

A description of the pharmacokinetics of first line anti-tuberculosis drugs and adjunctive corticosteroid therapy in the pericardial compartment and their effect on cytokine profiles in the pericardial compartment of patients with tuberculous pericarditis

Dr Justin Tapiwa Shenje

MBChB (University of Zimbabwe), MSc Clinical Epidemiology
(University of Pretoria)



Thesis presented for the Degree of DOCTOR OF PHILOSOPHY
in the Department of Medicine, Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

October 2024

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Table of Contents

A description of the pharmacokinetics of first line anti-tuberculosis drugs and adjunctive corticosteroid therapy in the pericardial compartment and their effect on cytokine profiles in the pericardial compartment of patients with tuberculous pericarditis i

Index of figures	vi
Index of tables	vi
Acknowledgements	vii
Abbreviations	x
Abstract	xii
1. CHAPTER 1: Introduction	1
1.1. Definition and pathogenesis of tuberculous pericarditis	1
1.2. Anatomy and function of the pericardium	1
1.3. Pathophysiology of TBP	4
1.4. The epidemiology of TBP	6
1.5. Clinical presentation	8
1.6. The utility of ECHO in TBP	13
1.7. Approach to diagnosis of TBP	15
1.8. Management of TBP	17
1.9. Anti-tuberculosis chemotherapy	18
1.10. Pharmacokinetics of anti-TB drugs	23
1.11. Evacuation of the pericardium	23
1.12. Other aspects to consider when managing patients with TBP	26
1.13. Adjunctive corticosteroid therapy in tuberculosis	27
1.14. Cytokines and adjunctive corticosteroid therapy	28
1.15. The effect of mycobacterial infection on endogenous corticosteroids	29
1.16. Outcome and prognosis of TBP	30
1.17. Introduction to pharmacokinetics as it applies to anti-TB chemotherapy	31
1.18. Non-compartmental and compartmental analyses	34
1.19. Hypothesis and motivation for study	36
1.20. References	38
2. Chapter 2: Rationale, aims and objectives	46
2.1. Rationale	46
2.2. Aims	48
2.3. Objectives	48
2.4. References	51
3. CHAPTER 3: Methods	53
3.1. Setting	53
3.2. Eligibility criteria	54
3.3. Pericardiocentesis	54
3.4. Data collection	55
3.5. Sample collection	55
3.6. Pre-analytical storage	56
3.7. Methods for measurement of drugs, cytokines and hormones	56
3.8. Ethical considerations	57
3.9. References	58
4. CHAPTER 4: Poor Penetration of Antibiotics Into Pericardium in Pericardial Tuberculosis	59
4.1. Introduction	59
4.2. Methods	60
4.2.1. Ethics	60

4.2.2.	Setting	61
4.2.3.	Case Definition	61
4.2.4.	Pilot Study	61
4.2.5.	Intensive Sampling	62
4.2.6.	Drug Assays	62
4.2.6.1.	Isoniazid concentrations	63
4.2.6.2.	Rifampicin, ethambutol pyrazinamide concentrations	63
4.2.7.	Pharmacokinetic Analysis	64
4.2.8.	Comparison of concentrations Achieved with Minimum Inhibitory Concentrations (MICs)	64
4.2.9.	Statistical Analysis	65
4.3.	Results	65
4.4.	Discussion	77
4.5.	References	82
5.	CHAPTER 5: Detectable prednisolone is delayed in pericardial fluid, compared with plasma of patients with tuberculous pericarditis: A pilot study	86
5.1.	Introduction	86
5.2.	Patients and methods	87
5.2.1.	Intensive PK sampling procedure	88
5.2.2.	Drug concentration assay	89
5.2.3.	Compartmental pharmacokinetics	89
5.2.4.	Statistical analysis	90
5.3.	Results	91
5.3.1.	Comparison of prednisolone concentrations based on naïve pooling	92
5.3.2.	Compartmental pharmacokinetic modelling based comparisons	94
5.4.	Discussion	97
5.5.	Limitations	99
5.6.	Conclusion	99
5.7.	References	101
6.	CHAPTER 6: Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study	104
6.1.	Introduction	104
6.2.	Materials and methods	105
6.2.1.	Patient populations	105
6.2.2.	Luminex multiplex assay for cytokines and chemokines	106
6.2.3.	Statistical analysis	106
6.3.	Results	107
6.4.	Discussion	115
6.5.	References	118
7.	CHAPTER 7: A description of the elevation of pericardial cortisol: cortisone ratio in patients with tuberculous pericarditis	120
7.1.	Introduction	120
7.2.	Materials and methods	123
7.2.1.	Sampling	125
7.2.2.	Endogenous glucocorticoid and pharmacological glucocorticoid analysis	126
7.2.3.	Measurement of cytokine concentrations	127
7.2.4.	Statistical analysis	128
7.3.	Results	128
7.3.1.	Cortisol measurements	129
7.3.2.	Cytokine measurements	130

7.3.2.1.	Comparison of cytokines in plasma in patients with TBP versus reference range derived from plasma of healthy controls	130
7.3.2.2.	Comparison of pericardial cytokines from patients with TBP with plasma cytokine of healthy controls.....	132
7.3.2.3.	Comparison of pericardial ,saliva, and plasma cytokines in patients with TBP	132
7.3.2.4.	Prednisolone and cortisol/cortisone ratio.....	133
7.4.	Discussion.....	134
7.5.	Conclusion	137
7.6.	Reference.....	138
8.	CHAPTER 8: Final discussion and recommendations.....	141
8.1.	Summary of the major findings.....	141
8.1.1.	The pharmacokinetics of anti-TB drugs in pericardial compartment	142
8.1.2.	The pharmacokinetics of prednisolone in the pericardial compartment	143
8.1.3.	The cytokine response of pericardial compartment.....	144
8.1.4.	The pericardial cortisol/cortisone ratio	146
8.2.	Strengths of the study	147
8.3.	Weaknesses of the study	147
8.4.	Recommendations for future areas of research.....	148
8.4.1.	Suggestions for further evaluation of the pharmacokinetics of anti-TB drugs in the pericardial compartment.....	148
8.4.2.	Suggestions for further research into the effect of effect of prednisolone on function of stimulated lymphocytes.....	149
8.4.3.	Suggestions for further research into the pharmacokinetic pharmacodynamic relationship between anti-TB drugs and clinical outcome	150
8.4.4.	Suggestions for further research into the pharmacokinetic pharmacodynamic relationship between prednisolone and cytokine concentration.....	150
8.4.5.	Suggestions research into alternative immune modulatory therapy for TBP	151
8.5.	Final conclusions.....	152
8.6.	References.....	153

Index of figures

Figure 1: The embryological development of the pericardial compartment.....	3
Figure 2: Anatomy of the pericardium.....	4
Figure 3: The relationship between pericardial effusion volume and intra-pericardial pressure	9
Figure 4: An ECHO of a pericardial effusion with variation of blood flow across the mitral value.....	11
Figure 5: Constrictive pericarditis on ECHO	12
Figure 6: Echocardiographic features suggestive of tuberculous pericarditis.....	14
Figure 7: Important pharmacokinetic parameters	32
Figure 8: The relationships between various compartments in the body	35
Figure 9: Rifampicin concentrations in plasma and pericardium	67
Figure 10: Rifampicin concentrations in relation to the MIC.....	70
Figure 11: Ethambutol concentrations in relation to the MIC.....	72
Figure 12: Isoniazid concentrations in relation to the MIC	74
Figure 13: Pyrazinamide concentrations.....	76
Figure 14: The pH of pericardial fluid and corresponding pyrazinamide MIC	77
Figure 15: Prednisolone concentration time graphs.....	93
Figure 16: The prednisolone pharmacokinetic model for plasma and pericardial fluid	97
Figure 17: The effect of prednisolone on cytokine concentrations	114
Figure 18: The number of participants screened and enrolled in each arm.....	124
Figure 19: Cortisol/ cortisone ratio in saliva, plasma, and pericardial fluid.....	130
Figure 20: The effect of prednisolone on the cortisol/ cortisone ratio	134
Figure 21: Dose response curve with optimal dose at E90	151

Index of tables

Table 1: A suggested algorithm for diagnosis of TBP in a South African setting	16
Table 2: South African Department of Health National Tuberculosis Management Guidelines 2014. ³¹	19
Table 3: South African Department of Health National TB program anti-TB fixed dose combination. ³¹	20
Table 4: Clinical demographic characteristics in pilot study patients.....	66
Table 5: Clinical and demographic characteristics in patients who underwent intensive pharmacokinetic sampling	68
Table 6: Baseline characteristics of participants who underwent intensive pharmacokinetic sampling.	90
Table 7: Compartmental model choices using information criteria.....	94
Table 8: Estimation of plasma and pericardial fluid prednisolone one compartment model parameters	95
Table 9: Patient Characteristics	108
Table 10: Median (IQR) of analytes in plasma	109
Table 11: Median (IQR) of analytes in pericardial fluid	110
Table 12: Median of analytes in saliva.....	111
Table 13: Median (IQR) analytes of combined (both prednisolone and placebo arms) in plasma, pericardial fluid and saliva	112
Table 14: Baseline characteristics of patients with TBP, allocated to either prednisolone or placebo, in addition to standard anti-TB therapy.....	129
Table 15: Plasma, Pericardial and Saliva Cytokine concentrations and their respective plasma reference ranges	131

Declaration of permission to include publications

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications:

1. Shenje J, Adimora-Nweke FI, Ross IL, et al. Poor penetration of antibiotics into pericardium in pericardial tuberculosis. *EBioMedicine* 2015; **2**(11): 1640-9. (Number of citations 27)
2. Shenje J, Gumbo T, Wiesner L, Ntsekhe M, Mayosi B, Ross I. Detectable prednisolone is delayed in pericardial fluid, compared with plasma of patients with tuberculous pericarditis: A pilot study. *IJC Heart & Vasculture* 2019; **22**: 105-10. (Number of citations 0)
3. Shenje J, Lai RP, Ross IL, et al. Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study. *IJC Heart & Vasculture* 2017; **18**: 104-8 (Number of citations 10)
4. Shenje JT, Raubenheimer P, Wiesner L, Ross I. A description of the elevation of pericardial cortisol: cortisone ratio in patients with tuberculous pericarditis. *Frontiers in Endocrinology*; **14**: 1399. (Number of citations 0)

Signature: _____  _____

Date: __30 October 2024__

Student Name: __Justin Tapiwa Shenje__

Student Number: __SHNJUS002__

Declaration

I, ...Justin Tapiwa Shenje, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of this has been, is being or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Date:

Acknowledgements

This body of work was made possible by support from many individuals who supported me in my quest for my PhD. I would like to acknowledge and thank all who contributed to this work and would like to give special mention to the following.

First and foremost, my main supervisor Prof Ian Ross for his patience, understanding and the rigor with which he helped with planning and reviewing my work. There were many obstacles along the way and your guidance and support helped overcome these challenges.

I would also like to thank the late Prof Bongani Mayosi for the opportunity to work on the Investigation of the Management of Pericarditis (IMPI) project. Prof Mayosi was also instrumental in helping secure funding and bringing together several key collaborative partners.

Some of the collaborative partners include Dr Tawanda Gumbo who played a key role in the population pharmacokinetic analysis for the anti-TB drugs and prednisolone, he also helped with planning of the whole project. Professor Lubber Wiesner was instrumental in development and validation of the High-performance liquid chromatography tandem mass spectrometry assay which was used to measure the following drugs and their metabolites rifampicin, ethambutol, isoniazid, pyrazinamide and prednisolone. While the measurement and analysis of cytokines and chemokines was done with the assistance of Professor Katlin Wilkinson.

This research project was a sub-study of the IMPI study, and I would like to thank the Cape Town IMPI site who played a major role in recruitment and follow-up of participants. The Cape Town IMPI site team included Prof Bongani Mayosi, Prof Mpiko Ntskhe, Dr Shaheen Pandie, Dr Mukesh Chibha, Sr Veronica Frances, Sr Unita

September, Marne Van De Merwer, Felicia Gili, Roxi Vergotine, Lwazi Mhlanti and Janine Saaiman. I would also like to thank the Groote Schuur Hospital staff particularly the Cardiac Catheterisation Laboratory, Coronary Care Unit and the registrars who referred participants to the study.

The research project was funded by South African Medical Research Council for self-initiated research from November 2012 to April 2015 and the Lily and Ernst Hausmann Research Trust to Bongani Mayosi, and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R01AI079497) awarded to Tawanda Gumbo.

Last but not least I would like to thank my friends and family for the unwavering support during this journey.

Abbreviations

11βHSD	11- β -hydroxysteroid dehydrogenase
Acp	Acyl carrier protein
ADA	Adenosine deaminase
AIC	Akaike information criteria
AUC	Area under the curve
BIC	Bayesian information criteria
BOV	Between observation variability
BSV	Between subject variability
CA	Compartmental analysis
CD4	Cluster of differential 4
CI	Confidence interval
C_{max}	Maximum concentration
CSF	Cerebrospinal fluid
CT	Computer tomography
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
E	Ethambutol
ECG	Electrocardiogram
ECHO	Echocardiogram
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme link immune sorbent assay
FDA	Food and Drug Administration
FDC	Fixed dose combination
H	Isoniazid
HAART	Highly active anti-retroviral therapy
HIV	Human immunodeficiency virus
HPA	Hypothalamus-pituitary-adrenal
HPLC-MS	High performance liquid chromatography tandem mass spectrometry
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12p40	Interleukin-12p40
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IMPI	Investigation of the Management of Pericarditis
INF-γ	Interferon gamma
IP-10	Induced protein-10
IQR	Interquartile range
IRIS	Immune reconstitution inflammatory syndrome
JVP	Jugular venous pressure
K3EDTA	Tri-potassium ethylenediamine tetra-acetic acid
K_a	Absorption rate constant
K_e	Elimination rate constant

LAM	Lipoarabinomannan
LTBI	Latent tuberculosis infection
MHC II	Major histocompatibility complex II
MIC	Minimum inhibitory concentration
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
Mtb	Mycobacterium tuberculosis
NADH	Nicotinamide adenine dinucleotide + hydrogen
NCA	Non-compartmental analysis
P	Plasma
PCR	Polymerase chain reaction
PF	Pericardial fluid
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/pharmacodynamic
POA	Pyrazinoic acid
QC	Quality control
R	Rifampicin
RH	Rifampicin/isoniazid
RHZE	Rifampicin/isoniazid/pyrazinamide/Ethambutol
RNA	Ribonucleic acid
S	Saliva
SD	Standard deviation
SLE	Systemic lupus erythematosus
TB	Tuberculosis
TBP	Tuberculous Pericarditis
TGF-β	Transforming growth factor beta
TH1	T helper 1 cells
T_{max}	Time to maximum concentration
TNF-α	Tumour necrosis factor alpha
USS	Ultra sound scan
WHO	World Health Organization
Z	Pyrazinamide

Abstract

Introduction

Tuberculous pericarditis is caused by *Mycobacterium tuberculosis* infection of the pericardium, which is associated with a higher mortality, compared with pulmonary tuberculosis. Constrictive pericarditis, a significant complication of tuberculous pericarditis, contributes to this elevated mortality. Adjunctive corticosteroid therapy with prednisolone, has been shown to reduce the incidence of constrictive pericarditis by more than 50%. However, this is associated with significant side effects, especially in patients with HIV co-infection. Constrictive pericarditis is only partially responsible for this increased mortality, whereas other factors including delayed diagnosis of tuberculosis, cardiac complications for example, pericardial tamponade and poor pericardial exposure of anti-TB drugs also likely contribute.

The pharmacokinetics of anti-tuberculous (TB) drugs within the pericardial space remain poorly understood and until now have relied on studies of pulmonary tuberculosis. While adjunctive corticosteroids have been shown to be beneficial, there are no available data on the pharmacokinetics of prednisolone in the pericardium, and uncertainty remains as to whether an empiric dose of 120 mg prednisolone is the optimal dose for reducing the incidence of constrictive pericarditis or whether it is detectable at the site of infection, to exert its pharmacodynamic effect. Combining anti-TB treatment and high doses of prednisolone are likely to have a profound effect on the immune response, therapeutic effect and cortisol milieu in the pericardium. I was therefore interested in investigating the impact of this therapeutic combination, through direct sampling of the pericardial fluid in patients with tuberculous pericarditis.

Methods

A total of 16 patients with newly diagnosed tuberculous pericarditis and a pericardial effusion requiring pericardial aspiration, were enrolled into the study. The patients were randomly assigned to 120 mg of prednisolone or matching placebo and were simultaneously started on first line World Health Organization (WHO) anti-tuberculosis treatment, namely, rifampicin, isoniazid, ethambutol and pyrazinamide. Intensive pharmacokinetic samples were collected at the following timepoints, just before administration of the anti-tuberculosis treatment and prednisolone or placebo and at 0.5 hour, 1 hour, 2 hour, 3 hour, 5 hour, 8 hour and 24 hour intervals thereafter for the first 24 hours. Intensive pharmacokinetic samples were collected from plasma, pericardial fluid and saliva. The pharmacokinetics of rifampicin, isoniazid, ethambutol and pyrazinamide in the pericardium were described so as to assess the adequacy of conventional doses of the WHO first line anti-TB treatment regimen. The study also described the pharmacokinetics of 120 mg of prednisolone and evaluated its effect on endogenous corticosteroids and cytokines. Furthermore, the study compared the immunological response to tuberculous pericarditis in plasma and pericardium.

Results

While pericardial drug exposures to isoniazid and pyrazinamide were comparable to those of plasma, there was a reduction in rifampicin and ethambutol exposure in the pericardium, which were 20%; ($p<0.0010$) and 55%; ($p<0.0010$) of their respective plasma drug exposures. The median pericardial rifampicin concentration was 0.125 mg/L, which was lower than the reference minimum inhibitory concentration (MIC) for *Mycobacterium tuberculosis* isolates, which was 0.208 mg/L ($p=0.0010$). The pharmacokinetic profile of prednisolone in the pericardium was similar to that of the

plasma, but there was a 1.60 hours delay ($p=0.0320$) in time to maximum concentration (T_{max}) in the pericardial space. Adjunctive corticosteroid therapy administered as a single dose of 120 mg of prednisolone, was associated with 83% reduction in median plasma interleukin-6 ($p=0.0360$), a 50% reduction in median plasma interleukin-8 ($p=0.0300$) and a 50% reduction in median pericardial interleukin-8 ($p=0.0400$). Prior to administration of glucocorticoids, the pericardial cortisol/cortisone ratio was twice that of plasma ($p=0.0050$). A similar elevation of the cortisol/cortisone ratio at the site of infection has previously been described in the bronchial lavage fluid of patients with pulmonary tuberculosis. Among those participants who did not receive glucocorticoids, pericardial cytokine interferon-gamma, tumour necrosis-alpha, interleukin-6, interleukin-8, interleukin-10, and interferon gamma-induced protein-10 concentration increases were multiples fold higher than their corresponding plasma concentrations, ($p=0.0011$), ($p=0.0043$), ($p=0.0009$), ($p=0.0001$), ($p=0.0054$) and ($p=0.0031$), respectively.

Conclusion

By directly sampling the pericardial fluid among patients with tuberculous pericarditis, I was able to gain novel insights, which have never previously been described. The reduced pericardial rifampicin and ethambutol exposures, below the required minimum inhibitory concentrations in the pericardial space, are of great concern, particularly, considering the relative importance of rifampicin in the treatment of drug sensitive tuberculosis. This warrants a revision of the dosing strategy, or a review of therapeutic regimen used in tuberculous pericarditis, to enhance efficacy. Prednisolone administered at a dose of 120 mg daily orally is detectable with high exposure in the pericardium and may explain the beneficial effect observed in tuberculous pericarditis

among HIV negative individuals, through its alteration in the cytokine milieu, and lower propensity to developing constrictive pericarditis. Additionally, the raised cortisol/cortisone ratio at the site of infection, the pericardium, compared with other matrices especially plasma may indicate an immunomodulatory role either to dampen an excessive or evoke an immunological response.

1. CHAPTER 1: Introduction

1.1. Definition and pathogenesis of tuberculous pericarditis

Tuberculosis pericarditis (TBP) is an infection of the membranous coverings of the heart by *Mycobacterium tuberculosis* (*Mtb*).¹ These *Mycobacteriae* are thought to enter the pericardial space either via retrograde lymphatic spread from the lungs or haematological dissemination.¹ *Mtb* is a slow growing resilient organism.² On average it takes about 15 to 20 hours for a single cell division.² The organism has a characteristic immunogenic, impervious and protective lipid rich cell wall, which contains mycolic acid.² This lipid rich cell wall allows the *Mycobacterium* to survive under adverse conditions.³ The *Mycobacteria* may survive phagocytosis and may even go on to replicate inside a macrophage.⁴ The immunogenic lipid rich cell wall initiates a cascade of inflammatory reactions, causing a constellation of debilitating symptoms.^{1,5}

1.2. Anatomy and function of the pericardium

The heart lies in a protected semi-mobile covering called the pericardial sac, which has an inner and outer layer.⁶ The outer fibrous parietal and inner serous visceral layer are separated by the pericardial space.⁶ The parietal pericardium consists of a layer of mesothelial cells with underlying dense layers of collagen fibres interspaced with scanty layers of elastic fibres, while the visceral pericardium has a layer of mesothelial cells, with an underlying thin layer of sparse collagen and elastic fibres.⁶

The pericardium is embryologically derived from mesoderm.⁶ Around the 17th day after fertilization, the mesoderm differentiates into a proximal thick plate of mesoderm, called the paraxial mesoderm and the thin lateral mesoderm called the lateral plate, as shown below in figure 1A.⁶ The lateral plate undergoes uneven growth, also known as dehiscence, creating small fluid filled sacks in the process, ultimately dividing the lateral plate into two layers, namely the parietal and visceral mesoderm layer as illustrated in figure 1B.⁶ Between these two layers lies the intra-embryonic coelom. The intra-embryonic coelom is then transformed into the pericardial, peritoneal and pleural cavity by cephalo-cordal and lateral flexion of the tri-laminar embryo, by the end of the fifth week.⁶ The pericardium then envelopes the primitive heart tube as it develops.⁶ Figure 1 shows the cephalo-cordal and lateral flexion with 1A and 1B illustrating changes in the sagittal plane, while 1C and 1D illustrate changes in the transverse planes.

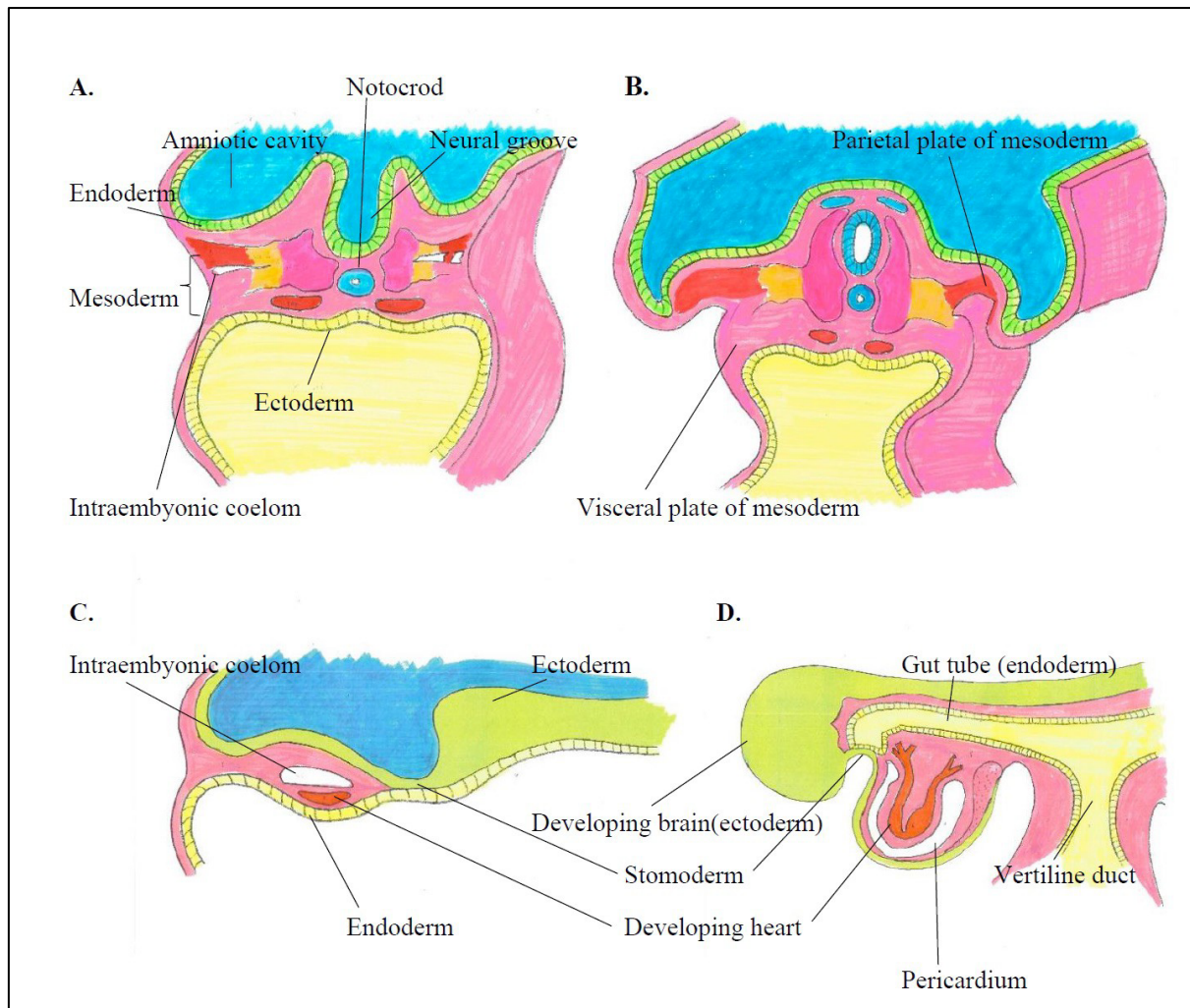


Figure 1: Illustrates the embryological development of the pericardial compartment. Adapted from "Notes on the anatomy, embryology, and histology of the pericardium" by Elias et al. The tri-laminar embryo undergoes cephalo-cordal and lateral flexion to form the pericardial cavity.⁶

Pericardial fluid acts as a lubricant, allowing the parietal and visceral surfaces to glide over each other, providing a protective cushion forming a closed compartment, as depicted in Figure 2.¹ The highlighted area in Figure 2 shows the parietal and visceral layers and the intervening pericardial space.⁷ The pericardium also anchors the heart in place and limits the spread of infection.⁸ Fluid secreted by the visceral pericardium is absorbed via the regional lymphatic nodes, namely the anterior-mediastinal,

posterior-mediastinal and tracheal nodes.⁸ Under physiological conditions, there are between 15 ml and 50 ml of fluid in the pericardial space.⁸

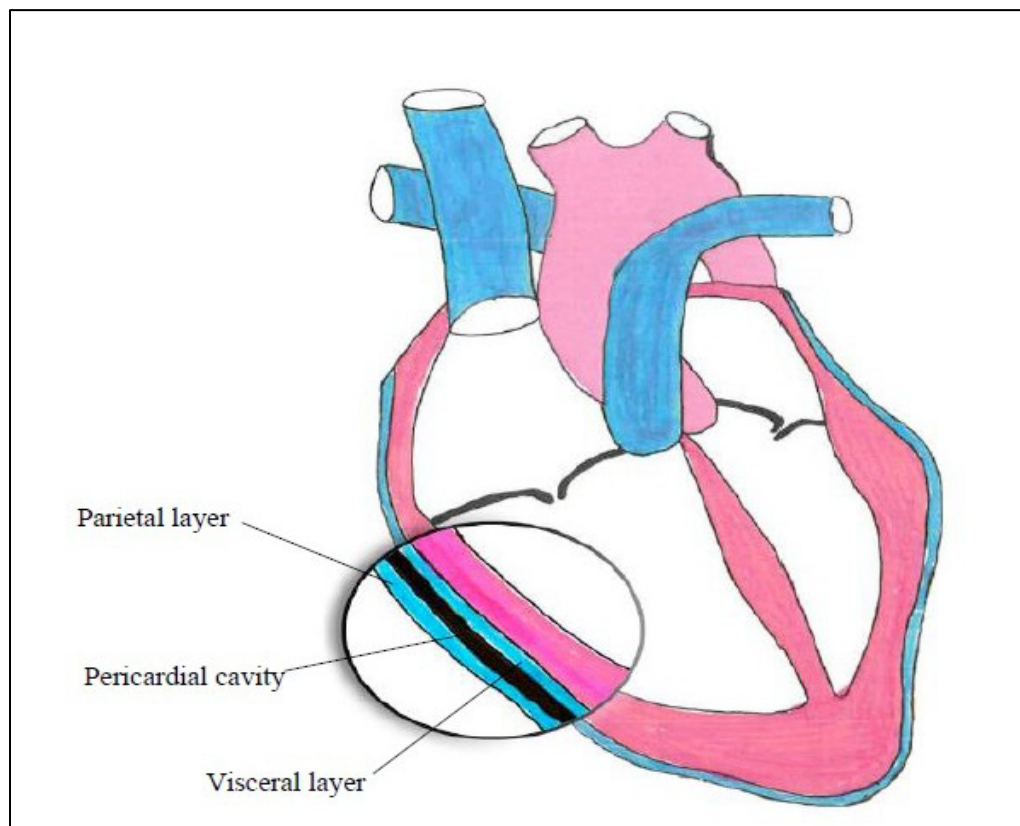


Figure 2: Anatomy of the pericardium highlighting the two pericardial layers with the pericardial cavity between them. Adapted from “Comprehensive Anatomy of the Pericardial Space and the Cardiac Hilum: Anatomical Dissections With Intact Pericardium” by Mori *et al.*⁷

1.3. Pathophysiology of TBP

There are three main manifestations of pericardial disease in TBP, these are pericardial effusion, effusive-constrictive pericarditis and constrictive pericarditis.⁹ A pericardial effusion is an abnormal accumulation of fluid in the pericardial space.¹⁰ Constrictive pericarditis is caused by long term inflammation of the pericardium, which leads to thickening and scarring of the pericardium.¹¹ By contrast, an effusive-constrictive pericarditis is a syndrome, characterised by both pericardial effusion and constrictive pericarditis.¹² An effusion is the most common presentation, occurring in

79.5% of patients with TBP. In a pericardial effusion associated with TBP, there is accumulation of an inflammatory exudate, as a result of an immunological response mobilised against *Mycobacteria* in the pericardial space.¹

The immune response is coordinated by T-helper 1 (TH1) cells, which release cytokines, in particular interferon gamma (IFN- γ) and interleukin 2 (IL-2), mobilising macrophages to form granulomas, with the purpose of eradicating *mycobacteria*.¹³ Cytokines are the backbone of the signalling network of the immune system.¹⁴ Once a macrophage comes in to contact with a *Mycobacterium bacillus*, there is macrophage activation via the compliment receptors 1, 3 and 4, and mannose receptor, triggering phagocytosis to kill the invading bacillus. The macrophage then presents the *Mycobacterium* antigen to CD4+ cells via the major histocompatibility complex (MHC) II, which activates the cell-mediated immune system.¹⁵ These cytokines stimulate macrophages, cause clonal expansion of T lymphocytes and recruitment of other cells of the immune system.¹⁶ This leads to granuloma formation which is an attempt to wall off the *Mycobacterium* creating a hostile environment, *inter alia* a low pH, reduced oxygen concentration, and an environment devoid of nutrients.³ The inflammatory process leaves in its wake necrotic cells and an inflammatory exudate.³ It is ironically the host's immunological response to *Mycobacterium tuberculosis*, which is responsible for the majority of the tissue damage caused by active tuberculosis (TB) disease.

Burgess *et al*, showed that raised plasma concentrations of the following cytokines; interferon gamma (IFN- γ), tumour necrosis alpha (TNF- α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6) and interleukin-10 (IL-10) and a decrease in interleukin-4 (IL-4) to undetectable levels in the pericardial fluid of patients with TBP, compared to patients with pericardial effusions, other than due to TB.¹⁷ These

cytokine changes were consistent with a shift towards a TH1 response. A similar trend was noted in plasma and bronchial lavage fluid of patients with pulmonary TB, but the magnitude of change in cytokine concentration was significantly higher in bronchial lavage fluid, compared to plasma.¹⁸ A study by Ntsekhe *et al*, demonstrated an association between elevated IL-10 and transforming growth factor beta (TGF- β) concentrations with effusive constrictive pericarditis in patients with TBP.¹⁹ Understanding the behaviour of certain cytokines in pericardial fluid and plasma during TBP, could be the key to uncovering the underlying immunological and pathological processes paving the way for the development of diagnostic and prognostic biomarkers, and targets for novel treatment interventions.

