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Role of matrix metalloproteinases in drug-resistant and drug-susceptible tuberculosis

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DECLARATION

The research presented in this dissertation was conducted at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), Nelson Mandela R University of KwaZulu-Natal, Durban, between April 2024 and April 2026. This work was undertaken under the supervision of Dr Aida Sivro, with co-supervision by Dr Thando Maseko. The dissertation represents the original work of the author and has not been submitted, in whole or in part, to any other university. All sources and contributions of others have been appropriately acknowledged in the text.

_____ __Date:_____

Azande Sthoko Gazu (Candidate)

As the candidate's supervisor, I have approved this thesis for submission.



May 11, 2026

Dr Aida Sivro (Supervisor)

_____ ___Date:_____

Dr Thando Maseko (Co-supervisor)

DECLARATION- PLAGIARISM

I, Azande Gazu, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original work.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons.
4. This dissertation does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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DEDICATION

This work is dedicated to my beloved mother **Thembelihle Mthembu**, whose unwavering love, strength, and sacrifices have been the foundation of my journey. Your constant support, encouragement, and belief in me have carried me through every challenge and milestone. Thank you for always standing by my side, for your patience, and for instilling in me the resilience and determination to pursue my dreams. This achievement is a reflection of your love and everything you have poured into my life. I also dedicate this to my late father, **Mthandeni Gazu**, whose memory continues to inspire me and whose love remains forever in my heart.

ACKNOWLEGDEMENT

First and foremost, I would like to give all glory and honour to God, Lord the Creator, whose grace, strength, and guidance carried me throughout this journey. In moments of doubt and difficulty, I was reminded that *"with God all things are possible"* (Matthew 19:26). His presence has been my source of perseverance and hope.

I would like to express my sincere and heartfelt gratitude to my supervisor, **Dr Aida Sivro**, for her exceptional guidance, patience, and unwavering support throughout this research journey. Your willingness to go above and beyond, mentorship, insightful feedback, and constant encouragement have not only shaped this work but have also contributed significantly to my growth as a researcher. I am truly grateful for the time, effort, and belief you have invested in me. I am equally grateful to my co-supervisor, **Dr Thando Maseko**, for her valuable input, guidance, and support. Thank you for holding my hand throughout. Your expertise, thoughtful advice, and encouragement have greatly enriched this study and strengthened my academic development. I deeply appreciate your contribution and the role you have played in bringing this work to completion.

To my beloved mother, **Thembelihle Mthembu**, words cannot fully express my gratitude. Thank you for your endless love, sacrifices, prayers, and belief in me. Your strength and support have been my foundation, and I am truly grateful for everything you have done for me.

To my close family, friends and church family (HRCC), I extend my heartfelt appreciation for your constant love, support, and encouragement throughout this journey. Your presence has truly meant so much to me. A special thanks to: **Lindelwa Nkosi, Valentine Shozi, Simamisa Mbanjwa, Ziyanda Mndiyata**. Thank you for your unwavering support, motivation, and the many moments of encouragement that kept me going.

Finally, and most importantly, to my partner, **Andiswa Phahla**, thank you for standing by my side through it all. Your patience, understanding, love, and constant encouragement sustained me during the most challenging times. This achievement is as much yours as it is mine.

I would also like to sincerely thank the CAPRISA 011 and CAPRISA 020 study participants for their time, willingness, and invaluable contributions to this research. My appreciation also goes to the CAPRISA team for their support and assistance throughout the study.

To everyone who contributed in any way to completing this thesis, I am sincerely grateful.

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List of Abbreviations

ACD	Acid citrate dextrose
ADCC	Antibody-dependent cellular cytotoxicity
AIDS	Acquired immunodeficiency syndrome
AM	Alveolar macrophages
ART	Antiretroviral therapy
APC	Antigen-presenting cell
BB-94	Batimastat
BMI	Body mass index
bnAbs	Broadly neutralizing antibodies
CAPRISA	Centre for the AIDS programme of research in South Africa
COVID-19	Coronavirus disease 2019
CTLs	Cytotoxic T-lymphocytes
CVD	Cardiovascular disease
DC	Dendritic cells
DR-TB	Drug-resistant Tuberculosis
DS-TB	Drug-susceptible Tuberculosis
EMC	Extracellular matrix
FGF	Fibroblast growth factor
GALT	Gut-associated lymphoid tissue
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HIV	Human immunodeficiency virus
IFN	Interferon
IGRA	Interferon-gamma release assay
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-10	Interleukin 10
iNOS	Inducible nitric oxide synthase
IP-10	Interferon gamma-induced protein 10
KZN	KwaZulu-Natal
LTBI	Latent TB infection
LPS	Lipopolysaccharide
MCHC	Major histocompatibility complex

MDR-TB	Multidrug-resistant
MIP-1α	Macrophage inflammatory protein 1 alpha
MIP-1β	Macrophage inflammatory protein 1 beta
MMP	Matrix metalloproteases
MTB	Mycobacterium-tuberculosis
NK	Natural killer
PAMPs	Pathogen-associated molecular patterns
PLWH	People living with HIV
pDCs	Plasmacytoid dendritic cells
PDGF-BB	Platelet-derived growth factor BB monomer
PTB	Pulmonary TB
RNS	Reactive nitrogen species
RR-TB	Rifampicin-resistant
ROS	Reactive oxygen species
SA	South Africa
TACE	Tumour necrosis factor-α
TB	Tuberculosis
Th1	T helper 1
Th2	T helper 2
Th17	T helper 17
TIMP	Tissue inhibitors of metalloproteases
TLRs	Toll-like receptors
TNF	Tumor necrosis factor
TST	Tuberculin skin test
VEGF	Vascular endothelial growth factor
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

Thesis Context

This thesis consists of four chapters, which contain the following:

Chapter 1 provides the overall background and context of the study. It introduces tuberculosis (TB) as a global public health challenge, with particular emphasis on disease pathogenesis and lung tissue damage. The chapter outlines the role of host immune responses, including matrix metalloproteinases (MMPs), in TB progression, drug-susceptible and drug-resistant TB, and TB/HIV co-infection. The research problem, study rationale, aims, and objectives are clearly defined, followed by the hypotheses and the significance of the study.

Chapter 2 describes the methodological approach used to address the study objectives.

Chapter 3 presents the main findings of this study, examining matrix metalloproteinase (MMP) expression in drug-susceptible and drug-resistant tuberculosis, with and without HIV co-infection. Associations between MMP levels and clinical outcomes, including lung cavitation and relevant clinical and immunological factors, are described. The results are presented in a manuscript-ready format submitted for publication to the journal *Tuberculosis*.

Chapter 4 summarizes the major findings of this study, describes its significance, outlines its limitations, and provides recommendations for future directions.

CHAPTER 1:
INTRODUCTION

ABSTRACT

Introduction: Tuberculosis (TB) remains a global health challenge. The severity of TB-related lung damage is a major determinant of treatment outcomes. Matrix metalloproteinases (MMPs) mediate extracellular matrix degradation, regulate leukocyte recruitment, and modulate cytokine and chemokine activity, thereby contributing to lung tissue destruction, cavitation, and inflammatory regulation during tuberculosis infection. This study investigated the role of MMPs in the pathogenesis of drug-resistant (DR)-TB and drug-susceptible (DS)-TB.

Methods: Plasma levels of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 during active TB were quantified in 128 plasma samples from DR-TB participants enrolled in the CAPRISA 020 (InDEX) study and 78 DS-TB participants in the CAPRISA 011 (IMPRESS) study in Durban, South Africa using multiplex immunoassays. Associations between MMP levels and lung cavitation, as a marker of disease severity, were analysed using logistic regression models in the total cohorts and stratified by HIV status. Correlations between MMPs and pro-inflammatory cytokines/chemokines were also examined.

Results: In univariable models, MMP-1 levels were significantly associated with the presence of lung cavitation in DS-TB, both in the overall cohort (OR 9.86, 95% CI 1.45–84.5, p=0.025) and among HIV co-infected individuals (OR 17.1, 95% CI 1.57–270, p=0.028). However, this association was attenuated in multivariable analysis adjusting for age, sex, body mass index (BMI), HIV status, smoking, and alcohol use (p=0.097). Similarly, plasma MMP-10 levels showed a significant association with lung cavitation in DR-TB in univariable analysis (OR 8.16, 95% CI 1.32–59.2, p=0.02), which was not sustained after multivariable adjustment. No significant associations were found between MMP levels and smear grade or time to culture conversion. There was a strong positive correlation between MMP-1 and IL-6, IL-8 and other inflammatory cytokines/chemokines, whereas other MMPs showed mixed positive and negative cytokine correlations.

Conclusion: These findings highlight the differences in involvement of MMPs in immunopathogenesis of DS- and DR-TB. MMP-1 was strongly linked to lung cavitation and pro-inflammatory cytokine networks in DS-TB, while MMP-10 showed a more modest association with cavitation in DR-TB. Together, these results indicate that MMPs may reflect host-driven tissue injury and inflammatory dysregulation rather than bacterial burden or treatment response. These findings warrant further validation through longitudinal, larger-cohort studies to clarify their mechanistic role in TB pathogenesis.

CHAPTER 1

1. Introduction

Human Immunodeficiency Virus (HIV) and tuberculosis (TB) are two of the most significant infectious diseases globally, each placing a substantial burden on public health systems (Rewari *et al.*, 2021). TB, caused by *Mycobacterium tuberculosis* (MTB), continues to be a major global health concern and remains one of the leading causes of death from an infectious agent (WHO, 2024). In South Africa, where both infections are highly prevalent, TB and HIV form a deadly syndemic: each pathogen accelerates the progression and worsens the clinical outcomes of the other, producing a clinical scenario characterised by synergistic interactions, increased TB disease severity, higher mortality, and greater diagnostic and treatment complexity (Gupta-Wright *et al.*, 2020)(Naidoo *et al.*, 2019). Although substantial progress has been made in understanding the individual pathogenesis of HIV and TB, the mechanisms underpinning their bidirectional enhancement and the immune dysregulation observed in co-infected individuals remain incompletely understood.

Matrix Metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that play crucial roles in various physiological and pathological processes, including tissue remodelling, inflammation, and immune responses (Rosenberg *et al.*, 1995). MMPs are known to have detrimental effects during TB, increasing TB spread and disease severity and emerging evidence implicates them in the pathogenesis of TB and HIV individually (Mastroianni and Liuzzi, 2007, Elkington *et al.*, 2011a). However, the specific roles of MMPs in the context of TB-HIV co-infection remain poorly understood.

Understanding the role of MMPs in TB-HIV co-infection is of paramount importance for several reasons. MMPs contribute to the breakdown of extracellular matrix components implicated in the tissue damage and fibrosis seen in both infections; they modulate immune responses by influencing the migration and activation of immune cells critical for controlling TB and HIV; and they may facilitate pathogen dissemination within the host, potentially influencing disease progression and transmission (Webster and Crowe, 2006).

