The immunopathogenesis and treatment of tuberculous pericardial effusions in a population with a high prevalence of infection with the human immunodeficiency virus

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and I have not previously in its entirety or in part submitted it at any University for a degree.

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SUMMARY

Mycobacterium tuberculosis (*M. tuberculosis)* accounts for more adult deaths than any other infectious agents. The present study included 162 patients with tuberculous pericarditis; 50% of the tuberculous pericarditis patients studied were human immunodeficiency virus (HIV) positive, compared to only 4.2% of patients who presented with non-tuberculous pericardial effusions. A steady year-to-year rise in HIV prevalence was observed in this 6-year study. Although the prognosis of pericardial tuberculosis (TB) is excellent with appropriate medical treatment, untreated pericardial TB has a mortality of 80-85%. It is thus important to diagnose tuberculous pericarditis efficiently. Traditionally, the diagnosis of pericardial TB is established by positive mycobacterial culture and/or histological evidence of necrotising granulomatous inflammation of the pericardium. Our study confirmed the insensitivity of pericardial fluid culture and pericardial biopsy in the diagnosis of pericardial TB, and at the time of clinical decision-making, results were usually not available. To overcome these difficulties, we explored various alternative strategies and this resulted in two diagnostic tools, namely a diagnostic rule and a diagnostic algorithm or classification tree.

By means of classification and regression tree analysis, we allocated a weighted diagnostic index to each of five independently predictive features (fever, night sweats, weight loss, serum globulin >40 g/L and peripheral blood leukocyte count $<10x10^9$/L). A total diagnostic index of 6 or more corresponded to 82-86% sensitivity and 76-87% specificity for a diagnosis of tuberculous pericarditis.

When possible, pericardial fluid should be aspirated to determine adenosine deaminase (ADA) levels and pericardial differential leukocyte counts. Fluid should also be sent for Gram stain and culture. The proposed diagnostic classification tree utilises the independently predictive attributes of pericardial adenosine deaminase levels, pericardial fluid lymphocyte/neutrophil ratios, peripheral leukocyte counts and the HIV status. Applying this prediction model to our entire data set of 233 patients resulted in 96% sensitivity and 97% specificity for the correct diagnosis of tuberculous pericarditis.

Generally, patients were critically ill at the time of enrolment; 90% of tuberculous pericarditis presented with echocardiographic features of cardiac tamponade. Echo-guided percutaneous pericardiocentesis with an indwelling catheter and intermittent daily aspiration was highly effective and safe. It is likely that the combination of this drainage technique and the early initiation of anti-tuberculous chemotherapy contributed to the almost complete absence of constriction in the patients studied, and our data do not support the routine use of adjunctive corticosteroids in patients with tuberculous pericarditis.

Tuberculous exudates result from a Th1 mediated immune response characterised by lymphocyte dominance, significantly elevated levels of gamma-interferon (IFN-γ) and undetectable levels of interleukin-4 (IL-4). IFN-γ levels were not influenced by HIV status in spite of the severely diminished pericardial CD4+ lymphocyte counts observed in this study. It is thus likely that in HIV positive patients IFN-γ production is partly maintained by activated CD8+ T cells, which were significantly elevated in HIV positive patients compared to HIV negative tuberculous pericarditis patients.

This finding underlines the importance of IFN-γ in the human immune response against *M. tuberculosis*. We also demonstrated that the presence of ADA in pericardial fluids reflects the activity of the cellular immune response. Both IFN-γ and ADA can be utilised as sensitive and specific diagnostic tools for pericardial TB.

OPSOMMING

Mycobacterium tuberculosis (*M. tuberculosis)* is verantwoordelik vir meer volwasse sterftes as enige ander infektiewe organisme. Die huidige studie sluit 162 pasiënte met tuberkuleuse perikarditis in; 50% van hierdie pasiënte was menslike immuungebrek virus (MIV) positief, in vergelyking met slegs 4.2% van pasiënte in wie nie-tuberkuleuse perikardiale effusies voorgekom het. 'n Bestendige jaar-tot-jaar toename in MIV voorkoms is waargeneem tydens hierdie 6-jaarlange studie. Alhoewel die prognose van perikardiale tuberkulose (TB) met toepaslike mediese behandeling uitstekend is, het onbehandelde perikardiale TB 'n mortaliteit van 80 – 85%. Dit is dus belangrik om tuberkuleuse perikarditis effektief te diagnoseer. Tradisioneel word die diagnose van perikardiale TB vasgestel deur middel van positiewe mikobakteriële kulture en/of histologiese bewys van nekrotiserende granulomateuse inflammasie van die perikardium. Ons studie bevestig die gebrek aan sensitiwiteit van perikardiale vogkulture en perikardiale biopsie in die diagnose van perikardiale TB, en ten tye van kliniese besluitneming was die resultate gewoonlik nog nie beskikbaar nie. Om hierdie probleme te oorkom, het ons verskeie alternatiewe strategieë ondersoek. Dit het gelei tot die ontwikkeling van twee diagnostiese hulpmiddels, naamlik 'n diagnostiese reël en 'n diagnostiese algoritme of klassifikasie vloeidiagram.

Deur middel van klassifikasie en regressie vloeidiagram analise, het ons 'n diagnostiese indeks toegeken aan elk van die vyf onafhanklike voorspelbare eienskappe (koors, nagsweet, gewigsverlies, serum globulien >40 g/L en perifere bloedleukosiettelling $<10x10^9$/L). 'n Totale diagnostiese indeks van 6 of meer het

ooreengestem met ’n sensitiwiteit van 82-86% en ’n spesifisiteit van 76-87% vir ’n diagnose van tuberkuleuse perikarditis.

Wanneer moontlik, behoort perikardiale vog geaspireer te word om adenosien deaminase (ADA) vlakke en perikardiale differensiële leukosiettellings te bepaal. Vog behoort ook gestuur te word vir Gramkleuring en kultuur. Die voorgestelde diagnostiese klassifikasie vloeidiagram gebruik die onafhanklike voorspelbare bydraes van perikardiale ADA vlakke, perikardiale vog limfosiet/neutrofiel verhoudings, perifere leukosiettellings en die MIV status. Toepassing van hierdie voorspellingsmodel op ons totale datastel van 233 pasiënte, het gelei tot 96% sensitiwiteit en 97% spesifisiteit vir die korrekte diagnose van tuberkuleuse perikarditis.

Oor die algemeen was die pasiënte ernstig siek met toetrede tot die studie, 90% van tuberkuleuse perikarditis het voorgedoen met eggokardiografiese kenmerke van kardiale tamponade. Eggogerigte perkutane perikardiosentese deur middel van ’n inblywende kateter en intermitterende daaglikse aspirasie was hoogs effektief en veilig. Die kombinasie van hierdie dreineringstegniek en die vroeë aanvang van anti-tuberkuleuse chemoterapie het waarskynlik bygedra tot die byna volledige afwesigheid van konstriksie in die pasiënte wat bestudeer is, en ons data steun nie die roetine gebruik van bykomende kortikosteroïede in pasiënte met tuberkuleuse perikarditis nie.

Tuberkuleuse eksudate is die gevolg van ’n Th1-gemedieerde immuunrespons, wat gekenmerk word deur limfosiet oorheersing, betekenisvol verhoogde vlakke van

gamma-interferon (IFN-γ) en onmeetbare vlakke van interleukin-4 (IL-4). IFN-γ vlakke is nie beïnvloed deur die MIV status nie, ten spyte van die erg verlaagde perikardiale CD4+ limfosiettellings waargeneem in hierdie studie. Dit is dus hoogs waarskynlik dat in MIV positiewe pasiënte die produksie van IFN-γ gedeeltelik gehandhaaf word deur geaktiveerde CD8+ T-selle, wat betekenisvol verhoog was in die MIV positiewe pasiënte, in vergelyking met MIV negatiewe tuberkuleuse perikarditis pasiënte. Hierdie bevinding beklemtoon die belang van IFN-γ in die menslike immuunrespons teen *M. tuberculosis.* Ons het getoon dat die teenwoordigheid van ADA in perikardiale vog die aktiwiteit van die sellulêre immuunrespons reflekteer. Beide IFN-γ en ADA kan gebruik word as sensitiewe hulpmiddels vir die diagnose van perikardiale TB.

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LIST OF ABBREVIATIONS

In addition to the conventional atomic symbols and S.I. units, the following abbreviations are used in this thesis:

ADA	adenosine deaminase
AIDS	acquired immunodeficiency syndrome
BACTEC	radiometric mycobacterial culture system
CART	classification and regression tree
CI	confidence interval
CNTD	connective tissue disaese
CTR	cardiothoracic ratio
CXR	chest X-ray
DOTS	directly observed therapy, short course
ECG	electrocardiograph
ELISA	enzyme-linked immunosorbent assay
FN	false negative
FP	false positive
HAART	highly active antiretroviral therapy
HIV	human immunodeficiency virus
JVP	jugular venous pressure
IL-1	interleukin-1
IL-4	interleukin-4
IL-10	interleukin-10
IFN-γ	interferon-gamma

LDH	lactate dehydrogenase
L/N ratio	lymphocyte/neutrophil ratio
MCTD	mixed connective tissue disease
MGIT	mycobacterial growth indicator tube
NK cell	natural killer cell
Non-TB	non-tuberculous
NPV	negative predictive value
PB	peripheral blood
Pc	pericardial
PCR	polymerase chain reaction
PPD	purified protein derivative
PPV	positive predictive value
RA	rheumatoid arthritis
ROC curve	receiver operating characteristic curve
S	serum
SLE	systemic lupus erythematosus
TB	tuberculosis
TNF-α	tumour necrosis factor-alpha
TN	true negative
TP	true positive
TST	tuberculin skin test
WBC	white blood cell count
WCC	white cell count
WHO	World Health Organization
ZN	Ziehl-Neelsen

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Chapter 1

TUBERCULOUS PERICARDITIS IN THE ERA OF THE HIV PANDEMIC

More than a hundred years after Robert Koch's discovery of *Mycobacterium tuberculosis* (*M. tuberculosis*) in 1882, tuberculosis (TB) remains a major cause of morbidity and mortality among the world's poor and those infected with the human immunodeficiency virus (HIV). It is estimated that one third of the world's population is infected with *M. tuberculosis*, which is transmitted by person-to-person spread via droplets inhaled by the susceptible host (Coovadia and Benatar, 1991; Raviglione *et al*, 1995). The acid-fast tubercle bacilli elicit a non-specific acute response and are ingested by macrophages in the lung. Due to cell mediated immunity the tubercle bacilli are confined intracellularly within granulomatous lesions where they can persist for years (latent infection). However, if not adequately contained, *M. tuberculosis* causes active tuberculous disease (Murray *et al*, 1990; Dolin *et al*, 1994; Raviglione *et al*, 1995). In sub-Saharan Africa, HIV infection rates among TB patients are, on average, over three times higher than those in the general population and the resurgence of TB has been explained by the high HIV seroprevalence (Narain *et al*, 1992; Cantwell and Binkin, 1997)

The World Health Organization (WHO) reported 8.8 million new cases of TB in 2003 (140 / 100 000 population), of which 3.9 million were smear positive and 674 000 co-infected with HIV (WHO report, 2005). An estimated 1.7 million people died from TB in 2003, including 229 000 individuals co-infected with HIV (WHO report, 2005).

In 2003, the TB incidence rate was falling or stable in five out of six WHO regions, but growing at 1% per year globally, due to the quickly rising incidence of TB in the African countries with high HIV prevalence rates (WHO report, 2005). In 2003, South Africa had a TB incidence of 536 cases/100 000 population per year and an estimated TB mortality of 73/100 000 population per year. In the age group 15-49 years the HIV seroprevalence in patients with active TB was estimated to be 61% (WHO report, 2005). In South Africa, the HIV infection rate among pregnant women attending antenatal services in 2003 was 27.9%, with variation among the nine provinces ranging from as high as 37.5% in KwaZulu-Natal to as low as 13.1% in the Western Cape Province (UNAIDS, 2004). By the end of 2003, an estimated 5.3 million South Africans (95% CI 4.5–6.2 million) were HIV positive, the largest number of individuals living with the virus in a single country (UNAIDS, 2004). This is the greatest health challenge facing South Africa and it fuels the TB epidemic and has serious social, economic and health implications.

THE NATURAL HISTORY OF HIV INFECTION

The principal cells targeted by HIV are CD4+ helper T cells and, to a lesser degree, cells of the monocyte-macrophage lineage (Fauci, 1993; Fauci *et al*, 1996). As a consequence of persistent viral replication there is a vicious cycle of immune activation and cytokine secretion that results in the depletion of CD4+ T cells, destruction of lymphoid tissue and the onset of life-threatening infections (Fauci, 1993; Fauci *et al*, 1996). TB is the leading cause of morbidity and mortality in HIV-infected South Africans (Badri *et al*, 2002). The progression of HIV infection can be conceptualised as three stages: a first phase of primary infection, a second clinically latent period when viral replication continues, and a third phase of advanced disease

(Fauci *et al*, 1996). Active TB can occur in any one of these stages (Badri *et al*, 2002). Primary infection generally refers to the period (few weeks to months) from initial infection until the immune response to HIV gains some measure of control over viral replication. Approximately 30-70% of newly infected individuals experience an acute mononucleosis-like syndrome with signs and symptoms during acute HIV infection that may include fever, malaise, rash, lymphadenopathy, pharyngitis, headache, diarrhoea and occasionally arthralgia and neurological manifestations (Tindall *et al*, 1991; Clark *et al*, 1991; Pantaleo *et al*, 1993). This constellation of features is referred to as the "acute retroviral syndrome". It is characterised by extremely high levels of plasma viraemia and a precipitous decline in CD4+ lymphocyte cell counts associated with a high risk for active TB (Clark *et al*, 1991; Piatak *et al*, 1993; Pantaleo *et al*, 1994; Badri *et al*, 2002). The initial high levels of HIV replication and plasma viraemia generally decrease with the appearance of the specific immune response and viral levels gradually stabilise within six months to one year at a virologic "set-point", which correlates with HIV-mediated disease progression (Mellors *et al*, 1995; Mellors *et al*, 1996). The relative stabilisation of the viraemia generally signifies the beginning of a clinically latent period that is characterised by chronic immune activation and persistent viral replication despite a lack of consistent signs or symptoms of disease (Pantaleo *et al*, 1996). During this phase, the number of circulating CD4+ lymphocyte T cells slowly declines by about 50-80 cells/μL per year, signalling the onset of progressive immunodeficiency. The pivotal role of cytotoxic T lymphocytes (CTL) in controlling virus replication continues throughout the latent phase of infection (Haynes *et al*, 1992; Haynes *et al*, 1996). Eventually, the effective CTL response declines and plasma viraemia escalates. Neutralising antibodies are also present

throughout the asymptomatic phase of disease, albeit at relatively low 1evels (Montefiori *et al*, 1996).

Advanced disease is characterised by an acquired immunodeficiency syndrome (AIDS) defining illness or a decline in the levels of circulating CD4+ lymphocyte T cells below 200 cells/μL (Centers for Disease Control, 1992). Plasma viraemia usually increases during this stage of disease (Pantaleo *et al*, 1996) and is associated with a sharp decrease in CD4+ lymphocyte T cell counts. The natural history and pathogenic processes of HIV infection are highly variable and are influenced by viral and human genetic factors, virulence of HIV variants and host immunologic response to the virus (Pantaleo *et al*, 1996; Haynes *et al*, 1996; Pantaleo *et al*, 1997).

EFFECT OF ACTIVE TB ON PROGRESSION OF HIV

Despite adequate anti-tuberculous therapy, many individuals co-infected with TB and HIV have an accelerated course of HIV disease and shortened survival (Whalen *et al*, 1995). Active TB provokes activation of the immune system, which results in high serum levels of pro-inflammatory cytokines and increased expression of cellular activation markers that facilitate HIV replication (Wallis *et al*, 1993; Whalen *et al*, 1995; Bouscarat *et al*, 1996)

THE EFFECTS OF HIV INFECTION ON CLINICAL TB

Due to the gradual loss of CD4+ T lymphocytes and the associated inadequate cellular immunity, HIV-infected individuals are at an extraordinarily high risk of developing clinical TB (Barnes *et al*, 1991). Effects of HIV include increased susceptibility to reactivation of latent tuberculous infection, rapid progression following primary

infection, increased risk of recurrent disease, increased re-infection rates and also a change in the clinical picture, including a higher proportion of extrapulmonary TB and involvement at more than one site, a lower rate of tuberculin skin test positivity, more frequent atypical chest radiographs, and a marked increase in mortality (Chaisson *et al*, 1987; Barnes *et al*, 1991; Snider and Roper, 1992; Barnes *et al*, 1993; Houston *et al*, 1994; Sonnenberg *et al*, 2001, Churchyard *et al*, 2003). In countries with a high TB incidence, recurrent TB accounts for a significant proportion of all cases (Weyer and Kleeberg, 1992; Churchyard *et al*, 2001) and HIV infection is the strongest risk factor for recurrent TB, especially in those with a low CD4+ lymphocyte count (Pulido *et al*, 1997; Johnson *et al*, 1997; Mallory *et al*, 2000; Sonnenberg *et al*, 2001). Recurrent TB results from recrudescence of disease with the original infecting organism or re-infection with a new strain of *M. tuberculosis* (Van Rie *et al*, 1999; Sonnenberg *et al*, 2001). HIV-negative persons infected with *M. tuberculosis* have an estimated 5-10% lifetime risk (0.2% per year) of progression to active TB (Dolin *et al*, 1994; Rieder *et al*, 1998) compared to a cumulative lifetime risk of 30% or more (2.6-13.3% per year) for HIV-infected individuals (Selwyn *et al*, 1989; Braun *et al*, 1991; Allen *et al*, 1992; Guelar *et al*, 1993; Antonucci *et al*, 1995; Mlika-Cabanne *et al*, 1995). The additional active TB cases lead to increased *M. tuberculosis* transmission within the community, thereby constituting an additional means by which HIV increases TB morbidity (Narain *et al*, 1992). The incidence of extrapulmonary TB in a community depends on two factors: (i) the prevalence of TB in that community and (ii) the degree of immune deficiency in TB infected individuals (Chaisson *et al*, 1987; Barnes *et al*, 1991; Mlika-Cabanne *et al*, 1995). In HIV negative patients tuberculous pericarditis was estimated to occur in 1-2% of instances of pulmonary TB (Larrieu *et al*, 1980). Since the early 1980s, the proportion

of patients with extrapulmonary TB has drastically increased as a result of the HIV epidemic (Harries, 1990; Narain *et al*, 1992). Several reports have documented the relationship between pericardial TB and HIV infection, with seropositivity being observed in 67-92% of patients (Cegielski *et al*, 1990; Pozniak *et al*, 1994; Maher and Harries, 1997). Tuberculous pericarditis usually presents with a history of dyspnoea, fever and features suggestive of congestive cardiac failure (Strang, 1984). In the context of HIV, congestive features could be caused by a number of other HIV-associated conditions, the most important of these being viral carditis, HIV-associated dilated cardiomyopathy, primary pulmonary hypertension with right ventricular failure and cor pulmonale secondary to chronic disease (Patel and Frishman, 1995; Barbaro 2001).

TUBERCULOUS PERICARDITIS

Although it is an important cause of morbidity and mortality in countries where TB is endemic, tuberculous pericarditis receives scant attention in the world literature. It is the most common cause of pericardial effusions among HIV-infected and underprivileged populations of sub-Saharan Africa (Desai, 1979; Strang, 1984; Cegielski *et al*, 1991; Cegielski *et al*, 1994; Maher and Harries, 1997), as well as Afro-Americans in the United States of America (Reynolds *et al*, 1992; Kwan *et al*, 1993; Lorell, 1997). In the Eastern Cape Province (Transkei region) of South Africa, tuberculous pericarditis is such a common cause of cardiac failure that it is known as "Transkei heart" (Strang, 1984). The HIV epidemic of sub-Saharan Africa is likely to worsen this situation (Fowler, 1991, Cantwell *et al,* 1992; Reynolds *et al*, 1992; Kwan *et al*, 1993; Hakim *et al*, 2000). It is important to diagnose tuberculous pericarditis efficiently because it is treatable. If left untreated, however, it has a mortality

approaching 85% as a result of cardiac tamponade, constriction or disseminated TB (Harvey and Whitehill, 1937; Desai, 1979).

Tuberculous pericardial disease most often develops as the result of the breakdown of TB-infected mediastinal lymph nodes, particularly those at the tracheobronchial bifurcation (Spodick, 1956; Rooney *et al*, 1970; Ortbals, 1979; Cherian *et al*, 2003a; Cherian, 2004). The spread is either direct or via lymph channels that merge at points where the parietal pericardium and the pleura separate (Cherian, 2004). Studies in humans (Eliskova *et al*, 1995), in macaque monkeys (Eliskova *et al*, 1992) and in dogs (Miller *et al*, 1988) demonstrated that lymphatic drainage of the pericardium is mainly to the anterior mediastinal tracheobronchial, lateropericardial, and posterior mediastinal lymph nodes and not into the hilar nodes. Computerised tomography (CT) of the chest may demonstrate enlarged mediastinal lymph nodes, which have been reported to occur in virtually 100% of patients with pericardial TB (Cherian *et al*, 2003b). Enlarged nodes disappeared or regressed with specific anti-tuberculous therapy (Cherian *et al*, 2003b).

Less commonly, pericardial TB results from breakdown and contiguous spread of a necrotic tuberculous lesion in the lung, pleura, or spine or from early haematogenous spread from the primary tuberculous infection (Peel, 1948; Auerbach, 1950; Cherian *et al*, 2003a).

Pathologically, a number of stages are recognised in the development of tuberculous pericarditis. The early stage is characterised by a fibrinous serosal exudate that contains lymphocytes. The middle phase manifests granuloma formation, and the

presence of viable acid-fast bacilli, and this is usually followed by absorption of the effusion, pericardial thickening, and proliferation of granulomata accompanied by caseating necrosis. At this stage, viable acid-fast bacilli are often no longer detected. In the late stage, fibrous pericarditis develops as the granulomatous reaction is replaced by fibrous tissue and collagen (Peel, 1948; Lorell, 1997; Nardell *et al*, 2004). These changes may be followed by the accumulation of cholesterol crystals and the development of pericardial calcification (Lorell, 1997). According to the literature, constrictive pericarditis develops ultimately in almost all patients with untreated tuberculous pericarditis and in up to half of the patients who receive anti-tuberculous chemotherapy (Schrire, 1959; Hageman *et al,* 1964; Long *et al,* 1989; Komsuoglu *et al,* 1994). Before the advent of effective chemotherapy, TB pericarditis was usually fatal, either in the acute stage due to cardiac tamponade or disseminated TB, or later as a result of constriction (Harvey and Whitehill, 1937). With the advent of effective anti-tuberculous chemotherapy in the 1940s, the mortality had decreased to about 35% of cases by 1970 (Shapiro, 1953; Schepers, 1962; Hageman *et al,* 1964; Rooney *et al,* 1970).

DIAGNOSIS OF PERICARDIAL TB

The clinical presentation is variable and includes acute pericarditis with or without effusion, cardiac tamponade, silent large pericardial effusion with a relapsing course, toxic symptoms with persistent fever, acute constrictive pericarditis, subacute constriction, effusive-constrictive, or chronic constrictive pericarditis (Permanyer-Miralda *et al*, 1985; Spodick, 1997).

The predominant symptoms of pericardial TB include dyspnoea, cough, chest pain, night sweats, orthopnoea, weight loss and ankle oedema. The most frequent signs are cardiomegaly, hepatomegaly, fever and tachycardia. Other findings may include pericardial rub, pulsus paradoxus, distended neck veins, pleural effusion and soft heart sounds (Schepers, 1962; Hageman *et al*, 1964; Rooney *et al*, 1970; Fowler and Manitas, 1973; Gooi and Smith 1978; Ortbals and Avioli, 1979; Desai, 1979; Fowler, 1991).

The prevalence of tuberculous pericarditis varies widely with geographic location and so the positive predictive value of characteristic clinical features varies as well (Trautner and Darouiche, 2001). In sub-Saharan African countries where TB and HIV co-infection is endemic, and where access to pericardial biopsy and microbiologic studies is difficult, symptoms and signs of pericardial effusion in HIV-infected individuals may be enough to initiate anti-tuberculous therapy (Cegielski *et al*, 1994; Pozniak *et al*, 1994; Strang, 1997). In other countries, however, numerous other infectious and non-infectious causes may present with similar features (Montgomerie *et al*, 1975) and further diagnostic work-up is indicated. Echocardiography is the definitive investigation for the presence of pericardial effusion, and is useful to distinguish tamponade from subacute constriction (Strang *et al*, 2004; Quarashi *et al*, 2005).

The tuberculin skin test is performed by intradermal injection of purified protein derivative (PPD). It was reported in all patients with pericardial TB in one report (Rooney *et al*, 1970) and in 239/240 in another (Strang *et al*, 1988). Although a positive tuberculin skin test result increases the suspicion of pericardial TB, it is

important to realise that a negative result does not exclude tuberculous disease, and the response to tuberculin may be affected by the time of presentation (Fowler and Manitas, 1973; Rooney *et al*, 1970; Alvarez and McCabe, 1984; Pozniak *et al*, 1994; Trautner and Darouiche, 2001). The tuberculin skin test may be false negative in 25–33% of tests (Sagrista-Sauleda *et al*, 1991) and false positive in 30–40% of patients (Fowler, 1991). The more sensitive and accurate enzyme-linked immunospot (ELISPOT) test detects T-cells specific for *M. tuberculosis* antigen (Ewer *et al*, 2003; Liebeschuetz *et al*, 2004). This test is, however, only useful to detect infection, and does not differentiate between latent infection and active disease.

Pericardial fluid smears for acid–fast bacilli are usually negative (Cherian, 2004). The reported diagnostic yield of pericardiocentesis in tuberculous pericarditis ranges from 29–77% (Rooney *et al*, 1970; Fowler and Manitas, 1973; Gooi and Smith, 1978; Quale *et al*, 1987; Strang *et al*, 1988; Sagrista-Sauleda *et al*, 1988; Fowler, 1991; Uthaman *et al*, 1997; Trautner and Darouiche, 2001). Mycobacterial culture may take up to eight weeks and results are usually only available after patients have already been discharged from hospital (Rooney *et al*, 1970; Fowler and Manitas, 1973; Trautner and Darouiche, 2001). Pericardial biopsy may have a better diagnostic yield than pericardial fluid culture, but is invasive and requires surgical expertise. Histological evidence of granulomatous inflammation with the demonstration of acid-fast bacilli would be a definite diagnostic criterion. The typical granuloma is, however, not always found and the pericardial biopsy may show non-specific findings, even when *M. tuberculosis* is found in the pericardial fluid (Strang *et al*, 1988; Cherian *et al*, 2003b; Cherian, 2004). Pericardial biopsy specimens should also be sent for mycobacterial culture and, where available, polymerase chain reaction (PCR) for

M. tuberculosis to optimise the diagnostic yield (Cegielski *et al*, 1997; Trautner and Daroiche, 2001).

Fibre-optic pericardioscopy facilitates visualisation of the parietal pericardium and epicardium on the anterior, posterior and inferior surfaces of the heart allowing selective biopsies beyond the small region of pericardium that is usually accessible with a subxiphoid incision (Maisch *et al*, 1992). Pericardioscopy with optically guided epicardial biopsy offers the potential for analysis of small tissue samples for myopericarditis using conventional histology as well as new molecular techniques of PCR and *in situ* hybridisation (Maisch, 1994; Ziskind *et al*, 1994). In patients with suspected but undocumented malignancy, pericardial biopsy increases the yield of a positive diagnosis of malignancy compared with pericardial fluid cytology alone (Maisch and Drude, 1992; Maisch, 1994; Ziskind *et al*, 1994). Nugue *et al* (1996) reported improved diagnostic accuracy for tuberculous pericarditis by using pericardioscopy and pericardial biopsy.

Analyses of the pericardial fluid specific gravity (>1.015), protein level (>30 g/L; pericardial fluid/serum protein ratio >0.5), pericardial fluid LDH (>200 U/L) and pericardial fluid/ serum LDH ratio >0.6) can separate exudates from transudates but are not directly diagnostic (Burgess *et al*, 2002a). Tuberculous pericardial fluid demonstrates high specific gravity, high protein levels, and high white cell count (Fowler, 1991). In suspected cases of pericardial TB, adenosine deaminase (ADA) activity, pericardial lysozyme and PCR analyses for *M. tuberculosis* may assist in confirming the diagnosis, whereas cytology and tumour markers (carcinoembryonic antigen [CEA], alpha-feto protein [AFP], carbohydrate antigens) are more important

for the diagnosis of suspected malignant disease (Meyers *et al*, 1997; Permanyer-Miralda *et al*, 1985; Garcia *et al*, 1994; Seo *et al*, 1993; Koh *et al*, 1994; Komsuoglu *et al*, 1995; Aggeli *et al*, 2000; Dogan *et al*, 1999; Lee *et al*, 2002). Differentiation of tuberculous and neoplastic effusions is virtually absolute with low levels of ADA and high levels of CEA (Koh *et al*, 1994). In one study, very high ADA levels suggested prognostic value for pericardial constriction (Komsuoglu *et al*, 1995). PCR assays for the detection of *M. tuberculosis* have been used to diagnose pericardial TB (Cegielski *et al*, 1997; Rana *et al*, 1999; Lee *et al*, 2002). Cegielski *et al* (1997) performed PCR with pericardial fluid and with pericardial biopsy specimens. PCR gave one false positive result for a patient with septic pericarditis (n=19). The sensitivity of PCR was higher with tissue specimens (12 out of 15; 80%) than with fluid specimens (2 out of 13; 15%; p=0.002). The diagnostic accuracy of PCR (tissue specimens) approached the results of conventional methods, but PCR was much faster than culture. The sensitivity of PCR with pericardial fluid was poor (Cegielski *et al*, 1997). False positive results remain a concern (Cegielski *et al*, 1997; Lee *et al*, 2002). Besides diagnosing TB, PCR analysis is also useful for the detection of cardiotropic viruses and to discern viral from autoreactive pericarditis (Maisch *et al*, 2002a). Perimyocardial tuberculous involvement is associated with high serum titres of antimyolemmal and antimyosin antibodies (Maisch *et al*, 1982). The use of interferon-gamma (IFN-γ) provided the basis for rapid and efficient diagnosis of pleural TB (Villegas *et al*, 2000) and of pericardial TB (Burgess *et al*, 2002b).

For the differentiation of tuberculous pericarditis from septic pericarditis, a pericardial fluid Gram stain has a specificity of 99%, but a sensitivity of only 38% for exclusion of bacterial infection in comparison to bacterial cultures (Maisch *et al*, 2004). In

suspected septic pericarditis, Gram staining and at least three cultures of pericardial fluid for aerobes and anaerobes as well as blood cultures are mandatory (Maisch *et al*, 2004). Pericardial fluid white blood cell (WBC) counts are highest in inflammatory diseases, particularly of bacterial and rheumatologic origin (Meyers *et al*, 1997).

DIFFERENTIAL DIAGNOSIS OF HIV-ASSOCIATED CARDIAC DISEASE

HIV-related cardiac disease is often masked or mimicked by pulmonary conditions, such as pulmonary TB, community acquired pneumonia or infection with *Pneumocystis jeroveci*. Radiological differentiation between cardiac failure and pulmonary disease is sometimes impossible. In patients with inappropriate tachycardia and/or increased cardio-thoracic ratio on chest radiograph, any form of ultrasonography may be useful to confirm the diagnosis of pericardial effusion and to evaluate its significance (Wragg and Strang, 2000; Quarashi *et al*, 2005). In industrialised countries echocardiographic evidence of pericardial effusion has been reported to be present in 22-53% of HIV-infected patients, and at autopsy in 15-59% of cases (Fink *et al,* 1984; Hecht *et al*, 1986; Anderson and Virmani, 1990; Heidenreich *et al*, 1995). It may be related to opportunistic infections, Kaposi's sarcoma or non-Hodgkin's lymphoma, but often a clear aetiology is not found (Heidenreich *et al*, 1995). Even in the USA tuberculous aetiology has to be considered in patients presenting with large pericardial effusions, especially in HIV-infected individuals and those that have come from or travelled to a TB endemic region (Reynolds *et al*, 1992; Kwan *et al*, 1993; Nardell *et al*, 2004). Pericardial TB needs to be differentiated from pericarditis caused by other opportunistic infections (bacterial, fungal, protozoal or viral) and also from acute HIV pericarditis, which presents like idiopathic pericarditis (Acierno, 1990; Reynolds *et al*, 1992; Kwan *et al*,

1993). Advances in pericardioscopy and optically directed pericardial and epicardial biopsy offer the potential for the diagnosis of both viral and tuberculous pericarditis using molecular techniques of *in situ* hybridisation and PCR (Satoh *et al*, 1993; Saatci *et al*, 1993; Maisch, 1994).

HIV-associated myocarditis may result in acute cardiac failure, arrhythmias, or conduction disturbances and the development of a chronic dilated cardiomyopathy (Herskowitz, 1996). HIV-associated cardiomyopathy is characterised by global systolic functional impairment with or without left ventricular dilatation (Magula and Mayosi, 2003). The cardiomyopathy is not related to any specific opportunistic infection and not associated with classic cardiac risk factors such as diabetes mellitus or hypertension. It has been postulated that the myocardial damage results from cytokines released by HIV-infected lymphocytes and monocyte-macrophages that invade the myocardium (Patel and Frishman, 1995; Herskowitz, 1996). Cardiomyopathy is associated with more advanced immunosuppression and the majority of patients with severe cardiomyopathy have CD4+ lymphocyte counts <200 cells/μL (Herskowitz, 1996; Barbaro, 2002; Nzuobontane *et al*, 2002). HIV-associated cardiomyopathy has also been linked to treatment with zidovudine (AZT) and to nutritional deficiencies (Hoffman *et al*, 1999; Barbaro, 2001). AZT may cause mitochondrial dysfunction by inhibiting mitochondrial deoxyribonucleic acid (DNA) replication (Lewis *et al*, 2000). Reversible cardiac dysfunction is associated with prolonged high-dose therapy with doxorubicin and interferon alpha (Rerkpattanapiatt *et* al, 2000) used to treat Kaposi's sarcoma, and foscarnet which is used to treat cytomegalovirus (CMV) infection (Bristow *et al*, 1978; Brown *et al*, 1993).

The prevalence of infective endocarditis in HIV-infected patients is similar to that in other patients who abuse intravenous drugs (Rerkpattanapiatt *et* al, 2000). Right-sided valves are predominantly affected and the most common pathogens are *Staphylococcus aureus*, *Candida albicans*, *Aspergillus fumigatus*, *Histoplasma capsulatum*, and *Cryptococcus neoformans* (Barbaro *et al*, 1998; Rerkpattanapiatt *et al*, 2000). Late stage HIV-disease with significant immunodeficiency is associated with an increased mortality (Rerkpattanapiatt *et* al, 2000). Non-bacterial thrombotic endocarditis (marantic endocarditis) is most common in patients with HIV-wasting syndrome. It is characterised by endocardial vegetations, consisting of platelets within a fibrin mesh with few inflammatory cells, and may cause systemic or pulmonary embolisation (Rerkpattanapiatt *et* al, 2000).

A strong association between HIV infection and primary pulmonary hypertension (PPHT) has been recognised (Rerkpattanapiatt *et* al, 2000), and this condition needs to be echocardiographically excluded in HIV-positive patients who present with features of right ventricular failure.

TREATMENT OF TUBERCULOUS PERICARDIAL EFFUSION

The goal of therapy for tuberculous pericarditis is not only to treat the acute symptoms of tamponade, but also to prevent progression from the effusive to the constrictive stage, in which a fibrotic and calcified pericardium entraps the heart (Desai, 1979; Fewell *et al*, 1971). The mortality rate in untreated acute effusive tuberculous pericarditis approaches 85% (Desai, 1979) and pericardial constriction occurs in 30–50% (Sagrista-Sauleda *et al*, 1988; Long *et al*, 1989). Three issues arise in the treatment of tuberculous pericarditis: (i) the duration of anti-tuberculous therapy, (ii)

the use of adjuvant corticosteroids, and (iii) the need for open surgical drainage versus closed pericardiocentesis. Various anti-tuberculous drug combinations of different time periods (6, 9 and 12 months) have been applied without clear differences in the treatment outcome (Sagrista-Sauleda *et al*, 1988; Strang *et al*, 1988; Fowler, 1991; Koh *et al*, 1994). Prevention of constriction in chronic pericardial effusion of undetermined aetiology by empiric anti-tuberculous treatment was not successful (Dwivedi *et al*, 1997), and therefore only patients with proven or highly likely tuberculous pericarditis should be treated with anti-tuberculous drugs. The use of adjuvant corticosteroids remains controversial (Strang *et al*, 1988; Alzeer and Fitzgerald, 1993; Senderovitz and Viskum, 1994; Mayosi *et al*, 2002; Ntsekhe *et al*, 2003; Strang *et al*, 2004; Yang *et al*, 2005). A meta-analysis of patients with effusive and constrictive tuberculous pericarditis (Mayosi *et al*, 2002; Ntsekhe *et al*, 2003) suggested that anti-tuberculous treatment combined with steroids might be associated with fewer deaths and less frequent need for pericardiocentesis or pericardiectomy, but that published trials were too few and too small to be conclusive. The Cochrane systematic review (last updated in 2002) included only four trials: two randomised controlled trials performed in the Transkei (Strang *et al*, 1987; Strang *et al*, 1988), one non-randomised trial (Schrire, 1959), and a fourth study involving only HIV-infected patients (Hakim *et al*, 2000). Pericardiectomy is indicated if, in spite of combination therapy, constriction develops (Maisch *et al*, 2004).

Standard management of pericardial effusion includes pericardiocentesis. Echocardiographically guided pericardiocentesis is safe and can be performed at the bedside (Tsang *et al*, 2002a; Strang *et al*, 2004). Echocardiography should identify the safest route where the pericardium can be entered intercostally or subcostally. After

attempted complete drainage the catheter is kept *in situ* and prolonged pericardial drainage is performed until the volume of effusion obtained by intermittent pericardial aspiration falls to <25 mL per day (Tsang *et al*, 2002b). The most serious complications of pericardiocentesis are laceration and perforation of the myocardium and the coronary vessels. In addition, patients can experience air embolism, pneumothorax, arrhythmias (usually vasovagal bradycardia) and puncture of the peritoneal cavity or abdominal viscera (Seferović *et al*, 2000). Internal mammary artery fistulas, acute pulmonary oedema and purulent pericarditis are rarely reported. The safety was improved with echocardiographic or fluoroscopic guidance. Recent large echocardiographic series reported an incidence of major complications of 1.3–1.6% (Tsang *et al*, 1998; Tsang *et al*, 1999; Tsang *et al*, 2002a; Tsang *et al*, 2002b).

The literature favours surgical fenestration to pericardiocentesis for the management of tuberculous pericarditis (Strang *et al*, 1988; Hakim *et al*, 2000; Trautner and Darouiche, 2001; Quraishi *et al*, 2005). The open procedure has the potential advantage that pericardial tissue is obtained for mycobacterial culture and histopathological diagnosis (Trautner and Darouiche, 2001).