1.4. The epidemiology of TBP

TBP constitutes 1% of all TB cases.²⁰ The incidence of TBP, as with pulmonary TB has been on a decline globally, until the advent of human immunodeficiency virus (HIV).² As with other forms of TB, the main catalysts for the spread of TB are poor ventilation, overcrowding, malnutrition and immune suppression.⁴ The HIV pandemic has changed the epidemiology of TB, with TB becoming more common in young and middle-aged adults.²¹ Even in high TB prevalence areas, the majority of individuals who are exposed to TB, do not develop active TB disease, as the infection is successfully contained by the immune system, leading to a state referred to as latent TB infection (LTBI).¹³ However, LTBI can later progress to active TB disease.²² The progression from LTBI to active TB disease is accelerated by compromise of the immune system.^{13,22}

In Southern Africa, the most common factor responsible for accelerating the progression of LTBI to active TB disease is concurrent HIV infection, with 65% of all TB cases having underlying HIV co-infection.²³ High TB HIV co-infection rate is of great concern, given that TB is more difficult to diagnose in HIV-positive patients than HIV-negative patients. Microbiological TB yield in HIV-positive patients is often low, and therefore smear microscopy often fails in this setting.²⁴ The co-existence of TB and HIV infection harbours a poorer prognosis than TB alone, necessitating prompt initiation of anti-tuberculous therapy.²⁵ Moreover, patients recently commenced on highly active anti-retroviral therapy (HAART), without any prior symptoms and signs of TB may develop symptoms of TB in keeping with the immune reconstitution inflammatory syndrome (IRIS).¹⁷ In the immune-compromised patient, *Mycobacterium* infection progresses unabated and once the immune system is restored by HAART, any underlying disseminated disease may evoke a massively exaggerated immune response causing IRIS.¹⁵

Before the emergence of HIV, TB predominantly affected the very elderly and young children. With the advent of HIV, the epidemiology has changed, as a greater number of young sexually-active people are affected which has public health implications, warranting regular screening for TB in all people living with HIV, irrespective of whether they are on HAART or not.²⁶ Similarly, it is essential to test for HIV in all patients diagnosed with TB, as those who test positive for HIV, also need to be initiated on HAART.^{27,28} The World Health Organization (WHO) recommends initiation of HAART on all HIV-positive individuals regardless of WHO clinical stage or CD4 cell count. In individuals with TB, HAART should be initiated within 2 weeks of commencing TB treatment.²⁸⁻³⁰ Patients with TBP and HIV co-infection are managed in a similar

manner to those with pulmonary TB and HIV co-infection, in as far as anti-tuberculous therapy is concerned .³¹

1.5. Clinical presentation

Patients with TBP present with a spectrum of severity ranging from being asymptomatic to manifesting with severe debilitating disease.³² TBP symptoms can either be non-specific (not confined to any particular organ system and also known as constitutional symptoms) or can be organ specific; in this case indicating pathology of the cardiovascular system. Non-specific symptoms of TBP may include fever, weight loss and night sweats.^{9,32} Organ specific symptoms include chest pain, palpitations, dyspnoea and orthopnoea, while organ specific signs are reduced blood pressure, tachycardia, tachypnoea, elevated jugular venous pressure, hepatomegaly and dependent oedema.³³

The presence of chest pain and cardiac compromise usually prompt the clinician to request a chest x-ray, which may show an enlarged cardiac silhouette, warranting further investigation with higher resolution imaging techniques including an ultrasound scan (USS), echocardiography (ECHO), computer tomography (CT) scan or magnetic resonance imaging (MRI). These investigations are of proven utility for the diagnosis of pericardial effusions.³⁴ The choice of imaging technique is usually determined by the available resources. ECHO is the imaging modality of choice, as it offers high resolution imaging, it is affordable, accessible and most importantly, permits a dynamic visualisation of the functional relationship between the pericardial effusion and the myocardium.³⁵

1.5.1. Pericardial effusion

As the pericardial effusion develops, there is progressive accumulation of pericardial fluid in the pericardial space. The pericardial space is a closed compartment, with some elasticity, indicating that it has the ability to accommodate an effusion, without any perceived effect on cardiac function.³⁶ Figure 3 below illustrates the relationship between intra-pericardial pressure versus volume of pericardial effusion, the increase in pericardial pressure is also dependent on the duration of pericardial fluid accumulation.³⁶ The pericardial reserve volume represents the pericardial effusion volume below the elastic limit of the pericardial compartment.³⁷ As illustrated in Figure 3, once the volume of the pericardial effusion exceeds the pericardial reserve volume, there is a steep rise in intra-pericardial pressure with every unit rise in pericardial volume.³⁷

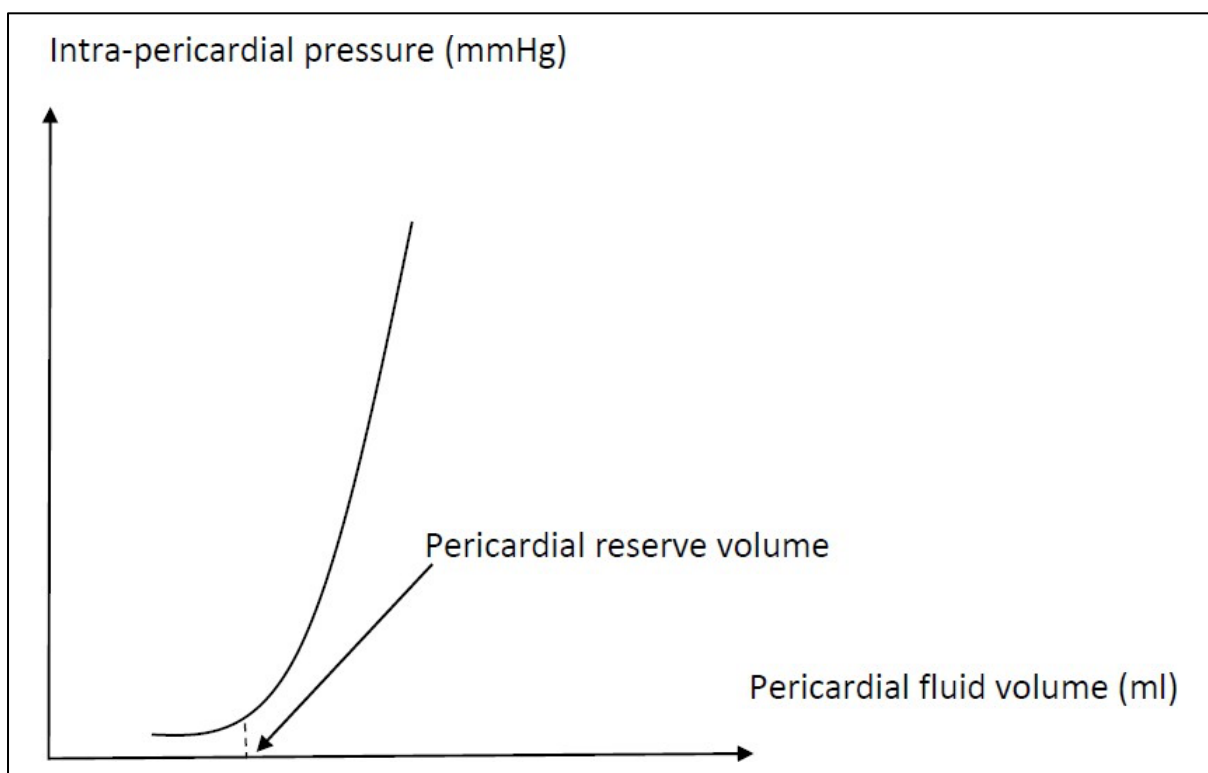


Figure 3: Displays the relationship between pericardial effusion volume and intra-pericardial pressure. Adapted from "Pathophysiology of Cardiac Tamponade" by Spodick.³⁷

As the intra-pericardial pressure rises, it reduces the maximum diastolic volume which in turn reduces the stroke volume.¹⁰ The heart is initially able to compensate for this reduction in stroke volume by an increase in heart rate, but the compensatory increase in heart rate has a limited effect, since a rapid heart rate further reduces the duration of diastole. Once the intra-pericardial pressure exceeds the early diastolic right ventricular pressure, the heart decompensates causing right ventricular collapse, giving way to a syndrome known as cardiac tamponade.¹⁰

Cardiac tamponade can be defined by the presence of Beck's triad, characterised by; low arterial pressure, distended neck veins and distant, muffled heart sounds.¹⁰ A patient with cardiac tamponade presents with; dyspnoea, pulsus paradoxus and elevated systemic venous pressure in the presence of a pericardial effusion.¹⁰ Cardiac tamponade is a life-threatening condition, which warrants urgent intervention in the form of evacuation of the pericardial effusion.^{10,38}

1.5.2. Constrictive pericarditis

TBP causes chronic inflammation, which sometimes results in fibrosis of the pericardium.¹¹ The fibrotic change results in a thicker and more rigid pericardium, giving rise to constrictive pericarditis. In the normal physiological state, the intra-thoracic pressure varies with respiration and this pressure change is transmitted to the chambers of the heart and the pulmonary veins.³⁹ The inspiration phase of the respiratory cycle reduces intra-thoracic pressure, while expiration increases intra-thoracic pressure. These pressure changes when transmitted to the chambers of the heart and the pulmonary veins, have a small, but real effect on cardiac output. In patients with constrictive pericarditis however, the rigid pericardium insulates the chambers of the heart from the intra-thoracic pressure variation, while the pulmonary

veins are spared, thereby reducing the pressure gradient between the pulmonary veins and the left ventricle, consequently causing a reduction in left ventricular filling.⁸ The reduction in left ventricular filling associated with inspiration, is coupled with an increase in right ventricular filling and during expiration, the opposite is observed; this process is known as right left inter-ventricular interdependence.⁴⁰ Right left ventricular interdependence leads to variation of velocity of blood flow across the mitral valve with respiration, as shown in Figure 4.

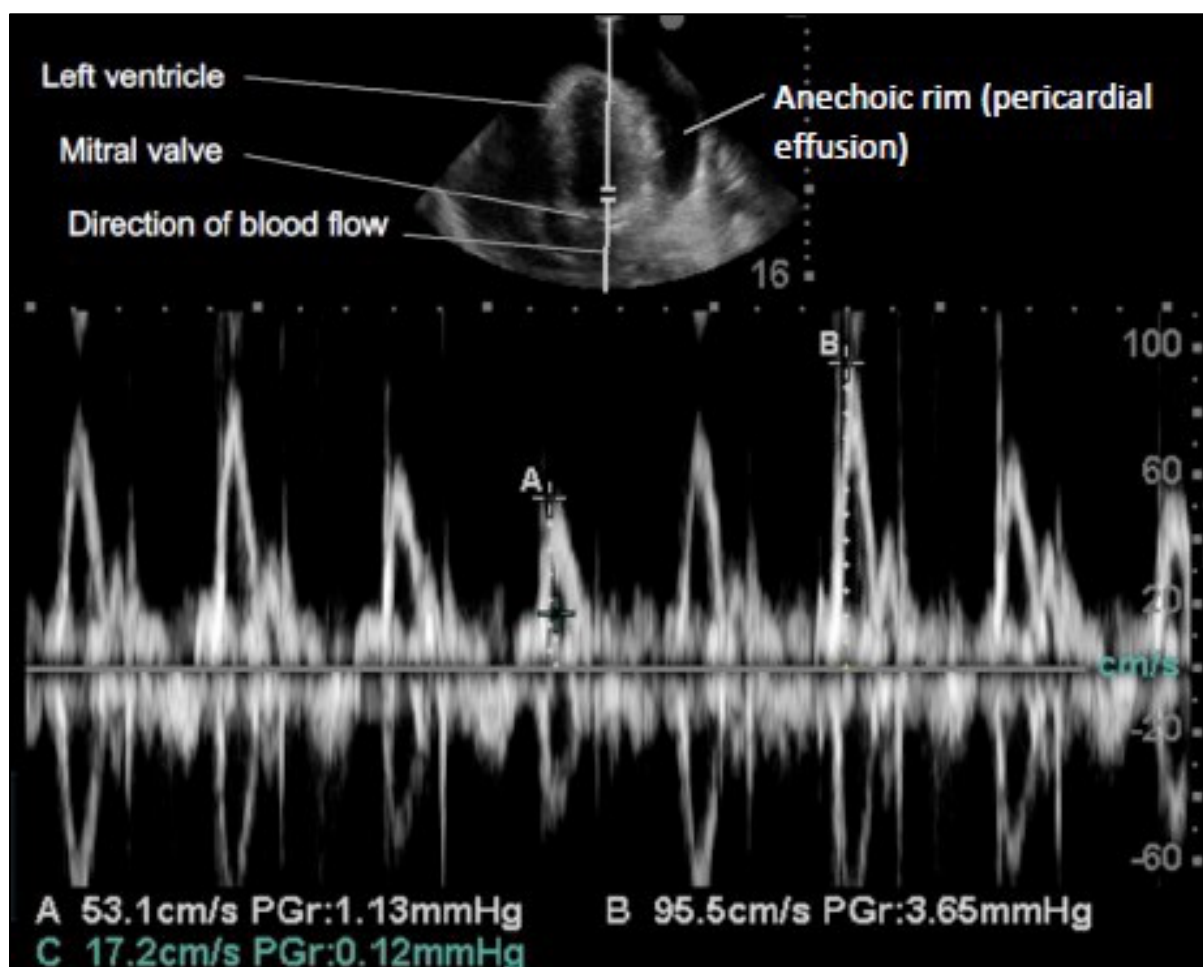


Figure 4: This is an ECHO showing the left ventricle of a patient with a large pericardial effusion, measuring the velocity of blood flow across the mitral valve. Each wave represents velocity of blood flow across the mitral valve during one cardiac cycle. The graph shows variation of velocity of blood flow across the mitral valve, this is evidenced by a greater than 25% variation in peak velocity with respiration, the velocity at time B is 95.5cm/s while at time A is 53.1cm/s.

The rigid pericardium also interferes with the second phase of diastole, by restricting the increase in volume that occurs during diastole and results in a high end-diastolic pressure, leading to a premature and an abrupt end of diastole.^{8,10} Constrictive pericarditis often presents with right ventricular failure, as evidenced by an elevated jugular venous pressure (JVP), peripheral oedema, hepatomegaly and ascites, pericardial knock (an early diastolic sound, caused by an abrupt end to diastolic filling and is diagnostic of constrictive pericarditis), sudden instantaneous split of the second heart sound on inspiration, with a small pericardial effusion in a structurally normal heart, as shown in Figure 5.³⁹



Figure 5: Shows the ECHO of a patient with constrictive pericarditis. ECHO shows pericardial thickening and an exaggerated septal shift towards the right ventricle.

1.5.3. Effusive-constrictive pericarditis

Patients with an effusive-constrictive pericarditis present with symptoms of both effusion and constriction. This phenomenon should be considered when a patient undergoes pericardiocentesis and the haemodynamic compromise persists, despite aspiration of pericardial fluid.⁴¹ Effusive-constrictive pericarditis is characterised by persistent elevated right atrial pressure, after intra-pericardial pressure has been reduced following pericardiocentesis,⁴¹ occurring in 15% of patients with TBP.²⁵ To confirm a diagnosis of effusive-constrictive pericarditis, one needs to show that the right atrial pressure has failed to drop by more than 50% or the right atrial pressure remains above 10 mmHg after intra-pericardial pressure has been reduced to nearly 0 mmHg.⁴² Pericardiocentesis and/ or cardiac catheterization are required to verify this diagnosis.⁴¹

1.6. The utility of ECHO in TBP

Echocardiography (ECHO) is a useful modality for the diagnosis and management of TBP. A pericardial effusion appears on ECHO, as free fluid around the heart with an anechoic rim around the heart, demonstrated in Figure 4.⁴⁰ Figure 6 also shows fibrinous strands and exudative coating (also known as shaggy coats), which if present is highly suggestive TBP, as opposed to other causes of pericarditis.⁴³ An ECHO is also useful to exclude other causes of an enlarged cardiac silhouette, for example, a dilated cardiomyopathy.³⁴ With large pericardial effusions, the restraining effect of the pericardium is lost, leaving the heart floating in an unrestricted fashion and as a result, oscillates superiorly and antero-medially as it contracts, a phenomenon known as “swinging heart”.⁴⁴ Right-left inter-ventricular interdependence appear on ECHO as right ventricular collapse, variation of blood flow across the mitral valve with respiration

and dilatation of the inferior vena cava.⁸ When variation in blood flow across the mitral valve between inspiration and expiration exceeds 25% and dilatation of the inferior vena cava exceeds 21 mm or inferior vena cava collapse is less than 40% of maximum inferior vena cava diameter when a patient is asked to sniff are present; this is diagnostic of pericardial tamponade.^{8,37}

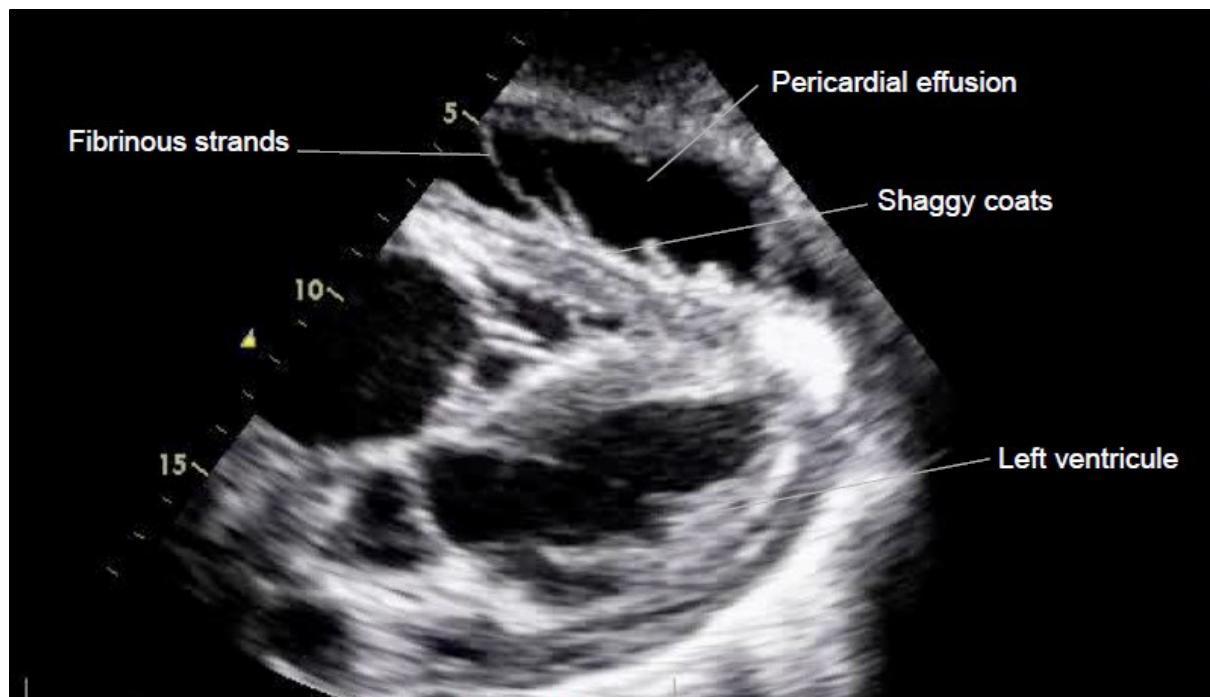


Figure 6: ECHO of a patient with tuberculosis pericarditis showing a large pericardial effusion, depicted by anechoic rim, shaggy coats and fibrinous strands.

An ECHO showing a thickened pericardium, restricting myocardial function is diagnostic of constrictive pericarditis.¹¹ A pericardial thickness greater than 3 mm is considered to be abnormally thickened.⁴⁵ Patients with constrictive pericarditis often have evidence of raised right-side diastolic pressure, which include; premature opening of the pulmonary valve, rapid early closure of the mitral valve, right atrial dilatation and inferior vena cava dilatation.⁴⁶ Right-left inter-ventricular interdependence can be visualized on ECHO, as diastolic septal bounce and variation of blood flow across the mitral valve with respiration.^{37,47,48} The term septal bounce is

used to describe when the inter-ventricular septum moves towards the left ventricle and back again in early diastole. This movement occurs as the right ventricle filling commences just before the left ventricle, resulting in inter-ventricular septal movement reflective of pressure differences between the two ventricles, which is accentuated during inspiration.⁴⁹

1.7. Approach to diagnosis of TBP

In high prevalence TB areas, for example, in Southern Africa, up to 69% of all pericardial effusions are due to TBP.⁵⁰ The gold standard confirmation modality of TBP, is microbiological evidence of *Mycobacterium tuberculosis* in the pericardium of an individual with a pericardial effusion. However, due to the difficulty in accessing pericardial fluid and the low yield of pericardial fluid in conventional TB diagnostic tests, a clinical diagnosis without microbiological confirmation is a more pragmatic approach. The use of validated diagnostic algorithms may be a surrogate for the diagnosis of TBP.⁵⁰ A number of diagnostic algorithms have been developed to standardise the approach to a clinical diagnosis of TBP, utilising clinical signs, biomarkers and laboratory results.³³ Researchers from Tygerberg Hospital in the Western Cape, South Africa, a high TB burden setting, developed an algorithm to aid diagnosis,³³ (Table 1) called the Tygerberg diagnostic algorithm. This combines several clinical features and readily available laboratory investigations to make a diagnosis of TBP in a resource poor setting. A score of 6 or more, suggests a diagnosis of TBP, with a sensitivity and specificity of 86% and 84%, respectively.³³ In contrast to the developed world, where only 4% of all pericardial effusions are due to TB, therefore, more rigorous microbiological evidence for the diagnosis of TBP is required, as malignancy is more likely to be the underlying cause.¹

Table 1: A suggested algorithm for diagnosis of TBP in a South African setting

Criteria	Score
Weight loss >10%	1
Night sweats	1
Temperature above 37.8°C	1
Blood leukocyte count <10 x 10 ⁹ /L	3
Serum globulin >40 g/L	3

Note: According to the Tygerberg diagnostic algorithm a patient has tuberculosis if they have a cumulative score of 6 or more.³³

On the other hand, a definitive diagnosis of TBP is made when *Mycobacteria* are isolated in pericardial fluid or when there is histological evidence of TB, by way of granuloma formation in the pericardium.^{9,23} The major limitations of relying on a microbiological and histological diagnosis of TBP, are firstly, a low diagnostic yield for isolating *Mycobacteriae* and identification of granuloma, and an inordinately prolonged processing time. Gene expert[®] is a cartridge based, automated polymerase chain reaction (PCR) diagnostic test, which has improved sensitivity and reduced time to diagnosis, demonstrating a sensitivity and specificity (95% confidence interval) of 63.8% (52.4% to 75.1%) and 100% (85.6% to 100%), respectively.^{51,52} The high cost of these cartridges and the complicated nature of the apparatus, limit widespread accessibility to all primary health-care facilities in South Africa.⁵¹ There are also other

diagnostic techniques, which are still under development for example, serum or urine lipoarabinomannan (LAM) and serum interferon gamma (IFN γ), which have shown promise with a sensitivity and specificity of 74% and 99%, and 81% and 85%, for each of the aforementioned tests, respectively.^{53,54} Ideally, a diagnostic modality which has a high yield, short processing time, is affordable and easy to use is required, but so far remains elusive.⁴

1.8. Management of TBP

The mainstay of treatment is anti-TB chemotherapy in TBP, as with other sites of TB. Percutaneous drainage of the effusion may also be necessary, depending on the size of the effusion and the severity of haemodynamic compromise.^{1,5} A pericardial effusion that is large enough to cause symptoms may require pericardiocentesis, which also provides an opportunity to confirm the diagnosis. The first line anti-TB chemotherapy agents are rifampicin, isoniazid, pyrazinamide and ethambutol.⁵ The pharmacokinetics of these drugs have been well described in plasma and show adequate exposure of all four drugs in plasma, as each of these drugs have minimum trough concentrations, which exceed the minimum inhibitory concentration (MIC).^{6,8} There have also been a number of studies, which have characterised the pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol in lung parenchyma and cerebrospinal fluid.^{9,37} However, the pharmacokinetics of these anti-TB drugs and adequacy or otherwise, in the pericardial compartment remain largely and more importantly, their impact on the course of disease in TBP are unknown.

There are unique challenges in the treatment of TBP, especially when considering percutaneous drainage, due to proximity to the heart. During evacuation of the

pericardial effusion or performing a pericardial biopsy, there is a risk of damaging the adjacent myocardium, which may lead to cardiac arrest and subsequently death.⁵⁵ Furthermore, the available body of literature suggests that TBP patients, especially those with underlying HIV infection, may have an associated myocarditis, which results in the pericardium being more friable and have increased susceptibility to inadvertent damage.⁵⁶

1.9. Anti-tuberculosis chemotherapy

The treatment of TB requires a combination of drugs with a view to reducing the likelihood of drug resistance. The WHO recommends the use rifampicin, isoniazid, pyrazinamide and ethambutol as the first-line treatment of TB.^{1,57} These four drugs target different aspects of the *Mycobacterium* namely; interfere with translation, inhibit the production of fatty acids, de-energize the membrane potential across the cell wall and disrupt synthesis of key components of the cell wall.⁵⁸⁻⁶¹ The treatment is administered for a minimum of six months, divided into the intensive phase, aimed at rapid killing of actively replicating *Mycobacteriae* and the continuation phase, which targets slow growing dormant persistors.⁵ The intensive phase consists of all four drugs for two months, while the continuation phase consists of rifampicin and isoniazid for a further four months.⁵⁷ The recommended doses of rifampicin, isoniazid, pyrazinamide and ethambutol used in the treatment of TBP, were adapted from those used in the treatment of pulmonary TB and are prescribed universally for all forms of TB, without any modification, regardless of the difference in absorption and clearance of these TB drugs at different sites of infection.¹ However, given the high mortality associated with TBP, it raises the question as to the extent to which the therapy is ideal. Anti-TB drugs and their respective desirable plasma drug concentrations, as

recommended by the South African Department of Health National TB program are shown in Table 2.³¹ To achieve the required drug concentrations of all four drugs means that patients have to ingest a large number of tablets, which can be challenging and burdensome. Table 3 shows some commonly used fixed-dose combinations, which help reduce the tablet number burden.³¹

Table 2: South African Department of Health National Tuberculosis Management Guidelines 2014.³¹

TB drug	Dose (mg/kg)	Dose range (mg/kg)
Rifampicin	10	8-12
Isoniazid	5	4-6
Pyrazinamide	25	20-30
Ethambutol	15	15-20

Table 3: South African Department of Health National TB program anti-TB fixed dose combination.³¹

Weight	Intensive phase	Continuation phase	
	RHZE (150,75,400,275)	RH (150,75)	RH (300,150)
30-37 kg	2 tablets	2 tablets	-
38-54 kg	3 tablets	3 tablets	-
55-70 kg	4 tablets	-	2 tablets
>70 kg	5 tablets	-	2 tablets

Note: RHZE: R=rifampicin, H= isoniazid, Z=pyrazinamide and E=ethambutol while RH stands for R=rifampicin and H=isoniazid. In parenthesis are the respective amounts of each drug in milligrams (mg) contained in each tablet of the fixed dose combination.

1.9.1. Rifampicin

Rifampicin is a bactericidal anti-TB drug, produced by *Norcadia mediterranei* and acts by inhibiting ribonucleic acid (RNA) polymerase, an enzyme, which transcribes deoxyribonucleic acid (DNA) to RNA.^{21,58} Rifampicin is well absorbed in the gastrointestinal system, with a time to maximum concentration of 2 hours. The WHO recommended dose reaches a maximum concentration of 7 µg/ml, which is well above the MIC of 0.2 µg/ml. Rifampicin is also predominantly protein-bound in the order of 60% to 90% .^{57,62} It is also a liver enzyme inducer and causes up-regulation of cytochrome-P-450 enzymes, particularly cytochrome 3A4, and as a result, reduces the effectiveness of other drugs including the combined oral contraceptive pill, co-

administered corticosteroid therapy and warfarin.^{62,63} The prominent side-effects are hepatotoxicity and red orange bodily secretions. Resistance to rifampicin occurs where there is a mutation of the β subunit of the RNA polymerase binding site for rifampicin.^{58,64}

1.9.2. Isoniazid

Isoniazid has bactericidal activity and is a pro-drug, which is converted to iso-nicotinic acyl-NADH complex, by a bacterial catalase-peroxidase enzyme, known as KatG.⁶⁵ Iso-nicotinic acyl-NADH complex then binds to and inhibits enoyl-acyl carrier protein (Acp) reductase, which produces enoyl-acyl carrier protein malonyl co-enzyme A (enoyl-AcpM), an essential substrate for fatty-acid synthase in the production of fatty-acids.^{59,65} Disruption of fatty-acid production, results in decreased cell wall lipid synthesis, reduced nucleic acid production and a generalised depression of metabolic activity of the Mycobacteria.⁵⁹ Isoniazid is well absorbed in the gastrointestinal system, with a time to maximum concentration (T_{max}) of 1 hour, with a maximum concentration (C_{max}) of 5 $\mu\text{g/ml}$. Isoniazid is metabolised by the liver, where it undergoes acetylation. There are genetic differences in the rate of acetylation, with fast and slow acetylators, giving rise to a bimodal distribution of the half-life of isoniazid, respectively.⁶⁶ Isoniazid half-life ranges between 1 hour and 4 hours.⁶⁷ The common side effects of isoniazid are rash, hepatitis and peripheral neuropathy.⁶⁸

1.9.3. Pyrazinamide

Pyrazinamide is a bacteriostatic drug, which acts in an acidic environment, for example, in foci of caseous necrosis including cavities of the lungs, usually as a

consequence of PTB.⁶⁹ This allows killing of semi-dormant *Mycobacteria* in the presence of inflammation, thereby shortening the duration of treatment from 9 months to 6 months.⁷⁰ Pyrazinamide is a pro-drug, which is converted to pyrazinoic acid (POA) by a *Mycobacterial* enzyme called pyrazinamidase.⁷⁰ The mechanism of action of this drug is not clearly understood, but it is thought that POA de-energises the cell membrane potential of the *Mycobacterium*, by interfering with transportation of fatty-acids across the cell membrane.⁶⁰ This theory is supported by pyrazinamide being more effective in an acid environment. Pyrazinamide is well absorbed in the gut with T_{max} of 1 hour and is well distributed throughout the body. It is metabolised in the liver and eliminated mainly via the kidneys.⁷¹ Side effects of pyrazinamide include nausea, vomiting, loss of appetite, muscle pain, joint pain and fatigue.⁷²

1.9.4. Ethambutol

Ethambutol is a bacteriostatic anti-TB drug, which acts by interfering with the formation of the apparently impervious lipid rich cell wall.⁶¹ The antimicrobial action results in the inhibition of the enzyme arabinosyl transferase, preventing synthesis of arabinogalactan a key component of the mycolic cell wall.⁷³ The resulting cell wall is more permeable and thus, more prone to the innate immune system of the host rendering the *Mycobacterium* unable to replicate. Ethambutol is well absorbed in the gut, unaffected by food, reaches a maximum concentration in 2 to 4 hours after ingestion and has a high volume of distribution.⁷⁴ Side effects of ethambutol may include ocular toxicity which manifests as visual impairment, nausea, vomiting, loss of appetite, rash, pruritis, headache and joint pain.⁷²

1.10. Pharmacokinetics of anti-TB drugs

The pharmacokinetics of the 4 standard anti-TB drugs rifampicin, isoniazid, pyrazinamide and ethambutol are well characterised in plasma, with limited data on the penetration of these agents in lung parenchyma, central nervous system through assessment of pleural spaces and cerebrospinal fluid (CSF), respectively.⁷⁵ Pulmonary TB constitutes the majority of TB cases, thus the pharmacokinetics are best explained in patients with PTB.⁴ The lung tissue is well perfused with a thin permeable endothelial basement membrane to facilitate gaseous exchange.⁷⁶ As a result, the pharmacokinetic profile of these four anti-TB drugs in the lung parenchyma closely resembles that of the plasma compartment.⁷⁷ The pericardial fluid differs from lung parenchyma, as it represents ultra-filtration of the plasma. We hypothesize that the pericardial drug concentrations, using standard anti-TB treatment are significantly lower than those of plasma and may represent sub-therapeutic concentrations. Therefore, there is the need to evaluate whether the doses recommended for pulmonary TB are both adequate and non-toxic for patients with TBP.²⁵

1.11. Evacuation of the pericardium

Evacuation of the pericardium is performed for both diagnostic and therapeutic indications and is usually reserved for patients with large pericardial effusions, usually located anterior to the heart, features of pericardial tamponade, or where there is diagnostic uncertainty as to the underlying cause of pericarditis, especially after failure to respond to initial therapy.³³ Pericardiocentesis using fluoroscopy and ECG guided Seldinger technique via the sub-xiphoid approach, is the preferred method for evacuation of the pericardium.⁷⁸ Fluoroscopy is a real-time x-ray imaging technique,⁷⁹

which is a minimally invasive procedure, performed under local anaesthesia.⁷⁸ The sub-xiphoid area is the preferred point of entry for pericardiocentesis, as it lies adjacent to the low-pressure right ventricle, which if perforated will have a lower lethal potential, compared to the left ventricle.⁸⁰

A wide-bore needle is inserted at the point of insertion with an attached syringe under negative pressure, while monitoring the fluoroscopy and ECG to ensure the needle does not reach the adjacent myocardium. The real-time images from fluoroscopy allow the operator to visualize the distance between the needle and the myocardium, while the ECG undergoes non-specific ST segment and T wave changes when the needle injures the myocardium, which is an indication to withdraw the needle.⁸¹ Once the needle enters the pericardial space, pericardial fluid enters the syringe as a result of the negative suction pressure. While holding the needle firmly to ensure that it does not move, the syringe is removed and a guidewire is threaded through the bore of the needle and with the guide-wire in place, the needle is removed.³⁸ A size 11 surgical blade is used to widen the incision to allow the dilator-introducer sheath to be inserted over the guide-wire. A pigtail catheter and a small-bore tube are inserted into the pericardium through the dilator-introducer sheath and the pericardial fluid is drained through the pigtail catheter.^{38,82}

According to Horowitz *et al*, pericardial effusions can be classified into small, moderate and large by their sizes, using ECHO.⁴³ A pericardial effusion, less than 10 mm in depth on ECHO, is considered small, while moderate effusions are between 10 mm and 20 mm and large effusions are larger than 20 mm.⁴³ Evaluation of the risks versus the benefits is important, before making a decision whether or not to perform

pericardiocentesis, as there is a real risk of injuring the myocardium, which ranges from 4% to 20% depending on the setting.⁸³ Facilities for example, with continuous ECG monitoring, fluoroscopy and echocardiography guidance have lower rates of myocardial injury because they can accurately assess the position of the needle during the aspiration procedure.⁸³

The risk of damaging the underlying myocardium is inversely proportional to the size of the pericardial effusion.^{43,78} Most clinicians would advocate pericardiocentesis for any patient with a large pericardial effusion, in the absence of any contraindications.^{43,80} Others advocate pericardiocentesis for a pericardial effusion greater than 10 mm during diastole, at the site of pericardial aspiration.⁸⁰ Certain well-resourced centres utilise pericardioscopy, which allows the clinician to visualise the pericardial cavity with an endoscope directly, permitting a pericardial biopsy, resulting in an improved diagnosis of TBP by 20%, compared to pericardiocentesis without direct visualisation.⁸⁴ Some pericardial effusions which are deemed too small for pericardiocentesis may require drainage for example, if the patient has cardiac tamponade, in such patients a pericardial window (an open surgical technique is used to gain access into the pericardium) is an alternative method of draining the pericardial effusion.⁸⁵ However, a pericardial window is a more invasive procedure, when compared to pericardiocentesis, requiring a general anaesthesia and longer recovery time.

1.12. Other aspects to consider when managing patients with TBP

HIV co-infection is common among patients with TBP, in South Africa 67 % of all patients with TBP are also HIV positive and as is the case with other forms of TB.³⁰ It is critical to test for HIV to direct further therapy.²³ Patients with HIV co-infection should be initiated on highly active anti-retroviral therapy (HAART). In patients with TB and HIV co-infection, anti-TB therapy should take precedence and HAART should be started once anti-TB treatment is tolerated,^{9,15} with the proviso that there are no contraindications, clinicians should aim to initiate HAART within 2 weeks of starting anti-TB treatment.²⁹ Anti-TB treatment is initiated followed by HAART in a sequential fashion, to assess whether TB therapy is tolerated and to avoid misconstruing drug side effects to the wrong drug.²⁹ It also has the benefit of minimising the risks for example, the immune reconstitution inflammatory syndrome (IRIS), which is a dysregulated and excessive immunological response to opportunistic infections, associated with a high mortality.^{15,29} Moreover, a patient with TBP should be commenced on HAART after the acute illness has improved, following adequate counselling and is aware of the implications of lifelong HAART for example, the need for meticulous adherence.²⁹

When there is a recurrent pericardial effusion, an alternative cause for the pericardial effusion should be sought, for example, lymphoma, systemic lupus erythematosus (SLE) or uraemic pericarditis. It is also important to exclude drug-resistant TB and ensure that there is adherence to anti-TB therapy.⁸⁶ Surgical pericardiectomy is the preferred treatment modality for constrictive pericarditis.⁸⁵ Medical management is reserved for patients in whom surgery is contraindicated and involves treatment with diuretics. Patients with constrictive pericarditis typically present with right heart failure and diuretics have been shown to alleviate symptoms by reducing pulmonary and

systematic venous congestion.⁸⁷ However, diuretics must be used with caution, as volume depletion may have an adverse effect on cardiac output. Corticosteroid therapy reduces the likelihood of constriction to 4%, compared with 8% in those without adjunctive corticosteroid therapy.^{23,88}

1.13. Adjunctive corticosteroid therapy in tuberculosis

Mycobacteria have a unique cell wall, which is highly immunogenic and confers protection on the bacilli, by the immune system. Mycolic acid and lipoarabinomannan (LAM) are the chief virulence factors found in the cell wall. These factors facilitate survival under hostile conditions and cause protracted stimulation of the immune cascade.^{89,90} The classical TB symptoms for example, night sweats and weight loss are caused by persistent stimulation of the immune system, hence attenuation of the inflammatory process by use adjunctive corticosteroid therapy has been explored in the management of the various forms of TB.⁸⁹

Prednisolone is a synthetic derivative of cortisol, an endogenous corticosteroid, diffuses across the cell membrane and binds to glucocorticoid receptors in the nucleus, where it down-regulates production of pro-inflammatory cytokines.^{91,92} While it may seem counter-intuitive to administer an agent that impedes the function of the body's defense, corticosteroids are intended to modulate, but not terminate the immune response and must be given together with effective anti-TB chemotherapy to ensure that uncontrolled proliferation of the offending organism does not occur.⁹³

The aim of adjunctive corticosteroid therapy is to find the right balance between pro-inflammatory and anti-inflammatory immune signal pathways.⁹⁴ The majority of

individuals infected with *Mycobacterium tuberculosis* do not develop active TB disease, as most individuals are able to mount appropriate immune responses, resulting in latent TB infection without tissue damage.⁹⁴ While corticosteroid therapy has not shown any benefit in most forms of TB, a significant benefit in TBP and tuberculous meningitis, where it has been associated with a reduction in mortality and neurological deficits,²³ Adjunctive corticosteroid therapy was associated with a lower likelihood of hospitalization during the follow-up period of 2 years, hazard ratio of 0.79, 95% confidence interval (95% CI) (0.63 – 0.99) and reduced incidence of constrictive pericarditis,²³ hazard ratio 0.56, 95% CI (0.36 – 0.87).^{23,93}

Prednisolone is used to treat a wide range of allergic reactions, inflammatory and autoimmune conditions.⁹⁵ As a widely used drug, effective drug monitoring strategies have been developed, notably it has been shown that saliva corticosteroid concentrations can be used as a surrogate marker for plasma corticosteroid concentrations.^{96,97} Monitoring of corticosteroid therapy using saliva is potentially non-invasive and convenient.⁹⁶ It is unknown whether pericardial prednisolone has a similar relationship to plasma, and saliva or whether saliva prednisolone is directly related to pericardial prednisolone, making it a potentially useful surrogate marker.

1.14. Cytokines and adjunctive corticosteroid therapy

The body of available literature has shown that several cytokines are elevated in patients with TBP namely, IFN- γ , TNF- α , IL-1, IL-2, IL-6 and IL-10.¹⁷ This has led to the hypothesis that regulation of pro-inflammatory cytokines is the mechanism by which corticosteroid therapy exerts its beneficial effect in patients with TBP. Meintjes *et al*, showed a decrease in plasma concentrations of interleukin (IL)-6, IL-10, IL-12

p40, interferon (IFN)- γ , and IFN- γ induced protein (IP)-10 in patients with TB IRIS, who were taking corticosteroid therapy.¹⁴ While IRIS and TBP are two distinct clinical entities, there are critical similarities; where the primary cause is TB, they are characterised by an increase in pro-inflammatory cytokines and may be responsive to corticosteroid therapy.¹⁵ It is important to ascertain whether the cytokine response to adjunctive corticosteroids demonstrated in IRIS, can be replicated in TBP. There are uncertainties in respect to the requisite dose of glucocorticoids needed to establish this beneficial response. By establishing a predictable dose response relationship between prednisolone and these cytokines of interest, a surrogate marker for the adequacy of adjunctive corticosteroid therapy, may be identified.