Despite the potential significance of MMPs in TB-HIV co-infection, research in this area is still in its infancy. Most studies have focused on individual aspects of TB or HIV pathogenesis, overlooking the complex interactions that occur during co-infection (Gupta-Wright *et al.*, 2020). Therefore, there is a pressing need for comprehensive investigations that examine the

expression, regulation, and functional consequences of MMP expression and regulation in TB-HIV co-infected individuals.

1.2. LITERATURE REVIEW

1.2.1. Tuberculosis

1.2.1.1 Mycobacterium tuberculosis

TB is caused by *Mycobacterium tuberculosis* (MTB), a slow-growing, aerobic, non-motile bacillus belonging to the *Mycobacterium tuberculosis* complex (MTBC), which also includes *M. bovis*, M. africanum, and several other closely related species (Forrellad *et al.*, 2013). MTB is characterised by a thick, lipid-rich cell wall composed of mycolic acids, arabinogalactan, and peptidoglycan, which confers acid-fastness, structural rigidity, and resistance to many conventional antibiotics and host defence mechanisms (Brennan and Nikaido, 1995). This unique envelope also contributes to the organism's slow replication rate (approximately 15–20 hours per generation) and its ability to persist intracellularly within host macrophages for extended periods.

The complete genome sequence of MTB (strain H37Rv) was published in 1998, revealing a genome of approximately 4.4 million base pairs encoding around 4,000 genes, including a large number involved in lipid metabolism, which underlies the organism's distinctive cell envelope biology (Cole *et al.*, 1998). MTB is transmitted almost exclusively via airborne droplet nuclei expelled by individuals with active pulmonary disease, and infection is established when inhaled bacilli reach the alveoli. Key virulence determinants include the ESX/type VII secretion systems (notably ESX-1), which facilitate phagosomal rupture and modulation of host immune responses, and various secreted and cell-wall-associated proteins that interfere with phagolysosome maturation, antigen presentation, and cytokine signalling (Forrellad *et al.*, 2013, Smith, 2003). These virulence mechanisms allow MTB to evade eradication by the innate immune system and establish the chronic, and frequently latent, infection described below.

1.2.1.2 TB- global and South African statistics

TB is a multisystem infectious disease with diverse clinical outcomes. It has long been the leading cause of death from a single infectious agent globally, a position it briefly lost to COVID-19 during the acute phase of the pandemic (2020–2022), when mortality from the novel virus rose sharply; as COVID-19 deaths have since tapered, TB has once again become the leading cause of death from a single infectious agent worldwide.. According to the 2024

World Health Organization (WHO) report, an estimated 10.8 million people developed TB in 2023, an increase from 10.6 million in 2022. This corresponds to approximately 134 new cases per 100,000 population. Although the global TB incidence rate rose by 0.2% between 2022 and 2023, this increase was markedly smaller than the 2.2% rise observed between 2021 and 2022. Among individuals diagnosed with TB in 2023, 6.1% were also living with HIV, underscoring the continued overlap between the two epidemics. The burden of TB remains unevenly distributed across regions: South-East Asia accounted for the highest proportion of cases (45%), followed by Africa (24%) and the Western Pacific (17%). Lower proportions were reported in the Eastern Mediterranean (8.6%), the Americas (3.2%), and Europe (2.1%).(WHO, 2024).

TB can affect anyone, regardless of age or gender. Adult men (aged 15 and older) had the highest number of cases, with about 5.8 million in 2022, accounting for 55% of the total (WHO, 2022). Adult women (aged 15 and older) had about 3.5 million cases (33%), and children (aged 0-14) had about 1.3 million cases (12%). Notifications of new TB cases in 2022 accounted for 70% of the estimated total, but this varied by age and gender: 72% for adult men, 75% for adult women, and 49% for children aged 0-14. Among children under 5, the figure was only 42% (WHO, 2022).

Drug-resistant TB (DR-TB) remains a major barrier to global TB control efforts. According to the 2024 WHO report, an estimated 400,000 (95% UI: 360,000–440,000) individuals developed multidrug-resistant (MDR-TB) or rifampicin-resistant TB (RR-TB) in 2023. Despite advancements in diagnostic tools and treatment options, only 44% (175,923) of these individuals were diagnosed and initiated on appropriate treatment. The success rate for treating MDR/RR-TB has improved to 68%, but significant challenges remain in ensuring universal access to rapid diagnostics and effective treatment regimens (WHO, 2024). In 2022, the proportion of MDR/RR-TB cases with pre-extensively drug-resistant TB (i.e., resistance to any fluoroquinolone tested) was around 18%. Since 1994, the WHO has systematically collected and analysed global drug-resistance surveillance data, providing essential insights into patterns and trends in TB resistance(Dean *et al.*, 2022).

1.2.1.3. TB pathogenesis

Pathogenesis of TB involves a complex interplay between the host's immune response and the bacteria's ability to evade it. After inhalation of MTB by the new host, the bacteria travel

through the respiratory system and ultimately reach the lungs (Leung, 1999). At this point, the innate immune system of the host becomes active and works to control the infection by internalizing the tubercle bacilli into alveolar macrophages. If the macrophages fail to inhibit or destroy the bacilli, the bacteria multiply in their intracellular environment, get released, and then get phagocytosed by other alveolar macrophages, resulting in a continuous cycle (Leung, 1999). Lymphocytes are then recruited to the site of infection, initiating a cell-mediated immune response in which a group of immune cells arrives to contain the bacteria and prevent further multiplication (Luies and Du Preez, 2020). At this stage, the host remains asymptomatic, and the TB bacteria may be eliminated completely or enter a state of latency within the granuloma (Schluger, 2005). However, in situations where the immune system is compromised, the disease can progress rapidly into active TB with clinical symptoms. Bacteria have the ability to evade clearance by preventing the fusion of phagolysosomes and sabotaging the host's immune response, thereby creating a favourable environment for MTB to thrive. This allows the bacteria to survive for extended periods of time, evading the immune system and existing in a state of non-replication or slow replication. In such instances, the individual remains asymptomatic and non-infectious, displaying latent infection (Luies and Du Preez, 2020).

TB involves a spectrum of disease, from MTB infection to active pulmonary TB disease. Although TB disease can be seen as a dynamic continuum, patients are often classified as having either latent TB infection (LTBI) or active TB disease for simplicity in clinical and public health settings(Pai *et al.*, 2016). An individual's status can shift depending on changes in immune response and underlying health conditions. Exposure to MTB can result in pathogen elimination through innate immune responses or acquired T cell immunity (Pai *et al.*, 2016). Individuals who eliminate the infection without T cell priming or memory may have negative tuberculin skin test (TST) or interferon-gamma release assay (IGRA) results. Some may eliminate the pathogen but retain a strong memory T cell response, resulting in positive TST or IGRA results, and will not benefit from LTBI treatment (Esmail *et al.*, 2014). If the pathogen is not eliminated, bacteria can persist in a dormant state, leading to a positive TST or IGRA results, which indicate T cell responses against MTB antigens. These patients should receive one of the recommended LTBI preventive therapies, typically 6–9 months of isoniazid. Patients with subclinical TB may not show symptoms but will test positive on cultures (usually smear-negative due to low bacillary load) (Ruhl *et al.*, 2020). Those with active TB disease show symptoms like cough, fever, and weight loss, with diagnosis confirmed through sputum smear,

culture, and molecular tests. Sometimes, active TB patients might test negative on TST or IGRA due to disease-induced anergy or immune suppression from conditions like HIV or malnutrition. Individuals with subclinical or active TB should follow a recommended treatment regimen for active TB, starting with an intensive phase of four drugs, followed by a continuation phase with two drugs (Pai *et al.*, 2016).

1.2.2 HIV

1.2.2.1 The Virus: HIV

HIV is an enveloped, single-stranded, positive-sense RNA retrovirus belonging to the genus *Lentivirus*. Two types exist: HIV-1 and HIV-2, with HIV-1 responsible for the vast majority of infections worldwide and characterised by greater transmissibility and a more rapid clinical course (Fanales-Belasio *et al.*, 2010). The virus was first isolated in 1983 from a patient with lymphadenopathy (Barré-Sinoussi *et al.*, 1983), and subsequent work established it as the causative agent of AIDS.

The HIV virion is roughly spherical, approximately 100–120 nm in diameter, and consists of a lipid envelope derived from the host cell membrane, studded with the viral glycoproteins gp120 and gp41, which mediate attachment and fusion with target cells. Beneath the envelope lies a matrix protein layer (p17) and a conical capsid (p24) enclosing two copies of the viral genomic RNA, along with the essential enzymes reverse transcriptase, integrase, and protease (Turner and Summers, 1999). The HIV genome comprises nine genes, including the three major structural/enzymatic genes *gag*, *pol*, and *env*, together with several regulatory and accessory genes (*tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*) that modulate viral replication, immune evasion, and pathogenesis (Freed, 2001).

Replication begins with gp120 binding to the CD4 receptor on target cells, followed by interaction with a chemokine co-receptor (CCR5 or CXCR4) that triggers gp41-mediated membrane fusion. Following entry, the viral RNA genome is reverse-transcribed into double-stranded DNA by reverse transcriptase a process notoriously error-prone and the principal driver of HIV's high genetic diversity and rapid emergence of drug resistance. The resulting proviral DNA is transported to the nucleus and integrated into the host chromosome by integrase, establishing a stable, and potentially latent, infection that persists for the lifetime of the cell (Freed, 2001). This capacity for latency is central to why HIV infection cannot currently be cured with antiretroviral therapy alone.

1.2.2.2 HIV- global and South African statistics

According to UNAIDS 2024, in 2023, about 39.9 million people worldwide were living with HIV, including 38.6 million adults and 1.4 million children. Women and girls made up 53% of this group. About 86% of people with HIV knew their status, leaving 5.4 million unaware (UNAIDS, 2024). Around 1.3 million new HIV infections occurred, significantly lower than the peak of 3.3 million in 1995. AIDS-related illnesses claimed 630,000 lives. On a positive note, 30.7 million people were receiving antiretroviral therapy (ART) by the end of 2023, a significant increase from 7.7 million in 2010, though still below the 2025 target of 34 million (UNAIDS, 2024). Women had higher treatment access (83%) compared to men (72%), and 84% of pregnant women had access treatment to prevent mother-to-child transmission. New HIV infections have dropped by 39% since 2010, but progress, has slowed in recent years. Since the start of the epidemic, 88.4 million people have contracted HIV, and 42.3 million have died from AIDS-related causes (UNAIDS, 2024).

