmentalization of Th1 cytokines to the site of disease in pleural tuberculosis [100, 115, 117]. However it is still controversial in how much the Th2 response is involved in immunity to *M. tuberculosis*. There is evidence of a Th2 pattern in peripheral blood of tuberculosis patients with elevated levels of IL-4 [118, 119]. Further Orme *et al.* demonstrated a CD4 T cell population producing IFN- $\gamma$  as a first response and later after 20 to 40 days a second population secreting IL-4 in mice, which implies a role for both pathways in immunity to *M. tuberculosis* [120]. Comparable results are demonstrated by Govender *et al.* who studied cytokine secretion patterns in peripheral blood and granulation tissue of HIV-infected and -uninfected patients with spinal tuberculosis. Increases of IL-6 and IL-12 in lymphocytes and at the same time increases in IL-4, IL-6, IL-10 and IL-12 secreting monocytes also suggest a mixed immune response in tuberculosis [121]. These conflictive findings imply that the immune response in tuberculosis seems to be more complicated than actually assumed.

In this study highly elevated levels of IFN- $\gamma$ , TNF and IL-1 $\beta$  were detected in cell free pericardial fluid which also supports the hypothesis of a predominant Th1 response in tuberculosis. However no typical Th2 cytokines such as IL-4 or IL-5 have been assessed in the present study. The findings are consistent with the studies done by Burgess *et al.* and Reuter *et al.* being the only studies so far evaluating cytokine profiles in tuberculous pericardial fluid. They also determined elevated concentrations of IFN- $\gamma$ , IL-1, IL-2 and TNF in pericardial fluid of tuberculous pericarditis patients and further compared them to pericardial effusions due to other causes where those levels were significantly lower [105, 110]. These authors further found elevated levels of IL-10 in all diagnostic groups which is also reflected in the results of this study, however levels of IL-10 were here moderately higher in serum compared to pericardial fluid of tuberculous pericarditis patients (Table 3, Table 4, Figure 11). Interleukin-10 is known to suppress the T cell response by impeding the production of pro-inflammatory cytokines such as TNF, IFN- $\gamma$  and IL-12 [101-103] which could also explain their higher levels in pericardial fluid where IL-10 is less concentrated. Although the pro-inflammatory cytokines are crucial for the local cellular immune response at the site of disease one has to keep in mind that this increase of pro-inflammatory markers may at the same time contribute to tissue destruction which is mainly mediated by TNF. This tissue damage seems to be inhibited by IL-10, since highest levels of pericardial IL-10 were observed by Reuter *et al.* in pericardial biopsies with least tissue necrosis [105] which suggests a tissue protective immunoregulatory role for IL-10. TGF- $\beta$  also being an immunoregulatory factor is known to suppress the IFN- $\gamma$  production by T cells [106] and therefore downregulates the inflammatory response. In the present study TGF- $\beta$  levels were higher in serum than pericardial fluid of tuberculous pericarditis patients (Table 3, Table 4, Figure 12). But these levels were still elevated compared to those of the control group. TGF- $\beta$  is not only an immunoregulatory factor, it is also associated with pleural and cardiac fibrosis which underlines its role as a pro-fibrotic mediator [107, 108]. In the present study TGF- $\beta$  levels in cell free pericardial fluid of patients presenting with a pure effusive form of tuberculous pericarditis were evaluated and compared to the corresponding TGF- $\beta$  cytokine levels of patients with effusive-constrictive tuberculous pericarditis which is marked by fibrotic strands shown in

the echocardiography. Surprisingly, levels did not differ between those two groups ( $p = 0.579$ ) as shown in Table 9.

Levels of IL-17 and IL-22 were elevated in the tuberculous pericarditis group compared to the control group, however it has to be noted that the amounts of IL-17 were very small in general with a maximum concentration of 12.75 pg/ml in pericardial fluid of one tuberculosis patient. IL-22 concentration was higher in pericardial fluid compared to serum (Table 3, Table 4, Figure 10). Elevated levels of IL-22 have been described in specimen of tuberculosis patients before such as bronchoalveolar lavage [96].

### 4.3 Cytokines and correlation with severity of disease

Only very few studies evaluated cytokine levels and correlated them with the actual clinical presentation of the tuberculosis patient. Dlugovitzky *et al.* correlated levels of IFN- $\gamma$ , IL-2, IL-4 and IL-10 with the degree of pulmonary involvement classified by chest X-rays. High levels of IL-2 and IFN- $\gamma$  were measured in patients with mild and moderate disease while patients with advanced disease had higher concentrations of IL-4 and IL-10 [122]. However no measurement of cytokines at the site of disease was performed, only serum levels were evaluated. Similar findings were reported by Sodhi *et al.* who measured IFN- $\gamma$  productions by PBMCs [123], they detected higher levels of IFN- $\gamma$  in HIV-infected patients with only pleuropulmonary tuberculosis compared to HIV-infected patients with tuberculosis outside the lung and pleura. Still, no cells from the site of disease were obtained for this study. Findings from the present study demonstrate high concentrations of IFN- $\gamma$  in pericardial fluid and therefore the site of disease compared to serum of the same patients (Table 3, Table 4, Figure 5). Correlating these levels in pericardial fluid with clinical data indicative of the severity of pericardial disease brought up a positive correlation of IFN- $\gamma$  in pericardial fluid and the size of the pericardial effusion in millilitre (Spearman  $r = 0.47$ ,  $p = 0.0168$ ). This indicates that IFN- $\gamma$  might not only be beneficial to the patient although it is needed for an effective immune response to *M. tuberculosis*. At the site of disease high levels may contribute to pathology by an uncontrolled inflammatory reaction, which is displayed by an accumulation of fluid in the pericardial space. Mediators which

usually counteract the inflammatory reaction such as IL-10 and TGF- $\beta$  were less concentrated at the site of disease compared to serum (Table 3, Table 4, Figure 11, Figure 12). Experiments by Hiraki *et al.* have shown that levels of IFN- $\gamma$  in tuberculous pleurisy were particularly elevated in patients with high fever [124], which also implies a potential harmful role for IFN- $\gamma$  and a usefulness of this cytokine as a marker of disease activity.