1.15. The effect of Mycobacterial infection on endogenous corticosteroids

An elevated cortisol/ cortisone ratio has been observed in bronchial lavage fluid of patients with pulmonary tuberculosis, while the corresponding plasma cortisol/ cortisone ratio remained unchanged.⁹⁸ This rise in cortisol/ cortisone ratio was shown to resolve with resolution of disease.⁹⁸ A similar trend has been noted in the skin of patients with leprosy, where skin biopsies showed an elevated cortisol/ cortisone ratio in skin with leprosy lesions when compared to normal skin.^{99,100} Mycobacterial infection appears to cause a shift in the cortisol-cortisone equilibrium from inactive cortisone towards active cortisol at the site of infection, while sparing the plasma compartment.¹⁰¹ This may represent a local endogenous immune modulatory mechanism. Local cortisol and cortisone concentrations are under the influence of 11-beta-Hydroxysteroid dehydrogenase type 1 (11HSD-1), which governs the shift in

equilibrium from cortisone to cortisol, 11-beta-Hydroxysteroid dehydrogenase type 2 (11HSD-2), which inactivates cortisol to the inactive substrate, cortisone. These enzymes are expressed in peripheral tissues, for example, endothelial cells.¹⁰¹ It remains to be determined, whether the pericardial cortisol/ cortisone ratio is also elevated in patients with TBP and whether this is associated with any changes in cytokine concentrations. Understanding the relationship between the pericardial cortisol/ cortisone ratio and cytokines concentration could help elucidate the immunological mechanisms underlying TBP and moreover, whether this relationship is either pro-inflammatory or anti-inflammatory or both.

1.16. Outcome and prognosis of TBP

TBP is associated with a high mortality and morbidity. A multi-centre prospective observational study, the Investigation of the Management of Pericarditis (IMPI) Africa registry, showed an overall 6 month mortality of 25% in TBP patients.^{9,25} Some of the reasons for this high mortality are HIV co-infection, overwhelming burden of TBP, lack of specialised treatment for TBP, a challenge in making a definitive diagnosis of TBP, and it is speculated that anti-TB chemotherapy may be less efficacious in this setting.⁵

TBP in the presence of an underlying HIV infection has a poorer prognosis than in patients without HIV co-infection.²⁵ When TBP patients were followed up as part of the IMPI Africa registry and stratified by HIV status, those with HIV co-infection had a 6-month mortality, of more than double those without HIV co-infection (40% vs 17%; $p=0.001$).²⁵

In South Africa, there are a lack of specialised services for TBP patients. Once the diagnosis has been made and the acute illness has been treated, patients are referred

to their local primary health-care clinics,¹⁰² which are not supported by specialised services for example, ECHO and pericardial aspiration, making a diagnosis of recurrent pericardial effusion and constrictive pericarditis challenging or the long-term outcome.

1.17. Introduction to pharmacokinetics as it applies to anti-TB chemotherapy

The mainstay of the management of TBP is anti-TB chemotherapy.¹ For the anti-TB drugs to have the greatest bacteriostatic and or bactericidal effect, these are required to penetrate the target site at sufficient concentrations.⁶² In the normal physiological state, the pericardial space is too small to access through pericardiocentesis. Consequently, there is a dearth of literature describing the pharmacokinetics of anti-TB drugs in this compartment. A pericardial effusion that requires drainage, provides a rare opportunity to gain access into the pericardial compartment and to potentially evaluate drug concentrations.⁴³

Figure 7 shows an example of a concentration time graph of an orally administered drug.¹⁰³ On administration of a drug, the drug concentration in the central compartment increases until it reaches a maximum concentration (C_{max}), where the rate of absorption of the drug equals the rate of elimination, the time taken to reach C_{max} is called time to maximum concentration (T_{max}).¹⁰⁴ Beyond the C_{max} the elimination rate exceeds the absorption rate and the concentration starts to decline, until the drug is completely eliminated or another dose is administered.¹⁰⁴ The minimum concentration (C_{min}) is the lowest drug concentration and is reached just prior to the next dose of drug being administered, and is also known as the minimum trough concentration.⁶²

The dosing schedule should be designed to ensure that the C_{min} is sufficiently high for the drug to exert a desired pharmacological effect.¹⁰⁵ Adequacy of the antibiotic dose is often determined by comparing the C_{min} to the minimum inhibitory concentration (MIC). The MIC is the minimum concentration of the antibiotic, which is required to impede replication of the target microbe. Anti-microbicide dosing is deemed adequate when the C_{min} is greater than the MIC. The area under the curve (AUC) is a measure of exposure to the drug in a given time interval and can be used as an alternative measure of adequacy of antibiotic dosing.^{104,106} Rifampicin, isoniazid and pyrazinamide AUC_{0-24} below 13 mg.h/L, 52 mg.h/L and 363 mg.h/L, respectively, for example, are predictors of poor treatment outcomes in pulmonary TB patients.⁷⁷

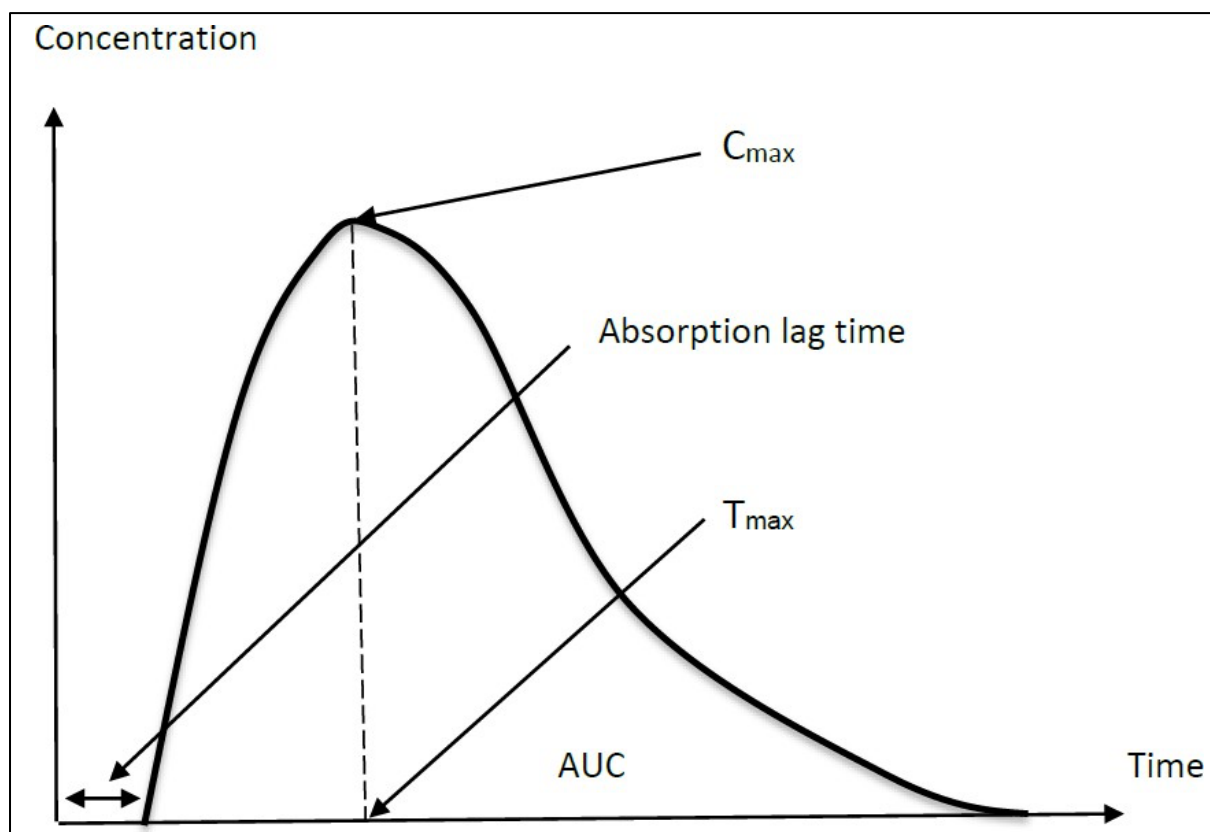


Figure 7: Shows concentration time graph of drug and some important pharmacokinetic parameters; C_{max} , T_{max} , AUC and absorption lag time. From administration of drug to T_{max} is the absorption phase

while from $T_{\max}$ to the time the drug is completely eliminated from the body is the elimination phase.¹⁰³

The rate of absorption depends on the nature of the drug and the route of administration.¹⁰⁷ Bioavailability can be defined as the proportion of the unaltered drug reaching the systemic circulation.¹⁰⁴ Drugs administered intravenously, instantaneously reach $C_{\max}$ and have a bioavailability of 100%, while drugs administered orally may at the outset, degrade in the gastrointestinal tract, long before it enters the systemic circulation and as a result, may have a lower bioavailability.¹⁰⁴ After absorption in the gastrointestinal system, the drug enters the portal circulation, passes via the liver, where metabolism starts before the drug enters the systemic circulation, a process called first-pass metabolism.¹⁰⁴ Rifampicin is an example of a drug which undergoes first-pass metabolism, as it is predominantly metabolised in the liver.¹⁰⁸ Absorption may also be affected by factors such as food, drug interactions, pathological conditions and physiological states, for example, isoniazid when taken with alcohol, has an increased risk of hepatotoxicity.^{62,104}

The absorption rate, $C_{\max}$, elimination rate and AUC can be used to effectively describe the absorption, metabolism and elimination of a given drug.¹⁰⁴ Statistical methods are used to objectively quantify these parameters, making it possible to compare the pharmacokinetic properties of different drugs in the same population of patients or to compare the same drug in different populations of patients.¹⁰⁴ To ensure that a patient receives an adequate dose of a drug the clinician must have an understanding of the factors which affect absorption, metabolism and elimination, otherwise the drug may be above or below the therapeutic range, resulting in harmful side effects or ineffective treatment due to under dosing.¹⁰⁵ For example, nevirapine, an anti-retroviral drug

when administered to adults has a lead-in dose of 200 mg for 14 days, which is then escalated to a maintenance dose of 400 mg daily.²⁹ Nevirapine causes liver enzyme induction of cytochrome P450 enzymes, thereby increasing its metabolism.¹⁰⁹ While a 200 mg daily dose is adequate in the first 14 days of treatment, after this lead-in period, a higher dose of 400 mg, is required to maintain adequate pharmacological plasma concentrations.¹⁰⁹

1.18. Non-compartmental and compartmental analyses

There are two methods, which are used to estimate pharmacokinetic parameters, namely non-compartmental (NCA) and compartmental analyses (CA). NCA views the body as a single compartment and uses the trapezium rule or integration to calculate the AUC.¹¹⁰ This is a simpler and more pragmatic approach, which does not make any assumptions about the population under investigation.⁵¹ While the NCA results cannot be generalised to other populations, these results are useful in obtaining initial estimates, which can be the foundation for more complicated pharmacokinetic models.¹¹⁰ However, NCA approach requires all patients to have identical time points, which is not always possible in a real world setting.¹¹⁰ More often than not during data collection, missing data arises, due to reasons beyond the researcher's control, resulting in discarding of data for patients with missing variables or adoption of complicated multiple imputation methods, which generate figures to replace these missing data.¹¹¹

CA gives a more accurate description of the fate of a drug in the human body.¹⁰⁴ This method views the human body as a series of interconnected compartments, each with its own absorption and elimination rates.¹⁰⁴ The plasma compartment is regarded as

the central compartment, which is central functionally, but not necessarily anatomically. As plasma transports the drug to and from the various parts of the body via the circulatory system, the concentration of the drug in both plasma and peripheral tissues changes over time. Using drug concentrations at certain time points after administration, one can employ differential equations to calculate the respective absorption and elimination rates of each compartment.¹⁰⁴

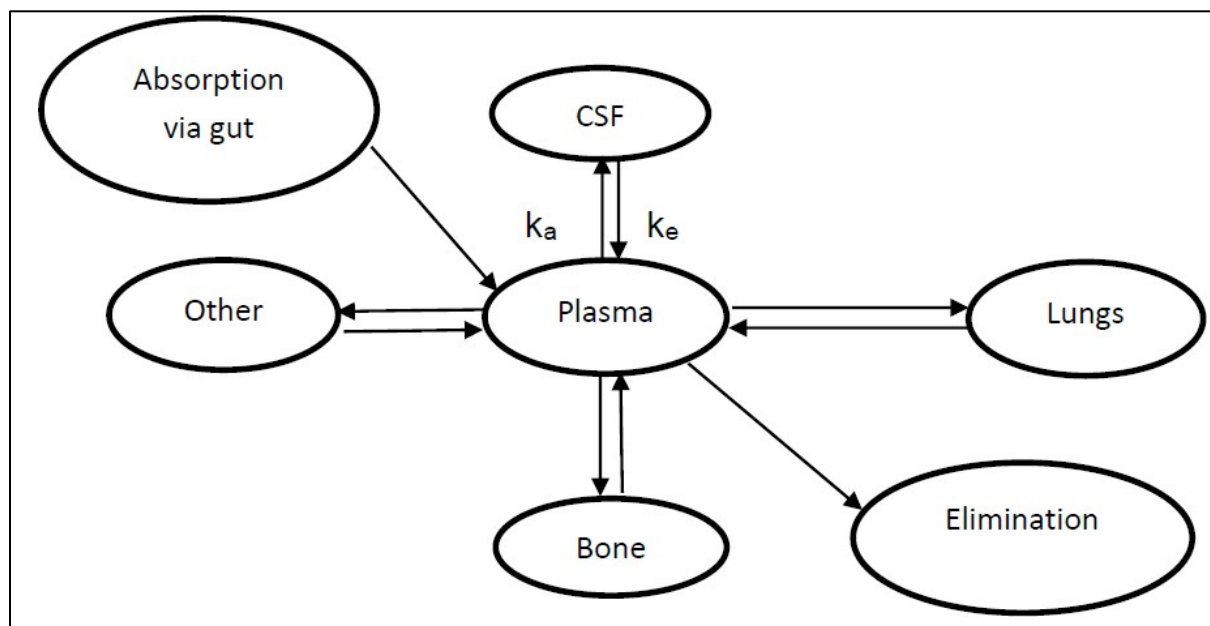


Figure 8: Showing the relationships between various compartments in the body for an orally ingested drug. Each compartment has its own absorption (k_a) and elimination (k_e) rate which are represented by the arrows.¹⁰⁴

This method has the added advantage of being able to estimate variability, both between occasion variability (BOV) and between subject variability (BSV).¹⁰⁴ When designing dosing schedules for large populations, understanding the relationship between BSV and certain covariates such as age, ethnicity or co-morbidity allows one to predict the pharmacokinetics of a drug in certain sub-populations.¹⁰⁵ This allows targeted dosing for specific sub-populations for example, patients with renal failure who may have a decreased drug clearance.¹⁰⁵

An advantage of population pharmacokinetics is that a model can be used to establish the relationship between the concentration time graph or pharmacokinetic profile of a drug to the physiological action of the drug. These are also known as pharmacokinetic pharmacodynamics (PK-PD) models and are important in early drug development, when trying to determine initial dosing schedule for larger clinical trials.¹⁰⁶ Lastly, CA is a flexible approach, which is well-adapted to dealing with missing data, hence there is no need to discard data of patients with missing variables, while ensuring efficient and maximum use of the available data.¹⁰⁴

1.19. Hypothesis and motivation for study

Given the high mortality associated with TBP in South Africa, it is essential for researchers in this region to prioritize research in TBP, with a view to improving patient outcomes.²⁵ We hypothesise that the management of TBP is inadequate given the high mortality associated with TBP, even when patients are commenced timeously on treatment. Anti-TB drug dosing schedule for TBP is derived from the treatment guidelines for pulmonary TB and not tailored to TBP. There is therefore a need to describe the pharmacokinetics of the anti-TB chemotherapy in the pericardial compartment of patients with TBP and if necessary, adapt the dose and /or dosing schedule for TBP.

Adjunctive corticosteroid therapy has already been shown to be beneficial in TBP, however, this literature is based on several studies which adopted different prednisolone doses and duration of treatment. The pharmacokinetics of prednisolone in plasma are well described generally, however, there are no data relative to the pharmacokinetics of prednisolone at the site of infection for example, the pericardial

space. We hypothesize that gaining a better understanding of the pharmacokinetics of prednisolone in the pericardial compartment, may determine the adequacy of dosing in the context of TBP, or at least confirming detection of prednisolone at the site of infection.

Regulation of endogenous glucocorticoid concentrations at tissue level is controlled by the cortisol-cortisone shuttle enzymes.¹⁰¹ Mycobacterial infections are associated elevated cortisol/ cortisone ratios in pulmonary TB and leprosy at the site of infection.^{98,100} We postulate that a similar rise in cortisol/ cortisone ratio will be found in the pericardial space of patients with TBP.

The pericardial space is mostly inaccessible, presenting a challenge for monitoring a response to treatment and predicting an outcome. There is also a need to explore the suitability of cytokines and endogenous glucocorticoids for potential use as biochemical surrogate markers for the effectiveness of adjunctive corticosteroids, potentially serving as pharmacodynamic markers.¹ It is hypothesised that by identifying a suitable biomarker, it may prove useful to determine the efficacy or otherwise of corticosteroid treatment in patients with TBP.

1.20. References

1. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
2. Ntsekhe M, Wiysonge CS, Gumedze F, et al. HIV infection is associated with a lower incidence of constriction in presumed tuberculous pericarditis: a prospective observational study. *PLoS One* 2008; **3**(6): e2253.
3. Smith I. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. *Clinical microbiology reviews* 2003; **16**(3): 463-96.
4. Sudre P, Ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. *Bulletin of the World Health Organization* 1992; **70**(2): 149.
5. Mayosi BM. Interventions for treating tuberculous pericarditis. *The Cochrane Library* 2002.
6. Elias H, Boyd L. Notes on the anatomy, embryology, and histology of the pericardium. II. *New York Medical College news & notes* 1960; **2**: 50-75.
7. Mori S, Hanna P, Dacey MJ, et al. Comprehensive anatomy of the pericardial space and the cardiac hilum: anatomical dissections with intact pericardium. *Cardiovascular Imaging* 2022; **15**(5): 927-42.
8. Spodick DH. The normal and diseased pericardium: current concepts of pericardial physiology, diagnosis and treatment. *Journal of the American College of Cardiology* 1983; **1**(1): 240-51.
9. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. *BMC infectious diseases* 2006; **6**(1): 2.
10. Spodick DH. Acute cardiac tamponade. *New England Journal of Medicine* 2003; **349**(7): 684-90.
11. Gimlette T. Constrictive pericarditis. *British heart journal* 1959; **21**(1): 9.
12. NTSEKhe M, Wiysonge CS, Commerford PJ, MAYOSI BM. The prevalence and outcome of effusive constrictive pericarditis: a systematic review of the literature. *Cardiovascular journal of Africa* 2012; **23**(5): 281-5.
13. Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. *Clinical and Developmental Immunology* 2010; **2011**.

14. Meintjes G, Skolimowska KH, Wilkinson KA, et al. Corticosteroid-modulated immune activation in the tuberculosis immune reconstitution inflammatory syndrome. *American journal of respiratory and critical care medicine* 2012; **186**(4): 369-77.
15. Meintjes G, Lawn SD, Scano F, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *The Lancet infectious diseases* 2008; **8**(8): 516-23.
16. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annual review of immunology* 2013; **31**: 475-527.
17. Burgess L, Reuter H, Carstens M, Taljaard J, Doubell A. Cytokine production in patients with tuberculous pericarditis. *The International Journal of Tuberculosis and Lung Disease* 2002; **6**(5): 439-46.
18. Jo E-K, Park J-K, Dockrell HM. Dynamics of cytokine generation in patients with active pulmonary tuberculosis. *Current opinion in infectious diseases* 2003; **16**(3): 205-10.
19. Ntsekhe M, Matthews K, Syed FF, et al. Prevalence, hemodynamics, and cytokine profile of effusive-constrictive pericarditis in patients with tuberculous pericardial effusion. *PLoS One* 2013; **8**(10): e77532.
20. Larrieu AJ, Tyers GFO, Williams EH, Derrick JR. Recent experience with tuberculous pericarditis. *The Annals of thoracic surgery* 1980; **29**(5): 464-8.
21. Karim QA, Karim SSA. The evolving HIV epidemic in South Africa. *International Journal of Epidemiology* 2002; **31**(1): 37-40.
22. Jasmer RM, Nahid P, Hopewell PC. Latent tuberculosis infection. *New England Journal of Medicine* 2002; **347**(23): 1860-6.
23. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
24. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *The Lancet* 2007; **369**(9578): 2042-9.
25. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *SAMJ: South African Medical Journal* 2008; **98**(1): 36-40.
26. Sharma S, Mohan A, Kadiravan T. HIV-TB co-infection: epidemiology, diagnosis & management. *Indian J Med Res* 2005; **121**(4): 550-67.
27. Maher D, Chaulet P, Spinaci S, Harries A. Treatment of tuberculosis: guidelines for national programmes. *Treatment of tuberculosis: guidelines for national programmes Second edition* 1997; (Ed. 2): 1-77.

28. Gilks CF, Crowley S, Ekpini R, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. *The Lancet* 2006; **368**(9534): 505-10.
29. Organization WH. HIV treatment and care: WHO HIV policy adoption and implementation status in countries: factsheet: World Health Organization, 2019.
30. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. *Heart failure reviews* 2013; **18**: 367-73.
31. Health SAdo. National Tuberculosis Management Guidelines 2014: Department of Health; 2014.
32. Joshi JM. Tuberculosis chemotherapy in the 21st century: Back to the basics. *Lung India: Official Organ of Indian Chest Society* 2011; **28**(3): 193.
33. Reuter H, Burgess L, Van Vuuren W, Doubell A. Diagnosing tuberculous pericarditis. *Journal of the Association of Physicians* 2006; **99**(12): 827-39.
34. Trautner BW, Darouiche RO. Tuberculous pericarditis: optimal diagnosis and management. *Clinical infectious diseases* 2001; **33**(7): 954-61.
35. Strang J. Echoes from the Third World—two-dimensional echocardiography in a developing country. *South African medical journal= Suid-Afrikaanse tydskrif vir geneeskunde* 1990; **77**(2): 85-91.
36. Mercé J, Sagristà-Sauleda J, Permanyer-Miralda G, Soler-Soler J. Should pericardial drainage be performed routinely in patients who have a large pericardial effusion without tamponade? *The American journal of medicine* 1998; **105**(2): 106-9.
37. Spodick DH. Pathophysiology of cardiac tamponade. *Chest* 1998; **113**(5): 1372-8.
38. De Carlini CC, Maggiolini S. Pericardiocentesis in cardiac tamponade: indications and practical aspects. *E-journal Cardiol Pract* 2017; **15**.
39. Myers RB, Spodick DH. Constrictive pericarditis: clinical and pathophysiologic characteristics. *American heart journal* 1999; **138**(2): 219-32.
40. Pepi M, Muratori M. Echocardiography in the diagnosis and management of pericardial disease. *Journal of Cardiovascular Medicine* 2006; **7**(7): 533-44.
41. Hancock E. Subacute effusive-constrictive pericarditis. *Circulation* 1971; **43**(2): 183-92.
42. Syed FF, Ntsekhe M, Mayosi BM, Oh JK. Effusive-constrictive pericarditis. *Heart failure reviews* 2013; **18**(3): 277-87.
43. HOROWITZ MS, SCHULTZ CS, STINSON EB, HARRISON DC, POPP RL. Sensitivity and specificity of echocardiographic diagnosis of pericardial effusion. *Circulation* 1974; **50**(2): 239-47.
44. Rizi HR, Kline RC, Thrall JH, et al. Pericardial effusion swinging heart phenomenon by dynamic radionuclide ventriculography. 1981.

45. Ling LH, Oh JK, Tei C, et al. Pericardial thickness measured with transesophageal echocardiography: feasibility and potential clinical usefulness. *Journal of the American College of Cardiology* 1997; **29**(6): 1317-23.
46. Engel PJ, Fowler NO, Tei C, et al. M-mode echocardiography in constrictive pericarditis. *Journal of the American College of Cardiology* 1985; **6**(2): 471-4.
47. Ling LH, Oh JK, Schaff HV, et al. Constrictive pericarditis in the modern era: evolving clinical spectrum and impact on outcome after pericardiectomy. *Circulation* 1999; **100**(13): 1380-6.
48. Santamore WP, Bartlett R, Van Buren SJ, Dowd MK, Kutcher MA. Ventricular coupling in constrictive pericarditis. *Circulation* 1986; **74**(3): 597-602.
49. Walker CM, Chung JH, Reddy GP. Septal bounce. *Journal of thoracic imaging* 2012; **27**(1): w1.
50. Reuter H, Burgess L, Doubell A. Epidemiology of pericardial effusions at a large academic hospital in South Africa. *Epidemiology & Infection* 2005; **133**(3): 393-9.
51. Hillemann D, Rüscho-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *Journal of clinical microbiology* 2011; **49**(4): 1202-5.
52. Pandie S, Peter JG, Kerbelker ZS, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon- γ in a high burden setting: a prospective study. *BMC medicine* 2014; **12**(1): 101.
53. Dheda K, Pooran A, Pai M, et al. Interpretation of Mycobacterium tuberculosis antigen-specific IFN- γ release assays (T-SPOT. TB) and factors that may modulate test results. *Journal of Infection* 2007; **55**(2): 169-73.
54. Doherty M, Wallis RS, Zumla A. Biomarkers for tuberculosis disease status and diagnosis. *Current opinion in pulmonary medicine* 2009; **15**(3): 181-7.
55. Day TA, Penn-Nicholson A, Luabeya AKK, et al. Safety and immunogenicity of the adjunct therapeutic vaccine ID93+ GLA-SE in adults who have completed treatment for tuberculosis: a randomised, double-blind, placebo-controlled, phase 2a trial. *The Lancet Respiratory Medicine* 2021; **9**(4): 373-86.
56. Sagar S, Liu PP, Cooper Jr LT. Myocarditis. *The Lancet* 2012; **379**(9817): 738-47.
57. Falzon D, Jaramillo E, Schünemann H, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respiratory Soc; 2011.
58. Campbell EA, Korzheva N, Mustaev A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell* 2001; **104**(6): 901-12.
59. Vilchèze C, Jacobs J, William R. The mechanism of isoniazid killing: clarity through the scope of genetics. *Annu Rev Microbiol* 2007; **61**: 35-50.

60. Zhang Y, Wade MM, Scorpio A, Zhang H, Sun Z. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. *Journal of Antimicrobial Chemotherapy* 2003; **52**(5): 790-5.
61. Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. *Antimicrobial agents and chemotherapy* 1989; **33**(9): 1493-9.
62. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrobial agents and chemotherapy* 2006; **50**(4): 1170-7.
63. Arbex MA, Varella MdCL, Siqueira HRd, Mello FAFd. Antituberculosis drugs: drug interactions, adverse effects, and use in special situations-part 1: first-line drugs. *Jornal Brasileiro de Pneumologia* 2010; **36**(5): 626-40.
64. Wehrli W. Rifampin: mechanisms of action and resistance. *Reviews of infectious diseases* 1983; **5**(Supplement_3): S407-S11.
65. Zhang Y, Young DB. Molecular mechanisms of isoniazid: a drug at the front line of tuberculosis control. *Trends in microbiology* 1993; **1**(3): 109-13.
66. Augustynowicz-Kopec E, Zwolska Z. Bioavailability factors of isoniazid in fast and slow acetylators, healthy volunteers. *Acta poloniae pharmaceutica* 2002; **59**(6): 452-6.
67. Kergueris M, Bourin M, Larousse C. Pharmacokinetics of isoniazid: influence of age. *European journal of clinical pharmacology* 1986; **30**(3): 335-40.
68. Schaberg T, Rebhan K, Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *European Respiratory Journal* 1996; **9**(10): 2026-30.
69. Steele MA, Des Prez RM. The role of pyrazinamide in tuberculosis chemotherapy. *Chest* 1988; **94**(4): 845-50.
70. Zhang Y, Shi W, Zhang W, Mitchison D. Mechanisms of pyrazinamide action and resistance. *Microbiology spectrum* 2013; **2**(4): 1.
71. Zhu M, Starke JR, Burman WJ, et al. Population pharmacokinetic modeling of pyrazinamide in children and adults with tuberculosis. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 2002; **22**(6): 686-95.
72. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. *American journal of respiratory and critical care medicine* 2003; **167**(11): 1472-7.
73. Beggs W. Ethambutol. *Mechanism of Action of Antibacterial Agents*: Springer; 1979: 43-66.

74. Zhu M, Burman W, Starke J, et al. Pharmacokinetics of ethambutol in children and adults with tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 2004; **8**(11): 1360-7.
75. Nuermberger E, Grosset J. Pharmacokinetic and pharmacodynamic issues in the treatment of Mycobacterial infections. *European Journal of Clinical Microbiology and Infectious Diseases* 2004; **23**(4): 243-55.
76. Valcke Y, Pauwels R, Van der Straeten M. Pharmacokinetics of antibiotics in the lungs. *European Respiratory Journal* 1990; **3**(6): 715-22.
77. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases* 2013; **208**(9): 1464-73.
78. Spodick D. The technique of pericardiocentesis. When to perform it and how to minimize complications. *The Journal of critical illness* 1995; **10**(11): 807-12.
79. Katada K, Kato R, Anno H, et al. Guidance with real-time CT fluoroscopy: early clinical experience. *Radiology* 1996; **200**(3): 851-6.
80. Callahan JA, Seward JB, Nishimura RA, et al. Two-dimensional echocardiographically guided pericardiocentesis: experience in 117 consecutive patients. *The American journal of cardiology* 1985; **55**(4): 476-9.
81. Kaye P. Myocardial contusion: emergency investigation and diagnosis. *Emergency Medicine Journal* 2002; **19**(1): 8-10.
82. Hingorani A, Bloomberg T. Ultrasound-guided pigtail catheter drainage of malignant pericardial effusions. *Clinical radiology* 1995; **50**(1): 15-9.
83. Kumar R, Sinha A, Lin MJ, et al. Complications of pericardiocentesis: a clinical synopsis. *International journal of critical illness and injury science* 2015; **5**(3): 206.
84. Nague O, Millaire A, Porte H, et al. Pericardioscopy in the etiologic diagnosis of pericardial effusion in 141 consecutive patients. *Circulation* 1996; **94**(7): 1635-41.
85. Strang J, Gibson D, Mitchison D, et al. Controlled clinical trial of complete open surgical drainage and of prednisolone in treatment of tuberculous pericardial effusion in Transkei. *The Lancet* 1988; **332**(8614): 759-64.
86. Maisch B, Ristic AD, Seferovic PM, Tsang TS. Interventional pericardiology: pericardiocentesis, pericardioscopy, pericardial biopsy, balloon pericardiotomy, and intrapericardial therapy: Springer Science & Business Media; 2011.
87. Imazio M, Brucato A, Mayosi BM, et al. Medical therapy of pericardial diseases: part II: noninfectious pericarditis, pericardial effusion and constrictive pericarditis. *Journal of Cardiovascular Medicine* 2010; **11**(11): 785-94.

88. Strang J, Gibson D, Nunn A, Kakaza H, Girling D, Fox W. Controlled trial of prednisolone as adjuvant in treatment of tuberculous constrictive pericarditis in Transkei. *The Lancet* 1987; **330**(8573): 1418-22.
89. Flynn JL, Chan J. Immunology of tuberculosis. *Annual review of immunology* 2001; **19**(1): 93-129.
90. Flynn JL, Goldstein MM, Chan J, et al. Tumor necrosis factor- α is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995; **2**(6): 561-72.
91. Lim HY, Müller N, Herold MJ, Van Den Brandt J, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. *Immunology* 2007; **122**(1): 47-53.
92. Tuckermann JP, Kleiman A, McPherson KG, Reichardt HM. Molecular mechanisms of glucocorticoids in the control of inflammation and lymphocyte apoptosis. *Critical reviews in clinical laboratory sciences* 2005; **42**(1): 71-104.
93. Alzeer A, FitzGerald J. Corticosteroids and tuberculosis: risks and use as adjunct therapy. *Tubercle and lung disease* 1993; **74**(1): 6-11.
94. Lawn SD, Wood R, Wilkinson RJ. Changing concepts of "latent tuberculosis infection" in patients living with HIV infection. *Clinical and developmental immunology* 2010; **2011**.
95. Cripp LH. Prednisone and prednisolone in the treatment of allergic diseases. *Journal of Allergy* 1956; **27**(3): 220-30.
96. Ross I, Lacerda M, Pillay T, et al. Salivary cortisol and cortisone do not appear to be useful biomarkers for monitoring hydrocortisone replacement in Addison's disease. *Hormone and Metabolic Research* 2016; **48**(12): 814-21.
97. Al-Habet S, Rogers H. Pharmacokinetics of intravenous and oral prednisolone. *British journal of clinical pharmacology* 1980; **10**(5): 503-8.
98. Baker RW, Walker BR, Shaw RJ, et al. Increased cortisol: cortisone ratio in acute pulmonary tuberculosis. *American journal of respiratory and critical care medicine* 2000; **162**(5): 1641-7.
99. Rook GA, Baker R. Cortisol metabolism, cortisol sensitivity and the pathogenesis of leprosy reactions. *Tropical Medicine & International Health* 1999; **4**(7): 493-8.
100. Andersson AK, Atkinson SE, Khanolkar-Young S, et al. Alteration of the cortisol–cortisone shuttle in leprosy type 1 reactions in leprosy patients in Hyderabad, India. *Immunology letters* 2007; **109**(1): 72-5.
101. Rook G, Baker R, Walker B, et al. Local regulation of glucocorticoid activity in sites of inflammation: Insights from the study of tuberculosis. *Annals of the New York Academy of Sciences* 2000; **917**(1): 913-22.

102. Organization WH, Initiative ST. Treatment of tuberculosis: guidelines: World Health Organization; 2010.
103. Makoid MC, Vuchetich PJ, Banakar UV. Basic Pharmacokinetics: Virtual University Press; 1996.
104. Bonate PL. Nonlinear models and regression. *Pharmacokinetic-Pharmacodynamic Modeling and Simulation* 2006: 93-124.
105. Davidian M. Introduction to statistical population modeling and analysis for pharmacokinetic data. Invited white paper for the International Workshop on Uncertainty and Variability in Physiologically Based Pharmacokinetic (PBPK) Models Retrieved from the Internet: URL: <http://www.epa.gov/ncct/uvpkm/files/Calibration—PreMeeting—Draft.pdf> (89 pages)[Retrieved on Jan 29, 2014]; 2006: Citeseer; 2006.
106. Goutelle S, Bourguignon L, Maire PH, Van Guilder M, Conte JE, Jelliffe RW. Population modeling and Monte Carlo simulation study of the pharmacokinetics and antituberculosis pharmacodynamics of rifampin in lungs. *Antimicrobial agents and chemotherapy* 2009; **53**(7): 2974-81.
107. Bergmann TK, Barraclough KA, Lee KJ, Staatz CE. Clinical pharmacokinetics and pharmacodynamics of prednisolone and prednisone in solid organ transplantation. *Clinical pharmacokinetics* 2012; **51**(11): 711-41.
108. Loos U, Musch E, Jensen J, Mikus G, Schwabe H, Eichelbaum M. Pharmacokinetics of oral and intravenous rifampicin during chronic administration. *Klinische Wochenschrift* 1985; **63**(23): 1205-11.
109. Conney A. Pharmacological implications of microsomal enzyme induction. *Pharmacological Reviews* 1967; **19**(3): 317-66.
110. Gabrielsson J, Weiner D. Non-compartmental analysis. Computational toxicology: Springer; 2012: 377-89.
111. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Bmj* 2009; **338**: b2393.

2. Chapter 2: Rationale, aims and objectives

2.1. Rationale

Tuberculous pericarditis is associated with poor treatment outcomes.¹ The pharmacokinetics of first line anti-TB drugs are largely unknown hence sub-therapeutic drug concentrations are a possible cause the poor outcomes. Furthermore, while adjunctive corticosteroid therapy with 120 mg of prednisolone has been shown to reduce incidence of constrictive pericarditis and reduce the duration of hospitalization, it is unknown whether this is the optimum dose or whether there is room to further escalate the dose.² This body of work seeks to characterize the pharmacokinetics of rifampicin, isoniazid, ethambutol, pyrazinamide and prednisolone in pericardial fluid and determine whether these doses are sufficient for maximal therapeutic effect.

While tuberculous pericarditis (TBP) is a relatively uncommon form of tuberculosis (TB),¹ this form of TB differs from pulmonary TB, due to its different clinical presentation and approach to diagnosis and management.² Patients with TBP commonly present with not only constitutional symptoms which are synonymous with TB, but also cardiac specific symptoms and signs due to the site of the *Mycobacterium* infection.²

The management of TBP has remained largely unchanged for over half a century, despite several major advances in the field of medicine and cardiology in particular, invention of echocardiography (ECHO), pharmacokinetics, interventional cardiology, pericardioscopy and electrophysiology.^{1,3,4} Anti-TB chemotherapy remains the cornerstone of TBP treatment.⁵

Adjunctive corticosteroid therapy and pericardiocentesis are only indicated in certain TBP patients.^{5,6} Mayosi *et al*, showed that adjunctive corticosteroid therapy reduced

incidence of constrictive pericarditis by 43% and reduced hospitalization by 18%, but due to the risk of HIV associated malignancies, only recommend adjunctive corticosteroid therapy for patients who are HIV negative.⁵ While pericardiocentesis is of diagnostic and therapeutic benefit,⁶ it is reserved for participants who have pericardial effusions, which are large enough for safe pericardiocentesis.⁷ The size deemed large enough for pericardiocentesis varies from one health facility to another and is dependent on the resources available and level of experience of a health facility.