THE PREVENTION AND TREATMENT OF TB

The reduction and prevention of pericardial TB depends on the global control of TB. Fuelled by the concomitant HIV pandemic, TB control measures rely more than ever on improved case finding, earlier treatment of smear positive individuals and better cure rates of the highest possible number of diseased individuals (Kochi, 1991). This includes those with extrapulmonary TB, which is more difficult to diagnose and treat and more frequently present in HIV positive than HIV negative patients (Nunn *et al*,

1991; Narain *et al*, 1992). TB control contributes to the elimination of poverty, especially in poorer countries (Department for International Development, 1997). Since TB affects the poorest societies disproportionately, the countries with the highest prevalence of the disease have the fewest resources to deal with it (Gwatkin and Guillot, 1998). Anti-tuberculous chemotherapy is an effective and affordable intervention that improves quality and duration of life. The WHO promotes the directly observed therapy, short-course (DOTS) strategy, which is based on political commitment, effective diagnosis and prioritisation of cases with the greatest need, standardised evidence-based protocols and a strategy to ensure that these strategies are properly adhered to, a regular supply of quality drugs is available, and an effective system for recording and reporting that focuses upon outcomes (Global Tuberculosis Programme, 1994). A total of 182 countries were implementing the DOTS strategy during 2003, and by the end of 2003, 77% of the world's population lived in countries covered by DOTS (WHO, 2005). In total, 17.1 million TB patients, including 8.6 million smear-positive patients, were treated in DOTS programmes between 1995 and 2003.

One of the keys to the prevention of TB is the early diagnosis and effective treatment of infectious patients who are coughing up TB bacilli. In order for treatment to be effective, it is essential that the correct combination of drugs be given for an appropriate period of time. Only actively replicating organisms are killed by chemotherapy, and differences in mycobacterial metabolic rate are associated with differences in mycobacterial susceptibility to anti-tuberculous drugs. To prevent the emergence of drug-resistant mutants, anti-tuberculous therapy should always consist of at least two effective drugs (Haas and Des Prez, 1995). Short-course regimens are

divided into an initial or bactericidal phase and a continuation or sterilising phase. First-line anti-tuberculous drugs include isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA), ethambutol (EMB) and streptomycin (SM). Therapy should not be stopped unless one is certain that the disease is controlled and the smears/cultures are negative. The treatment regimens of the South African National TB Control Programme have been summarized in Tables 1.1 and 1.2. New adult patients (including those with extrapulmonary TB) require treatment for a full six months and "retreatment" patients for a full eight months.

THE USE OF ADJUNCTIVE CORTICOSTEROIDS IN TB

Corticosteroids have been shown to be useful in fulminant miliary disease, obstructive lymphadenopathy, adrenal TB and in patients with tuberculous meningitis stages 2 and 3 (Prasad, 2000). Their benefit in pleurisy is minimal (Ferrer, 1997; Morehead, 1998) and their role in pericardial disease is unclear (Hakim *et al*, 2000; Mayosi *et al,* 2002; Ntsekhe *et al*, 2003; Strang *et al*, 2004; Yang *et al*, 2005).

Table 1.1 Regimen 1 for the treatment of new adult TB patients (before 2004)

Intensive phase: 2 months	**Body weight under 50 kg**	**Body weight over 50 kg**
RMP / INH / PZA 120/80/250 mg FCT	4 tablets	5 tablets
EMB 400 mg tablet	2 tablets	3 tablets
Daily number of tablets	6	8
Continuation phase: 4 months		
RMP / INH 150/100 mg FCT	3 tablets	-
RMP / INH 300/150 mg FCT	-	2 tablets
Daily number of tablets	3	2

RMP = rifampicin

INH = isoniazid

PZA = pyrazinamide

EMB = ethambutol

FCT = fixed combination tablet

Table 1.2 Regimen 2 for the retreatment of adult TB patients (before 2004)

Intensive phase: 2 months	**Body weight under 50 kg**	**Body weight over 50 kg**
Streptomycin (intramuscular injection)	750 mg	1000 mg
RMP / INH / EMB 120/75/300 mg FCT	3 tablets	4 tablets
PZA 500 mg tablet	2 tablets	3 tablets
Daily number of tablets	5	7
Third month		
RMP / INH / EMB 120/75/300 mg FCT	3 tablets	4 tablets
PZA 500 mg tablet	2 tablets	3 tablets
Daily number of tablets	5	7
Continuation phase: 5 months		
RMP / INH / EMB 120/75/300 mg FCT	3 tablets	4 tablets
Daily number of tablets	3	4

RMP = rifampicin

INH = isoniazid

PZA = pyrazinamide

EMB = ethambutol,

FCT = fixed combination tablet

TREATMENT OF TB IN HIV-INFECTED PATIENTS

Treatment of TB in patients with HIV infection is extremely effective when begun promptly and regimens contain rifampicin (Small *et al,* 1991; Chaisson *et al,* 1996). The use of highly active antiretroviral therapy (HAART) in the treatment of patients co-infected with TB and HIV is problematic because there are potential complex drug interactions, overlapping adverse reactions, potential non-adherence due to the pill burden, and drug malabsorption (Burman and Jones, 2001). Despite these potential problems, HAART substantially reduces new AIDS events and death in co-infected patients (Badri *et al*, 2002; Dheda *et al*, 2004). Those with CD4+ lymphocyte counts <100 cells/μL have a high event risk during the intensive phase of anti-tuberculous treatment (Dheda *et al*, 2004). Paradoxical deterioration due to the immune reconstitution inflammatory syndrome (IRIS) has been reported to occur in 11-36% of patients with TB who start HAART (Narita *et al*, 1998; Wendel *et al*, 2001). Secondary preventive therapy with INH reduces TB recurrence in HIV infected patients: the absolute impact seems to be greatest among individuals with low CD4+ lymphocyte counts (Churchyard *et al*, 2003).

Chapter 2

PATIENTS AND METHODS

The primary aim of the study was to optimise the management of patients with tuberculous pericarditis. To achieve this we analysed the hospital based epidemiology of pericardial disease, studied various aspects of the immunopathogenesis of pericardial tuberculosis (TB), evaluated a variety of diagnostic tests and conducted a randomised controlled trial to establish the optimal therapeutic management of patients with tuberculous pericarditis. During the period from February 1995 to June 2001, all adolescent and adult patients presenting to the Cardiology Unit with large pericardial effusions (defined as epi-pericardial separation of more than 10 mm) were included in this study and followed for one year. All patients gave written informed consent for participation in the study, which was approved by the Ethics Committee of Stellenbosch University. Demographic, clinical and echocardiographic data were obtained at baseline.

DIAGNOSTIC PROTOCOL

All patients had a full clinical assessment, a structured history was taken and a standardised diagnostic work-up (Table 2.1) was performed. A positive TB contact was defined as prolonged exposure to another person with active TB or individuals on TB treatment. Prolonged exposure included working, co-habiting and/or socialising with an infectious person for more than one month.

Table 2.1. Diagnostic protocol for patients requiring pericardiocentesis

Pericardial fluid
Adenosine deaminase activity (ADA)
Lactate dehydrogenase (LDH)
Total protein
Glucose
Cytology
Differential white blood cell count
Gram / Ziehl-Neelsen (ZN) stain and direct microscopy
Culture (BACTEC™) for TB, fungi and bacteria
Peripheral blood
Renal function, liver function and thyroid function tests (T_4 and TSH)
Full blood count cell count and differential white blood cell count
ELISA tests for HIV (screening and confirmatory)
CD4+ and CD8+ lymphocyte count (HIV positive patients)
C-reactive protein (CRP),
Antinuclear factor (ANF)
Rheumatoid factor (RF)
Antistreptolysin–O titre (ASOT)
Blood culture (BACTEC™) for bacteria, fungi and TB
Other investigations
Chest radiograph (CXR) - posterior-anterior and lateral views
Echocardiography (M-mode and four chamber view)
Electrocardiography (12-lead surface ECG)
Sputum collection for Gram and ZN staining, microscopy and culture
Tuberculin skin tests with purified protein derivative (n=52)
Pericardial biopsy and histopathology (n=36)

All patients with large pericardial effusions were subjected to CXR, 12-lead surface ECG (MAC, Marquett Electronics, inc; Milwakee, Wisconsin; paper speed 25 mm/sec) and a two-dimensional echocardiographic study (Hewlett Packard, Sonos 2000 Phased Array Imaging System). Sputum was collected on three consecutive days, stained with auramine or Ziehl-Neelsen (ZN) stain and examined by fluoromicroscopy or direct microscopy. Sputum was also cultured for *M. tuberculosis*. Evaluation of the pericardial fluid included: (i) differential WBC count; (ii) cytopathological analysis; (iii) total protein; (iv) lactate dehydrogenase (LDH), and (v) adenosine deaminase (ADA) activity.

A sample of the aspirated pericardial fluid (5-10 mL) was sent to the Department of Microbiology for Gram and ZN staining and microscopic examination. In addition, an aliquot of pericardial fluid (7 mL) was injected into a BACTEC^{TM} medium immediately after completion of the pericardiocentesis procedure and cultured routinely in an automated radiometric BACTEC^{TM} MGIT^{TM} 960 system (Becton Dickenson and Co, Hood USA). In addition, we evaluated the diagnostic utility of polymerase chain reaction (PCR) for the detection of *M. tuberculosis* and measuring interferon-gamma (IFN-γ) in pericardial aspirates.

The demographic and clinical data were recorded prospectively and hospital records were reviewed to reach a diagnosis. Effusions were classified into diagnostic groups according to pre-determined criteria.

ECHOCARDIOGRAPHY

Echocardiography was used to determine the following: (i) the location of the effusion (anterior, apical, posterior, inferior, circumferential), (ii) the size of the effusion by measuring the epi-pericardial distance (mm), (iii) the presence of epi- and pericardial thickening and measuring the maximal pericardial thickness (mm), (iv) the presence of tamponade and/or constriction, and (v) the safest route for the pericardiocentesis procedure. A standard protocol was used with examinations in the left parasternal long and short axes, apical two and four chamber and subcostal views. Additional views were dictated by the requirements of the individual case. Patients who gave informed consent were treated by echocardiographically guided pericardiocentesis under local anaesthesia. The term "large pericardial effusion" refers to the pericardial effusions that were echocardiographically characterised by >10 mm separation between the pericardium and the epicardium during diastole. Tamponade was defined by the presence of predetermined echocardiographic and /or clinical features. The echocardiographic features were defined as: (i) inversion of >30% of the right atrial wall during late diastole and/or early systole, and/or (ii) inward motion of the right ventricular wall in early diastole persisting after mitral valve opening (Gubermann *et al*, 1981). Apart from these echocardiographic findings, the patient was also considered to have tamponade if he/she complained of dyspnoea and had at least three of the following five physical signs that were relieved by pericardiocentesis: (i) tachycardia (ventricular rate >100/min); (ii) hypotension (systolic blood pressure <100 mm Hg); (iii) pulsus paradoxus (>10 mm Hg decrease in peak systolic pressure on inspiration); (iv) positive Kussmaul's sign (accentuation of jugular venous distension during inspiration), and/or (v) elevation of the jugular venous pressure ≥4 cm above the sternal angle.

BASELINE BIOCHEMISTRY

Pericardial aspirates obtained by echo-guided pericardiocentesis were analysed for biochemistry. Biochemical parameters on pericardial fluid and serum were determined using a multi-channel analyser (Bayer Technicon DAX 48). Total protein concentration (g/L) was estimated using the biuret method and albumin concentration (g/L) was measured using bromocresol green (both spectrophotometric methods). LDH concentration (U/L) was measured using an enzymatic ultra-violet optimised method. Pericardial aspirates were classified as exudates if they fulfilled more than one of the following criteria: protein level >30 g/L, pericardial fluid/serum protein ratio >0.5, pericardial fluid LDH >200 U/L, and pericardial fluid/ serum LDH ratio >0.6 (Burgess *et al*, 2002a).

DETERMINATION OF ADA

ADA activity (U/L) was determined according to the method described by Giusti (1974). Adenosine is deaminated by ADA and the free ammonia is deaminated by Berthelot's reaction. One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 μmol of ammonia per minute from adenosine at standard assay conditions. The enzyme is stable for at least 24 hours at 25°C, 7 days at 4°C and 3 months at -20°C (Ellis and Goldberg, 1970; Heinz, 1984).

PERICARDIAL FLUID MICROSCOPY AND CULTURE

Pericardial aspirates were obtained by closed pericardiocentesis procedure and a sample of the effusion was sent to the Department of Microbiology for Gram and ZN staining and microscopic examination. An aliquot of 7 mL of pericardial fluid was

injected into a bottle of BACTEC™ medium immediately after completion of the pericardiocentesis procedure and cultured routinely in an automated radiometric BACTEC™ MGIT™ 960 system (Becton Dickenson and Co, Hood USA), which uses added materials for detection of mycobacterial growth by radiometric or colorimetric systems. The MGIT Mycobacteria Growth Indicator Tube contains 7 mL of modified Middlebrook 7H9 broth base with growth supplement and PANTA antibiotic mixture. All types of clinical specimens, pulmonary as well as extrapulmonary (except blood), were processed for primary isolation in the MGIT tube.

SPUTUM MICROSCOPY AND TB CULTURE

When possible, three morning samples of expectorated sputum were examined by fluoromicroscopy after preparation and auramine-rhodamine staining. Sputum samples were prepared and fixed to slides in methanol for one minute. These were stained with auramine for 20 minutes, and rinsed thereafter with water. The slides were decolourised with 0.5% acid-alcohol solution for two minutes, followed by rinsing with water. The slides were counterstained with potassium permanganate for 30 seconds, followed by rinsing with water. Excess water was drained from slides and they were placed on a hotplate to dry. Auramine positive slides were ZN stained to confirm the presence of acid-fast bacilli (AFB). Smear negative sputum specimens were cultured for TB using an automated BACTEC 12B radiometric broth with and without PANTA antibiotic supplement.

TUBERCULIN SKIN TESTING

During the first 18 months of the study, tuberculin skin testing was part of the standardised diagnostic protocol. Initially patients underwent tuberculin skin testing with purified protein derivative (PPD). An experienced operator injected 0.1 mL of PPD intradermally; tests were interpreted 48-72 hours after administration of antigen and the transverse diameter of induration was measured (mm). An interim analysis performed at the end of the first 18 months suggested that tuberculin skin testing and pericardial biopsy did not contribute to the decision-making process and were therefore removed from the standardised diagnostic protocol.

PERICARDIAL FLUID CYTOLOGY

A sample of the pericardial effusion (5-50 mL) was sent to the Department of Anatomical Pathology for cytological analysis. The pericardial fluid aspirates were fixed by addition of an equal amount of 50% ethanol. The fluid was spun down and smears were prepared from sediments using routine methods (Wiener *et al*, 1991). Each specimen was stained according to the Papanicolaou method. Fluids that arrived at the laboratory in an unfixed state were used to prepare air-dried smears and these were stained according to the May-Grünwald-Giemsa method. The slides were then mounted and examined microscopically. The different cells were counted per hundred cells on various fields throughout the slide. A total of 300 cells were counted and then an average taken. The differential white blood cell counts were reported as percentages.

PERICARDIAL HISTOPATHOLOGY

During the first 18 months of the study pericardial biopsy was part of the standardised diagnostic protocol. A biopsy was performed when an aetiological diagnosis could not be made within seven days after initial pericardiocentesis or alternatively as a therapeutic procedure in all patients in whom adequate drainage could not be achieved by closed pericardiocentesis. Thereafter, patients were only referred for surgical pericardiocentesis when therapeutically indicated, including patients with loculated or posterior effusions, recurring effusions, or worsening effusive–constrictive pericarditis.

A pericardial biopsy was taken of accessible tissue under general anaesthesia. The biopsy tissue was sent for histopathological evaluation. One sample of each specimen was formalin-fixed (using 10% buffered formalin solution) and processed, using routine histochemical techniques. Each piece of tissue was sectioned and stained with haematoxylin-eosin (H&E) and ZN stains. Two independent histopathologists who were not aware of the final diagnosis examined the mounted sections.

FLOW CYTOMETRIC PHENOTYPIC LEUKOCYTE ANALYSES

By using fluorescent labelled antibody, a variety of cell surface markers are recognised and specific cells can be identified easily and effectively. The presence and quantity of immune cells in the pericardial fluid was determined and compared to that of the peripheral circulation. We analysed the phenotypes of lymphocyte subpopulations, using specific monoclonal antibodies with colour conjugates, including fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridine chlorophyll

(Per-CP) and allophycocyanin (APC; Becton Dickinson, Bactlab Systems). The following panels were included:

- CD45-PerCP/ CD3-FITC/ CD4-APC/ CD8-PE for total T Lymphocytes, T-helper cells (Th cells) and cytotoxic (or suppressor) T cells and
- CD45-PerCP/ CD3-FITC/ CD19-APC/ CD16+56-PE for T cells, B cells and natural killer cells (NK cells).

The monoclonal antibodies were diluted 1:4 and 20 μL of the dilution added to 50 μL of pericardial fluid or whole blood. After incubation in the dark for 15 minutes at room temperature, the cells were incubated with 450 μL of a 1:10 dilution of Fluorescent Activated Cell Sorter (FACS) Lysing Solution (Becton Dickinson, Bactlab Systems) for an additional 10 minutes in the dark. The Lysing Solution helps to fix the surface epitopes and optimise the permeabilisation process. After centrifugation for 5 minutes at 1800 revolutions per minute (rpm), the cells were washed with 2 mL phosphate-buffered saline, and fixed with 500 μL of a fixing buffer, consisting of a 1:20 dilution of formalin with phosphate-buffered saline. The tubes were kept at 4°C in the dark and analysed within one week. Analysis was performed in a flow cytometer (FACScan, Becton Dickinson, Bactlab Systems), equipped with the analytical software programme, Lysis II. The cells were counted by the flow cytometer and results were expressed as percentage of the cells positive for the antigen detected by the monoclonal antibodies.

DETERMINATION OF CYTOKINE CONCENTRATIONS

At the time of pericardiocentesis, pericardial fluid was collected on ice and frozen within 30 minutes at -70°C for the analysis of cytokines. The concentration of a variety of cytokines was determined on pericardial fluid and serum samples using

commercially available immunoassays developed by Biotrak™ (Amersham Pharmacia Biotech, UK).

Determination of interleukin-4

The Biotrak™ human interleukin-4 [(h)IL-4] ELISA system (Amersham Pharmacia Biotech, UK) provides a simple, specific, reliable and precise quantitative determination of (h)IL-4 in plasma and serum. The assay system is based on a solid phase ELISA that utilizes an antibody for (h)IL-4 bound to the wells of a micro titre plate, together with a biotinylated antibody to (h)IL-4 and streptavidin conjugated to HRP. The assay employs the quantitative "sandwich" enzyme immunoassay technique. An antibody specific for (h)IL-4 has been coated on the micro titre plate provided in the kit. Samples (50 μL serum) are pipetted into the wells along with biotinylated antibody reagent (50 μL) and incubated for 2 hours (25°C). If present, the (h)IL-4 is bound by the immobilised antibody and the biotinylated antibody. After washing away any unbound sample proteins and biotinylated antibody, a streptavidin-HRP conjugate (100 μL) is added to the wells and the plate incubated for 30 minutes (25°C). Any (h)IL-4, which was bound by both the immobilised and the biotinylated antibody during the first incubation, will be bound by the streptavidin conjugate. Following a wash to remove unbound conjugate, a tetramethylbenzidine substrate solution (100 μL) is added to the wells and during the incubation of 30 minutes (25°C), colour develops in proportion to the amount of (h)IL-4 bound in the initial step. In addition to the samples to be assayed, a series of wells is prepared using known concentrations of the (h)IL-4 standard (0-400 pg/mL). A curve, plotting the optical density at 450 nm versus the concentrations of the standard wells, is prepared.

By comparing the optical density of the samples to this standard curve, the concentration of the (h)IL-4 in the unknown samples is then determined.

Determination of interleukin-10

The Biotrak™ human interleukin-10 [(h)IL-10] ELISA system (Amersham Pharmacia Biotech, UK) provides a simple, specific, reliable and precise quantitative determination of (h)IL-10 in plasma and serum. This solid phase ELISA assay is done in exactly the same way as the assay described for Biotrak™ human interleukin-4, but instead of using an antibody for (h)IL-4, it utilizes an antibody for (h)IL-10. In addition to the samples to be assayed, a series of wells is prepared using known concentrations of the (h)IL-10 standard (0-600 pg/mL). A curve, plotting the optical density at 450 nm versus the concentrations of the standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (h)IL-10 in the unknown samples is then determined.

Determination of tumour necrosis factor-alpha

The Biotrak™ human tumour necrosis factor-alpha [(h)TNF-α] ELISA system (Amersham Pharmacia Biotech, UK) provides a simple, specific, reliable and precise quantitative determination of (h)TNF-α in plasma and serum. This solid phase ELISA assay is done in exactly the same way as the assay described for Biotrak™ human interleukin-4, but instead of using an antibody for (h)IL-4, it utilizes an antibody for (h)TNF-α. In addition to the samples to be assayed, a series of wells is prepared using known concentrations of the (h)TNF-α standard (0-1000 pg/mL). A curve, plotting the optical density at 450 nm versus the concentrations of the standard wells, is

prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (h)TNF-α in the unknown samples is then determined.

Determination of gamma-interferon

The Biotrak™ human gamma-interferon [(h)IFN-γ] ELISA system (Amersham Pharmacia Biotech, UK) provides a simple, specific, reliable and precise quantitative determination of (h)IFN-γ in plasma and serum. This solid phase ELISA assay is done in exactly the same way as the assay described for Biotrak™ human interleukin-4, but instead of using an antibody for (h)IL-4, it utilizes an antibody for (h) IFN-γ. In addition to the samples to be assayed, a series of wells is prepared using known concentrations of the (h)IFN-γ standard (0-1000 pg/mL). A curve, plotting the optical density at 450 nm versus the concentrations of the standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (h)IFN-γ in the unknown samples is then determined.

PCR FOR *M. TUBERCULOSIS*

A commercially available PCR assay was used, namely the Roche Amplicor™ PCR for *M. tuberculosis* test for detection of the IS6110 sequence of *M. tuberculosis* in pericardial fluid specimens applying standard techniques and procedures (Carpentier *et al,* 1995; Yuen *et al,* 1993; Shah *et al,* 1998). Aliquots of pericardial fluid were frozen and stored at –70°C and batches of 12 consecutive effusions were analysed by PCR.

PREDETERMINED DIAGNOSTIC CRITERIA

Each diagnostic group was defined by the presence of a pericardial effusion and one or more criteria in the absence any other obvious cause associated with pericardial effusions:

Tuberculous pericarditis

- Isolation of *M. tuberculosis* from the drained pericardial effusion or pericardial biopsy specimen;
- Demonstration of granulomatous inflammation on histopathological examination of the pericardial biopsy sample;
- Presence of a lymphocytic pericardial exudate and identification of *M. tuberculosis* in sputum, pleural fluid or lymph node aspirate by ZN stain / culture, and a sustained response to anti-tuberculous therapy;
- Presence of a lymphocytic pericardial exudate and demonstration of necrotising granulomatous inflammation on histopathological examination of extracardiac tissue, and a sustained response to anti-tuberculous therapy (TB therapy), and/or
- Presence of a lymphocytic pericardial exudate and a chest X-ray suggestive of active TB, compatible clinical features, and a good response to TB therapy.

Septic pericardial effusions

- Pericardial effusions associated with acute febrile illness and responsiveness to antibiotic treatment or identification of the organism in the pericardial fluid by Gram stain and/or culture;

- Polymorphonuclear pericardial exudate in the presence of other obvious infectious conditions (e.g. pneumonia) in the absence of any other cause associated with pericardial effusions.

Malignant pericardial effusions

- Presence of cytological and / or histological evidence of a neoplastic process involving the pericardium, and / or
- Evidence of an extracardiac malignancy with exclusion of other cause known to be associated with pericardial effusions.

Uraemic pericarditis

- Pericardial effusion in the presence serum creatinine ≥ 300 μmol/L and urea >30 mmol/L) in the absence of other causes known to cause pericarditis, and / or
- Presence of pericardial effusion in dialysis patient known with renal failure, in the absence of other causes associated with pericardial effusion.

Pericardial effusions associated with cardiac surgery or trauma

- Pericardial effusion occurring within three months post-cardiac surgery

  or,
- Pericardial effusion occurring within three months after chest trauma in

  the absence of any other obvious cause for pericardial effusion.

Pericardial effusion associated with congestive cardiac failure:

- Presence of a pericardial transudate in combination with echocardiographic evidence of dilated ventricles and decreased ejection fraction <40%.

Pericarditis associated with systemic inflammatory and connective tissue diseases

- Systemic lupus erythematosus (according to the American College of Rheumatology [ACR] criteria);
- Mixed connective tissue disease (according to ACR criteria);
- Rheumatoid arthritis (according to the ACR criteria);
- Scleroderma (according to the ACR criteria);
- Acute rheumatic fever (according to the revised Duckett-Jones criteria).

Pericardial effusion associated with hypothyroidism

- Defined as those pericardial effusions occurring in the presence of biochemical evidence for hypothyroidism, i.e. serum thyroxine (T4) <10 mmol/L and thyroid stimulating hormone (TSH) levels > 5.6 pmol/L.

Idiopathic pericarditis

- Defined as those pericardial effusions for which after full diagnostic work-up there was no demonstrable cause.

Pericardial effusions of indeterminate origin

- Patients having multiple superimposed diseases or effusions of unknown origin, that is all possible aetiological causes could not be excluded.

THERAPEUTIC MANAGEMENT PROTOCOL

Each patient underwent echocardiographically guided pericardiocentesis and daily intermittent drainage by indwelling pigtail catheter. After initial complete drainage, the pigtail catheter was kept *in situ* to allow daily intermittent drainage and it was only removed when the daily aspirate amounted to less than 100 mL or when it became blocked. Previous studies have demonstrated that the use of extended catheter drainage is associated with decreased risk of recurrence of effusion and use of surgery for management (Kopecky *et al,* 1986; Tsang *et al,* 1998; Tsang *et al,* 1999).

For the pericardiocentesis, patients were usually positioned supine with the head and upper body slightly elevated. In patients experiencing orthopnoea due to the supine position, the echocardiographic examination and the pericardiocentesis was performed with the patient sitting upright. The ideal entry site for the drainage procedure was the point on the body surface where the effusion is closest to the ultrasound transducer and the fluid accumulation is maximal. The distance from the skin to the pericardial space was assessed and the needle trajectory defined by the angulation of the handheld transducer. A straight trajectory was selected that avoided vital structures such as the liver, myocardium and the lung. The intended point was marked on the skin and the direction of the ultrasound beam noted. A wide area around the intended puncture site was cleaned with an antiseptic solution (povidone-iodine followed by chlorhexidine) and this area was subsequently covered with a transparent plastic sheet. Pericardiocenteses were initiated with a 16- or 18-gauge, sheathed “intracath” needle with an attached syringe after infiltrating the skin with local (2% lignocaine) using a 20-gauge needle. The “intracath” needle was positioned at the predetermined entry site, held at the intended angle and then slowly

advanced in the direction of the fluid-filled space while exerting gentle negative pressure by the attached syringe. To prevent potential injury of the neurovascular bundle, which runs along the inferior margin of each rib, the needle was always introduced in proximity of the upper margin of the rib. On entering the fluid, the needle was advanced by a further 2 mm before the Teflon sheath was pushed over the needle and the steel core withdrawn, so that only the sheath remained in the fluid space. A flexible guide wire was inserted through the sheath. After making a small stab incision of the skin at the entry site, a dilator and introducer sheath (6 to 8 French) were advanced over the wire into the pericardial sac. Predilatation of the chest wall passage facilitated insertion of the introducer sheath-dilator and minimised burring of the sheath tip. The guide-wire and dilator were removed and only the sheath left in the pericardial sac. In our view, the introducer sheath technique is superior to direct catheter passage over the guide-wire, because it prevents potential dislodging of the guide wire by the catheter tip. Once the introducer sheath and guide wire were placed successfully in the pericardial space, a 6 or 7 French thin-walled pigtail angiocatheter (65 cm) was inserted. The sheath could then be withdrawn leaving only the pigtail catheter in the pericardial space that needed to be drained. The catheter was attached to a 50-mL syringe via a three-way stopcock for initial complete drainage by manual suction. When no further fluid passed, echocardiography was repeated to confirm successful and satisfactory removal of fluid. Antiseptic ointment was applied to the entry site and after covering the catheter with a sterile dressing it was secured carefully to the chest wall.

After the pericardiocentesis patients were admitted to a general medical ward where their condition could be monitored clinically, but without continuous ECG

monitoring. While the catheter was *in situ*, the patients were encouraged to be ambulatory and upper body movement was usually not restricted. The patients were monitored clinically for an increase in the volume of, or a change in, appearance of the aspirated fluid, the development of acute chest pain and a change in the vital signs, such as development of tachycardia, hypotension, tachypnoea or fever. Following the initial pericardiocentesis, pericardial fluid was aspirated aseptically through the three-way stopcock at 24-hour intervals, or more frequently, if clinically indicated. After the aspiration, heparinised saline solution (3-5 mL of a solution of 5000 IU of heparin diluted in 200 mL of normal saline) was injected to prevent catheter blockage. Maintaining an aseptic technique, the syringe and tap were covered at the end of the procedure with a sterile latex glove to avoid contamination. A new, sterile glove was applied after each daily aspiration. The catheter was removed when the daily amount of aspirated fluid had decreased to less than 100 mL and the patient was haemodynamically stable. Echocardiography was not routinely performed at this stage. Pericardial fenestration and open drainage were only considered if the closed pericardiocentesis failed to alleviate cardiac compression satisfactorily or if the daily aspirate continued to exceed 100 mL after five days of intermittent drainage.

Empiric anti-tuberculous chemotherapy was commenced if after rigorous investigation no alternative diagnosis could be reached within the first seven days after the pericardiocentesis. Anti-tuberculous treatment was initiated in hospital and after discharge continued at the TB clinic closest to the affected individual's home. The National TB Control Programme guidelines were followed and treatment regimens for new adult patients and retreatment patients are summarised in Tables 1.1.and 1.2. The clinical response was evaluated over a period of one year and follow-

up visits were scheduled for one month, three months, six months and one year after the pericardiocentesis. Patients were reminded by letter and/or telephonically about their follow-up date and received a travel allowance for attending their appointment. Patients who were found to be HIV positive were referred to the Infectious Diseases Clinic at Tygerberg Academic Hospital for staging and treatment. Each patient was started on oral cotrimoxazole as primary prophylaxis for *Pneumocystis carinii.* Due to unavailability of highly active antiretroviral therapy (HAART) at our hospital at the time of this study, none of the patients was treated with HAART.

Patients with non-tuberculous pericarditis were treated according to their aetiological diagnosis. Septic pericarditis was treated with broad-spectrum antibiotics until specific culture results and sensitivity patterns were known. Two patients with purulent pericarditis required surgical drainage. Uraemic patients were referred to the renal unit for dialysis. Patients with pericardial effusions associated with connective tissue diseases were managed in collaboration with the rheumatology unit and their management was primarily directed at the underlying cause and included corticosteroids and chloroquine in the majority of cases. Patients with neoplastic pericardial involvement were referred for further management to the most appropriate specialists, including oncologists, radiotherapists, surgeons, pulmonologists and haematologists. Depending on the individual situation, management varied between chemotherapy, radiotherapy, palliative care or a combination thereof.

STATISTICAL ANALYSIS

Statistical analysis was done with the Mann-Whitney U, the Wilcoxon two-sample, the Kruskall-Wallis One-Way ANOVA, the Bonferroni two-way ANOVA, and Chi-

squared tests. A p-value < 0.05 was considered statistically significant. The correlation between two variables was plotted on a scatter plot and the Pearson product moment method or Spearman rank correlation coefficients were used to express the relationship. The significance of the correlation was denoted by a p-value < 0.05. All statistical analyses were done using Statistica version 7.0.

For the determination of the utility of the various diagnostic tests we included only those patients who had a valid test result and/or had not been exposed to anti-tuberculous therapy and/or any drugs associated with the development of pericarditis during a period of three months preceding pericardiocentesis. The results of the various laboratory and diagnostic tests were compared between patients with tuberculous effusions and patients with non-tuberculous patients, and the diagnostic value of the observation studied was assessed in terms of the sensitivity, specificity, positive predictive vale (PPV), negative predictive value (NPV), and diagnostic efficiency, expressed as percentage. Sensitivity was defined as TP/(TP+FN) x 100; specificity as TN/(TN+FP) x 100; PPV as TP/(TP+FP) x 100, NPV as TN/(TN+FN) x 100, and diagnostic efficiency was defined as (TP+TN)/(TP+FP+TN+FN) x 100, where TP = true positive, TN = true negative, FP = false positive and FN = false negative. These were compared by means of receiver operating characteristic (ROC) curves (Beck and Schultz, 1986) and the cut-off value that maximised the true-positive rate was selected.

Chapter 3

EPIDEMIOLOGY OF LARGE PERICARDIAL EFFUSIONS AT TYGERBERG HOSPITAL

Tuberculosis (TB) is the leading cause of pericarditis in South Africa and a number of other developing countries (Desai, 1979; Strang 1984; Cegielski 1990; Fowler, 1991; Hakim *et al*, 2000). This is in contrast to first world countries where TB is responsible for less than 4% of acute pericarditis (Fowler, 1991). In spite of economic developments and the availability of effective chemotherapy, the burden of TB is increasing. This increase has been partially attributable to the spread of human immunodeficiency virus (HIV) and is characterised by an increasing proportion of extrapulmonary cases (Narain *et al*, 1992). Estimates by the Medical Research Council's National Tuberculosis Research Program put the burden of TB in South Africa for 2001 at 323 342 new cases, 41.1% of which were infectious and 52.5% of which were also HIV positive (Weyer and Fourie, 2001). The purpose of this study was to establish the prevalence of large, clinically significant pericardial effusions in the Western Cape Province of South Africa. Furthermore, we wanted to determine the incidence of various types of effusions, in particular tuberculous pericarditis in relation to demography, potential risk factors of lifestyle and HIV co-infection.

PATIENTS AND METHODS

A prospective study was carried out at Tygerberg Hospital, South Africa. Patients presenting with large pericardial effusions between February 1995 and June 2001 were enrolled. All patients gave written informed consent for participation in the study,

which was approved by the Ethics Committee of Stellenbosch University. A pericardial tap was performed under echocardiographic guidance through a pigtail catheter and fluid sent for biochemistry, microbiology, cytology and differential white cell count. Each patient also underwent an HIV test. Patients were allocated to diagnostic groups according to pre-determined criteria (Chapter 2). Patients were specifically asked about their smoking habits, alcohol consumption as well as their employment status, including the type of work they were doing. In the case of women, excessive use of alcohol was defined as a regular weekly intake of more than 14 units of alcohol and in men, more than 21 units of alcohol per week. A person was considered "employed" if he/she was working in return for wages. A person >15 years, who was not employed but was available for work and had the desire to work, was categorised as "unemployed". For the purpose of this study we expanded the definition of unemployment to include those who were not available for work, such as pensioners and people receiving disability grants. Students, whether still at school or at a tertiary institution, were categorised as "students" and were not considered to be economically active. At the time of opening a hospital folder, patients declared their income and these figures were used to categorise enrolled individuals into various income groups according to the quintiles contained in the income and expenditure survey and the October household survey (Hirschowitz and Orkin, 1996). Patients were classified as having had TB if they had previously been treated for TB. A positive TB contact was defined as prolonged exposure to another person with active TB or individuals on TB treatment. Prolonged exposure included working in the same room as, and/or co-habiting with, an infectious person.

RESULTS

A total of 233 consecutive patients were enrolled in this study. Eighty-four patients (36.1%) were HIV positive; 81 of these (96.4%) had tuberculous pericarditis, two (2.4%) had septic pericarditis and one (1.2%) had uraemic pericarditis, probably secondary to HIV associated renal disease. Only two patients were not tested for HIV; these were classified as HIV negative for the purposes of this study. There were 132 males (56.7%) and 101 females (43.3%) that presented with large pericardial effusions. The majority of patients were black (n=121; 51.9%), followed by patients of mixed racial ancestry (n=101; 43.3%). Only 4.7% of the study population was Caucasian (n=11). There was a high rate of HIV infection amongst black patients; 55.6% (n=30) of black females were HIV positive, compared to 13 females (27.7%) of other ethnic origin; 43.3% (n=29) of black males were HIV positive compared to 18.5% (n=12) of males of other ethnic origin. There was only one white male who was HIV positive.

The prevalence of HIV coinfection increased from year to year, beginning with ten cases in the first year of the study and amounting to 19 cases in 2000, as presented in Figure 3.1. The age at presentation ranged from 13 to 85 years, the mean (SD) was 38.0 (14.6) years. More than 65.0% of the study population and 84.5% of the HIV positive individuals were aged between 15 and 39 years.

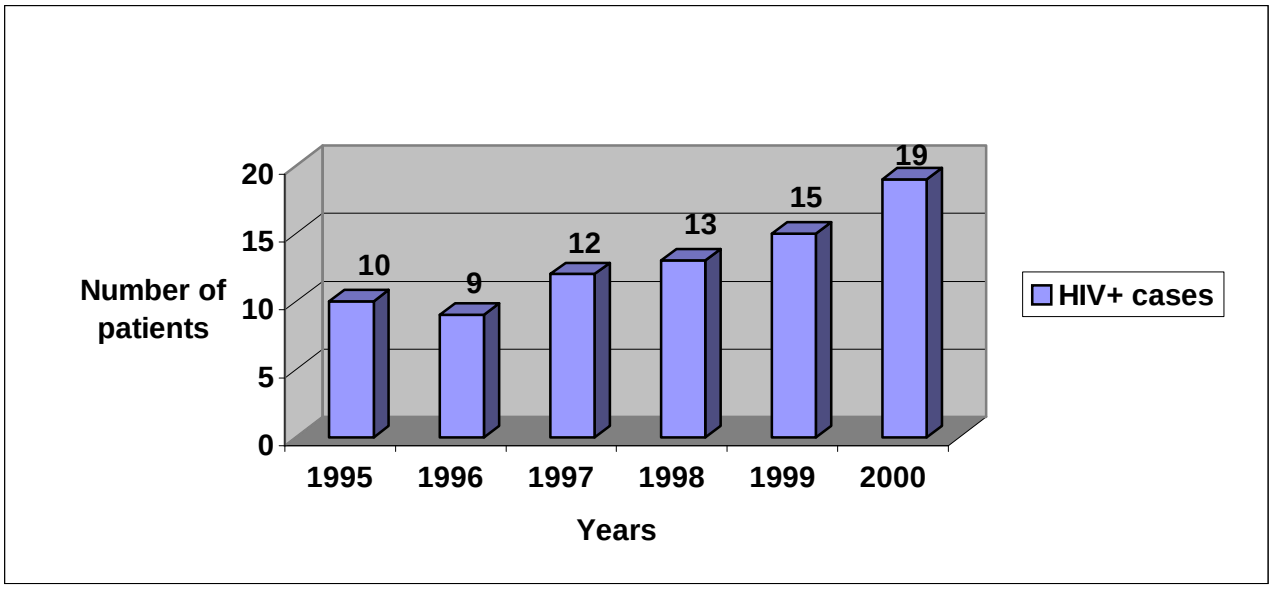

Figure 3.1. Annual number of newly diagnosed HIV positive tuberculous pericarditis patients seen at Tygerberg Hospital, 1995-2000

HIV+ = human immunodeficiency virus positive

The mean (SD) age of the HIV positive patients was significantly lower ($p<0.05$) than the HIV negative patients [31.9 (8.4) years versus 41.6 (14.1) years]. In addition, HIV positive females were significantly younger ($p<0.05$) than their HIV positive male counterparts [29.2 (7.2) years versus 34.0 (9.1) years]. The demographic data have been summarised in Table 3.1.