#### **4.4 Impact of HIV-infection on tuberculosis and tuberculous pericarditis**

Tuberculosis and co-infection with HIV remains a major health problem especially in sub-Saharan Africa. In dually infected patients extrapulmonary sites such as the pericardium are more often involved than in HIV-seronegative patients [125]. This further involvement frequently results in a more complicated form of tuberculosis which is reflected in having difficulties with the diagnosis and treatment. Patients suffering from tuberculosis and HIV-infection are assumed to have alterations in cytokine production which lead to a more severe form of tuberculosis. However this is still a subject of some debate. Zhang *et al.* reported reduced proliferative and Th1 responses of PBMC from dually infected patients compared to tuberculosis HIV-seronegative patients. Nevertheless, the responses of the HIV-infected individuals were not associated with an enhanced production of Th2 cytokines (IL-4 and IL-10) [126]. Impaired IFN- $\gamma$  production by dually infected patients was also demonstrated in a whole blood cytokine assay in another study [127]. In contrast to those findings Silveira *et al.* could mark no difference in proliferative responses between tuberculosis and dually infected patients with circulating CD4<sup>+</sup> T cells > 200 cells/mm<sup>3</sup> [128]. Other experiments also determined no significant difference between IFN- $\gamma$  levels in tuberculous pleural and pericardial effusions of HIV-infected and HIV-uninfected tuberculosis patients [105, 129]. This is consistent with measurements of IFN- $\gamma$  in the present study, since no significant differences could be detected in pericardial fluid of HIV-infected and -uninfected patients ( $p = 0.16$ ), (Table 6, Table 7). Interestingly, concentrations of IFN- $\gamma$  even tended to be higher in the HIV-seropositive group as shown in Table 6.



However the relatively small number of HIV-uninfected patients ( $n = 9$ ) compared to HIV-infected individuals ( $n = 26$ ) has to be considered when interpreting these results. Elevated levels of IFN- $\gamma$  in HIV-infected patients could be interpreted in a way that the required specific immune response is induced even in immunocompromised patients. However, highly elevated levels of IFN- $\gamma$  in patients co-infected with HIV and *M. tuberculosis* compared to patients only infected with *M. tuberculosis* have been reported by Hodson *et al.* [130] and despite the high levels of secreted IFN- $\gamma$  co-infected patients were unable to control the bacterial replication [130]. Higher levels of IFN- $\gamma$  were rather contributed to the immune response to HIV than *M. tuberculosis* which was reflected in a positive correlation between IFN- $\gamma$  and the viral load in pleural fluid. These contradictory perceptions emphasize the complexity of the immune response to tuberculosis in HIV-infected individuals.

Focusing on other cytokines than IFN- $\gamma$  in the present study, significantly higher levels of IL-1 $\beta$  were measured in serum and cell free pericardial fluid of HIV-uninfected patients (Table 6, Table 7), although the secretion of pro-inflammatory cytokines such as IL-1 $\beta$  is believed to be up-regulated during HIV-infection [131]. The concentration of IL-6 however was elevated in serum and cell free pericardial fluid of HIV-infected patients compared to HIV-seronegative individuals ( $p = 0.05$ ), (Table 6, Table 7). The mean level of IL-6 in pericardial fluid of dually infected patients was 1253.52 pg/ml (SD 378.46) compared to 848.18 pg/ml (SD 751.90) in CFF of HIV-uninfected patients. Related results have been demonstrated by Bal *et al.* who reported a significantly higher IL-6 secretion by PBMCs of dually infected patients [132]. As mentioned in the introduction IL-6 plays an ambiguous role in the immunity to *M. tuberculosis* for on the one hand it seems to be necessary in keeping tuberculosis under control [94], but on the other hand it is able to suppress the T cell response and to inhibit the production of TNF and IL-1 $\beta$  [91, 93]. This is consistent with the findings in this study showing elevated levels of IL-1 $\beta$  in HIV-uninfected patients where IL-6 is less concentrated.

Highly elevated levels of IL-6 can further be detrimental to the patient as high levels of TNF and IL-6 are believed to increase the HIV burden [133] which underscores the various interactions between *M. tuberculosis* and HIV resulting in

complicated forms of tuberculosis and difficulties in treatment of dually infected patients.

#### 4.5 Usefulness of IFN- $\gamma$ as a diagnostic marker for tuberculosis

Several studies have been undertaken which evaluated the usefulness of IFN- $\gamma$  and *M. tuberculosis* specific T cells producing IFN- $\gamma$  as diagnostic tools for tuberculosis. These studies agree that IFN- $\gamma$  is a useful marker for the diagnosis of tuberculosis even in different body fluids. IFN- $\gamma$  could be measured in pleural fluid of patients presenting with tuberculous pleurisy [27, 129], even in immunocompromised patients IFN- $\gamma$  related diagnosis of tuberculosis could be rapidly established [129, 134]. Further it has been investigated that measurements of IFN- $\gamma$  in pleural fluid could be supportive in distinguishing between tuberculous pleural effusions and effusions with a nontuberculous etiology such as malignancy or parapneumonic pleurisy since levels of IFN- $\gamma$  were always highest in effusions of tuberculosis patients [135, 136]. Involvement of other body parts in tuberculosis infection could also be demonstrated by detection of IFN- $\gamma$  or IFN- $\gamma$  producing T cells for instance in CNS [26], peritoneal [137] or pericardial tuberculosis [78]. Burgess *et al.* reported higher concentrations of IFN- $\gamma$  in pericardial fluid of tuberculous pericarditis patients compared to malignant and infectious effusions other than tuberculosis. A cut-off value of 200 pg/L for IFN- $\gamma$  resulted in a sensitivity and specificity of 100% for the diagnosis of tuberculous pericarditis [78, 105]. Measurements of IFN- $\gamma$  in pericardial fluid of tuberculous pericarditis patients in the present study are consistent with the findings of already published studies, since IFN- $\gamma$  levels were elevated in pericardial fluid of the tuberculosis group compared to the control subjects ( $p = 0.065$ ) where IFN- $\gamma$  was below the minimum range of detection. Also concentration of IFN- $\gamma$  was significantly higher in pericardial fluid of the tuberculosis patients compared to their serum (Table 3, Table 4, Figure 5) which supports the suggestion that T lymphocytes migrate to the site of infection to release IFN- $\gamma$  so as to enable macrophages to kill mycobacteria efficiently [84]. Such high levels at the site of disease reflect a strong cellular immune response. Villena *et al.* found similarly elevated IFN- $\gamma$  levels in pleural fluid of HIV-infected and -uninfected tuberculosis patients [129].

Comparable findings are reported by Reuter *et al.* who measured similar IFN- $\gamma$  concentrations in pericardial fluid of HIV-seropositive and -seronegative tuberculosis patients [105]. This indicates that IFN- $\gamma$  can be used as a diagnostic tool for tuberculosis in immunocompetent and immunocompromised patients.