1.2.2.3 HIV pathogenesis

HIV is mainly spread through unprotected vaginal, anal, and oral sex. It can also be transmitted by blood transfusions, sharing of contaminated needles, and from an infected and untreated mother to her child during pregnancy, childbirth, and breastfeeding. After HIV enters the body through mucous membranes, it spreads to nearby lymph nodes and then into the bloodstream, reaching different parts of the body such as the brain, lungs, and gut-associated lymphoid tissue (GALT) (Haase, 2010).

The development of HIV infection involves three key processes: (i) the ability to infect $CD4^+$ T lymphocytes and macrophages, which is determined by the expression of the HIV cell receptor CD4 (Dalgleish *et al.*, 1984, Klatzmann *et al.*, 1990), and a member of the chemokine receptor family acting as a co-receptor, namely, CCR5, CXCR4, or alternative receptors (Calado *et al.*, 2010). (ii) the establishment of latently infected cells harbouring the HIV genome integrated into the chromosomal DNA of the cell (Siliciano and Siliciano, 2022); and (iii) the induction of immune hyperactivation throughout infection, leading to accelerated immune senescence (Paiardini and Müller-Trutwin, 2013). These three features have both local and systemic consequences, leading to lifelong infection, irreversible $CD4^+$ T lymphocyte depletion and dysfunction, and immune senescence and exhaustion.

The pathogenesis of HIV involves a complex interplay of immune responses and viral mechanisms leading to the progressive depletion of $CD4^+$ T cells and the development of acquired immunodeficiency syndrome (AIDS) (Wong *et al.*, 2020). HIV infection inhibits T helper 1 (Th1) cell activity and increases T helper 2 cell (Th2) activity, resulting in a shift from Th1 to Th2 (Becker, 2004). This shift is responsible for decreased expression of IL-2 and IFN-γ and increased expression of cytokines such as IL-4, IL-5, and IL-13. The depletion of $CD4^+$ T cells severely compromises the host's immune response, leaving the individual vulnerable to opportunistic infections such as TB (Doyle *et al.*, 2015). Mechanistically, HIV-1 causes $CD4^+$ T-cell loss through multiple forms of programmed cell death. In activated, productively infected (permissive) $CD4^+$ T cells, cell death occurs predominantly via caspase-3-dependent apoptosis. In contrast, most quiescent, non-permissive bystander $CD4^+$ T cells in lymphoid tissue undergo caspase-1-dependent pyroptosis triggered by abortive infection. Pyroptosis is a lytic, highly inflammatory form of programmed cell death that promotes release of pro-inflammatory mediators and contributes to chronic immune activation (Doitsh *et al.*, 2014). Furthermore, HIV infection leads to chronic inflammation, which triggers the immune response and may increase the likelihood of developing comorbidities similar to those of aging, such as cardiovascular disease, fractures, and kidney disease (Van Epps and Kalayjian, 2017).

1.2.3 TB-HIV co-infection statistics

In 2023, an estimated 662,000 new TB cases were co-infected with HIV, representing 6.1% of all new TB cases globally (UNAIDS, 2024). This proportion has been steadily declining in recent years. The highest prevalence of TB-HIV co-infection was observed in Africa, particularly in the southern regions, where the proportion of people living with HIV (PLWH) TB cases exceeded 50%. This trend is likely attributable to the high HIV prevalence, suboptimal TB control measures, and structural vulnerabilities within healthcare systems in these areas.

Globally, TB was responsible for approximately 1.25 million deaths in 2023, including 161,000 among individuals living with HIV. Although TB-related mortality was higher among HIV-negative individuals, co-infection with HIV remains a significant risk factor for poor clinical outcomes. Among PLWH TB deaths, 48% occurred in adult males, 36% in adult females, and 16% in children and adolescents, highlighting demographic disparities in disease burden.

The global coverage of HIV testing among individuals diagnosed with TB remained at 80% in 2023, consistent with the previous year. Regional variations were noted, with the highest testing coverage reported in Africa (90%) and Europe (94%). These findings underscore the ongoing efforts to integrate TB and HIV care, which are critical for improving patient outcomes and reducing TB-HIV-associated morbidity and mortality. (UNAIDS, 2024)

1.2.3.1. TB-HIV co-infection pathogenesis

TB/HIV co-infection is a significant health concern, particularly in high-burden countries like South Africa. The co-dependent relationship between HIV and TB leads to a more severe health impact, known as a syndemic, resulting in increased mortality rates and treatment challenges (Olivier and Luies, 2023, Bruchfeld *et al.*, 2015). In high-burden settings, HIV is the most important risk factor for developing active TB, significantly increasing the susceptibility to TB infection (Herbert *et al.*, 2023). PLWH have up to 20 times higher risk of developing active TB compared to those without HIV infection. The synergy between HIV and TB accelerates immunological deterioration, leading to increased inflammation, oxidative stress, and metabolic dysfunction, which can be further exacerbated by factors like ART-induced mitochondrial damage. Co-infected individuals face unique challenges in treatment, requiring a comprehensive approach that integrates effective anti-TB treatment, concurrent ART, and management of comorbidities to improve outcomes. WHO recommends that ART should be started as soon as possible, within two weeks of initiating TB treatment, regardless of CD4 count, except when signs and symptoms of meningitis are present. There were an estimated 37,000 HIV/TB coinfections and 5,900 deaths in the Region in 2020. Among PLWH newly enrolled in care 39 % received preventive treatment (WHO, 2023).

1.2.4. Host immune responses in TB infection and disease progression

The spectrum of TB commonly includes presumed latent MTB infection, incipient TB, subclinical TB, active disease, and post-tuberculosis lung disease. Upon entry into the body, innate immune cells, such as neutrophils, macrophages, natural killer (NK) cells, and dendritic cells (DCs), rapidly initiate immune response mechanisms in coordination with adaptive immune cells, including CD4+ T cells, CD8+ T cells, and B cells, to mount an immune defense (Figure 1) (Zhuang *et al.*, 2024).

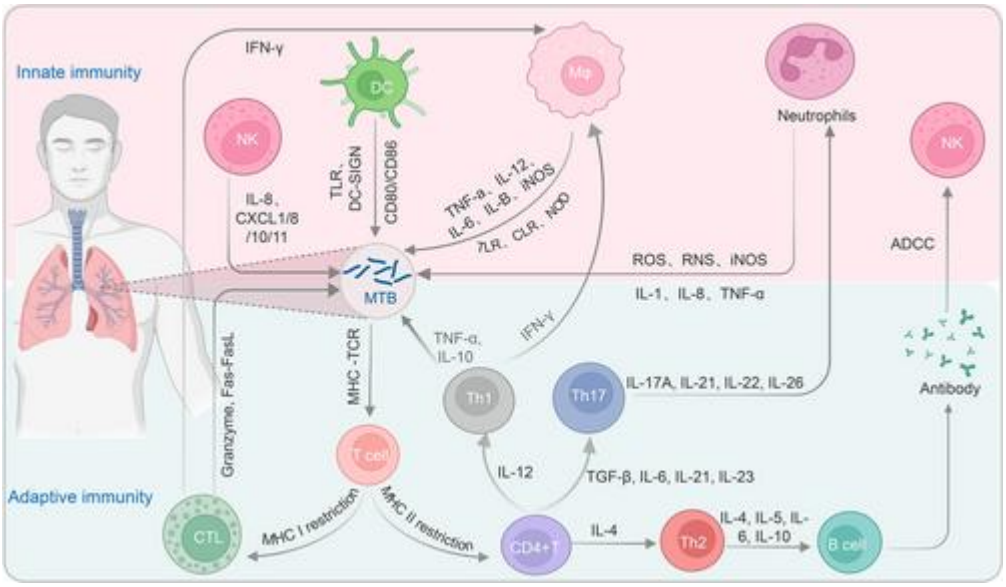

Figure 1: The immune response to Mycobacterium tuberculosis infection. Upon entering the respiratory tract and reaching the lung tissue, MTB triggers the activation and recruitment of neutrophils, macrophages, natural killer cells, and dendritic cells within the host. These cells engulf and digest MTB, releasing antimicrobial substances such as tumour necrosis factor-alpha (TNF-α), interleukin-12 (IL-12), interleukin-10 (IL-10), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) to clear the infection. Additionally, antigen-presenting cells (APCs) like DCs and B cells process and present MTB antigens to T cells, leading to T cell activation. Activated $CD4^+$ T cells differentiate into different subsets of helper (Th) cells that mediate apoptosis of MTB-infected cells, while activated $CD8^+$ T cells, also known as cytotoxic T lymphocytes (CTLs), can induce the clearance of MTB through pathways involving granule enzymes or the Fas/FasL pathway. The immune response against MTB infection involves a delicate balance of pro-inflammatory and anti-inflammatory cytokines, reactive oxygen species (ROS), and reactive nitrogen species (RNS), as well as the involvement of major histocompatibility complex (MHC) molecules and Toll-like receptors (TLRs) (Zhuang et al., 2024). Adapted from (Zhuang et al., 2024).

1.2.4.1. Innate Immune responses

Innate immune cells are the first to encounter MTB, and their response dictates the course of infection (Liu *et al.*, 2017). The initial phase of MTB infection is crucial, as it is during this stage that innate immune responses in the lung play a vital role in restricting bacterial growth and preventing disease development (Khan *et al.*, 2016). When innate cells detect MTB by identifying pathogen-associated molecular patterns (PAMPs) via TLRs and nucleotide-binding oligomerization domain receptors (NLRs), a local inflammatory response occurs

(Kleinnijenhuis *et al.*, 2011). However, while these innate immune mechanisms are essential, they are insufficient to prevent progression of MTB infection to active disease (Mayer-Barber and Barber, 2015). Once phagocytes are infected with MTB or exposed to MTB antigens, they can migrate to regional lymph nodes and prime $CD4^+$ and $CD8^+$ T cells, which play crucial roles in adaptive immune responses and the development of immunity against MTB (Sankar and Mishra, 2023).

1.2.4.2 Macrophages

Macrophages, derived from monocytes, are white blood cells found throughout the body, responsible for recognizing MTB, releasing cytokines, and phagocytosing and killing bacteria. During MTB infection, lung macrophages are the primary cells that initially encounter MTB (Weiss and Schaible, 2015). Upon MTB interaction, alveolar macrophages (AM) are activated through pattern recognition receptors (PRRs), leading to the release of inflammatory cytokines such as TNF-α, IL-12, IL-1β, etc (Drage *et al.*, 2009, Ochoa *et al.*, 2023). This activation process involves the recognition of MTB components by surface receptors such as toll-like receptor 2 (TLR2), TLR4, NOD2, and Dectin-1 on macrophages. These cytokines help recruit and activate other immune cells, including additional macrophages, T cells, and neutrophils. This response leads to the formation of a granuloma, which is a cluster of immune cells that forms around the MTB to contain and isolate the infection (Zhuang *et al.*, 2024).