Socio-economic and social history data

Eighty individuals (including five housewives) were employed (34.3%), whereas 115 patients (49.4%) were unemployed. A further 15 patients were students (6.4%), 13 received a state pension (5.6%) and 10 received a state disability grant (4.3%). A total of 54.9% of individuals in the age groups 15-49 years was unemployed. The prevalence of HIV amongst unemployed individuals was 49.0% compared to 30.0% amongst employed individuals. 65.0% of HIV positive patients were unemployed, whereas 31.0% of the HIV infected were employed and 4.0% were students. The difference between employment status in HIV positive and HIV negative patients was statistically significant ($p<0.005$).

The majority of unemployed HIV positive individuals had never been employed and their unemployment status was not the result of being ill due to HIV infection. The majority of patients categorised as employed were in the low-income brackets of less than R6868 (≈USD 1000) per year. Only 24 (10.3%) patients earned more than R6868 per year, including three patients who earned more than R52801 per year. None of these patients were infected with either HIV or TB.

Table 3.1. Patient demographics

	Male (n=132)		Female (n=101)	
	HIV positive	**HIV negative**	**HIV positive**	**HIV negative**
Number	n=41 (31%)	n=91 (69%)	n=43 (43%)	n=58 (57%)
Mean (SD) age (years)	34.0 (9.1)	39.9 (13.9)	29.9 (7.2)	45.0 (14.5)
Ethnic origin				
- African black	29 (70.7%)	38 (41.8%)	30 (69.8%)	24 (41.4%)
- Mixed race	11 (26.8%)	46 (50.5%)	13 (30.2%)	3 (51.7%)
- Caucasian	1 (2.4%)	7 (7.7%)	0 (0%)	31 (5.3%)

HIV = human immunodeficiency virus

SD = standard deviation

Thirty-seven patients (15.9%) had previously received anti-tuberculous therapy. Of the 84 HIV positive patients, 9.5% had previously had TB, compared to 19.5% of HIV negative patients. Fifty-three individuals (22.7%) gave a history of a positive TB contact, including 23 of the 84 HIV positive patients (27.4%) and 30 of the 149 HIV negative patients (20.1%). Amongst the tuberculous pericarditis patients, excessive amounts of alcohol were consumed by similar percentages of HIV positive and of HIV negative individuals (35.9% versus 34.9%; p=0.89), whereas the percentage of smokers amongst the HIV negative subgroup tended to be higher than percentage of smokers in the HIV positive patient population (45.8% versus 43.6%).

Aetiology of large pericardial effusions

Tuberculous pericarditis was the most common cause of pericardial effusions (69.5%, n=162), while malignancy accounted for 9.4% (n=22) of all effusions. The aetiological data are presented in Figure 3.2. The group of "miscellaneous" effusions (n=20) included the following: idiopathic (n=7), post-traumatic (n=5), post-surgical (n=2) and multifactorial/uncertain, including congestive cardiac failure and underlying sepsis (n=6). The incidence of the various types of pericardial effusions differed according to age. TB was the most common cause of pericardial effusions in all age groups, but its prominence decreased in those older than 50 years. It accounted for 83.0% of all pericardial effusions in the age group 15-29 years and for 78.0% of those aged 30-39 years. The HIV prevalence in these TB patients was found to be 57.6% and 69.4%, respectively. This strong correlation with HIV diminished with increased age; the prevalence of HIV among tuberculous effusions was 44.0% in the 40-49 year age group, 12.5% in the 50-59 year group and 0% in the age group older than 60 years. Most patients who presented with malignancy were in the 50-59 year

age group, where it accounted for 32.0% of cases. TB was the most prevalent cause for pericardial effusions amongst the most marginalised groups, accounting for 52.5% (n=53) of effusions from patients of mixed race and 90.1% (n=109) of effusions from black patients. Amongst males, TB was the aetiological agent in 75.8% (n=100) of pericardial effusions, whereas TB accounted for 61.4% (n=62) of pericarditis amongst females. Half of all tuberculous effusions occurred in individuals that were co-infected with HIV, and 96.4% of the 84 HIV positive individuals required pericardiocentesis because of TB (n=81). Of note was the difference observed between females with tuberculous effusions and their male counterparts with regards to HIV.

In the female TB group, 67.7% of those affected were HIV positive, whereas in the male TB group, only 39.0% were co-infected with HIV.

Malignancy (18 out of 22 malignant effusions) and connective tissue disease (CNTD; 10 out of 12 patients with CNTD) were more frequently diagnosed in individuals of Caucasian or mixed racial ancestry. Nine cases of CNTD (75.0%) were diagnosed in women, whereas uraemic pericarditis was seen more frequently in males (n=7; 58.3%).

i) Tuberculous pericarditis

TB accounted for 69.5% of the 233 effusions (n=162), including 100 (61.7%) males and 62 (38.3%) females. Eighty-one patients (50.0%) were HIV positive, including 42 females (51.9%) and 39 males (48.1%). Of the HIV negative tuberculous patients, a significantly higher proportion ($p<0.01$) was male (75.3%) than female (24.7%).

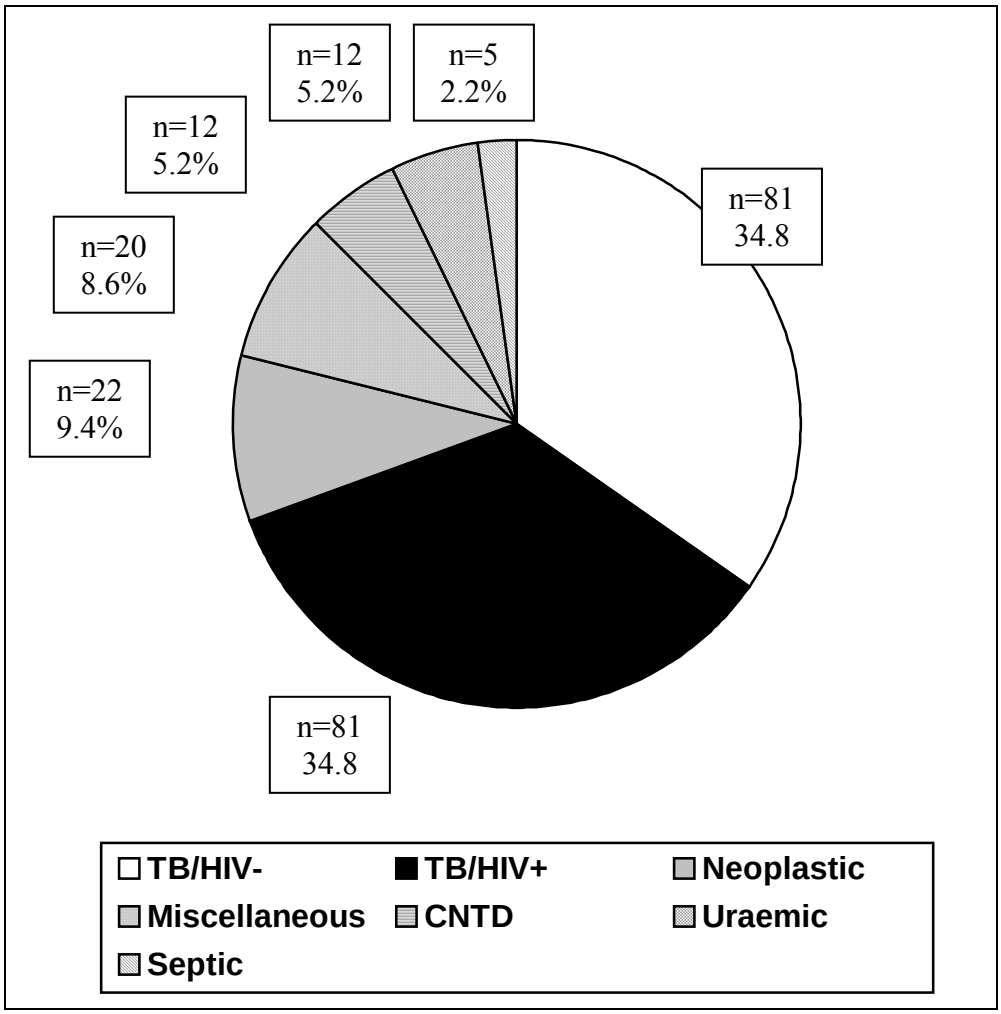

Figure 3.2. Aetiological causes of large pericardial effusions

TB = tuberculosis

HIV- = human immunodeficiency virus negative

HIV+ = human immunodeficiency virus negative

CNTD = connective tissue disease

The mean (SD) age at presentation differed significantly between HIV positive and HIV negative patients with TB [31.9 (8.4) years versus 39.7 (15.9) years; ($p<0.05$)]. The difference in age was most striking among the group of black females, in whom the mean (SD) age at presentation was 44.7 (16.1) years in HIV negative patients compared to 29.3 (7.8) years in HIV positive patients.

The one-year mortality rate in this group of patients was 17.3%, and was significantly higher in the HIV positive patients (22.2%) compared to those not infected with HIV (12.3%).

ii) Malignant pericardial effusions

Twenty-two patients had pericardial effusions due to an underlying malignancy. The majority of these patients had bronchus carcinomas (n=7), including small cell carcinoma (n=1), squamous cell carcinoma (n=1) and unspecified bronchus carcinoma (n=5). Haematological malignancies accounted for three effusions, including T-cell lymphoma (n=1), diffuse large cell lymphoma (n=1) and chronic myelomonocytic leukaemia (CMML; n=1). Another patient known with B cell lymphoma developed a pericardial effusion secondary to septicaemia and complicated by uraemia; this patient was included under the miscellaneous group. Other malignancies associated with pericardial effusions included adenocarcinoma (n=3), breast carcinoma (n=2), cervical cancer (n=1), neuroendocrine tumour (n=1), thymoma (n=1), neuroblastoma (n=1), multicentric liver carcinoma (n=1), mesothelioma (n=1), and unspecified (n=1). The mean (SD) age of this group was 49.7 (13.8) years; 14 of the 22 patients (63.6%) were older than 50 years of age.

Despite the high prevalence of HIV in the study population, none of these patients was coinfected with HIV. In addition, none of the other classically HIV-associated malignancies (such as Kaposi's sarcoma and anal carcinoma) was found. Twenty (out of 22) patients with neoplastic pericardial effusions died within one year of diagnosis; the one-year mortality rate for this group was 90.9%.

iii) Pericarditis associated with CNTD

Twelve patients had underlying CNTD, including systemic lupus erythematosus (SLE, n=7), scleroderma (n=1), mixed connective tissue disease (MCTD, n=1), rheumatoid arthritis (RA, n=1) and acute rheumatic fever (n=2). Although not classical CNTD, acute rheumatic fever and RA were included in this group, as these diseases may have similar presentations to SLE with regards to constitutional symptoms and involvement of the joints and pericardium. The two patients with acute rheumatic fever were 13 and 15 years old, respectively, while the male patient with RA was 64 years old.

The mean age (SD) at presentation for this group was 32.6 (14.8) years and the mortality was 33.3%. None of the deaths (three patients with SLE and one with RA) was clearly attributable to the pericardial disease *per se*.

iv) Septic pericarditis

Only five of the 233 patients (2.1%) were diagnosed with septic pericarditis, four of whom were immunocompromised: two were infected with HIV, one had underlying B-cell lymphoma and the fourth patient was a known diabetic with a history of chronic alcohol abuse. In three of these cases, the diagnosis was based on positive

cultures: *Staphylococcus aureus* (n=2) and Group B salmonella species (n=1). The remaining two cases had negative cultures, probably attributable to the fact that both were receiving treatment with broad-spectrum antibiotics. The mean (SD) age of this diagnostic group was 43.0 (25.6) years. The one-year mortality rate for this group was 80.0%, and 100.0% in those infected with HIV.

v) Uraemic pericarditis

Twelve patients were classified as having uraemic pericarditis; one of them was HIV positive and had no evidence of opportunistic infection or TB. The mortality in this group was high (42.0%), and all the deaths occurred in males of mixed racial ancestry. The mean age (SD) at death was 50.2 (13.7) years.

vi) Miscellaneous group

In seven patients, large effusions followed cardiac trauma or pericardiotomy, including sustained blunt trauma secondary to motor vehicle accidents (n=2), previous stab wounds to the chest (n=3), post-aortic valve replacement surgery (n=1) and coronary artery bypass grafting (n=1). Five additional patients had large pericardial effusions that appeared to be idiopathic in nature.

DISCUSSION

Pericardial effusions can result from a number of disease processes. The clinical presentation can be variable, ranging from asymptomatic effusions discovered incidentally by chest X-ray to life-threatening emergencies associated with cardiac tamponade (Corey *et al*, 1993). Numerous studies have reported on the aetiologies of pericardial effusions (Krikorian and Hancock, 1978; Permanyer-Miralda *et al*, 1985;

Corey *et al*, 1993); however, the majority are retrospective reviews undertaken in first world countries.

The incidence of the various types of pericardial effusions at Tygerberg Hospital differs significantly from developed countries. TB, which accounts for less than 4% of all cases in first world countries (Fowler, 1991), was the most prevalent cause in this study accounting for 69.5% of all pericardial effusions. It was especially significant amongst blacks and patients of mixed racial ancestry, accounting for 90.1% and 52.5% of all effusions, respectively. TB was diagnosed in only one Caucasian patient. Conversely, pericardial effusions of non-tuberculous origin were seen more frequently in Caucasians (90.9% of effusions) and to a lesser extent in those of mixed racial ancestry. These data confirm previous reports indicating significant ethnic differences in the incidence and prevalence of TB in South Africa (Styblo, 1976; Wulfsohn and Küstner, 1985). There are, however, no data indicating that certain ethnic groups have increased genetic susceptibility to activation of latent tuberculous infection. A study performed in Puerto Rico, which involved the follow up of a large number of persons in a BCG vaccination trial, showed no major differences in the incidence of TB between black and white tuberculin reactors (Comstock *et al*, 1974).

Although not excluding the possibility of genetic factors, this present study demonstrates clearly that socio-economic factors contribute significantly to the racial distribution observed. TB is more prevalent in poor nations, especially affecting individuals living in poverty. In the present study only 5.8% of African blacks had an individual income in excess of R6868 (≈USD 1000) per annum. Although total household income was not specified, the impression was that this would not have

been much higher. In keeping with these results and according to data contained in the income and expenditure survey, black households are the poorest in the Western Cape Province (South African Communication Service, 1995; Hirschowitz and Orkin, 1996). Other indicators of deprivation, such as access to drinking water and sanitation, also suggest a relationship between race and socio-economic status. In the Western Cape, 37.0% of black households, compared to 77.0% of mixed and 99.0% of white households, have running tap water inside their dwelling; flush toilets are found in almost all white (99.0%) households, but in only 68.0% of mixed and 30.0% of black households (South African Communication Service, 1995; Hirschowitz and Orkin, 1996). The correlation between poverty and TB is probably based on the two most important elements of tuberculous disease; firstly the risk of becoming infected, and secondly the inactivity of the cellular immune system associated with poor nutrition, alcohol abuse and the feeling of hopelessness, which are all prevalent in impoverished communities. The high risk of infection is due to the late diagnosis of smear positive individuals (source), and the proximity and frequency of exposure related to the sharing of sleeping areas in overcrowded households (Rieder *et al*, 1989).

The risk of TB is now confounded by HIV co-infection (Narain *et al*, 1992; Cegielski *et al*, 1994; Maher and Harries, 1997). In this study 36.1% of the study population was HIV positive, whereas the seroprevalence amongst those with TB was 50.0% ($p<0.05$). Of the 84 HIV positive individuals in the study, 94.6% presented with tuberculous pericarditis and only 5.4% with other pericardial disease. This finding of dual infection is a major problem in sub-Saharan Africa. The World Health Organization (WHO) estimated that of the 9.4 million people with dual infection of

TB and HIV, 6.6 million (70.0%) live in sub-Saharan Africa (Department of Health, 2000; World Health Organisation, 2003). In South Africa, the incidence rate of TB is 686 per 100 000 per year, of which 52.5% are estimated to be coinfected with HIV (Weyer and Fourie, 2001). In the Western Cape, the TB incidence is estimated to be 932 per 100 000 per year, but the HIV prevalence (36.5%) amongst TB patients is significantly lower than the national average (Weyer and Fourie, 2001).

HIV, due to its ability to destroy the immune system, has frequently been implicated as a principal cause for the recent resurgence of TB worldwide. Furthermore, in many developing countries, TB has now emerged as the most common opportunistic disease associated with HIV infection (Narain *et al*, 1992). In our study group, 50.0% of all patients presenting with tuberculous pericardial effusions were HIV positive. The difference in age between the HIV positive and HIV negative TB groups was statistically significant ($p < 0.01$), the mean (SD) ages of the two groups being 31.9 (8.4) years and 39.7 (15.9) years, respectively. In this study, the highest prevalence of HIV was found in those that are most severely socio-economically deprived, namely African black females.

In addition to an association between ethnicity and HIV infection, this study revealed a statistically significant association between unemployment and HIV infection. The overwhelming majority of those who were unemployed had never been employed; the high level of unemployment was thus not due to the effects of HIV disease. Overall, the level of unemployment observed in our study population was significantly higher than the official 19.0% unemployment level for this province (Hirschowitz and Orkin, 1996). Differences with regards to both population group and gender have been

described in Western Cape unemployment patterns. In 1995, 45.0% of black women were unemployed compared to 23.0% of black men; in comparison, 14.0% of white females and only 3.0% of white men were unemployed, respectively (South African Communication Service, 1995). In keeping with previous studies, the majority of unemployed individuals were in the 15-34 years age groups (Hirschowitz and Orkin, 1996). It is, however, important to note that although blacks are most severely affected by poverty (and dual infection), it should not distract from the high degree of unemployment and poverty experienced by people of mixed racial background.

A previous experience of TB according to the history did not contribute significantly to the development of tuberculous pericarditis in patients with HIV. This finding could be interpreted to mean that previous infection had indeed occurred less frequently in those with HIV co-infection and that tuberculous pericardial disease occurs in these patients as part of rapidly progressive primary disease. Alternatively, it could mean that the diagnosis of TB was made less frequently in the HIV positive group of which the majority were blacks - many of whom who had grown up in the Eastern Cape with poor access to health services. In this group, the pericardial effusion could have occurred as a result of reactivation of previously undiagnosed disease. Patients with HIV co-infection were also significantly younger than the HIV negative patients and extrapulmonary TB is more likely to occur during a first (potentially diagnosable) episode of tuberculous disease in those infected with HIV.

Tobacco smoking and alcohol abuse were not encountered more frequently in patients with tuberculous compared to non-tuberculous effusions. This data must, however, be reviewed cautiously because data were not collected from a matched group of

controls. A study performed in Shanghai found the incidence of TB higher (relative risk 2.2) among smokers than non-smokers after adjustment for age, sex, type of work, history of contact, and area of housing (Yu and Peng, 1989). The high level of alcohol abuse is particularly disturbing. A case-control study conducted in Mamre in the Western Cape found an association between alcohol problems in the household and TB with an odds ratio adjusted for employment status of 2.2 (Coetzee *et al*, 1988). It is thus not sufficient to state that TB is caused by *M. tuberculosis.* The association between poverty and TB has long been recognised, both locally and internationally (Dormer and Wiles, 1946; Hinman *et al*, 1976).

CONCLUSION

The multifactorial conditions resulting in tuberculous disease involve an interaction between agent, host and environment. There are risk factors associated with becoming infected, and others that determine the breach in the cellular immunity that allows infection to progress to disease. South African statistics have for many years indicated major ethnic differences in the prevalence of TB infection, and similar differences are seen in the incidence (notification rate) of TB, suggesting that the latter are due mainly to differences in the risk of infection. In this study, 50% of all the tuberculous pericarditis patients studied were found to be HIV positive, compared to only 4.2% of patients presenting with non-tuberculous aetiology. HIV-infected tuberculous pericarditis patients were significantly younger than the HIV negative TB patients, and the highest prevalence of HIV and TB co-infection was found in the socio-economically most deprived group, namely African females. The steady rise in the number of HIV and TB co-infected patients observed in this study may reflect progressively increasing numbers of new cases of HIV infection, however, it may also

demonstrate how the maturation of the HIV epidemic results in more cases of clinically ill individuals. After initial infection, the human immune system becomes increasingly destroyed and the longer the epidemic lasts, the more clinical cases of HIV associated disease will be seen. This has ominous social, medical and economic implications for the country and in particular, the Western Cape, and places considerable pressure on the existing fragile and overstretched health services in sub-Saharan Africa where more than 70.0% of the world's co-infection with HIV and TB occurs.

Chapter 4

THE ROLE OF HISTOPATHOLOGY IN DIAGNOSING TUBERCULOUS PERICARDITIS

Tuberculosis (TB) is the leading cause of pericarditis in South Africa and other developing countries (Fowler, 1991; Cegielski *et al*, 1994; Hakim *et al*, 2000). This is in stark contrast to first world countries where TB accounts for less than 4% of pericardial disease (Permanyer-Miralda *et al*, 1985). The incidence of tuberculous pericarditis in sub-Saharan Africa is increasing as a result of the human immunodeficiency virus (HIV) endemic, and this trend is likely to occur in other parts of the world where the spread of HIV is leading to a resurgence of TB (Cegielski *et al*, 1990; Cegielski *et al*, 1994; Maher and Harries, 1997). Tuberculous pericarditis accounts for up to 100% of HIV positive patients presenting with pericardial effusions in African countries (Cegielski *et al*, 1994; Pozniak *et al*, 1994), but for only 0-15% of corresponding patients in the USA (Eisenberg *et al*, 1992; Reynolds *et al*, 1992; Kwan *et al*, 1993; Hsia and Ross, 1994).

A definitive diagnosis of tuberculous pericarditis is made by isolating the tubercle bacillus from the pericardial fluid and/or pericardial biopsy, yet pericardial TB is often not identified because of the difficulty in isolating the organism (Fowler, 1991; Zayas *et al*, 1995). Histological features of granulomatous inflammation and caseous necrosis are highly suggestive of TB. In the absence of demonstrable tubercle bacilli, these findings may also be present in chronic pericardial disease secondary to fungal

infections and rheumatoid arthritis (RA; Kumar and Robbins, 1997; Underwood, 2000).

Although the influence of HIV with regards to the epidemiology of extrapulmonary TB is well documented (Cegielski *et al*, 1990; Narain *et al*, 1992; Cegielski *et al*, 1994; Pozniak *et al*, 1994; Maher and Harries, 1997), little is known about the effects of HIV on the pathogenesis, histomorphology and likelihood of establishing a definite diagnosis in an HIV positive individual with tuberculous pericarditis. The purpose of this study was thus to establish the influence of HIV on the histomorphological features of patients presenting with tuberculous pericardial effusions and to determine the diagnostic efficiency of pericardial histology compared to other diagnostic tests.

PATIENTS AND METHODS

A prospective study was carried out at Tygerberg Academic Hospital. During the period from February 1995 to June 2001, all patients presenting to the Cardiology Unit with large pericardial effusions (defined as epi-pericardial separation of more than 10 mm) and having open pericardial biopsies under general anaesthesia were included in this study. Each patient underwent a comprehensive diagnostic work-up as described in Chapter 2. One sample of each pericardial biopsy specimen was fixed with 10% solution of buffered formalin, processed, paraffin-embedded and sectioned using routine technology. Samples were stained with haematoxylin-eosin (H&E) and Ziehl-Neelsen (ZN) before being examined independently by two histopathologists. The predetermined diagnostic criteria are summarised in Chapter 2. The utility of various diagnostic tests to confirm tuberculous pericarditis were evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative

predictive value (NPV) and diagnostic efficiency expressed as a percentage as described in Chapter 2.

RESULTS

During the period from February 1995 to June 2001, a total of 233 patients presented to the Cardiology Unit with large pericardial effusions; however, open pericardial biopsies under general anaesthesia were performed in only 36 patients. Of these, 25 patients had pericardial biopsies for diagnostic purposes and 11 required surgical pericardial fenestration for therapeutic reasons. Based on predetermined criteria, tuberculous pericarditis was identified in 25 patients, five of whom were HIV-positive. The eleven cases of non-tuberculous effusions included one patient with septic pericarditis who was HIV-positive. The other cases included uraemia (n=2), rheumatoid arthritis (n=1), T-cell lymphoma (n=1), adenocarcinoma (n=1) and idiopathic or "indeterminate" pericarditis (n=5). All of these patients tested negative for HIV. The histopathological patterns identified for pericarditis are summarised in Table 4.1.

Tuberculous pericarditis

The histopathological features in tuberculous pericardial effusions included non-necrotising granulomatous pericarditis (Figure 4.1.a), necrotising granulomatous pericarditis (Figure 4.1.b), purulent pericarditis (Figure 4.1.c), fibrotic pericarditis (Figure 4.1.d), fibrinous pericarditis and serous pericarditis, respectively. Twelve biopsies (48% of tuberculous effusions) demonstrated necrotising granulomatous inflammation; four of these were ZN positive. Three other specimens showed granulomatous inflammation without any evidence of necrosis (ZN negative).

Table 4.1. Histopathological findings in patients with large pericardial effusions

Histopathological finding	Tuberculosis (n=25)					Non-TB (n=11)
	Culture (+) TB		Culture (-) TB		Total TB	
	HIV-	HIV+	HIV-	HIV+		
Granulomatous, necrotising; ZN+	3		1		4	
Granulomatous, necrotising; ZN-	4	1	3		8	
Purulent; ZN+		1			1	
Granulomatous, no necrosis; ZN-	2			1	3	
Fibrotic	1		1		2	1
Serofibrinous	2		3	1	6	5
Serous				1	1	2
Purulent; ZN-						1
Neoplastic deposits						2

ZN = Ziehl-Neelsen

Figure 4.1. Variety of histopathological findings in tuberculous pericarditis

Panel demonstrates variety of histopathological findings in tuberculous pericarditis, including (1a) Non-necrotising granulomatous pericarditis with a Langhans giant cell (thin arrow), epithelioid macrophages (arrowhead) and small lymphocytes (thick arrow); (1b) Granulomatous pericarditis showing granulomata with central necrosis (arrow) against a background of granulation tissue; Insert demonstrates strongly positive ZN stain for acid-fast bacilli; (1c) Purulent pericarditis showing severe acute inflammatory cell infiltrate (arrow); (1d) Healed pericarditis showing dense fibrocollagenous tissue (arrow).

Unless indicated otherwise haematoxylin and eosin stain; 40 x magnification.

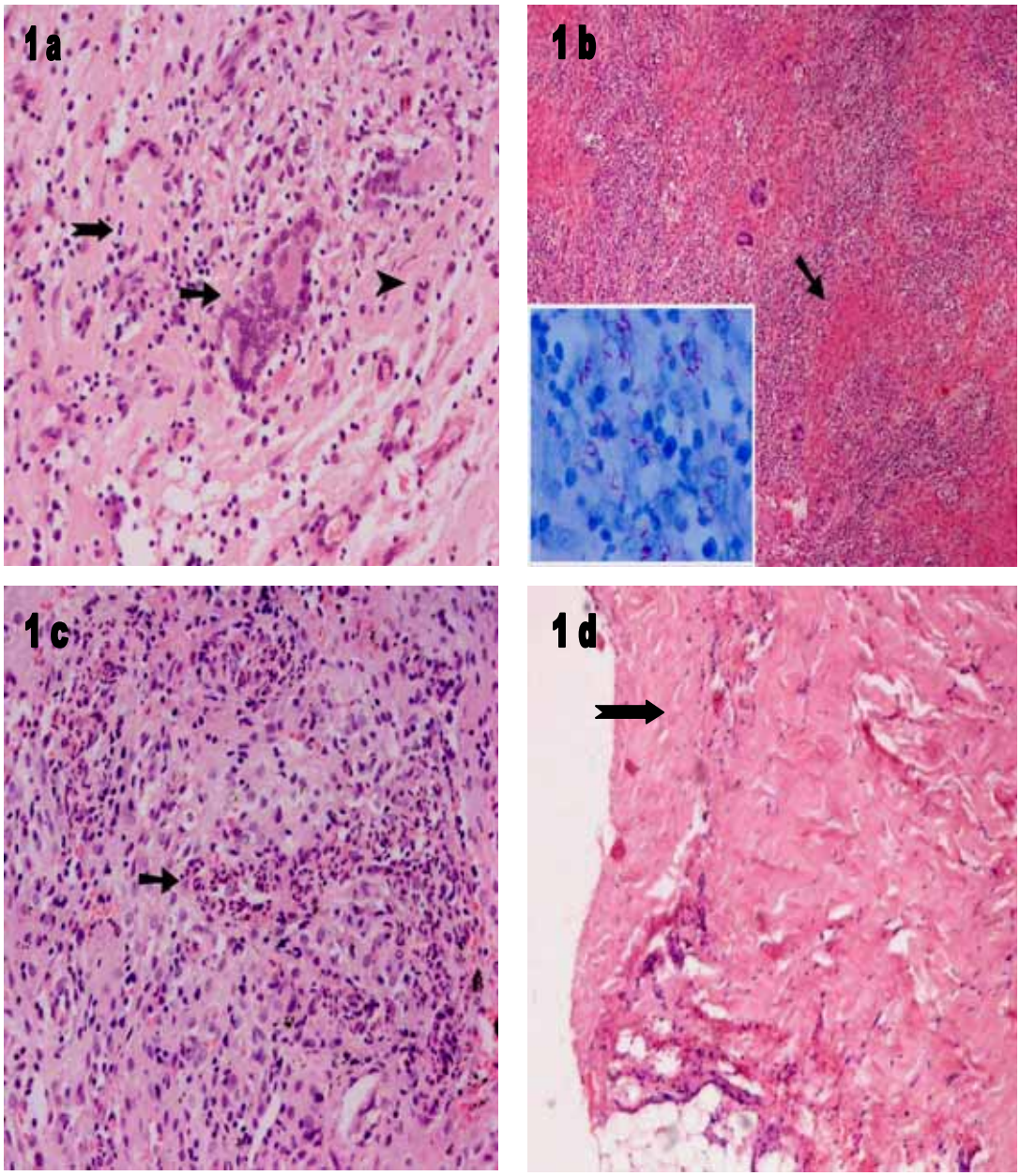

Figure 4.1. Variety of histopathological findings in tuberculous pericarditis

A further biopsy (taken from an HIV-positive individual with a CD4+ lymphocyte count of 39 cells/μL) showed an infiltrate of acute inflammatory cells suggestive of purulent pericarditis, the histology in this case was ZN positive. The pericardial fluid culture was positive for *M. tuberculosis* and negative for other bacterial or fungal growth. Other histopathological findings included fibrotic pericarditis (n=2), serofibrinous pericarditis (n=6) and serous pericarditis (n=1), respectively. Fibrotic pericarditis was characterised by plaque-like fibrinous thickening of the serosal membranes and multiple layers of fibrocollagenous tissue in the absence of any calcifications.

Influence of HIV on tuberculous pericarditis

Thirteen of the 20 HIV negative tuberculous effusions (65%) demonstrated granulomatous lesions compared to two of the five HIV positive cases (40%). In 11 out of the 13 HIV negative cases (85%), the granulomatous inflammation was accompanied by central necrosis, whereas necrosis was present in only one (50%) of the two HIV positive cases with granulomatous inflammation. Serofibrinous pericarditis was demonstrated in five HIV negative patients (25%) and in one HIV positive patient (20%), respectively. In the HIV positive patients, all cases of granulomatous inflammation (n=2) and serofibrinous pericarditis (n=1) were associated with CD4+ lymphocyte cell counts >200 cells/μl, whereas the histopathological features of serous pericarditis (n=1) and purulent pericarditis (n=1) were restricted to patients with CD4+ lymphocyte cell counts of 44 and 39 cells/μl, respectively. Fibrotic pericarditis was not seen in HIV-positive individuals.

Non-tuberculous pericarditis

In the non-tuberculous pericardial effusions the histopathological classes included serofibrinous pericarditis (Figure 4.2.a), serous pericarditis (Figure 4.2.b), neoplastic pericarditis (Figures 4.2.c and 4.2.d), purulent pericarditis and fibrotic pericarditis. The histopathological descriptions of the non-tuberculous pericarditis cases are presented in Table 4.2. Serofibrinous pericarditis was the most common histopathological class and was present in uraemic pericarditis (n=2), rheumatoid arthritis (RA) (n=1) and idiopathic pericarditis (n=2), respectively, whereas granulomatous inflammation was not seen in non-tuberculous pericardial effusions.

The diagnostic utility of pericardial biopsy

Various histopathological criteria were tested for the diagnosis of TB; the presence of granulomatous inflammation (with or without necrosis) and/or ZN positivity was found to yield the best results. This corresponded to a sensitivity, specificity, PPV, NPV, and diagnostic efficiency of 64%, 100%, 100%, 55%, and 75%, respectively. There were nine false negative but no false positive results.

DISCUSSION

In the current study, only six out of 36 (16.7%) patients were HIV positive; in five cases, the effusion was secondary to TB and in the remaining case, septic pericarditis was diagnosed. This finding is not representative of the population; HIV-associated pericardial effusions are present in 50% of patients presenting with large pericardial effusions in this population (Chapter 3). Pericardial biopsies were performed for (a) diagnostic purposes if no diagnosis was made within one week and (b) for therapeutic purposes when surgical fenestration was required. The low rate of HIV positive

Figure 4.2. Variety of histopathological findings in non-tuberculous pericarditis

Panel demonstrates variety of histopathological findings in non-tuberculous pericarditis, including (2a) Fibrinous pericarditis showing a fibrinous exudate (arrow) with underlying organisation against an inflammatory background (arrowhead); (2b) Serous pericarditis with chronic inflammatory cells and dilated blood vessels; (2c) Pericardial infiltration by T-cell lymphoma showing an infiltrate of small blue tumour cells; insert demonstrates uptake for immunohistochemical stain for CD3 (T-cell marker); and (2d) Metastatic adenocarcinoma of the pericardium.

Unless indicated otherwise haematoxylin and eosin stain; 40 x magnification.

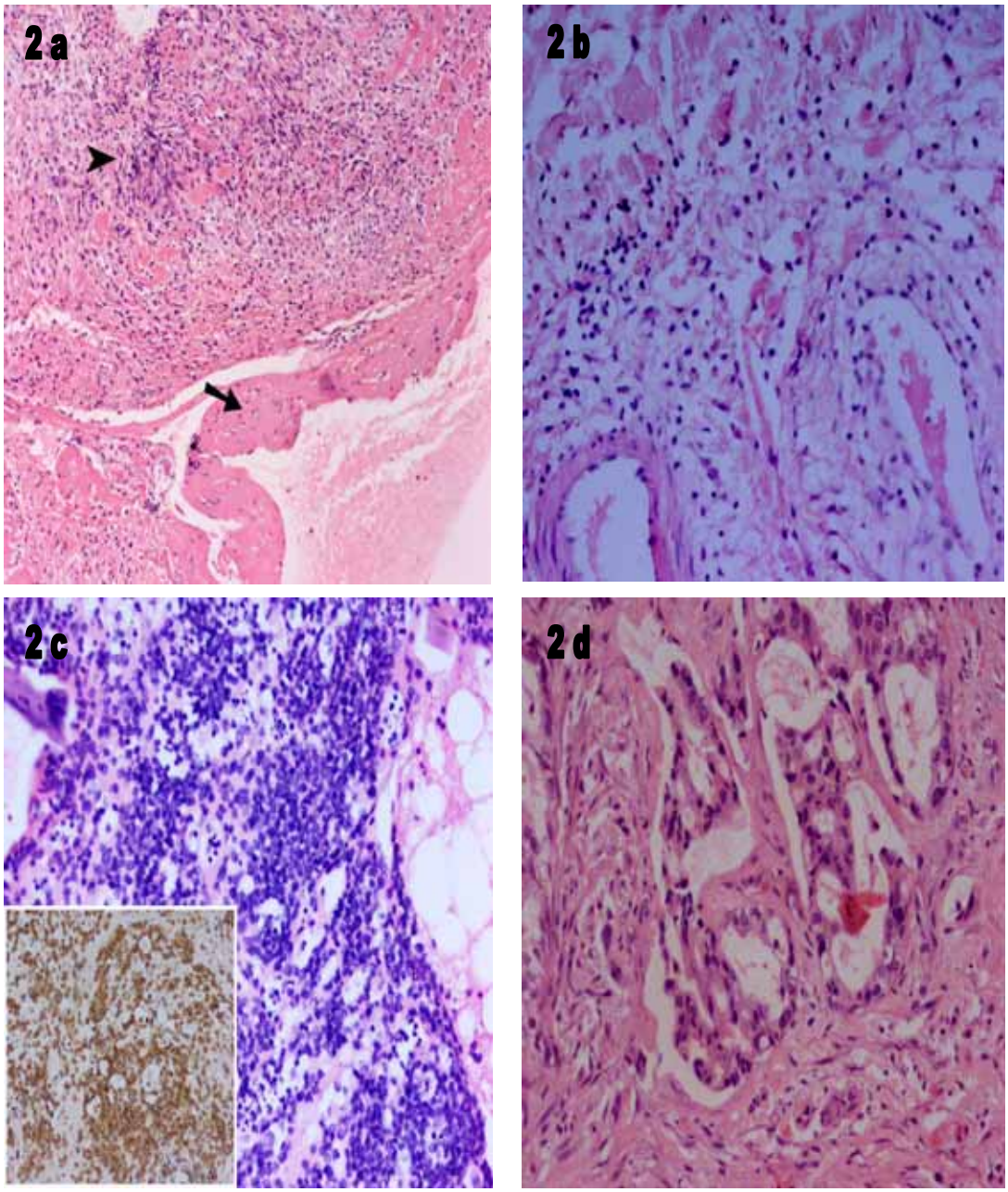

Figure 4.2. Variety of histopathological findings in non-tuberculous pericarditis

Table 4.2. Histopathological description of cases of non-tuberculous pericarditis

Histological finding	Septic (n=1)	Uraemic (n=2)	Neoplastic (n=2)	RA (n=1)	Other (n=5)	Total (n=11)
Purulent, ZN -	1					1
Neoplastic deposits			2			2
Healed					1	1
Serofibrinous		2		1	2	5
Serous					2	2

RA = rheumatoid arthritis

ZN - = Ziehl-Neelsen negative

patients being referred for pericardial biopsies in this setting can probably be attributable to (a) tendency for treating physicians to diagnose and treat tuberculous pericarditis more readily in HIV positive than in HIV negative patients in this setting and (b) an unwillingness of surgeons to perform these procedures in HIV positive patients unless truly indicated. Pericardial effusions are recognised as one of the early presenting features of HIV infection in sub-Saharan Africa (Cegielski *et al*, 1990; Taelman *et al*, 1990). In developed countries, large pericardial effusions in HIV positive patients are often idiopathic (Heidenreich *et al*, 1995), while in sub-Saharan Africa, the disease is caused by TB in as many as 100% of cases (Cegielski *et al*, 1994; Longo-Mbenza *et* al, 1997).