Recently published studies support the effectiveness of IFN- $\gamma$  by meta-analyzing diagnostic studies using IFN- $\gamma$  or by comparing methods in the diagnosis of pleural tuberculosis [138, 139]. Jiang *et al.* conducted a meta-analysis of 22 studies so as to determine the accuracy of IFN- $\gamma$  in the diagnosis of pleural tuberculosis [138], the summary estimate of sensitivity was 89% (95% CI 87-91%) and of specificity was 97% (95% CI 96-98%). These results imply a superiority even to the ELISpot assay which demonstrated a higher sensitivity of 95% but at the same time a poorer specificity of 76% for pleural fluid [27]. However Jiang *et al.* also calculated the negative likelihood ratio in the meta-analysis which was found to be 0.11 [138]. This means that if the IFN- $\gamma$  assay result was negative, there would still be a probability of approximately 10% of this patient having a pleural effusion due to infection with *M. tuberculosis*, therefore the diagnosis of tuberculosis still cannot be excluded if the IFN- $\gamma$  assay result is negative. This is reflected by measurements of IFN- $\gamma$  in pericardial fluid of this study, in 14 patients no IFN- $\gamma$  could be detected although other test results were positive or clinical symptoms likely to be due to tuberculosis were displayed. A disadvantage of using IFN- $\gamma$  as a diagnostic parameter for tuberculosis is certainly the cost of this test. Sharma *et al.* evaluated the cost-effectiveness of IFN- $\gamma$  and ADA activity as parameters for infection with *M. tuberculosis*. Measuring IFN- $\gamma$  was more sensitive than detection of ADA (97.1%, 91.4% respectively), however the cost of IFN- $\gamma$  detection for one patient equaled the costs of full antituberculous treatment for six patients [140]. Although IFN- $\gamma$  is a sensitive and quite specific parameter for the diagnosis of tuberculosis it may not be affordable for every clinic and other diagnostic tests are still necessary.

#### 4.6 Role of TNF in the diagnosis of tuberculosis

Tumor necrosis factor is a crucial factor for granuloma formation and therefore for the containment of tubercle bacilli [87]. The role of TNF in the diagnosis of tuberculosis has been assessed in various studies. Elevated levels of TNF have been measured in pleural fluid of patients presenting with tuberculous pleurisy [135, 141-144]. However TNF is not specific for tuberculosis, it is also produced in course of other diseases such as rheumatoid pleurisy [144] or parapneumonic effusions [135] for instance. But its usefulness in the diagnosis of tuberculosis can be improved by measuring ADA activity additionally to TNF [141, 143]. Burgess *et al.* observed highly elevated levels of TNF in pericardial effusions due to tuberculosis and non-tuberculous infections compared to effusions due to malignancy [110]. This is consistent with the findings of the present study, highly elevated levels of TNF in pericardial fluid of tuberculosis patients compared to the control subjects ( $p > 0.0001$ ) were determined (Table 3, Table 5, Figure 6). In patients with tuberculosis TNF concentration was higher in pericardial fluid than in serum (Table 3, Figure 6) indicating that TNF is mainly produced at the site of disease where it is needed for the immune response against *M. tuberculosis*. Levels of TNF at the site of disease did not differ between HIV-infected and -uninfected patients (Table 7) which was also expected since macrophages are the major source of TNF and they are not affected by HIV-infection in the same way as CD4+ T cells.

#### 4.7 Conclusions, limitations of the study

In summary, the results of the present study demonstrate the compartmentalization of pro-inflammatory cytokines (IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6 and IL-22) to the site of disease in pericardial tuberculosis. Anti-inflammatory cytokines (TGF- $\beta$  and IL-10) were concentrated in serum when compared to the pericardial fluid. Differences in cytokine pattern in HIV-seropositive and -seronegative patients were also evaluated. Higher levels of IL-6 were found in cell free pericardial fluid of HIV-infected patients. Concentrations of IL-1 $\beta$  were higher in serum and cell free pericardial fluid of HIV-uninfected patients.

Further this study suggests a potentially harmful role of IFN- $\gamma$  in pericardial tuberculosis. High levels of IFN- $\gamma$  in pericardial fluid were associated with a larger pericardial effusion. This is the first study assessing the correlation of cytokine mediators in pericardial fluid and disease severity in pericardial tuberculosis and demonstrating a probable harmful role for IFN- $\gamma$ . More studies need to be done in order to really evaluate those findings.

Knowing the cytokine pattern at the site of disease in pericardial tuberculosis may help in diagnosing tuberculous pericarditis. Diagnosis confirmed by culture may take several weeks and clinical and therapeutic decisions have to be made beforehand. Further, tuberculous pericarditis often responds well to antituberculous treatment which emphasizes the importance of an early diagnosis.

One limitation of this study is the very small number of HIV-seronegative patients ( $n = 9$ ) compared to HIV-seropositive patients ( $n = 26$ ). Conclusions drawn from differently elevated levels of cytokines in these two groups of patients have to be treated with care. Another limitation of this study is the application of the Bonferoni correction. It is considered a somewhat rigorous statistical instrument, however a correction of the correlation p-values seems to be necessary to avoid a positive correlation due to coincidence which becomes more probable the more comparisons are done.

#### **4.8 Future prospects derived from this study**

In this study elevated levels of IFN- $\gamma$  in cell free pericardial fluid were demonstrated. It is yet uncertain which subsets of cells are responsible for the production of IFN- $\gamma$ . It may be hypothesized that *M. tuberculosis* specific T cells which have been recruited to the site of disease are the major source of IFN- $\gamma$ . However this still needs to be evaluated by applying ELISpot assays on pericardial fluid. Also further work includes the analysis of the phenotype of lymphocyte subsets present in pericardial fluid by flow cytometry and the analysis of the proteins which are expressed in the RNA of the pericardial fluid cells in order to better understand the immunopathogenesis of pericardial tuberculosis.

The control group consisted of patients undergoing open heart surgery for bypasses and valve reconstruction or replacements who did neither have tuberculosis nor HIV. Cytokine levels in pericardial fluid differed significantly between tuberculous pericarditis patients and the control group. However more studies need to be undertaken assessing pericardial fluid of other forms of pericarditis so as to rate the diagnostic usefulness of those cytokines measured in the present study for pericardial tuberculosis. Elevated levels of IL-6 were described in a case of rheumatoid pericarditis and in another case of POEMS syndrome (plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy of various forms, monoclonal gammopathy, skin changes) [145, 146] and are therefore not specific for tuberculous pericardial effusions. Pankuweit *et al.* suggested the demonstration of IFN- $\gamma$ , IL-6 and IL-8 in pericardial fluid and their lack in serum as helpful in discriminating autoreactive and lymphocytic pericardial effusions [147]. Burgess *et al.* measured IFN- $\gamma$ , TNF, IL-1, IL-2, IL-4, IL-6 and IL-10 in pericardial effusions due to tuberculosis, malignancy and infectious diseases other than tuberculosis and found significant differences in cytokine levels between those three groups, one of them being the strongly elevated level of IFN- $\gamma$  in the tuberculous pericarditis group [110].

The application of an IFN- $\gamma$  ELISpot has shown promising results in the diagnosis of pleural tuberculosis [27] and has also been successfully applied in a case of tuberculous pericarditis [25]. This test would allow a more rapid diagnosis and therefore earlier therapeutic decisions than the usual confirmation of tuberculous pericarditis by culture. No quicker test such as a point of care test for pericardial tuberculosis is available up today. This test would also require the retrieval of pericardial fluid by pericardiocentesis. Due to echocardiographic guidance the procedure of pericardiocentesis has become safer, but still complications such as puncture of the myocardium, a coronary artery, accidental puncture of the stomach, lung or liver, needle induced arrhythmias, infection of the pericardium, vasovagal hypotension and the incidence of pneumopericardium or hemothorax may occur. An overall complication rate of 5% has been described by two studies [148, 149]. Risks have to be calculated carefully. It is a relatively safe procedure when performed by a trained surgeon. However other diagnostic ways have to be taken into consideration as well.