1.2.4.3 Neutrophils

Neutrophils, also called polymorphonuclear leukocytes, are among the most common granulocytes in the peripheral blood, typically comprising 50-70% of circulating leukocytes. They are also known as professional phagocytes and their functions include chemotaxis and phagocytosis (Malech *et al.*, 2014). When MTB enters the alveoli via the respiratory tract, neutrophils are among the first cells to be recruited to the site of infection, and they form the first line of defense against MTB (Borkute *et al.*, 2021). Neutrophils are released from the bone marrow into the bloodstream and then enter peripheral tissues under the influence of chemotactic stimuli such as chemokines and cytokines produced by the host through interactions involving the selectin family of C-type lectin glycoproteins (Kobayashi and DeLeo, 2009). Once at the site of infection, neutrophils directly recognize and engulf MTB by interacting with the pathogen's components, such as lipopolysaccharide (LPS), lipoproteins, lipoteichoic acid, and flagella, through surface receptors, including Toll-like receptors (TLR1, TLR2, TLR4-10, and others) (Hayashi *et al.*, 2003). Neutrophils employ both oxygen-

dependent and oxygen-independent killing pathways to eliminate engulfed MTB. The oxygen-dependent killing pathway primarily involves reactive oxygen species and reactive nitrogen species (such as nitric oxide and peroxynitrite) (Su *et al.*, 2019). Upon engulfment of MTB by neutrophils, released reactive oxygen species (ROS) and iNOS can kill the bacteria by producing high levels of nitric oxide (NO) (Sarr *et al.*, 2018). In the oxygen-independent killing pathway, neutrophil granules play a crucial role in inhibiting or killing MTB. Cationic proteins, defensins, and permeability proteins are the major bactericidal substances (Alcantara *et al.*, 2023). Furthermore, activated neutrophils secrete various chemotactic factors, such as interleukin-8 (IL-8), CXCL8, CXCL1, CXCL10, and CXCL11, which amplify their own recruitment and directed chemotaxis, synergistically working with other immune cells to enhance phagocytosis and bactericidal functions, thus enhancing MTB clearance (Warren *et al.*, 2017).

1.2.4.4 NK cells

NK cells, derived from bone marrow lymphoid progenitor cells, play a crucial role in clearing infected and tumour cells. They are key effector cells of innate immunity, with unique characteristics such as MHC-unrestricted recognition, and are therefore able to act early during MTB infection. In humans, mature NK cells can be classified into different subsets ($CD56^{dim}$ and $CD56^{bright}$) based on the variable expression of their surface antigens (Cooper *et al.*, 2001). For instance, peripheral blood NK cells express low levels of CD56 and the IgG Fc receptor FcγRIII (CD16), whereas $CD56^{bright}$ NK cells are predominantly found in the lymph nodes. $CD56^{bright}$ NK cells display constitutive high- and intermediate-affinity IL-2 receptors and can produce cytokines, whereas $CD56^{dim}$ NK cells express high levels of CD16 and exhibit potent cytotoxicity (Caligiuri, 2008). The specific mechanisms underlying NK cell activities involve a balance between inhibitory signals transmitted by surface inhibitory receptors and activating signals transmitted by activating receptors, as well as the coordination of cytokine signalling (Bald *et al.*, 2020). During MTB infection, NK-cell activity is governed by the balance of inhibitory and activating signals. Reduced or altered expression of MHC class I on some infected cells can diminish inhibitory signalling through NK-cell receptors, thereby facilitating NK-cell activation. In addition, natural cytotoxicity receptors (NCRs), particularly NKp44, can recognize selected mycobacterial cell-wall components, including arabinogalactan–peptidoglycan and mycolic acids, promoting cytokine production. Activated NK cells secrete

IFN-γ and IL-22, which support antimycobacterial immunity, and can eliminate infected cells through perforin/granzyme-mediated cytotoxicity and death-receptor pathways such as Fas/FasL (Abebe, 2021, Esin *et al.*, 2013, Liu *et al.*, 2017). Moreover, NK cells can also be activated through antibody-dependent cell-mediated cytotoxicity, leading to the secretion of inflammatory cytokines and chemokines, and the modulation of effector cells, which in turn influence the adaptive immune response. For example, IL-2 promotes the growth, differentiation, and survival of T cells and enhances the cytotoxic activity of NK cells. IFN-γ activates macrophages, increasing their phagocytic and bactericidal activity, while also promoting the differentiation of Th1 cells and enhancing NK cell cytotoxicity. TNF-α plays a crucial role in promoting inflammation and can induce apoptosis in certain cells; it activates macrophages, enhancing their ability to kill intracellular pathogens, and recruits neutrophils to infection sites. These cytokines collectively modulate the immune response, impacting the control and progression of TB. The mechanisms of action of NK cells impact the survival of MTB within granulomas and regulate the functions of multiple immune effector cells, ultimately affecting the host's adaptive immune response. This comparable with dendritic cells demonstrates their ability to shape the immunological microenvironment and modulate the functions of different effector cells, ultimately aiding in the host defence against MTB.

1.2.4.5. DC cells

DCs are a subtype of highly efficient APCs that originate from myeloid progenitors. They play a crucial role in antigen uptake, processing, and presentation. DCs are currently the only APCs capable of activating naive T cells, initiating adaptive immune responses. They act as a crucial link between innate and adaptive immunity. DCs are widely distributed in human tissues, except for the brain, and can be classified into immature DCs (iDCs) and mature DC (mDCs) based on their stage of development (Collin and Bigley, 2018). During MTB infection, iDCs use PRRs such as (TLR2 and TLR4, and DC-SIGN) on their surface to mediate the internalization of MTB antigens and subsequent phagocytosis of MTB (Ehlers, 2010). After phagocytosing MTB, iDCs upregulate the chemokine receptor CCR7, which enables them to migrate from peripheral inflammatory tissues to secondary lymphoid tissues via the bloodstream or lymphatic circulation under the guidance of CCR7 (Liu *et al.*, 2019). This process of migration is when iDCs mature. mDCs then present processed MTB antigens to T cells, initiating a specific immune response. In contrast, mDCs upregulate the costimulatory molecules CD80/CD86, which activate T cells and promote their differentiation toward $CD4^+$ T cells (Fucikova *et al.*, 2019). Furthermore, $CD4^+$ T cells, under the influence of cytokines

secreted by mDCs (such as IL-12, TNF-α, and IFN-α), differentiate into Th1 cells. The production of IFN-γ by Th1 cells can further activate macrophages, enhancing their ability to eliminate MTB. Thus, DCs play a critical role in the defence against MTB infection by both innate and adaptive immunity.

1.2.4.6. Adaptive immunity T cell responses in TB

T-cell responses are central to control of MTB, particularly when the bacillus survives within macrophages (Pai *et al.*, 2016, Sia Jonathan and Rengarajan, 2019). Following inhalation, antigen-presenting cells transport MTB antigens to draining lymph nodes, where naïve T cells are primed and differentiate into effector subsets (Zhuang *et al.*, 2024). $CD4^+$ T helper 1 (Th1) cells are considered crucial in MTB response, as they produce IFN-γ and other mediators that activate infected macrophages to enhance phagolysosomal maturation and antimicrobial effector pathways, thereby restricting intracellular bacterial growth (Sia Jonathan and Rengarajan, 2019, Pai *et al.*, 2016). $CD4^+$ T cells also provide help for the maintenance of $CD8^+$ T-cell responses and support the organisation and function of granulomas, the hallmark structures that contain infection (Pai *et al.*, 2016). $CD8^+$ T cells contribute by recognising infected cells and exerting cytotoxic activity (e.g., perforin/granzyme pathways and death-receptor signalling), as well as by producing cytokines such as IFN-γ; these functions can limit bacterial replication and remove heavily infected cells (Zhuang *et al.*, 2024). Additional $CD4^+$ T-cell lineages, including Th17 cells, promote recruitment of innate effector cells through IL-17/IL-22-driven chemokine responses and may be important for early mucosal immunity, although excessive Th17-associated inflammation can contribute to disease pathology (Vyas and Goswami, 2017). To prevent immunopathology, regulatory mechanisms such as regulatory T cells and inhibitory receptors (e.g., PD-1) dampen effector responses; however, overactive regulation or T-cell exhaustion can impair bacterial control and favour progression to active disease (Dorhoi and Kaufmann, 2014, Vyas and Goswami, 2017). Overall, protective immunity in TB reflects a balance between strong T-cell-mediated macrophage activation and cytotoxicity on the one side, and controlled inflammation that preserves lung tissue integrity on the other (Dorhoi and Kaufmann, 2014).

Humoral immunity in TB:

Although TB disease is controlled primarily by cell-mediated immunity, there is increasing evidence that humoral immunity contributes to host defence and influences disease outcomes.

During MTB infection, B cells can accumulate in and around granulomas and form lymphoid-like structures, where they may influence local immune organisation through cytokine production and antigen presentation. Their role is not limited to making antibodies: B cells can also act as antigen-presenting cells, supporting T-cell priming and differentiation, and they shape inflammation directly by secreting both pro- and anti-inflammatory cytokines; a balance that ultimately affects whether the immune response stays protective or tips into immunopathology (Pai *et al.*, 2016, Dorhoi and Kaufmann, 2014, Rijnink *et al.*, 2021). Antibodies contribute to antimycobacterial activity via Fc-dependent effector mechanisms, including opsonisation that enhances phagocytosis, promotion of phagolysosomal maturation, and activation of innate effector cells through Fc receptors; these processes can alter macrophage responses to intracellular bacilli and influence bacterial containment (Rijnink *et al.*, 2021). At mucosal surfaces, antibody isotypes and glycosylation patterns may affect Fc receptor engagement and downstream inflammatory signalling, potentially supporting early control while limiting tissue damage (Rijnink *et al.*, 2021). Importantly, humoral responses in TB are heterogeneous: antibody titres and specificities often differ between latent infection and active disease, and some antibody features correlate with disease severity rather than protection, highlighting that not all humoral responses are beneficial (Pai *et al.*, 2016, Rijnink *et al.*, 2021). Overall, current evidence suggests that B cells and antibodies act as modulators of TB immunity—enhancing bacterial control in some contexts while also contributing to inflammation and pathology in others—underscoring the need to consider both cellular and humoral arms of adaptive immunity when interpreting immune correlates of risk and designing host-directed interventions (Dorhoi and Kaufmann, 2014, Rijnink *et al.*, 2021).