The diagnosis of TB as the aetiological cause is important. Without specific treatment, the average survival was 3.7 months and only 20% were alive at six months (Desai, 1979). On the other hand, the prognosis is excellent with appropriate medical treatment (Long *et al*, 1989; Cherian *et al*, 2003a). A definitive diagnosis of tuberculous pericarditis is made by isolation of the tubercle bacillus from the pericardial fluid and/or pericardial biopsy, yet pericardial TB is often not identified because of the difficulty in isolating the causative organism (Fowler, 1991; Zayas *et al*, 1995). Culture of tubercle bacilli from pericardial fluid can range from 30% to 75%, depending on the culture medium (Rooney *et al*, 1970; Gooi and Smith, 1978; Strang *et al*, 1991), while pericardial tissue established the diagnosis in up to 83% of cases (Rooney *et al*, 1970; Quale *et al*, 1987). These studies were performed in populations that did not have a high incidence of TB. In the current study, histopathological examination led to a definitive diagnosis of tuberculous pericarditis (caseating granulomata or tubercle bacilli on biopsy sample) in only 11 of the 20

HIV-negative tuberculous pericardial effusions (55%) and two of the five HIV-positive patients (40%). Histological analysis played an important role in diagnosing malignant and septic pericarditis, respectively (100% for each group). In spite of its relatively poor sensitivity in diagnosing tuberculous pericarditis (58%), histology nevertheless had a better diagnostic efficiency (71%) than pericardial effusion culture (sensitivity 52%, diagnostic efficiency 69%). By including all cases of granulomatous inflammation as being diagnostic of tuberculous pericarditis, the sensitivity and diagnostic efficiency improved to 64% and 75%, respectively. This may be of importance in areas where HIV/TB co-infection is endemic. Our study revealed that HIV does not only exert its impact on the presence of caseating necrosis, but also on the presence of granuloma formation.

We observed histological features in tuberculous pericarditis that have not, to our knowledge, previously been described. This included a ZN positive case resembling purulent pericarditis in a patient with a CD4+ lymphocyte cell count of 39 cells/μL, and a case of serous pericarditis in an individual with a CD4+ lymphocyte cell count of 44 cells/μL, respectively. The cases that demonstrated granulomatous inflammation and the case of serofibrinous pericarditis were associated with CD4+ lymphocyte cell counts >200 cells/μL. CD4+ lymphocytes are of particular importance in orchestrating the delayed hypersensitivity response that results in granuloma formation observed in TB (Sanchez *et al*, 1994), but which was in the present study absent in 60% of HIV positive patients. Although these effects of HIV have not previously been described for tuberculous pericarditis, our finding is consistent with observations that when atypical clinical, laboratory, and radiographic manifestations of TB do occur in HIV-infected persons, it usually implies that the degree of HIV-

induced immunosuppression is severe; i.e. CD4+ lymphocyte cell counts <200 cells/μL (Saks and Posner, 1992; Jones *et al*, 1993; Mlika-Cabanne *et al*, 1995a; Mlika-Cabanne *et al*, 1995b). Our findings demonstrated that depletion of CD4+ lymphocyte cells results in an altered immune response, which leads to diminished granuloma formation. It is likely that this affects the containment of bacillary spread and is thus responsible for the high frequency of extrapulmonary TB in immunodeficient individuals (Narain *et al*, 1992; Barnes *et al*, 1993). HIV prevalence rates are significantly higher among patients with extrapulmonary TB than among those with pulmonary TB (Narain *et al*, 1992).

The major disadvantage of pericardial biopsy is the need for general anaesthesia, the discomfort caused by the surgical procedure and the potentially longer duration of hospitalisation recorded in the present study. The greatest utility of pericardial histology is its high level of specificity, and by using the new techniques of pericardioscopy and optically guided pericardial biopsy, better tissue can be sampled while avoiding general anaesthesia and surgery (Little and Ferguson, 1986; Wurtz *et al*, 1992; Maisch, 1994). Although pericardioscopy offers new approaches for obtaining diagnostic information in patients with suspected malignant or infectious pericardial disease (Maisch, 1994; Endrys *et al*, 1988), these techniques have mainly been used in countries where only the minority of effusions are caused by TB, and the usefulness of this diagnostic procedure still needs to be evaluated in larger number of patients with tuberculous pericarditis.

Chapter 5

OPTIMISING THE DIAGNOSTIC PROTOCOL IN SUSPECTED TUBERCULOUS PERICARDITIS

The prompt treatment of tuberculous pericarditis saves lives (Strang, 1984). Effective treatment requires a rapid and accurate diagnosis of pericardial fluid tuberculosis (TB), but this is often difficult (Koh *et al*, 1994). Ziehl-Neelsen (ZN) stained smears of pericardial have poor sensitivity for the detection of *Mycobacterium tuberculosis* (*M. tuberculosis*), while culture is too slow and insensitive to aid clinical decision-making (Strang, 1984; Koh *et al*, 1994; Cherian, 2004). Pericardial biopsy, which is invasive and requires technical skills, is often also not diagnostic (Sagrista-Sauleda *et al,* 1988; Fowler, 1991; Koh *et al*, 1994). Clinicians thus have to rely heavily on clinical features of pericardial TB to initiate anti-tuberculous therapy (Cegielski *et al*, 1994; Pozniak *et al*, 1994; Maher and Harries, 1997). The potential for toxic effects and the duration of anti-tuberculous chemotherapy mandate diagnostic specificity. It is thus important to identify which clinical and basic laboratory features should be used to predict tuberculous aetiology. A diagnostic algorithm or scoring system based on these predictors might improve the diagnostic accuracy. Multivariate logistic regression has been used to model the clinical predictors of tuberculous meningitis in 251 adults (Thwaites *et al*, 2002). The researchers developed a simple diagnostic rule that resulted in 86% sensitivity and 79% specificity for the diagnosis of tuberculous meningitis (Thwaites *et al*, 2002).

When diagnostic infrastructure and resources are less problematic, suspected cases of tuberculous pericarditis may be diagnosed with the aid of polymerase chain reaction (PCR) analysis for *M. tuberculosis* (Cegielski *et al,* 1997; Lee *et al*, 2002), adenosine deaminase (ADA) activity (Koh *et al*, 1994; Komsuoglu *et al*, 1995; Burgess *et al*, 2002b) and pericardial interferon-gamma (IFN-γ) levels (Burgess *et al*, 2002b). The effect of human immunodeficiency virus (HIV) infection on ADA levels in patients with TB is controversial (Hsu *et al*, 1993; Burgess *et al,* 2002b), and the impact of HIV infection on the production of IFN-γ has not been evaluated. Cytology has been used for the diagnosis of pleural TB (Spriggs and Boddington, 1960; Yam, 1967, Gresham, 1989), and although it has been shown to be useful for the diagnosis of pericardial malignancy (Reyes *et al*, 1982; Pozniak *et al*, 1986; Di Bonito *et al*, 1990; Wiener *et al*, 1991; Nugue *et al*, 1996), the utility of cytology for the diagnosis of pericardial TB has not been studied. We aimed to develop a strategy that would optimise the diagnostic efficiency of available diagnostic tests and test these prospectively in a population with a high prevalence of HIV.

PATIENTS AND METHODS

The general diagnostic work-up and management of patients are summarised in Chapter 2. ADA activity (U/L) was determined in all pericardial fluid specimens according to the method described by Giusti (1974). This is a calorimetric method based on measurement of the formation of ammonia by Berthelot's reaction, which is produced when ADA acts on excess adenosine. One unit of ADA is defined as the amount of enzyme required to release one μmol of ammonia per minute from adenosine at standard assay conditions. The enzyme is stable for at least 24 hours at 25°C, seven days at 4°C and three months at -20°C (Ellis and Goldberg, 1970; Heinz,

1984). For cytopathological analyses pericardial fluid specimens (5-10 mL) were fixed by adding an equal amount of 50% ethanol. Smears were prepared from sediments by routine methods and stained according to the Papanicolaou method. Aliquots of pericardial fluid were collected on ice, frozen within 30 minutes and stored at -70^0C for the analysis of IFN-γ and the diagnostic application of polymerase chain reaction (PCR) technology. A commercially available PCR assay (Roche Amplicor™ PCR for *M. tuberculosis*) was used for the detection of the IS6110 sequence of *M. tuberculosis* in pericardial fluid specimens applying standard techniques and procedures. IFN-γ concentration was determined by enzyme-linked immunosorbent assay (ELISA) as described in Chapter 2.

The methodology used for the statistical analyses is summarised in Chapter 2. In addition, we developed a TB prediction model as a diagnostic aid by means of a statistical approach called "classification and regression tree" (CART) analysis. The classification trees were developed by consideration of all the variables separately. The range of each variable was divided into two groups to obtain the best separation between patients with tuberculous pericarditis and those with non-tuberculous pericarditis. The division corresponding to the best separation was selected. The resulting subsets of cases were then partitioned independently in turn. The process was done recursively, until a stopping condition was satisfied. Node deviance, which measures node heterogeneity was set to 0.1 to stop the tree growing process, and subsets smaller than 10 were not partitioned further. The sample was randomly divided into a training set (101 patients) and a test set (63 patients). The prediction model was then derived from the training set, and used on the test set to determine sensitivity and specificity values. The optimum cut-off for the total diagnostic index

(by which to classify a patient has having tuberculous pericarditis) was found by use of receiver operating characteristic (ROC) curves (Beck and Schultz, 1986).

RESULTS

During the study period, 233 patients presented to Tygerberg Hospital with large pericardial effusions requiring pericardiocentesis. These included 101 (43.0%) females and 132 (67.0%) males. Tuberculous pericarditis accounted for 162 effusions (69.5%), malignant effusions for 22 (9.4%), effusions associated with connective tissue diseases for 12 (5.2%), septic pericarditis for 5 (2.1%), and "other" effusions for 32 (13.7%). In total, 84 patients were HIV positive, including 81 patients who had pericardial TB (50.0% of TB patients). The epidemiology of these effusions has been described in Chapter 3. Eleven of the 162 tuberculous patients had been on anti-tuberculous therapy for more than 48 hours at the time of pericardial aspiration; all of these were HIV negative.

Microbiological/histopathological diagnosis of pericardial TB

"Definite" TB (histological and/or microbiological evidence for TB) was diagnosed in 118 (73%) of 162 patients classified as having tuberculous pericarditis. These included three patients with a positive pericardial fluid Ziehl-Neelsen (ZN) smear, 91 patients with a positive pericardial effusion TB culture, 16 patients with a pericardial biopsy that was diagnostic of TB, 32 patients with a positive ZN smear and/or culture, and 16 patients with a positive TB culture and/or histology in one or more extracardiac site. Positive TB cultures were obtained from pleural fluid (n=8), peritoneal fluid (n=2), blood (n=3), lymph node aspirate (n=3) and skin biopsies (n=2). Twenty-three patients had TB demonstrated in more than one site. Thirty-six

pericardial biopsy specimens were evaluated histologically. Of these, 15 biopsies demonstrated granulomatous inflammation; 12 accompanied by caseating necrosis. A biopsy specimen from an HIV positive patient resembled acute purulent pericarditis; a diagnosis of pericardial TB was based on the presence of numerous acid-fast bacilli (AFB). Of the remaining 20 biopsies, 17 biopsies were non-diagnostic (non-specific features) and three biopsies were diagnostic for non-tuberculous disease, including septic pericarditis, T cell lymphoblastic lymphoma and pericardial adenocarcinoma

Clinical and echocardiographic features

Patients who presented with tuberculous pericarditis were significantly younger than patients who presented with non-tuberculous pericardial disease. The clinical features as observed at the time of admission are summarised in Table 5.1. Significant differences ($p<0.05$) between patients with tuberculous and patients with non-tuberculous effusions were observed with regards to the presence of fever, night sweats, weight loss, cough, dyspnoea, and lymphadenopathy. Univariate analysis comparing HIV positive with HIV negative patients demonstrated an increased frequency of fever ($p=0.02$), weight loss ($p<0.00001$), and lymphadenopathy ($p=0.00015$) in the HIV positive TB group compared to HIV negative pericardial TB patients (Table 5.1.). In addition, oral candidiasis was significantly more frequent in the HIV positive TB group than in the HIV negative TB patients (16 versus 2 cases; $p=0.004$). The echocardiographic features are summarised in Table 5.2.

Table 5.1. Univariate analysis comparing clinical features as observed at admission

	TB/HIV- n=81	TB/HIV+ n=81	p	TB n=162	Non-TB n=71	p
Fever	65%	85%	0.02	75%	52%	0.0006
Night sweats	56%	68%	0.16	62%	30%	<0.0001
Weight loss	64%	94%	<0.0001	79%	44%	<0.0001
Cough	87%	93%	0.32	90%	69%	0.0002
Dyspnoea	80%	93%	0.25	86%	73%	0.03
Orthopnoea	44%	32%	0.08	38%	41%%	0.71
Chest pain	30%	23%	0.22	27%	51%	0.001
Lymphadenopathy	22%	51%	<0.001	36%	20%	0.009
Pleural effusion	42%	34%	0.25	38%	41%	0.71
Tachycardia	72%	76%	0.91	74%	54%	0.003
Soft heart sounds	75%	74%	0.99	75%	55%	0.003
↑ JVP ≥4cm	80%	76%	0.17	78%	70%	0.20
Pulsus paradoxus	24%	30%	0.34	27%	16%	0.15
Hypotension	5%	7%	0.25	6%	6%	0.92
Hepatomegaly	63%	60%	0.78	62%	45%	0.02
Ankle oedema	42%	34%	0.32	38%	47%	0.24

↑ JVP = elevated jugular venous pressure

Table 5.2. Univariate analysis of echocardiography findings at admission

	TB/HIV- n=81	TB/HIV+ n=81	p	TB n=162	Non-TB n=71	p
Tamponade	90%	90%	1.0	90%	78%	0.04
Pericardium >5mm	63%	72%	0.34	67%	46%	0.02
Fibrin strands	60%	68%	0.44	65%	46%	0.02

TB = tuberculosis

HIV- = human immunodeficiency virus negative

HIV+ = human immunodeficiency virus positive

Non-TB = non-tuberculous

Significant differences were noted between the echocardiographic features of tuberculous pericarditis compared to non-tuberculous pericardial effusions regarding the presence of tamponade (p=0.03), pericardial thickness ≥5mm (p=0.02), and the presence of fibrinous strands in the pericardial space (p=0.02). Seven patients were diagnosed with effusive-constrictictive pericarditis, including five patients with tuberculous pericarditis (one of them HIV positive).

Macroscopically, pericardial aspirates could be classified into pus, haemorrhagic or straw-coloured effusions. Tuberculous effusions were more frequently haemorrhagic than non-tuberculous effusions (72% versus 59%; p=0.04). Thirteen pericardial effusions resembled pus, including cases of septic pericarditis (n=4), tuberculous pericarditis (n=8) and one case of systemic lupus erythematosus (SLE).

Biochemistry and haematology results

The biochemistry results have been summarised in Table 5.3. Application of Light's criteria (Light *et al*, 1972) classified all tuberculous pericardial aspirates as exudates. Patients who presented with tuberculous pericarditis had significantly higher serum protein, serum globulin and pericardial protein levels than patients who presented with non-tuberculous pericarditis ($p<0.001$ for each of these variables). Using a serum globulin concentration >40 g/L as a cut-off level for the diagnosis of tuberculous pericarditis resulted in an odds ratio of 15.1 ($p<0.001$), whereas a pericardial total protein level >39 g/L resulted in an odds ratio of 3.42 (p=0.005). Serum protein, serum globulin and pericardial fluid protein levels were significantly higher in HIV positive patients who presented with pericardial TB than HIV negative tuberculous pericarditis patients ($p<0.01$ for each of these variables). Peripheral blood and

pericardial fluid haematology results are summarised in Table 5.4. Peripheral blood total leukocyte counts, neutrophil, lymphocyte and monocytes numbers were significantly lower in patients diagnosed with tuberculous aetiology compared with patients who presented with non-tuberculous pericarditis ($p<0.01$ for each variable), and significant differences were demonstrated between HIV positive and HIV negative pericardial TB patients for each of these variables ($p<0.05$). Using a peripheral blood total leukocyte count $<10x10^9$ cells/L as a cut-off level for the diagnosis of pericardial TB resulted in the respective odds ratio of 12.6 ($p<0.01$). Pericardial fluid total leukocyte counts and pericardial fluid neutrophil counts were significantly higher in non-tuberculous effusions than in tuberculous exudates, which were in turn characterised by significantly higher lymphocyte counts than those found in non-tuberculous cases. Various pericardial lymphocyte/neutrophil (pc-L/N) ratios, were evaluated as cut-off level for the diagnosis of pericardial TB and, based on ROC curves, the best results were obtained at a pericardial fluid L/N ratio ≥1.0, resulting in the corresponding sensitivity, specificity, PPV, NPV and diagnostic efficiency of 73%, 78%, 86%, 61%, and 75%, respectively.

Clinical prediction model for TB diagnosis

The sample set used for deriving the prediction model consisted of 164 patients, who had valid test results, including 110 patients with definite TB (67%) and 54 patients with non-tuberculous pericarditis (33%). Five admission variables were identified that were independently predictive for tuberculous pericarditis and a diagnostic index was calculated for each of these as presented in Table 5.5.

Table 5.3. Comparison of biochemistry results

	TB/HIV- n=64	TB/HIV+ n=78	p	TB n=142	Non-TB n=61	p
S-protein (g/L)	72 (9.8)	78 (8.6)	<0.001	75 (9.0)	65 (11.4)	<0.001
S-globulin (g/L)	43 (7.7)	51 (6.4)	<0.001	47 (7.1)	35 (8.2)	<0.001
S-albumin (g/L)	30 (2.1)	27 (2.2)	0.46	28 (2.2)	30 (2.4)	0.09
S-albumin/S-globulin	0.67 (0.25)	0.48 (0.21)	<0.001	0.56 (0.23)	0.83 (0.29)	<0.001
Pc-protein (g/L)	52.1 (11.9)	60.2 (14.9)	0.001	56.2 (8.0)	47.2 (17.9)	<0.001
Pc-protein/S-protein	0.72 (0.16)	0.79 (0.15)	0.08	0.76 (0.15)	0.73 (0.18)	0.27
Pc-LDH/S-LDH	3.86 (5.06)	2.40 (1.62)	0.01	3.2 (3.31)	4.0 (6.2)	0.26
CRP (mg/L)	109 (84.9)	121 (79.0)	0.46	115 (82)	90 (85.7)	0.04

Results expressed as mean (standard deviation)

S = serum

Pc = pericardial fluid

LDH = lactate dehydrogenase

CRP = C-reactive protein

Table 5.4. Comparison of haematology results

	TB/HIV- n=64	TB/HIV+ n=78	p	TB n=142	Non-TB n=61	p
PB-leukocytes[a]	7.9 (3.37)	6.7 (4.15)	0.01	7.33 (3.76)	12.6 (4.8)	<0.001
PB-neutrophils	5.68 (1.34)	4.92 (0.91)	0.01	5.38 (1.31)	8.87 (1.56)	<0.001
PB-lymphocytes	1.33 (0.61)	0.93 (0.53)	0.03	1.13 (0.57)	2.01 (2.23)	<0.001
PB monocytes	0.55 (0.19)	0.24 (0.12)	0.01	0.40 (0.16)	0.69 (0.25)	0.001
Pc- leukocytes	2.69 (2.33)	1.85 (1.52)	0.03	2.27(1.92)	4.62 (3.81)	0.001
Pc % neutrophils	28.4 (22.5)	35.9 (25.5)	0.05	32.2 (24.0)	55.8 (24.6)	0.01
Pc % lymphocytes	52.1 (25.5)	39.4 (22.5)	0.03	45.5 (25.2)	25.6 (22.4)	0.01

Results expressed as mean (standard deviation)

[a] cells $x10^9/L$

PB = peripheral blood

Pc = pericardial fluid

Table 5.5. Odds ratios and weighted diagnostic index for admission variables

Admission variable	Odds ratio	Weight	Diagnostic index
Weight loss	6.15	0.13	1
Night sweats	4.16	0.09	1
Fever	7.71	0.16	2
Serum globulin >40 g/L	15.09	0.31	3
Leukocyte count $<10x10^9/L$	12.76	0.26	3

The first three variables are yes/no variables, whereas the remaining two (serum globulin and peripheral blood leukocyte count) are measurements for which optimal threshold values were derived using classification tree analysis. For each of these five factors the odds ratio for having TB (when the factor is present) was calculated and these odds ratios were used to calculate the respective weight and the diagnostic index (DI) for each variable in the following manner:

$$weight = \frac{odds\ ratio}{sum\ of\ \ all\ \ odds\ \ ratios} \quad \text{and} \quad DI = weight \times 10$$

The total diagnostic index was calculated for each patient according to the formula: DI (weight loss) + DI (night sweats) + DI (fever) + DI (serum globulin) + DI (peripheral blood leukocyte count). The optimum cut-off for the total diagnostic index was found by use of an ROC curve (Figure 5.1.).

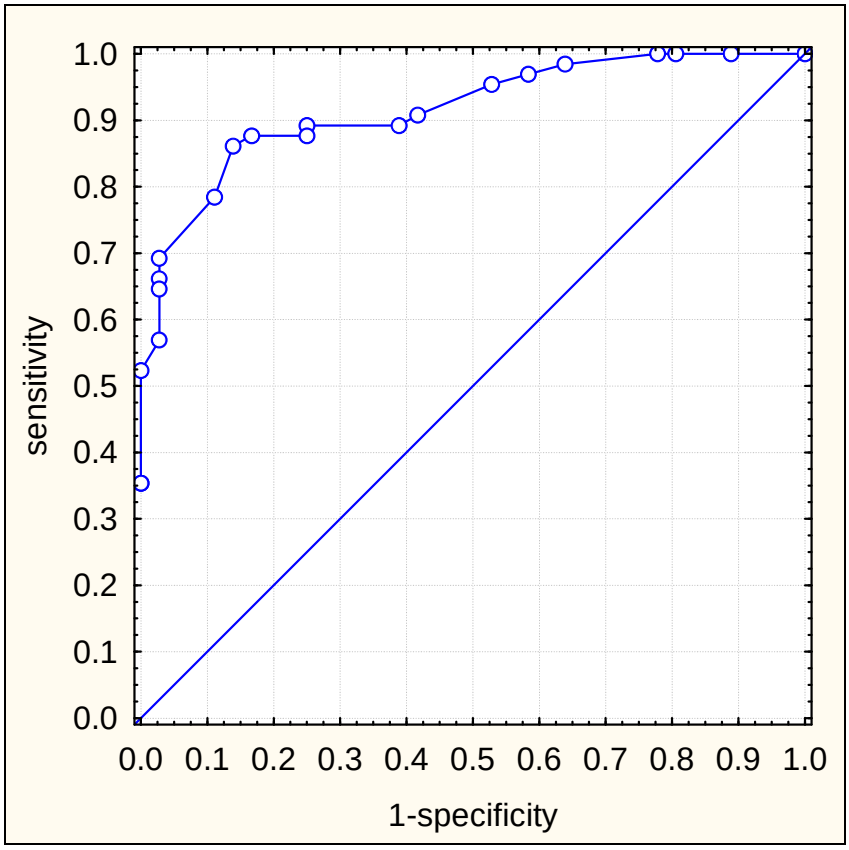

Figure 5.1. Receiver operating characteristic (ROC) curve determining the optimal total diagnostic index for diagnosing pericardial TB

The prediction model was derived from the training set (n=101), and predictions made for the test set (n=63) to determine sensitivity and specificity. The best diagnostic efficiency for diagnosing TB corresponded in the training and in the test set to a total DI of 6 (point on graph closest to top left hand corner). Results for the prediction model applied to the training data demonstrated 86% sensitivity and 87% specificity for the diagnosis of TB, whereas application to the test set resulted in 82% sensitivity and 76% specificity. Our suggested diagnostic rule was therefore: if the patient has a total diagnostic index score of 6 or more, he or she has tuberculous pericarditis, and if the patient has a score of less than 6 he or she has non-tuberculous pericarditis.

Pericardial fluid adenosine deaminase activity

The corresponding median (range) adenosine deaminase (ADA) activity for patients with tuberculous (HIV negative), tuberculous (HIV positive), malignancy, septic pericarditis, connective tissue disease and other non-tuberculous pericardial effusions are presented in Table 5.6. Various levels of pericardial fluid ADA activity were evaluated as a cut-off level for the diagnosis of pericardial TB and, based on ROC curves (Beck and Schultz, 1986), the best results were obtained at a cut-off level of 40 U/L.

Table 5.6. Pericardial ADA activity in various diagnostic groups of pericarditis

Diagnostic groups	n	Adenosine deaminase activity (U/L)		p
		Mean	95% Confidence Intervals	
Tuberculous pericarditis				
HIV negative	75	79.6	69.7 – 89.5	
HIV positive	76	76.3	66.6 – 86.0	1.00
Malignant pericardial effusion	20	39.3	20.0 – 58.6	0.007
Uraemic pericarditis	9	21.1	-7.7 – 49.8	0.004
Septic pericarditis	4	102.3	59.2 – 145.4	1.00
Connective tissue disease	8	31.1	0.6 – 61.6	0.06
Other pericardial effusions	20	30.0	10.7 – 49.3	0.0002

*p-value established by Bonferroni test, notifying difference between diagnostic groups from HIV negative tuberculous pericarditis.

In total, 13 (out of 71) patients with non-tuberculous effusions had levels of ADA activity exceeding 40 U/L. These false positives included patients with septic pericarditis (n=4; corresponding ADA activities of 49.1, 55.6, 138.6 and 165.8 U/L, respectively), one case of systemic lupus erythematosus (SLE; corresponding ADA 49 U/L), rheumatoid arthritis (n=1; corresponding ADA 65.3 U/L), post-traumatic pericarditis (n=1; corresponding ADA 47.5 U/L), pericarditis of unknown origin (n=1; corresponding ADA 50.4 U/L), non-haematological malignancies (n=3); and haematological malignancies (n=2). The malignancies included pericardial adenocarcinoma (ADA 47.5 U/L), squamous cell carcinoma (ADA 46.0 U/L), undifferentiated carcinoma (ADA 50.9 U/L), lymphoblastic T cell lymphoma (ADA 166.0 U/L) and chronic myelomonocytic leukaemia (ADA 102.5 U/L). Using the cut-off level of 40 U/L resulted in 22 false negative pericardial effusions, 12 of these occurred in HIV negative and ten in HIV positive patients, respectively. Nine of the 12 HIV negative patients were on anti-tuberculous therapy at the time of pericardiocentesis, whereas no HIV positive patient was on active anti-tuberculous therapy at the time of pericardial aspiration. An additional two HIV negative patients were also on anti-tuberculous medication; in these two the corresponding pericardial ADA activity levels were 75.0 U/L and 81.0 U/L, respectively. The corresponding median (range) ADA activity for HIV negative TB patients on anti-tuberculous therapy, HIV negative TB patients not on anti-tuberculous therapy, HIV positive TB patients and patients with non-tuberculous pericardial effusions is presented in Table 5.7. In three patients with tuberculous pericarditis no specific cause could be identified for the low pericardial ADA activity; the corresponding ADA activity for these three cases was 22 U/L, 24 U/L and 34 U/L, respectively.

After exclusion of all patients on anti-tuberculous therapy and all those patients categorized as pericarditis of unknown cause, an ADA activity cut-off level of 40 U/L resulted in the corresponding sensitivity, specificity, PPV, NPV and diagnostic efficiency of 90%, 74%, 90%, 76% and 86%, respectively.

Pericardial fluid gamma-interferon concentration

The corresponding mean (SD) IFN-γ concentrations for HIV negative tuberculous, HIV positive tuberculous and non-tuberculous effusions were 787 (115) pg/mL, 624 (103) pg/mL, and 27 (19) pg/mL, respectively. The difference in IFN-γ concentration between tuberculous exudates and non-tuberculous effusions was highly significant ($p<0.0001$), whereas no difference was seen between HIV positive and HIV negative tuberculous groups ($p=0.89$). IFN-γ levels were detectable in only three of the non-tuberculous effusions, including a case of staphylococcal sepsis (corresponding IFN-γ concentration of 28.9 pg/mL), a case of metastatic adenocarcinoma (42.9 pg/mL), and the remaining case was caused by diffuse large cell lymphoma (39.4 pg/mL). Various levels of pericardial fluid IFN-γ concentration were evaluated as a cut-off level for the diagnosis of pericardial TB and, based on ROC curves, best results were obtained at a cut-off level of 50 pg/mL. Using a cut-off level of 50 pg/mL as being diagnostic for tuberculous pericarditis resulted in 92% sensitivity, 100% specificity and a positive predictive value of 100% for pericardial TB.

Table 5.7. Pericardial ADA activity in tuberculous and non-tuberculous pericarditis

Diagnostic groups	n	ADA activity (U/L)		p
		Mean	95% Confidence intervals	
Tuberculous pericarditis				
HIV negative (not on ATC)	64	88.9	78.5 – 99.3	
HIV negative (on ATC)	11	24.9	0.1 - 50.1	0.000004
HIV positive	76	76.3	66.6 – 86.0	0.48
Non-tuberculous pericarditis	61	36.6	25.9 – 47.3	0.001

ADA = adenosine deaminase activity

ATC anti-tuberculous chemotherapy

*p-value established by Bonferroni test, notifying difference between diagnostic groups from HIV negative tuberculous pericarditis not on ATC

Polymerase chain reaction for *Mycobacterium tuberculosis*

In this study PCR for *M. tuberculosis* was used on pericardial fluid samples of 48 consecutive patients, including 33 patients with tuberculous pericarditis and 15 patients with non-tuberculous effusions. Positive PCR results were obtained for four "definite" tuberculous effusions and for six "probable" tuberculous effusions, but for none of the non-tuberculous effusions. Inclusion of the "probable" TB cases in the evaluation of the diagnostic utility of PCR resulted in the corresponding sensitivity, specificity, PPV, NPV and diagnostic efficiency of 30%, 100%, 100%, 31%, and 52%, respectively.

Cytopathology

Cytopathology results were available for 202 patients, including 146 patients who were diagnosed with pericardial TB and 56 patients who presented with non-tuberculous pericarditis. Ten out of 146 TB patients were using anti-tuberculous chemotherapy at the time of the pericardiocentesis and were excluded from further analyses. Each pericardial fluid specimen (n=192) was classified into one specific cytopathological category on the basis of cytodiagnostic criteria (Spriggs and Boddington, 1960; Yam, 1967; Pettersson, 1982; Gibas *et* al, 1986; Gresham, 1989) as summarised in Table 5.8.

Table 5.8. Cytopathological classification for various diagnostic groups

Diagnosis	n	Benign	Purulent	Non-purulent	Tuberculous	Malignant
Tuberculous	136	7 (5%)	10 (8%)	108 (79%)	11 (8%)	
HIV negative	61	3 (5%)	4 (7%)	48 (79%)	6 (10%)	
HIV positive	75	4 (5%)	6 (8%)	60 (80%)	5 (7%)	
Non-tuberculous	56	11 (20%)	6 (11%)	25 (45%)	2 (4%)	12 (21%)
Malignant	19	1 (5%)		5 (26%)	1 (5%)	12 (63%)
CNTD	9	3 (33%)	1 (11%)	4 (44%)	1 (11%)	
Uraemia	8	2 (25%)		6 (75%)		
Septic	4		4 (100%)			
Traumatic	7	1 (14%)		6 (86%)		
Idiopathic	5	2 (40%)		3 (60%)		
Other	4	2 (50%)	1 (25%)	1 (25%)		

CNTD = connective tissue disease

Three of the 192 effusions were ZN positive, including one purulent and two non-purulent inflammatory effusions; all three were found in HIV positive individuals and results were verified by positive TB culture. None of these effusions fulfilled the cytodiagnostic criteria for the category "tuberculous" effusion. In the 136 tuberculous pericarditis patients (diagnosed according to predetermined criteria; Chapter 2), the most common cytopathological diagnosis was "non-purulent inflammatory effusion", which was demonstrated in 79% of TB patients. Pericardial fluid lymphocyte/neutrophil (L/N) ratios >3:1 were demonstrated in 53% of HIV negative pericardial effusions and in 20% of HIV positive tuberculous effusions, and significant numbers of mesothelial cells were present in the majority of pericardial aspirates obtained from pericardial TB patients. The pericardial aspirates of 13 patients were classified as "tuberculous" cytology (Figure 5.2.a), including 11 patients with a diagnosis of pericardial TB (according to predetermined criteria; Chapter 2), one case of SLE, and one case of histologically confirmed lymphoblastic T cell lymphoma. Cytopathological review of the lymphoma patient's aspirate revealed cytological features suggestive of a haematological malignancy (Figure 5.2.b). Thirty-six pericardial biopsy samples were available for comparative analysis between histopathological diagnosis and cytopathological diagnosis. Fifteen of the 36 biopsies demonstrated granulomatous inflammation. The cytological classification in these 15 cases included "non-purulent inflammatory" effusion (n=13) and "tuberculous" effusion (n=2). Two cases of serofibrinous pericarditis and one case of pericardial lymphoma corresponded to the cytological category "tuberculous" effusion. Using the cytodiagnostic category "tuberculous" effusion as measure of diagnosing pericardial TB resulted in 11% sensitivity and 85% specificity.

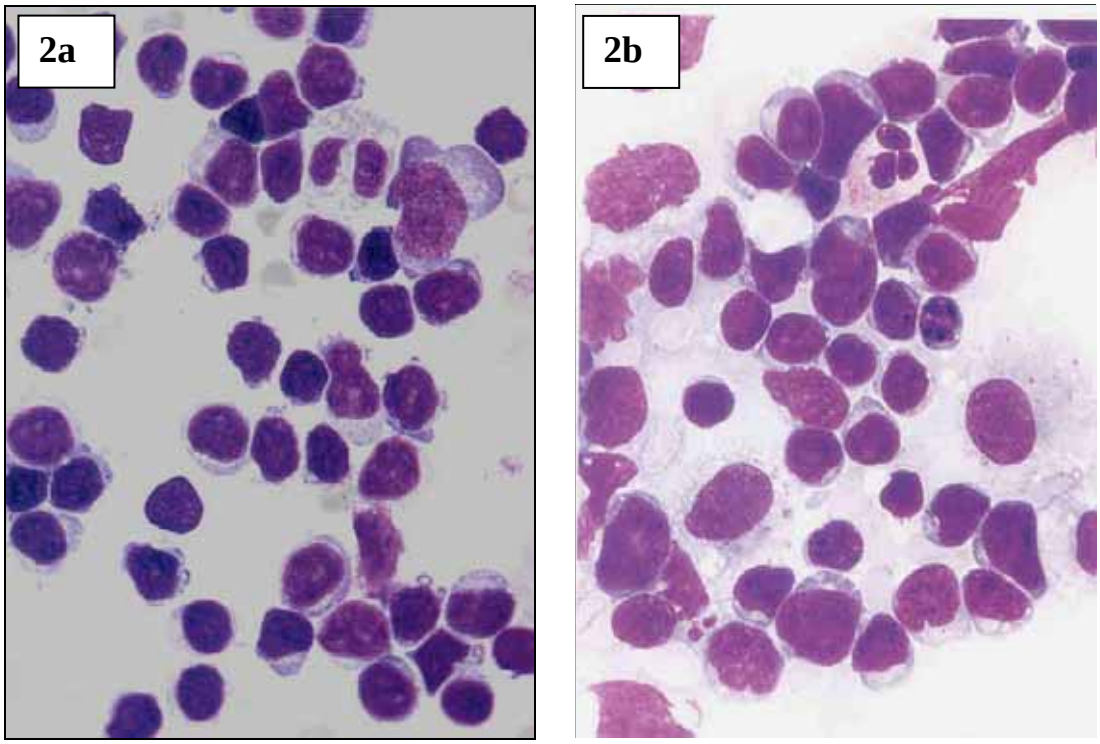

Figure 5.2. Cytopathology smears of patients with large pericardial effusions

Tuberculin skin testing

Tuberculin skin testing was performed in 52 consecutive patients, including 36 patients with tuberculous pericarditis (12 HIV positive) and 16 patients with non-tuberculous pericarditis. The aetiological causes for the non-tuberculous pericarditis group included malignancy (n=6), septic pericarditis (n=2), uraemic pericarditis (n=2), post-traumatic pericarditis (n=2), rheumatoid arthritis (n=1), scleroderma (n=1) and systemic lupus erythematosus (n=1). Different cut-off levels for diameter of skin induration were tested and the best diagnostic efficiency for diagnosing TB (including HIV positive patients) was obtained at a diameter ≥10 mm. At this cut-off level, tuberculin skin tests were positive in 32 out of 36 tuberculous pericarditis patients (89% "true positive") and in seven out of 16 patients with non-tuberculous effusions (44% "false positive") resulting in the corresponding sensitivity, specificity, PPV, NPV and diagnostic efficiency of 89%, 56%, 82%, 69%, and 79%, respectively. At a cut-off level (diameter) ≥15 mm of skin induration, the respective sensitivity, specificity, PPV, NPV and DE was 43%, 93%, 93%, 38%, and 57%.

Diagnostic classification tree

Based on the predictive sensitivity and specificity for the diagnosis of TB, we identified four parameters that form the basis of the diagnostic classification tree that we developed as an aid for the diagnosis of pericardial TB (Figure 5.3.). Application of the patient data set resulted in 96% sensitivity and 97% specificity for the diagnosis of pericardial TB (Table 5.9.). The utility of various tests for the diagnosis of TB, including pericardial effusion culture, pericardial histology, PCR, IFN-γ and ADA activity were evaluated and the results are summarised in Table 5.9.