In summary, future work should include the assessment of cytokine profiles in pericarditis of different origins and the usefulness of ELISpot in diagnosing tuberculous pericarditis. Also, more HIV-uninfected patients should be included so as to assess the impact of HIV-infection on pericardial tuberculosis. Analyzing highly expressed proteins in the RNA of peripheral blood and pericardial fluid cells may also help to gain a better understanding of the immunopathogenesis of pericardial tuberculosis next to the analysis of the phenotype of lymphocyte subsets at the site of disease. In future, this extended knowledge may help to diagnose tuberculous pericarditis more rapidly and to prevent severe complications, which should be reflected in a better outcome for patients with pericardial tuberculosis.

## 5 Summary

Tuberculous pericarditis accounts for 60% of all pericarditis cases in Africa compared to only 4% in more industrialized countries. There is considerable urgency in establishing the correct diagnosis since severe complications such as constrictive pericarditis and tamponade can occur which are life-threatening to the patient. The aim of our study was to gain a better understanding of the immunopathology of pericardial tuberculosis which may bring benefit in diagnosing tuberculous pericarditis and also in treatment of pericardial tuberculosis.

Forty patients presenting with tuberculous pericardial effusion were enrolled in this study at Groote Schuur Hospital, Cape Town, Republic of South Africa. Pericardial fluid samples and blood samples for serum specimen were taken. Most of the patients agreed to voluntary HIV testing. Additionally, 26 control patients who did not have tuberculosis nor HIV undergoing open heart surgery for bypass operations or valve replacements were recruited to this study. Cytokines were measured in serum and cell free pericardial fluid of all patients by ELISA. Cytokines of interest were on the one hand pro-inflammatory cytokines IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6, IL-17 and IL-22 and on the other hand anti-inflammatory cytokines TGF- $\beta$  and IL-10. Cytokine levels were correlated to clinical data indicative of left ventricular dysfunction and pericardial disease severity such as ejection fraction, effusion size, right atrial pressure, degree of arterial paradox, E/A-ratio, NYHA-stage, opening pressure at pericardiocentesis and left ventricular shortening fraction (LVSF). Further, patients were split up into groups according to HIV status, the clinical form of pericarditis and NYHA-stage so as to determine whether cytokine levels differed between those groups.

Pro-inflammatory cytokines IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6 and IL-22 were significantly higher in pericardial fluid of the tuberculous pericarditis patients compared to their serum. Levels of these cytokines in pericardial fluid were highly elevated compared to the control subjects. Interleukin-17 concentration was evenly distributed in serum and pericardial fluid of tuberculous pericarditis patients however still elevated compared to the control group. Still, measured amounts of IL-17 were generally very small. Anti-inflammatory cytokines TGF- $\beta$  and IL-10 were elevated in serum compared to the site of disease of tuberculosis patients.



Some levels of cytokines differed between HIV-seropositive and HIV-seronegative individuals: Higher levels of IL-6 in pericardial fluid of HIV-infected patients were observed ( $p = 0.047$ ). Further higher levels of IL-1 $\beta$  in serum and pericardial fluid of HIV-seronegative individuals were determined.

By correlating cytokine levels and clinical data indicative of disease severity a positive correlation of IFN- $\gamma$  and the actual size of the pericardial effusion in milliliter could be established (Spearman  $r = 0.47$  and  $p = 0.0168$ ). Cytokine concentrations did not differ between patients with NYHA-stage II/III and NYHA-stage IV. No correlation between ECG data indicating a severe pericarditis and cytokine concentrations could be established, but a higher concentration of TGF- $\beta$  in serum of patients with atrial fibrillation could be detected compared to serum of patients with a normal sinus rhythm ( $p = 0.04$ ). Patients presenting with a pure effusive form of pericarditis had a higher level of IL-17 in CFF compared to patients with an effusive-constrictive form of pericarditis ( $p = 0.01$ ).

The inflammatory immune response mediated by IFN- $\gamma$  has various important aspects for the patient. On the one hand the adaptive immune response mediated by IFN- $\gamma$  is needed to control the infection with *M. tuberculosis* and further assaying pericardial fluid levels of IFN- $\gamma$  is a very useful diagnostic tool for the rapid diagnosis of tuberculous pericarditis. But on the other hand high levels of IFN- $\gamma$  in the pericardial fluid are associated with severity of pericardial disease. The magnitude of the inflammatory reaction is correlated to the size of the pericardial effusion and may thus determine the risk of a hemodynamically relevant pericardial tamponade.

## 6 Deutsche Zusammenfassung

Die tuberkulöse Perikarditis ist eine lebensbedrohliche Form der extrapulmonalen Tuberkulose. Circa 60% aller Perikarditis-Fälle in Afrika sind tuberkulöser Genese während in weiter entwickelten Ländern nur etwa 4% durch Tuberkulose hervorgerufen werden. Die Inzidenz der Tuberkulose und somit auch der tuberkulösen Perikarditis ist in Afrika ansteigend. Als Hauptursache wird hierfür die HIV-Epidemie angesehen. Für den Patienten ist die frühestmögliche Diagnose und Einleitung der Therapie entscheidend, denn es gilt schwerwiegende und lebensbedrohliche Komplikationen zu vermeiden. Zu den Hauptkomplikationen der tuberkulösen Perikarditis zählen die Perikard-Tamponade und das Auftreten von Fibrinsträngen im Perikardspalt, die zur Konstriktion führen (pericarditis constructiva). Über die Immunopathogenese der tuberkulösen Perikarditis ist wenig bekannt. Daher war es das Ziel dieser Studie die Immunopathogenese der tuberkulösen Perikarditis auch in Zusammenhang mit der klinischen Präsentation des Patienten besser zu verstehen und Erkenntnisse zu erlangen, die hilfreich für die Diagnostik und Behandlung dieser Erkrankung sein können.

Vierzig Patienten mit tuberkulöser Perikarditis und einem Perikarderguss größer als 10 mm wurden in diese Studie aufgenommen. Jeder dieser Patienten unterzog sich einer klinischen Untersuchung, Thorax-Röntgen, Elektrokardiogramm, Echokardiographie und der Entnahme von Blutproben und Perikardflüssigkeit unter Perikardiozentese. Der Großteil der Patienten stimmte einem HIV-Test zu. Außerdem wurden noch 26 Kontrollpatienten, die Koronarbypässe, Herzklappenersatz oder – rekonstruktionen erhielten, rekrutiert. In Serumproben und Perikardflüssigkeit wurden Konzentrationen von Zytokinen mittels ELISA gemessen. Folgende Zytokine wurden dabei bestimmt: IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6, IL-17 und IL-22 mit pro-inflammatorischen Eigenschaften und IL-10 und TGF- $\beta$  mit anti-inflammatorischen Eigenschaften. Überdies wurden die Zytokinlevel der Patienten mit tuberkulöser Perikarditis mit klinischen Daten korreliert, die Auskunft über die Funktion des linken Ventrikels und somit auch über das Ausmaß der Krankheit geben. Zu diesen Daten zählen Ejektionsfraktion, Größe des Perikardergusses, der Druck im rechten Vorhof, Ausmaß des Pulsus paradoxus, der E/A-Quotient, NYHA-Stadium des Patienten, der Öffnungsdruck des Perikards

unter Perikardiozentese, die LVSF (left ventricular shortening fraction) und EKG-Daten, die eine schwere Perikarditis anzeigen.