1.5. Host immune responses in HIV

1.5.1. Innate immune response

Upon HIV exposure and entry across mucosal surfaces, the host generates an immediate innate response that can be protective but may also facilitate viral spread by recruiting susceptible target cells. Early after transmission, HIV interacts with (and infects) cells of the mononuclear lineage and triggers pathogen-recognition receptors (PRRs) on innate immune cells, initiating inflammatory and antiviral programmes (Mogensen *et al.*, 2010). A key feature of innate recognition is sensing of viral nucleic acids by endosomal Toll-like receptors (TLRs) and cytosolic sensors. HIV-derived single-stranded RNA can be detected by endosomal TLR7/8,

which activates downstream signalling (including NF-κB and interferon regulatory factors) and results in secretion of pro-inflammatory cytokines (e.g., IL-6, IL-1β, TNF-α) and type I interferons (Mogensen *et al.*, 2010). These cytokines promote local inflammation and leukocyte recruitment, shaping early immune responses but also increasing the pool of activated $CD4^+$ $CCR5^+$ T cells that HIV preferentially infects (Mogensen *et al.*, 2010). In addition to nucleic acid sensing, viral glycoproteins and particle capture by C-type lectin receptors can promote dissemination. For example, dendritic cells (DCs) can bind HIV through DC-SIGN and transport virions to lymphoid tissue, enhancing "trans-infection" of activated T cells, while B cells can bind virions through complement receptor interactions, further supporting spread (Mogensen *et al.*, 2010). Thus, early innate events can simultaneously restrict infection and unintentionally create conditions that favour viral propagation.

Dendritic cells are central to this process because they bridge innate and adaptive immunity. They sample viral material, present antigen, and provide co-stimulation and cytokines that shape downstream T-cell responses. Recent work emphasises that innate activation in some contexts is associated with a smaller and more transcriptionally suppressed HIV reservoir in spontaneous controllers, suggesting that early innate responses can influence long-term viral set points and reservoir configuration (Huber *et al.*, 2024). In HIV controllers, multiple innate cell types—including DCs, natural killer (NK) cells, monocytes, and γδ T cells—have been linked to early containment of viral replication and maintenance of durable suppression (Huber *et al.*, 2024). Mechanistically, DCs sense viral nucleic acids via PRRs and can cross-present HIV antigens to prime cytotoxic $CD8^+$ T cells; they also produce cytokines that activate NK cells and other effectors (Huber *et al.*, 2024, Mogensen *et al.*, 2010). However, HIV can also impair DC function: infection and exposure to viral proteins may reduce antigen-presenting capacity and alter cytokine output, with downstream consequences for T-cell priming and immune regulation (Mogensen *et al.*, 2010). The balance between DC-driven antiviral immunity and DC-mediated viral dissemination is therefore a key determinant of disease trajectory.

NK cells provide rapid cytotoxic and cytokine-mediated antiviral activity and are increasingly recognised as important contributors to early HIV control. NK cells can be activated indirectly by cytokines produced by DCs and monocytes (including type I interferons, IL-12, and IL-15), and they have effector functions before strong HIV-specific $CD8^+$ T-cell responses fully develop (Huber *et al.*, 2024). During acute HIV infection, NK-cell activity has been reported to correlate with viral replication dynamics, highlighting their potential role in early

suppression (Huber *et al.*, 2024). NK cells can also respond through antibody-dependent cellular cytotoxicity (ADCC), in which antibodies bound to viral antigens engage Fc receptors on NK cells, triggering degranulation and killing of infected cells (Huber *et al.*, 2024). In addition, NK cells produce antiviral cytokines (notably IFN-γ) that can enhance macrophage antimicrobial functions and support Th1-polarised responses, while chemokines produced by NK cells may reduce infection by competing for CCR5 co-receptor binding (Mogensen *et al.*, 2010). Importantly, innate immune activation is not universally beneficial: chronic or excessive stimulation can contribute to immune activation and tissue damage, and HIV itself may exploit inflammatory signalling pathways to support replication (Mogensen *et al.*, 2010). Therefore, effective innate immunity in HIV requires careful balance—early, robust antiviral activation that limits infection and spread, followed by timely regulation to avoid persistent inflammation and immune dysfunction.

1.5.2 T cell responses to HIV

The adaptive T-cell response is central to partial control of HIV replication but is progressively undermined by chronic antigen exposure and sustained immune activation. Following infection, HIV proteins are processed and presented on MHC class I, enabling recognition and killing of infected cells by HIV-specific $CD8^+$ T cells. In acute infection, $CD8^+$ T cells contribute to the initial decline in viremia through cytotoxic mechanisms (perforin/granzyme and death-receptor pathways) and secretion of antiviral cytokines such as IFN-γ and TNF-α, while β-chemokines (e.g., MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5) can limit new infections by competing for CCR5 (Gulzar and Copeland, 2004). However, durable viral control requires more than the presence of HIV-specific T cells. Viral controllers have strong, high-quality $CD8^+$ responses characterized by favourable antigen recognition (including protective HLA restriction and targeting of conserved/constrained epitopes), robust proliferative capacity, sustained cytotoxicity, and tissue localization near sites of persistence (Rutishauser and Trautmann, 2022). In most individuals, persistent viremia and inflammation drive a gradual transition toward T-cell exhaustion, a dysfunctional state marked by progressive loss of function (often beginning with reduced IL-2 production and proliferative capacity, followed by diminished cytotoxicity and polyfunctionality) together with increased expression of inhibitory receptors, particularly PD-1, with deeper dysfunction associated with co-expression of additional checkpoints (e.g., LAG3 and TIM3) (Fenwick *et al.*, 2019). In HIV

infection, PD-1 expression on HIV-specific $CD4^+$ and $CD8^+$ T cells is associated with higher viral load, lower CD4 counts, and impaired effector function; although ART reduces antigen burden and can partially decrease inhibitory receptor expression, functional defects can persist and have been linked to earlier viral rebound after treatment interruption (Fenwick *et al.*, 2019). Notably, progressive $CD4^+$ T-cell depletion is a hallmark of HIV infection and is disproportionately severe in the gastrointestinal tract (gut-associated lymphoid tissue), where early loss of mucosal $CD4^+$ T-cell populations (including Th17/Th22 subsets important for barrier integrity) disrupts mucosal immunity and contributes to microbial translocation, which further fuels chronic immune activation (Haase, 2010, Getaneh *et al.*, 2023, Lau *et al.*, 2025). Even under suppressive ART, inflammatory cytokines and immune activation can remain elevated and are associated with impaired antigen-induced activation and cytokine responses of memory CD4 T cells, including reduced vaccine recall responses and disrupted Type I and Type III interferon signalling pathways (Kießling *et al.*, 2024). Collectively, these data support a model in which HIV-specific $CD8^+$ T cells can constrain replication, but persistent antigen and inflammation progressively erode both CD8 effector activity and CD4 T-cell help (including IL-2/Th1-supportive functions), contributing to immune exhaustion, reduced recall functionality, and failure of immune containment in the absence of ART (Kießling *et al.*, 2024, Catalfamo *et al.*, 2012, Fenwick *et al.*, 2019).

1.5.3 Humoral response to HIV

During acute HIV infection, the earliest antibodies to appear target Env, mainly gp41 and gp120. These early responses rarely neutralize circulating virus, but they are not without effect: through Fc-mediated mechanisms, particularly antibody-dependent cellular cytotoxicity (ADCC), they can still promote killing of infected cells once Fc receptors on effector cells such as NK cells are engaged (Baum *et al.*, 1996, Tomaras *et al.*, 2008). This is supported by in vitro work showing ADCC activity when plasma or purified IgG from acutely infected patients is combined with NK cells from healthy donors, pointing to a role for non-neutralizing antibodies in early immune pressure (Forthal *et al.*, 2001).Yet HIV evades this pressure with remarkable efficiency, using extensive Env sequence diversity, glycan shielding, and conformational masking of key epitopes to keep replicating even in the presence of bound antibody.

Broadly neutralizing antibodies (bnAbs) emerge later, and only in a subset of PLWH, after prolonged antigenic stimulation drives extensive B-cell maturation. Because they target

relatively conserved regions of Env, bnAbs can neutralize a wide range of HIV-1 strains, which is why they remain a major focus of both prevention and cure-oriented research. Most current bnAb-induction vaccine strategies try to prime the rare B cells capable of becoming bnAb precursors, then steer their maturation with a sequence of Env-based immunogens designed to work around viral diversity and immune escape (Kumar *et al.*, 2023). bnAbs are not limited to classical neutralization; their Fc-dependent functions, including ADCC, may also matter for therapies aimed at clearing infected cells through immune-mediated mechanisms. Importantly, HIV pathogenesis and persistence are strongly shaped by mucosal immunology: the gastrointestinal tract is a major site of HIV replication and immune dysfunction, and ongoing mucosal damage and inflammation can influence both B-cell function and the quality of antibody responses (Lau *et al.*, 2025). Together, these observations support a model in which humoral immunity applies meaningful immune pressure on HIV, but is inactivated by viral evolution. Still, understanding and leveraging neutralizing and Fc-mediated antibody functions remains central to HIV vaccine design and antibody-based interventions.

1.6. Host immune responses in TB/HIV co-infection

Host immune responses in TB/HIV co-infection are shaped by HIV-driven $CD4^+$ T-cell depletion and chronic immune activation. $CD4^+$ T cells are central to antimycobacterial immunity because they sustain Th1 cytokine production (notably IFN-γ and TNF-α), activate infected macrophages, and support granuloma organisation; therefore, HIV-associated loss and dysfunction of $CD4^+$ T cells compromise M control and are strongly associated with increased risk of progression to active TB (Shao *et al.*, 2016, Gunawan *et al.*, 2026). In addition, early depletion of mucosal $CD4^+$ T cells and disruption of gut barrier function promote microbial translocation, which amplifies systemic inflammation and activation of multiple immune subsets (including monocytes, pDCs, NK cells, and T and B cells), increasing turnover and apoptosis and contributing to immune dysregulation (Getaneh *et al.*, 2023, Gunawan *et al.*, 2026). This hyperactivated and ineffective immune response can impair granuloma integrity, facilitating MTB dissemination and increasing macrophage death and granuloma breakdown that precede reactivation (Bocchino *et al.*, 2000, Bell *et al.*, 2016, Gunawan *et al.*, 2026).