Table 5.9. Utility of various diagnostic tests

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DE (%)
Pericardial effusion culture positive	52	100	100	48	56
Histopathology (granulomatous and/or ZN+)	64	100	100	55	75
Pericardial ADA activity ≥40 U/L	87	89	95	72	88
Pericardial lymphocyte/ neutrophil ratio ≥1	73	79	86	61	75
Interferon-γ ≥50 pg/mL	92	100	100	85	94
PCR for *M. tuberculosis* positive	30	100	100	31	52
Diagnostic classification tree (excluding "probable" TB patients)	96	97	98	94	96
Diagnostic classification tree (including "probable" TB patients)	96	97	98	92	96

PPV = positive predictive value

NPV = negative predictive value

DE = diagnostic efficiency

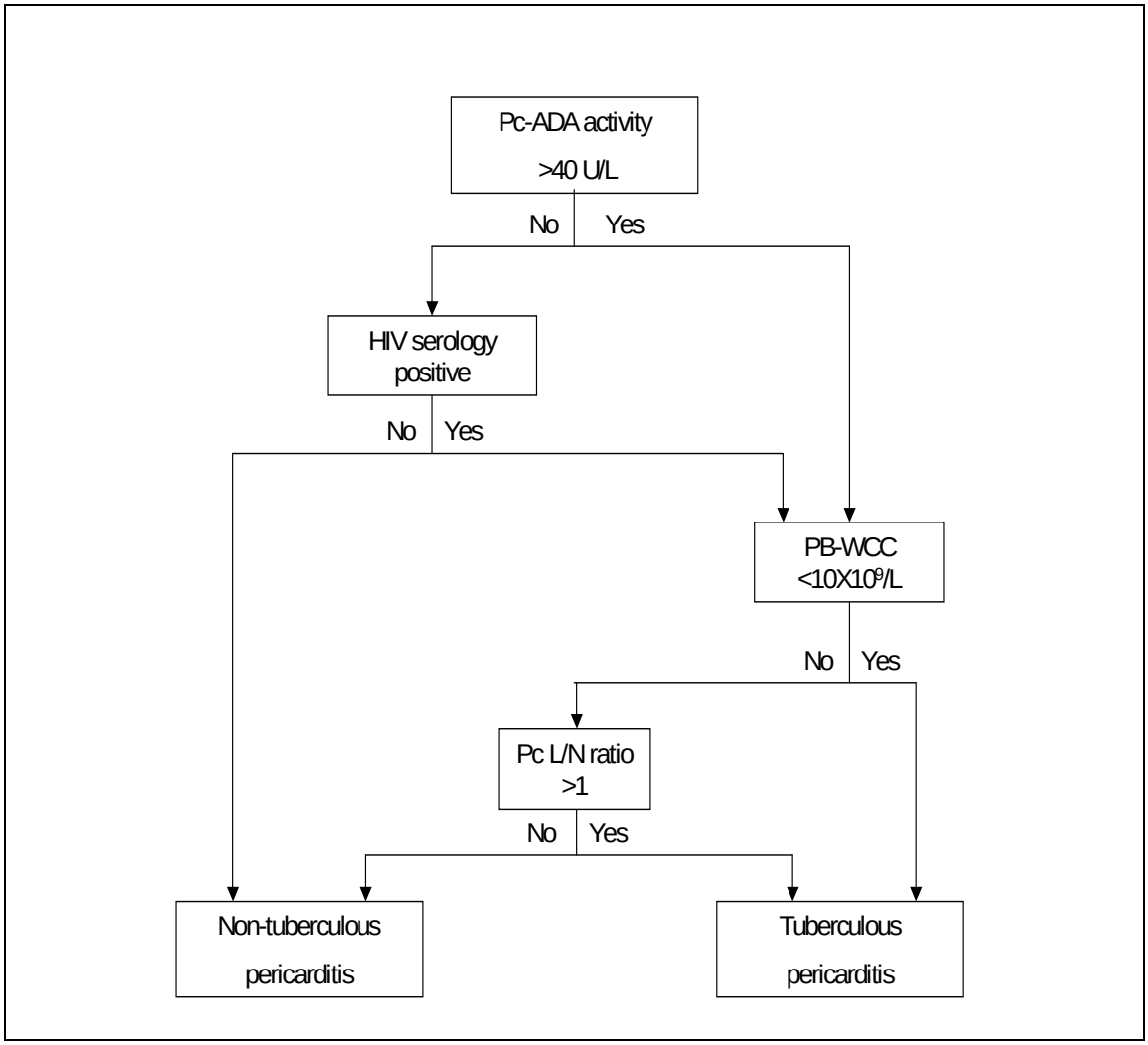

Figure 5.3. Classification tree developed for the diagnosis of pericardial TB

Pc-ADA = pericardial adenosine deaminase

PB-WCC = peripheral blood white cell count

Pc L/N ratio = pericardial lymphocyte / neutrophil ratio

DISCUSSION

The diagnosis of tuberculous pericarditis is important, because without specific treatment, the average survival is 3.7 months and the mortality approaches 85% at six months (Desai, 1979). Our study confirmed the insensitivity of the traditional diagnostic tools used for diagnosing pericardial TB. Pericardial biopsy specimens taken from the tuberculous group demonstrated caseating granulomatous inflammation in about 50% of biopsy samples and non-specific histopathological findings were common, even when *M. tuberculosis* was found in the pericardial fluid. Although mycobacterial culture was much more sensitive than fluid smear, it yielded positive results in only 56% of patients diagnosed with pericardial TB. In our study the microbiological diagnosis was significantly augmented by culturing *M. tuberculosis* from extracardiac sites, including sputum (n=32), pleural fluid (n=8) and lymph node aspirate (n=3). Pleural TB and peripheral tuberculous lymphadenitis are the two most common forms of extra-pulmonary TB (Light, 1994), and both are commonly seen in patients with pericardial TB. In one series, 50% of patients with tuberculous pericarditis had necropsy evidence of pleural effusion due to tuberculous pleuritis (Rooney *et al*, 1979), and peripheral lymphadenopathy affecting the cervical glands has been reported in 13%–28% with tuberculous pericarditis (Desai, 1979; Cherian, 2004). Persistent generalised lymphadenopathy is a common manifestation of HIV positive patients (Jacobson *et al*, 1991; Pantaleo *et al*, 1994), however, when the enlarged lymph nodes are >1cm in diameter and their distribution not symmetrical, disseminated TB needs to be considered. Tuberculous lymphadenopathy is characterised by caseation and "matting" on palpation, and the adenopathy clears or regresses on specific therapy (Cherian *et al*, 2003b). The diagnostic workup of patients with suspected pericardial TB should thus include sputum smears in patients

with a productive cough and lymph node aspiration when possible. However, culture results take long and rarely influence decision-making.

We documented high levels of ADA in tuberculous pericardial effusions. In HIV negative TB patients the most notable cause for low pericardial ADA levels was the concomitant use of anti-tuberculous chemotherapy at the time of pericardiocentesis, suggesting that anti-tuberculous therapy influences ADA activity by one or more of the following mechanisms: (i) it effects the cell turnover of pericardial macrophages and T lymphocytes, thereby reducing ADA levels, and/or (ii) it increases the metabolic breakdown of ADA (possibly by rifampicin's enzyme inducing effect on hepatic metabolism), and/or (iii) anti-tuberculous therapy suppresses ADA activity directly.

Pericardial lymphocyte counts were higher and pericardial neutrophil counts significantly lower in patients with tuberculous effusions compared to patients who presented with non-tuberculous effusions, whereas median ADA activity was significantly higher in tuberculous than in non-tuberculous exudates. This implies that in tuberculous effusions lymphocytes (and possibly macrophages) play the major role in the production and release of ADA as has been demonstrated in the case of tuberculous pleural exudates (Valdes *et al*, 1996). ADA is a polymorphic enzyme that is involved in purine metabolism and metabolises the deamination of adenosine to inosine and ammonium (Van der Weyden and Kelley, 1976). There are two principal isoenzymes of ADA, namely ADA_1 and ADA_2. ADA_1 is found in all cells, with the highest activity in lymphocytes and monocytes (Ungerer *et al*, 1992), whereas ADA_2 exists only in monocytes (Ungerer *et al*, 1992; Gakis, 1996). The elevated ADA levels

found in tuberculous exudates are thought to result from cell-mediated immunity (Blake and Berman, 1982), and HIV infection has been implicated as a cause for low ADA levels in patients with TB (Hsu *et al*, 1993). In our study ADA activity was significantly more elevated in tuberculous than in non-tuberculous pericardial effusions. Pericardial ADA levels were not affected by HIV infection, and contrary to a previous report (Hsu *et al*, 1993), the diagnostic utility measuring ADA activity was not diminished by underlying HIV. Based on ROC curves (Beck and Schultz, 1986), the best diagnostic results were obtained at a pericardial ADA cut-off level of 40 U/L, which corresponded to a sensitivity, specificity, PPV, NPV, and diagnostic efficiency of 90%, 74%, 90%, 76% and 86%, respectively.

The use of pericardial ADA levels in a patient with suggestive clinical features provides a rapid and accurate means of diagnosing tuberculous pericarditis, especially in high-prevalence areas. If a high ADA level is found, septic pericarditis and haematological neoplastic diseases need to be excluded by differential white blood cell, direct microscopy, Gram stain, bacterial culture, and cytological analysis, all of which should be performed routinely on patients with large pericardial effusions. If these tests are negative, the diagnosis will in all likelihood be TB, especially in countries where this infection is endemic or in those infected with HIV. If a low level of ADA is found, non-tuberculous causes are likely, and further tests such as cytology or pericardial biopsy are indicated to determine the cause.

The cytopathological analysis of tuberculous pericardial exudates demonstrated two distinct differences between pericardial and pleural tuberculous exudates. First, mesothelial cells were present in the majority of tuberculous pericardial aspirates, and

second, almost 30% of tuberculous pericardial exudates displayed neutrophilic dominance, whereas tuberculous pleural exudates are characterised by prominent lymphocytosis and virtually complete absence of mesothelial cells (Spriggs and Boddington, 1960; Yam, 1967, Gresham, 1989). Consequently, strict applicarionof the cytodiagnostic criteria that had been developed for the diagnosis of pleural TB resulted in a low diagnostic yield for pericardial TB. However, the finding of pericardial lymphocytosis was nevertheless 73% sensitive and 79% specific for a diagnosis of pericardial TB. The literature is vague about pericardial lymphocytosis; it is mentioned but has to the best of our knowledge not been quantified (Harvey and Whitehill, 1937; Hopewell, 1994; Cherian *et al*, 2004). Our study illustrated the useful role of pericardial cytology for the diagnosis of malignant effusions (Reyes *et al*, 1982; Pozniak *et al*, 1986; Di Bonito *et al*, 1990), and as a screening tool for septic pericarditis. Finding a purulent exudate does however not exclude TB, and has also been described for SLE, rheumatoid arthritis, and various fungal infections (Gresham, 1989, Meyers *et al*, 1997). Further investigations such as Gram stain, bacterial culture and TB culture are necessary. If possible, determining IFN-γ levels would be the ideal investigation. Significantly elevated levels of IFN-γ were demonstrated in tuberculous pericarditis compared to non-tuberculous pericardial effusions ($p<0.00005$), and importantly, concentrations never exceeded 50 pg/mL in any of the non-tuberculous effusions. Using a cut-off level of 50 pg/mL as being diagnostic for tuberculous pericarditis resulted in 92% sensitivity, 100% specificity and a diagnostic efficiency of 94% and the diagnostic usefulness was not influenced by HIV infection. The underlying immunological principle has resulted in the development of the highly sensitive and accurate enzyme-linked immunospot (ELISPOT) test that detects IFN-γ producing T-cells specific for *M. tuberculosis* antigen (Ewer *et al*, 2003; Liebeschuetz

et al, 2004). The ELISPOT test is very useful to detect tuberculous infection, but it does not differentiate between latent infection and active disease, which is a major problem for use in TB endemic areas. The diagnostic utility of currently available IFN-γ assays is unfortunately seriously limited by technical and financial constraints.

Less sensitive, but related to the ELISPOT test is tuberculin skin testing where a T cell mediated immune response to an intradermally injected dose of purified protein derivative (PPD) is quantified by measuring the diameter of skin induration 48-72 hours after injection. Our study confirmed lack of specificity for the diagnosis of active TB. At a cut-off level of 10 mm skin induration we observed 89% sensitivity and 56% specificity for correct diagnostic classification. The high proportion of "false positive" tests (44% of patients with non-tuberculous effusions) is similar to previously reported rates of 30–40% (Fowler, 1991). A strongly positive tuberculin skin test increases the suspicion of pericardial TB, but it is important to realise that a negative result (11% of TB patients in our study) does not exclude tuberculous disease, and the response to tuberculin may be affected by HIV infection, malnutrition and the time of presentation (Fowler and Manitas, 1973; Rooney *et al*, 1970; Pozniak *et al*, 1994; Trautner and Darouiche, 2001).

The use of PCR for the detection of *M. tuberculosis* provides a test with high specificity for the diagnosis of pericardial TB; however, we observed a low diagnostic sensitivity of 32%. This surprisingly poor sensitivity has also been reported in tuberculous pleural effusion (Vlaspolder *et al*, 1995) and pericardial effusion studies (Cegielski *et al*, 1997), where corresponding sensitivity ranged between 15-20% and specificity between 96-100%, respectively. Explanations for the poor sensitivity of

PCR in tuberculous exudates include poor specimen preparation, the presence of inhibitors such as fibrin, haemoglobin, as well as low numbers of tubercle bacilli or their DNA in the specimens (Kox *et al,* 1994; Ferrer, 1997). Sensitivity is much better when pericardial tissue is used for analysis by PCR analysis (Cegielski *et al*, 1997; Rana *et al*, 1999). Recently, concerns have been raised about false positive results with PCR (Lee *et al*, 2002). Overall, PCR has not provided the answers to the quest for a sensitive, specific and cost-effective tool for the diagnosis of TB.

For 2003, the WHO reported 8.8 million new cases of TB and an estimated 1.7 million TB deaths, including 229 000 coinfected with HIV (WHO report, 2005). The majority of TB cases come from of South East Asia, however most cases of HIV and TB co-infection were reported in sub-Saharan Africa (WHO report 2005). These regions are characterised by serious lack of financial resources and almost complete absence of diagnostic infrastructure (Cegielski *et al*, 1994; Maher and Harries, 1997; Thwaites *et al*, 2002). Our study demonstrates the potential usefulness of a basic diagnostic rule that assists clinical decision-making and could be applied in resource poor settings. We developed this diagnostic aid by identifying independently predictive clinical features and basic laboratory tests. In our study, the features of pericardial effusion and cardiac compression (jugular distension, soft heart sounds, pulsus paradoxus, Kussmauls's sign, ankle oedema, hypotension) were similarly distributed among tuberculous patients and non-tuberculous patients; this concurs with previous reports in the literature (Schepers, 1962; Hageman *et al*, 1964; Rooney *et al*, 1970; Fowler and Manitas, 1973; Gooi and Smith 1978; Ortbals and Avioli, 1979; Desai, 1979; Fowler, 1991).

Univariate analysis of the admission variables suggested a set of potentially discriminative clinical features, including cough, fever, night sweats, weight loss and lymphadenopathy. In addition, patients with tuberculous pericarditis have higher serum globulin levels than non-tuberculous patients, and will usually not present with peripheral blood leukocytosis. Multivariate logistical regression and CART analyses identified five of these features that were independently predictive of distinction between tuberculous and non-tubercuous pericarditis, namely fever, night sweats, weight loss, globulin level and peripheral leukocyte count (Table 5.5). Based on the respective odds ratio for each of these five variables, we developed a weighted score or diagnostic index that if added together would amount to a potential maximum score of 10 (Table 5.5). By using a ROC curves we demonstrated the best diagnostic efficiency for the training and the test set at a total score of 6. Results for the prediction model applied to the training data demonstrated 86% sensitivity and 87% specificity for the diagnosis of TB, whereas application to the test set resulted in 84% sensitivity and 78% specificity. Our suggested diagnostic rule was therefore: if the patient has a total diagnostic index score of 6 or more, he or she has tuberculous pericarditis, and if the patient has a score of less than 6 he or she has non-tuberculous pericarditis. In spite of the simplicity of the rule, the diagnostic results are similar or better than those reported for culture or pericardial histology (Strang 1984; Fowler, 1991; Zayas *et al*, 1995). Major advantages of this proposed diagnostic rule include the non-invasiveness, availability, cost-effectiveness and the rapidity of the laboratory tests. However, the predictive value of this diagnostic rule may be influenced by the prevalence of HIV and TB. In a setting with a substantially different TB and HIV prevalence than that of the Western Cape, these predictors should not be used without prospective evaluation in those settings.

The strength of association between HIV infection and tuberculous pericarditis cannot be overemphasised. Pericardial effusion is recognised as one of the early presenting features of HIV infection in sub-Saharan Africa (Cegielski *et al*, 1990; Taelman *et al*, 1990; Cegielski *et al*, 1994), and the disease has been ascribed to TB in as many as 100% of cases (Cegielski *et al*, 1994; Longo-Mbenza *et al*, 1997). In our series, TB caused 94% of cases, and only three out of 84 HIV positive patients were diagnosed with non-tuberculous aetiology, including two cases of septic pericarditis. Univariate analysis demonstrated significantly higher frequency of lymphadenopathy, weight loss, and oral candidiasis in HIV positive TB patients compared with the HIV negative group. The differential diagnosis of pericarditis in HIV positive individuals includes other opportunistic infections (bacterial, fungal, protozoal or viral), Kaposi's sarcoma, lymphoma, uraemia, and idiopathic pericarditis (Acierno, 1990; Reynolds *et al*, 1992; Kwan *et al*, 1993). In our experience septic pericarditis was the most likely non-tuberculous cause, and it was characterised by a pericardial fluid lymphocyte/neutrophil ratio <0.5, peripheral blood leukocyte counts $>10x10^9$/L, peripheral blood neutrophil counts $>7x10^9$/L and pericardial leukocyte count $>2x10^9$/L.

By combining the predictive attributes of four widely available laboratory tests into a diagnostic classification tree (Figure 5.3.) we achieved our aim of developing a tool that is sensitive, specific, rapid and relatively inexpensive. HIV serology, pericardial ADA activity, peripheral blood leukocyte count and pericardial differential leukocyte do not require sophisticated technology and can be performed in rural hospitals. ADA is an enzyme that is stable for at least 24 hours at 25°C, 7 days at 4°C and 3 months at -20°C (Ellis and Goldberg, 1970; Heinz, 1984). The major advantage of determining

ADA activity lies in the fact that it can be determined within hours by a simple hand-method requiring only a spectrophotometer (Burgess *et al*, 2002b). It is thus possible to perform this analysis in basic peripheral and rural laboratories. Application of the entire patient data set to the classification tree resulted in a sensitivity of 96%, a specificity of 97%, and a diagnostic efficiency of 96%, and the diagnostic utility was not influenced by inclusion or exclusion of the patients with "probable TB". In settings where it is not possible to measure ADA activity and whenever pericardiocentesis cannot be done we suggest the use of the diagnostic rule, using a score of 6 or more as a predictor of pericardial TB. The likelihood of TB increases with an increase in the total diagnostic index. The predictive value of characteristic clinical features and diagnostic tests used for the determination of tuberculous aetiology is influenced by the prevalence of HIV and TB infection in the population studied. In areas of substantially different HIV and TB prevalence to those of Southern Africa the diagnostic classification tree and the diagnostic rule should not be used until prospectively assessed for diagnostic accuracy in such an area.

When available, IFN-γ is the diagnostic tool of choice and should be used to aid in the rapid diagnosis of pericardial TB. It can be used effectively in combination with ADA and pericardial cytology to confirm or exclude contentious cases. The diagnostic utility of currently available IFN-γ assays is however, seriously limited by technical and financial constraints.

Chapter 6

THE ROLE OF ELECTROCARDIOGRAPHY AND RADIOGRAPHY IN PERICARDIAL DISEASE

Echocardiography is the most useful test for the diagnosis of pericardial disease, but where not available, electrocardiography and chest radiography (CXR) have to be alternatives. Several studies have found the 12-lead electrocardiogram (ECG) to be helpful in the diagnosis of pericarditis. Electrocardiographic changes have been described in 50-70% of patients presenting with pericarditis (Fowler, 1992). The presence of micro-voltage has been reported to correlate with a large effusion size and the presence of electrical alternans and PR segment depression was found to be specific for the presence of cardiac tamponade (Eisenberg *et al*, 1996; Spodick, 1997). The majority of studies have been conducted in patients with characteristics that differ from our population. In published studies, tuberculous pericarditis was rare, whereas the majority of pericardial effusions in our hospital are attributable to TB. The classical finding on chest X-ray (CXR) is an enlarged globular cardiac shadow with a clear margin due to impaired movement of the distended pericardium (Commerford and Strang, 1991). It is not clear how the CXR assists in diagnosing the underlying cause for large pericardial effusions. According to the literature, less than one third of patients with tuberculous pericardial effusions have any evidence of active pulmonary tuberculosis (TB) on CXR (Strang *et al*, 1988; Lorell, 1997).

The aim of this study was thus to evaluate the usefulness of electrocardiography and chest radiography in patients presenting with large pericardial effusions.

PATIENTS AND METHODS

All new patients that were referred between February 1995 and June 1999 and had echocardiographically confirmed large pericardial effusions, with an epi-pericardial separation of more then 10 mm, underwent the following diagnostic work up: history, physical examination, 12-lead surface ECG (MAC, Marquett Electronics, Inc; Milwakee, Wisconsin; paper speed 25 mm/sec), CXR with postero-anterior and lateral views, and two dimensional echocardiographic studies (Hewlett Packard, Sonos 2000 Phased Array Imaging System). The location (anterior, apical, posterior, inferior, circumferential), measured epi-pericardial distance (mm), presence and amount of epicardial thickening (mm), presence of fibrin strands, signs of tamponade (at least 30% of the right atrial wall inverted during late diastole/early systole, inward motion of the right ventricular wall in early diastole persisting after mitral valve opening), and presence and localization of constriction were determined, followed by pericardiocentesis and drainage by an indwelling pig-tail catheter. The amount of drained effusion was measured. Four groups of patients were identified according to the amount that was drained, namely < 499 mL, 500-999 mL, 1000-1499 mL, > 1500 mL, respectively.

The admission CXR was studied in detail by a radiologist and two physicians, all of whom were blinded to the clinical diagnosis. The following radiological parameters were noted: the cardio-thoracic ratio (CTR), presence of pericardial or pleural calcifications, presence of pleural effusions, mediastinal lymphadenopathy, pulmonary mass lesion, features of disseminated TB, alveolar infiltrates and evidence of cavitatory lesions. The admission ECG was evaluated by two physicians with regards to various parameters, including rate, rhythm, and presence of micro-voltage,

electrical alternans, PR segment abnormalities, J-ST segment abnormalities, and T wave abnormalities. Micro-voltage was diagnosed when all limb lead complexes were <5 mm, and or all precordial leads complexes <10 mm.

RESULTS

Poor quality radiographs and those of patients with "probable" TB were excluded. A radiologist and two physicians who were not aware of the aetiological diagnosis evaluated 129 radiographs, including the radiographs of 90 patients with tuberculous pericardial effusions and 39 individuals with non-tuberculous pericardial effusions. The aetiology of these non-tuberculous effusions included malignancy (n=17), septic pericarditis (n=3), uraemic pericarditis (n=6), systemic lupus erythematosus (n=5), rheumatoid arthritis (n=1), scleroderma (n=1), post-traumatic pericarditis (n=4), and idiopathic pericarditis (n=2). Forty-three of the subjects were HIV positive, including 41 patients who presented with tuberculous pericarditis and two patients who were diagnosed with pericardial sepsis. The median (range) CD4+ lymphocyte count of the HIV positive patients was 266 (34–1006) cells/μL. The echocardiographic data are presented in Table 6.1. There was no significant difference between the HIV positive and HIV negative patients with regards to the amount of pericardial fluid drained or the echocardiographic findings. All 129 radiographs revealed enlarged cardiac silhouettes (cardio-thoracic ratio >0.55). The radiographic findings are summarised in Table 6.2. In ten tuberculous pericarditis patients, the CTR was >0.75. Eighteen patients who presented with non-tuberculous pericarditis (46%) and 45 of the pericardial TB patients (50%) had radiological evidence of pleural effusions, with no significant difference between HIV positive and negative individuals. In the tuberculous group 12 patients presented with bilateral effusions compared to 15

Table 6.1. Echocardiographic data of patients presenting with pericarditis

Parameter	TB/HIV+ (n=41)	TB/HIV– (n=49)	Non-TB (n=39)
Presence of tamponade	37 (90%)	44 (90%)	30 (78%)
Presence of fibrin strands	26 (63%)	32 (65%)	16 (41%)
Effusive constriction	2 (5%)	3 (6%)	2 (5%)
Pericardial thickness (mm)	6.7	6.8	6.3
Volume of aspirate (mL) * [Median (range)]	816 (250 – 1 800)	832 (250 – 2 700)	678 (80-1450)

* Volumes not available for 7 patients (HIV+: n=1, HIV-: n=2, non-TB: n=4)

Table 6.2. Summary of CXR findings of patients presenting with pericarditis

Variable	TB/HIV+ (n=41)	TB/HIV– (n=49)	Non-TB (n=39)
CTR > 0.55 and ≤ 0.75	39 (95%)	41 (84%)	39 (100%)
CTR > 0.75	2 (5%)	8 (16%)	0 (0%)
Pleural effusion	20 (49%)	25 (51%)	18 (46%)
Left-sided pleural effusion	7 (17%)	8 (16%)	6 (15%)
Right-sided pleural effusion	8 (20%)	10 (20%)	7 (18%)
Bilateral pleural effusion	5 (12%)	7 (14%)	5 (13%)
Mediastinal lymphadenopathy	5 (12%)	2 (4%)	2 (5%)
Disseminated TB	2 (5%)	2 (4%)	0 (0%)
Alveolar infiltrates with cavitation	2 (5%)	5 (10%)	0 (0%)
Alveolar infiltrates without cavitation	2 (5%)	2 (4%)	1 (3%)
Bronchopneumonia	1 (2%)	2 (4%)	2 (5%)
Consolidation pneumonia	2 (5%)	0 (0%)	1 (3%)
Pulmonary mass lesion	0 (0%)	0 (0%)	2 (5%)

CTR = cardio-thoracic ratio

patients with left-sided and 18 with right-sided pleural effusions. In HIV positive TB patients, the median (range) CD4+ lymphocyte count was significantly higher in patients with pleural effusions than in those without pleural effusions [312 (34–1006) cells/μL versus 144 (39–445) cells/μL, respectively; $p < 0.05$].

It was generally difficult to evaluate the hilar region, especially on the left side, due to the cardiac enlargement and displacement of anatomical structures. Two patients who presented with malignant pericardial effusions demonstrated radiographic evidence for mediastinal lymphadenopathy. Among the tuberculous patients, four were identified with right-sided mediastinal lymphadenopathy and three had bilateral mediastinal node enlargement; five of these seven patients were HIV positive. Seven patients had radiographic evidence of cavitatory disease and six of these were found to be sputum smear positive for *M. tuberculosis.* A total of 27 patients (30%) presented with radiographic features suggestive of active pulmonary TB, and a further six individuals diagnosed with tuberculous pericardial effusions had radiological features of pulmonary fibrosis suggestive of previous TB (6.7%). Four TB patients had calcified nodules suggestive of a healed Ghon focus (4.4%).

The amount of pericardial fluid drained at initial pericardiocentesis correlated with the radiographic finding of cardiac enlargement, as summarised in Table 6.3. In patients displaying a CTR between 0.55 and 0.75, the median (range) amount of fluid drained by pericardiocentesis was 795 (250–1800) mL, whereas in those with a CTR > 0.75, it amounted to 1605 (1400–2700) mL. Two of the HIV-infected TB patients had a CTR > 0.75 compared with eight TB patients who were HIV negative. No pericardial calcifications were noted on CXR.

Table 6.3. Cardio-thoracic ratio and amount of pericardial aspirate in patients presenting with pericardial effusion (n=122)

Amount of pericardial aspirate (mL)		Mean (SD) cardio-thoracic ratio	n*
Range	Mean (SD)		
<500	398.8 (82.6)	0.68 (0.12)	32
500 – 999	703.6 (124.8)	0.69 (0.17)	55
1 000 – 1 499	1 223.1 (96.7)	0.72 (0.16)	26
>1 500	1 770.0 (226.4)	0.77 (0.18)	9

* Volumes not available for seven patients (HIV+: n=1, HIV-: n=2; non-TB: n=4)

Electrocardiography

We evaluated the 12-lead surface ECG of 88 patients who presented with tuberculous pericarditis patients. Thirty-nine of these 88 patients were HIV positive patients (44%), and there were no significant differences in the amount of drained effusions, the ECG or echocardiographic findings between the HIV positive or and the HIV negative TB patients. The majority presented with a sinus tachycardia (73%), and there was no correlation between the size of the effusion and the rate or rhythm. Thirty-three patients (38%) had micro-voltage in the extremity and/or precordial leads and were found to have significant larger effusions than patients without micro-voltage (918 mL versus 740 mL; Table 6.4.).

Seventy-four patients (84%) presented with ECG changes of chronic pericarditis characterised by iso-electric PR segments and T-wave flattening/inversion compared to 14 patients who demonstrated ECG changes compatible with acute or subacute pericarditis (iso-electric or depressed PR segment with or without ST segment elevation; Spodick, 1997). Patients who presented with the ECG changes of chronic pericarditis were more likely to have larger effusions than those presenting with acute or subacute ECG changes.

The presence of micro-voltage (<5mm) in the extremity leads was 45% sensitive and 75% specific for the presence of cardiac tamponade compared with a sensitivity of 25% and a specificity of 69% when micro-voltage (<10mm) was present in the precordial leads alone.

Table 6.4. ECG parameters in correlation to amount of pericardial aspirate

ECG parameter	Amount aspirated (mL)	Number of patients
Micro-voltage in extremity leads	874	20 (23%)
Micro-voltage in precordial leads	1175	4 (5%)
Micro-voltage in both	903	9 (10%)
No micro-voltage	740	55 (63%)
PR-segment depression	654	24 (27%)
Electrical alternans	827	43 (49%)
Tachycardia	825	69 (78%)

DISCUSSION

In many TB endemic areas clinicians do not have access to echocardiography, but when available, the sonographic detection of intrapericardial fibrinous strands and the presence of prominent pericardial thickening is suggestive of tuberculous aetiology. Liu *et al* (2001) went as far as describing these features diagnostic of pericardial TB. In our series, fibrinous strands and pericardial thickening were generally more prominent and occurred more frequently in tuberculous disease, but were also demonstrated in cases of septic, malignant and post-traumatic pericardial effusion. However, when echocardiography is not available, the diagnosis of pericardial effusion needs to be based on clinical features, chest radiography and in some cases electrocardiography.

Enlargement of the cardiac silhouette on CXR does not usually occur until ≥250 mL of fluid have accumulated in the pericardial space (Lorell, 1997). Classically, the presence of a pericardial effusion is suspected when a rapid increase in the size of the cardiac silhouette is seen in the presence of clear lung fields. In some cases, the heart may assume a globular or "water bottle" shape, blurring the contours along the left cardiac border and obscuring the hilar vessels (Strang, 1984; Lorell, 1997). The parietal pericardial and epicardial fat layers are normally 1-2 mm apart. The presence of an effusion may result in more marked separation of the pericardial fat lines, apparent on high-quality frontal or lateral chest films in about 25% of patients with pericardial effusions (Carsky *et al*, 1980). The fact that all our patients had enlarged cardiac silhouettes confirms that there is a good correlation between the sonographically confirmed diagnosis of large pericardial effusions and CXR. A study

conducted in Harare, Zimbabwe also found that 100% of patients with tuberculous pericarditis had a CTR > 55% (Hakim *et al*, 2000).

The 50% proportion of tuberculous patients in this study with radiological evidence of pleural effusions is higher than the 30% previously reported (Strang *et al*, 1988), but in keeping with necropsy data. Rooney *et al* (1970) found that 50% of patients with tuberculous pericarditis had necropsy evidence of pleural effusion due to tuberculous pleuritis. In our study, right-sided pleural effusions occurred slightly more frequently than left-sided effusions. These results differ from previous studies in which it was found that pericarditis is more strongly associated with left-sided pleural effusions (Spodick, 1973; Weiss and Spodick, 1983). These studies were, however, not performed in TB endemic areas. The effusions seen in the present study could have developed either as a result of congestion or as part of a pleural immune response against tubercular antigens (Ferrer, 1997). In our study, mycobacteria were cultured from the pleural fluid of six individuals.

Median CD4+ lymphocyte counts were found to be higher in those HIV positive patients who presented with pleural effusions (median CD4+ lymphocyte count of 312 cells/μL) than those that did not have pleural effusions (median CD4+ lymphocyte count of 144 cells/μL). In a similar study, the prevalence of pleural effusion in tuberculous HIV patients with CD4+ lymphocyte counts greater than 200 cells/μL was 27%, while it was only 10% in HIV patients with TB and CD4+ lymphocyte counts of less than 200 cells/μL (Jones *et al*, 1993). It is possible that a more vigorous immune response seen in patients with higher CD4+ lymphocyte cell

counts is responsible for pleural effusions that are larger and more easily detectable by chest radiograph.

The radiological evidence of active pulmonary TB in 30% of our patients confirms previous results (Strang *et al*, 1988). Although this finding emphasises the importance of chest radiography in the search for supportive evidence for tuberculous aetiology, it also demonstrates the relative insensitivity theory. The correlation between cavitatory TB and smear positivity is well documented (Beyers, 1991). Four of the six patients with mediastinal lymphadenopathy were HIV positive. Generalised lymphadenopathy was noted in more than half of the HIV positive patients in the current study and is related to B cell and CD8+ lymphocyte cell hyperactivity caused by HIV (Jacobson *et al*, 1991). Individuals with dual infection of TB and HIV are particularly prone to significant mediastinal lymphadenopathy (Chaisson *et al*, 1987; Barnes *et al*, 1991; Saks and Posner, 1992; Barnes *et al*, 1993; Jones *et al*, 1993). Mediastinal lymphadenopathy is much better visualised by chest CT (computerised tomography) than by chest radiograph, and detects enlarged mediastinal lymph nodes in virtually 100% of patients with pericardial TB (Cherian *et al*, 2003b). Enlarged nodes disappeared or regressed with specific anti-tuberculous therapy (Cherian *et al*, 2003b).

The presenting ECG in patients diagnosed with tuberculous pericarditis is generally considered to show non-specific changes, usually T wave inversion (Rooney *et al,* 1970; Strang, 1984; Cegielski *et al,* 1994). Changes of acute pericarditis have been reported in 9% of patients, while micro-voltage was found in 34% (Rooney *et al,* 1970). In our study, most patients (84%) presented with ECG changes of chronic pericarditis, and 38% of our patients had micro-voltage in either the extremity, or the

precordial or both leads. Five patients (6%) presented with atrial fibrillation, confirming previous reports (Meyers *et al,* 1993; Spodick, 1997). Uncomplicated acute pericarditis does not produce significant rhythm disturbances unless there is underlying cardiac disease, either pre-existing or myocarditis.

In our study, the presence of micro-voltage in the extremity leads was 45% sensitive and 75% specific for the presence of cardiac tamponade and presence of micro-voltage in extremity leads corresponded to a sensitivity of 25% and a specificity of 69%, which supports published data. Eisenberg *et al* (1996) reviewed the electrocardiograms of 136 patients with echocardiographically diagnosed pericardial effusions and found that low voltage was associated with moderate and large effusions and the presence of tamponade, whereas Meyers *et al* (1993) demonstrated a weak correlation between QRS voltage and effusion size. In our study, cardiac tamponade was less likely in the absence of micro-voltage than when it was present.

CONCLUSION

In developing countries, where TB is usually more prevalent than in industrialized nations, CXR plays an important role in the identification of large pericardial effusions. The degree of radiographic cardiomegaly correlates well with the amount of pericardial fluid aspirated at the time of pericardiocentesis, and a chest radiograph may contribute to the diagnosis of coexistent pulmonary TB. Radiographic evidence of mediastinal lymphadenopathy is suggestive of co-infection with HIV. The presence of micro-voltage on ECG points towards the presence of a large pericardial effusion. Importantly, in the absence of micro-voltage, it is unlikely that the patient suffers from cardiac tamponade. Our study demonstrates that CXR and electrocardiography

can be useful screening tools for the diagnosis of pericardial effusion and aid patient management.

Chapter 7

THE USE OF ADJUNCTIVE CORTICOSTEROIDS IN THE MANAGEMENT OF TUBERCULOUS PERICARDIAL EFFUSIONS

Pericardial disease accounts for about 10% of all patients who are hospitalised for cardiac impairment in developing countries (Maharaj, 1991), the most common cause being tuberculosis (TB). Before the introduction of anti-tuberculous drugs and improved anaesthetic and surgical techniques, tuberculous pericarditis was usually fatal (Harvey and Whitehill, 1937). The mortality has since decreased to between 17% and 40% (Rooney *et al*, 1970; Desai, 1979; Bhan, 1980).

Tuberculous pericarditis is routinely treated with pericardiocentesis (Harvey and Whitehill, 1937) and chemotherapeutic agents (including isoniazid, pyrazinamide, rifampicin and ethambutol; Department of Health, 1996). Although national guidelines recommend six months of intensive therapy (Department of Health, 1996), some experts recommend longer regimens ranging from nine (Sagrista-Sauleda *et al*, 1988; Fowler, 1991) to 12 months (Koh *et al*, 1994). Uncertainty exists as to whether the treatment duration should be altered in human immunodeficiency virus (HIV) positive individuals. Higher rates of side effects from standard treatment regimens and higher relapse rates have been described in these patients (Harries, 1990), although this finding has not been consistent (Harries, 1990; de Cock *et al*, 1992).

The use of adjunctive corticosteroid therapy, together with anti-tuberculous drugs administered as standardized directly observed short-course treatment (DOTS), remains topical in the treatment of tuberculous pericarditis. Rapid improvement of symptoms and reduced mortality has been suggested following the use of oral adjunctive corticosteroids (Rooney *et al*, 1970; Bhan, 1980; Strang *et al*, 1987; Strang *et al*, 1988; Spodick, 1994). However, their use in HIV positive individuals may increase the risk of bacterial and viral infections (Gill *et al*, 1989; Eliott *et al*, 1992; Clifford *et al*, 1993). The risk of systemic infection may be reduced by the delivery of steroids directly into the epicardial space. Quigg *et al* (1985) successfully treated patients with uraemic pericardial effusions by pericardiocentesis with an indwelling catheter followed by a stat instillation of a nonresorbable steroid, triamcinolone hexacetonide, into the pericardial space. This procedure has not previously been tested in patients presenting with tuberculous pericarditis.

The aims of this study were: (i) To assess the role of adjunctive corticosteroid therapy with regards to mortality and the prevention of constrictive pericarditis; (ii) To compare the administration of conventional oral corticosteroid therapy with intrapericardial triamcinolone hexacetonide; and (iii) To assess the impact of HIV co-infection on the clinical course of patients with tuberculous pericardial effusions, as well as the effects of therapy in these patients.

PATIENTS AND METHODS

Patients presenting to the Cardiology Unit of Tygerberg Academic Hospital, Western Cape, South Africa with large tuberculous pericardial effusions requiring diagnostic and/or therapeutic pericardiocentesis from February 1997 to June 2000 were screened

for possible enrolment into this randomised trial. The study was approved by the Ethics Committee of Stellenbosch University and was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent and received counselling for HIV antibody testing. Baseline demographic, clinical, echocardiographic and electrocardiographic data were also obtained.

Patient selection and management

All patients met the following inclusion criteria: (a) age 13-75 years; (b) large pericardial effusion on echocardiography (epi-pericardial distance >10 mm); (c) pericardial aspirate with protein content >30 g/L and (d) pericardial fluid adenosine deaminase (ADA) activity >35 U/L.

Exclusion criteria are summarised in Table 7.1. Patients with CD4+ lymphocyte counts <200 cells/μL were excluded due to uncertainty as to what the effects of corticosteroids would be on immunocompromised patients with TB with regards to risk for disseminated disease. In addition, patients presenting with signs of constrictive pericarditis or requiring pericardial surgery within the first five days of admission were excluded.