Die pro-inflammatorischen Zytokine IFN- $\gamma$ , TNF, IL-1 $\beta$ , IL-6 und IL-22 waren im Perikarderguss höher konzentriert im Vergleich zum Serum der Patienten mit tuberkulöser Perikarditis. Auch waren die jeweiligen Level höher als in der Kontrollgruppe. IL-17 erschien im Serum und Perikarderguss der Tuberkulosepatienten gleichermaßen leicht erhöht, jedoch unterschieden sich diese Konzentrationen von den niedrigeren Leveln der Kontrollgruppe. Die anti-inflammatorischen Zytokine IL-10 und TGF- $\beta$  waren dagegen im Serum höher konzentriert als im Perikarderguss. Die Tuberkulosegruppe wurde in HIV-infizierte und nicht infizierte Patienten eingeteilt und die Serum- und Perikardergusspiegel der Zytokine wurden miteinander verglichen. IL-6 zeigte höhere Werte im Perikarderguss HIV-infizierter Patienten (Tabelle 6, Tabelle 7). IL-1 $\beta$  dagegen schien in Serum und Perikarderguss HIV-seronegativer Patienten höher konzentriert zu sein (Tabelle 6, Tabelle 7).

Die Level der Zytokine im Serum und Perikarderguss der Tuberkulosepatienten wurden nun mit klinischen Daten korreliert, die Hinweise auf die Schwere der Erkrankung gaben. Unsere Daten zeigten eine positive Korrelation zwischen der Konzentration von IFN- $\gamma$  im Perikarderguss und der Größe des Perikardergusses in Millilitern (Spearman  $r = 0.47$ ,  $p = 0.0168$ ). Außerdem wiesen Patienten mit Vorhofflimmern höhere Werte von TGF- $\beta$  im Serum auf als Patienten mit einem normalen Sinusrhythmus ( $p = 0.04$ ). Die Tuberkulosepatienten wurden überdies entsprechend der klinischen Form der Perikarditis in eine Gruppe mit Perikarderguss und eine Gruppe mit Perikarderguss und Konstriktion eingeteilt. Die Gruppe ohne Konstriktion zeigte höhere Werte von IL-17 im Perikarderguss im Vergleich zur anderen ( $p = 0.01$ ).

Die durch IFN- $\gamma$  vermittelte Entzündungsreaktion bei der tuberkulösen Perikarditis hat zwei bedeutende Aspekte für die betroffenen Patienten: Die adaptive IFN- $\gamma$  vermittelte Immunreaktion ist für die Kontrolle der Infektion mit *M. tuberculosis* essentiell. Die Analyse von IFN- $\gamma$  im Perikarderguss bietet daher eine geeignete Methode zur raschen Diagnose der tuberkulösen Perikarditis. Andererseits korrelieren hohe Konzentrationen von IFN- $\gamma$  im Perikarderguss mit dem Ausmaß

der Entzündungsreaktion der Perikarditis und könnten ein prognostischer Marker für die Entwicklung einer hämodynamisch relevanten Perikardtamponade sein.

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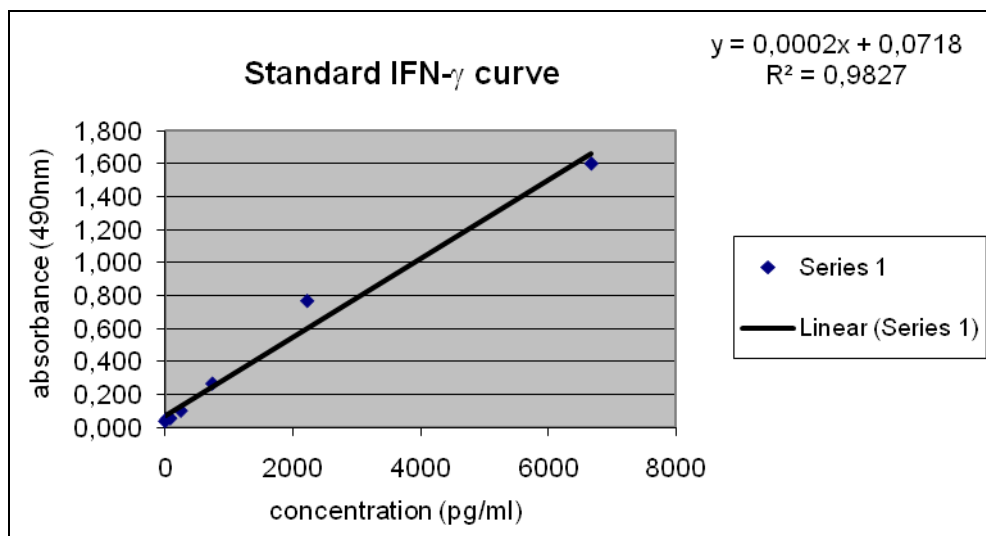
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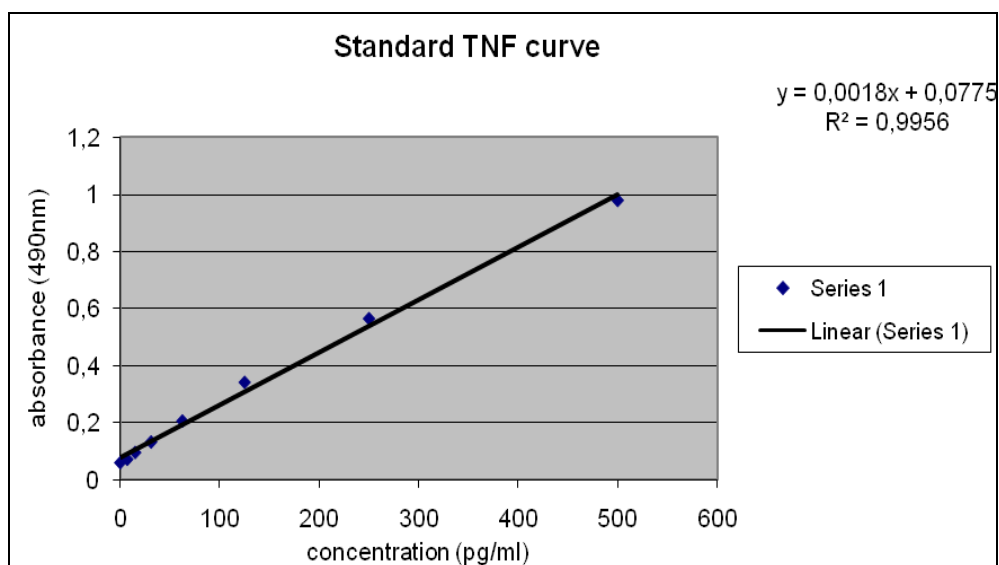
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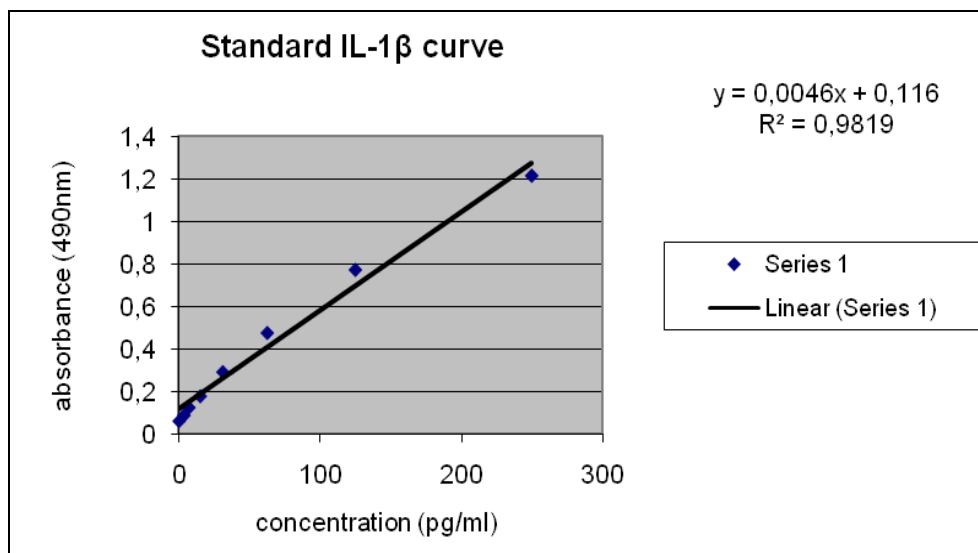
## 8 Supplements