Given the high mortality and morbidity associated with TBP, there is a need to find more innovative ways to improve the management of TBP.⁸

There is extensive literature on the pharmacokinetics of rifampicin, ethambutol, pyrazinamide and isoniazid in plasma, but the pharmacokinetics of these drugs in the pericardial compartment, remains so far unexplored.^{9,10} Understanding the relationship between drug concentrations in the pericardial compartment and the more accessible plasma compartment may assist researchers with the prediction of pharmacokinetics of anti-TB therapy in the pericardial compartment of patients in whom pericardiocentesis is not possible and may in the future optimize the dosing schedule of anti-TB chemotherapy for this indication.

Objectively quantifying the clinical benefits of corticosteroid therapy is a challenge; therefore, identifying a surrogate, which reflects immunological response at a cellular level, could serve as a biochemical marker. Cytokines and endogenous corticosteroids are possible candidates for such surrogate makers.

Elevated serum or bronchial alveolar lavage cortisol/ cortisone ratios have been observed at the site of Mycobacterial infection, and this rise has been shown to revert to normal with resolution of disease.^{11,12} This interesting phenomenon has potential utility as a surrogate of disease severity and or treatment of response. However, it remains unknown whether a similar rise in cortisol/ cortisone ratio occurs in pericardial fluid of patients with TBP.

Enzymes regulating peripheral cortisol and cortisone concentrations, specifically 11-beta-hydroxysteroid dehydrogenase type 1 (11HSD-1) and 11-beta-hydroxysteroid dehydrogenase type 2 (11HSD-2),¹³ are influenced by cytokines, for example, tumour necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β), favouring cortisol in the

cortisol-cortisone equilibrium.¹⁴ Therefore, it is essential to ascertain if there is an increase in the pericardial cortisol/ cortisone ratio in TBP patients and to determine if this rise correlates with the expected changes in cytokine concentrations. Additionally, by confirming the shift in equilibrium towards the formation of cortisol in pericardial fluid in patients with TBP, this will reaffirm our understanding of yet another inflammatory condition and this may lend itself to future manipulation, if it is deemed harmful or insufficient.

2.2. Aims

The overall aims of this study are to describe the pharmacokinetics of rifampicin, ethambutol, pyrazinamide, isoniazid and prednisolone, examined in the pericardial space in patients with TBP, with a view to determining how these relate to the other more accessible plasma compartment. The study also aims to determine the pharmacokinetic profiles of prednisolone in the pericardial compartment and to evaluate how it relates to the plasma and saliva compartments. This study will also explore the effect of corticosteroid therapy on cytokine concentrations in plasma, pericardial fluid and saliva. The study also aims to examine endogenous cortisol and its metabolite cortisone in the 3 matrices namely, plasma, pericardial space and saliva.

2.3. Objectives

2.3.1. To describe the pharmacokinetics of rifampicin, ethambutol, pyrazinamide and isoniazid in TBP:

- i. To describe the pharmacokinetics of rifampicin, ethambutol, pyrazinamide and isoniazid in the pericardial space.

- ii. To describe the relationship of rifampicin, ethambutol, pyrazinamide and isoniazid pharmacokinetics in the pericardial and plasma matrices.
- iii. To determine whether pharmacologically adequate concentrations of rifampicin, ethambutol, pyrazinamide and isoniazid are achieved in the pericardial compartment, as defined by area under the curve (AUC), maximum concentration ($C_{\max}$) and minimum inhibitory concentration (MIC).

2.3.2. Describe the pharmacokinetics of prednisolone in TBP patients:

- i. To describe the pharmacokinetics of prednisolone in the pericardial space in patients receiving therapeutic doses of prednisolone.
- ii. To describe the relationship between the pharmacokinetics of prednisolone in three compartments; plasma, pericardial space and saliva.
- iii. To assess whether salivary prednisolone concentration relates to pericardial prednisolone concentration.

2.3.3. Describe the effect of prednisolone on cytokine response in TBP patients:

- i. Determine the effect of prednisolone on plasma, pericardial and saliva concentrations of the following cytokines and chemokines; IFN- γ , IL-1 α , IL-1 β , IL-6, IL-10, IL-12p40, TNF- α , IL-8, IP-10, caspase 3, caspase 8 and caspase 9.
- ii. Evaluate whether there was a difference in cytokine responses between the three compartments namely, plasma, pericardial fluid and saliva.

2.3.4. Describe pericardial, plasma and saliva endogenous glucocorticoids and cytokine concentrations in patients with TBP.

- i. Describe cortisol/cortisone ratio, IFN- γ , IL-1 α , IL-1 β , IL-6, IL-10, IL-12p40, TNF- α , IL-8, IP-10, caspase 3, caspase 8 and caspase 9 concentrations in the pericardium, plasma and saliva of patients with TBP.
- ii. Correlate pericardial cortisol/ cortisone ratio, IFN- γ , IL-1 α , IL-1 β , IL-6, IL-10, IL-12p40, TNF- α , IL-8, IP-10, caspase 3, caspase 8 and caspase 9 concentrations to those of plasma and saliva.

Chapter 3 details a description of the study setting, study methods and ethical considerations. Chapter 4 describes the pharmacokinetics of rifampicin, isoniazid, pyrazinamide and ethambutol in plasma and pericardial fluid and describes the relationship between these two compartments. Chapter 5 determines the pharmacokinetics of prednisolone in plasma, pericardial fluid and saliva. Chapter 6 evaluates the effect of prednisolone on cytokine profiles in plasma, pericardial fluid and saliva. Chapter 7 describes the pericardial endogenous glucocorticoids and cytokine concentrations in relation to plasma and saliva compartments. Finally, chapter 8 summarizes the major findings of all the studies, which are part of this body of work, and will provide recommendations, which will impact on the management on TBP effusions. It is hoped that this body of work will contribute to altering the management of TBP and will improve the morbidity and mortality of TBP patients in a resource limited setting with a high burden of TB.

2.4. References

1. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
2. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. *BMC infectious diseases* 2006; **6**(1): 2.
3. Schrire V. Experience with pericarditis at Groote Schuur Hospital, Cape Town. *South African Medical Journal* 1959; **33**(9).
4. Strang J, Nunn A, Johnson D, Casbard A, Gibson D, Girling D. Management of tuberculous constrictive pericarditis and tuberculous pericardial effusion in Transkei: results at 10 years follow-up. *Qjm* 2004; **97**(8): 525-35.
5. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
6. Krikorian JG, Hancock EW. Pericardiocentesis. *The American journal of medicine* 1978; **65**(5): 808-14.
7. HOROWITZ MS, SCHULTZ CS, STINSON EB, HARRISON DC, POPP RL. Sensitivity and specificity of echocardiographic diagnosis of pericardial effusion. *Circulation* 1974; **50**(2): 239-47.
8. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *SAMJ: South African Medical Journal* 2008; **98**(1): 36-40.
9. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases* 2013; **208**(9): 1464-73.
10. McIlleron H, Wash P, Burger A, Norman J, Folb PI, Smith P. Determinants of rifampin, isoniazid, pyrazinamide, and ethambutol pharmacokinetics in a cohort of tuberculosis patients. *Antimicrobial agents and chemotherapy* 2006; **50**(4): 1170-7.
11. Baker RW, Walker BR, Shaw RJ, et al. Increased cortisol: cortisone ratio in acute pulmonary tuberculosis. *American journal of respiratory and critical care medicine* 2000; **162**(5): 1641-7.
12. Andersson AK, Atkinson SE, Khanolkar-Young S, et al. Alteration of the cortisol–cortisone shuttle in leprosy type 1 reactions in leprosy patients in Hyderabad, India. *Immunology letters* 2007; **109**(1): 72-5.

13. Rook G, Hernandez-Pando R. Pathogenetic role, in human and murine tuberculosis, of changes in the peripheral metabolism of glucocorticoids and antiglucocorticoids. *Psychoneuroendocrinology* 1997; **22**: S109-S13.
14. Rook G, Baker R, Walker B, et al. Local regulation of glucocorticoid activity in sites of inflammation: Insights from the study of tuberculosis. *Annals of the New York Academy of Sciences* 2000; **917**(1): 913-22.

3. CHAPTER 3: Methods

3.1. Setting

According to the World Health Organization Global Tuberculosis 2018 report, the overall incidence of tuberculosis (TB) in South Africa was 520 per 100 000.^{1,2} Tuberculous pericarditis (TBP) makes up 1% of all TB cases.³ Most TB cases are diagnosed at primary health-care facilities, while TBP is more likely to be diagnosed at district and tertiary hospitals, as they require investigation modalities, which have limited availability for example, chest x-rays, fluoroscopy, echocardiography (ECHO) and electrocardiograms (ECG) .⁴

This was a pilot study which recruited newly diagnosed patients with TBP from Groote Schuur Hospital, South Africa, a tertiary hospital situated in the southern district of the city of Cape Town. Groote Schuur Hospital has a specialized cardiology unit, which has experience in managing TBP for example, echocardiography services, cardiac catheterization laboratory and an acute coronary care ward.⁵ These facilities permit appropriate diagnosis and management of TBP. Groote Schuur Hospital provides a controlled environment for safe pericardiocentesis and a ward, which offers intensive monitoring after pericardiocentesis.⁵ This body of work was a sub-study of a randomized control trial, which investigated the use of adjunctive prednisolone and *Mycobacterium pranii* in the management of TBP (IMPI) study.⁶ The IMPI study was a large clinical trial, which recruited 1400 patients from 19 sites across the African continent.

3.2. Eligibility criteria

Participants who were diagnosed with TBP and had a large pericardial effusion, requiring pericardiocentesis were considered suitable for enrolment in my study. A pericardial effusion with a depth of greater than 10 mm, was deemed large enough to require pericardiocentesis as defined by Horowitz *et al.*⁷ A diagnosis of TBP was made on the basis of a pericardial effusion with evidence of TB. Participants were categorized as having either a definite or probable diagnosis of TB. A definite diagnosis of TBP was made when there was bacteriological confirmation of *Mycobacterium tuberculosis* in the pericardial fluid either positive GeneXpert® or culture, whereas a probable diagnosis of TBP was made when there were clinical features, biochemical biomarkers, which were suggestive of TB or when there was confirmation of *Mycobacterium* at another clinical site. To ensure consistency in the manner in which clinicians made the diagnosis of probable TBP, the Tygerberg diagnostic algorithm or an elevated adenine deaminase (ADA) equal to or greater than 40 units/ml were used to guide clinicians in making a diagnosis of probable TBP.^{8,9}

3.3. Pericardiocentesis

Pericardiocentesis was performed independently by me in the cardiac catheterization laboratory, under fluoroscopy and electrocardiography guidance. The Seldinger technique was used to gain access to the pericardial space, subsequently a pig tail catheter was used to drain the pericardial fluid to avoid damaging the myocardium. The pericardial effusion was drained until there was no residual pericardial fluid. The pig tail catheter was left *in-situ*, combined with a one-way valve to prevent air entering

the pericardial space, reducing the likelihood of infection and prevention inadvertent leakage of the pericardial fluid between sample collection.

3.4. Data collection

Once a participant was enrolled the following data were collected; demographic information which included age and sex. The study also collected basic anthropometric data including weight and height. Clinical data including symptoms, duration of illness, mode of TBP diagnosis (definite or probable TBP), presence of HIV co-infection, CD4 counts if appropriate, were also collected. The cardiovascular status was assessed using; the New York Heart Association functional classification, pulse, blood pressure, and ECHO findings (which included depth of pericardial effusion, left ventricular ejection fraction and pericardial thickness). The following laboratory investigations were performed including hemoglobin, serum creatinine, total serum protein, serum albumin and pericardial fluid ADA were also collected.

3.5. Sample collection

Baseline plasma, pericardial fluid and salivary samples were collected just before administration of the prednisolone or placebo. Subsequent samples of all three matrices were collected at 0.5 hour, 1 hour, 2 hour, 3 hour, 5 hour, 8 hour and 24 hour intervals, after administration of either prednisolone or equivalent placebo.

Where possible, the first dose of prednisolone was administered contemporaneously with the initial dose of anti-TB therapy, but where this was not possible, the time at which the anti-TB drugs were administered was obtained from the medication administration charts in the hospital notes. On sampling the pericardial space, an initial 2 ml of fluid was discarded to remove pericardial fluid left in the pig tail catheter during the previous sample collection. Saliva samples were obtained using a saliva collection device, called a Sarstedt salivette®, which is a highly absorbent sponge-like device, on which the participant was asked to chew for 30 seconds. Prior to the collection of each salivary sample participants were asked to rinse their mouths with water. On completion of the sample collection the participant was then discharged on TB treatment and was followed up on an outpatient basis.

3.6. Pre-analytical storage

Each plasma sample was centrifuged for 10 minutes at 1500 revolutions per minute and the supernatant was collected and stored in a -80°C freezer. The saliva and pericardial fluid samples were stored, without being centrifuged

3.7. Methods for measurement of drugs, cytokines and hormones

High performance liquid chromatography tandem mass spectrometry (HPLC-MS) was used to measure the concentrations of rifampicin, isoniazid, pyrazinamide, ethambutol, prednisolone, prednisone, cortisol, cortisone and their respective metabolites. The assays for rifampicin, isoniazid, pyrazinamide and ethambutol were validated in plasma and pericardial fluid. On the other hand, prednisolone, prednisone, cortisol and cortisone were validated in plasma, pericardial fluid and saliva. The

samples were processed by the Division of Clinical Pharmacology, University of Cape Town using an AB SCIEX API 4000 instrument.

The cytokines included tumour necrosis alpha (TNF- α), interferon gamma (IFN- γ), interleukin 1 β (IL-1 β), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 12 (IL-12), interleukin 22 (IL-22) transforming growth factor beta (TGF- β) and interferon gamma-induced protein 10 (IP-10), which were measured using Luminex Milliplex kits® (HCYTOMAG-60 K, Millipore, St Charles, MO, USA) and are custom made enzyme linked immune multiplexed cytokine arrays (ELISA) kits. While Caspases 3, 8 and 9 were measured using human *in vitro* ELISA kits from Abcam (Cambridge, UK).

3.8. Ethical considerations

The study adhered to the 59th World Medical Association General Assembly, October 2008 Declaration of Helsinki and was approved by the University of Cape Town Human Research Ethics Committee (ethics reference number 102/2003).¹⁰ Only patients older than 18 years of age were enrolled into the study and all patients were requested to provide written informed consent before taking part in the study. The consent forms were available in English, Afrikaans and Xhosa.

3.9. References

1. Organization WH. Global tuberculosis report 2018. 2018. *Geneva: World Health* 2019.
2. Health SAdo. National Tuberculosis Management Guidelines 2014: Department of Health; 2014.
3. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
4. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. *BMC infectious diseases* 2006; **6**(1): 2.
5. Schrire V. Experience with pericarditis at Groote Schuur Hospital, Cape Town. *South African Medical Journal* 1959; **33**(9).
6. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
7. HOROWITZ MS, SCHULTZ CS, STINSON EB, HARRISON DC, POPP RL. Sensitivity and specificity of echocardiographic diagnosis of pericardial effusion. *Circulation* 1974; **50**(2): 239-47.
8. Reuter H, Burgess L, Van Vuuren W, Doubell A. Diagnosing tuberculous pericarditis. *Journal of the Association of Physicians* 2006; **99**(12): 827-39.
9. Burgess LJ, Reuter H, Carstens ME, Taljaard JF, Doubell AF. The use of adenosine deaminase and interferon- γ as diagnostic tools for tuberculous pericarditis. *Chest* 2002; **122**(3): 900-5.
10. Williams JR. The Declaration of Helsinki and public health. *Bulletin of the World Health Organization* 2008; **86**: 650-2.

4. CHAPTER 4: Poor Penetration of Antibiotics Into Pericardium in Pericardial Tuberculosis

4.1. Introduction

Tuberculous pericarditis (TBP) is a relatively uncommon medical condition, accounting for approximately 1% of all tuberculosis (TB) cases.¹⁻³ Despite treatment, one in every four patients dies within six months of being diagnosed, and close to one in two patients with human immunodeficiency virus (HIV) co-infection, do not survive six months.¹ The main causes of death were either pericarditis or disseminated TB, which accounted for more than 42% of deaths in a large study of pericarditis, involving 1400 patients.⁴ These outcomes were encountered despite adherence to treatment in about 90% of patients, and are thus attributable to primary failure of standard therapy. The reasons for therapy failure are unclear, but could include high bacterial burden, concurrent immunodeficiency, or treatment factors that could be summarized as antimicrobial pharmacokinetics and/or pharmacodynamics (PK/PD).^{5,6} PK/PD science relates drug concentration of antibiotics at site of infection to microbial kill and resistance emergence; non-protein bound concentrations are central to pharmacodynamics effects.⁵⁻⁸

First-line anti-TB drugs exert their effect by binding directly to the bacterial targets inside the *Mycobacterium tuberculosis* (*Mtb*) cells. Only non-protein bound drug concentrations enter bacteria. Therefore, in TBP, each antibiotic must penetrate several layers of tissue to achieve adequate free-drug concentrations in the pericardial

space. The antibacterial effect of rifampicin, isoniazid, ethambutol and pyrazinamide is closely linked to specific peak concentration, 0–24 hour area under the concentration–time curve (AUC_{0-24}), and minimum inhibitory concentration (MIC).⁹⁻¹³ Utilizing standard treatment of pulmonary TB, recommended doses barely achieve these target concentrations.⁹⁻¹³ Moreover, despite administration of recommended doses, patient pharmacokinetic variability leads substantial proportions of patients to metabolize some of the drugs more extensively than others, who may end up on effective mono-therapy, therapy failure, and acquired drug resistance.^{11,14,15} It is unknown to what extent these drugs penetrate into pericardial space, or the variability thereof. We performed a pilot study to identify the rifampicin concentrations in paired plasma and pericardial fluid samples, followed by a second more intensive pericardial fluid pharmacokinetic sampling study for all four anti-TB drugs. We hypothesize that first line anti-TB treatment at standard doses which was designed for pulmonary TB, is inadequate for TBP and could be the impetus of poor outcomes associated with TBP. The findings of these studies are reported herein.

4.2. Methods

4.2.1. Ethics

Ethics approval was obtained from the University of Cape Town Human Research Ethics Committee prior to commencement of the study. Informed consent forms were available in isiXhosa, Afrikaans, and English and registered at clinicaltrials.gov (NCT00810849).

4.2.2. Setting

Both the pilot and intensive sampling pharmacokinetic sub-studies were performed at Groote Schuur Hospital in Observatory, Cape Town, South Africa. The pilot study was performed between June and December 2011. The intensive pharmacokinetic sampling study was performed between October 2012 and June 2014.

4.2.3. Case Definition

Patients 18 years or older, were enrolled if they had definite or probable pericardial TB, based on a TB pericarditis case definition.^{1,4,16,17} Definite TB pericarditis patients were those with confirmed pericardial TB, based on either the presence of acid and alcohol fast bacilli on microscopy of pericardial fluid, presence of *Mtb* by culture or by nucleic acid test in pericardial fluid or tissue, or histologic evidence of caseating granulomata. Probable TB pericarditis patients did not have evidence of these microbiological, nucleic acid, or histological features, in pericardial fluid or tissue, but had evidence of TB at another anatomical location and/ or presence of a lymphocytic pericardial exudate with elevated adenine deaminase or a Tygerberg TB Pericarditis Diagnostic Index Score ≥ 6 .^{1,4,16,17}

4.2.4. Pilot Study

The pilot study sample size was set based on precedence of some of our prior pharmacokinetic studies of anti-TB agents.¹⁸ Consecutive patients scheduled to undergo pericardiocentesis as part of their routine care, provided informed written consent. Patients had the four-drug standard therapy regimen administered under supervision of a study nurse, after which they underwent pericardiocentesis. At the time of study, most patients were treated with Rifafour®, each containing rifampicin

150 mg, isoniazid 75 mg, pyrazinamide 400 mg, and ethambutol 275 mg fixed dose combination (FDC). Without clashing with patients' scheduled time for pericardiocentesis, the timing of drug therapy was set to allow for sampling time points scattered through the 24-hour dosing interval. At each of several time points, simultaneous blood and pericardial fluid samples were drawn, placed on ice, processed, and stored at -80°C .

4.2.5. Intensive Sampling

Demographic data, clinical information and laboratory data were collected, including age, sex, weight, HIV status, and concomitant medication. Serum total protein and globulin were taken at the time of enrolment. Echocardiography, followed by fluoroscopy guided pericardiocentesis, using the Seldinger technique was performed. The pericardial effusion was drained to “dryness” (to the point of no residual drainage) and a pigtail catheter was left *in-situ*. Antibiotic therapy was administered under direct supervision, after which simultaneous blood and pericardial fluid samples were collected at 0 minutes (before drug administration), 30 minutes, 1, 2, 3, 5, 8 and 24 hour post-dose in heparinized tubes. The samples were also collected in ethylenediamine tetra-acetic acid (EDTA), centrifuged at 2000 relative centrifugal force for 10 minutes, after which supernatant was collected and stored at -80°C .

4.2.6. Drug Assays

The determination of concentrations in plasma and pericardial fluid was accomplished using validated tandem mass spectrometry high performance liquid chromatography-tandem mass spectrometry (HPLC-MS). The methods were validated in plasma and cross validated in pericardial fluid.¹⁹

4.2.6.1. Isoniazid concentrations

For quantification of isoniazid in 20 μ L of tripotassium ethylenediaminetetraacetic acid (K3EDTA) human plasma, protein precipitation with 0.1% acetic acid in acetonitrile was followed by chromatographic separation on a Kinetex Hilic C18, 2.6 μ m column. For quantification of pyrazinamide from 20 μ L human plasma, protein precipitation was achieved using a mixture of methanol and acetonitrile, and chromatographic separation on an Atlantis T3 C18, 3 μ m column under isocratic conditions.

4.2.6.2. Rifampicin, ethambutol pyrazinamide concentrations

For rifampicin and ethambutol quantification from 20 μ L plasma, protein precipitation was achieved with acetonitrile, followed by separation on a Discovery C18, 5 μ m, column for rifampicin, and a Phenomenex Onyx Monolith C18 (100 $\times$ 3.0 mm) column for ethambutol. An AB Sciex API 3000 mass spectrometer was operated in the multiple reactions monitoring mode, assessing the transitions 138.1 mass-to-charge ratio (m/z) to 79.1 m/z for isoniazid, 124.0 m/z to 81.4 m/z for pyrazinamide, 823.4 m/z to 791.4 m/z for rifampicin, and 205.2 m/z to 116.2 m/z for ethambutol.

The assays were validated over the concentration range of 0.102 μ g/mL to 26.0 μ g/mL for isoniazid, 0.203 μ g/mL to 81.1 μ g/mL for pyrazinamide, 0.117 μ g/mL to 30 μ g/mL for rifampicin, and 0.0844 μ g/mL to 5.40 μ g/mL for ethambutol. The between-day mean percentage accuracies for isoniazid were 101.0%, 103.2%, and 96.0%, those for pyrazinamide were 96.5%, 95.5% and 98.2%, for rifampicin 102.2%, 99.6% and 101.7%, and those for ethambutol were 98.5%, 101.3% and 99.0% at the high, medium, and low-quality control (QC) levels, respectively. The level of precision had

a coefficient of variation (% CV) of less than 13% at low, medium, and high QC levels for all analytes.

4.2.7. Pharmacokinetic Analysis

Previous studies of several hundred patients in the same Cape Town population as well as from elsewhere in the past have identified rifampicin, isoniazid, and pyrazinamide as one compartment model drugs, and ethambutol as a two compartment drug.^{11,20} The pharmacokinetics of each of these drugs was modelled and individual drug exposures were estimated in ADAPT version 5, 2009 software (Biomedical Simulations Resource, University of Southern California), assuming the same number of compartments identified in past studies. To identify clearance and volume of distribution in the pericardial space, the pericardial drug concentrations were modelled separately in ADAPT and the full 24-hour concentration–time curve identified from the pharmacokinetic model. These were then used to calculate the full AUC₀₋₂₄ in the pericardium.

4.2.8. Comparison of concentrations Achieved with Minimum Inhibitory Concentrations (MICs)

We compared drug concentrations achieved in pericardium to their MICs encountered in drug susceptible *Mtb* isolates in Cape Town, based on previous publications.^{13,21,22} Isolates from previous studies were used as a large number is needed in order to accurately identify the correct MIC distribution. Moreover, the non-protein bound peak-to-MIC and AUC-to- MIC ratios of the four first-line drugs determine microbiological and clinical outcomes, and because it is a central tenant of PK/ PD science to adjust

or calculate the non-protein bound concentrations at site of infection, we calculated the non-bound drug concentrations in pericardial fluid assuming 90% of rifampicin and 30% of ethambutol are protein bound.^{5-13,23-27} These drugs are bound to albumin.²⁷ In normal pericardial fluid, albumin/ total protein ratios are similar to plasma; the albumin ratio is higher in the pericardial fluid exudate during infection.^{28,29} Since isoniazid and pyrazinamide are only 10% protein bound, we used the total concentrations for these drugs.^{25,27}

4.2.9. Statistical Analysis

A non-parametric Mann–Whitney test was used to compare peak, AUC and clearances in pericardial fluid and in plasma. Similarly, a non-parametric Mann–Whitney test was used to compare the medians of non-protein bound concentrations and MICs.

4.3. Results

Sixteen patients were enrolled in the pilot study. The clinical and demographic features of these patients are shown in Table 4. Ten (63%) patients were male. Of the 16 patients, 10 were HIV-infected, with the mean CD4+ count of 307 as shown in Table 4. All patients, apart from one, received four fixed drug combination (FDC) anti-TB tablets (one patient received individual tablets, corresponding to each of the components of FDC), equivalent of rifampicin 600 mg a day, isoniazid 300 mg a day, pyrazinamide 1600 mg a day, and ethambutol 1100 mg a day. Blood and pericardial fluid were collected commencing at time 0 hour to 24 hours after the dose, as summarized in Table 4.

Table 4: Clinical demographic characteristics in pilot study patients

Variable	Mean	Range
Age in years	35.2	19.2-48.9
Time from dose to sample collection (hr)	4.82	0-24
Diastolic blood pressure (mmHg)	69.6	60-90
Haemoglobin (g/L)	9.7	5.9-14.3
White cell count $\times 10^3$ cells/ μ L	5.73	3.6-9.8
CD4+ cells/ml	307	263 ^a
Adenosine deaminase (U/L)	56.9	70-133.2
Pericardial fluid total protein (g/L)	56.9	38-75

^a standard deviation

The paired plasma and pericardial rifampicin concentrations are shown in Figure 9. The difference is illustrated in the figure and summarized by ratio of rifampicin pericardial fluid concentration to plasma concentration of 0.19 ± 0.33 for paired samples. Figure 10E compares the distribution of all the non-protein bound rifampicin concentrations to rifampicin MICs in Western Cape clinical isolates, and shows that none of the pericardial concentrations, at any sampling time-point, was above the MICs. The median concentration was 0.125 mg/L and was lower than the median MIC 0.208 mg/L ($p=0.001$). However, in the pilot study, the timing of sampling relative to the antibiotic dose was variable, which could explain some of the inter-individual variability of the results.

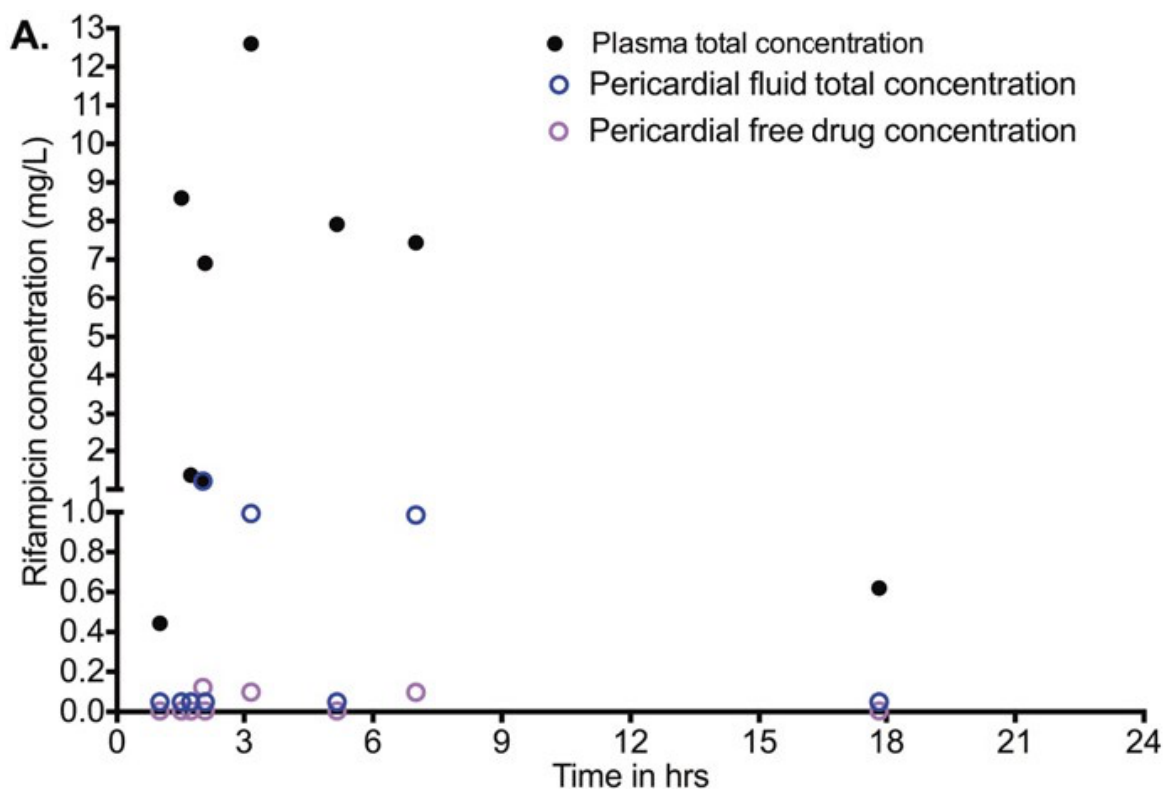


Figure 9: Rifampicin concentrations in plasma and pericardium in pilot study. The figure shows that, at all-time points the total rifampicin pericardial concentrations were lower than in the paired plasma. Moreover, derived pericardial free drug concentrations were even lower. In B, when all the concentrations at any time point were compared to the Minimum Inhibitory Concentrations (MICs), virtually all were below the MICs. Thus, no non-protein bound concentration was higher than MIC in pericardial space.

The pilot study results were used to identify the ideal sampling times to assess rifampicin and companion drug concentrations over a 24-hour period. Eleven patients were enrolled in this more intensive pharmacokinetic sampling study. Their clinical and demographic characteristics are shown in Table 5. Five patients were allocated to prednisolone, while the rest received placebo. All eleven patients received four FDC tablets, equivalent of rifampicin 600 mg, isoniazid 300 mg, ethambutol 1100 mg and pyrazinamide 1600 mg daily. One patient was also on antiretroviral therapy.

Table 5: Clinical and demographic characteristics in patients who underwent intensive pharmacokinetic sampling

Parameter	Mean or proportion	Range of %
Demographic and clinical characteristics		
Sex: male	5	45%
Age in years	37.02	23.98-58.76
Weight in kg	58.36	40-82
Acid-fast bacilli on microscopy	9	82
HIV infected	8	73
Duration of symptoms in weeks	26.77	3-60
Blood counts and concentrations		
CD4+ count cells/mL	251	42-874
CD4+ $\leq$ 200 cells/mL	7	64%
Creatinine μ mol/L	87.00	20-257
Globulin in g/L	47.55	30-56
Pericardial fluid concentrations		
Total protein g/L	61.18	50-70
Adenosine deaminase U/L	77.70	25.90-133.00

The rifampicin concentrations achieved in patients in both the plasma and pericardium are shown in Figure 10A. The time to peak concentration in pericardial fluid was a

median (range) of 8.18 (6.85–15.40) hours, compared to 3.44 (2.76–3.77) hours in blood ($p<0.0001$). The form of the concentration time curves in Figure 10A, is characterized by high plasma peak concentrations, but very low and flattened peak concentrations in the pericardium. This is due to both delay and decrease in penetration from the plasma into pericardial fluid, and higher rifampicin clearance from pericardial fluid, compared to plasma (Figure 10A–B). All patients apart from one had higher clearance in pericardium than in plasma (Figure 10B), and the median clearances differed significantly ($p=0.002$). The non-protein bound rifampicin concentrations in pericardial fluid are also shown in Figure 10A–D, which demonstrates very low effective rifampicin concentrations in pericardial fluid. A comparison of free rifampicin peak concentrations to MICs encountered in isolates is shown in Figure 10E, which shows that the median rifampicin peak concentrations in pericardial fluid, were lower than the median MIC ($p=0.001$).

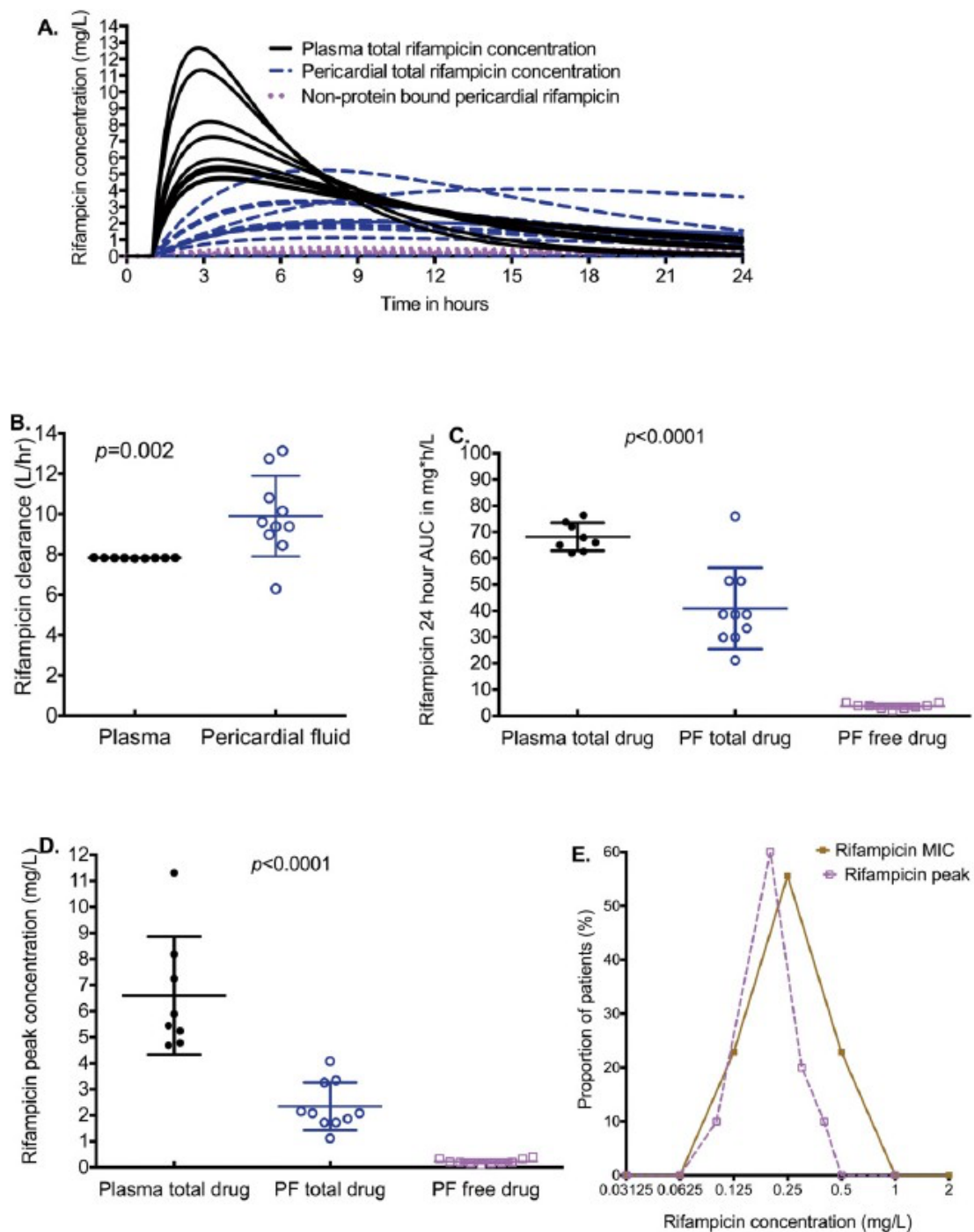


Figure 10: Rifampicin concentrations in the blood and pericardial fluid. The p -values shown throughout are for the Mann–Whitney test and compared the total drug concentrations in plasma versus pericardial fluid, and not the free drug. A. The concentration–time profiles of rifampicin in pericardium and in the blood over a 24-hour dosing interval. The time to maximum concentration was longer in pericardial fluid, while the peak concentrations were blunted. B. Clearance of rifampicin from pericardial fluid (PF) and plasma for each patient. The median clearance from the blood was 7.82 (range: 7.79–7.84) L/h while that in pericardial fluid was 9.49 (range: 6.30–13.14) L/h. C. The 0–24 hour AUC for total drug concentrations was lower in pericardial fluid than in plasma; the free drug concentrations were even lower. D. Total rifampicin peak concentrations achieved in pericardial fluid are lower than in plasma; free concentrations are substantially lower. E. Comparison of free rifampicin peak concentrations to minimum inhibitory concentrations (MICs) demonstrates that the maximum

concentrations achieved in pericardial fluid are lower than MICs. Thus, as the concentrations are cleared from the pericardium, the differences between drug concentration and MIC rapidly widens.