Although ART reduces HIV viremia and improves overall CD4+ T-cell recovery, it often does not fully normalize immune activation or restore protective pulmonary effector memory $CD4^+$

T-cell (T_{EM}) responses, which are important for local control of MTB. In a rhesus macaque LTBI/SIV co-infection model, concurrent anti-TB therapy (weekly isoniazid/rifapentine, 3HP) plus cART improved clinical and microbiological outcomes and reduced bacterial burden, but lung immune reconstitution remained incomplete, with persistent activation/exhaustion markers, ongoing inflammatory signatures (including type I interferon programmes), and persistent depletion of $CD4^+$ T_{EM} cells (Sharan *et al.*, 2025). These findings align with human observations that HIV/TB co-infection is characterised by heightened immune activation and altered cytokine networks, which can persist despite viral suppression and contribute to ongoing TB risk (Bell *et al.*, 2016, Gunawan *et al.*, 2026). TB treatment can expand or restore detectable MTB-specific $CD4^+$ responses in vivo, even when baseline frequencies are reduced in co-infected individuals (Siddiqui *et al.*, 2015). However, effective antimycobacterial immunity also depends on functional T-cell help for $CD8^+$ responses; MTB co-infection has been associated with impaired HIV-specific and MTB-relevant T-cell functionality, consistent with exhaustion and reduced protective capacity (Chetty *et al.*, 2015). Consequently, adjunctive approaches aimed at reducing residual immune activation and supporting restoration of protective Th1/Th17 and tissue-resident memory responses (e.g., IL-21-based strategies evaluated in preclinical models) have been proposed, but require further validation in humans(Micci *et al.*, 2015, Sharan *et al.*, 2025).

1.7. Role of inflammation in TB pathogenesis

Findings from previous CAPRISA cohorts provide strong clinical evidence that inflammatory pathways are not only a feature of TB disease but are also associated with key outcomes, including lung tissue destruction, microbiological response to therapy, and risk of recurrent disease. In the CAPRISA 020 InDEX drug-resistant TB cohort, higher pre-treatment plasma IL-6 levels were independently associated with the presence of lung cavitation after adjustment for demographic and clinical factors (including HIV status), supporting a link between systemic inflammation and the immunopathology in cavitary disease, transmission risk and poor outcomes (Maseko *et al.*, 2023). These data position IL-6 as a clinically relevant marker of inflammatory pathology in TB in a high-burden setting.

Published CAPRISA studies also suggest that inflammatory mediator profiles during active TB relate to bacterial clearance during treatment. Using specimens from the CAPRISA 011 IMPRESS trial, (Rambaran *et al.*, 2021) evaluated plasma cytokines/chemokines measured

during active pulmonary TB and reported associations with early microbiological response: higher IP-10 was associated with higher odds of culture conversion by 8 weeks (including among PLWH), and higher MCP-3 and IL-6 were associated with a shorter time to culture conversion in adjusted models (Rambaran *et al.*, 2021). Importantly, because cavitation reflects a destructive inflammatory phenotype, these results collectively highlight that inflammatory pathways can track with both disease severity and treatment response, underscoring the need to interpret inflammation as a context-dependent component of TB pathogenesis rather than as detrimental or beneficial.

Beyond active disease, CAPRISA studies in PLWH individuals on ART demonstrate that persistent systemic inflammation is linked to future TB risk. In the CAPRISA TRuTH cohort of ART-treated PLWH with prior pulmonary TB who were followed longitudinally for recurrence, plasma IL-6 and markers in the IL-1 axis (IL-1β and IL-1Rα) were associated with increased risk of TB recurrence, whereas IFN-β was associated with reduced risk in multivariable analyses, suggesting that specific inflammatory and interferon programmes may differentially relate to protection versus pathology (Sivro *et al.*, 2017). Extending this work, (Pillay *et al.*, 2021) reported that additional markers consistent with microbial translocation and endothelial activation—including lipopolysaccharide-binding protein (LBP) and soluble ICAM (sICAM)—were associated with increased odds of recurrent TB, alongside TGF-β3, and that LBP correlated with IL-6, supporting a model in which gut barrier dysfunction and chronic immune activation contribute to TB susceptibility even in the setting of ART (Pillay *et al.*, 2021). Taken together, CAPRISA findings refine the role of inflammation in TB pathogenesis by linking defined circulating inflammatory mediators to (i) destructive lung pathology (cavitation), (ii) microbiological response to therapy, and (iii) longer-term risk of recurrent TB, particularly in HIV co-infection.

1.8. MMPs Structure and function

MMPs constitute a family of zinc-dependent endopeptidases involved in degrading extracellular matrix (ECM) components and regulating various physiological processes. There are different types of MMPs, each with its own function, and there are currently 23 known human MMPs. The most well-known MMPs include MMP-1, MMP-2, and MMP-9. Structurally, MMPs typically contain several conserved domains, including a pro-domain, catalytic domain, and hemopexin-like domain (Mastroianni and Liuzzi, 2007, Woessner, 1991)

(Figure 2). The pro-domain maintains MMPs in an inactive zymogen form, preventing premature activation and enzymatic activity. The pro-domain is cleaved upon activation, revealing the active catalytic site responsible for ECM degradation (Visse and Nagase, 2003). The catalytic domain contains the zinc-binding motif essential for MMP activity, while the hemopexin-like domain facilitates substrate recognition and binding. MMPs are classified into subgroups based on structural and functional characteristics, with distinct substrate specificities and cellular localization patterns (Nagase *et al.*, 2006). These subgroups of MMPs include: collagenases (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinases (MMP-2, and MMP-9), stromelysins (MMP-3, MMP-10 and MMP-11), matrilysin, and elastase (MMP-7, and MMP-12) (Khokha *et al.*, 2013). MMPs have the ability to regulate the activity of cytokines and chemokines by cleaving and converting them into more potent forms or inactivating them, which consequently modulates inflammatory cell recruitment (Khokha *et al.*, 2013). MMPs are controlled by proteins called tissue inhibitors of metalloproteinases (TIMPs), which bind to them and stop their activity (Brew *et al.*, 2000). TIMPs are also linked to the development of fibrosis (Nagase *et al.*, 2006), a common feature of TB healing (Hunter, 2011). Understanding the structural organization of MMPs is critical for elucidating their role in physiological and pathological processes and designing specific inhibitors targeting MMP activity.

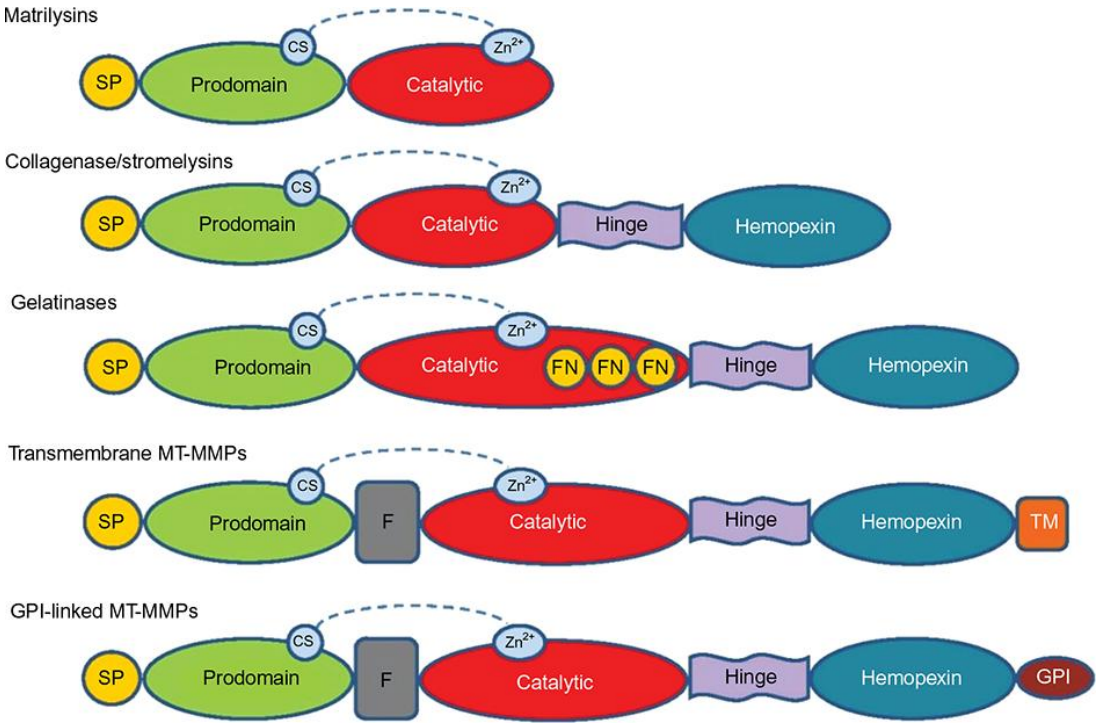

Figure 2:The structure of MMP groups with SP, propeptide, catalytic, and hemopexin domains. The active site of zinc bound to the catalytic domain and the CS can be found in all

MMPs. Gelatinase FN repeats are found in the catalytic domain of gelatinases. A furin-cleavage site (F) between the pro-domain and catalytic domain can be found in MT-MMPs. Some MMPs have a TM domain attached to the hemopexin domain (MT-MMP). Other MT-MMPs have GPI-anchor domain. CS, cysteine switch; FN, fibronectin; GPI, glycosylphosphatidylinositol; MMP, matrix metalloproteinase; MT-MMP, membrane type-matrix metalloproteinase; SP, signal peptide; TM, transmembrane. Adapted from (Jaoude J, 2016).

1.9. Role of MMPs in TB

In TB, MMPs are important mediators of immunopathology because they remodel the lung ECM, regulate leukocyte trafficking, and modify cytokine/chemokine activity. In pulmonary TB, dysregulated MMP activity contributes to collagen and ECM degradation, which results in lung tissue destruction and cavitation—pathological processes that can increase bacillary burden in airways and thereby facilitate transmission (Elkington *et al.*, 2011a, Rohlwink *et al.*, 2019b). This same matrix-destructive activity can also drive lung remodelling that contributes to longer-term respiratory morbidity after TB (post-TB lung disease), reinforcing interest in MMP-focused host-directed strategies that aim to limit immunopathology while preserving protective immunity (Loader *et al.*, 2026). MMP expression in TB is influenced by bacterial burden and inflammatory cytokines and is regulated through signalling pathways such as NF-κB, p38, and MAPK (Sabir *et al.*, 2019). Human and experimental data implicate specific MMPs in distinct TB disease phenotypes: MMP-1 (a key interstitial collagenase) has been linked to collagen breakdown and lung tissue destruction in pulmonary TB (Elkington *et al.*, 2011b), whereas MMP-9 has been associated with TB meningitis pathogenesis via effects on blood–brain barrier integrity and has also been implicated in granuloma formation and inflammatory cell recruitment in some models (Parasa *et al.*, 2017, Rohlwink *et al.*, 2019b). Clinical studies report elevated circulating or site-of-disease MMP levels in active TB, and in some settings MMP concentrations correlate with radiographic severity and/or cavitation, supporting their role as biomarkers of tissue-destructive inflammation (Kumar *et al.*, 2018, Walker *et al.*, 2012b). These findings underscore that MMP dysregulation is a key component of TB pathophysiology and a plausible target for host-directed therapy aimed at limiting matrix destruction and improving post-TB lung outcomes