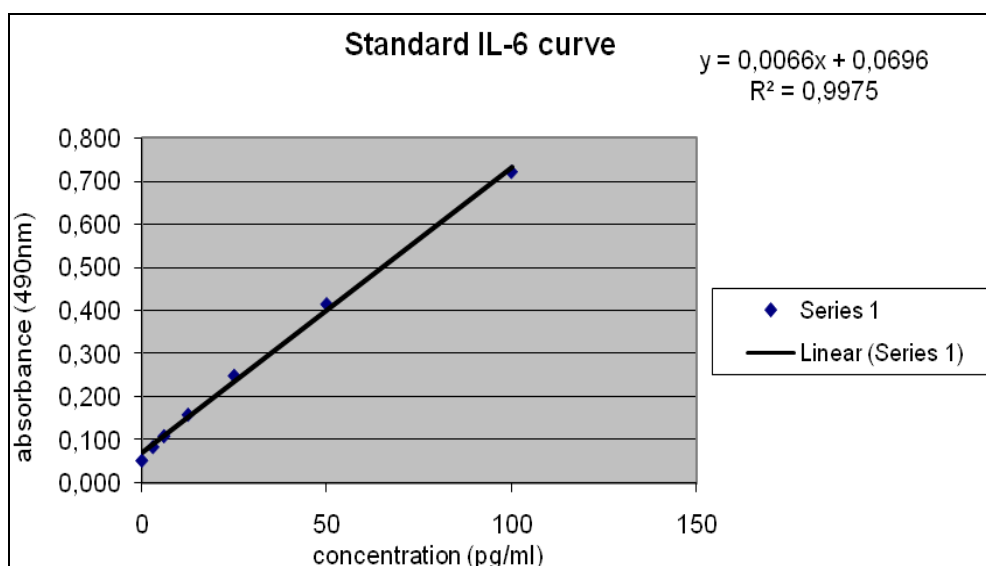
**Figure 14: Standard curve of the interferon- $\gamma$  (IFN- $\gamma$ ) enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 143.5 pg/ml (8396.0 pg/ml)**



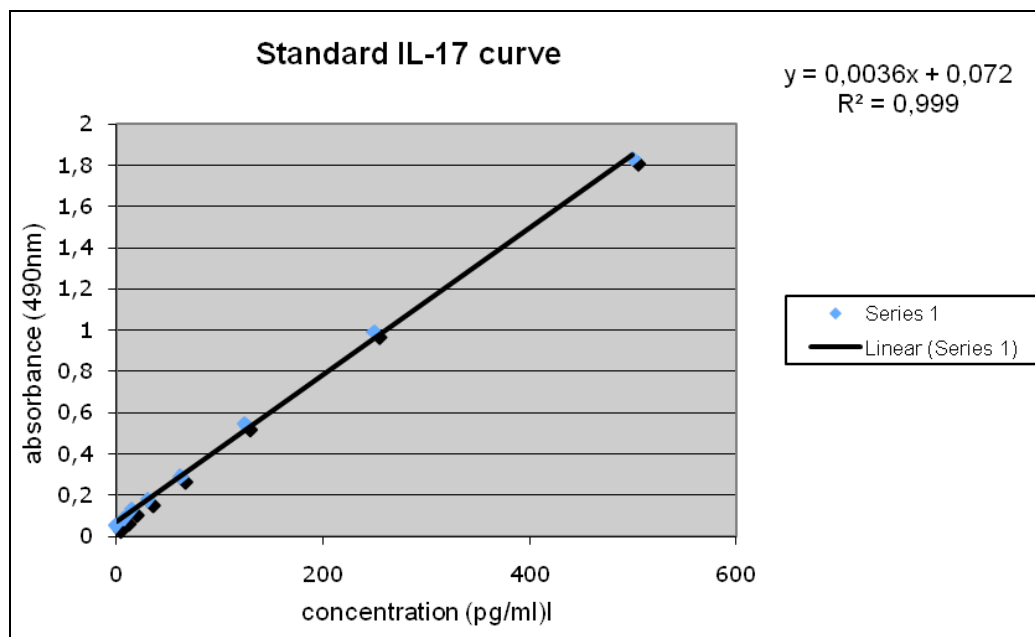
**Figure 15: Standard curve of the tumor necrosis factor (TNF) enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 10 pg/ml (500.3 pg/ml)**