Ethambutol concentrations determined in pericardial fluid, are shown in Figure 11A. The figure shows significantly lower ethambutol concentrations were achieved in pericardium, compared to plasma ($p<0.001$). Figure 11A demonstrates time to maximum concentration between plasma and pericardial fluid and are similar in conformation. Despite the lower clearance rates in pericardium (Figure 11B), the ethambutol AUC_{0-24} in the pericardium was lower than plasma (Figure 11C). In addition, peak concentrations were also lower in pericardium (Figure 11D), compared with plasma. The lower peak and AUC_{0-24} in pericardial fluid compared to plasma, despite the lower clearance in pericardial fluid, are likely due to poor penetration. As a result, as shown in Figure 10E, the non-protein bound ethambutol concentrations were below previously encountered MICs.

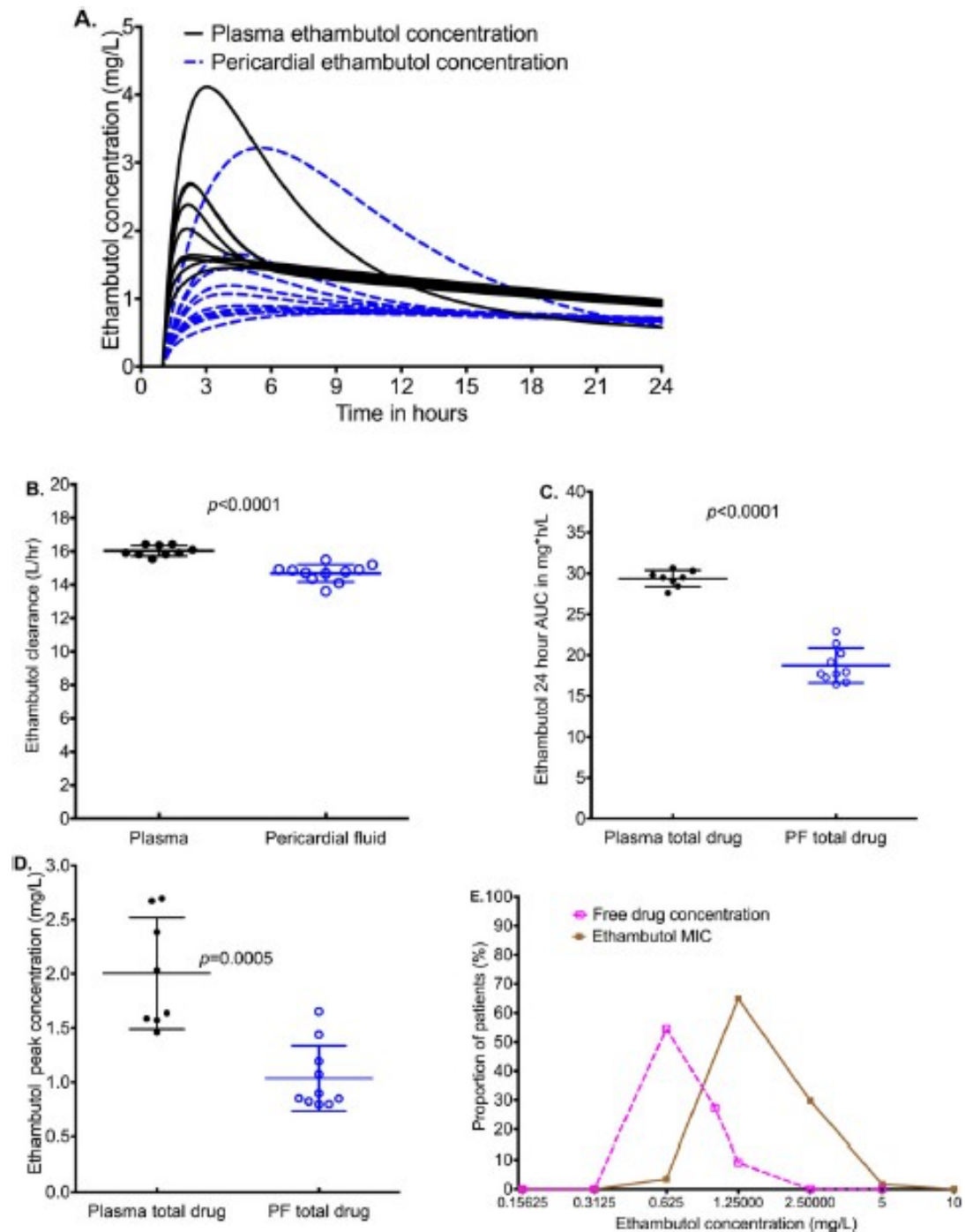


Figure 11: Ethambutol concentrations in plasma and pericardial fluid (PF). The concentration time profiles of ethambutol total concentration over 24 hours demonstrate reduced penetration of ethambutol. Clearance from pericardium was slightly lower than plasma (15.91 versus 14.77 L/h). Despite the lower clearance from pericardial fluid, the median AUC_{0-24} in pericardial fluid was 17.83 mg*hr/L, compared to 29.51 mg*hr/L in plasma, a 40% reduction. The median total ethambutol peak concentration was 0.87 mg/L in pericardial fluid compared to 1.84 mg/L in plasma and was thus 53% lower. Since ethambutol is 30% protein bound, this means the free peak concentration in pericardium is a median of 0.61 mg/L. The non-protein bound peak ethambutol concentrations were lower than MICs for most patients.

On the other hand, isoniazid concentrations in the pericardial fluid approximated those in the plasma in conformation, including time to maximum concentration (Figure 12A). The concentration–time curves, peak concentrations, clearances, and AUCs did not differ between the pericardial space and plasma (Figure 12A–D). As isoniazid is only 10% protein bound, these total concentrations approximate free drug concentrations. A comparison between isoniazid peak concentrations and previously encountered MICs which is shown in Figure 12E, demonstrates that the isoniazid peak concentrations in pericardial fluid were above MIC. The magnitude of the median peak concentration was 64-fold higher, compared with median MIC ($p<0.001$).

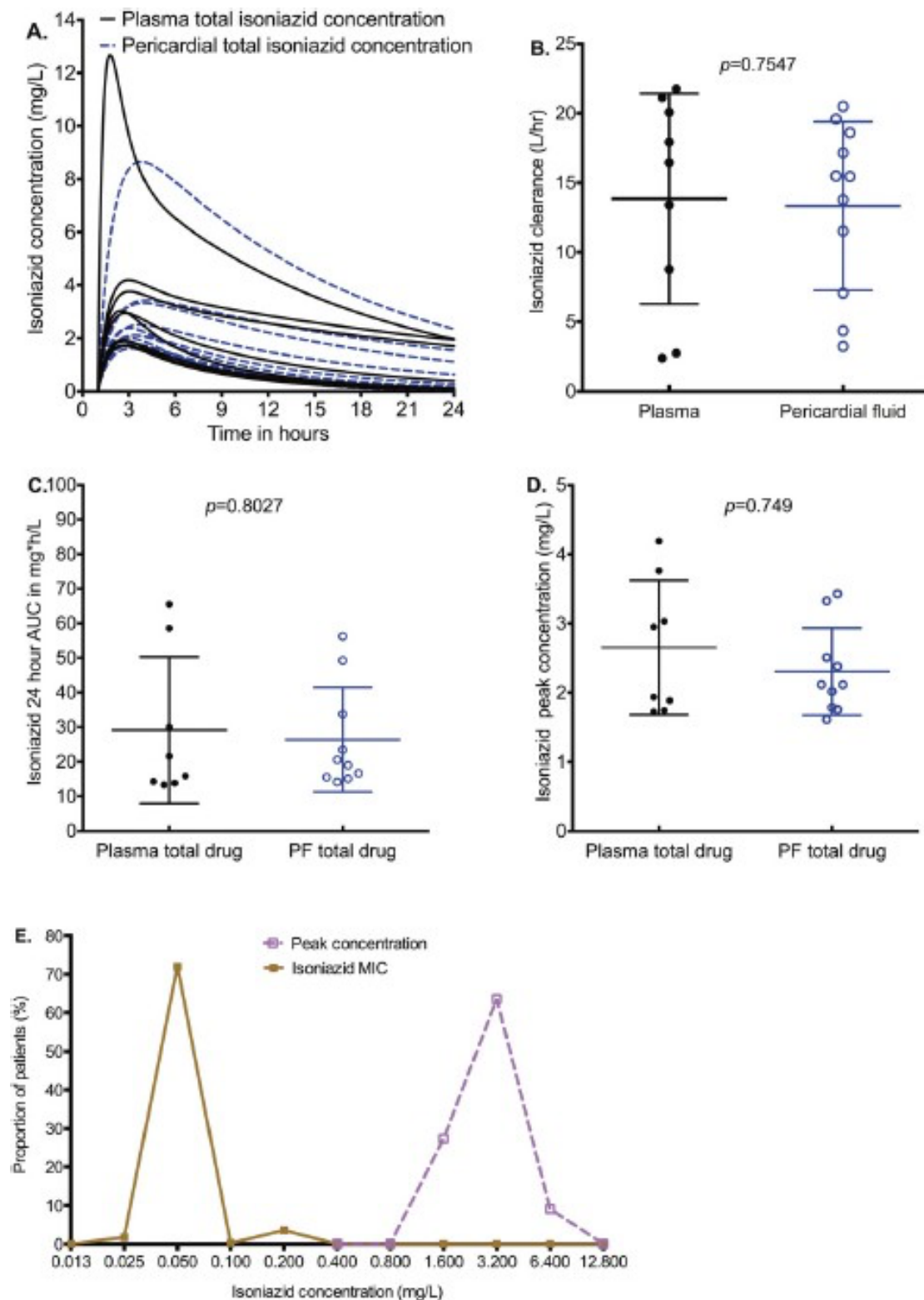


Figure 12: Isoniazid concentrations in plasma and pericardial fluid (PF). The concentration time profiles of total concentration over 24 hour demonstrate matching concentration. Clearance from pericardium was similar between plasma and pericardial fluid. The median pericardial fluid AUC₀₋₂₄ in pericardial fluid was 19.78mg*h/L compared to 18.75 mg*h/L in plasma. The median total pericardial peak concentration was 2.12 mg/L in pericardial fluid and 2.44 mg/L in plasma, virtually the same. Isoniazid penetrated well into pericardial fluid and achieved concentrations of at least 64-fold higher than MICs.

Pyrazinamide concentrations in pericardial fluid also closely tracked those in plasma. However, Figure 13A shows that there was a delay to peak concentration in pericardial fluid, compared to plasma. Nevertheless, similar peak concentrations were ultimately achieved (even though delayed); yet the AUCs were similar (Figure 13B–D). Pyrazinamide requires acidic conditions for bactericidal effect on *Mtb* but has no bactericidal effect where the ambient pH ≥ 6.8 .^{30,31} MIC decreases as pH falls, based on the Henderson–Hasselbach relationship.³¹ We measured the pH in pericardial fluid of 10 of the 11 patients, using pH probes. The between-test % coefficient of variation (CV) in pH measurements at 8 different times for the same sample was $\pm 3.27\%$, which corroborates precision with respect to the pH measurements. The pH results for the several measurements in each patient's sample are shown in Figure 14A. The mean $\pm$ (standard deviation) pericardial fluid pH was 7.34 ± 0.11 , similar to physiological pH. Since pyrazinamide MICs are performed at pH 5.9, the actual MICs at pH 7.34, were calculated using the Henderson–Hasselbach equation as described by Zhang *et al.*^{21,31,32} Figure 14B shows that the pyrazinamide peak concentrations were about 40-times lower than the pH-adjusted MICs, with a median peak of 42.93 mg/L versus a median MIC of 800 mg/L ($p < 0.0001$).

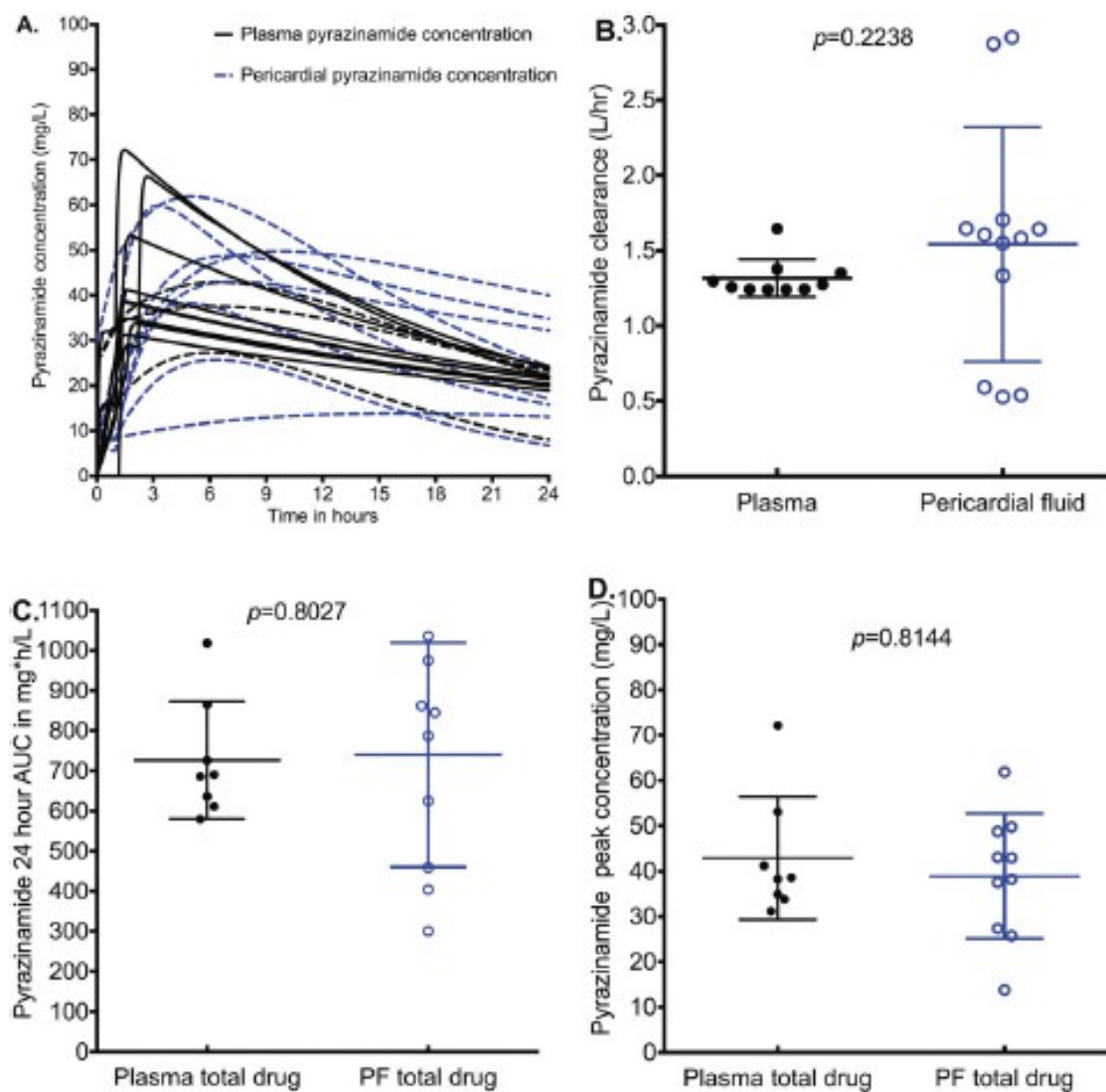


Figure 13: Pyrazinamide concentrations in plasma and pericardial fluid. While pericardial fluid pyrazinamide peak concentration was somewhat delayed, it nevertheless reached the same level as in plasma. Pyrazinamide clearance from the pericardium was similar to that from plasma. The median

pericardial fluid AUC₀₋₂₄ in pericardial fluid was 815.6 mg·h/L compared to 687.90 mg·h/L in plasma.

The median total pericardial peak concentration was 38.45 mg/L in pericardial fluid and 40.59 mg/L in plasma.

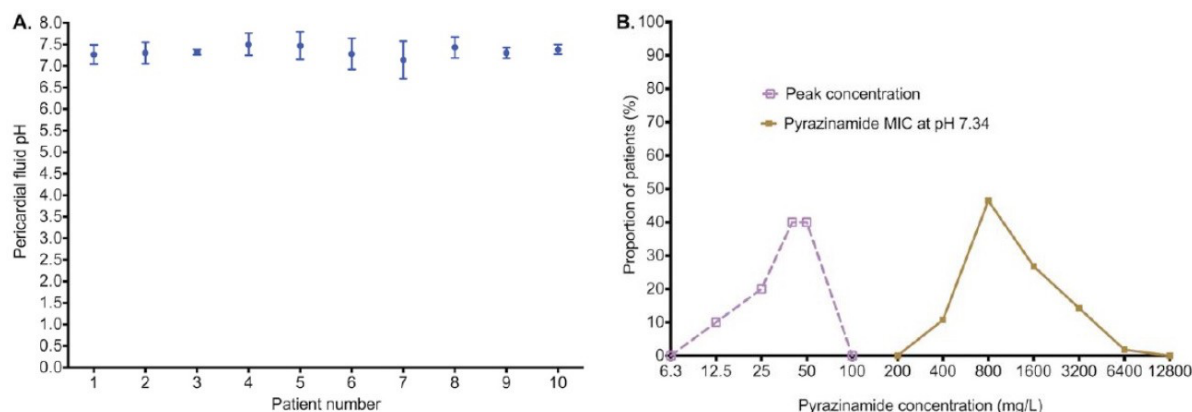


Figure 14: The pH of pericardial fluid and pyrazinamide MICs. A. The pH in pericardial fluid in each patient was measured 8 times in triplicate; shown are the mean values and standard deviation. The D'Agostino and Pearson omnibus normality test p-value was 0.991, indicating that the pH values in the pericardial fluid were normally distributed. All the pH values were greater than 7.0 and were similar to the pH of 7.365 in the plasma, which is slightly alkaline. B. Pyrazinamide MICs measured at pH 5.9 were used calculate the actual MICs at physiological pH based on the relationship between the active moiety pyrazinoic acid (POA; anion POA⁻) and HPOA in the Handersen–Hasselbach equation (POA $P_{K_a} = 2.9$), as demonstrated by Zhang et al. (2002). At pH 5.90 the % of the HPOA is 0.1% compared to 0.00363% at pH 7.34, a 27.54-fold change, which is the factor used to calculate the MIC value at pH 7.34 based on that identified at pH 5.9. This leads to an MIC with a median 18.6-fold higher than the median peak concentrations; indeed, the highest pyrazinamide peak concentration was 4-fold lower than the lowest MIC.

4.4. Discussion

We have identified poor penetration and rapid clearance of rifampicin in the infected space of TB pericarditis. There was delayed and decreased penetration from the blood into pericardial fluid, as demonstrated by both the peak concentration achieved and the time to maximum concentration. The reasons for this are unclear, but could include thickened pericardial layers, or fibrosis, among several possibilities.

The rapid clearance, compared with plasma is surprising, given the well-known extraction from the blood and metabolism by hepatocytes of rifampicin. We speculate that this may be due to an active physiological process, such as transport out of the

pericardium. The consequences, however, are concerning, given that rifampicin, in concert with pyrazinamide, is the primary drug associated with sterilizing effect in the standard TB treatment regimen. We found that the non-protein bound peak concentrations achieved in pericardium were below MICs encountered in Cape Town clinical historical *Mtb* isolates.¹³ AUCs were also very low, which implies that the AUC-to-MIC ratios were also low. Therefore, there is likely to be little bactericidal effect of rifampicin at concentrations achieved with the current doses in the standard regimen.^{5,7,9,11,13} Given the separation between non-protein bound peak and MICs, and the relationship of peak-to-MIC ratios for optimal rifampicin kill and suppression of resistance,^{7,9,11} it is unlikely that escalating the rifampicin dose alone would increase peak-to-MIC ratio to ranges that could provide sterilizing effect in TB pericarditis, without inducing toxicity, thus presenting a challenge in treating this condition.

Pyrazinamide concentrations were approximately similar to those of plasma. However, this is substantially lower than the concentrations associated with effective bactericidal effect at the site of infection. In pulmonary epithelial lining fluid, pyrazinamide is concentrated 17–22-fold, compared to plasma, which allows the pyrazinamide to achieve the peak concentrations and AUC-to-MIC ratios associated with optimal sterilizing effect in cavitary TB.^{11,24,33} Optimal efficacy is achieved at a pyrazinamide AUC-to-MIC ratio of ~210 at site of infection based on hollow fibre system model of TB, which has a quantitative predictive accuracy of 94% of clinical therapeutic values.^{11,24,34,35} The hollow fibre system model is an *in vitro* method used to simulate *in vivo* PK/ PD relationship of a given drug.³⁶ Median AUC-to-MIC ratios were ≤ 1 after adjusting for pH. Similarly, the median peak concentration to MIC ratio is unlikely to produce much bactericidal effect even under optimal pH conditions.^{7,24} These are all

the more concerning given that pyrazinamide has no antimicrobial effect at physiological pH and only works at a pH below 6.0.^{24,30-32} Direct measurement of pericardial fluid pH in our patients revealed that the pH was slightly alkaline and similar to physiological pH. At this pH, we expect little or no pyrazinamide effect on *Mtb* in pericardial fluid. Since rifampicin concentrations are also low, and the sterilizing effect of the current regimen is driven by the synergistic effect of these two drugs, this suggests that there may be little to no sterilizing effect during treatment of pericardial TB.^{11,13}

Ethambutol concentrations were also lower in pericardial fluid compared to plasma concentrations, due to reduced penetration. Similar to rifampicin, pyrazinamide, and isoniazid, ethambutol penetration from plasma into pericardial fluid varied by as much as 50% between patients. This additional variability needs to be taken into account when deciding on optimal doses. The result of the low ethambutol concentrations, when added to low rifampicin and pyrazinamide concentrations, suggesting that the drug concentrations of the majority of drugs in the regimen is suboptimal.

Isoniazid concentrations in pericardium appear to be the most favourable. Similar concentrations to those in plasma were achieved, resulting in peak and AUCs in the range associated with positive outcomes in other types of TB.⁷ In addition, the clearance was similar to that of plasma; thus, isoniazid pharmacokinetics in this compartment can easily be inferred from those in the blood. From the data identified in this pilot study, the doses of isoniazid for the treatment of TB pericarditis likely warrant no alteration.

These data imply that the current rifampicin, isoniazid, ethambutol, and pyrazinamide combination regimen may fail to provide an optimal therapeutic effect. Isoniazid alone seems to penetrate the pericardial fluid to achieve adequate concentrations. It has been shown previously that at low rifampicin concentrations such as those in pericardial fluid, isoniazid antagonizes rifampicin efficacy in a concentration-dependent fashion.^{13,33,37} Thus one consequence of a low rifampicin concentration, but adequate isoniazid concentration could be drug antagonism, further compromising the regimen. It is of concern that our current anti-TB drug regimen is of limited utility in TB pericarditis, based on the poor penetration of the key sterilizing effect drugs, potentially explaining the high mortality rates from TB pericarditis and disseminated TB, encountered despite receiving therapy. Given that we have now shown that proven TB pericarditis is a high bacillary burden disease, with about 10⁶ colony forming units per millilitre, and that TB pericarditis mortality is higher at CD4+ counts below 200 cells/mL,³⁸ it means that we are unlikely to derive benefit from the immune system to sterilize the pericardial space of this large bacterial burden. It is in this context that antibiotics with high efficacy are needed. Nevertheless, attempts at dose optimization for these drugs should still be made. We propose that a new regimen specific to TB pericarditis that is developed using the PK/ PD aspects specific to TB pericarditis, is needed. We therefore suggest that there is a need for drugs that are rapidly bactericidal, which sterilize the *Mtb* encountered in TB pericarditis, within weeks rather than months.

Our study has several limitations, including its small sample size. It is possible that with larger studies different results could be obtained. However, given the requirement to leave a pigtail catheter *in-situ* for the intensive pharmacokinetic sampling, it was

important to limit the number of patients exposed to this procedure. Thus, our results should be interpreted with caution. However, given our results, and that rifampicin was studied in a total of 27 patients, showing the same results, it is very likely that the same results will be obtained in larger studies. We did not measure the MICs directly from our patients in this study. However, given the small numbers of patients, the number of *Mtb* isolates would have been too few to identify an adequate distribution, may still have had to rely on MICs from larger studies. The use of steroids could also have altered the ability of antibiotics to penetrate into pericardium. However, there were no apparent differences in antibiotic concentrations between patients who received steroids and those who did not. Additionally, there was a wide variation in plasma and pericardial isoniazid concentrations among the study population. A single-nucleotide polymorphism of the N-acetyltransferase-2 (NAT2) gene has a marked effect on isoniazid metabolism.¹⁰ Unfortunately, the study did not collect pharmacogenetic information in order to determine NAT2 gene polymorphism status.

In summary, this is the first study of its kind to measure anti-TB drug concentrations in pericardial fluid. The study also related these concentrations to protein binding, and the pH encountered in TB pericardial fluid. Concentrations of rifampicin, ethambutol and pyrazinamide were substantially lower relative to MICs.

4.5. References

1. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
2. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. *BMC infectious diseases* 2006; **6**(1): 2.
3. Cherian G. Diagnosis of tuberculous aetiology in pericardial effusions. *Postgraduate medical journal* 2004; **80**(943): 262-6.
4. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
5. Gumbo T, Angulo-Barturen I, Ferrer-Bazaga S. Pharmacokinetic-pharmacodynamic and dose-response relationships of antituberculosis drugs: recommendations and standards for industry and academia. *The Journal of infectious diseases* 2015; **211**(suppl_3): S96-S106.
6. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clinical infectious diseases* 1998: 1-10.
7. Pasipanodya J, Gumbo T. An oracle: antituberculosis pharmacokinetics-pharmacodynamics, clinical correlation, and clinical trial simulations to predict the future. *Antimicrobial agents and chemotherapy* 2011; **55**(1): 24-34.
8. Zeitlinger MA, Derendorf H, Mouton JW, et al. Protein binding: do we ever learn? *Antimicrobial agents and chemotherapy* 2011; **55**(7): 3067-74.
9. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. *Antimicrobial agents and chemotherapy* 2007; **51**(11): 3781-8.
10. Gumbo T, Louie A, Liu W, et al. Isoniazid bactericidal activity and resistance emergence: integrating pharmacodynamics and pharmacogenomics to predict efficacy in different ethnic populations. *Antimicrobial agents and chemotherapy* 2007; **51**(7): 2329-36.
11. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases* 2013; **208**(9): 1464-73.
12. Srivastava S, Musuka S, Sherman C, Meek C, Leff R, Gumbo T. Efflux-pump—derived multiple drug resistance to Ethambutol Monotherapy in Mycobacterium tuberculosis and the

pharmacokinetics and pharmacodynamics of Ethambutol. *The Journal of infectious diseases* 2010; **201**(8): 1225-31.

13. Chigutsa E, Pasipanodya JG, Visser ME, et al. Impact of nonlinear interactions of pharmacokinetics and MICs on sputum bacillary kill rates as a marker of sterilizing effect in tuberculosis. *Antimicrobial agents and chemotherapy* 2015; **59**(1): 38-45.
14. Srivastava S, Pasipanodya JG, Meek C, Leff R, Gumbo T. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *The Journal of infectious diseases* 2011; **204**(12): 1951-9.
15. Pasipanodya JG, Srivastava S, Gumbo T. Meta-analysis of clinical studies supports the pharmacokinetic variability hypothesis for acquired drug resistance and failure of antituberculosis therapy. *Clinical Infectious Diseases* 2012; **55**(2): 169-77.
16. Reuter H, Burgess L, Van Vuuren W, Doubell A. Diagnosing tuberculous pericarditis. *Journal of the Association of Physicians* 2006; **99**(12): 827-39.
17. Pandie S, Peter JG, Kerbelker ZS, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon- γ in a high burden setting: a prospective study. *BMC medicine* 2014; **12**(1): 101.
18. Hall RG, Swancutt MA, Meek C, Leff RD, Gumbo T. Ethambutol pharmacokinetic variability is linked to body mass in overweight, obese, and extremely obese people. *Antimicrobial agents and chemotherapy* 2012; **56**(3): 1502-7.
19. Zhou Z, Wu X, Wei Q, et al. Development and validation of a hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the simultaneous determination of five first-line antituberculosis drugs in plasma. *Analytical and bioanalytical chemistry* 2013; **405**(19): 6323-35.
20. Hall RG, Swancutt MA, Meek C, Leff R, Gumbo T. Weight Drives Caspofungin Pharmacokinetic Variability in Overweight and Obese People: Fractal Power Signatures Beyond 2/3 or 3/4. *Antimicrobial agents and chemotherapy* 2013; AAC. 01490-12.
21. Gumbo T, Chigutsa E, Pasipanodya J, et al. The pyrazinamide susceptibility breakpoint above which combination therapy fails. *Journal of Antimicrobial Chemotherapy* 2014; **69**(9): 2420-5.
22. Gumbo T, Pasipanodya JG, Wash P, Burger A, McIlleron H. Redefining multidrug-resistant tuberculosis based on clinical response to combination therapy. *Antimicrobial agents and chemotherapy* 2014; **58**(10): 6111-5.
23. Ambrose PG, Bhavnani SM, Rubino CM, et al. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clinical Infectious Diseases* 2007; **44**(1): 79-86.

24. Gumbo T, Dona CSS, Meek C, Leff R. Pharmacokinetics-pharmacodynamics of pyrazinamide in a novel *in vitro* model of tuberculosis for sterilizing effect: a paradigm for faster assessment of new antituberculosis drugs. *Antimicrobial agents and chemotherapy* 2009; **53**(8): 3197-204.
25. Tawanda G. Chemotherapy of tuberculosis, Mycobacterium avium complex disease and leprosy. Goodman and Gilman's The Pharmacological basis of the therapeutics. McGraw Hill Med; 2011.
26. te Brake LH, Ruslami R, Later-Nijland H, et al. Exposure to total and protein-unbound rifampin is not affected by malnutrition in Indonesian tuberculosis patients. *Antimicrobial agents and chemotherapy* 2015; **59**(6): 3233-9.
27. Woo J, Cheung W, Chan R, Chan H, Cheng A, Chan K. *In vitro* protein binding characteristics of isoniazid, rifampicin, and pyrazinamide to whole plasma, albumin, and α -1-acid glycoprotein. *Clinical biochemistry* 1996; **29**(2): 175-7.
28. Ben-Horin S, Shinfeld A, Kachel E, Chetrit A, Livneh A. The composition of normal pericardial fluid and its implications for diagnosing pericardial effusions. *The American journal of medicine* 2005; **118**(6): 636-40.
29. Milevoj Kopcinovic L, Culej J. Pleural, peritoneal and pericardial effusions—a biochemical approach. *Biochemia medica: Biochemia medica* 2014; **24**(1): 123-37.
30. McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environments *in vitro*. *American review of tuberculosis* 1954; **70**(4): 748-54.
31. Zhang Y, Permar S, Sun Z. Conditions that may affect the results of susceptibility testing of Mycobacterium tuberculosis to pyrazinamide. *Journal of medical microbiology* 2002; **51**(1): 42-9.
32. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. *The international journal of tuberculosis and lung disease* 2003; **7**(1): 6-21.
33. Conte JE, Golden JA, Duncan S, McKenna E, Zurlinden E. Intrapulmonary concentrations of pyrazinamide. *Antimicrobial agents and chemotherapy* 1999; **43**(6): 1329-33.
34. Gumbo T, Pasipanodya JG, Nuermberger E, Romero K, Hanna D. Correlations between the hollow fiber model of tuberculosis and therapeutic events in tuberculosis patients: learn and confirm. *Clinical Infectious Diseases* 2015; **61**(suppl_1): S18-S24.
35. Gumbo T, Pasipanodya JG, Romero K, Hanna D, Nuermberger E. Forecasting accuracy of the hollow fiber model of tuberculosis for clinical therapeutic outcomes. *Clinical Infectious Diseases* 2015; **61**(suppl_1): S25-S31.
36. Klopogge F, Hammond R, Kipper K, Gillespie SH, Della Pasqua O. Mimicking *in-vivo* exposures to drug combinations *in-vitro*: anti-tuberculosis drugs in lung lesions and the hollow fiber model of infection. *Scientific reports* 2019; **9**(1): 1-8.

37. Srivastava S, Sherman C, Meek C, Leff R, Gumbo T. Pharmacokinetic mismatch does not lead to emergence of isoniazid-or rifampin-resistant *Mycobacterium tuberculosis* but to better antimicrobial effect: a new paradigm for antituberculosis drug scheduling. *Antimicrobial agents and chemotherapy* 2011; **55**(11): 5085-9.
38. Pasipanodya JG, Mubanga M, Ntsekhe M, et al. Tuberculous pericarditis is multibacillary and bacterial burden drives high mortality. *EBioMedicine* 2015; **2**(11): 1634-9.

5. CHAPTER 5: Detectable prednisolone is delayed in pericardial fluid, compared with plasma of patients with tuberculous pericarditis: A pilot study

5.1. Introduction

Tuberculous pericarditis (TBP) results from infection of the pericardium by *Mycobacterium tuberculosis* (*Mtb*).¹ TBP accounts for about 1% of all tuberculosis (TB) cases, but 26% of patients die within six months of diagnosis, rising to approximately 40% in those with human immunodeficiency virus (HIV) co-infection and signs of advanced immunosuppression.² About 60% of TBP patients have a pericardial effusion requiring aspiration.³ Pericardial aspiration may be indicated in patients with large pericardial effusions, especially if cardiac tamponade is present.⁴ The mainstay of the management of TBP is anti-TB chemotherapy, which may be augmented by adjunctive corticosteroids in those without advanced HIV related immunosuppression.^{3,4} Adjunctive corticosteroids have been shown to reduce the incidence of constrictive pericarditis, hasten resolution of symptoms, and reduce the incidence of hospitalization.³

Prednisolone is the preferred glucocorticoid in this setting.⁵ *Mtb* infection leads to pericardial inflammation, which is thought to be responsible for many of the debilitating symptoms patients experience in TBP.¹ Adjunctive prednisolone attenuates this inflammatory response. However, there is a lack of consensus on the optimal dose for adjunctive corticosteroid therapy. Part of the problem is that the degree of prednisolone penetration into the pericardium is unknown. Drug pharmacokinetics and penetration concentration gradients into TB lesions by antibiotics are major determinants of

microbial and clinical outcomes.⁶⁻¹⁰ Pericardiocentesis represents a rare opportunity to explore the relationships between the prednisolone concentrations in the pericardial compartment and plasma, which have not previously been determined. However, we were also interested in finding less invasive sampling procedures to use as future surrogates of serum and pericardial fluid prednisolone concentrations. The pharmacokinetics of prednisolone have been characterized in plasma and saliva.⁵

Prednisolone administered orally is rapidly absorbed into the plasma compartment, with a time to maximum concentration ($T_{\max}$) of 1– 3 hours,¹¹ and is 80% protein-bound, predominantly by globulin and albumin.^{12,13} Unbound prednisolone diffuses passively across cell membranes to the nucleus, where it binds to glucocorticoid receptor type II, forming a complex, which subsequently adheres to the glucocorticoid response element, down regulating transcription of pro-inflammatory cytokines.^{14,15} This down-regulation of pro-inflammatory pathways is concentration-dependent. The aim of the study was to identify the prednisolone penetration into pericardial fluid, and saliva.

5.2. Patients and methods

Our study was a sub-study of the now published Investigation of the Management of tuberculous Pericarditis (IMPI) trial, which sought to evaluate the effect of adjunctive prednisolone on the 6 months incidence of the combined outcome of mortality tamponade constrictive pericarditis of patients with TBP.³ In this sub-study, all participants were required to provide written informed consent. The study was approved by the University of Cape Town Research Ethics Committee and was registered with clinicaltrials.gov (NCT00810849). All patients with TBP over the age of

18 years, presenting to a tertiary hospital in Cape Town as part of IMPI study were invited to participate in the sub-study. Patients who had received corticosteroids within the previous month and those who had been on TB treatment for more than a week were excluded. The sub-study was restricted to patients who required pericardiocentesis, as part of their standard of care to relieve hemodynamic compromise caused by the pericardial effusion.³ Participants were categorized as definitive TBP or probable TBP on the basis of pre-specified published criteria by Pandie *et al.*¹⁶ A definite diagnosis required microbiological evidence of *Mtb* infection in the pericardium, whereas a probable diagnosis was based on the presence of a lymphocyte predominant exudate with raised adenosine deaminase (ADA) or a Tygerberg diagnostic index score of 6 or greater.¹⁶

5.2.1. Intensive PK sampling procedure

All participants had a clinical history and physical examination performed and baseline investigations which included a full blood count, serum urea, and electrolytes. An echocardiogram confirmed the presence of a pericardial effusion and determined whether the pericardial effusion exceeded a cross-sectional diameter of 10 mm, hence amenable to aspiration. Participants underwent fluoroscopy and electrocardiogram (ECG) guided pericardial aspiration, using local anaesthesia and the Seldinger technique, during which a pig-tail catheter was left in the pericardial space for 24 hours, to permit ongoing drainage of residual pericardial fluid. A dose of 120 mg prednisolone was administered orally under supervision after pericardiocentesis.¹⁰ Patients who were randomized to the control arm, received anti-TB therapy without prednisolone. All patients continued their usual concomitant medication, for example their antiretroviral treatment. Following pericardial aspiration and within 5 minutes after the

administration of either prednisolone or placebo, a baseline plasma, pericardial fluid and saliva sample were collected and subsequently at the following time points; baseline, 0.5-, 1-, 2-, 3-, 5-, 8- and 24 hours for a total of 8 time-points in each patient for each matrix. Plasma, pericardial and saliva samples were stored at -80°C .