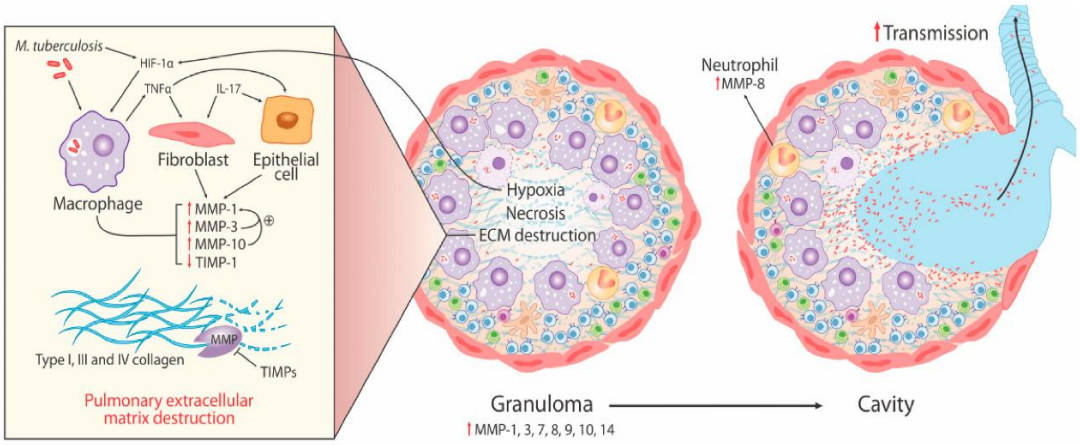

Figure 3: Role of Metalloproteinases in the destruction of the extracellular matrix (ECM) during pulmonary tuberculosis (pTB). Lung granulomas in TB patients contain infected macrophages (purple), dendritic cells(beige), T (blue) and B (green) lymphocytes, and neutrophils (orange). These cells secrete various MMPs, including MMP-1, -3, -7, -9, and -10, which contribute to a proteolytic cascade. Adapted from (Rohlwink *et al.*, 2019b).

MMPs contribute directly to ECM destruction within pulmonary TB granulomas and cavities (Figure 3). Granulomatous lesions contain infected macrophages and recruited immune cells (including neutrophils, T cells, and B cells) that can produce MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-10, supporting a proteolytic cascade that drives matrix breakdown, necrosis, and cavitation (Rohlwink *et al.*, 2019a). MMP-1 is a major collagenase implicated in degradation of fibrillar collagens, and neutrophil-derived MMP-8 can also contribute to collagen destruction; together, these enzymes have been linked to cavitation and tissue-remodelling phenotypes in TB (Walker *et al.*, 2022, Ong *et al.*, 2015). Local microenvironmental factors may further amplify this response: hypoxia can increase MMP expression via hypoxia-inducible factor (HIF)-1α signalling in myeloid cells, and MTB-driven cytokine networks (including TNF-α) can enhance MMP expression in both immune and stromal cells, such as lung fibroblasts (Rohlwink *et al.*, 2019a). Because MMPs are tissue-destructive effectors, inhibiting MMP activity is being explored as a host-directed approach. Doxycycline (a licensed broad-spectrum MMP inhibitor) suppresses TB-driven MMP secretion in experimental systems, and a small phase II randomised trial reported suppression of systemic and respiratory MMPs and a reduction in cavity volume with adjunctive doxycycline, supporting further evaluation of MMP inhibition to limit TB immunopathology (Walker *et al.*, 2012b, Miow *et al.*, 2021).

1.10. Role of MMPs in HIV

MMPs have been implicated in HIV pathogenesis, where an imbalance between MMPs and their endogenous inhibitors (TIMPs) may contribute to HIV-associated tissue injury and immune dysfunction (Mastroianni and Liuzzi, 2007). During HIV infection, inflammatory signalling can increase MMP expression and activity, promoting ECM remodelling and degradation of matrix barriers. This can facilitate leukocyte trafficking across endothelial and basement-membrane structures and may support dissemination of HIV-infected mononuclear cells, thereby linking MMP dysregulation to ongoing viral spread and disease progression (Mastroianni and Liuzzi, 2007). Clinically, elevated MMP levels—particularly MMP-1, MMP-2 and MMP-9—have been reported in PLWH and in some studies correlate with lower $CD4^+$ T-cell counts and markers of immune dysregulation (Sabir *et al.*, 2019). Beyond effects on cell migration and tissue remodelling, MMP activity has been associated with HIV-related comorbidities, including neurocognitive impairment and cardiovascular disease, consistent with broader roles of MMPs in inflammatory tissue damage (Mastroianni and Liuzzi, 2007, Cabral-Pacheco *et al.*, 2020).

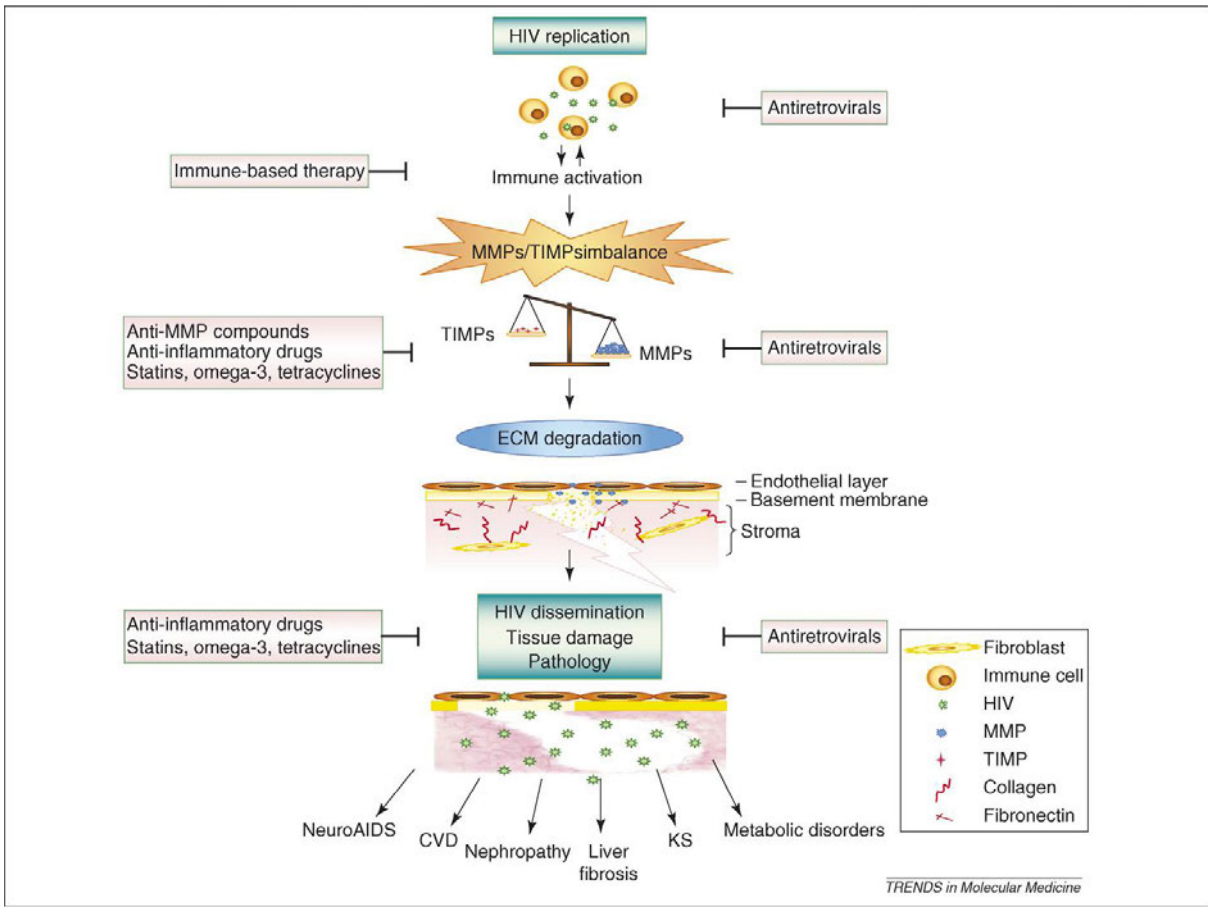

Figure 4: The role of MMPs in HIV. This figure illustrates the progression of HIV-induced tissue damage through a sequence of molecular and cellular events. HIV replication activates

the immune system, leading to an imbalance between MMPs and TIMPs. This imbalance favours MMP activity, resulting in ECM degradation, which compromises the integrity of the endothelial layer, basement membrane, and stroma. Consequently, this degradation facilitates HIV dissemination, tissue damage, and pathology. Potential therapeutic interventions include the use of antiretroviral drugs to inhibit HIV replication, immune-based therapies to modulate immune activation, and anti-MMP compounds or anti-inflammatory drugs such as statins, omega-3 fatty acids, and tetracyclines to restore MMP/TIMP balance and mitigate ECM degradation. The downstream effects of uncontrolled HIV dissemination and tissue damage include NeuroAIDS, cardiovascular disease (CVD), nephropathy, liver fibrosis, Kaposi's sarcoma (KS), and metabolic disorders. The schematic also highlights key cell types and molecular components involved, including fibroblasts, immune cells, MMPs, TIMPs, collagen, and fibronectin. Adapted from (Mastroianni and Liuzzi, 2007)

These observations have prompted interest in host-directed approaches that modulate matrix remodelling alongside standard antiretroviral therapy (Figure 4). Interventions that reduce upstream immune activation and/or restore the MMP–TIMP balance could help limit ECM damage and downstream organ-specific complications; however, targeting MMPs in HIV remains to be established and requires careful evaluation given the physiological roles of MMPs in normal tissue repair (Cabral-Pacheco *et al.*, 2020, Mastroianni and Liuzzi, 2007).