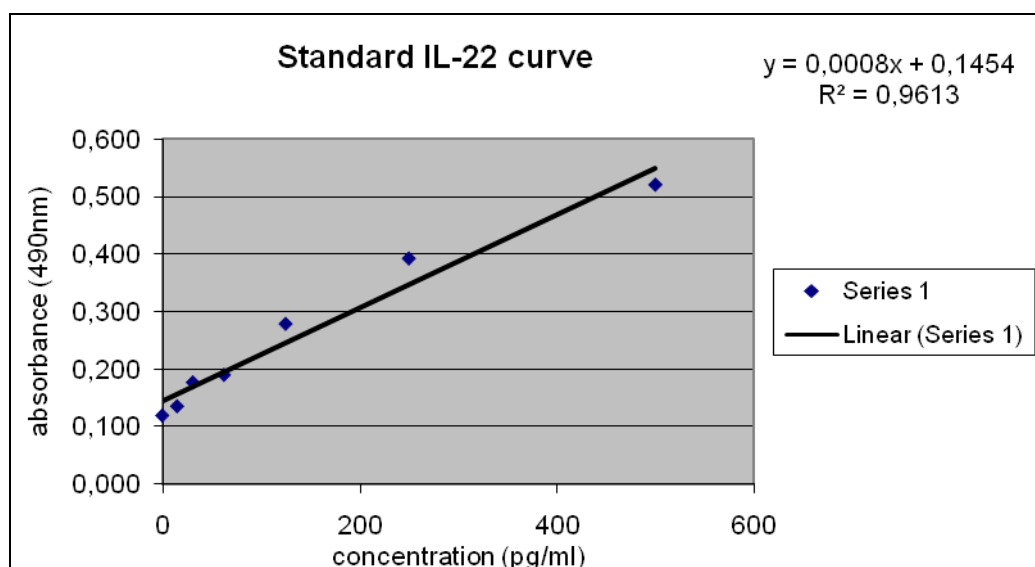
**Figure 16: Standard curve of the IL-1 $\beta$  enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 2.28 pg/ml (238.7 pg/ml)**



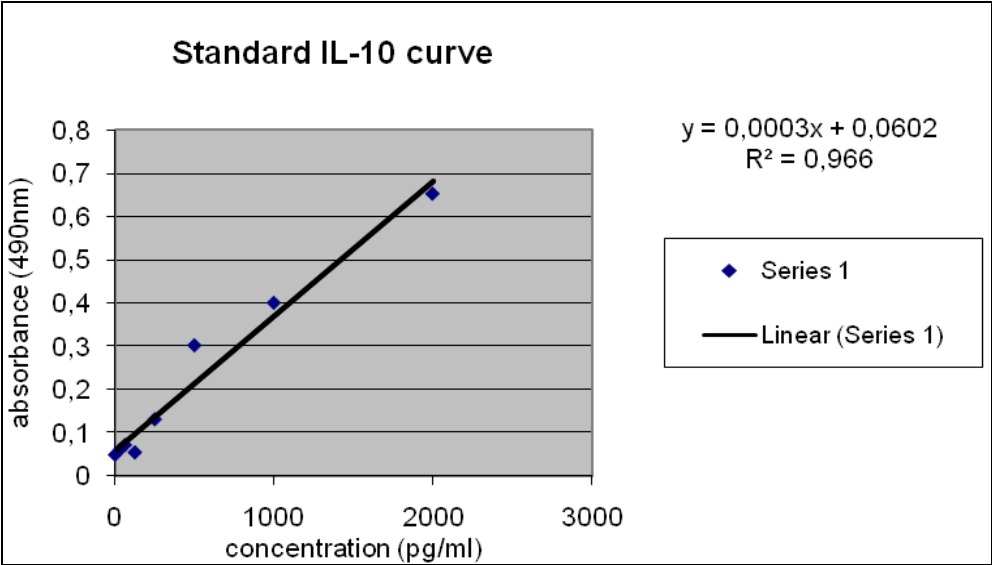
**Figure 17: Standard curve of the IL-6 enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 2.18 pg/ml (132.41 pg/ml)**



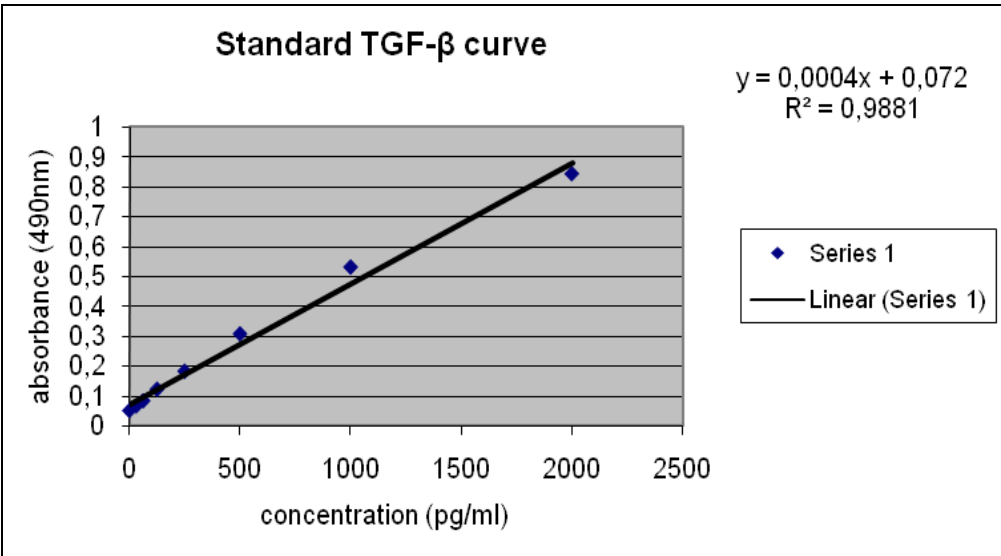
**Figure 18: Standard curve of the IL-17 enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 4.0 pg/ml (489.0 pg/ml)**



**Figure 19: Standard curve of the IL-22 enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 39.5 pg/ml (710.13 pg/ml)**



**Figure 20: Standard curve of the IL-10 enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 6.67 pg/ml (1976.67 pg/ml)**



**Figure 21: Standard curve of the TGF-β enzyme-linked immunosorbent assay (ELISA), lower (and upper) detection limit 25.0 pg/ml (1928.8 pg/ml)**