Table 7.1. Summary of ineligible patients who presented with tuberculous pericarditis (n=38)

Exclusion criteria	Number of patients (n=38)	Baseline constriction (n=6)	Deaths (n=16)
CD4+ lymphocyte cell count <200 cells/μL	21	3	4
Lymphocyte count <0.8 x 10^9/L	4	0	3
DTB / lymphocyte count <0.8 x 10^9/L	1	0	1
Early death/lymphocyte count <0.8 x 10^9/L	1	0	1
Early pericardial surgery	5	2	1
Blocked drainage tube	1	0	1
Left ventricular ejection fraction <35%	2	1	2
Severe fibrotic lung disease	3	0	3

DTB = disseminated tuberculosis

The pericardial effusion was drained by echocardiographically guided aspiration via an indwelling pigtail catheter (Louw *et al*, 2002; Reuter and Doubell, 2002); the intention of this procedure was complete pericardial drainage. Chest radiography (CXR) was performed thereafter to exclude a pneumothorax and to evaluate the lung fields. The patient was then admitted into a general medical ward. The catheter was kept *in situ* to allow daily intermittent drainage and was removed when the daily aspirate was <100 mL, when it became blocked, or when there was evidence of localised skin infection. During hospitalisation, patients were examined twice daily for fever, change in haemodynamic status, and clinical evidence of localised skin and/or pericardial infection.

A standard short course anti-tuberculous regimen was initiated according to national guidelines, namely a combination of rifampicin, isoniazid, pyrazinamide and ethambutol for two months, followed by rifampicin and isoniazid for a further four months (Department of Health, 1996). An unblinded, independent physician then randomised patients to one of the following groups: (i) Triamcinolone group: 200 mg (5 mL) intrapericardial triamcinolone hexacetonide (n=17); (ii) Prednisone group: Oral prednisone plus intrapericardial placebo (5 mL 0.9% saline solution; n=16). Oral prednisone was started at 60 mg/day for four weeks, followed by 30 mg/day for four weeks, 15 mg/day for two weeks and 5 mg/day for one week; (iii) Placebo group: 5 mL intrapericardial 0.9% saline (n=24).

Triamcinolone was injected directly into the pericardium just prior to the removal of the indwelling catheter. Due to limited resources, an oral placebo was not used in conjunction with the intrapericardial triamcinolone.

Patients were discharged on anti-tuberculous therapy and pyridoxine, with or without adjunctive prednisone. HIV positive patients also received daily oral cotrimoxazole and multivitamin supplement; due to the prevailing national policy at the time of this study, none of these patients received antiretroviral therapy. The diagnostic protocol and methodology of the laboratory investigations have been described in Chapter 2.

Follow-up assessment

Patients were assessed one month after discharge and thereafter at three-monthly intervals for a minimum of one year. At follow-up visits, patients were assessed clinically for features of ongoing TB, immunodeficiency, pericardial disease (effusion, tamponade and constriction), side-effects of medication (anti-tuberculous drugs and prednisone), general sense of well-being and functional capacity. Therapeutic response was assessed as: (i) absence, improvement or worsening of exercise tolerance, dyspnoea, cough, ankle swelling, abdominal discomfort, chest pain, fever, night sweats and/or weight loss; (ii) absence, improvement or worsening of oedema, ascites, hepatomegaly, pleural effusion, raised jugular venous pressure (JVP), tachycardia, hypotension, pulsus paradoxus, Kussmaul's sign, pericardial rub and/or pericardial knock; (iii) evidence of infection at the puncture site and/or the pericardial space; and (iv) echocardiographic evidence of persisting or reaccumulated pericardial fluid or pericardial constriction and the subsequent need for pericardiectomy. Echocardiography was performed at baseline, at the first follow-up, and thereafter as clinically indicated.

Outcome measures

The primary end point was all cause mortality. Secondary endpoints included: (i) death attributed to pericarditis; (ii) disability related to pericardial disease at one year follow-up visit, where disability is defined as a history of restricted physical activity (using New York Heart Association [NYHA] functional class), (iii) development of effusive constriction, and (iv) development of fibrous constrictive pericarditis requiring pericardiectomy. The diagnosis of constrictive pericarditis was made on the basis of a combination of clinical and echocardiographic features (Strang, 1984; Feigenbaum, 1986). The collected data was analysed by statistical methods described in Chapter 2.

RESULTS

A total of 134 patients were admitted to Tygerberg Academic Hospital with large pericardial effusions requiring pericardiocentesis between February 1997 and June 2000. Ninety-five individuals (70.9%) were diagnosed with pericardial TB, 47 of whom tested HIV positive (49.5%). Based on pre-determined eligibility criteria, 57 patients (60.0%) were enrolled into the study. The age of study subjects ranged from 17 to 66 years, and included 23 females and 34 males. Reasons for patient ineligibility are shown in Table 7.1. The mean (SD) follow up period was 14.2 (2.3) months.

Forty of the 57 patients (70.0%) had microbiological and/or histological evidence of TB; the remaining 17 patients (30.0%) were diagnosed by clinical and supportive laboratory data. Twenty-one of these patients (37.0%) were HIV positive. Baseline demographic, radiographic, electrocardiographic and echocardiographic findings were

similar between the three treatment groups, as were baseline diagnostic and other laboratory findings (including Adenosine deaminase [ADA] results).

Clinical endpoints and complications

Complications and observations of trial patients at follow-up are presented in Table 7.2. All patients had a good clinical response to initial pericardiocentesis. The duration of hospitalisation ranged from four to 30 days; although the hospitalisation duration was slightly lower in the two steroid groups compared to the placebo group, the difference was not statistically significant. There were no significant differences in the number of patients in each group that experienced minor complications such as localised pain, leakage at wound site and local skin infection. Two patients developed transient jaundice but still completed six months of anti-tuberculous therapy. Thirteen cases of infection were recorded during the follow-up period, including oral candidiasis (n=5), local skin infection (n=4), herpes labialis (n=2) and varicella zoster (n=2). These events occurred more frequently in the combined corticosteroid group (n=9; 27.2%) than in the placebo arm (n=4; 16.7%; p=0.07).

Two male patients (both HIV negative) developed effusive-constrictive pericarditis, which was diagnosed clinically and confirmed echocardiographically at the first follow up. Both patients were over 40 years of age with a positive smoking history. In addition, both patients had evidence of pulmonary infiltrates on CXR and had been investigated for pulmonary TB in the month preceding pericardial aspiration, suggesting the possibility of a more chronic form of pericardial TB. One patient (prednisone group) underwent surgical pericardial fenestration at six weeks, followed by total pericardiectomy at four months, and was entirely well at his one-year follow-

up. The other patient (triamcinolone group) had relatively mild features and refused surgery. He was managed conservatively, and the features of constriction improved progressively and had subsided by the one-year follow-up. Echocardiography demonstrated pericardial thickening, which was still present at the two-year follow-up, but not accompanied by features of constriction.

Table 7.2. Complications and observations of trial patients at follow-up (n=57)

	Prednisone (n=16)	Triamcinolone (n=17)	Placebo (n=24)
Complications in hospital			
Local pain	3	3	4
Repeat pericardiocentesis	1	0	1
Leakage at skin insertion	1	1	2
Local skin infection	0	1	1
Duration of hospitalisation			
Mean (SD) days	9.9 (5.1)	10.5 (4.4)	11.2 (5.3)
Range in days	4-21	4-19	4-30
Complications as out-patient			
Herpes labialis	1	0	1
Varicella zoster	1	1	0
Local skin infection	1	1	0
Effusive constriction	1	1	0
Fibrous constriction	1	0	0
Left ventricular aneurysm	0	1	0
Surgical intervention			
Pericardial fenestration	1	0	0
Total pericardiectomy	1	0	0
Status at 1-year follow-up			
Reduced level of activity	2	2	3
Raised JVP	1	1	2
Hepatomegaly	0	1	0
Failure to attend follow-up visit	4	1	4

JVP = jugular venous pressure

Seven patients (12.0%) complained of reduced levels of activity at their one-year follow up. Three of these patients (42.9%; all HIV positive) had no cardiac abnormalities and tests for disseminated TB and other opportunistic infections were negative. The other four patients (57.1%; of which one was HIV positive) were found to have an increased jugular venous pressure. Echocardiography confirmed cardiac abnormalities: two had pericardial thickening but no definitive evidence of constriction; the other two had left ventricular impairment with mild to moderate mitral and tricuspid regurgitation. The distribution of complications was similar between the three treatment arms (Table 7.2). No deaths were recorded for the study population, but nine patients (16.0%) failed to attend their follow-up appointments at six and 12 months. None of these patients were admitted to Tygerberg Academic Hospital or any other public hospitals in the region for a period of two years after enrolment and all efforts to trace them failed. In addition, their names did not appear on the National Death Registry. The age distribution of these "non-attendees" ranged from 17 to 58 years. Four of the nine patients were HIV positive with baseline CD4+ lymphocyte counts of 392, 214, 256, and 243 cells/μL, respectively.

An analysis of the outcomes of the 38 TB patients that were excluded from the randomised trial is presented in Table 7.1. All of these patients were treated by closed pericardiocentesis and concurrent early initiation of anti-tuberculous therapy. Patients with effusive constrictive pericarditis were treated by pericardial fenestration and adjunctive oral prednisone based on published recommendations (Strang *et al*, 1987; Strang *et al*, 1988).

DISCUSSION

The present study suggests that echocardiographically guided closed pericardiocentesis with intermittent daily aspiration in combination with early initiation of anti-tuberculous therapy results in excellent outcomes in patients with effusive pericardial TB, irrespective of HIV status. Only two cases of effusive constrictive pericarditis developed during the first six months after enrolment. Both patients did well; one required total pericardiectomy due to symptomatic constriction, whereas the other improved without surgical intervention. The study was, unfortunately, underpowered to detect significant effects of adjunctive corticosteroids and a multi-centered study is required to assess larger number of patients in order to evaluate the potential benefit of adjunctive corticosteroids.

The study also demonstrated that secondary skin infection occurs rarely when an aseptic technique is applied and no differences in the frequency of skin sepsis were noted between the various treatment groups. The incidence of systemic infections or HIV-associated complications such as opportunistic infections or malignancies was also similar in the three treatment categories. The use of adjunctive corticosteroids led to shortened hospitalisation with a mean (SD) duration of days in hospital for the prednisone, triamcinolone and placebo groups of 9.9 (5.1), 10.5 (4.4) and 11.2 (5.3), respectively. Differences between the two adjunctive corticosteroid and the placebo groups were statistically insignificant.

The results of this study support the use of routine anti-tuberculous therapy for six months in both HIV positive and negative patients. There were no associated cases of multi-drug resistance, reactivation or dissemination of TB. HIV infected patients had

very similar outcomes to those that were HIV negative and based on our results, there is no reason to support prolonged treatment duration in individuals who have CD4+ lymphocyte counts > 200 cells/μL.

Contrary to a number of other reports (Strang *et al*, 1987; Strang *et al*, 1988; Alzeer and Fitzgerald, 1993; Senderovitz and Viskum, 1994), the use of adjunctive steroids did not result in improved clinical outcome in patients with effusive tuberculous pericarditis. Our findings are in accordance with a systematic review concluding that prednisone has no clear beneficial effect in patients with tuberculous pericarditis (Mayosi *et al*, 2002). Effusive-constrictive pericarditis was an exclusion criterion in the present study, whereas other studies have described features suggestive of established or threatening effusive-constrictive pericarditis (Strang *et al*, 1987; Strang *et al*, 1988). In the Transkei, effusive constriction was considered to be a more common clinical variety than pericardial effusion or classical fibrous constrictive pericarditis (Commerford and Strang, 1991). It is thus necessary to recognize that different interventions may be required for specific clinical phases of the same disease. In an observational study, patients who had only 80-100 mL of fluid aspirated before treatment with high doses of oral prednisolone (including four weeks of 120 mg daily) and where standard TB therapy was initiated, had rapid resolution of their effusions (Strang, 1994). A potential place for adjunctive corticosteroids may thus be for those patients who present relatively late and have features of effusive-constrictive disease at presentation (Strang *et al*, 1987; Strang *et al*, 1988), or those in whom pericardiocentesis is unsuccessful (Lorell, 1997). It has also been suggested that corticosteroids should be reserved for critically ill patients with recurrent large effusions who do not respond to pericardial drainage and anti-tuberculous drugs

alone. In view of the results of our study, no benefit was observed by using intrapericardial triamcinolone and we would thus recommend the use of oral corticosteroids in these cases.

The present study also suggests that the mortality of HIV infected individuals (CD4+ lymphocyte counts >200 cells/μL) does not differ significantly from those who are HIV negative. This contradicts earlier reports suggesting a significantly higher mortality due to the increased likelihood of disseminated TB (Pozniak *et al*, 1994). In the present study, adjunctive steroids resulted in a tendency towards a higher number of minor infections; however, none of the patients developed oesophageal candidiasis, cryptococcal meningitis or other more serious opportunistic infections, malignancies, septicaemia, or pneumonia.

Other researchers have found potentially harmful side effects related to the use of steroids in HIV infected individuals, including a higher incidence of bacterial infections, herpes simplex and herpes zoster reactivation, and potentially the development of Kaposi's sarcoma (Schulhafer *et al*, 1987; Gill *et al*, 1989; Eliott *et al*, 1992). In a non-randomised study of TB in HIV positive patients, Elliott and colleagues (1992) reported increased risk for herpes zoster and Kaposi's sarcoma in patients treated with prednisone, but in the study of Hakim and colleagues (2000), Kaposi's sarcoma occurred only in patients who were not on steroids. None of the HIV patients in the present study developed constriction and it has been postulated that the rate of constriction is reduced by HIV infection (Hakim *et al*, 2000). Hakim and colleagues (2000) observed a trend towards improved survival but no reduction in the occurrence of constriction in the adjunctive steroid group. In keeping with our

results, the latest version of the Cochrane systematic review (which includes Hakim's study) revealed that the results of all published studies are still inconclusive and that there is uncertainty regarding the effectiveness of steroids in patients with tuberculous pericarditis (Mayosi *et al*, 2002; Ntsekhe *et al*, 2003).

Our study has a number of weaknesses, most notably the fact that 16.0% of patients did not attend their 6-month and/or one-year follow-up visits and that data on mortality and constriction may therefore be incomplete. The poor follow-up rate probably reflects the impact of migratory lifestyles on health care in poorer socio-economic regions of South Africa. It could also be argued that an oral placebo should have been used in combination with the intrapericardial placebo. At the time of study planning, the role of corticosteroids in HIV infected patients was unclear and it was decided to expose only those with CD4+ lymphocyte counts >200 cells/μL. Twenty-one potential patients were not enrolled due to this reason. An analysis of these patients confirmed an increased rate of morbidity and mortality due to non-cardiac complications. There was a dramatically increased mortality in patients that had been excluded from the randomised trial on the basis of low CD4+ and/or total lymphocyte counts. Retrospectively it could be argued that these patients should have been included in the study.

CONCLUSION

Intrapericardial and systemic corticosteroids were well tolerated, but did not improve the outcome in these selected patients. The standard six-month treatment regimen was effective regardless of HIV infection. Unfortunately, this study was underpowered to detect significant effects of adjunctive corticosteroids. Further placebo-controlled

trials are warranted but should be conducted at several centres using the same protocol, as single centre studies are unlikely to answer these questions. Patients with effusive constrictive disease should be included, as well as those with CD4+ lymphocyte counts <200 cells/μL. At present, corticosteroids should be reserved for critically ill patients with recurrent large effusions who do not respond to pericardial drainage and anti-tuberculous drugs alone (Lorell, 1997).

Chapter 8

THE MANAGEMENT OF PATIENTS WITH LARGE PERICARDIAL EFFUSIONS

Tuberculous pericarditis is a life-threatening form of extrapulmonary tuberculosis (TB) and presents either as pericardial effusion or as constrictive pericarditis (Strang, 1984, Strang *et al*, 2004). Recently, a rapid rise in the incidence of tuberculous pericarditis has been observed in countries with a high prevalence of human immunodeficiency virus (HIV) infection (Cegielski, 1994; Maher and Harries, 1997). The first goal in the treatment of large pericardial effusions is effective drainage of the pericardial space to decrease compression of the cardiac chambers. This needs to be followed by specific therapy directed at the underlying aetiological cause of the pericardial disease. In the case of tuberculous pericarditis, anti-tuberculous therapy has reduced the mortality of TB from about 85% (Harvey and Whitehill, 1937) to between 17% and 40% (Rooney *et al*, 1970; Desai, 1979; Bhan, 1980). The use of adjuvant corticosteroids for the prevention of constriction and TB-related death remains controversial (Strang *et al*, 1988; Alzeer and Fitzgerald, 1993; Senderovitz and Viskum, 1994; Hakim *et al*, 2000; Mayosi *et al*, 2002; Strang *et al*, 2004). If constriction develops, pericardiectomy is usually indicated (Maisch *et al*, 2004).

Standard management for pericardial effusion includes pericardiocentesis, ideally under guidance by echocardiography or fluoroscopy (Tsang *et al*, 1998; Tsang *et al*, 1999; Tsang *et al*, 2002a; Tsang *et al*, 2002b; Strang *et al*, 2004). Echocardiography identifies the safest route where the pericardium can be entered intercostally or

subcostally (Tsang *et al*, 2002b). The most serious complications of pericardiocentesis include laceration and perforation of the myocardium and the coronary vessels, air embolism, pneumothorax, arrhythmias (usually vasovagal bradycardia), and puncture of the peritoneal cavity or abdominal viscera (Seferović *et al*, 2000).

We have reported on our experience of pericardiocentesis with extended intermittent pericardial drainage in 170 patients, including 116 patients who presented with tuberculous pericardial effusion (Louw *et al*, 2002). Contrary to the perception that the HIV positive patients would suffer more infective complications, no cases of local skin or pericardial sepsis were encountered in any of the 54 HIV-infected patients studied (Louw *et al*, 2002). We are now reporting the thirty-day and one-year outcomes of 233 patients who presented with large pericardial effusions, focusing on the 162 patients who presented with pericardial TB.

METHODS AND PATIENTS

All 233 pericarditis patients described in Chapter 3 are included in this review. The predetermined diagnostic criteria, the diagnostic work-up and standardised management protocol have been described in Chapter 2. Patients were assessed one month after discharge and thereafter at three-monthly intervals for a minimum of one year. Therapeutic response was assessed regarding improvement or worsening of admission clinical features, evidence of infection at the puncture site and/or the pericardial space, and evidence of persisting effusion or pericardial constriction. Echocardiography was performed at baseline, at first follow-up, and thereafter as clinically indicated. Echocardiographic diagnosis of cardiac tamponade was made when, in the presence of pericardial effusion, the following were noted: inversion of

≥30% of the right atrial wall during late diastole/early systole, and/or inward motion of the right ventricular wall in early diastole that persisted after mitral valve opening (Gubermann *et al*, 1981). The methods used for statistical analysis are summarised in Chapter 2.

RESULTS

Altogether, 233 patients were treated by echo-guided pericardiocentesis. Of these, 162 patients (100 males and 62 females) were diagnosed with pericardial TB; "definite" TB was diagnosed in 118 patients, whereas 44 presented with "probable" TB. Eleven patients were on anti-tuberculous treatment at the time of pericardiocentesis; these patients are included in this review. Eighty-four patients were HIV positive, including 81 patients with tuberculous pericarditis, two patients with pericardial sepsis and one patient with uraemic pericarditis. The mean (SD) age at presentation differed significantly between HIV positive and HIV negative patients with pericardial TB [31.9 (8.4) years versus 39.7 (15.9) years; ($p<0.05$)]. The mean (SD) CD4+ lymphocyte count for HIV positive patients was 215 (202) cells/μL. Echocardiographic evidence of tamponade was found in 197 cases (87%). Chest wall puncture site was chosen in 204 of the 233 patients (88%), and the subcostal approach in the remaining 29 patients (12%). The median (range) volume of pericardial fluid drained at initial pericardiocentesis was 791 (80-2770 mL). The majority of pericardial effusions had a haemorrhagic macroscopic appearance (66.1%), 25% of effusions were straw-coloured and 3% resembled pus. A pigtail catheter was left *in situ* for intermittent daily drainage. The pericardiocentesis related complications are summarised in Table 8.1.

Table 8.1. Complications in 233 pericardiocenteses

	TB / HIV+ n=81	TB / HIV- n=81	Non-TB n=71
Minor complications			
Local pain	15 (19%)	12 (15%)	15 (21%)
Catheter removed inadvertently	2	3	2
Repeat pericardiocentesis	3	4	1
Catheter blockage	3	3	2
Leakage at skin insertion	3	4	2
Local skin infection	3	1	
Local bleeding	1	1	
Disconnection of system	1	2	1
Major complications			
Tamponade post-tap (non-fatal)		1	1
Intrapericardial sepsis	1	1	
Thrombus left ventricle		1	
Pneumothorax		1	

The most common minor complication (n=42) was local pain at the site of catheter insertion. In four (2.4%) patients local skin infection necessitated removal of the catheter; three of them were HIV positive and had CD4+ lymphocyte counts <200 cells/μL. No patient developed a pneumothorax during catheter insertion, but one patient developed a pneumothorax during catheter removal, which was managed by intercostal under-water tube drainage. Although the 30-day mortality rate was 11.6%, no death was attributable to the pericardiocentesis procedure.

Anti-tuberculous treatment was initiated in hospital and continued at the TB clinic closest to a patient's home. TB treatment was tolerated well and the six months course was adequate for both HIV positive and HIV negative patients; there were no relapses or need for prolonged treatment in any of the patients. Three patients presented with liver toxicity resulting in brief interruption and successful staggered reintroduction of treatment. HIV positive patients also received daily oral cotrimoxazole. Nine patients with complicated pericardial effusions (namely recurrent pericardial effusions, loculated pericardial effusions and/or constrictive pericarditis) received oral prednisone according to published guidelines (Strang *et al*, 1987; Strang *et al*, 1988; Lorell, 1997). In addition, 33 patients received corticosteroids as part of a randomised trial, as reported in Chapter 7. Non-tuberculous effusions were treated according to underlying cause. Septic pericarditis was treated with broad-spectrum antibiotics until the causative organism was identified by culture. Two patients with septic pericarditis underwent pericardial fenestration.

Thirty-six patients underwent pericardial surgery; 19 of these had diagnostic pericardial biopsies, 17 patients underwent therapeutic pericardial fenestration; one of them had a total pericardiectomy after developing fibrous constrictive pericarditis. The indications for the therapeutic biopsies are summarised in Table 8.2. Two tuberculous pericarditis patients died postoperatively while still in theatre. Both of them had echocardiographic evidence of poor left ventricular function; one of them was HIV positive. The mean (SD) duration of hospitalisation was 18.7 (4-57) days for surgically treated patients compared to 10.6 (4.9) days for the "pericardiocentesis-only" group ($p<0.01$). One patient underwent total pericardiectomy for constrictive pericarditis performed after two months of anti-tuberculous therapy; he was well at one-year follow-up.

Table 8.2. Therapeutic indications for pericardial surgery

	Tuberculous pericarditis n=11	**Non-tuberculous pericarditis n=6**
Recurrent effusion	3	1
Effusive constriction	4	2
Loculated effusion	1	2
Daily drainage >100 mL	2	0
Obstructed tube	1	1
Fibrous constriction*	1	0

* One patient had pericardial fenestration and total pericardiectomy was performed after 2 months of anti-tuberculous therapy

The 30-day all-cause mortality was higher in patients with non-tuberculous pericardial disease than in patients with pericardial TB (20% versus 8.0%; $p<0.01$). The causes of 30-day mortality in the pericardial TB group are presented in Table 8.3. The one-year all-cause mortality data are summarised in Table 8.4. The one-year all-cause mortality was significantly higher in patients who presented with non-tuberculous pericardial effusions than in the tuberculous pericarditis patients, namely 60.0% versus 17.3% ($p<0.001$), and in the tuberculous pericarditis group it was higher for HIV positive (22.2%) than for HIV negative patients (12.3%; $p=0.04$). The causes of the one-year mortality observed in pericardial TB patients are summarised in Table 8.5.

Table 8.3. Causes of 30-day mortality in patients presenting with pericardial TB

	Case No.	Age (years)	HIV	Cause of death
<24 hours	85	25	+	Drug abuse, pneumonia, septicaemia and respiratory failure (day 1)
24 hours to 7 days	74	43	-	Disseminated TB, sudden hypotensive episode followed by cardiac arrest (day 5)
	97	40	+	Loculated pericardial effusion with tamponade and constrictive features, died pre-operatively due to massive gastrointestinal haemorrhage (day 4)
	143	36	+	Unexpected sudden cardiac arrest, echo showed no constriction or loculated effusion (day 4)
	181	28	+	Cerebral toxoplasmosis, status epilepticus, septicaemia, uraemia, progressive deterioration, no pericardial cause noted on repeat echo (day 5)
	188	54	-	Disseminated TB, sudden deterioration in spite of daily pericardial drainage (day 4)
	203	31	+	Underlying muscular dystrophy, cardiac arrest (day 2)
7 to 30 days	83	53	-	Post TB bronchiectasis, cor pulmonale, reactivation of TB, cardio-respiratory failure (day 20)
	126	30	-	Chronic pulmonary fibrosis, cavitation, smear positive pulmonary TB, cardio-respiratory failure (day 10)
	138	53	-	Large residual effusion with tamponade and features of constriction – died due acute peri-operative cardiac insufficiency (day 16)
	157	35	+	Underlying cardiomyopathy, removal of obstructed catheter on day 2. Cardiac arrest 9 days later (day 11)
	173	47	+	CD4+ lymphocyte count 43 cells/μL, severe diarrhoea, vomiting, tender abdomen, dysentery, septicaemia (day 17)
	181	29	+	CD4+ lymphocyte count 9 cells/μL, severe pain in left flank, delirious, diarrhoea, septicaemia (day 9)

Table 8.4. Mortality according to diagnostic groups

	30-day mortality	One-year mortality
Tuberculosis (n=162)	13 (8%)	28 (17%)
Malignancy (n=22)	5 (23%)	20 (91%)
Uraemic (n=12)	3 (33%)	5 (42%)
Septic (n=5)	3 (60%)	4 (80%)
Other (n=32)	3 (9%)	13 (41%)
Total	27 (11.6%)	70 (30.0%)

Table 8.5. Causes of one-year mortality in tuberculous pericarditis patients

	n	HIV negative (n=10)	HIV positive (n=18)
Disseminated TB	3	1	2
Effusive constriction / Tamponade	6	3	3
Fibrotic pulmonary disease	3	3	
Systemic non-tuberculous infection	9	1	8
Underlying cardiomyopathy	2	1	1
Gastrointestinal haemorrhage	1		1
Underlying muscular dystrophy	1		1
Unknown	3	1	2

Three pericardial TB patients (two of which were HIV positive) died during the first 60 days after pericardiocentesis as a result of disseminated TB; one of them had interrupted anti-tuberculous therapy. HIV-related non-tuberculous infectious complications that resulted in death included *Pneumocystis carinii* pneumonia (n=2), cerebral toxoplasmosis with status epilepticus (n=1), cryptococcal meningitis (n=1), abdominal sepsis (n=1) and septicaemia (n=3). Low CD4+ lymphocyte counts were associated with increased mortality risk; 75% of patients that died during the first year of follow-up had an admission CD4+ lymphocyte count <200 cells/μL, whereas admission CD4+ lymphocyte counts <200 cells/μL were observed in only 45% of "one-year survivors". The corresponding mean (SD) CD4+ lymphocyte count for survivors was 237.2 (212.4) cells/μL compared to 155.1 (188.6) for non-survivors ($p<0.01$).

DISCUSSION

Large pericardial effusions are usually the manifestation of underlying disease, and the 60% mortality of the non-tuberculous group reflects the seriousness of the conditions that resulted in patients requiring therapeutic pericardiocentesis. Although the prognosis was significantly better for tuberculous pericardial disease, effective management of tuberculous pericarditis is nevertheless of utmost importance. Without specific treatment, the reported average survival was 3.7 months and only 20% were alive at six months (Desai, 1979). In our experience the majority of patients who presented to the echocardiography department with suspected pericardial TB are critically ill and have large pericardial effusions; 90% of those enrolled into this study had echocardiographic evidence of tamponade. On average, more than 800 mL of fluid was drained at the initial drainage procedure. The

community based epidemiology and clinical presentation of pericardial TB may differ significantly from our observations in hospitalised patients in a tertiary referral centre. However, in our experience echo-guided pericardiocentesis with extended intermittent drainage resulted in effective relief of cardiac tamponade and over period of seven years in almost complete absence of fibrous constrictive pericarditis. Six patients (2.6%) had major complications related to the pericardiocentesis. We observed a very low incidence of recurrence and only nineteen were referred for therapeutic pericardial surgery indicating a pericardiocentesis success rate of 92%. Procedural success rates are high in most series, generally more than 90%, whereas complication rates are approximately 4% (Callahan *et al*, 1985; Kopecky *et al*, 1986; Tsang *et al*, 1998). In our study it was equally safe and effective in tuberculous pericardial effusions as in non-tuberculous pericardial effusions and our results are similar to series reported from settings where only the minority of patients have pericardial TB (Tsang *et al*, 2002a; Tsang *et al*, 2002b).

Patients were referred for pericardial surgery, whenever pericardiocentesis failed to adequately drain loculated effusions or if echocardiography was suggestive of effusive-constrictive pericarditis. As was to be expected, the duration of hospitalisation was significantly longer for the surgically treated patients than for the pericardiocentesis group because it was reserved for critically ill patients and during the first 18 months of the study for those in whom a diagnosis could not be reached within the first seven days. Besides the 19 diagnostic biopsies, pericardial fenestration was performed on 17 patients for therapeutic indications. The post-operative 30-day mortality for these 36 individuals was 5.6% in comparison to a 30-day mortality for surgical drainage of malignant pericardial effusions of 19.4%

(Piehler *et al*, 1985). More specifically, complete pericardiectomy, partial pericardiectomy, and subxiphoid or anterior transthoracic window were associated with 30-day mortality rates of 37.5, 23.8, and 8.6%, respectively (Tsang *et al*, 1999). In the present series, two patients died while awaiting surgery. One of them had a massive upper gastrointestinal haemorrhage, whilst the other, a critically ill HIV positive patient died from tamponade which could not be adequately relieved by catheter drainage. Most patients can be adequately treated by pericardiocentesis. The technique is safe even in very sick and unstable patients, and does not usually need to be performed in theatre. In selected stable patients, it can even be performed on an outpatient basis (Drummond *et al*, 1998).

Effusive-constrictive pericarditis preceded fibrous constriction in the two patients who developed this complication. One of them refused surgery. Fortunately, he improved over a period of two years and thus had transient constrictive pericarditis, which is a rare but important entity, since pericardiectomy is not indicated for these patients (Haley *et al*, 2004). Possibly, his favourable outcome was related to the installation of intrapericardial triamcinolone. The other constrictive pericarditis patient was treated successfully by total pericardiectomy; he was well at one-year follow-up. Pericardiectomy for constrictive pericarditis has a mortality rate of 6–25% (Culliford *et al*, 1980; Robertson and Mulder, 1984; Aagaard and Haraldsted, 1984; Siefert *et al*, 1985; Palatianos *et al*, 1985; McCaughlin *et al*, 1985; Cameron *et al*, 1987; Bashi *et al*, 1988; Astrudillo and Ivert, 1989; Nataf *et al*, 1993; Tirilomis *et al*, 1994; Ling *et al*, 1999; Ufuk *et al*, 2003). Major complications include acute peri-operative cardiac insufficiency (low-output syndrome) and ventricular wall rupture (Sunday *et al*, 1999). The low-output syndrome occurs in 14–28% of patients in the

immediate postoperative period, and the major risk factors predictive of in-hospital mortality and low-output syndrome include presurgically unrecognised presence of myocardial atrophy or myocardial fibrosis (Reinmuller *et al*, 1993), the degree of preoperative disability (functional Class III or IV) and the severity of constriction as indicated by marked elevation of right atrial/right ventricular end-diastolic pressure (Tirilomis *et al*, 1994; McCaughlin *et al*, 1985, Siefert *et al*, 1985). In effusive-constrictive pericarditis, pericardiocentesis usually fails to alleviate the features of haemodynamic compromise, and subtotal or complete pericardiectomy is not possible because the pericardium cannot be stripped from the epicardium. In our experience, patients with effusive-constrictive pericarditis required surgical fenestration to drain the fibrinous material from the pericardial space, and to break down pericardial adhesions. This did not, however, address the main problem, namely the presence of a stiffened epicardium which causes the constriction and cannot be removed at this stage of the disease, because a skin has not yet formed that would allow surgical stripping. Once the fibrous skin becomes echocardiographically evident, total pericardiectomy should be performed in symptomatic patients before pericardial calcifications have formed or the onset of cardiac cachexia (Lorell, 1997; Maisch *et al*, 2004).

An unfortunate proportion of deaths is attributable to disseminated TB (Harvey and Whitehill, 1937; Gooi and Smith, 1978), and despite the availability of anti-tuberculous chemotherapy, the mortality of disseminated TB is as high as 20-30% (Munt 1971; Vijayan, 2000). The HIV epidemic has considerably altered the frequency and descriptive epidemiology of disseminated TB (Narain *et al*, 1992; Barnes *et al*, 1993). Disseminated TB, which arises from the inadequacy in

containing tuberculous infection, occurs more frequently and may be more difficult to diagnose in HIV positive individuals (Chaisson *et al*, 1987; Barnes *et al*, 1993). Because of the multisystem involvement in disseminated TB, the clinical manifestations are protean. Presenting symptoms are dominated by systemic effects, particularly fever, weight loss, anorexia and weakness (Munt 1971; Grieco and Chmel, 1974 Sahn and Neff, 1974; Prout and Benatar, 1980; Vijayan, 2000). Approximately 25% of patients with tuberculous pericarditis have evidence of other organ involvement at the time pericarditis is diagnosed, particularly pleuritis and lymphadenitis (Harvey and Whitehill, 1937; Gooi and Smith, 1978). The degree of immunodeficiency and risk for disseminated TB and other serious opportunistic infections and death correlates with CD4+ lymphocyte cell counts (Barnes *et al*, 1993; Mellors *et al*, 1997). In our study, 75% of HIV positive patients who died during the first year of follow-up had admission CD4+ lymphocyte counts <200 cells/μL compared to only 45% of the HIV positive one-year survivors, and the majority of deaths was caused by opportunistic infections, septicaemia and disseminated TB.

The cornerstone of the treatment of HIV is highly active antiretroviral therapy (HAART), which has been shown to reduce the mortality and the morbidity of people living with advanced HIV disease, decreasing death rates in Europe by at least fivefold (Palella *et al*, 1998; Mocroft *et al*, 1998). The goal of HAART is maximal and durable viral suppression to enable preservation and restoration of the immune system (Mellors *et al*, 1997). During the course of our study, HAART was not available at Tygerberg Hospital. The recently improved accessibility of HAART will hopefully result in improved prognosis of the HIV-infected TB patients. The use of

HAART in the treatment of patients co-infected with TB and HIV may be problematic because there are potential complex drug interactions, overlapping adverse reactions, potential non-adherence due to the pill burden, and drug malabsorption (Burman and Jones, 2001). Despite these potential problems, HAART substantially reduces new acquired immunodeficiency syndrome (AIDS) events and death in co-infected patients (Badri *et al*, 2002; Dheda *et al*, 2004). Those with CD4+ lymphocyte counts <100 cells/μL have a high event risk during the intensive phase of anti-tuberculous treatment (Dheda *et al*, 2004). Paradoxical deterioration due to the immune reconstitution inflammatory syndrome (IRIS) has been reported to occur in 11-36% of patients with TB who start HAART (Narita *et al*, 1998; Wendel *et al*, 2001). Secondary preventive therapy with isoniazid reduces TB recurrence in HIV infected patients: the absolute impact seems to be greatest among individuals with low CD4+ lymphocyte cell counts. (Churchyard *et al*, 2003).

CONCLUSION

Before the advent of anti-tuberculous chemotherapy in 1945, tuberculous pericarditis was often rapidly fatal, with a mortality rate of 80-85% (Harvey and Whitehill, 1937; Desai, 1979). Our series of 162 consecutive patients demonstrates that the risk for constriction and cardiac death can be very effectively reduced by using echo-guided pericardiocentesis with extended intermittent drainage and early initiation of anti-tuberculous therapy, and this drainage technique is safe and effective irrespective of the patient's HIV status. The one-year all-cause mortality was higher in HIV positive patients than in HIV negative patients. The majority of deaths were caused by non-cardiac disease and CD4+ lymphocyte counts <200 cells/μL predicted a poor prognosis.

Chapter 9

THE INFLUENCE OF HIV INFECTION ON THE CYTOKINE PRODUCTION IN PATIENTS WITH TUBERCULOUS PERICARDITIS

The human immune response eliminates microbial pathogens through an inflammatory response that may be harmful to host tissue. In tuberculosis (TB), tissue necrosis and pleural exudates are characteristic manifestations of the cytokine mediated inflammatory response (Barnes *et al*, 1991). A large number of TB related studies has focused on the T helper1/T helper2 (Th1/Th2) response and has shown that the Th1 cytokine profile is associated with successful elimination of tubercle bacilli (Collins and Kaufmann, 2001; Barnes and Wizel, 2000; Jo *et al*, 2003). Cytokines produced by Th1 and Th2 subsets reciprocally regulate the functions of each other. Interferon-gamma (IFN-γ) inhibits the proliferation of Th2 cells, but not of Th1 cells (Gajewski and Fitch, 1988). Within the complex immunoregulatory response to mycobacterial infection, IFN-γ and tumour necrosis factor-alpha (TNF-α) are of particular importance because of their potential anti-mycobacterial effects.

In 2002 we reported on the cytokine production in 30 pericardial effusions (Burgess *et al*, 2002c). Various pericardial fluid cytokine concentrations were measured, including IFN-γ, TNF-α, interleukin-1 (IL-1), IL-2, IL-4, IL-6, and IL-10. Our results indicated a Th1 dominant immune response in the tuberculous pericarditis group and we concluded that tuberculous pericardial effusions result from a delayed

hypersensitivity reaction that is orchestrated by Th1 lymphocytes in collaboration with activated macrophages. Activated T lymphocytes produce a number of cytokines, including IFN-γ, IL-2 and IL-10. Of these, IFN-γ plays the leading role in the anti-mycobacterial response. However, both IFN-γ and IL-2 enhance the recruitment and activation of monocytes-macrophages in the pericardial space, which results in the production of IL-1 and TNF-α (Burgess *et al*, 2002c). This first phase of our study included only four HIV positive tuberculous pericarditis patients. To determine the influence of HIV infection on the cytokine production in patients with tuberculous pericarditis we have subsequently evaluated the cytokine production in a further 26 patients, including an additional 18 HIV positive patients with tuberculous pericardial effusion.

PATIENTS AND METHODS

At the time of pericardiocentesis, an aliquot of pericardial fluid was collected on ice and frozen within 30 minutes at -70°C for the analysis of cytokines. Fifty-six pericardial specimens, ten serum samples and five pericardial fluid samples from normal controls were included in this sub-study. Control patients were evaluated by obtaining pericardial fluid during routine open-heart surgery by open pericardial aspiration. The following cytokines were measured: IL-4, IL-10, TNF-α and IFN-γ by using an enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Amersham Pharmacia Biotech, UK; Chapter 2). The laboratory and statistical methods are presented in Chapter 2.

RESULTS

The concentrations of various cytokines were determined in 56 pericardial effusions. The diagnostic causes of these pericardial effusions included TB (n=43), malignancy (n=6), septic pericarditis (n=5) and systemic lupus erythematosus (SLE; n=2). The malignant group included two patients with metastatic adenocarcinoma, one with undifferentiated bronchus carcinoma, one with small cell carcinoma, one with lymphoma and one with neuroblastoma. Twenty-four patients were HIV positive, including 22 who presented with tuberculous pericarditis and two who presented with septic pericarditis. The mean (SD) CD4+ lymphocyte count for these HIV positive patients was 214 (200) cells/μL. The cytokine concentrations found in the 56 pericardial effusions are summarised in Table 9.1. In the five normal controls, the concentrations of all cytokines tested were below the detectable minimum range.