5.2.2. Drug concentration assay

Prednisolone concentrations were determined with a liquid chromatography tandem mass spectrometry assay, developed in the Division of Clinical Pharmacology, University of Cape Town. The assay was validated in plasma, and cross-validated in blank pericardial fluid and artificial saliva. Samples were processed with a liquid-liquid extraction method using ethyl acetate, followed by high performance liquid chromatography with MS/MS detection using an AB SCIEX API 4000 instrument. An Agilent Zorbax-SB Phenyl Rapid Resolution HT $1.8\ \mu\text{m}$, $2.1 \times 100\ \text{mm}$ analytical column was used. Prednisolone and prednisolone-d8 (internal standard), were monitored at mass transitions of the protonated precursor ions $m/z\ 361.2$ and $m/z\ 369.3$ to the product ions $m/z\ 147.2$ and $m/z\ 150.2$, respectively. The calibration curves fitted quadratic (weighted by $1/\text{concentration}^2$) regressions over the ranges $1.95\ \text{ng/ml}$ to $1000\ \text{ng/ml}$. A 5-fold dilution was validated for samples above the upper limit of quantification.

5.2.3. Compartmental pharmacokinetics

Prednisolone concentrations were modelled using ADAPT 5 (Biomedical Simulations Resource, California, USA) software of D'Argenio et al.¹⁷ We used the maximum likelihood expectation maximization algorithm. We modelled the concentrations using a one-compartment and a two-compartment model with first-order input and

elimination, as described in our prior publications.¹⁸⁻²¹ Akaike information criteria (AIC), Bayesian information criteria (BIC) and parsimony were then used to choose the best number of pharmacokinetic compartments. The model-derived concentration-time profiles were then used to identify the 0–24 hour area under the concentration-time curves (AUC₀₋₂₄).

5.2.4. Statistical analysis

The statistical analysis was done using GraphPad Prism version 7, 2016 (Graphpad, California, USA). The non-parametric Mann-Whitney and Fisher's exact test were used to compare baseline characteristics between prednisolone and placebo arms and to compare the pericardial and plasma prednisolone C_{max} and T_{max}. The median and proportions were used as the measures of central tendency.

Table 6: Baseline characteristics of participants who underwent intensive pharmacokinetic sampling.

Parameters	Median (range) or proportion (%) in all	Median or proportion in placebo, N=5	Median or proportion in treatment, N=5	<i>p</i> -value
Demographic and clinical parameters				
Gender: male	4(40%)	2(40%)	2(40%)	1.0
Age in years	30(24–59)	29 (24.1–56.3)	31 (24.0–58.8)	0.999
Weight in kg	60(40–82)	53 (40–82)	66 (52–72)	0.278
Prednisolone dose mg/kg	N/A	0	1.81 (1.67–2.31)	N/A
Positive Mtb	7(70%)	4(80%)	3(60%)	0.431

HIV	7(70%)	4(80%)	3(60%)	0.431
Proportion on HAART	1(14%)	1(20%)	0(0%)	N/A
Plasma				
CD4 count in cell/m3	149(42–874)	159 (50–485)	139 (42–874)	0.999
Creatinine in µmol/L	78(20–257)	80 (20–257)	65 (43–97)	0.547
Globulin in g/L	51(30–56)	46 (30–57)	55 (36–56)	0.999
Pericardial fluid				
Total protein in g/L	62(50–70)	58 (54–67)	66 (55–70)	0.175
Adenosine deaminase in U/L	57(26–133)	87 (52.5–133)	51 (25.9–119.4)	0.190

5.3. Results

A total of 37 potential participants were screened for the study. Ten participants met all inclusion criteria and were enrolled, while 27 participants were excluded; 14 did not require pericardiocentesis, 11 did not provide consent for this sub-study and 2 participants withdrew consent, due to discomfort of pigtail catheter. The patients' demographic and clinical characteristics are shown in Table 6. Seven patients had microbiological confirmation of TBP and 3 had probable TBP. Seven participants had HIV co-infection and were referred for initiation of anti-viral treatment following enrolment. There were no differences between baseline characteristics in respect of selected clinical and biochemical factors for the treatment and placebo groups.

5.3.1. Comparison of prednisolone concentrations based on naïve pooling

All five participants randomized to prednisolone had prednisolone concentrations above limits of detection in saliva, plasma and pericardial fluid, while the prednisolone concentrations were below limits of detection in all three matrices in participants who were assigned to placebo. The prednisolone concentrations versus time plots are shown in five patients Figure 15A. Prednisolone was rapidly absorbed into plasma and was detectable within 30 minutes of administration. The shape of the pericardial fluid concentration-time profile versus plasma was consistent with system hysteresis as evidenced by the relationship between the C_{max} and T_{max} , among the three matrices shown in Figure 15B and C. The ratio of the pericardial to plasma and salivary to plasma C_{max} (pericardial/ plasma or saliva/plasma) were median 0.28 with range 0.12 to 0.82 and median 0.59 with a range of 0.20 to 0.99 respectively. Figure 15 shows a rapid rise in prednisolone concentration plasma and saliva, with a higher permeation in plasma, compared to saliva while the rise in pericardial concentration was more circumspect, but more sustained. The relative peaks were highest in plasma, followed by saliva and lastly pericardial fluid.

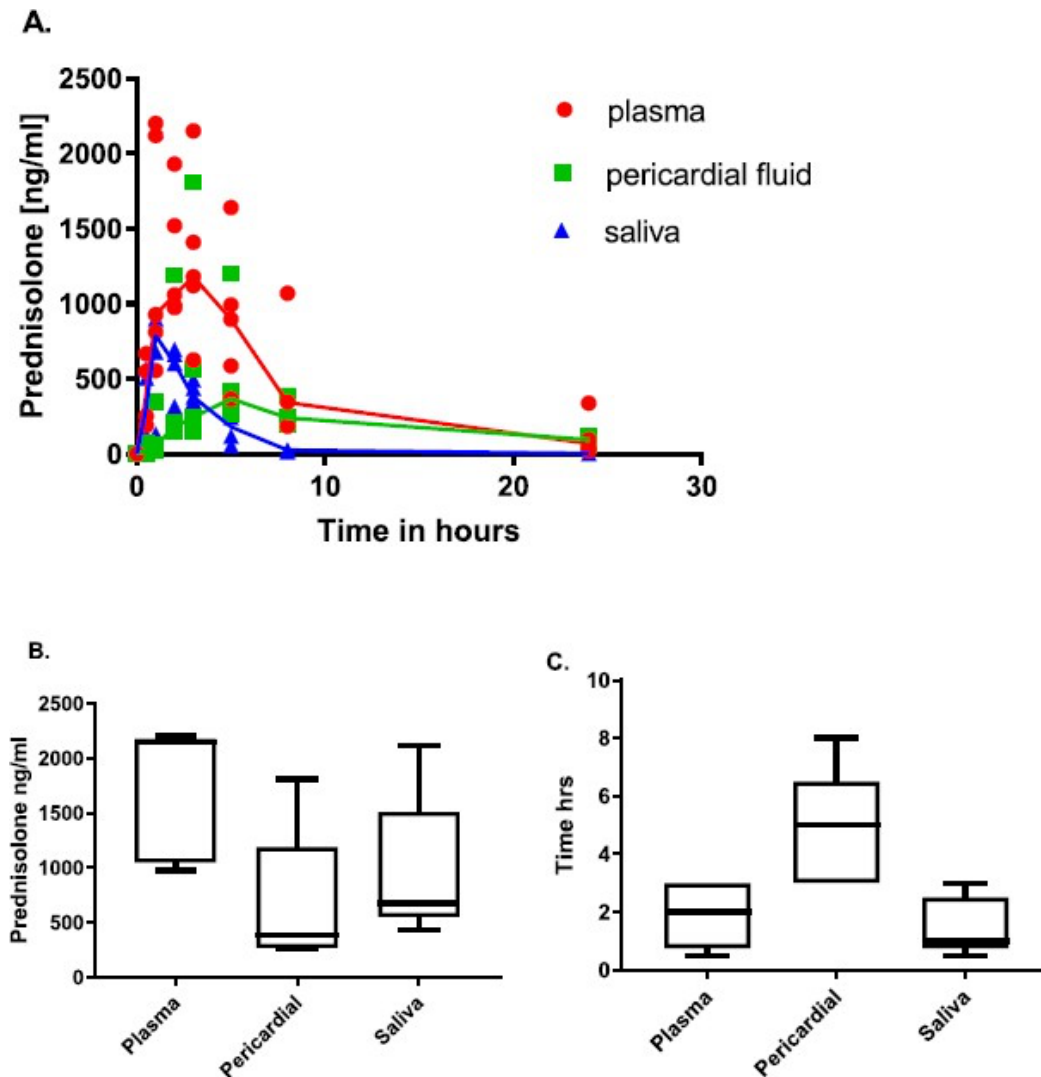


Figure 15: Naïve pooled prednisolone concentrations over a 24-hour period. A. Prednisolone concentration time graphs over a 24-hour period for each of the five participants who were assigned to the treatment arm with prednisolone. The plasma, pericardial fluid and saliva matrices were represented by red, green and blue, respectively. Each matrix was represented by a line of best fit and the data points for each of the 5 participants. B. The box and whisker plot comparing the prednisolone C_{max} of the three compartments; plasma, pericardial fluid and saliva. The median plasma, pericardial and saliva median C_{max} was 2150.0 $\mu\text{g/ml}$, 389.0 $\mu\text{g/ml}$ and 679.0 $\mu\text{g/ml}$ respectively. Plasma C_{max} was higher than pericardial C_{max} ($p=0.032$). Plasma C_{max} was higher than saliva C_{max} ($p=0.032$). There was no difference between pericardial and saliva prednisolone C_{max} ($p=0.151$). C. The box and whisker plot comparing the prednisolone T_{max} of the three compartments; plasma, pericardial fluid and saliva. The median plasma, pericardial and saliva median T_{max} was 2.0 hours, 5.0 hours and 1.0 hours respectively. Pericardial T_{max} was higher than plasma T_{max} ($p=0.048$). Pericardial T_{max} was higher than saliva T_{max} ($p=0.024$). There was no difference between plasma and saliva prednisolone T_{max} ($p=0.810$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.3.2. Compartmental pharmacokinetic modelling-based comparisons

Given the system hysteresis for the plasma concentrations and pericardial concentrations, we modelled each compartment separately from each other. Table 7 shows that based on Akaike Information Criteria and Bayesian information criteria, a one compartment model best explained plasma concentrations, consistent with what has been reported in the literature in larger studies of prednisolone plasma pharmacokinetics.^{14,22} Prednisolone pericardial concentrations were also best explained by a one-compartment model.

Table 7: Compartmental model choices using information criteria.

Matrix	Number of compartments in model	Akaike information criteria	Bayesian information criteria
Plasma	One	80.011	92.453
	Two	87.866	106.530
Pericardial fluid	One	-35.243	-22.800
	Two	-27.200	-8.536

The prednisolone plasma and pericardial pharmacokinetic parameter estimates for the one compartment model are shown in Table 8. The table shows that the clearance and apparent volume were similar in pericardial fluid and plasma, and by parsimony, are considered as a single pharmacokinetic compartment. The major differences were in the absorption rate constant (K_a), a constant value to describe the rate and extent

of which a drug is absorbed into a compartment expressed in units of time^{-1} .²³ As it applies to this experiment, it is the rate of prednisolone absorption from the gastrointestinal tract, the site of administration. The plasma K_a of 0.693 h^{-1} in Table 8 translates to an absorption half-life of exactly 1.0 hour into plasma, while the pericardial K_a of 0.0735 h^{-1} translates to an absorption half-life of 9.431 hours into pericardial fluid. Thus, the rate of absorption into pericardial fluid differed between plasma and pericardial fluid. In addition, Table 8 means that the largest between patient variability among all pharmacokinetic parameters, measured as % coefficient of variation (CV), was the 99.59% that was encountered in pericardial fluid K_a . Thus, the physiological basis for the prednisolone system hysteresis curves, was a slow rate of absorption into pericardial fluid versus a fast rate into plasma.²⁴

Table 8: Estimation of plasma and pericardial fluid prednisolone one compartment model parameters

Parameter	Plasma		Pericardial fluid	
	Mean	Standard deviation	Mean	Standard deviation
Clearance (L/h)	17.2	7.86	13.7	0.483
Volume of distribution (L)	37.6	1.54	25.7	1.58
Absorption constant (h^{-1})	0.693	0.039	0.0735	0.0732

The pharmacokinetic-model derived $T_{\max}$ is shown in Figure 16C, which shows that in all cases the paired values for each patient demonstrated greater $T_{\max}$ in pericardial fluid compared to plasma, with medians of 2.49 hours versus 4.09 hours ($p=0.032$). The pharmacokinetic model-derived prednisolone $C_{\max}$ and AUC_{0-24} in plasma and the pericardial fluid were used to derive the drug penetration ratios, with results shown in Figure 16D. The median pericardial fluid-to plasma $C_{\max}$ ratio was 0.218 with between-patient % CV of 96.93%. The median pericardial fluid-to-plasma AUC_{0-24} ratio was 0.793 (95% confidence interval: 0.419 to 1.269) with between-patient % CV of 40.55%. Thus, while the peak concentration in pericardial fluid was lower than in plasma, the total daily drug concentration (AUC_{0-24}) was comparable between the two matrices, which means although delayed, most prednisolone eventually reaches pericardial fluid.

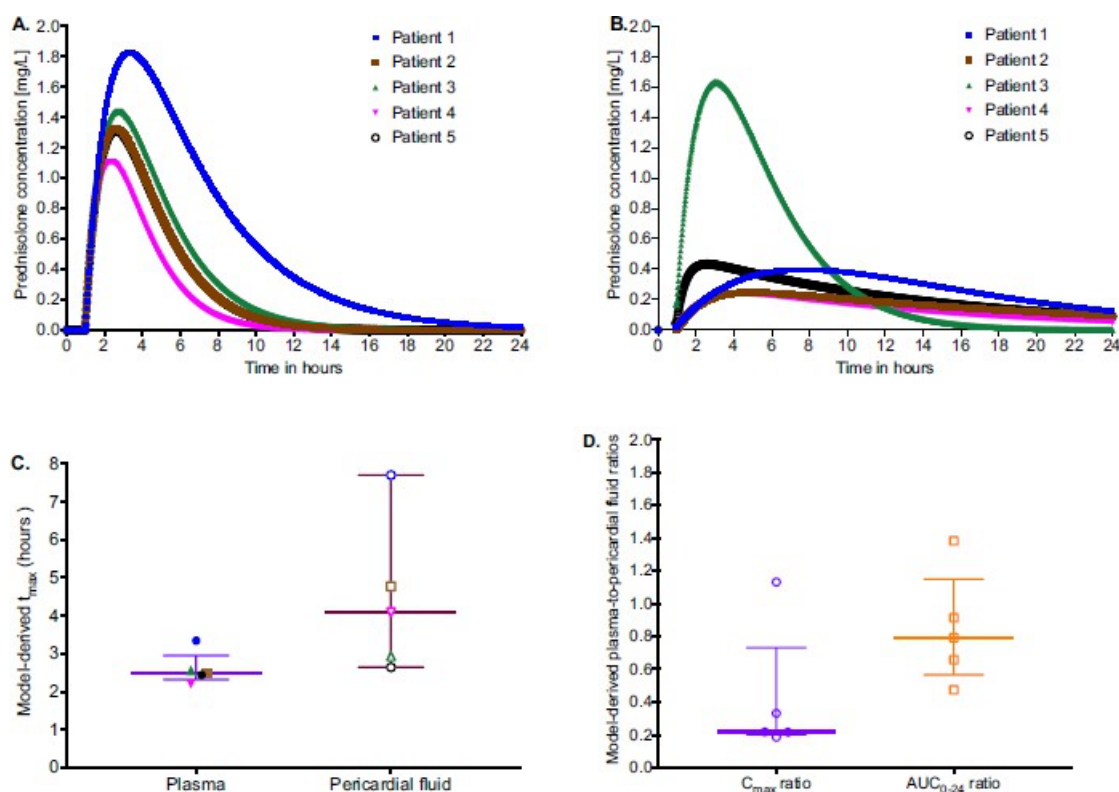


Figure 16: The prednisolone pharmacokinetic model for plasma and pericardial fluid. A. Pharmacokinetic model-predicted concentration-time curves for plasma. There was rapid absorption of prednisolone into the plasma compartment followed by a rapid elimination. B. Pharmacokinetic model-predicted concentration-time curves for pericardial fluid, shows delayed time to peak concentration due to reduced absorption into pericardial fluid compartment. C. Pharmacokinetic model-derived T_{max} are shown paired for each patient [colour coded], with closed symbol in plasma and open symbol in pericardial fluid. D. Pharmacokinetic model-derived pericardial fluid-to-plasma concentrations in each patient, demonstrate a low peak concentration penetration ratio but an AUC_{0-24} with confidence intervals crossing 1.

5.4. Discussion

We managed in the first instance to quantify prednisolone concentrations in pericardial fluid in patients with TBP. We found that the compartmental pharmacokinetic parameters for a one compartment model were similar between pericardial fluid and plasma, so that these two matrices were likely one pharmacokinetic compartment.²⁵ Prednisolone metabolism and clearance is by 11- β -hydroxysteroid dehydrogenase

(11 β HSD), in the liver and kidneys, which could explain the similar clearances between blood and pericardial fluid.^{26,27} Similarly, the volumes demonstrate that the plasma and pericardial fluid in reality form a single “bucket”. We found that there was a delayed entry into the pericardial fluid, resulting in a shape best described by system hysteresis. System hysteresis occurs when the output (pericardial fluid concentrations) lags behind the input (in this case drug dose and plasma concentrations), a concept first described in electromagnetism.²⁴ This resulted in pericardial fluid prednisolone peak concentrations that were only 21% those in plasma. However, AUC₀₋₂₄ ratios had a 95% confidence interval that crossed 1, another factor consistent with system hysteresis: dependence of the system on history (history being the input and plasma AUCs that were achieved earlier). The therapeutic implications are as yet unclear, and will depend on the prednisolone anti-inflammatory effect. If efficacy is peak concentration-driven then efficacy will be compromised, however if it is AUC or time above threshold driven then efficacy will not be compromised. In the latter case, it would not be necessary to sample pericardial fluid concentrations, as plasma AUC₀₋₂₄ based calculations would be good surrogates for those in pericardial fluid. Second, we also found that the saliva prednisolone concentration time profile was more closely aligned to that of plasma than that of the pericardial matrix, which means that there was little delay to peak concentration between the plasma and saliva. This less invasive method using saliva could help with a more convenient way for pharmacokinetic sampling.²⁸ However, there was reduced prednisolone penetration into saliva for both C_{max} and AUC₀₋₂₄. Thus, if saliva is to be used as a surrogate for plasma, a correction factor would need to be applied for both concentrations; if it is to be used as a surrogate for pericardial fluid concentration then a correction factor will need to be calculated for AUC₀₋₂₄. A possible drawback of using saliva as a surrogate

for pericardial concentrations of prednisolone is the potential for contamination. There is also concern that saliva prednisolone may contain residual prednisolone left over from ingestion of prednisolone and may result in over-estimation of saliva pharmacokinetic profiles.^{28,29}

5.5. Limitations

Our study has several limitations, including a small sample size limiting the power of the study. Second, the study participants received a very high dose of prednisolone 120 mg, whereas previous TBP studies used lower doses of prednisolone and thus our findings may not be generalisable to administration of lower doses.^{13,14} Additionally, the findings of this study may not be generalizable to other conditions requiring prednisolone therapy. Third, it is also not understood what effect if any the inflamed pericardium has on pericardial concentrations of prednisolone. Other serosa have been shown to be more permeable to prednisolone when inflamed, as for example, the meninges during meningitis.^{30,31} Third, it remains to be determined in clinical studies the prednisolone concentration threshold in plasma and pericardium, which reduce inflammation and associated with prevention of constrictive pericarditis.^{9,32,33} If these were known, then the optimal dose could be determined, based on the penetration ratios that we identified.

5.6. Conclusion

In conclusion, we have demonstrated [1] that prednisolone penetrates into pericardium in a delayed fashion, [2] that the AUC₀₋₂₄ in pericardial fluid approximates that in plasma, and [3] that saliva can be a surrogate matrix for prednisolone pharmacokinetic

sampling, but AUC_{0-24} measures would need a correction factor to approximate those in pericardial fluid.

5.7. References

1. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
2. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *SAMJ: South African Medical Journal* 2008; **98**(1): 36-40.
3. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
4. Mayosi BM. Interventions for treating tuberculous pericarditis. *The Cochrane Library* 2002.
5. Teeninga N, Guan Z, Freijer J, et al. Monitoring prednisolone and prednisone in saliva: a population pharmacokinetic approach in healthy volunteers. *Therapeutic drug monitoring* 2013; **35**(4): 485-92.
6. Dheda K, Lenders L, Magombedze G, et al. Drug penetration gradients associated with acquired drug resistance in tuberculosis patients. *American journal of respiratory and critical care medicine* 2018; (ja).
7. Dheda K, Gumbo T, Maartens G, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The lancet Respiratory medicine* 2017; **5**(4): 291-360.
8. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases* 2013; **208**(9): 1464-73.
9. Swaminathan S, Pasipanodya JG, Ramachandran G, et al. Drug concentration thresholds predictive of therapy failure and death in children with tuberculosis: bread crumb trails in random forests. *Clinical Infectious Diseases* 2016; **63**(suppl_3): S63-S74.
10. Shenje J, Adimora-Nweke FI, Ross IL, et al. Poor penetration of antibiotics into pericardium in pericardial tuberculosis. *EBioMedicine* 2015; **2**(11): 1640-9.
11. Pickup M. Clinical pharmacokinetics of prednisone and prednisolone. *Clinical pharmacokinetics* 1979; **4**(2): 111-28.
12. Frey F. Kinetics and dynamics of prednisolone. *Endocrine reviews* 1987; **8**(4): 453-73.
13. Rose JQ, Yurchak AM, Jusko WJ. Dose dependent pharmacokinetics of prednisone and prednisolone in man. *Journal of pharmacokinetics and biopharmaceutics* 1981; **9**(4): 389-417.
14. Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clinical pharmacokinetics* 1990; **19**(2): 126-46.
15. Wilckens T. Glucocorticoids and immune function: physiological relevance and pathogenic potential of hormonal dysfunction. *Trends in pharmacological sciences* 1995; **16**(6): 193-7.

16. Pandie S, Peter JG, Kerbelker ZS, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon- γ in a high burden setting: a prospective study. *BMC medicine* 2014; **12**(1): 101.
17. D'argenio DZ, Schumitzky A. A program package for simulation and parameter estimation in pharmacokinetic systems. *Computer programs in biomedicine* 1979; **9**(2): 115-34.
18. Hall RG, Swancutt MA, Meek C, Leff R, Gumbo T. Weight Drives Caspofungin Pharmacokinetic Variability in Overweight and Obese People: Fractal Power Signatures Beyond 2/3 or 3/4. *Antimicrobial agents and chemotherapy* 2013; AAC. 01490-12.
19. Hall RG, Swancutt MA, Gumbo T. Fractal geometry and the pharmacometrics of micafungin in overweight, obese, and extremely obese people. *Antimicrobial agents and chemotherapy* 2011; **55**(11): 5107-12.
20. Hall R, Pasipanodya J, Swancutt M, Meek C, Leff R, Gumbo T. Supervised Machine-Learning Reveals That Old and Obese People Achieve Low Dapsone Concentrations. *CPT: pharmacometrics & systems pharmacology* 2017; **6**(8): 552-9.
21. Hall R, Pasipanodya J, Meek C, Leff R, Swancutt M, Gumbo T. Fractal geometry-based decrease in trimethoprim-sulfamethoxazole concentrations in overweight and obese people. *CPT: pharmacometrics & systems pharmacology* 2016; **5**(12): 674-81.
22. Al-Habet S, Rogers H. Pharmacokinetics of intravenous and oral prednisolone. *British journal of clinical pharmacology* 1980; **10**(5): 503-8.
23. Upton RN, Mather LE, Runciman WB, Nancarrow C, Carapetis RJ. The use of mass balance principles to describe regional drug distribution and elimination. *Journal of pharmacokinetics and biopharmaceutics* 1988; **16**(1): 13-29.
24. Ewing JA. I. Experimental researches in magnetism. *Proceedings of the Royal Society of London* 1885; **38**(235-238): 58-62.
25. Burton ME. Applied pharmacokinetics & pharmacodynamics: principles of therapeutic drug monitoring: Lippincott Williams & Wilkins; 2006.
26. Vermeulen A. The metabolism of 4-¹⁴C prednisolone. *Journal of Endocrinology* 1959; **18**(3): 278-91.
27. Rusvai E, Naray-Fejes-Toth A. A new isoform of 11 beta-hydroxysteroid dehydrogenase in aldosterone target cells. *Journal of Biological Chemistry* 1993; **268**(15): 10717-20.
28. Ross I, Levitt N, Van der Walt J-S, et al. Salivary cortisol day curves in Addison's disease in patients on hydrocortisone replacement. *Hormone and Metabolic Research* 2013; **45**(01): 62-8.

29. Ross I, Lacerda M, Pillay T, et al. Salivary Cortisol and Cortisone do not Appear to be Useful Biomarkers for Monitoring Hydrocortisone Replacement in Addison's Disease. *Hormone and Metabolic Research* 2016; **48**(12): 814-21.
30. Duma R, Berry A, Smith S, Baggett J, Swabb E, Platt T. Penetration of aztreonam into cerebrospinal fluid of patients with and without inflamed meninges. *Antimicrobial agents and chemotherapy* 1984; **26**(5): 730-3.
31. Holdiness MR. Cerebrospinal fluid pharmacokinetics of the antituberculosis drugs. *Clinical pharmacokinetics* 1985; **10**(6): 532-4.
32. Modongo C, Pasipanodya JG, Magazi BT, et al. Artificial intelligence and amikacin exposures predictive of outcome in multidrug-resistant tuberculosis patients. *Antimicrobial agents and chemotherapy* 2016: AAC. 00962-16.
33. Srivastava S, Pasipanodya JG, Ramachandran G, et al. A long-term co-perfused disseminated tuberculosis-3D liver hollow fiber model for both drug efficacy and hepatotoxicity in babies. *EBioMedicine* 2016; **6**: 126-38.

6. CHAPTER 6: Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study

6.1. Introduction

Tuberculosis (TB) is the most common opportunistic infection in HIV-1 infected persons in Sub-Saharan Africa. Tuberculous pericarditis (TBP) is an inflammation of the pericardium caused by *Mycobacterium tuberculosis*. It is a life-threatening extra-pulmonary form of TB that results in accumulation of fluid around the heart, potentially leading to constriction. In developed countries it accounts for approximately 5% of all cases of acute pericarditis. In Sub-Saharan Africa, 90% of pericardial effusions in HIV-1 co-infected patients are related to TBP.¹ Mortality in these HIV positive patients is up to 40% despite anti-tuberculosis therapy, pericardial drainage, or pericardiectomy.² The recently completed Investigation of the Management of Pericarditis (IMPI), a large clinical trial examining the effect of prednisolone or *Mycobacterium indicus pranii* or both added to the standard regimen of isoniazid, rifampicin, ethambutol and pyrazinamide showed, that neither standard anti-TB therapy alone nor the addition of prednisolone to chemotherapy resulted in a clinically satisfactory mortality reduction, however the prednisolone group had a lower rate of constrictive pericarditis and fewer hospitalisations, compared to placebo.³

TBP is characterised by a compartmentalized pro-fibrotic immune response and in response, we hypothesised that prednisolone had a suppressive effect on the concentration of inflammatory and potentially pro-fibrotic cytokines in the pericardium.⁴ In this pilot study, the effect of prednisolone on inflammatory markers in pericardial fluid, plasma and saliva, in a subset of patients was evaluated from the above clinical

trial. It was hoped that our understanding of immunological mechanisms at the disease site would be enhanced, which could inform future targeted interventions. Specific analytes were selected based on recent analyses of differentially abundant inflammatory markers (at both RNA and protein level) between blood and pericardial fluid compartments.⁴ Additionally, the hypothesis that inflammation induced cell death in the pericardial compartment would be in part due to apoptosis was evaluated, which is initiated by two major pathways: the extrinsic (through ligation of death receptors or TNF receptors) and intrinsic pathways (mitochondria mediated).⁵

For example, caspase 8 activation is an essential early step in the induction of the apoptosis by the extrinsic pathway, while caspase 9 activation is part of the intrinsic pathway, both leading to activation of caspase 3.⁶ We therefore also assessed the effect of prednisolone on the concentration of caspases 3, 8 and 9 in the pericardial fluid and plasma, hypothesising that the concentrations of these cytokines may be influenced by prednisolone, due to its inherent anti-inflammatory action.

6.2. Materials and methods

6.2.1. Patient populations

Patients were recruited from Groote Schuur Hospital, Cape Town, South Africa as part of an intensive pharmacokinetic sampling study, as described previously.^{3,7} Ethics approval for these studies was obtained from the Faculty of Health Sciences Human Research Ethical Committee at the University of Cape Town (Reference numbers 102/2003, 402/2005, 289/2007) and as published.^{3,7} Briefly, patients were eligible for inclusion in the trial, if they were 18 years of age or older, had a pericardial effusion

requiring pericardiocentesis confirmed by echocardiography, had evidence of definite or probable TBP and commenced on anti-tuberculosis treatment for less than a week prior to enrolment and provided written informed consent for inclusion in this study. Pericardial fluid, plasma and saliva were obtained from 14 patients at multiple time points, following randomisation to receiving prednisolone or matched placebo. Pericardial samples were collected through a catheter, which was left *in-situ* in the pericardial space for 24 hours post-pericardiocentesis. Due to missing samples, the concentration of cytokines, chemokines and caspases at 0, 8 and 24 hours were evaluated.

6.2.2. Luminex multiplex assay for cytokines and chemokines

Mediators analysed in undiluted plasma (P), pericardial fluid (PF) and saliva (S) samples included interferon gamma (IFN- γ), interleukin-1 α (IL-1 α), IL-1 β , IL-6, IL-10, IL-12p40, TNF, IL-8 and interferon gamma-induced protein-10 (IP-10), using customized Milliplex™ kits (HCYTOMAG-60 K, Millipore, St Charles, MO, USA) on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA) as described.⁸ Caspases 3, 8 and 9 in undiluted pericardial fluid and plasma were measured using human *in vitro* ELISA kits from Abcam (Cambridge, UK), following the manufacturer's recommendations.

6.2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 7, 2016 (Graphpad, California, USA). The distribution of the data was assessed using the D' Agostino and Pearson normality test. Due to missing data, some results were not paired. Between group comparisons of unpaired non-normally distributed data were analysed, using the Mann-Whitney U test. No correction for multiple comparisons was performed.

6.3. Results

Of the 14 patients, 9 received matched-placebo and 5 received 120 mg prednisolone daily for one week. Patient characteristics are shown in Table 9. Nine patients had definite TBP (3 received prednisolone and 6 received matched placebo, whereas 5 had probable TBP (2 prednisolone versus 3 placebo). The pericardial protein measured in pericardial fluid was median 61 g/l (IQR 55–67.5) in the definite TBP patients and median 66 g/l (IQR 56–76) in the probable TBP patients, respectively. The adenosine deaminase (ADA) was median 87.3 U/l (IQR 43–127) and 51 U/l (32.8–67.6) in the definite and probable patients, respectively. There were no significant differences between these groups. There was also no difference between age and weight between the groups and sex composition was male/ female 5/ 4 and 2/ 3 for the placebo and prednisolone groups, respectively. Seven out of 9 patients in the placebo and three out of 5 patients in the prednisolone group were HIV infected. Only two patients were receiving antiretroviral treatment at the time of the study, both of whom received matched placebo (Table 9). Median CD4 counts were not different between the groups (240 cells/mm³ for the placebo versus 139 cells/ mm³ for the prednisolone group). Since the findings indicated that neither HIV-1 co-infection status, nor CD4 count or pericardial fluid *Mycobacterium tuberculosis* culture result affected the compartmentalized pro-fibrotic immune response,⁴ we combined patients with and without HIV infection for analysis, as well as patients with definite and probable TBP.

Table 9: Patient Characteristics

Patient number	Age (years)	Weight (kg)	CD4 (cells/ μ l)	Creatinine (μ mol/l)	Globulin (g/l)	Pericardial protein (g/l)	Adenine deaminase (units/l)
1	28	53	115	76	46	54	Haemolysed
2	29	40	50	20	45	54	83
3	24	66	42	43	56	68	57
4	56	82	485	121	30	62	133
5	31	72	319	82	36	55	119
6	51	53	159	80	56	50	53
7	24	45	321	257	56	60	92
8	27	66	135	45	55	70	26
9	44	52	139	65	56	66	51
10	59	66	874	97	38	62	33
11	45	70	721	109	47	76	32
12	33	47	116	71	49	61	130
13	25	76	*	73	*	55	38
14	27	*	255	41	74	76	68

*Missing data

Results are summarised in Tables 10, 11, 12 and 13, indicating the median and interquartile range (IQR) of all analytes measured in plasma, pericardial and saliva samples at the tested time points. Since samples were collected before prednisolone administration, we combined the baseline data to analyse the effect of compartmentalization: higher concentrations of IFN- γ , IL-10, IL-1 β , IL-6, IL-8, IP-10 and TNF were found at the disease site, compared to plasma at day 0. Interestingly, IL-1 α and IL-12p40 was mostly detectable in the saliva samples, which also contained

elevated concentrations of IL-1 β , IL-8 and IP-10, compared to plasma. Only IL-6 concentrations were higher in the plasma, compared to saliva ($p=0.03$) at baseline.