**Table 10: Correlations of cytokine levels with clinical data, findings are listed as Spearman r and p-values**

cytokines	fluid volume		right atrial pressure		pulsus paradoxus		opening pressure at pericardiocentesis		LVEF		LVSF		E/A-ratio	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
CFF IFN- $\gamma$	0.41	0.0168	0.35	0.28	0.36	0.21	0.3	0.63	-0.054	5.35	0.043	5.77	-0.24	1.87
serum TNF	-0.043	5.6	0.14	3.08	0.3	0.56	0.086	4.48	-0.044	5.71	-0.026	6.28	-0.2	2.48
CFF TNF	-0.031	5.95	0.028	6.16	0.2	1.82	0.16	2.73	0.057	5.33	-0.11	4.14	-0.054	5.65
serum IL-1 $\beta$	0.1	3.71	0.34	0.35	0.15	2.87	0.11	3.71	0.055	5.37	0.2	2.17	-0.34	0.75
CFF IL-1 $\beta$	0.22	1.26	0.013	6.58	-0.19	1.96	0.069	4.97	0.091	4.38	-0.15	3.11	-0.2	2.48
serum IL-6	0.043	5.58	0.19	2.03	0.08	4.52	0.076	4.7	0.23	1.47	0.19	2.23	-0.24	1.79
CFF IL-6	-0.22	1.3	0.077	4.66	0.21	1.6	0.043	5.67	0.071	4.91	0.072	4.93	-0.22	2.03
serum IL-17	0.036	5.79	-0.057	5.27	0.1843	2.02	-0.12	3.48	-0.124	3.49	-0.159	2.81	0.303	0.99
CFF IL-17	0.131	3.0	-0.091	4.25	-0.002	6.93	-0.268	0.88	0.04	5.79	0.054	5.44	-0.014	6.62
serum IL-22	-0.038	5.74	0.44	0.07	0.24	1.23	0.43	0.07	0.36	0.31	0.32	0.61	-0.15	3.29
CFF IL-22	0.064	4.91	0.2	1.77	0.06	5.14	0.37	0.22	0.23	1.38	0.17	2.5	-0.11	4.21
serum IL-10	-0.17	2.1	0.42	0.07	0.23	1.33	0.47	0.07	0.12	3.58	0.15	3.09	-0.077	5.08
CFF IL-10	0.19	1.68	0.21	1.75	0.14	2.94	0.41	0.14	0.1	4.08	0.16	2.82	-0.2	2.55
serum TGF- $\beta$	0.075	4.55	-0.12	3.57	-0.44	0.07	-0.11	3.85	-0.28	0.82	-0.15	3.13	-0.17	3.02
CFF TGF- $\beta$	0.17	2.1	-0.2	1.75	-0.14	3.01	-0.14	2.87	0.051	5.43	0.053	5.48	0.055	5.58

**Table 11: Correlations of clinical electrocardiogram (ECG) data and cytokine levels in serum and cell free pericardial fluid (CFF), findings are listed as Spearman r and p-values**

cytokines	beats per minute		max. R in I.II.III in mV		number of leads with a negative T		ST-elevation in mV	
	r	p	r	p	r	p	r	p
CFF IFN- $\gamma$	0.21	0.25	-0.2	0.25	0.14	0.44	-0.24	0.18
serum TNF	-0.2	0.28	0.047	0.8	-0.036	0.84	0.0028	0.99
CFF TNF	-0.23	0.2	-0.0095	0.96	0.18	0.32	0.061	0.74
serum IL-1 $\beta$	-0.089	0.63	-0.25	0.18	-0.039	0.83	-0.16	0.38
CFF IL-1 $\beta$	0.012	0.95	-0.02	0.91	0.3	0.09	0.18	0.32
serum IL-6	-0.149	0.4	-0.071	0.69	0.285	0.1	-0.058	0.75
CFF IL-6	0.24	0.17	-0.199	0.26	0.158	0.37	0.129	0.47
serum IL-17	-0.029	0.87	0.078	0.66	-0.2	0.25	-0.009	0.96
CFF IL-17	0.13	0.45	0.17	0.33	-0.08	0.65	0.041	0.82
serum IL-22	0.033	0.85	-0.06	0.74	-0.12	0.49	-0.018	0.92
CFF IL-22	0.25	0.16	-0.17	0.34	0.1	0.56	0.053	0.76
serum IL-10	0.0045	0.98	-0.35	0.05	0.049	0.76	0.0097	0.96
CFF IL-10	-0.075	0.69	0.0051	0.98	0.025	0.89	-0.19	0.3
serum TGF- $\beta$	0.071	0.69	0.25	0.16	-0.2	0.27	0.15	0.41
CFF TGF- $\beta$	0.11	0.56	0.04	0.82	-0.27	0.13	-0.12	0.5

**Table 12: Cytokine levels according to heart rhythm, concentrations are given as median pg/ml (interquartile range)**

<b>cytokines</b>	<b>sinus rhythm</b>	<b>atrial fibrillation</b>	<b>p-value</b>
<b>CFF IFN-<math>\gamma</math></b>	1308.0 (0.0 - 3798.0)	873.0 (0.0 - 3288.0)	p = 0.86
<b>serum TNF</b>	0.0 (0.0 - 44.0)	18.0 (0.0 - 44.0)	p = 0.16
<b>CFF TNF</b>	22.0 (0.0 - 101.0)	27.0 (0.0 - 78.0)	p = 0.80
<b>serum IL-1<math>\beta</math></b>	0.0 (0.0 - 0.0)	0.0 (0.0 - 48.0)	p = 0.47
<b>CFF IL-1<math>\beta</math></b>	0.0 (0.0 - 9.5)	0.6 (0.0 - 7.0)	p = 0.91
<b>serum IL-6</b>	0.0 (0.0 - 40.8)	0.0 (0.0 - 0.0)	p = 0.19
<b>CFF IL-6</b>	1263.0 (897.6 - 1525.0)	1326.0 (1054.0 - 1576.0)	p = 0.79
<b>serum IL-17</b>	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	p = 0.94
<b>CFF IL-17</b>	0.0 (0.0 - 0.0)	0.0 (0.0 - 5.3)	p = 0.55
<b>serum IL-22</b>	29.0 (1.4 - 46.0)	63.0 (16.0 - 137.0)	p = 0.07
<b>CFF IL-22</b>	74.0 (39.0 - 173.0)	125.0 (25.0 - 168.0)	p = 0.80
<b>serum IL-10</b>	93.0 (0.0 - 547.0)	156.0 (0.0 - 1255.0)	p = 0.77
<b>CFF IL-10</b>	103.0 (62.0 - 293.0)	61.0 (0.0 - 158.0)	p = 0.19
<b>serum TGF-<math>\beta</math></b>	52.0 (0.6 - 200.0)	185.0 (165.0 - 265.0)	<b>p = 0.04</b>
<b>CFF TGF-<math>\beta</math></b>	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	p = 0.57

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## 10 Curriculum Vitae



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2006 until present: Medical dissertation at the Research Center Borstel, Germany, in collaboration with the Institute of Infectious Disease and Molecular Medicine (IIDMM) at the University of Cape Town, South Africa, Title: “Cytokine pattern in pericardial tuberculosis – Correlations with disease severity”

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*03/2009:* Congress of the German Society for Pneumology and Respiratory Medicine in Mannheim, Germany



## 11 Publications

### Journal article:

Kerryn Matthews, Katalin A. Wilkinson, Barbara Kalsdorf, Teri Roberts, Andreas Diacon, Gerhard Walzl, Janine Wolske, Mpiko Ntsekhe, Faisal Syed, James Russell, Bongani M. Mayosi, Rodney Dawson, Keertan Dheda, Robert J. Wilkinson, Willem A. Hanekom, Thomas J. Scriba, "Predominance of interleukin-22 over interleukin-17 at the site of disease in human tuberculosis", *Tuberculosis (Edinb)*. 2011 Nov;91(6):587-93. Epub 2011 Jul 20

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