Table 9.1. Cytokine concentrations in various pericardial effusions

	TB / HIV+ (n=22)	**TB / HIV- (n=21)**	**Non-tuberculous (n=13)**
Interleukin-4	0.4 (n=1)	*	*
Interleukin-10	85.0 (30.3)	176.3 (31.9)	152.0 (33.9)
Tumour necrosis factor-α	2.4 (1.9)	8.7 (2.0)	5.3 (2.3)
Interferon-γ	623.6 (103.3)	786.7 (115.4)	27.0 (18.9)

Results expressed as mean (SD) pg/mL

* Concentration below minimum detectable range

The concentration of IFN-γ was significantly higher in the tuberculous pericardial effusions than in non-tuberculous effusions ($p < 0.00001$; Figure 9.1.). IFN-γ levels were detectable in only three of the 13 non-tuberculous effusions, including two malignant effusions and one septic pericardial effusion; in each of these effusions the IFN-γ concentration was <50 pg/mL. The concentration of TNF-α was elevated in all diagnostic categories; TNF-α levels tended to be lower in HIV positive TB patients than in HIV negative patients with pericardial TB ($p=0.09$; Figure 9.2.). Elevated levels of IL-10 were found in all three groups; the differences in IL-10 concentrations were not statistically significant ($p=0.11$; Figure 9.3.). With the exception of one HIV positive TB patient (corresponding IL-4 concentration, 0.4 pg/mL), the concentration of IL-4 was below the minimum range in all other patients. Fourteen of the 56 patients underwent pericardial biopsy, including nine HIV negative TB patients, three HIV positive TB patients, one HIV positive patient with septic pericarditis and one patient who had adenocarcinoma involving the pericardium. The comparison between histological findings and cytokine concentrations are summarized in Table 9.2. Nine of the 12 tuberculous effusions were associated with histological evidence of necrotising granulomatous inflammation; the other three biopsies showed non-necrotising granulomata. The histology of the case of septic pericarditis demonstrated an acute inflammatory cellular infiltrate, whereas the pericardial biopsy taken from the patient with the malignant effusion showed adeno-carcinomatous infiltration. IFN-γ levels exceeded 700.0 pg/mL in ten out of the twelve TB cases, whereas in the cases of septic pericarditis and adenocarcinoma, the corresponding IFN-γ levels were 28.9 pg/mL and 42.4 pg/mL, respectively. No significant difference in IFN-γ levels was noted regarding cases of granulomatous inflammation with or without necrosis ($p=0.48$). The concentration of IL-10 exceeded 600.0 pg/mL in one pericardial fluid

sample corresponding to granulomatous inflammation without tissue necrosis, whereas the lowest level (15.0 pg/mL) corresponded to extensive tissue necrosis. The corresponding mean (SD) IL-10 levels tended to be lower in effusions associated with necrosis than those without necrosis, at levels of 110.0 (116.2) pg/mL and 257.9 (198.5) pg/mL, respectively (p=0.18). The corresponding mean (SD) TNF-α concentrations for tuberculous effusions associated with tissue necrosis and tuberculous effusions without tissue necrosis were 12.4 (4.4) pg/mL and 11.2 (7.1) pg/mL, respectively (p=0.89).

Table 9.2. Comparison between pericardial cytokine levels and histology

Cytokine [a]	Necrosis (+) n=8	Necrosis (-) n=3	Purulent n=1	Malignant n=1
Interleukin-10	110.0 (116.2)	257.9 (198.5)	390.1	163.4
Tumour necrosis factor-α	12.4 (4.4)	11.2 (7.1)	22.4	0.5
Interferon-γ	966.0 (386)	775.7 (223.4)	28.9	42.4

[a] Values expressed as mean (SD) pg/mL

Necrosis (+) = necrotising granulomatous inflammation

Necrosis (-) = non-necrotising granulomatous inflammation

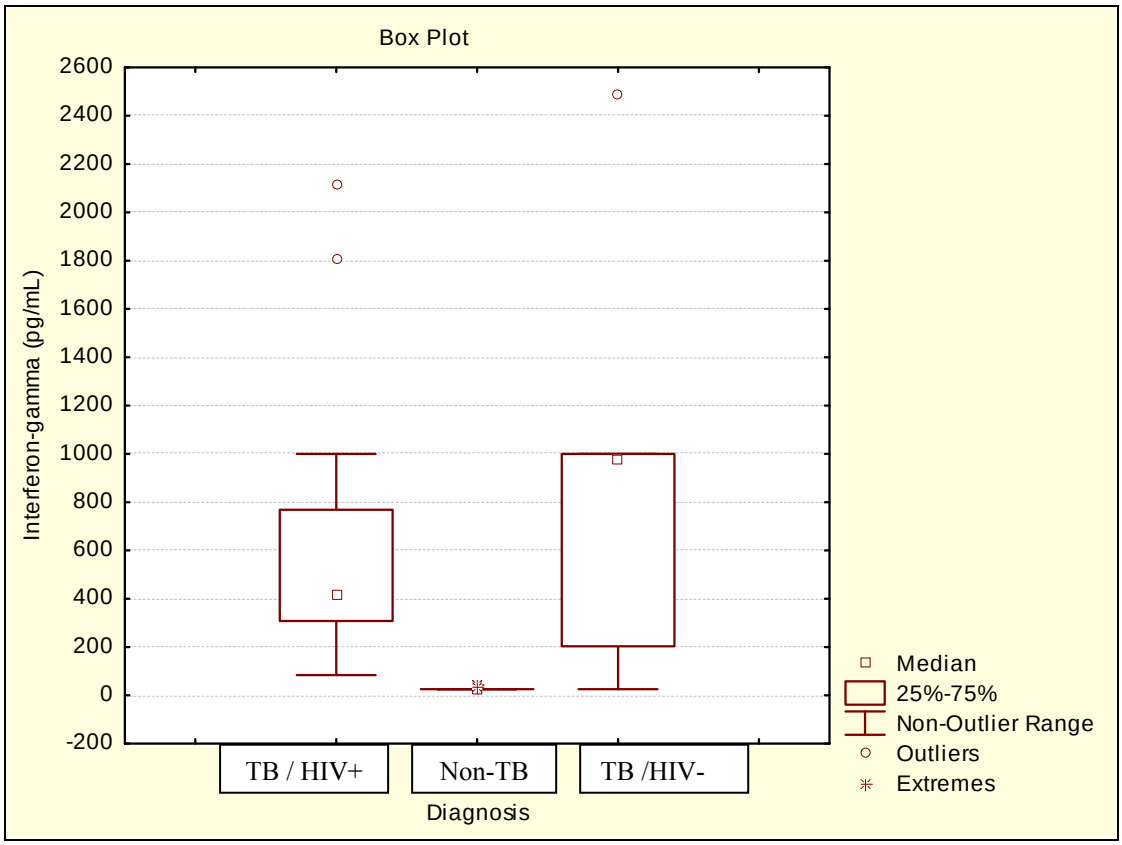

Figure 9.1. Distribution of interferon-γ in pericardial effusions

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

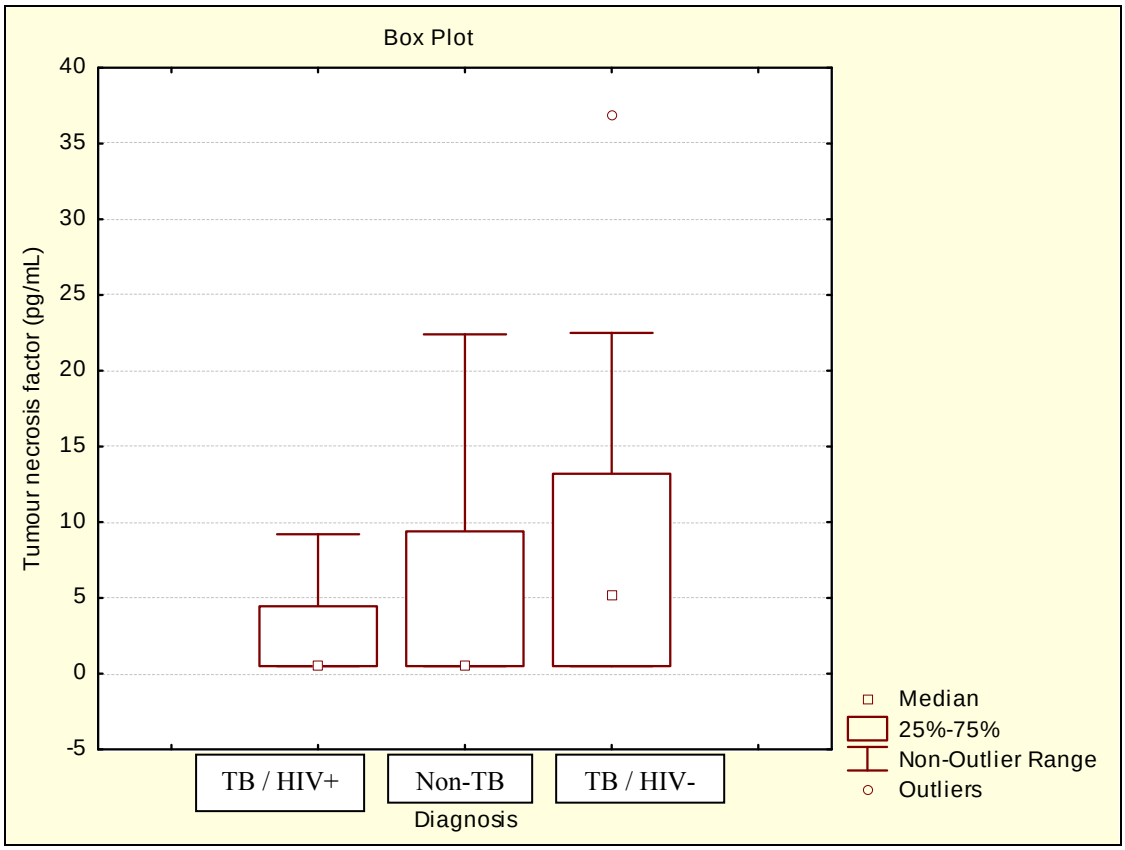

Figure 9.2. Distribution of tumour necrosis factor-α in pericardial effusions

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

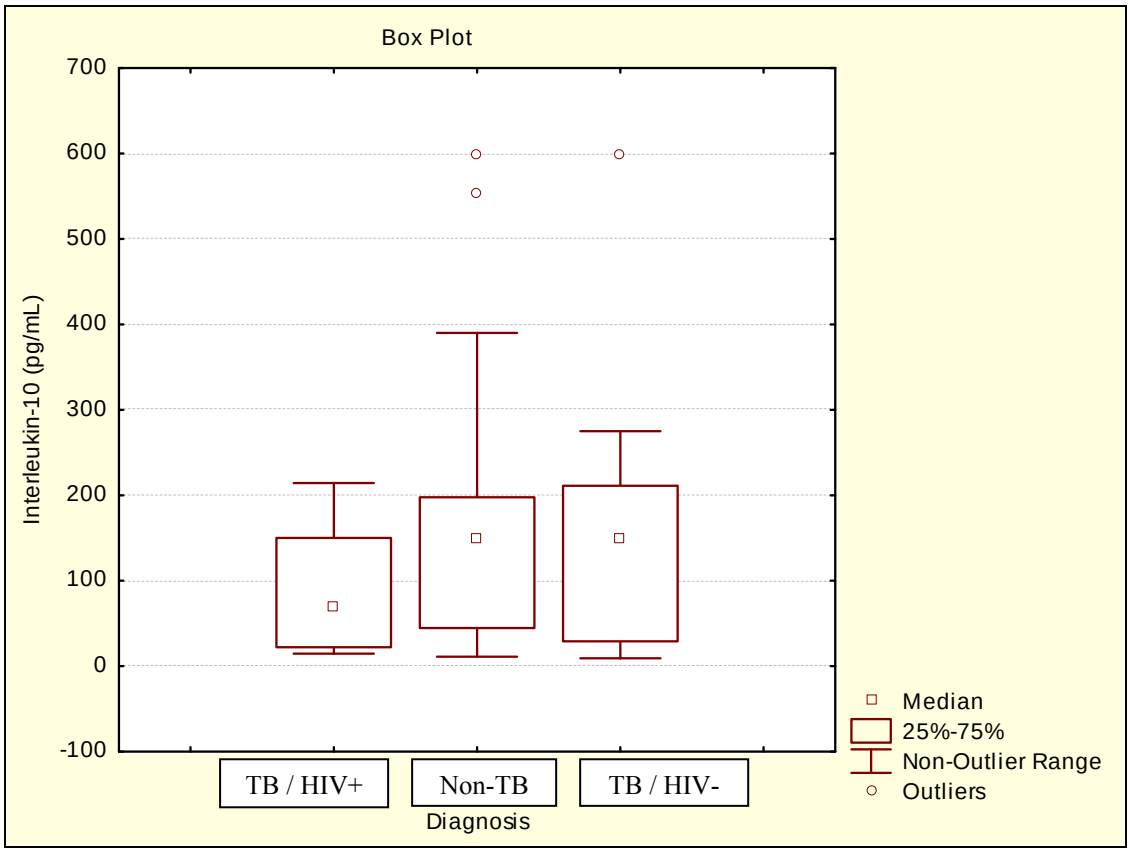

Figure 9.3. Distribution of interleukin-10 in pericardial effusions

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

DISCUSSION

The clinical outcome of infection with *M. tuberculosis* depends on the efficacy of cell-mediated immunity rather than humoral immunity. Persons with defective cell-mediated immunity, such as those with HIV infection, are at markedly increased risk for TB (Barnes *et al*, 1991). The immune response to TB is, however, a double-edged sword that may contribute to both clearance of infection and tissue damage, or in the case of pericardial TB to cardiac tamponade or constrictive pericarditis. .

In our study, a highly significant difference in the pericardial levels of IFN-γ was noted between tuberculous effusions and effusions caused by SLE, septic pericarditis or malignancy ($p < 0.00005$). This finding emphasizes the importance of IFN-γ in the pathogenesis of tuberculous pericarditis and supports an important role in the protective immune response against infection with *M. tuberculosis.* No significant correlation could be demonstrated between pericardial lymphocyte counts and IFN-γ concentration. Interestingly, IFN-γ levels were not significantly affected by HIV infection. This indicates that the production of IFN-γ does not depend on the total number of lymphocytes, but rather on specific activated subpopulations. IFN-γ is encoded by a single gene and produced by a number of T lymphocytes, including CD8+ cells, γδ cells, Th1 and Th0 CD4+ lymphocyte cells, but not Th2 CD4+ lymphocytes (Barnes *et al*, 1992; Farrar and Schreiber, 1993). IFN-γ is a major role player in the differentiation and activation of monocytes and macrophages (Snapper and Paul, 1987; Farrar and Schreiber, 1993). It promotes their action by enhancing antigen-presenting activity, and by the production of hydrogen peroxide, which facilitates intracellular elimination of mycobacteria (Farrar and Schreiber, 1993). In addition to this direct promotion of cellular immunity and delayed hypersensitivity,

IFN-γ suppresses the production of IL-4 by Th2 cells, and it antagonises the actions of IL-4 on B cells (Finkelman *et al*, 1990). Under the influence of IFN-γ and IL-2, activated monocytes and macrophages produce TNF-α (Cuturi *et al,* 1987; Vassalli, 1992).

In our series, pericardial fluid TNF-α concentrations tended to be lower in HIV positive patients. Although the production of TNF-α depends primarily on activated monocytes and macrophages (Cuturi *et al,* 1987), it is, in part, also produced by B lymphocytes (Sung *et al*, 1988), T lymphocytes (Steffen *et al*, 1988), natural killer (NK) cells (Vassalli, 1992), mast cells, polymorphonuclear leukocytes, keratinocytes and tumour cells (Vassalli, 1992). TNF-α increases macrophage phagocytic capacity and enhances mycobacterial killing by human macrophages *in vitro.*

Concentrations of IL-4 were indeterminably low in all but one HIV positive TB patient. In the presence of elevated levels of IFN-γ, this provides further evidence that tuberculous effusions result from a Th1 dominant cytokine response (Gajewski and Fitch, 1988; Mosman an Sad, 1996). Our data demonstrate that this Th1 dominant cellular response takes place irrespective of HIV status, even when peripheral blood CD4+ lymphocyte cell numbers are as significantly reduced as in the present study, where HIV positive tuberculous pericarditis patients were found to have a mean (SD) CD4+ lymphocyte cell count of 214 (200) cells/μL at admission. .

The Th1-dominant immune response is essential for successful elimination of tubercle bacilli, but it may result in detrimental tissue damage (Barnes and Wizel, 2000; Collins and Kaufmann, 2001; Jo *et al*, 2003). In this regard the correlation of

pericardial histology and cytokine levels produced interesting results, although the significance of our observations is unclear due to the small number of pericardial biopsy specimens. We noted that IFN-γ levels exceeded 800 pg/mL in nine out of the 12 specimens that demonstrated granulomatous lesions, and all of these were associated with elevated levels of TNF-α, ranging from between 8.4 to 36.8 pg/mL. These observations support the notion that in response to infection with *M. tuberculosis* an interaction between IFN-γ and TNF-α results in a delayed hypersensitivity reaction and the development of granulomatous lesions (Ando *et al,* 1972; Nathan *et al,* 1983; Phillip and Epstein, 1986; Esparza *et al,* 1987; Kindler *et al,* 1989). In addition to the association between granulomatous lesions and IFN-γ levels, we noted a tendency towards an inverse relationship between IL-10 levels and tissue damage seen on pericardial biopsy specimens. The highest IL-10 level (exceeding 600 pg/mL) corresponded with a biopsy that demonstrated granulomatous inflammation without tissue necrosis, whereas the lowest IL-10 concentration (15 pg/mL) accompanied a biopsy revealing extensive tissue necrosis. The corresponding mean (SD) IL-10 levels for effusions with or without histological evidence of tissue necrosis were 110.0 (116.2) pg/mL and 257.9 (198.5) pg/mL, respectively (p=0.18). The small numbers in the present study preclude definitive conclusions; nevertheless, our findings are in keeping with reports that suggest a tissue protective immunoregulatory role for IL-10 (Novelli *et al,* 1991; Barnes *et al,* 1993; Othieno *et al*, 1999; Shaw *et al*, 2000). IL-10 down-regulates the Th1 immune response and thereby protects the TB-infected host against excessive tissue damage (Barnes *et al,* 1993).

It has been suggested that a "switch" from a Th1 cytokine phenotype to a Th2 phenotype is a critical step in the progression of HIV disease (Clerici and Shearer, 1993; Maggi *et al,* 1994). Our data do not support this; our HIV infected patients demonstrated a classical Th1 cytokine response characterised by high IFN-γ levels and undetectable levels of IL-4, despite advanced HIV disease and significant CD4+ lymphocyte cell depletion.

Although chemotherapy remains the mainstay of anti-tuberculous therapy, the use of adjunctive immunotherapeutic modalities is attractive, particularly in patients with drug-resistant TB or in those that are severely immunocompromised. Our data indicate that IFN-γ is of primary importance in the human defence mechanism against infection with *M. tuberculosis* and the administration of IFN-γ should enhance mycobacterial elimination. Administration of IFN-γ to lepromatous leprosy patients decreases the bacillary burden, but increases the frequency of erythema nodosum leprosum (Sampaio *et al*, 1992). Therapeutic use of recombinant cytokines is limited by high costs and by toxicity of the large parenteral doses required to attain effective tissue concentrations. The administration of thalidomide, which acts as a TNF-α antagonist has been used to treat erythema nodosum leprosum (Sampaio *et al*, 1992), and has experimentally been used in the management of tuberculous meningitis. Another immunomodulatory approach could involve administering neutralising antibodies to immunosuppressive cytokines such as IL-4. Further research is indicated to explore the potential use of immunomodulatory responses in patients who are co-infected with TB and HIV.

Chapter 10

ADENOSINE DEAMINASE – MORE THAN A DIAGNOSTIC TOOL

In countries where tuberculosis (TB) is endemic, the increase in adenosine deaminase (ADA) activity observed in the pericardial fluid of patients with TB has been used to establish the diagnosis of tuberculous pericarditis (Telenti *et al*, 1991: Koh *et al*, 1994; Komsuoglu *et al*, 1995). ADA is a polymorphic enzyme that is involved in purine metabolism. It catalyses the deamination of adenosine and deoxyadenosine to produce inosine and deoxyinosine, respectively (Van der Weyden and Kelley, 1976). ADA plays a role in the differentiation of lymphoid cells (Barton and Goldschneider, 1979; Shore *et al*, 81), and the maturation of monocytes to macrophages (Fischer *et al*, 1976). The presence of ADA in pericardial and other body fluids reflects the activity of the cellular immune response in the respective compartment and, in particular, the activation of T lymphocytes and macrophages (Ocaña *et al*, 1983; Petterson *et al*, 1984).

In order to improve the understanding of factors that influence the ADA activity in large pericardial effusions, we determined whether ADA activity levels correlated with pericardial fluid leukocyte counts, peripheral CD4+ lymphocyte counts, pericardial cytokine concentrations and/or histopathological features of tuberculous pericarditis.

PATIENTS AND METHODS

ADA activity (U/L) was determined in all pericardial fluids specimens according to the method described in Chapter 2. Peripheral blood and pericardial fluid leukocyte counts were routinely done. Statistical analysis was done as summarised in Chapter 2. The correlation between two variables was plotted on a scatter plot and Spearman rank correlation coefficients were used to express the relationship. The significance of the correlation was denoted by a p-value <0.05.

RESULTS

During the study period, 233 patients presented to Tygerberg Hospital with large pericardial effusions requiring pericardiocentesis, including 162 patients who were diagnosed with tuberculous pericarditis (Chapter 3). We documented high levels of ADA in tuberculous pericardial effusions (Chapter 5). The most notable cause for low pericardial ADA levels in patients with tuberculosis pericarditis was the concomitant use of anti-tuberculous chemotherapy at the time of pericardiocentesis, and eleven patients who were taking anti-tuberculous therapy at the time of pericardiocentesis were excluded from analyses. The pericardial fluid ADA activity and peripheral blood differential leukocyte counts have been summarised in Table 10.1. Pericardial ADA activity was significantly elevated in tuberculous pericardial effusions compared to other diagnostic classes ($p<0.01$). HIV infection did not have a significant influence on ADA levels in the tuberculous effusions ($p=0.48$; Table 10.1). In tuberculous pericardial effusions, lymphocytes dominated the inflammatory cellular infiltrate, and the corresponding median (range) pericardial lymphocyte/neutrophil ratios for tuberculous (HIV negative), tuberculous (HIV

Table 10.1. Summary of differential leukocyte counts and pericardial fluid ADA activity in patients presenting with pericardial effusion

	TB / HIV- *n=64*	**TB / HIV+** *n=76*	***p***	**TB** *n=140*	**Non-TB** *n=61*	***p***
PB leukocytes[a]	7.8 (3.4)	6.6 (4.2)	0.01	7.3 (3.8)	12.5 (4.8)	<0.01
PB neutrophils	5.6 (2.9)	4.8 (3.8)	0.01	5.3 (3.4)	8.9 (6.1)	<0.01
PB lymphocytes	1.1 (0.6)	0.9 (1.1)	0.03	1.0 (0.8)	1.8 (2.3)	<0.01
PB monocytes	0.55 (0.19)	0.24 (0.16)	0.01	0.40 (0.18)	0.69 (0.25)	<0.01
Pc- leukocytes	2.7 (2.3)	1.8 (1.5)	0.03	2.3(1.9)	4.6 (3.8)	<0.01
Pc % neutrophils [b]	28.4 (22.5)	35.9 (25.5)	0.05	32.2 (24.0)	55.8 (24.6)	0.01
Pc neutrophils	1.0 (1.8)	0.8 (0.7)	0.88	0.9 (1.3)	2.6 (1.8)	<0.01
Pc % lymphocytes	52.1 (25.5)	39.4 (22.5)	0.03	45.5 (25.2)	25.6 (22.4)	<0.01
Pc lymphocytes	1.3 (0.9)	0.8 (0.6)	0.03	1.0 (0.8)	0.6 (0.6)	0.01
Pc % macrophages	19.4 (20.3)	18.9 (13.7)	0.89	19.2 (17.0)	13.3 (12.8)	0.03
Pc macrophages	0.38 (0.44)	0.34 (0.28)	0.60	0.36 (0.36)	0.48 (0.78)	0.06
Pc-ADA[c]	89 (35)	76 (38)	0.48	82 (37)	36 (29)	<0.01

[a] Number of cells expressed as mean (SD) x 10^9 / L

[b] Percentages expressed as mean (SD) %

[c] Pericardial adenosine deaminase activity expressed as mean (SD) U/L

PB = peripheral blood

Pc = pericardial fluid

positive) and non-tuberculous pericardial effusion were 2.7 (0.2-74.0), 1.9 (0.1-7.5) and 0.4 (0.1-2.2), respectively.

The corresponding Spearman rank correlation coefficient between pericardial ADA activity and pericardial total leukocyte, neutrophil, lymphocyte and macrophage counts were $r = 0.30$ ($p = 0.07$); $r = 0.25$ ($p = 0.13$); $r = 0.31$ ($p = 0.07$); and $r = 0.14$ ($p = 0.59$), respectively. No significant correlation was demonstrable between ADA activity and peripheral blood leukocyte counts. However, a significant association was noted between high levels of ADA activity and high pericardial leukocyte, neutrophil and lymphocyte counts. In the tuberculous pericardial exudates, the corresponding median (range) pericardial fluid leukocyte, neutrophil and lymphocyte counts were significantly higher in effusions with ADA activity ≥40 U/L than in pericardial effusions with ADA levels <40 U/L ($p<0.05$ for each), whereas in non-tuberculous effusions only the total leukocyte and neutrophil counts were higher in high ADA (≥40 U/L) effusions than in low ADA (<40 U/L) effusions (Table 10.2.).

Table 10.2. Relationship between pericardial fluid leukocytes numbers and pericardial fluid ADA activity

Subgroups	Adenosine deaminase activity		p
	ADA <40 U/L	ADA ≥40 U/L	
Tuberculous effusions			
Pericardial total leukocytes[a]	1220 (470-2210)	2580 (400-10260)	0.03
Pericardial neutrophils	53 (15-88)	918 (158-7377)	0.02
Pericardial lymphocytes	721 (180-1768)	1140 (99-2654)	0.03
Non-tuberculous effusions			
Pericardial total leukocytes	1655 (220-360)	4341 (80-24140)	0.02
Pericardial neutrophils	812 (103-1787)	2843 (24-19336)	0.04
Pericardial lymphocytes	584 (51-1520)	260 (56-1497)	0.18

[a] Results expressed as median (range) cells x 10^6/L

p-value established by two-way ANOVA

Correlation between ADA and peripheral blood CD4+ lymphocyte cells

No significant correlation between pericardial ADA activity and peripheral blood CD4+ lymphocyte cell counts could be demonstrated ($r = 0.17$; $p = 0.20$). However, at very low CD4+ lymphocyte cell counts there was a distinct tendency towards low levels of ADA activity. The corresponding median (range) CD4+ lymphocyte cell counts for patients with ADA activity <30 U/L, ADA activity <40 U/L and ADA activity ≥ 40 U/L were 59.0 (6.0-115.0) cells/μL, 183.0 (6.0-578.0) cells/μL and 219.0 (25.0-1006.0) cells/μL, respectively.

In the HIV negative tuberculous patients and the non-tuberculous patients the corresponding correlation between pericardial ADA activity and peripheral CD4+ lymphocyte cell counts was $r = -0.89$ ($p = 0.04$) and $r = -0.42$ ($p = 0.57$), respectively.

Correlation between ADA and pericardial cytokines

Various cytokine concentrations were evaluated in pericardial effusion patients (n=56; Chapter 9). Significant correlation was demonstrable between pericardial fluid ADA activity and pericardial TNF-α concentration of patients with tuberculous pericardial effusions ($r=0.63$; $p=0.001$; Figure 10.1). No significant correlation could be demonstrated between pericardial ADA activity and pericardial IFN-γ ($r=0.33$, $p=0.13$), nor a correlation between pericardial ADA and pericardial IL-10 concentration ($r=0.06$, $p=0.76$). There was also no significant correlation between pericardial ADA levels and cytokines in the non-tuberculous pericardial effusion patients.

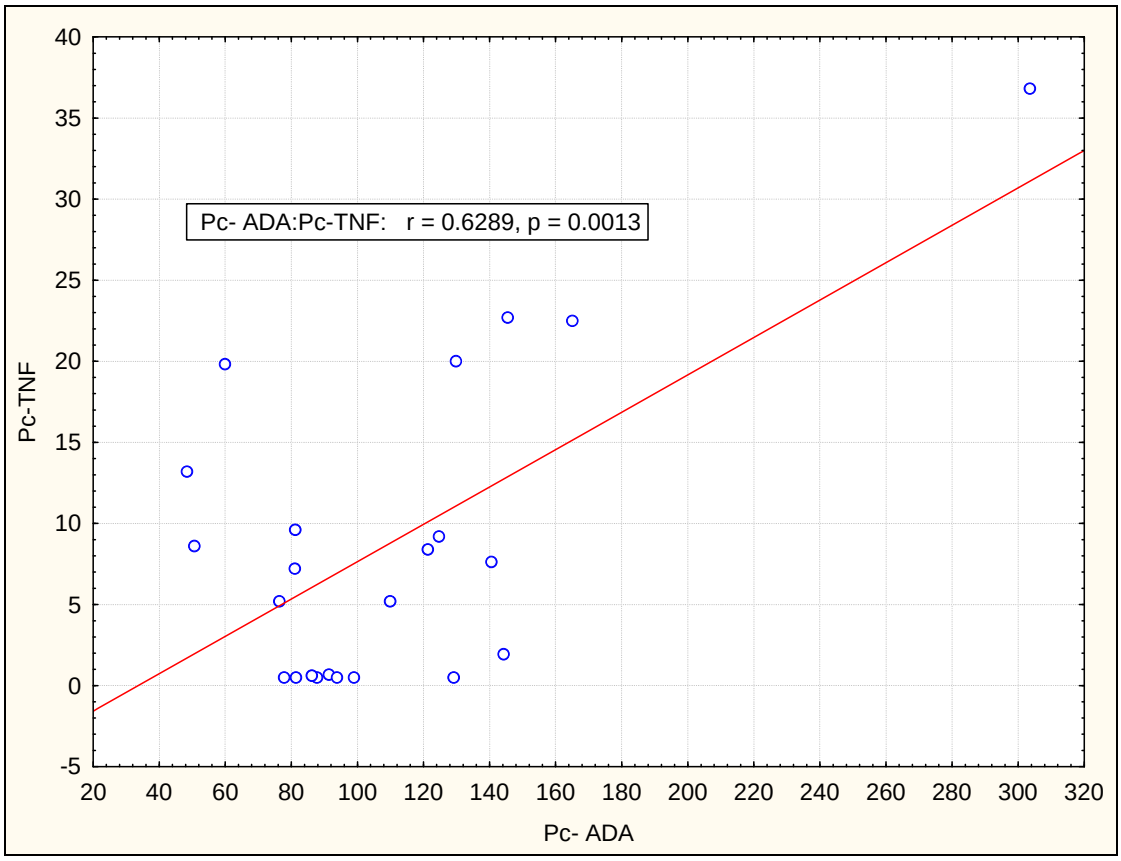

Figure 10.1. Correlation between pericardial ADA activity and concentration of tumour necrosis factor-α in tuberculous pericarditis

Pc-TNF = pericardial tumour necrosis factor-α (pg/mL)

Pc-ADA = pericardial adenosine deaminase activity (U/L)

Correlation between ADA and pericardial histology

A total of 25 tuberculous pericarditis patients underwent pericardial biopsy; five of these patients were HIV positive. Fifteen biopsies (60%) demonstrated granulomatous inflammation; 12 of these were accompanied by caseating necrosis. The remaining ten biopsies demonstrated no characteristic histomorphological features of TB. For patients with pericardial TB, the corresponding mean (SD) ADA level for granulomatous necrotising, granulomatous non-necrotising, and non-granulomatous pericarditis was 125 (77) U/L, 105 (56) U/L, and 95 (53) U/L, respectively (p=0.62). The lowest ADA activity was associated with serofibrinous pericarditis {corresponding mean (SD) of 67 (78) U/L}.

DISCUSSION

In South Africa, the majority of patients presenting with large pericardial effusions have pericardial TB (Desai, 1979; Strang, 1984. The present study confirms that pericardial TB is responsible for approximately 70% of the large pericardial effusions seen at Tygerberg Academic Hospital. We documented high levels of ADA in tuberculous pericardial effusions. The ADA activity was not significantly lower in HIV positive patients than in HIV negative TB patients and the correlation between peripheral CD4+ lymphocyte counts and pericardial ADA activity levels was non-significant. However, a relationship between severe peripheral CD4+ lymphocyte cell depletion and low ADA levels was noted. Seven of the 10 HIV positive patients with low ADA tuberculous effusions (ADA activity <40 U/L) had corresponding CD4+ lymphocyte cell counts < 200 cells/μL. The difference between CD4+ lymphocyte cell counts associated with pericardial ADA activity < 40 U/L and pericardial ADA

activity ≥40 U/L was minimal [corresponding counts being 183.0 (6.0-578.0) cells/μL and 219.0 (25.0-1006.0) cells/μL, respectively (p=0.8)], whereas in the group of tuberculous patients with pericardial ADA activity < 30 U/L the corresponding median (range) CD4+ lymphocyte cell count being 59.0 (6.0-115.0) cells/μL, was significantly decreased (p=0.04). These results suggest that severe CD4+ lymphocyte cell depletion may result in low ADA activity levels, probably by impeding the CD4+ lymphocyte dependent cellular immune response against mycobacterial antigens in the pericardial space.

Pericardial exudates with an ADA activity ≥40 U/L were associated with higher total leukocyte and higher neutrophil counts than pericardial effusions with ADA activity <40 U/L. In the group of patients with tuberculous pericarditis, pericardial exudates with ADA activity ≥40 U/L were also characterised by higher lymphocyte counts than tuberculous exudates with ADA activity <40 U/L. No specific correlation between ADA levels and macrophage counts could be demonstrated. The lack of correlation between pericardial ADA and the number of pericardial lymphocytes is in keeping with numerous studies that could not demonstrate any correlation or were inconclusive (Ocaña *et al*, 1983; Baganha *et al*, 1990; Bovornkitti *et al*, 1991). However, pericardial neutrophil counts were lower for tuberculous effusions than for non-tuberculous effusions, whereas median ADA activity was significantly higher in tuberculous than in non-tuberculous exudates, implying that lymphocytes (and possibly macrophages) play the major role in the production and release of ADA in tuberculous effusions; this supports data from tuberculous pleural exudates (Valdes *et al*, 1996). It has been demonstrated that lymphocytes and macrophages contain similar levels of ADA activity, and that this is much higher than in other cell types or

tissues (Ungerer *et al*, 1994). Our study does not add additional information on the potential contribution of macrophages to the ADA activity in tuberculous exudates. Tuberculous exudates with ADA activity <40 U/L were accompanied by significantly lower pericardial neutrophil counts than effusions with ADA activity ≥40 U/L, suggesting that neutrophils also contribute to ADA activity. In non-tuberculous effusions, neutrophils are probably the major source of ADA, although lymphocytes may also contribute (Ungerer *et al*, 1994). In our study, the contribution of neutrophils to ADA activity in non-tuberculous effusions was most notable in the case of septic pericarditis. However, significantly higher pericardial leukocyte counts are "required" to produce similar levels of ADA activity.

Two molecular forms of ADA have been described, namely ADA_1 and ADA_2 (Hischhorn and Ratech, 1980). Although ADA_1 is found in all cells, its highest activity is in lymphocytes and macrophages, whereas the ADA_2 isoenzyme appears to originate from monocytes and macrophages (Ungerer *et al*, 1992). ADA_1 and ADA_2 seem to function together to ensure the homeostasis of adenosine and 2'deoxygenase in monocytes-macrophages, and a small proportional increase in ADA_2 may result in a rapid increase in the levels of 2'deoxygenase in these monocytes-macrophages (Gakis, 1996). Seto *et al* (1986) demonstrated that 2'deoxygenase is deleterious for nucleic acid, and this has led to the postulation that ADA may play an important role in the defensive mechanisms of monocytes-macrophages against cellular micro-organisms (Gakis, 1996).

In the present study, ADA levels tended to be higher in cases that showed histological evidence of granulomatous inflammation than cases with serofibrinous pericarditis,

suggesting that the cells involved in the development and maintenance of granulomatous lesions (mainly activated macrophages and T lymphocytes) are also responsible for the elevated ADA activity in the pericardial fluid, as has been previously suggested (Ocaña *et al*, 1983; Petterson *et al*, 1984). The correlation noted between pericardial ADA activity and pericardial TNF- α suggests that there is an interaction between the locally produced cytokines and the demonstrable ADA activity. The question whether ADA production is influenced directly or indirectly by cytokines, and specifically by which cytokines, warrants further research.

Chapter 11

THE EFFECT OF HIV ON LYMPHOCYTE SUBPOPULATIONS IN PERICARDIAL TB

The protective immune response to active tuberculosis (TB) depends on the presence and activity of macrophages, as well as CD4+ lymphocyte cells and CD8+ T lymphocytes, which are responsible for the orchestrated production of cytokines (Cho *et al*, 2000; Rook and Zumla, 2001; Collins and Kaufmann, 2001; Boom *et al*, 2003). Pathologically, a number of stages are recognized in the development of tuberculous pericarditis. The early stage is characterised by a fibrinous serosal exudate that contains lymphocytes. The middle stage manifests granuloma formation, and the presence of viable acid-fast bacilli. This is followed by absorption of the effusion, pericardial thickening, and proliferation of granulomata often accompanied by caseating necrosis. At this stage, viable acid-fast bacilli are often no longer detected. In the late stage, fibrous pericarditis develops as the granulomatous reaction is replaced by fibrous tissue and collagen (Peel, 1948; Lorell, 1997; Nardell *et al*, 2004). These changes are followed by the accumulation of cholesterol crystals and the development of pericardial calcification (Lorell, 1997).

In tuberculous pleural effusions lymphocytes are the predominant cell type, accounting for more than 70% of the total white cell count (Spriggs and Boddington, 1960; Yam, 1967, Gresham, 1989), and these cells are mainly of the CD4+ lymphocyte phenotype (Bergroth *et al,* 1987; Tsukaguchi *et al*, 1999; Bergroth *et al,* 1987). To the best of our knowledge, the composition of the phenotypically

heterogeneous inflammatory cellular infiltrate has not previously been described in tuberculous pericarditis. To improve our understanding of the protective immune response against *M. tuberculosis* and to assess the influence of HIV on the inflammatory cells that participate in the immune response that results in pericardial effusion, we evaluated the composition of the cellular infiltrate in patients with tuberculous pericarditis.