Table 10: Median (IQR) of analytes in plasma

Plasma cytokines	Baseline		8 hrs		24 hrs	
	Prednisolone	Placebo	Prednisolone	Placebo	Prednisolone	Placebo
INF-γ	84 (28-231)	13 (5-118)	72 (14-102)	16 (4-117)	37 (9-259)	11 (3-83)
IL-1α	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
IL-1β	0 (0-0)	0 (0-0)	0 (0-5)	0 (0-0)	0 (0-5)	0 (0-0)
IL-6	23 (16-61)	35 (19-88)	4 (0-15)	49 (19-67)	6 (6-73)	44 (17-74)
IL-10	4 (0-7)	4 (0-7)	0 (0-8)	6 (0-10)	0 (0-7)	1 (0-3)
IL-12p40	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
TNF-α	33 (21-53)	26 (12-55)	23 (15-25)	22 (14-94)	19 (19-57)	20 (17-68)
IL-8	23 (11-33)	25 (5-72)	10 (10-25)	20 (8-99)	28 (16-54)	19 (16-30)
IP-10	82 (40-158)	86 (36-305)	52 (33-72)	68 (36-214)	49 (21-59)	47 (24-288)
Caspase 3	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-109)	0 (0-470)	0 (0-0)
Caspase 8	0 (0-2)	0 (0-0)	2 (1-4)	2 (0-3)	2 (0-2)	0 (0-0)
Caspase 9	24 (19-40)	20 (18-34)	49 (7-55)	23 (19-45)	25 (14-34)	20 (15-22)

Table 11: Median (IQR) of analytes in pericardial fluid

Pericardial cytokines	Baseline		8 hrs		24 hrs	
	Prednisolone	Placebo	Prednisolone	Placebo	Prednisolone	Placebo
INF-γ	1585 (533-6363)	2536 (553-3687)	1436 (383-2903)	1347 (298-3485)	838 (278-2696)	927 (482-2619)
IL-1α	0 (0-149)	0 (0-111)	0 (0-213)	2 (0-18)	0 (0-42)	8 (0-124)
IL-1β	0 (0-109)	18 (7-292)	0 (0-177)	14 (8-74)	0 (0-42)	54 (14-185)
IL-6	9277 (8533-9969)	8850 (7243-10254)	9166 (8269-9390)	9097 (8058-9483)	8797 (8102-9755)	8695 (6377-9394)
IL-10	28 (22-47)	22 (17-83)	37 (25-56)	29 (11-125)	24 (13-31)	16 (8-46)
IL-12p40	0 (0-3)	0 (0-10)	0 (0-0)	0 (0-3)	0 (0-0)	1 (0-18)
TNF-α	180 (107-469)	213 (99-291)	218 (142-483)	182 (96-353)	265 (141-394)	175 (83-299)
IL-8	3725 (1512-9987)	9327 (1903-10855)	4635 (2872-9677)	2603 (2334-9106)	1834 (1576-2890)	7067 (4180-9957)
IP-10	1435 (846-1537)	1348 (790-1887)	1495 (1050-1701)	1462 (657-1972)	1245 (865-1335)	1758 (908-1944)
Caspase 3	134 (0-250)	88 (0-403)	193 (0-193)	72 (0-216)	48 (0-137)	168 (0-684)
Caspase 8	6 (5-14)	7 (5-20)	8 (6-13)	10 (8-17)	6 (4-9)	11 (4-21)
Caspase 9	31 (24-82)	36 (30-91)	38 (26-61)	41 (31-70)	32 (24-42)	48 (32-61)

Table 12: Median of analytes in saliva

Saliva cytokines	Baseline		8 hrs		24 hrs	
	Prednisolone	Placebo	Prednisolone	Placebo	Prednisolone	Placebo
INF-γ	18 (10-30)	15 (0-23)	17 (17-35)	16 (13-17)	10 (1-36)	15 (11-23)
IL-1α	1758 (980-8567)	6473 (722-12416)	341 (336-5752)	1399 (401-13354)	390 (56-3341)	2890 (531-7632)
IL-1β	26 (15-663)	30 (17-146)	12 (5-20)	14 (4-56)	11 (2-14)	26 (17-105)
IL-6	10 (5-29)	11 (0-29)	5 (1-10)	8 (4-30)	6 (1-11)	10 (2-12)
IL-10	21 (15-57)	3 (0-22)	37 (4-40)	4 (1-37)	28 (2-63)	5 (0-77)
IL-12p40	31 (0-48)	11 (0-29)	37 (10-86)	0 (0-2)	23 (0-88)	0 (0-23)
TNF-α	13 (10-47)	59 (6-210)	14 (7-20)	22 (4-110)	12 (3-15)	26 (5-56)
IL-8	325 (88-1257)	265 (156-486)	52 (32-55)	140 (22-234)	44 (3-117)	163 (94-361)
IP-10	1061 (201-2420)	1184 (67-2361)	139 (45-762)	345 (90-1095)	92 (30-552)	376 (240-2661)
Caspase 3	nd	nd	nd	nd	nd	nd
Caspase 8	nd	nd	nd	nd	nd	nd
Caspase 9	nd	nd	nd	nd	nd	nd

nd - not detected

Table 13: Median (IQR) analytes of combined (both prednisolone and placebo arms) in plasma, pericardial fluid and saliva

Cytokines	Pericardial fluid	Plasma	Saliva
INF-γ	2061 (648-3285)	28 (10-116)	17 (10-23)
IL-1α	0 (0-62)	0 (0-0)	3393 (949-11180)
IL-1β	15 (0-114)	0 (0-0)	28 (18-144)
IL-6	9064 (8225-10000)	23 (16-61)	11 (2-28)
IL-10	26 (21-65)	4 (0-7)	15 (1-25)
IL-12p40	0 (0-5)	0 (0-0)	18 (0-39)
TNF-α	197 (100-354)	33 (21-53)	15 (9-80)
IL-8	7670 (1841-10434)	23 (11-33)	295 (134-961)
IP-10	1414 (1031-1646)	82 (40-158)	1123 (167-2316)
Caspase 3	93 (0-313)	0 (0-0)	nd
Caspase 8	7 (5-16)	0 (0-2)	nd
Caspase 9	35 (30-73)	24 (19-40)	nd

nd - not detected

Caspase 3, 8 and 9 concentrations were higher in pericardial fluid, when compared to plasma ($p=0.02$; 0.0001 and 0.03, respectively for the three measured caspases).

Prednisolone significantly decreased the concentration of IL-6 by 8 hours in plasma, compared to the patients who received placebo (Figure 17A, $p=0.036$, Mann Whitney U test). In pericardial fluid, prednisolone significantly reduced IL-8 concentrations by 24 hours (Figure 17B, $p=0.03$, Mann Whitney U test), compared to placebo treated patients. There was a trend towards decreased IL-1 β concentrations, however significance was not reached due to low numbers ($p=0.06$, not shown). Finally in saliva, we found a significant reduction in concentration of both IL-8 and IL-1 β by 24 hours of treatment in the prednisolone group (Figure 16C, $p=0.04$, Mann Whitney U test; and Figure 17D, $p=0.027$, Mann Whitney U test), compared to the placebo group. The concentration of caspase 3, 8 and 9 did not appear to be affected by prednisolone in any of the compartments evaluated.

A comparing baseline pericardial IL1 β , IL-6 and IL8 concentrations between participants on placebo versus those on prednisolone

Cytokines	Placebo concentrations (range) pg/ml	Treatment concentration (range) pg/ml	<i>P</i>-value
IL-1β	18(7-292)	0(0-109)	0.1111
IL-6	8850(7243-10254)	9277(8533-9969)	0.4293
IL-8	9327(1903-10855)	3725(1512-9987)	0.1490

6.4. Discussion

In the largest clinical trial evaluating the effect of adjunctive prednisolone therapy showing no effect on the primary composite of death, cardiac tamponade requiring pericardiocentesis or constrictive pericarditis, prednisolone reduced the incidence of constrictive pericarditis and the incidence of hospitalisations.³ Here it was hypothesised that the anti-inflammatory effects of prednisolone may manifest in reduced measurable concentrations of inflammatory cytokines and chemokines. Our findings suggest that the beneficial effect of prednisolone may be associated with the suppression of inflammatory mediators IL-6, IL-8 and IL-1 β .

These results are consistent with previous findings that treatment of tuberculosis may be associated with the immune reconstitution inflammatory syndrome (TB-IRIS),⁹ and the administration of prednisone reduced the duration of hospitalisation and the number of outpatient therapeutic procedures, compared with placebo. At the same time, IL-6, IL-10, IL-12 p40, TNF, IFN- γ , and IP-10 concentrations significantly decreased in the serum of prednisone, but not placebo, treated patients.¹⁰ In a separate study, adjunctive corticosteroid therapy modifies the inflammatory profile of those who develop TB-IRIS, with lower concentrations of IFN- γ , IP-10, TNF, IL-6, IL-8, IL-10, IL-12p40, and IL-18.¹¹ Thus, the beneficial effects of prednisone appear to be mediated via suppression of predominantly pro-inflammatory cytokine responses of innate immune origin, similar to the findings in this study. Earlier reports revealed that cell-death enrichment factors were elevated in pericardial fluid, and it was hypothesised that TB antigen specific activated T cells, which enter the pericardium and release INF- γ die,⁴ thus potentially activating the inflammasome pathway, resulting in pyroptosis and release of IL-1 β , ultimately leading to more cell death.

Additionally, a trend towards decreased IL-1 β concentrations in the pericardial fluid of prednisolone treated patients is demonstrated, as well as higher concentrations of caspases 3, 8 and 9 in the pericardial fluid, thereby supporting a hypothesis for compartmentalized inflammation induced apoptosis in the pericardial fluid.

This study is limited by the small sample size; therefore, the statistical significance of the findings in some instances may be weak. The small sample size further limited our capacity to analyse the results based on the HIV status of the patients, since it has been described that HIV infection is associated with a lower incidence of pericardial constriction in patients with presumed TBP,¹² and IL-13-secreting CD4 T cells, which are reduced by HIV, regulate fibrogenesis directly, through stimulating collagen synthesis by fibroblasts and indirectly by promoting TGF- β 1 production by macrophages.¹³ As a previous study showed that neither HIV-1 co-infection status, nor CD4 count nor pericardial fluid *Mycobacterium tuberculosis* culture result, affected the compartmentalized pro-fibrotic immune response with respect to the selected analytes we measured, HIV status *per se* was unlikely to have affected the cytokine measurements.⁴ Additional limitation of this pilot study is that pericardial fluid sampling was limited to within 24 hours of initiation of prednisolone therapy, due to safety concerns, resulting from leaving the pericardial catheter in the pericardial space for a prolonged period of time.

These findings are of interest as TBP remains the leading cause of constrictive pericarditis in Africa. Treatment of chronic pericardial tuberculosis leading to constriction is pericardiectomy, which is associated with high mortality and morbidity. Moreover, cardiac surgery is not widely available in Africa,¹⁴ thus adjunctive

prednisolone is an attractive alternative to reduce the incidence of constrictive pericarditis. Potentially, measurement of inflammatory markers, which are easily obtainable through sampling plasma or saliva, may in the future be beneficial, either for monitoring a response to prednisolone or for prognostication.

6.5. References

1. Lazaros G, Tousoulis D. Tuberculous pericarditis: a complex puzzle to put together. *EBioMedicine* 2015; **2**(11): 1570-1.
2. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *SAMJ: South African Medical Journal* 2008; **98**(1): 36-40.
3. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
4. Matthews K, Deffur A, Ntsekhe M, et al. A compartmentalized profibrotic immune response characterizes pericardial tuberculosis, irrespective of HIV-1 infection. *American journal of respiratory and critical care medicine* 2015; **192**(12): 1518-21.
5. Divangahi M, Behar SM, Remold H. Dying to live: how the death modality of the infected macrophage affects immunity to tuberculosis. *The New Paradigm of Immunity to Tuberculosis*: Springer; 2013: 103-20.
6. Creagh EM. Caspase crosstalk: integration of apoptotic and innate immune signalling pathways. *Trends in immunology* 2014; **35**(12): 631-40.
7. Shenje J, Adimora-Nweke FI, Ross IL, et al. Poor penetration of antibiotics into pericardium in pericardial tuberculosis. *EBioMedicine* 2015; **2**(11): 1640-9.
8. Marais S, Wilkinson KA, Lesosky M, et al. Neutrophil-associated central nervous system inflammation in tuberculous meningitis immune reconstitution inflammatory syndrome. *Clinical infectious diseases* 2014; **59**(11): 1638-47.
9. Meintjes G, Wilkinson RJ, Morroni C, et al. Randomized placebo-controlled trial of prednisone for paradoxical TB-associated immune reconstitution inflammatory syndrome. *AIDS (London, England)* 2010; **24**(15): 2381.
10. Meintjes G, Skolimowska KH, Wilkinson KA, et al. Corticosteroid-modulated immune activation in the tuberculosis immune reconstitution inflammatory syndrome. *American journal of respiratory and critical care medicine* 2012; **186**(4): 369-77.
11. Conesa-Botella A, Meintjes G, Coussens AK, et al. Corticosteroid therapy, vitamin D status, and inflammatory cytokine profile in the HIV-tuberculosis immune reconstitution inflammatory syndrome. *Clinical infectious diseases* 2012; **55**(7): 1004-11.
12. Ntsekhe M, Wiysonge CS, Gumedze F, et al. HIV infection is associated with a lower incidence of constriction in presumed tuberculous pericarditis: a prospective observational study. *PLoS One* 2008; **3**(6): e2253.
13. Wynn TA. Fibrotic disease and the TH 1/TH 2 paradigm. *Nature Reviews Immunology* 2004; **4**(8): 583-94.

14. Mutyaba AK, Balkaran S, Cloete R, et al. Constrictive pericarditis requiring pericardiectomy at Groote Schuur Hospital, Cape Town, South Africa: causes and perioperative outcomes in the HIV era (1990-2012). *The Journal of thoracic and cardiovascular surgery* 2014; **148**(6): 3058-65. e1.

7. CHAPTER 7: A description of the elevation of pericardial cortisol: cortisone ratio in patients with tuberculous pericarditis

7.1. Introduction

Cortisol is the major endogenous glucocorticoid hormone and is fundamental to the stress response.¹ Central to this function are its anti-inflammatory effects that reduce the potentially self-destructive effects of the host's immune response during an infection.² The suprachiasmatic nucleus of the hypothalamus regulates cortisol concentrations via the hypothalamic–pituitary–adrenal (HPA) axis, through its action on controlling the circadian rhythm and being sensitive to negative feedback inhibition.³ However, during acute stress or illness, plasma cortisol concentrations rise, and there is a loss of the diurnal variation.⁴ The elevated cortisol and disruption of the diurnal variation are thought to be due to the stimulation of the HPA axis, via the nerve fibres, lymphoid tissue, and the sympathetic medullary axis.⁵ Pro-inflammatory cytokines, for example, interleukin-1 (IL-1), also increase the production of corticotropin release hormone.⁵ Cortisol has anti-inflammatory effects, which it exerts by binding to the glucocorticoid receptor and by suppressing the aforementioned receptor function and suppress production of IL-1 β , IL-6, IL-8, interferon gamma-induced protein-10 (IP-10), IL-12, and inducible nitric oxide synthase by neutrophils and macrophages.⁵⁻⁷

Studies on tuberculosis (TB) have rarely examined the relationship between cortisol and cortisone at the site of infection. Baker *et al.*, for example, have demonstrated the elevated cortisol/ cortisone ratios in patients with pulmonary TB, yet this ratio is decreased with recovery.⁸ Moreover, with active PTB, the cortisol/ cortisone ratio is higher in bronchial lavage fluid, compared with that in plasma. This is supportive of a shift in the cortisol-cortisone shuttle activity in favour of the production of cortisol at the site of infection.^{8,9}

TB pericarditis (TBP) is a chronic granulomatous inflammatory disease, caused by infection of the pericardium by *Mycobacterium tuberculosis*.⁸ The immunological response to TBP leads to an accumulation of an inflammatory exudate in the pericardial space,¹⁰ followed by constrictive pericarditis in the short and long terms, respectively.¹¹ About 8% of patients with TBP develop constrictive pericarditis, a condition that causes pericardial fibrosis, leading to stiffening of the pericardium, and compromises cardiac function.¹² Immunomodulatory therapy with adjunctive corticosteroids is associated with a 40% reduction in constrictive pericarditis,¹³ emphasizing the importance of understanding the relationship between glucocorticoids and the immune system. As the site of infection in TBP is the pericardium and its inflammatory milieu,¹⁰ sampling the contents of the pericardial space should serve as a direct substrate from which the interaction between glucocorticoids and its respective inflammatory response can be evaluated. In addition, obtaining samples from this anatomical locale is impossible, except under unusual circumstances, for example, TBP, where there is a substantial volume of fluid surrounding the heart, as routine sampling of this space in the absence of fluid will invariably cause cardiac injury.¹⁴ A systematic study of pericardial glucocorticoids and cytokine responses in TBP has never previously been performed, and studies

evaluating this interaction in TBP will be pivotal in shaping our understanding of the changes that occur during this infective process, where, otherwise, it would have relied upon indirect evidence.

We previously published data on cytokine concentrations in the pericardium, plasma, and saliva of patients with TBP, in which we found that adjunctive corticosteroid therapy reduced saliva IL-1 β , plasma IL-6, and pericardial IL-8 concentrations.¹⁵ As changes in cytokine concentrations are expected to occur in inflammatory and infective conditions,^{8,9} these concentrations may be altered in response to an infected milieu, or it may be the result of changes in the cortisol/ cortisone ratio at the site on infection, dampening the inflammatory response.⁸

We hypothesized that there is a shift in the cortisol-cortisone shuttle enzyme activity, resulting in escalated cortisol concentrations, leading to a rise in the cortisol/ cortisone ratio in patients infected with TBP, particularly of the pericardium. We further hypothesize that the rise in the cortisol/ cortisone ratio is potentiated by a rise in pro-inflammatory cytokines at the site of infection, leading to the suppression of cortisone and relatively greater concentrations of cortisol.

Our objectives were, therefore, to describe the pericardial cortisol/ cortisone ratio, a site that has never been previously evaluated for excursions of cortisol and cortisone, given its relative inaccessibility in patients with TBP, and to compare it to the more accessible plasma and saliva compartments, respectively, and to evaluate whether this rise corresponds to a rise in pericardial pro-inflammatory cytokines in the pericardium.

7.2. Materials and methods

The study was approved by the University of Cape Town Human Research Ethics Committee and adhered to the Declaration of Helsinki 2013 version 7.0.¹⁶ Patients with TBP participated in a randomized control trial consisting of standard TB therapy with prednisolone or matched placebo: the Management of Tuberculous Pericarditis (IMPI) trial, Clinical Trials Registry Number: NCT00810849.¹³ Only a small subset of the participants of the aforementioned trial was included in this sub-study if they presented to Groote Schuur Hospital in Cape Town, South Africa (a single site), from October 2012 to August 2014 (inclusive) and had a pericardial effusion amenable to pericardiocentesis. Figure 1 shows the number of participants who were screened, enrolled, and allocated prednisolone or matching placebo in the sub-study. Participants who agreed to participate in the IMPI trial were also invited to take part in this sub-study, in which a pigtail pericardial drain was left *in situ* for 24 hours to sample pericardial fluid continually, and their endogenous glucocorticoids and prednisolone concentrations were assessed serially over a 24-hour period. Patients were eligible for inclusion, if they were 18 years of age or older; had a pericardial effusion confirmed by echocardiography; had evidence of definite or probable TBP, according to the Tygerberg diagnostic score; and had less than 1 week of anti-tuberculous therapy.^{17,18} Participants were commenced on World Health Organization (WHO)–recommended first-line anti-TB chemotherapy, consisting of rifampicin, isoniazid, ethambutol, and pyrazinamide, and randomized at a ratio of 1:1 to receive either adjunctive 120 mg of prednisolone or a matching placebo.

CONSORT Flow Diagram

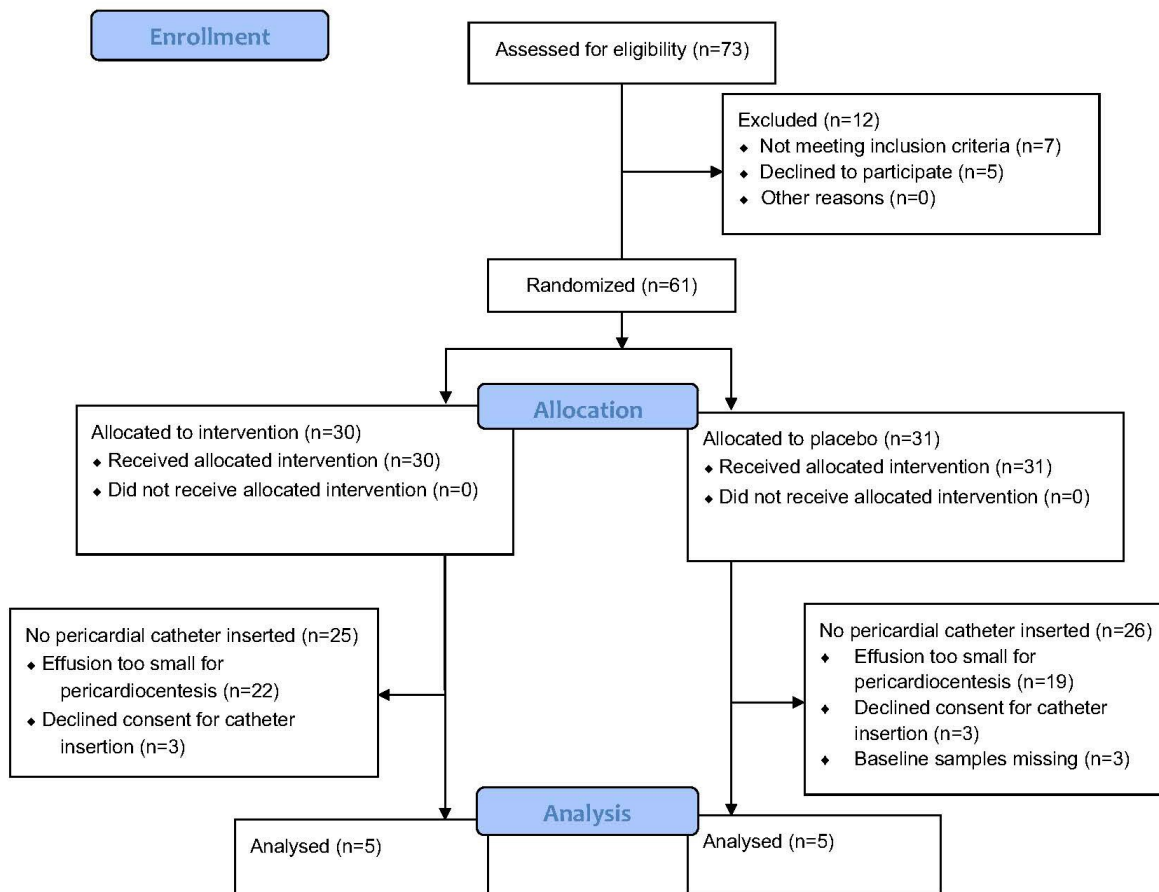


Figure 18: The number of participants screened and enrolled in each arm and the number of participants excluded from the analysis and the reason for exclusion.

The active dose of prednisolone was administered orally as a single dose, comprising 6 tablets, each containing 20 mg of prednisolone, produced by Cadila Pharmaceuticals Ahmedabad, India, which also manufactured matching (in appearance and number) inactive placebo comprising sugar and starch. Either prednisolone or placebo was administered immediately after the baseline samples were taken for saliva, plasma, and pericardial fluid and subsequently administered after a period of 24 hours and then daily for 28 days.

7.2.1. Sampling

Pericardial fluid, plasma, and saliva samples were then collected at baseline, before the administration of prednisolone, and then at the following time intervals after the administration of prednisolone or placebo: 0.5, 1, 2, 3, 5, 8, and 24 hours, following insertion of the pigtail catheter. In view of the serial nature of the samples, a decision was made to collect single samples at each time point, rather than duplicate. Baseline venous blood, saliva, and pericardial fluid samples were collected simultaneously at the bedside, following pericardiocentesis (as described above). Venous blood was extracted from a peripheral vein. Saliva was collected using Sarstedt salivettes® (Nümbrecht, Germany). The swab remained inside the mouth for a period of 2 to 3 minutes while gently chewing it and then was replaced in the salivette tube. Blood and pericardial fluid were collected in ethylenediamine tetra acetic acid (EDTA) tubes, each with a minimum of 4 ml, which were subsequently centrifuged at 2 000 g for 10 minutes, and 1 ml of supernatant was pipetted into each cryotube and performed in duplicate. The cryotubes containing pericardial fluid and plasma were immediately stored at -80°C in dedicated trays for subsequent analysis in a single batch. The

salivettes were also stored at -80°C to preserve stability, also using trays for vertical storage. On the day of analysis, samples were thawed and centrifuged at 1 500 g for 15 minutes. Time of day was recorded for each participant but differed in each case as patients presented at different times to the hospital, as they were extremely unwell in each case. The time intervals were logged for subsequent sampling and administration of prednisolone or placebo. The continuous monitoring of the freezer revealed no accidental temperature fluctuations.

7.2.2. Endogenous glucocorticoid and pharmacological glucocorticoid analysis

High performance liquid chromatography–tandem mass spectrometry (HPLC-MS) was used to measure cortisol and cortisone concentrations in all our serial samples. Cortisol, cortisone, prednisolone, and prednisone concentrations were also determined by HPLC-MS assay, developed in the Division of Clinical Pharmacology, University of Cape Town, using the methodology described by the Food and Drug Administration (FDA) guidance for industry for bioanalytical method validation.^{19,20} The assay was initially validated in plasma and then cross-validated in pericardial fluid and saliva. Charcoal stripped plasma, pericardial fluid, and saliva were used as controls. The limit of detection of assays was 0.5833 nmol/L; for concentrations that were measured below detection limits, a value of half the detection limit was assigned to allow calculation of relative ratios.

The assays were validated according to the FDA guidelines.²⁰ Samples were processed with a liquid-liquid extraction method using ethyl acetate, followed by HPLC-MS detection using an AB SCIEX API 4000 instrument. An Agilent Zorbax-SB Phenyl Rapid Resolution HT 1.8 μm , 2.1 $\times$ 100 mm analytical column was used.

Cortisol, cortisone, prednisolone, and prednisone were monitored at mass transitions of the protonated precursor ions at 363.2, 361.2, 361.2, and 359.2 to the product ions at 121.2, 163.1, 147.2, and 147.1, respectively. Cortisol-d4, cortisone-d8, prednisolone-d8, and prednisone-d4 stable isotope-labelled internal standards were used and monitored at mass transitions of the protonated precursor ions at 367.3, 369.3, 369.3, and 363.2 to the product ions 121.2, 168.2, 150.2, and 317.3, respectively. The calibration curves fitted quadratic (weighted by 1/concentration (2)) regressions over the ranges from 5.38 to 2758.6 nmol/L in plasma, pericardial fluid, and saliva.

7.2.3. Measurement of cytokine concentrations

Milliplex™ kits (HCYTOMAG-60 K, Millipore, St. Charles, MO, USA) and the Bioplex200 reader from Bio-Rad were used to measure the following cytokines: IFN- γ , IL-1 α , IL-1 β , IL-6, IL-10, IL-12p40, TNF- α , IL-8, and IP-10, in pericardium, plasma, and saliva. When performing the cytokine analyses, a minimum volume of 250 μ l was required. Samples were batched and analysed on a single occasion. We did not perform our own validation study for pericardial fluid and saliva, but employed the same methodology outlined by the manufacturer for the plasma cytokines that we analyzed.²¹

7.2.4. Statistical analysis

Descriptive statistics were used to summarize patient data, namely, frequency and percentage for categorical data and median with interquartile range (IQR) for continuous data. The differences between participant allocated to placebo and those allocated to prednisolone were compared using the Mann–Whitney U-test and were performed using GraphPad Prism version 9.2.0 (San Diego, CA) and StataCorp version 17.0 (College Station, TX). Comparisons among the three sampled compartments (plasma, pericardial fluid, and saliva) were performed using the Friedman test, with post-hoc analysis between individual groups using the Wilcoxon test and were performed using SPSS version 28.0 (Chicago, IL). We reported *p*-values of less than 5% to represent significance.

7.3. Results

The participants screened and subsequently enrolled into this sub-study are shown in Figure 18. Ten participants were enrolled, five of whom were men. Six participants were HIV positive, and only one participant was on anti-retroviral therapy at the time of enrolment. Four of the six (67%) HIV-positive participants had CD4 counts below 200 cells/mm³. Five participants had a diagnosis of confirmed TBP (50%), whereas the remaining participants had a diagnosis of probable TBP. All participants were of black African ancestry, except one who was of mixed ancestry. As shown in Table 14, there were no differences between participants allocated to placebo versus those allocated to prednisolone.

Table 14: Baseline characteristics of patients with TBP, allocated to either prednisolone or placebo, in addition to standard anti-TB therapy

Variable	Total (N=10)	Placebo (N=5)	Treatment (N=5)	p-value, Placebo vs treatment
Age (years)	38 (25-52)	45 (25-51)	31(28-44)	0.7540
Sex, proportion of males	5(50%)	2 (40%)	3 (60%)	0.5485
Weight (kg)	66 (53-72)	70 (53-73)	66 (52-72)	0.5970
HIV positive	6(60%)	3 (60%)	3 (60%)	1.000
On HAART	1(10%)	1 (20%)	0 (0%)	0.3173
CD4 count (cells/mm³)	319 (139-485)	403 (240 -603)	139 (135-319)	0.2207
Creatinine (μmol/L)	82 (65-105)	115 (95-189)	65 (45-82)	0.0635
Serum globulin (g/L)	55 (38-56)	52 (39-56)	55 (38-56)	1.000
Pericardial protein (g/L)	66 (62-68)	65 (56-72)	66 (62-68)	0.9021
Pericardial Adenine deaminase (IU/L)	53 (33-92)	72 (42-113)	51 (33-57)	0.4724
Definitive TBP	5(50%)	2 (40%)	3 (60%)	0.5485

Categorical data represented in n (%) and continuous data represented in median (interquartile range)

7.3.1. Cortisol measurements

At baseline, the median (IQR) cortisol concentration in the pericardium was 303 (258–385) nmol/L, plasma cortisol was 443 (379–523) nmol/L, and salivary cortisol was 20 (10–32) nmol/L. The median (IQR) cortisone concentration was 15.0 (0.6–21.7) nmol/L, 49 (35–58) nmol/L, and 37 (25–55) nmol/L, in pericardium, plasma, and saliva, respectively. The relevant median (IQR) cortisol/ cortisone ratios in the three

compartments were significantly different from each other at 20 (13–445) in the pericardium, 9.1 (7.4–12.1) in plasma, and 0.4 (0.3–0.8) in saliva (Figure 19).

Plasma, Pericardial and Saliva Cortisol/ Cortisone Ratios

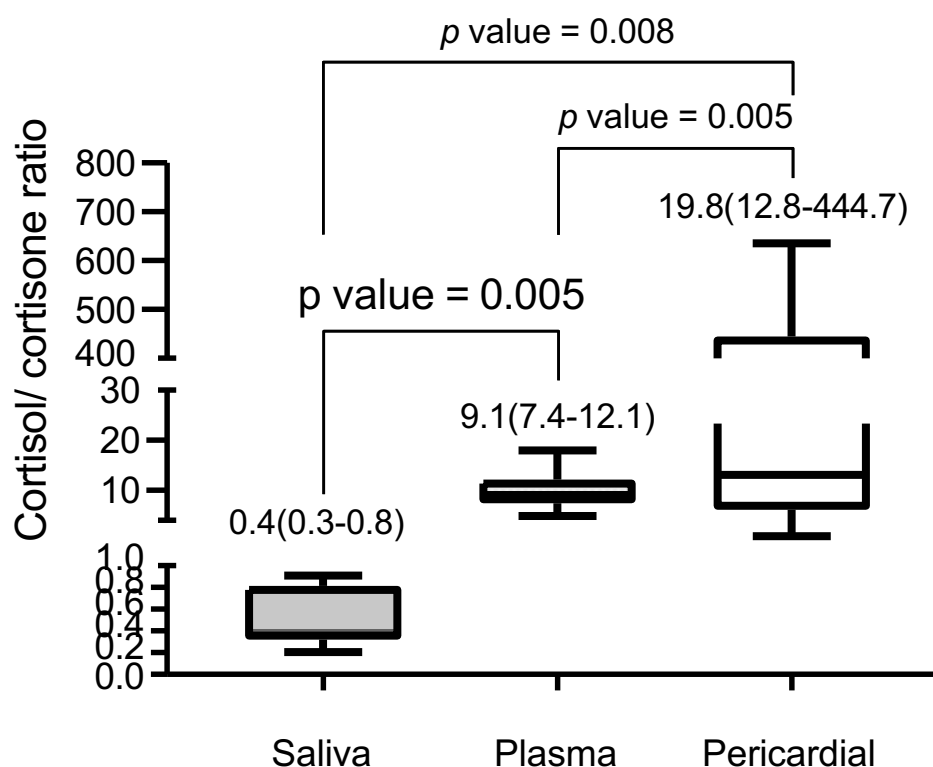


Figure 19: The baseline median (interquartile range) of the cortisol/ cortisone ratio in saliva, plasma, and pericardial fluid was 0.4 (0.3–0.8), 9.1 (7.4–12.1), and 19.8 (12.8–444.7), respectively. The p-value indicates the pairwise comparison for the post-hoc Wilcoxon test.

7.3.2. Cytokine measurements

7.3.2.1. Comparison of cytokines in plasma in patients with TBP versus reference range derived from plasma of healthy controls

The cytokine concentrations in the three compartments, pericardium, plasma, and saliva are shown in Table 15. The plasma cytokine concentrations were compared with their respective reference ranges, as determined in healthy individuals' plasma,

as shown in Table 15. The plasma cytokine concentrations of IL-1 α , IL-1 β , IL-8, IL-10, IP-10, and IL-12P40 were comparable with their reference ranges,²²⁻²⁵ whereas TNF- α (2-fold), IL-6 (10-fold), IL-8 was (5-fold), and IFN- γ (200-fold) are greater than their reference ranges.²¹

Table 15: Plasma, Pericardial and Saliva Cytokine concentrations and their respective plasma reference ranges

Cytokine	Plasma Reference range pg/ml Median(10th%tile-90th%tile)²¹	Plasma median (IQR) pg/ml	Pericardial median (IQR) pg/ml	Saliva median (IQR) pg/ml	p-value Friedman test
IFN- γ	0.1(0.1-0.3)	28(11-163)	2 061(690-2 765)	18(10-23)	0.0011
TNF- α	14(11-19)	33(22-58)	197(92-430)	16(10-80)	0.0043
IL-1 α	0(0-0)	0(0-0)	0(0-62)	2999(979-9910)	0.0002
IL-1 β	0(0-0)	0(0-0)	8(0-114)	31(21-180)	0.0039
IL-6	3.4(1.3-10)	23(18-75)	8 816(8 225-9 953)	11(5-32)	0.0009
IL-8	4.4(2.4-6.5)	23(14-31)	8 840(1 841-11 635)	401(93-1256)	0.0001
IL-10	4.8(3.7-8.8)	4(0-9)	34(23-77)	17(2-36)	0.0054
IP-10	64(33-103)	82(33-155)	1 414(1 002-1 574)	1061(201-2420)	0.0031
IL-12P40	0(0-0)	0(0-0)	0(0-1)	24(0-48)	0.0037

Source of recommended plasma reference ranges for the following cytokines.²¹ The Friedman test was used to evaluate whether there is a difference between cytokine concentrations in all three compartments.

7.3.2.2. Comparison of pericardial cytokines from patients with TBP with plasma cytokine of healthy controls

Due to the absence of normal reference ranges for pericardial cytokine concentrations, the pericardial cytokine concentrations were compared with their respective plasma reference ranges, as determined in healthy individuals' plasma, which are also shown in Table 15. Pericardial IL1 α , IL-1 β , and IL-12P40 were similar to their respective plasma reference ranges, whereas pericardial cytokine INF- γ (log3), TNF- α (10-fold), IL-6 (log3), IL-8 (log3), IL-10 (7-fold), and IP-10 (20-fold) are higher than their respective plasma reference ranges.²²⁻²⁵ Saliva IFN- γ (log-2), IL-1 α (log3), IL-1 β (30-fold), IL-6 (log3), IL-8 (log3), IL-10 (3-fold), IP-10 (20-fold), and IL-12P40 (20-fold) are lower than their reference ranges, respectively, whereas TNF- α was comparable to its respective reference range.²¹

7.3.2.3. Comparison of pericardial, saliva, and plasma cytokines in patients with TBP

For the different compartments, there were marked differences in cytokine concentrations. For example, pericardial IL-6 was 600-fold greater than saliva and plasma concentrations. In addition, INF- γ was about 100-fold greater in the pericardium, compared with saliva and plasma concentrations; however, the latter two were similar. TNF- α was greatest in pericardium and was eight-fold greater than that of saliva and plasma concentrations, respectively. Saliva IL-1 α , by contrast, was elevated by a magnitude of log3, compared with plasma and pericardial concentrations, which were similar. IL-1 β was elevated in saliva, lower in pericardium,

and least in plasma. The relative proportion of IL-8 concentrations in plasma, pericardium, and saliva was 1:20:400. The concentrations of IL-10 were similar in pericardium and saliva, but IL-10 was notably lower in plasma. IP-10 was similar in pericardium and saliva but was 15-fold lower in plasma. Saliva IL-12P40 was considerably higher, compared with plasma and pericardial IL-12P40, with the latter two matrices being virtually undetectable.

7.3.2.4. Prednisolone and cortisol/cortisone ratio

The cortisol/ cortisone ratio was highest in pericardial fluid median (IQR) 20 (13–445), followed by plasma median (IQR) of 9.1 (7.4–12) and then lowest in saliva median (IQR) of 0.4 (0.3–0.8). In the placebo arm, the cortisol and cortisone concentrations were unchanged throughout the 24-hour period in all three matrices, suggesting a high degree of reproducibility. In participants allocated to prednisolone, there was a rapid (from as early as 3 hours) and sustained reduction of cortisol and cortisone concentration for all three compartments.

The cortisol/ cortisone ratios in the placebo arm did not change significantly in all the matrices over the 24 hour period, as shown in Figure 20A. Figure 20B shows that, after a period of 5 hours in patients who received prednisolone, the pericardial cortisol/ cortisone ratio dramatically increased followed by a decrease after 8 hours, whereas these ratios in plasma and saliva were unchanged over the 24 hour period.

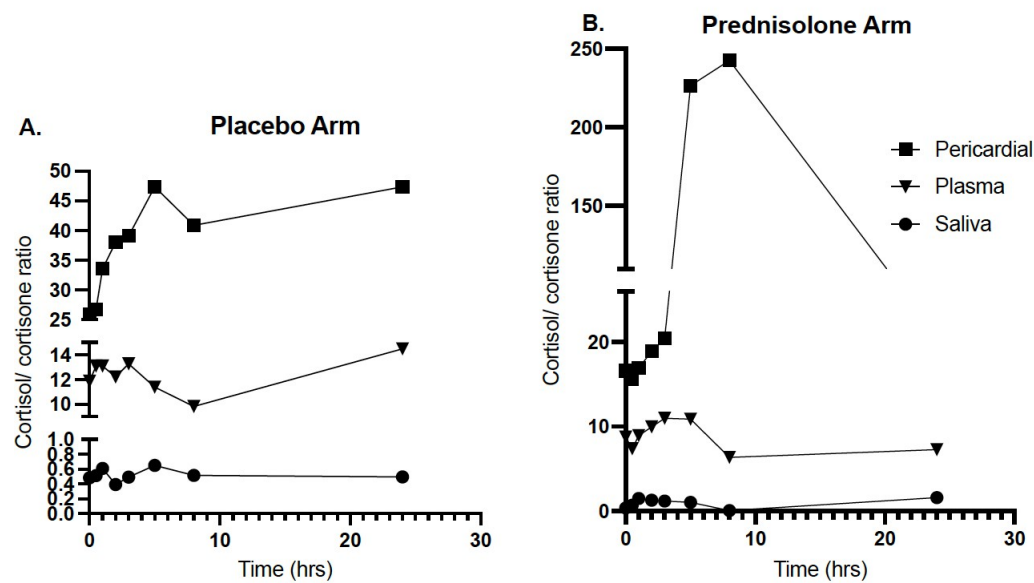


Figure 20: The cortisol/ cortisone ratio over a 24-hour period. (A) The cortisol/ cortisone ratio in participants allocate to placebo. (B) The ratio in participants allocated to prednisolone.

7.4. Discussion

This is the first examination of pericardial cortisol and cortisone concentrations and their relationship to cytokine concentrations in patients with TBP. We found that the pericardium has a high concentration of cortisol, close to that of the plasma compartment and, by contrast, much higher than in the salivary compartment, but a lower cortisone concentration. This resulted in an elevated pericardial cortisol/ cortisone ratio, when compared with plasma and saliva. In addition, most key cytokines measured were markedly elevated in the pericardium versus that of plasma.

Local cortisol-to-cortisone equilibriums are mostly dependent on the activity of local expression of the enzyme 11HSD-1 and 11HSD-2. In salivary glands, for example, there is a well-established high expression of 11HSD-2, resulting in local conversion of cortisol to cortisone and a low cortisol/ cortisone ratio.²⁶ In view of the absence of

direct evidence in the setting of TBP, we speculate that there is an increased expression of 11HSD-1 in the endothelial cells of the pericardial vasculature where it is expressed,^{8,9} but this has not yet been proven. Furthermore, our hypothesis is that this is driven by the increased local concentrations of pro-inflammatory cytokines such as TNF- α and IL-1 β . Moreover, our conjecture is that the aforementioned proinflammatory cytokines result in a shift in favour of pericardial cortisol rather than cortisone, compared with plasma.^{27,28}

These findings are consistent with the findings of *Baker et al.*, who showed an elevated cortisol/ cortisone ratio in bronchoalveolar lavage fluid in patients with pulmonary TB despite a normal central control of glucocorticoid production, in keeping with altered peripheral metabolism at the site of infection.⁸

We do not know whether the altered local glucocorticoid equilibrium is potentially beneficial, harmful, or not of pathogenic relevance at all. TB is an inflammatory disease, and much of the damage in TB is due to an inflammatory response.¹⁰ The elevated cortisol/ cortisone ratio may be of benefit in patients with TBP, through paring down the inflammatory response. Adjunctive corticosteroid therapy has been prescribed for the treatment of TBP patients, with the view to curtailing the inflammatory response.¹² *Baker et al.*, however, speculated that increased active glucocorticoids in the lungs of patients with pulmonary TB could be harmful; it could, for example, explain the increased production of TGF- β and IL-10 in human TB and the impaired macrophage function. Either way, manipulation of the cortisol/ cortisone equilibrium with, for example, 11HSD-1 inhibitors could be beneficial or harmful in patients with TBP and would need further investigation.

Previous work has shown that providing supraphysiological doses of prednisolone in a clinical study of TBP has conferred clinical benefit in HIV-negative patients, by reducing incidence of hospitalisation and constrictive pericarditis, a lethal complication.¹³ Our study was not designed to determine the clinical benefit but to determine its action on suppression of endogenous glucocorticoids in all compartments. Administering supraphysiologic prednisolone led to cortisol and cortisone suppression in all three compartments, plasma pericardium, and saliva. Plasma and saliva cortisol and cortisone suppression in response to administration of supra-physiological glucocorticoids have been well documented;^{29,30} however, this is the first time that it has been demonstrated in the pericardium. Administration of prednisolone caused the pericardial cortisol/ cortisone ratio to initially increase then decline; this is despite a rapid and sustained decline in pericardial cortisol. The median pericardial cortisone concentrations from 5 hours onward dropped to below 1 nmol/L; therefore, according to L'Hospital's Rule,³¹ a ratio approaches infinity as the function of the denominator approaches zero, and, thus, the rise in pericardial cortisol/ cortisone ratio may be artifactually high, due to the extremely low pericardial cortisone concentrations. The later decline in cortisol/ cortisone ratio is due to the extremely low cortisol concentrations at later timepoints. Overall, our data support that glucocorticoid therapy using prednisolone suppressed pericardial cortisol and cortisone concentrations in a similar manner to plasma and saliva cortisol and cortisone concentrations. In addition, the subsequent follow-up data during the 24-hour period for those individuals who received prednisolone, corroborates the reliability of the data in Figure 20.

It is apparent that the vast majority of our participants were of black African ancestry. Black and white women did not differ in the study evaluating the HPA axis using the

24-hour urinary excretion of cortisol and dexamethasone suppressibility matched for age, weight, body mass index, and body surface area.³² Despite the majority of our participants being of black African ancestry, it is likely that these data are generalizable to other ethnic population groups.

However, the study had a number of weaknesses, it is composed of a small sample size, and sampling was limited to the first 24-hour time period. It is likely that additional physiological responses occurred beyond this period, for example, during recovery. This study did not undertake cortisol-cortisone shuttle enzyme expression studies, thus relying on indirect evidence that it conferred an immune modulatory response, which warrants confirmation in the future. The participants were mainly of black African ancestry; therefore, there is uncertainty whether these findings are generalizable to other populations. We did not perform our own validation study on the cytokines but used the prescribed reference values. This represents an additional weakness of our study. Moreover, the reference ranges in plasma may not be applicable to pericardial fluid, thus invoking another degree of uncertainty in the interpretation of these data.

7.5. Conclusion

This pilot study was able to show a greater inflammatory response in the pericardium of patients with TBP, as evidenced by the following elevated proinflammatory cytokines including INF- γ , TNF- α , IL-6, and IL-8. In the pericardium, there was also a raised cortisol/ cortisone ratio, compared with plasma and saliva. In light of the previously documented evidence for the beneficial effect of adjunctive corticosteroids in patients with TBP, the elevated endogenous pericardial cortisol/ cortisone ratio may have a partial immunomodulatory and potentially, an immunostimulatory effect.

7.6. Reference

1. Young EA, Abelson J, Lightman SL. Cortisol pulsatility and its role in stress regulation and health. *Frontiers in neuroendocrinology* 2004; **25**(2): 69-76.
2. Gupta P, Bhatia V. Corticosteroid physiology and principles of therapy. *The Indian Journal of Pediatrics* 2008; **75**(10): 1039.
3. Yeager MP, Pioli PA, Guyre PM. Cortisol exerts bi-phasic regulation of inflammation in humans. *Dose-response* 2011; **9**(3): dose-response. 10-013. Yeager.
4. Yeager MP, Guyre P, Munck A. Glucocorticoid regulation of the inflammatory response to injury. *Acta Anaesthesiologica Scandinavica* 2004; **48**(7): 799-813.
5. Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology* 2005; **5**(3): 243-51.
6. Lim HY, Müller N, Herold MJ, Van Den Brandt J, Reichardt HM. Glucocorticoids exert opposing effects on macrophage function dependent on their concentration. *Immunology* 2007; **122**(1): 47-53.
7. Tuckermann JP, Kleiman A, McPherson KG, Reichardt HM. Molecular mechanisms of glucocorticoids in the control of inflammation and lymphocyte apoptosis. *Critical reviews in clinical laboratory sciences* 2005; **42**(1): 71-104.
8. Baker RW, Walker BR, Shaw RJ, et al. Increased cortisol: cortisone ratio in acute pulmonary tuberculosis. *American journal of respiratory and critical care medicine* 2000; **162**(5): 1641-7.
9. Rook G, Baker R, Walker B, et al. Local regulation of glucocorticoid activity in sites of inflammation: Insights from the study of tuberculosis. *Annals of the New York Academy of Sciences* 2000; **917**(1): 913-22.
10. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005; **112**(23): 3608-16.
11. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *South African Medical Journal* 2008; **98**(1): 36-40.
12. Ntsekhe M, Wiysonge C, Volmink J, Commerford P, Mayosi B. Adjuvant corticosteroids for tuberculous pericarditis: promising, but not proven. *Qjm* 2003; **96**(8): 593-9.

13. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
14. Imazio M, Adler Y. Management of pericardial effusion. *European heart journal* 2013; **34**(16): 1186-97.
15. Shenje J, Lai RP, Ross IL, et al. Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study. *IJC heart & vasculature* 2018; **18**: 104-8.
16. Association GAotWM. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *The Journal of the American College of Dentists* 2014; **81**(3): 14.
17. Burgess LJ, Reuter H, Carstens ME, Taljaard JF, Doubell AF. The use of adenosine deaminase and interferon- γ as diagnostic tools for tuberculous pericarditis. *Chest* 2002; **122**(3): 900-5.
18. Reuter H, Burgess L, Van Vuuren Wa, Doubell A. Diagnosing tuberculous pericarditis. *Journal of the Association of Physicians* 2006; **99**(12): 827-39.
19. Ionita IA, Fast DM, Akhlaghi F. Development of a sensitive and selective method for the quantitative analysis of cortisol, cortisone, prednisolone and prednisone in human plasma. *Journal of Chromatography B* 2009; **877**(8-9): 765-72.
20. FDA F. Guidance for industry: bioanalytical method validation. <http://www.fda.gov/cder/Guidance/4252f1.pdf> 2001.
21. Onlink. Validation Data. 14 April 2023 2023. <https://olink.com/content/uploads/2023/02/olink-flex-validation-data.pdf>.
22. Köksal D, Unsal E, Poyraz B, et al. The value of serum interferon-gamma level in the differential diagnosis of active and inactive pulmonary tuberculosis. *Tuberk Toraks* 2006; **54**(1): 17-21.
23. Strackowski M, Dzieńis-Strackowska S, Stępień A, Kowalska I, Szelachowska M, Kinalska I. Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor- α system. *The Journal of Clinical Endocrinology & Metabolism* 2002; **87**(10): 4602-6.

24. Sekiyama K, Yoshiba M, Thomson A. Circulating proinflammatory cytokines (IL-1 β , TNF- α , and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clinical & Experimental Immunology* 1994; **98**(1): 71-7.
25. Sarris AH, Kliche K-O, Pethambaram P, et al. Interleukin-10 levels are often elevated in serum of adults with Hodgkin's disease and are associated with inferior failure-free survival. *Annals of Oncology* 1999; **10**(4): 433-40.
26. Zhang Q, Chen Z, Chen S, Xu Y, Deng H. Intraindividual stability of cortisol and cortisone and the ratio of cortisol to cortisone in saliva, urine and hair. *Steroids* 2017; **118**: 61-7.
27. Andersson AK, Atkinson SE, Khanolkar-Young S, et al. Alteration of the cortisol–cortisone shuttle in leprosy type 1 reactions in leprosy patients in Hyderabad, India. *Immunology letters* 2007; **109**(1): 72-5.
28. Escher G, Galli I, Vishwanath BS, Frey BM, Frey FJ. Tumor necrosis factor α and interleukin 1 β enhance the cortisone/cortisol shuttle. *The Journal of experimental medicine* 1997; **186**(2): 189-98.
29. Ross I, Lacerda M, Pillay T, et al. Salivary cortisol and cortisone do not appear to be useful biomarkers for monitoring hydrocortisone replacement in addison's disease. *Hormone and Metabolic Research* 2016; **48**(12): 814-21.
30. MEIKLE AW. Dexamethasone suppression tests: usefulness of simultaneous measurement of plasma cortisol and dexamethasone. *Clinical endocrinology* 1982; **16**(4): 401-8.
31. Osgood WF. The Law of the Mean and the Limits. *Annals of Mathematics* 1898: 65-78.
32. Yanovski JA, Yanovski SZ, Gold PW, Chrousos GP. Differences in the hypothalamic-pituitary-adrenal axis of black and white women. *The Journal of Clinical Endocrinology & Metabolism* 1993; **77**(2): 536-41.

8. CHAPTER 8: Final discussion and recommendations

8.1. Summary of the major findings

As tuberculous pericarditis (TBP) is associated with poorer clinical outcomes, when compared to other forms of tuberculosis (TB),¹ this body of work sought to gain more insight, by obtaining objective biochemical and inflammatory measures at the site of infection. Sampling the pericardial space has yielded novel insights on the pharmacokinetics and immunological process that take place in the pericardial space of patients with TBP. Prior to this body of work very little was known about the pathophysiological changes, which occur in the pericardial compartment of patients with TBP. It was proposed that the pericardial compartment as a substrate, may inform us of the factors associated with a poor prognosis in TBP.¹⁻³

This body of work described the pharmacokinetic (PK) profile of first-line anti-TB drugs namely; rifampicin, ethambutol, pyrazinamide and isoniazid in the pericardial compartment and compared this to the PK profile in the plasma compartment.² It is of concern that of these aforementioned four drugs, only isoniazid achieved sufficient pericardial concentrations required for a therapeutic effect, raising doubt as to the efficacy of this pharmacotherapeutic regimen.² This study also described the PK profiles of prednisolone,⁴ a synthetic corticosteroid used for adjunctive corticosteroid therapy in patients with TBP, with the purpose of reducing an inflammatory response. Pericardial prednisolone concentrations were shown to be comparable to those of plasma and prednisolone, but there was a delay of prednisolone reaching adequate concentrations in the pericardial space.⁴

The cytokine profiles of the pericardial compartment in TBP were studied and the effect of adjunctive corticosteroid therapy on pericardial cytokine concentrations, were evaluated, which demonstrated that there were different cytokine responses among the plasma, pericardial and saliva compartments. Caspases 3, 8 and 9 were higher in the pericardial compartment, compared to the plasma and saliva compartments.⁵ Importantly, this study also showed that adjunctive corticosteroid therapy was associated with lower plasma interleukin (IL) 6, lower saliva IL-1 β and a lower IL-8 in saliva and pericardial fluid,⁵ providing a proof of concept that glucocorticoids may have had an anti-inflammatory effect in TBP.

It has already been established that the cortisol/ cortisone ratio is elevated at the site of infection in patients with pulmonary TB and this body of work has for the first time demonstrated a similar rise in the pericardium of patients with TBP.⁶ Importantly, the elevated cortisol/ cortisone ratio was associated with elevation of the following pericardial cytokines, INF- γ , TNF- α , IL-6, and IL-8.

8.1.1. The pharmacokinetics of anti-TB drugs in pericardial compartment

Anti-TB chemotherapy comprising rifampicin, isoniazid, pyrazinamide and ethambutol is the cornerstone of the management of drug sensitive TBP.⁷ In order to derive adequate benefit from anti-TB chemotherapy, all four drugs should ideally exceed their respective minimum inhibitory concentrations (MIC) at the site of infection, namely the pericardial space.⁸ This study described for the first time that the PK profiles of these four drugs in the pericardial space and revealed that pericardial concentrations of rifampicin and ethambutol were lower than their corresponding plasma concentrations and in both instances were both below their respective MICs,² and by implication

resulted in sub-optimal anti-mycobacterial activity. The pericardial concentration to MIC ratio standard deviation (SD) for rifampicin was 0.19 (0.33), while that of ethambutol was 0.55 (0.22).²

While pyrazinamide pericardial concentrations were similar to plasma pyrazinamide concentrations, pericardial pyrazinamide concentrations were nonetheless, below the MIC.² Pyrazinamide concentrations in the epithelial lining fluid of the lung is about 17-fold higher than plasma concentrations and as a result exceeds the MIC in lung tissue. This is by contrast, ideal for the treatment of pulmonary tuberculosis (TB), but inadequate for TBP.⁹ Furthermore, pyrazinamide bactericidal activity is pH-dependent and is optimal in acidic conditions below a pH of 6.8.¹⁰ The average pericardial fluid pH of the participants in the study was 7.43, standard deviation 0.11,² signifying that the relatively high pH is likely to further compromise the bactericidal effect of pyrazinamide.

Only isoniazid reached optimal therapeutic concentrations in the pericardial compartment, possibly accounting for the likely poor outcome associated with TBP.² These findings cumulatively suggest that patients with TBP, who are on standard first-line anti-TB are receiving inadequate treatment.¹¹

8.1.2. The pharmacokinetics of prednisolone in the pericardial compartment

Prednisolone has been used empirically for the treatment of TBP, with one important study showing a 40% reduction in incidence of constrictive pericarditis and a 20% reduction in the incidence of hospitalization.¹² It is a potent, synthetic corticosteroid, which has been widely used for its anti-inflammatory effect for over 60 years.¹³ As prednisolone has had benefit among HIV negative patients with TBP, determining

pericardial pharmacokinetic data in TBP are critical. The PK of prednisolone in plasma and saliva have been well described, yet until now the pericardial fluid prednisone PK data have been enigmatic.^{14,15}

This study showed that the PK of prednisolone in plasma, saliva and pericardial fluid are all described by a similar one compartment model.⁴ The main difference was that the PK profile of prednisolone in the pericardial compartment had a slower absorption rate, when compared to that of plasma, which indicates that there is a lag in absorption in the pericardial compartment. The ratio of pericardial/ plasma peak prednisolone concentration was a median of 0.28 with a range from 0.12 to 0.82.⁴ Despite this marked difference in peak concentrations, the AUC₀₋₂₄ in the respective compartments were comparable. It remains unknown in this context, which parameter, either peak concentration or AUC₀₋₂₄ is a better predictor of adequate prednisolone dosing and should be explored in future studies. This study contributes substantially to the scientific body of evidence by providing novel data, which for the first time can corroborate detection of prednisolone in the pericardial space and moreover, has established a basic PK model for prednisolone in TBP, on which further studies can be based.

8.1.3. The cytokine response of pericardial compartment

In patients with active TB disease, there is a profound inflammatory response to immunogenic *Mycobacterium tuberculosis* (*Mtb*), which culminates in granuloma formation.¹⁶ A cascade of cytokines is released by various cells of the immune system to trigger different elements of the innate and adaptive immune system.¹⁷ Previous evidence has shown that adjunctive corticosteroid therapy reduces the incidence of

constrictive pericarditis and reduces hospitalisation, due to its immunomodulatory effect.^{12,18} By contrast, very little is known in respect of the immunological responses in patients with TBP. The pericardial compartment is not easily accessible. It requires invasive investigations to evaluate it and by implication, it was considered whether plasma and saliva can be used as a surrogate substrate from which to investigate the pericardial space. Moreover, by establishing a dose response relationship between adjunctive corticosteroids and changes in cytokine concentrations, it was hoped to derive appropriate doses, for adjunctive corticosteroid therapy. Unfortunately, this remains an area for which future research should be undertaken.

This novel study demonstrated that there were different cytokine responses in different compartments. Pericardial fluid had higher caspases 3, 8 and 9 concentrations, when compared to plasma and saliva.¹⁹ Caspase 3 is a pro-apoptotic caspase, which is also known as the death protease or executioner caspase.²⁰ Apoptosis is an active process, which induces controlled cell death, allowing for the dismantling of infected cells without further spread of infection, as observed with necrosis.²¹ On the other hand, caspase 8 and 9 inhibit caspase 3 activity.^{22,23} Caspase 8 sends a pro-survival signal, which suppresses apoptosis, while caspase 9 cleaves caspase 3.^{22,23} Given that the pericardial space is the site of infection, it is understandable that there is a greater impetus for apoptosis rather than inflammation in a bid to safely dispose of infected cells and this in turn is accompanied by measures to regulate this pro-apoptotic drive.

The major consequences of corticosteroid therapy on the cytokine profiles, were that patients had lower plasma interleukin (IL) 6, lower saliva IL-1 β and a lower IL-8 in saliva and pericardial fluid, compared with those patients who did not receive corticosteroids.¹⁹ IL-6 is a multi-functional cytokine, which is pro-inflammatory,

promotes B cell maturation and acts as a link between the innate and adaptive immune system.²⁴ Dysregulation of IL-6 is associated with chronic inflammatory disease.²⁴ IL-1 β a pro-inflammatory cytokine, which has been implicated in inflammatory pain and autoimmune disease.²⁵ The activated inflammasome leads to secretion of IL-1 β , promoting pyroptosis, which is also known as inflammatory cell death.²⁵ The inflammasome is a multiprotein complex, which detects pathogen, other stressors and triggers release of cytokines.²⁶ IL-8 is a pro-inflammatory cytokine, which induces chemotaxis and activation of neutrophils and monocytes.²⁶ Lower concentrations of these three pro-inflammatory cytokines IL-6, IL-1 β and IL-8 supports the hypothesis that the therapeutic effect of corticosteroid therapy in patients with TBP is derived from the anti-inflammatory properties. These data for the first time provide an analysis that adjunctive corticosteroid therapy may have influenced the cytokine milieu in pericardial fluid, which could confer improved clinical outcomes.

8.1.4. The pericardial cortisol/cortisone ratio

The elevated pericardial cortisol/cortisone ratio reaffirms our initial premise, that there is an association between Mycobacterial infection and a localised elevated cortisol/cortisone ratio.^{6,27} As with previous studies, this rise spared compartments which were anatomically distant from the site of infection, namely, the plasma and saliva compartments. This is the first time this association has been demonstrated in patients with TBP. Due to the localised nature of this response, it is likely driven by the 11-beta-hydroxysteroid dehydrogenase (11HSD) enzymes, which regulates the equilibrium between cortisol and cortisone at tissue level. TNF- α and IL-1 β are known to affect the 11HSD type 2 enzyme, by shifting the equilibrium towards production of cortisol.²⁸ This is of great importance as our patients had elevated pericardial TNF- α and IL-1 β

concentrations, providing a plausible reason for their elevated cortisol/ cortisone ratio. Given the immune modulatory effect of cortisol in the pericardium, by either dampening an excessive immunological response or inducing an inflammatory reaction, it is premature to determine whether it is overwhelmingly beneficial or harmful.

8.2. Strengths of the study

The major strengths are that it was a prospective study, involving rich sampling and detailed analysis of several novel parameters in TBP. A detailed evaluation of pharmacokinetics of anti-tuberculous therapy was undertaken, a pharmacokinetic model of corticosteroids was generated, an analysis of inflammatory cytokines and a longitudinal clinical evaluation of these patients, providing an overall, comprehensive assessment, which has never been undertaken previously, in this condition. In order to have accomplished this, each of these patients underwent invasive pericardiocentesis and were followed up repeatedly to determine interval changes.

8.3. Weaknesses of the study

It is acknowledged that the sample size was small with only 14 participants, and 11 undergoing PK analysis, representing a pilot study.² The pharmacokinetic analyses of both the anti-TB drugs and prednisolone were limited to the first dose PK, as opposed to steady state PK, which could have provided more longitudinal data in respect of the dynamic equilibria.^{2,29} Rifampicin for example, causes liver enzyme induction, which occurs 10 to 14 days into treatment and further alters the PK profile of drugs, which are metabolized by cytochrome P450 enzyme by increasing their

metabolism. Considering the impact of anti-TB therapy, the PK profiles of the drugs of interest could be influenced in the medium term.

It would have been preferable to measure T lymphocytes in response to antigen stimulation also known as poly-functional lymphocytes.³⁰ The later approach has the advantage of being able to ascertain the number and functionality of T lymphocytes.

8.4. Recommendations for future areas of research

While this work is entirely novel, there are several areas in which it can be expanded upon. This study represented a series of pilot studies, and much of this work will need to be confirmed in a larger population with TBP.

8.4.1. Suggestions for further evaluation of the pharmacokinetics of anti-TB drugs in the pericardial compartment

There is a need for a steady state pharmacokinetic study of rifampicin, ethambutol, isoniazid and pyrazinamide. A steady state is reached once a drug PK profile has reached a dynamic equilibrium, occurring after 3 to 5 half-lives, which is often due to enzyme saturation.²⁹

The suboptimal rifampicin and ethambutol pericardial concentrations, which were identified require a safety and efficacy study of an anti-TB drug regimen with higher rifampicin and ethambutol doses. Rifampicin doses as high as 20 mg per kg, which is double the standard dose have been shown to be tolerable, paving the way potentially for improved therapeutic efficacy for TBP, but this would need to be tested to confirm safety and tolerability in the future.³¹ There is less of a margin for increasing the

ethambutol dose, as is it associated with optic neuritis.³² It is hypothesised, after conducting this study that optimal doses of rifampicin and ethambutol can reduce morbidity and mortality of TBP, comparable with pulmonary TB. Even if higher doses are associated with improved efficacy, there is a need to re-evaluate the risk benefit of a regimen, containing higher doses of rifampicin and ethambutol and their attending adverse effects.

8.4.2. Suggestions for further research into the effect of effect of prednisolone on function of stimulated lymphocytes

Future studies may require a more robust measure of immunological responses than plasma, saliva and pericardial cytokine concentrations. One measure of immunological response is evaluation of polyfunctional cells. Quantifying the number of T lymphocytes and determining the variety and magnitude of cytokines produced by each T lymphocyte population, in response to antigen stimulation, can be performed using flow-cytometry.³⁰ Immunosuppressive therapy has its downside, as it may leave the participant vulnerable to opportunistic infections, which in our population of HIV-positive patients seems counterintuitive.³³ It is therefore essential to determine the optimal level of immune modulation, which limits the harmful effects of the immune response to *Mycobacterium*, without leaving participants immunosuppressed. It is hoped that a better understanding of the immunological response on exposure to *Mycobacterium* can help find the optimal magnitude of immunomodulation, required from adjunctive corticosteroid therapy.

8.4.3. Suggestions for further research into the pharmacokinetic pharmacodynamic relationship between anti-TB drugs and clinical outcome

In addition to describing the pharmacokinetics of anti-TB drugs in the pericardial compartment, it is of great importance to determine the association of the effect of the PK and clinical outcomes, utilising pharmacokinetic pharmacodynamic (PK/PD) models.

8.4.4. Suggestions for further research into the pharmacokinetic pharmacodynamic relationship between prednisolone and cytokine concentration

This study was able to show that 120 mg of prednisolone suppressed plasma IL-6, saliva IL-1 β and saliva and pericardial fluid IL-8.⁴ It remains uncertain whether the dose of 120 mg is optimal and up to now has represented empiric dosing. There is a need for dose titration studies for prednisolone on the magnitude of reduction of each of the three cytokines. A dose response curve using a PK-PD model can be represented by a sigmoid $E_{\max}$ curve as shown in Figure 21 below.³⁴ The optimal dose varies from drug to drug, but the dose at which the drug exerts 90% of its efficacy (E_{90}),

is often used as a marker of optimal dose. A dose response curve would determine the ideal dose of prednisolone for managing patients with TBP.

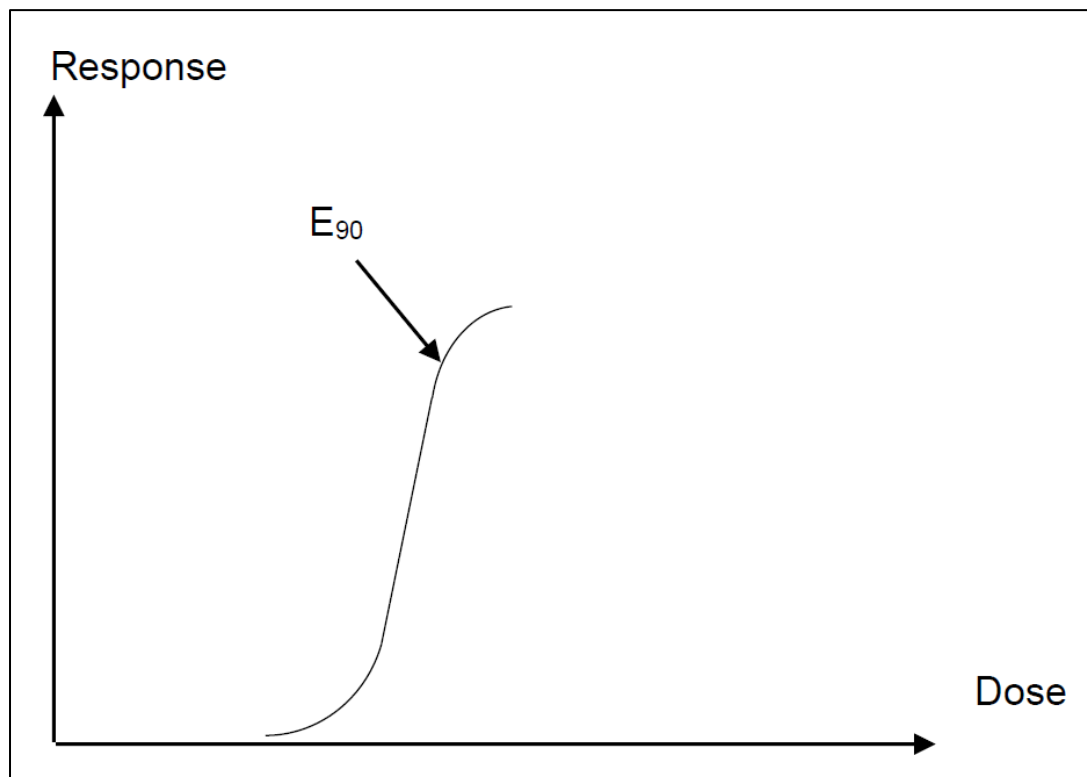


Figure 21: Dose response curve with optimal dose at E90

8.4.5. Suggestions research into alternative immune modulatory therapy for TBP

Adjunctive corticosteroid therapy in patients with TBP reduces incidence of constrictive pericarditis and reduces the need for hospitalisation, which are nevertheless associated with significant incumbent risks,¹² particularly among people with HIV co-infection.¹² Therefore, exploring alternative immune modulatory strategies for patients with TBP may represent a novel approach, which needs to be examined in the future. Pioglitazone for example, is a peroxisome proliferator-activated receptor- γ (PPAR γ) agonist.³⁵ and significantly increases both 11HSD1 activity and mRNA expression,³⁵

potentially increasing peripheral cortisol. The clinical benefit of this novel approach needs to be investigated in a randomised placebo-controlled trial.

8.5. Final conclusions

Patients who receive first line anti-TB chemotherapy at World Health Organization (WHO) recommended doses may be receiving suboptimal doses of rifampicin, pyrazinamide and ethambutol for their TBP and revision of these drug doses is probably warranted with more supportive evidence. Adjunctive corticosteroid therapy with 120 mg of pericardial prednisolone had a comparable AUC_{0-24hr} to that of plasma, but the maximum concentration was significantly lower in pericardial fluid, when compared to plasma. The significance of the lower maximum concentration in the pericardium remains unknown, but nevertheless had demonstrable alteration in the cytokine milieu, confirming its anti-inflammatory action. There was a significantly different cytokine response between plasma, pericardial compartment and saliva. Plasma IL-6, saliva IL-1 β and pericardial and saliva IL-8 were lower in patients who received 120 mg of prednisolone, compared to patients who did not receive prednisolone. Patients with TBP are at risk of considerable morbidity and mortality and every effort should be made to improve the outcomes for these patients, without relying on the data from patients with pulmonary tuberculosis. This body of work contributes to our understanding of the limitations in managing this condition and paves the way for future research in this area.

8.6. References

1. Mayosi BM, Wiysonge CS, Ntsekhe M, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. *South African Medical Journal* 2008; **98**(1): 36-40.
2. Shenje J, Adimora-Nweke FI, Ross IL, et al. Poor penetration of antibiotics into pericardium in pericardial tuberculosis. *EBioMedicine* 2015; **2**(11): 1640-9.
3. Gumbo T, Angulo-Barturen I, Ferrer-Bazaga S. Pharmacokinetic-pharmacodynamic and dose-response relationships of antituberculosis drugs: recommendations and standards for industry and academia. *The Journal of infectious diseases* 2015; **211**(suppl_3): S96-S106.
4. Shenje J, Gumbo T, Wiesner L, Ntsekhe M, Mayosi B, Ross I. Detectable prednisolone is delayed in pericardial fluid, compared with plasma of patients with tuberculous pericarditis: A pilot study. *IJC Heart & Vasculature* 2019; **22**: 105-10.
5. Shenje J, Lai RP, Ross IL, et al. Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study. *IJC heart & vasculature* 2018; **18**: 104-8.
6. Baker RW, Walker BR, Shaw RJ, et al. Increased cortisol: cortisone ratio in acute pulmonary tuberculosis. *American journal of respiratory and critical care medicine* 2000; **162**(5): 1641-7.
7. Health SAdo. National Tuberculosis Management Guidelines 2014: Department of Health; 2014.
8. Falzon D, Jaramillo E, Schunemann HJ, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. *Eur Respir J* 2011; **38**(3): 516-28.
9. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *The Journal of infectious diseases* 2013; **208**(9): 1464-73.
10. McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environments *in vitro*. *American review of tuberculosis* 1954; **70**(4): 748-54.
11. Committee MRCTCT. Long-term chemotherapy in the treatment of chronic pulmonary tuberculosis with cavitation. *Tubercle* 1962; **43**: 201-67.
12. Mayosi BM, Ntsekhe M, Bosch J, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *New England Journal of Medicine* 2014; **371**(12): 1121-30.
13. Criepp LH. Prednisone and prednisolone in the treatment of allergic diseases. *Journal of Allergy* 1956; **27**(3): 220-30.
14. Frey BM, Frey FJ. Clinical pharmacokinetics of prednisone and prednisolone. *Clinical pharmacokinetics* 1990; **19**(2): 126-46.
15. Rose JQ, Yurchak AM, Jusko WJ. Dose dependent pharmacokinetics of prednisone and prednisolone in man. *Journal of pharmacokinetics and biopharmaceutics* 1981; **9**(4): 389-417.

16. Rubin EJ. The granuloma in tuberculosis—friend or foe? *New England Journal of Medicine* 2009; **360**(23): 2471-3.
17. Peters W, Ernst JD. Mechanisms of cell recruitment in the immune response to *Mycobacterium tuberculosis*. *Microbes and infection* 2003; **5**(2): 151-8.
18. Strang J, Gibson D, Nunn A, Kakaza H, Girling D, Fox W. Controlled trial of prednisolone as adjuvant in treatment of tuberculous constrictive pericarditis in Transkei. *The Lancet* 1987; **330**(8573): 1418-22.
19. Shenje J, Lai RP, Ross IL, et al. Effect of prednisolone on inflammatory markers in pericardial tuberculosis: A pilot study. *IJC Heart & Vasculature* 2017.
20. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell death & differentiation* 1999; **6**(2): 99-104.
21. Behar S, Martin C, Booty M, et al. Apoptosis is an innate defense function of macrophages against *Mycobacterium tuberculosis*. *Mucosal immunology* 2011; **4**(3): 279-87.
22. O'Donnell MA, Perez-Jimenez E, Oberst A, et al. Caspase 8 inhibits programmed necrosis by processing CYLD. *Nature cell biology* 2011; **13**(12): 1437-42.
23. Kuida K. Caspase-9. *The international journal of biochemistry & cell biology* 2000; **32**(2): 121-4.
24. Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. *Trends in molecular medicine* 2008; **14**(3): 109-19.
25. Ren K, Torres R. Role of interleukin-1 β during pain and inflammation. *Brain research reviews* 2009; **60**(1): 57-64.
26. Holmes WE, Lee J, Kuang W-J, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. *Science* 1991; **253**(5025): 1278-80.
27. Andersson AK, Atkinson SE, Khanolkar-Young S, et al. Alteration of the cortisol–cortisone shuttle in leprosy type 1 reactions in leprosy patients in Hyderabad, India. *Immunology letters* 2007; **109**(1): 72-5.
28. Rook G, Hernandez-Pando R. Pathogenetic role, in human and murine tuberculosis, of changes in the peripheral metabolism of glucocorticoids and antiglucocorticoids. *Psychoneuroendocrinology* 1997; **22**: S109-S13.
29. Burger D, Agarwala S, Child M, Been-Tiktak A, Wang Y, Bertz R. Effect of rifampin on steady-state pharmacokinetics of atazanavir with ritonavir in healthy volunteers. *Antimicrobial agents and chemotherapy* 2006; **50**(10): 3336-42.
30. Wilkinson KA, Wilkinson RJ. Polyfunctional T cells in human tuberculosis. *European journal of immunology* 2010; **40**(8): 2139-42.

31. Jindani A, Borgulya G, de Patiño IW, et al. A randomised Phase II trial to evaluate the toxicity of high-dose rifampicin to treat pulmonary tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 2016; **20**(6): 832-8.
32. Chan R, Kwok A. Ocular toxicity of ethambutol. *Hong Kong Medical Journal* 2006; **12**(1): 56.
33. Segal BH, Sneller MC. Infectious complications of immunosuppressive therapy in patients with rheumatic diseases. *Rheumatic disease clinics of north america* 1997; **23**(2): 219-37.
34. Dragalin V, Hsuan F, Padmanabhan SK. Adaptive designs for dose-finding studies based on sigmoid e max model. *Journal of Biopharmaceutical Statistics* 2007; **17**(6): 1051-70.
35. Vagnerová K, Loukotová J, Ergang P, Musílková J, Mikšík I, Pácha J. Peroxisome proliferator-activated receptor- γ stimulates 11 β -hydroxysteroid dehydrogenase type 1 in rat vascular smooth muscle cells. *Steroids* 2011; **76**(6): 577-81.