PATIENTS AND METHODS

To evaluate the phenotypic lymphocyte subpopulations, 24 pericardial fluid and corresponding peripheral blood specimens were evaluated by flow cytometry, as described in Chapter 2. The blood and pericardial fluid was collected at the time of initial pericardiocentesis. The diagnoses were made according to predetermined criteria. None of the patients was receiving anti-tuberculous therapy at the time of presentation. The methodology used for statistical analysis has been summarised in Chapter 2.

RESULTS

Phenotypic differentiation of lymphocyte subpopulations was performed on 24 pericardial fluid and corresponding peripheral blood specimens. The diagnostic causes of the pericardial effusions (as per predetermined diagnostic criteria) included TB (n=18), malignancy (n=2), septic pericarditis (n=2), and systemic lupus erythematosus (SLE; n=2). Eleven patients were HIV positive; all presented with pericardial TB and none of them was previously known with HIV. The results of the phenotypic lymphocyte differentiation in peripheral blood and in pericardial fluid specimens are summarised in Table 11 .1. and Table 11.2., respectively.

Table 11.1. Phenotypic lymphocyte differentiation in peripheral blood samples of pericarditis patients

	TB / HIV+ n=11	**TB / HIV-** n=6	**Non-TB** n=7	***p***
Leukocytes[a]	6.5 (4.1)	7.6 (3.2)	12.1 (4.6)	<0.01
Neutrophils	4.8 (3.4)	5.5 (2.7)	8.9 (6.1)	<0.01
Monocytes	0.22 (0.17)	0.54 (0.17)	0.72 (0.34)	<0.01
Lymphocytes	1.10 (1.34)	1.33 (0.60)	1.72 (1.40)	0.03
% CD3+ lymphocytes[b]	79.2 (29.8)	74.5 (41.3)	75.0 (38.5)	0.16
% CD4+ lymphocytes	25.1 (6.4)	36.8 (24.6)	42.7 (24.3)	0.01
% CD8+ lymphocytes	56.1 (6.7)	39.2 (29.7)	26.7 (25.3)	<0.01
% CD19+ lymphocytes	11.6 (6.4)	7.3 (4.5)	12.9 (4.1)	0.25
% CD16+/56+ cells	10.1 (2.9)	13.6 (2.8)	8.7 (2.6)	0.47
CD4+/CD8+ cell ratio	0.41 (0.37)	1.12 (1.75)	2.50 (1.53)	<0.01

[a] Number of cells expressed as mean (SD) x 10^9/L

[b] Percentages expressed as mean (SD) %

P value established by Kruskall-Wallis One-Way ANOVA test on ranks

Table 11.2. Phenotypic lymphocyte differentiation in pericardial fluid samples of pericarditis patients

	TB / HIV+ n=11	**TB / HIV-** n=6	**Non-TB** n=7	***p***
Leukocytes[a]	1.8 (1.5)	2.7 (2.3)	4.6 (3.8)	<0.01
Neutrophils	0.8 (0.7)	1.0 (1.8)	2.6 (1.8)	<0.01
Macrophages	0.34 (0.28)	0.38 (0.44)	0.48 (0.78)	0.07
Lymphocytes	0.86 (0.56)	1.34 (0.91)	0.70 (0.85)	0.03
% CD3+ lymphocytes[b]	91.8 (3.5)	86.7 (4.0)	80.0 (2.1)	0.05
% CD4+ lymphocytes	25.3 (12.9)	50.2 (26.9)	58.0 (23.9)	<0.01
% CD8+ lymphocytes	61.0 (12.8)	37.3 (13.7)	19.5 (10.7)	<0.01
% CD19+ lymphocytes	1.3 (0.6)	5.7 (1.7)	4.3 (2.7)	0.02
% CD16+/56+ cells	1.1 (0.4)	3.8 (6.5)	3.9 (4.5)	0.03
CD4+/CD8+ cell ratio	0.46 (0.31)	1.66 (1.36)	4.46 (2.08)	<0.01

[a] Number of cells expressed as mean (SD) x 10^9/L

[b] Percentages expressed as mean (SD) %

P value established by Kruskall-Wallis One-Way ANOVA test on ranks

Peripheral blood

The lymphocyte subpopulations differed significantly in patients presenting with pericardial TB compared with patients with non-tuberculous pericarditis. The percentage of CD4+ lymphocyte cells and the total number of natural killer (NK) cells was significantly lower in patients with tuberculous pericarditis than in patients with non-tuberculous effusions ($p<0.01$ for both variables), whereas the percentage of CD8+ lymphocyte cells was higher in patients with tuberculous effusions than in those with non-tuberculous aetiology ($p<0.01$).

The distribution of lymphocyte phenotypes differed also significantly between the HIV positive TB group and the HIV negative tuberculous pericarditis patients, as indicated in Table 11 .1. The corresponding mean (SD) CD4+ lymphocyte cell counts for HIV positive TB, HIV negative TB and non-tuberculous effusions were 214.5 (210.3) cells/μL, 635.4 (482.5) cells/μL, and 538.7 (264.9) cells/μL, respectively. In addition, the peripheral blood CD4+lymphocyte/CD8+ lymphocyte cell ratios were significantly higher in HIV positive TB patients than in both HIV negative TB patients and patients presenting with non-tuberculous effusions ($p<0.01$ for both groups; Figure 11.1.).

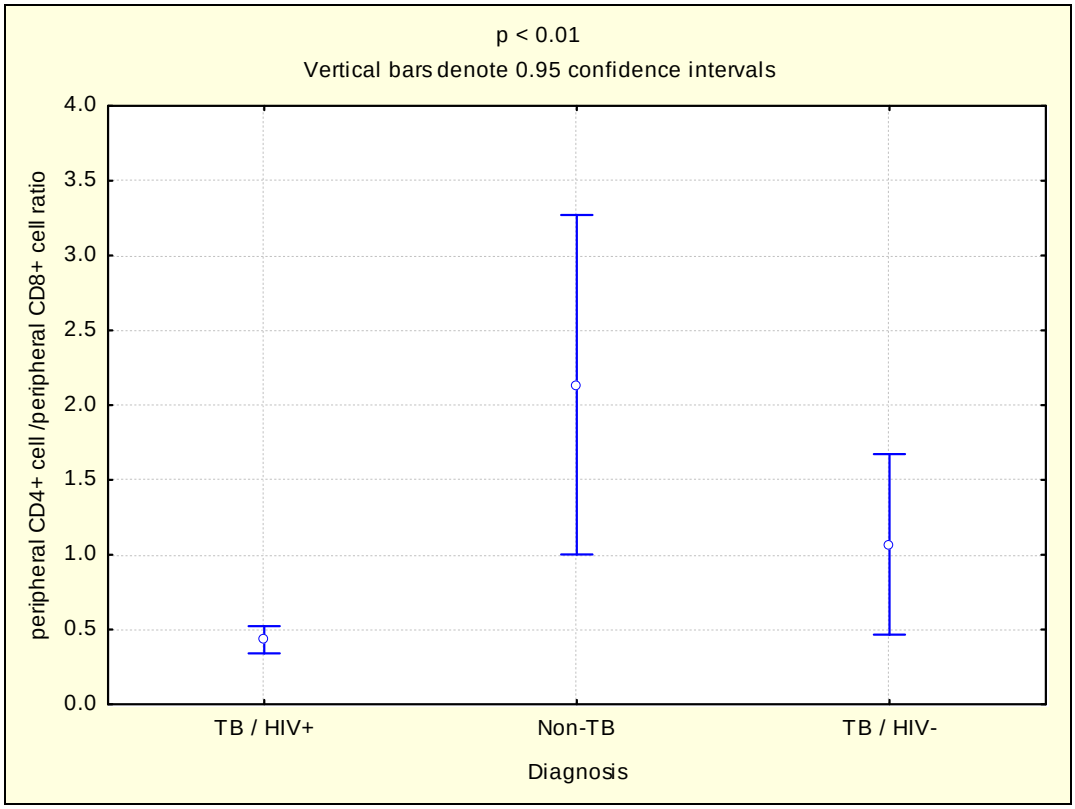

Figure 11.1. Distribution of peripheral blood CD4+ cell / CD8+ cell ratio

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

Pericardial fluid

Lymphocytes dominated the pericardial fluid cellular infiltrate in patients with tuberculous pericarditis in comparison to effusions caused by sepsis, malignancy or SLE, in which neutrophils were the dominant type of leukocyte. We also observed significant differences in the composition of lymphocyte subpopulations between patients with tuberculous pericarditis and patients who presented with non-tuberculous pericarditis (Table 11.2).

In comparison with HIV negative patients with tuberculous pericarditis, HIV positive TB patients were found to have a lower percentage of CD4+ lymphocyte cells ($p<0.01$; Figure 11.2.), a lower percentage of NK cells (CD16+/56+ cells; $p=0.01$; Figure 11.3.), a lower percentage of B lymphocyte cells (CD19+ cells; $p=0.03$; Figure 11.4.), and a lower CD4+ cell/CD8+ cell ratio ($p < 0.01$), however, the percentage of CD8+ lymphocyte cells was higher in HIV positive TB patients than in the HIV negative TB group ($p< 0.01$; Figure 11.5).

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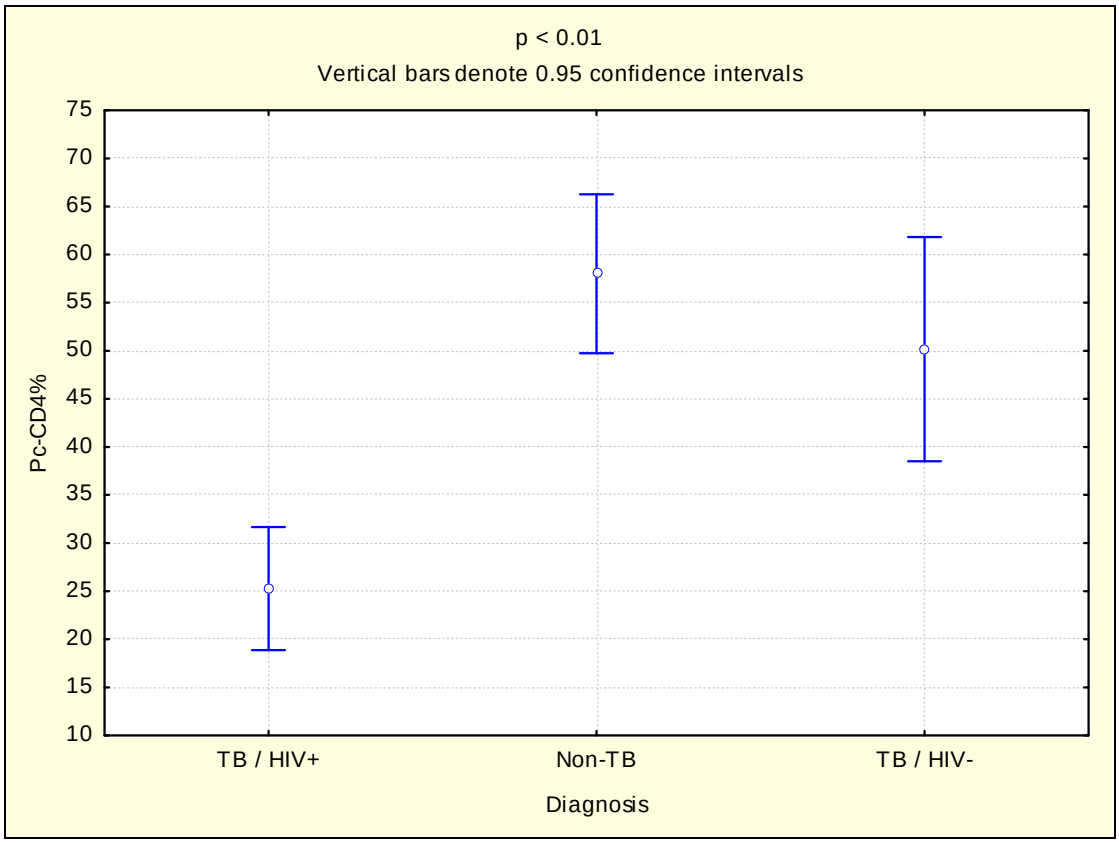

Figure 11.2. Distribution of pericardial CD4+ lymphocyte cells as percentage of pericardial lymphocytes

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

Pc = pericardial

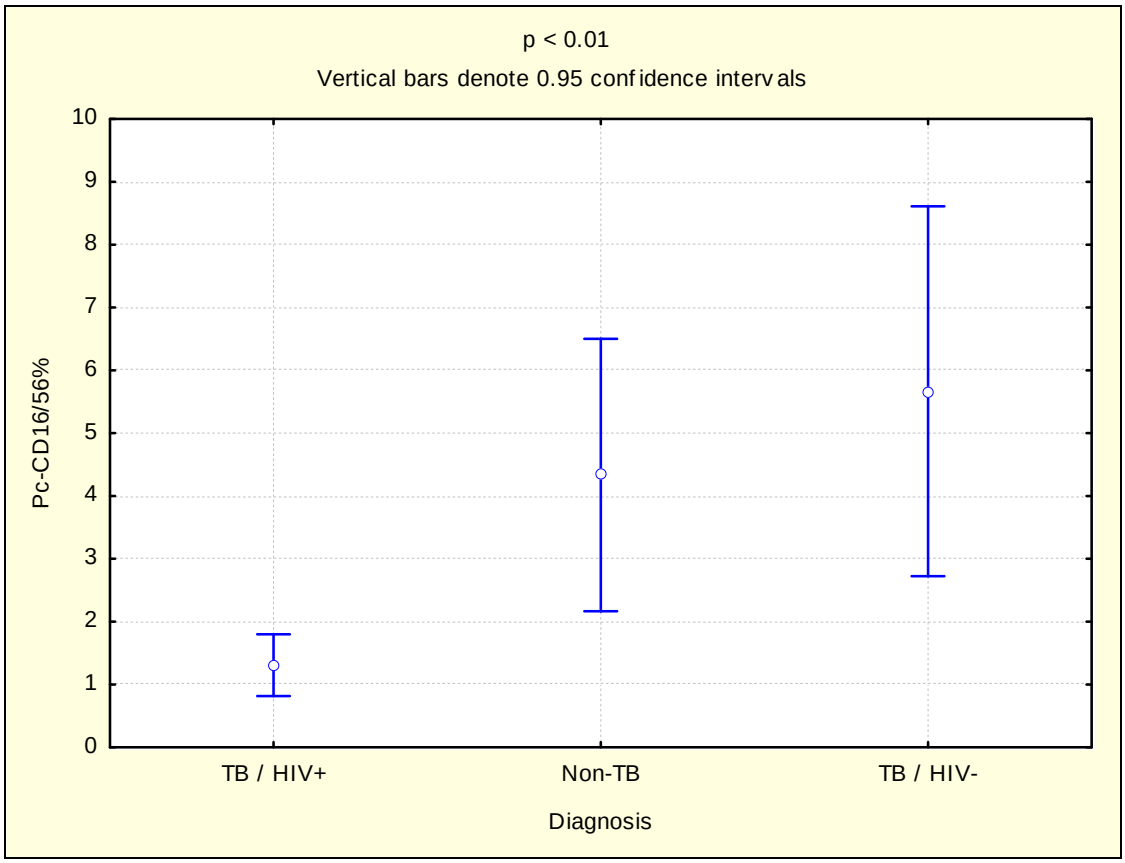

Figure 11.3. Distribution of pericardial CD16+/56+ cells as percentage of pericardial lymphocytes

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

Pc = pericardial

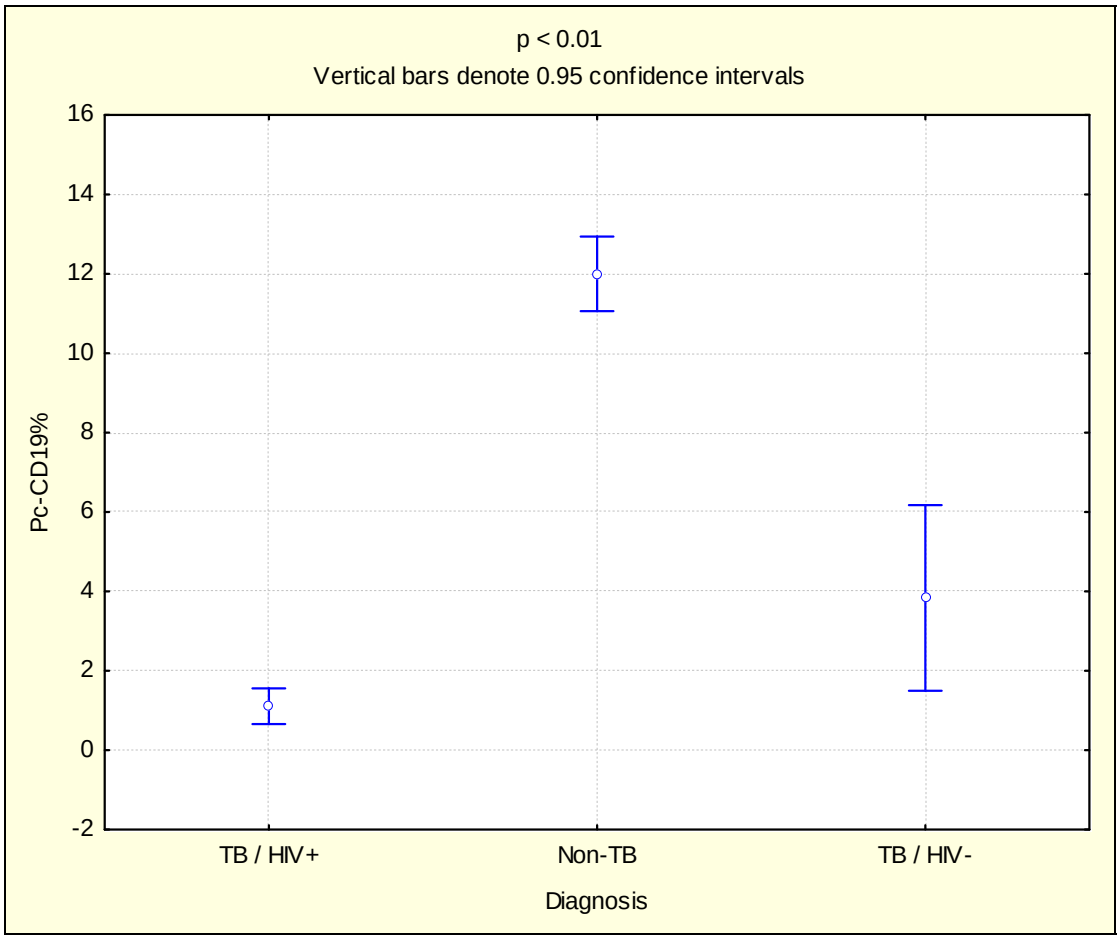

Figure 11.4. Distribution of pericardial CD19+ cells as percentage of pericardial lymphocytes

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

Pc = pericardial

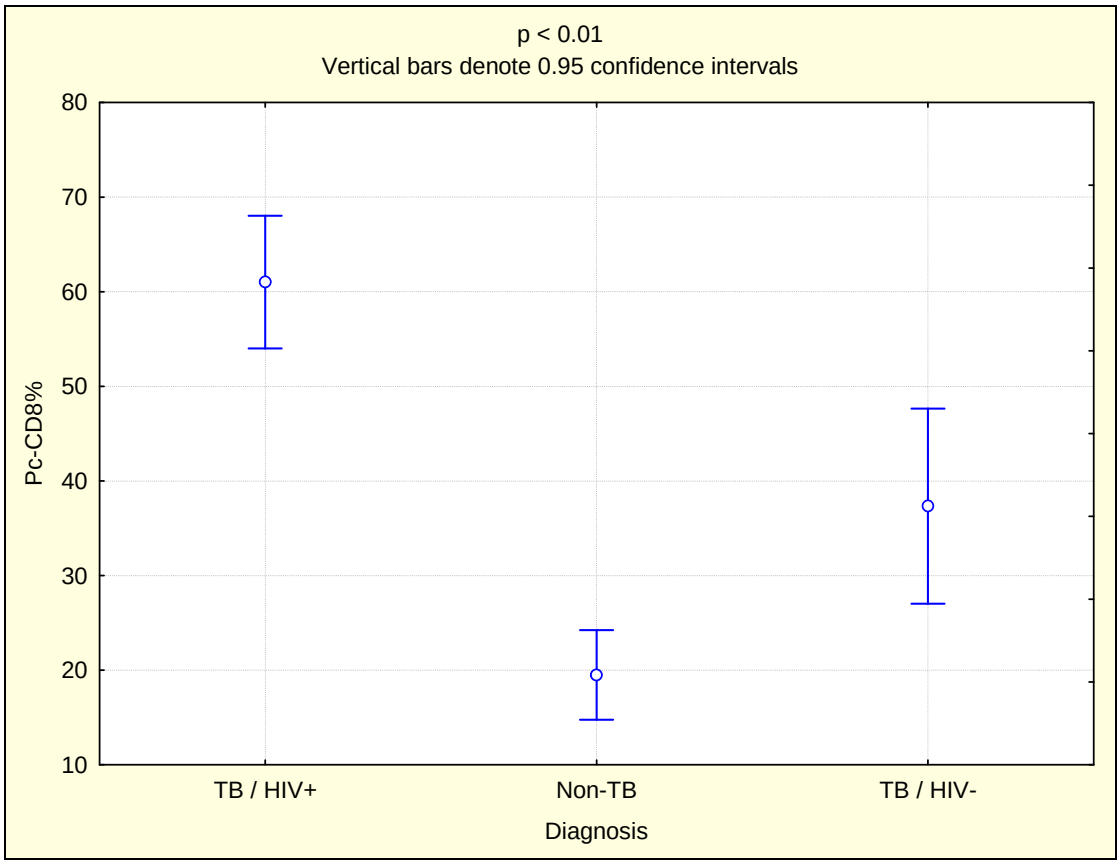

Figure 11.5. Distribution of pericardial CD8+ cells as percentage of pericardial lymphocytes

TB = tuberculosis

HIV+ = human immunodeficiency virus positive

HIV- = human immunodeficiency virus negative

Non-TB = non-tuberculous

Pc = pericardial

DISCUSSION

The central immunological feature of HIV disease is the loss of lymphocytes. In infected individuals, the rate of CD4+ lymphocyte loss reflects the balance between ongoing cellular destruction and an inadequate repopulation from progenitor cells. Infected CD4+ lymphocytes may be depleted through direct cytopathic effects of the virus, by virus-specific cytotoxic lymphocytes, or by antibody-dependent cell mediated cytotoxicity (Fauci, 1988; Siliciano, 1996). However, impaired regeneration of mature CD4+ lymphocyte cells from the precursor cell pool also contributes to the ultimate depletion of these cells.

In our study, significantly decreased numbers of peripheral blood CD4+ lymphocytes and monocytes were demonstrated in HIV positive TB patients compared to both HIV negative TB patients and patients with non-tuberculous disease. This was accompanied by significantly elevated CD8+ lymphocytes in the HIV infected individuals compared with those not infected with HIV. The lymphocyte phenotypes in the pericardial fluid reflected the phenotypic distribution observed in the peripheral blood. The dominant features were severely diminished presence of CD4+ lymphocytes and a predominance of CD8+ cells in HIV positive tuberculous effusions in contrast to HIV negative tuberculous patients in whom CD4+ lymphocytes dominated the inflammatory cell infiltrate. Previous studies that did not include HIV positive patients described CD4+ lymphocyte cell dominance in pleural fluid of patients with tuberculous pleurisy (Bergroth *et al*, 1987; Rossi *et al,* 1987; Ribera *et al*, 1988; Fontes *et al,* 1990). CD8+ lymphocyte dominance in tuberculous pericardial exudates of HIV positive individuals has, to the best of our knowledge, not previously been described but is consistent with the dominance of CD8+ lymphocyte cells in the

peripheral circulation of HIV positive patients (Margolick *et al,* 1995), as well as in bronchoalveolar lavage samples of HIV-infected individuals (Venet *et al*, 1985; Young *et al*, 1985). As CD4+ lymphocyte cells are lost in HIV positive individuals, CD8+ lymphocyte cells increase in a seemingly compensatory fashion, resulting in a constant level of total CD3+ lymphocyte cells (Margolick *et al,* 1995). The depletion of NK cells in tuberculous exudates of HIV positive patients was consistent with the diminished number of NK cells found in the peripheral blood of HIV positive patients with tuberculous pericarditis in this present study.

In spite of significant differences in the composition of the inflammatory cellular infiltrate, pericardial fluid cytokine levels were affected less markedly than expected. The difference in pericardial IFN-γ levels of HIV positive and HIV negative TB patients was negligible and no demonstrable correlation was found between pericardial lymphocyte counts and the corresponding IFN-γ levels in tuberculous effusions (Chapter 9). The contribution of CD4+ lymphocyte cells in the delayed hypersensitivity response and protection against TB is well described, including the interaction between IFN-γ secreting CD4+ lymphocyte cells and macrophages (Sanchez *et al*, 1994). In our study pericardial fluid CD4+ lymphocyte cell numbers were about fivefold higher in the HIV negative than the HIV positive TB group (mean [SD] 785.5 [166.9] cells/μL versus 145.5 [123.3] cells/μL); the negligible difference in IFN-γ level between the HIV positive and HIV negative tuberculous groups is thus unexpected. It is likely that a large proportion of the IFN-γ found in HIV positive patients originated from CD8+ lymphocyte cells, which dominated the cellular infiltrate, and in addition to cytolysis, contribute significantly to the host defence against TB by secreting protective cytokines such as IFN-γ and TNF-α (Cooper *et al*,

1997; Laochumroonvorapong *et al*, 1997; Sousa *et al*, 1999; Smith and Dockrell, 2000). IFN-γ and other cytokines are, in part, produced by gammadelta (γδ) T lymphocytes and CD4 CD8 double negative T lymphocyte cells (Mosmann and Moore, 1991; Abehsira-Amar *et al*, 1992; Chehimi and Trinchieri, 1994; Boom, 1999; Tsukaguchi, 1999). Of these, γδ T-lymphocytes seem to play an important role in the human anti-tuberculous immune response (Barnes *et al,* 1992).

In addition to the severe depletion of CD4+ lymphocyte cells HIV and TB co-infected individuals were characterised by decreased numbers of NK cells and B lymphocytes; only CD8+ lymphocyte cell numbers were maintained or elevated. It is possible that the widespread cytopenia observed in this study in HIV positive patients who presented with pericardial TB resulted from a combination of depressed haematopoiesis caused by HIV infection of CD34+ progenitor cells (Folks *et al*, 1988; Terstappen and Loken, 1990) and disturbed maturation of pluripotent haematopoietic stem cells (Folks *et al*, 1988). In the present study, peripheral monocytes were significantly diminished in the HIV positive TB patients compared to HIV negative individuals with TB, whereas the number and proportion of pericardial macrophages was similar in HIV positive and HIV negative individuals with pericardial TB. Monocytes and tissue macrophages are both susceptible to HIV infection (Rich *et al*, 1992), but due greater numbers of CD4+ lymphocyte molecules on peripheral blood monocytes than tissue macrophages these cells are more prone to be affected by HIV (Rich *et al*, 1992).

In patients who were co-infected with HIV and TB, CD8+ T lymphocyte cell numbers were elevated in the peripheral blood and pericardial cellular infiltrate, whereas the

numbers of other lymphocyte phenotypes, neutrophils and monocytes were significantly reduced. The observed cytopenia probably results from infection of haematopoietic stem cells and the release of endogenous cytokines within the haematopoietic micro-environment that affect the proliferation and differentiation of haematopoietic cells in the bone marrow and thymus (Kunzi *et al*, 1995). In spite of the decreased number of CD4+ lymphocyte cells and other types of leukocytes, tuberculous effusions of HIV positive individuals nevertheless demonstrated a Th1 dominant cytokine profile, characterised by indeterminably low levels of IL-4 and elevated levels of IFN-γ.

Chapter 12

GENERAL CONCLUSIONS

More than a hundred years after Robert Koch's discovery of the tubercle bacillus, tuberculosis (TB) remains a major cause of morbidity and mortality, accounting for more deaths of adults than any other infectious agent. More than 8 million new cases of TB were reported to the World Health Organization in 2003 (WHO Report, 2005), and millions are co-infected with the human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis (M. tuberculosis;* Dye *et al*, 1999; WHO Report, 2005). A rapid increase in TB cases coincident with the HIV epidemic has been reported for sub-Saharan Africa (WHO Report, 2005). The effects of HIV include a higher proportion of extrapulmonary TB (Narain *et al*, 1992, Barnes *et al*, 1993), including an increase in the incidence of tuberculous pericarditis (Cegielski *et al*, 1990; Cegielski *et al*, 1994; Magula and Mayosi, 2003). In our study, 50% of all the tuberculous pericarditis patients were HIV positive, compared to only 4.2% of patients presenting with non-tuberculous aetiology. The number of newly diagnosed HIV and TB co-infected individuals showed a year-to-year increase. HIV-infected tuberculous pericarditis patients were significantly younger than the HIV negative TB patients, and the highest prevalence of HIV and TB co-infection was found in the socio-economically most deprived group, namely African black females. The steady rise in the number of HIV and TB co-infected patients observed in this study reflects progressively increasing numbers of new cases of HIV infection, but it may also partly be due to maturation of the HIV epidemic resulting in more cases of clinically affected individuals.

The diagnosis of TB as the aetiological cause is important. Without specific treatment, the reported survival was 3.7 months and only 20% were alive at six months (Desai, 1979). On the other hand, the prognosis is excellent with appropriate medical treatment. Echocardiography provides the most efficient way to determine the presence of pericardial effusion. However, in developing countries echocardiography is not always available. We demonstrated how chest radiography and electrocardiography (ECG) assist in the diagnostic process under such circumstances. Our findings indicate good correlation between the degree of radiographic cardiomegaly and the amount of pericardial fluid aspirated at the time of pericardiocentesis. In a substantial number of cases, radiography also contributes to the diagnosis of coexistent pulmonary TB, whereas radiographic evidence of mediastinal lymphadenopathy suggests co-infection with HIV. The presence of micro-voltage on ECG points towards the presence of a large pericardial effusion, whereas absence of micro-voltage almost always excluded it.

A definitive diagnosis of tuberculous pericarditis is made by isolation of the tubercle bacillus from the pericardial fluid and/or pericardial biopsy, yet pericardial TB is often not identified because of the difficulty in isolating the causative organism. In the present study pericardial effusion cultures were positive in 56% of cases, and culture results usually only became available after management decisions had already been taken. Histopathological examination led to a definitive diagnosis of tuberculous pericarditis (caseating granulomata or tubercle bacilli on biopsy sample) in only 11 of the 20 HIV-negative tuberculous pericardial effusions (55%) and two of the five HIV-positive patients (40%). Pericardial biopsy is invasive, adds the risk of anaesthesia, requires technical skills and may result in longer hospitalisation. As a result of these

difficulties and the lack of other diagnostic resources it is important to utilise alternative strategies. The potential for toxic effects and the duration of anti-tuberculous chemotherapy mandate diagnostic specificity. In addition, numerous other infectious and non-infectious causes present with similar clinical features, thus sensitive and accurate diagnostic tools are required.

We evaluated a number of diagnostic strategies and used our results to develop tools to optimise the diagnostic efficiency of available tests. We propose a basic diagnostic rule that is simple and effective and can be applied in areas where TB and HIV co-infection is endemic, and where access to pericardial biopsy, microbiologic studies and other diagnostic tests is difficult. In patients with large pericardial effusions five features were identified that are independently predictive of tuberculous pericarditis, namely fever, night sweats, weight loss, serum globulin >40 g/L and peripheral blood leukocyte count $<10x10^9$/L. Based on these variables, we developed a TB prediction model by means of a statistical approach called "classification and regression tree" (CART) analysis. A weighted diagnostic index was allocated to each of the five variables and by adding these up a total diagnostic index (score) was obtained, ranging between 0-10. The optimum cut-off for the total diagnostic index (by which to classify a patient has having tuberculous pericarditis) was found using receiver operating characteristic (ROC) curves (Beck and Schultz, 1986). The diagnostic rule's best diagnostic efficiency for diagnosing TB corresponded in a training and in a test set to a total diagnostic index of 6. Results for the prediction model applied to the training data demonstrated 86% sensitivity and 87% specificity for the diagnosis of TB, whereas application to the test set resulted in 82% sensitivity and 76% specificity. Our suggested diagnostic rule was therefore: if the patient has a total diagnostic index

score of 6 or more, he or she has tuberculous pericarditis, and if the patient has a score of less than 6, he or she has non-tuberculous pericarditis. Our study demonstrates how basic clinical and laboratory features can help in the diagnosis of tuberculous pericarditis. In a setting with a similar HIV and TB prevalence to that of the Western Cape Province a diagnostic score of 6 or more will suffice to initiate empiric anti-tuberculous therapy, whereas a score of less than 6 warrants further investigations. It needs to be emphasised that the usefulness of the diagnostic rule will depend on the prevalence of TB and HIV infection in a particular setting and we suggest that it should not be used in areas of substantially different TB and HIV prevalence to those of Southern Africa until prospectively assessed for diagnostic accuracy in such a setting.

For areas with more sophisticated diagnostic infrastructure, we propose determining pericardial fluid adenosine deaminase (ADA) activity as the diagnostic test of choice. Using a pericardial fluid ADA activity >40U/L as a cut-off level for the diagnosis of pericardial TB resulted in 87% sensitivity and 89% specificity for the diagnosis of tuberculous pericarditis, and it was equally efficient in HIV positive as in HIV negative tuberculous pericarditis patients. In addition to the diagnostic usefulness, it provides the diagnosis rapidly and cost-effectively. It should, however, be noted that a number of other diseases are also associated with elevated pericardial ADA levels, including effusions caused by malignancy or by non-tuberculous bacterial infection. In the majority of cases, these aetiological causes could be differentiated by pericardial fluid cytology, differential white cell count, Gram stain and bacterial culture. We developed a diagnostic classification model or diagnostic algorithm that assisted in differentiating between tuberculous and non-tuberculous effusions and is

based on the predictive attributes of pericardial ADA levels, pericardial lymphocyte / neutrophil ratios, peripheral leukocyte counts and HIV status. Applying this prediction model to our entire data set of patients (n=233) resulted in 96% sensitivity and 97% specificity for the correct diagnosis of tuberculous pericarditis. As for the diagnostic rule, we suggest that this model should also not be used in areas of substantially different TB and HIV prevalence to those of Southern Africa until prospectively assessed.

Our study suggests that determining pericardial gamma-interferon levels provides the optimal diagnostic results; a cut-off level of 50 pg/mL resulted in 92% sensitivity and 100% specificity for the diagnosis of tuberculous pericarditis. However, before initiating anti-tuberculous therapy, pericardial fluid and sputum specimens should be sent for culture to exclude other bacterial, fungal and parasitic pericardial infections. Evidence of clinical and radiological improvement should be assessed at each visit and culture results checked regularly. Failure to improve must prompt a search for an alternative diagnosis.

The most urgent step in the therapeutic management of individuals with pericardial effusions causing haemodynamic compromise is effective drainage to relieve or prevent cardiac tamponade. We recommend echocardiographically guided percutaneous pericardiocentesis with an indwelling catheter and intermittent daily aspiration. Skin sepsis and intrapericardial sepsis are rare, even in patients with severe immunodeficiency, provided that diligent care is taken of the patients with indwelling pericardial catheters. We recommend that an indwelling catheter should not be kept *in situ* for more than five days. Indications for removal of the catheter include any one of

the following (i) aspirate < 100 mL per day; (ii) catheter blockage not amenable to flushing with heparinised sterile saline and/or iii) any of signs of intrapericardial or skin sepsis.

Anti-tuberculous chemotherapy is effective in the treatment of pericardial TB. In our experience, a rifampicin-containing short course of six months resulted in excellent cure rates in patients with tuberculous pericarditis and we did not observe any relapses. It is possible that the efficient drainage procedure and early initiation were responsible for the almost complete absence of pericardial constriction observed during this study. Intrapericardial and systemic corticosteroids were well tolerated, but did not improve the outcome in our patients. At present, corticosteroids should be reserved for critically ill patients with recurrent large effusions who do not respond to pericardial drainage and anti-tuberculous drugs alone (Lorell, 1997).

The one-year all-cause mortality was higher in HIV positive patients than in HIV negative patients. The majority of deaths were caused by non-cardiac opportunistic infections and septicaemia with CD4+ lymphocyte counts <200 cells/μL predicting a poor prognosis. At the time of the study highly active antiretroviral therapy (HAART) was not available at Tygerberg Hospital and further research is warranted to investigate if the provision of HAART results in improved prognosis in HIV positive patients with tuberculous pericarditis.

Tuberculous pericardial effusion results from the protective immune response to *M. tuberculosis*, which is characterised by elevated levels of pericardial fluid gamma-interferon (IFN-γ) and tumour necrosis factor-alpha (TNF-α). This response is

primarily orchestrated by CD4+ lymphocytes that produce IFN-γ and results in a cascade of monocyte recruitment and macrophage differentiation that results in the development and maintenance of granulomata, which are the hallmark of delayed hypersensitivity. Activated macrophages produce TNF-α which, in synergy with IFN-γ, results in further attraction and activation of monocytes and macrophages, producing and releasing a number of factors that participate in the immune response. Th2 lymphocyte activity is suppressed by the high levels of IFN-γ and this is reflected by the low levels of interleukin-4 (IL-4) in the pericardial fluid of patients presenting with tuberculous pericarditis. Our study supports an immunoregulatory, tissue protective role for IL-10.

In our study we observed significantly lower CD4+ T lymphocyte counts accompanied by increased CD8+ T lymphocyte counts in the peripheral blood and pericardial fluid of patients who were co-infected with HIV and TB compared to HIV negative TB patients. In addition, HIV infection also affected the numbers of B lymphocyte, NK cells, neutrophils, and monocytes. The observed widespread cytopenia probably results from HIV infection of haematopoietic stem cells and the release of endogenous cytokines within the haematopoietic micro-environment that affect the proliferation and differentiation of haematopoietic cells in the bone marrow and thymus. We found that the tuberculous effusions in HIV infected individuals were characterised by a Th1 dominant cytokine profile in spite of the significantly decreased numbers of pericardial fluid CD4+ lymphocytes. In these patients IFN-γ, may be produced by both CD8+ lymphocytes and γδ T lymphocytes, which are closely linked to anti-mycobacterial immunity.

The presence of adenosine deaminase (ADA) in pericardial fluids reflects the activity of this cellular immune response. We demonstrated significantly elevated ADA activity in tuberculous pericardial fluid compared to non-tuberculous disease. The elevated ADA levels reflect the prominence of lymphocytes and macrophages in the immune response to tuberculous infection. Granulomatous pericarditis, which is characterised by the presence of differentiated macrophages and prominent lymphocyte infiltration tended to be associated with higher ADA activity than serofibrinous pericarditis. In addition, we were also able to demonstrate a correlation between pericardial ADA levels and pericardial TNF-α, which is mainly produced by monocyte-macrophages. We identified two factors that predicted low ADA activity in tuberculous exudates, namely severe depletion of CD4+ lymphocytes (<100 cells/μL) and the use anti-tuberculous therapy.

In conclusion, tuberculous exudates result from a Th1 mediated immune response characterised by elevated levels of IFN-γ and TNF-α. The anti-tuberculous immune response is furthermore accompanied by high serum globulin levels, relatively low peripheral leukocyte counts and pericardial lymphocytosis. We have demonstrated how these immunological features can be utilised to diagnose pericardial TB effeciently and thereby improve the patient care.

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