1.11. Role of MMPs in TB-HIV Co-infection

MMP dysregulation has been implicated in TB/HIV co-infection, but available data suggest that the direction and clinical consequences of MMP changes depend on HIV disease stage, inflammatory environment, and treatment context (Ravimohan *et al.*, 2016, Kumar *et al.*, 2018, Loader *et al.*, 2026). Co-infection and lesion physiology are increasingly recognised as important modifiers of MMP biology: microenvironmental features common in TB lesions—including hypoxia, acidosis, and metabolic stress—can influence inflammatory signalling networks and protease activity, with implications for tissue destruction and post-TB lung sequelae (Loader *et al.*, 2026). In advanced HIV-associated TB (e.g., low CD4 counts), (Walker *et al.*, 2012b) reported that concentrations of several MMPs (including MMP-1, MMP-2, MMP-8, and MMP-9) were lower in induced sputum from HIV/TB co-infected patients than in HIV-negative TB, consistent with a blunted inflammatory/granulocytic response in the airway compartment. Importantly, lower airway MMP levels in advanced HIV should not be

interpreted as a lack of pathology; rather, they likely reflect altered immune cell recruitment and localisation in immunosuppressed disease, which is itself associated with poor containment of *M*TB and dissemination. As immune function is restored with ART, however, the balance can shift toward heightened inflammatory immunopathology. In this setting, MMP-driven matrix degradation becomes more prominent: (Ravimohan *et al.*, 2016) demonstrated that MMPs are elevated in TB-associated immune reconstitution inflammatory syndrome (TB-IRIS) and that these increases are linked to impaired lung function, supporting a role for MMPs as effectors of tissue damage during inflammatory rebound. Consistent with this concept, (Sharan *et al.*, 2025) identified persistent immune activation signatures in the pulmonary compartment during MTB/SIV co-infection despite concurrent therapy, including immune activation modules that include MMP-9, underscoring ongoing inflammatory pathways that may contribute to tissue remodelling even when microbiological control improves.

Neutrophils are an important source of MMP-8 and MMP-9, and these MMPs often track with systemic neutrophil-related inflammation in TB. In a clinical study, (Alisjahbana *et al.*, 2022) reported that MMP-8 and MMP-9 levels were associated with neutrophil counts, supporting a neutrophil contribution to their secretion in pulmonary disease. They also observed differences in leukocyte profiles and MMP patterns in TB/HIV co-infection compared with TB alone, highlighting that lymphocytopenia and altered innate cell dynamics in HIV can reshape the protease signature. Together, these findings support a model in which TB/HIV co-infection does not simply increase or decrease MMPs uniformly; instead, HIV-related immunosuppression may reduce airway MMP signals in advanced disease, whereas immune reconstitution and inflammatory rebound (including TB-IRIS) can be characterised by increased MMP activity that contributes to matrix destruction and worse pulmonary outcomes (Alisjahbana *et al.*, 2022, Ravimohan *et al.*, 2016, Walker *et al.*, 2012b).

1.12. Clinical use of MMP inhibitors

From the 1990s to the early 2000s, synthetic inhibitors of MMPs (MMPIs) were studied in various cancer types (Winer *et al.*, 2018). MMPs have been recognized for their clinical relevance, particularly in the context of cancer treatment and cardiovascular diseases like atherosclerosis. The therapeutic potential of MMPIs has been explored due to their role in tumour invasion, metastasis, and pathological tissue remodeling (Myasoedova *et al.*, 2018, Curran and Murray, 2000). More recently, there has been renewed interest in MMP inhibition

in infectious diseases—particularly TB—where MMP-driven matrix destruction contributes to cavitation and post-TB lung disease and where MMP-targeted host-directed therapies have shown proof-of-concept in preclinical studies and early clinical evaluations (Loader *et al.*, 2026).

Xu *et al*. investigated the role of various MMP inhibitors in improving the efficacy of standard TB treatment. This study highlighted several MMP inhibitors, including Marimastat, Batimastat, Prinomastat, Sb-3ct, and a specific MMP-9 inhibitor. Marimastat is a broad-spectrum MMP inhibitor that was developed as an antineoplastic drug candidate. Studies have shown that Marimastat significantly enhances the effectiveness of frontline TB drugs like isoniazid (INH) and rifampicin (RIF). This synergistic effect was observed in both cellular and animal models, specifically in C57BL/6J mice and in bone marrow-derived macrophages from these mice. The ability of Marimastat to boost the efficacy of TB drugs highlights its potential as an adjunctive therapy in TB treatment (Xu *et al.*, 2018). However, it has not been approved by the FDA due to its side effects and limited efficacy.

Batimastat (BB-94) was the first MMP inhibitor widely studied in animal models of cancer (Drumnond *et al.*, 1999). It is a broad-spectrum hydroxamic acid-based zinc metalloproteinase inhibitor that inhibits both MMP (including MMP-1, -2, -3, -7, and -9) and members of the ADAM family of enzymes, such as TNF-α leaving enzyme (TACE) (Rasmussen and McCann, 1997). These enzymes are involved in the regulation of the inflammatory processes in TB. BB-94 shares a similar structure with Marimastat, but has lower water solubility (Xu *et al.*, 2018). In a TB study, BB-94 showed a synergistic effect with INH, suggesting its potential use as an adjunctive therapy for TB in animal studies (Xu *et al.*, 2018). BB-94 has been developed as a cancer treatment to prevent metastasis by inhibiting MMPs. Its clinical use has been limited due to poor bioavailability when administered orally, and it is generally used in research contexts (Parsons *et al.*, 1997).

Prinomastat, a structurally unrelated broad-spectrum MMP inhibitor, was designed to inhibit several MMPs (including MMP-2, MMP-3, MMP-9, MMP-13 and MMP-14) involved in tumor growth and metastasis. Clinically, it has been tested in cancer trials but faced challenges due to side effects and limited efficacy (Bissett *et al.*, 2005). In the TB study, Prinomastat did not exhibit the same level of synergy with TB drugs as Marimastat and BB-94, indicating that it may have less potential for TB treatment compared to the other inhibitors (Xu *et al.*, 2018).

Sb-3ct is a more selective MMP inhibitor that primarily targets MMP-9 and MMP-2. MMP-9 and MMP-2 play critical roles in cancer and other diseases, such as neuroinflammatory conditions and cardiovascular diseases. In a TB study, Sb-3ct demonstrated a notable enhancement of INH's bactericidal activity, suggesting that it could be a valuable adjunct in TB therapy by specifically inhibiting MMPs that contribute to the pathology of the disease (Xu *et al.*, 2018).

The study also mentioned a specific MMP-9 inhibitor, which was designed to target MMP-9 selectively. MMP-9 is involved in various pathological processes, including cancer metastasis, cardiovascular disease, and chronic inflammatory conditions. Clinically, selective MMP-9 inhibitors have been investigated for their potential to treat these conditions. In the TB study, the specific MMP-9 inhibitor significantly enhanced the efficacy of INH, indicating its potential utility in improving TB treatment outcomes by targeting a key enzyme involved in the disease's progression (Xu *et al.*, 2018)

Periostat, a sub-antimicrobial dose formulation of doxycycline, is an FDA-approved MMP inhibitor used to treat periodontal disease in the United States. Doxycycline is the only licensed MMP inhibitor and broad-spectrum tetracycline antibiotic that inhibits MMP activity (Sang *et al.*, 2006). It suppresses MTB-induced MMP secretion in cellular models (Walker *et al.*, 2012a) and limits collagen destruction by MTB-induced MMPs (Ong *et al.*, 2015, Walker *et al.*, 2017). Doxycycline treatment at a dose of 20 mg twice daily inhibits MMP activity in patients with periodontal disease (Gapski *et al.*, 2009), and improves lung function in inflammatory lung disorders, such as chronic obstructive pulmonary disease (COPD) (Dalvi *et al.*, 2011) and asthma (Bhattacharyya *et al.*, 2012). In a guinea pig model of TB, doxycycline monotherapy reduced the lung mycobacterial burden in a dose-dependent manner (Walker *et al.*, 2012a), and in a mouse model of TB, MMP inhibition improved drug efficacy. Studies in mice have shown that doxycycline has high organ penetration and reaches higher concentrations in both necrotic and cellular TB lesions than in plasma (Gelhaus *et al.*, 2013). Miow *et al*. conducted a phase II randomized controlled trial, which was an exploratory study to investigate the effects of adjunctive doxycycline to standard anti-TB treatment in 30 patients with pulmonary TB. The study found that doxycycline specifically suppresses systemic MMP-1 and respiratory MMP-1, -8, and -9, inhibits matrix destruction in respiratory samples of patients with pulmonary TB, and reduces pulmonary cavity volume. However, larger studies on adjunctive doxycycline to limit TB immunopathology are merited (Miow *et al.*, 2021)

Study rationale:

TB remains a major global health problem and is further complicated by the emergence and ongoing transmission of DR-TB as well as the high prevalence of HIV co-infection in many high-burden settings. A major determinant of morbidity, mortality, and transmission is the extent of TB-associated lung destruction, particularly cavitation. MMPs are host proteases that degrade ECM components and can modulate inflammatory signalling, thereby mediating the tissue remodelling processes that contribute to cavitation. However, despite increasing evidence that MMP-driven matrix destruction is important in TB immunopathology, important gaps remain in understanding how circulating MMP profiles differ between DS-TB and DR-TB, and whether these profiles associate with cavitary disease similarly in people with and without HIV co-infection.

To address this gap, this study leverages two well-characterized CAPRISA cohorts from Durban, South Africa—CAPRISA 011 (IMPRESS; DS-TB) and CAPRISA 020 (InDEX; DR-TB)—to quantify plasma levels of MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 during active TB and to test their associations with radiographic lung cavitation, including stratified analyses by HIV status. By comparing MMP patterns across DS-TB and DR-TB and relating them to a clinically meaningful marker of disease severity, this work aims to refine the understanding of host pathways linked to lung destruction. Identifying MMP signatures associated with cavitation could support the development of improved risk stratification tools and strengthen the rationale for host-directed strategies that target tissue-destructive inflammation as adjuncts to standard TB therapy, particularly in the context of DR-TB, where treatment is prolonged, and outcomes are often poor.

Hypothesis:

We hypothesize that dysregulated MMP expression patterns will be associated with TB disease severity and poor treatment outcomes.

Overall Aim

The main aim of this study is to characterise the role of MMPs in the pathogenesis of DR- and DS- TB

Objectives:

1. To analyse the plasma expression levels of MMPs in individuals with DR- and DS-TB with and without HIV co-infection

2. To assess the impact of MMPs on TB disease severity as measured by the presence of lung cavitation

3. To evaluate the correlation between MMP expression levels and TB progression and treatment response

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CHAPTER 2:
METHODOLOGY

CHAPTER 2:

2. METHODS

2.1 Study Ethics

CAPRISA 011 (Clinicaltrials.gov, NCT02114684) was approved by the Medicines Control Council of South Africa (MCC Ref: 20130510). This study was reviewed and approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) [BFC029/13]. The CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX) was approved by the UKZN BREC, [BFC584/16] and registered with the South African Clinical Trials Register (NCT03237182). The nested study presented in this thesis obtained ethics from the UKZN Biomedical Research Ethics Committee (BREC/00007522/2024).

2.2. Study Cohort:

The plasma samples for this study were obtained from the CAPRISA 020 and CAPRISA 011 cohorts. The CAPRISA 020 Individualized M(X) Drug-Resistant TB Treatment Strategy study (InDEX) cohort was a randomized controlled clinical trial that assessed whether a gene-derived individualized treatment approach in participants with drug-resistant TB improved treatment success. Participants enrolled in the InDEX study were recruited from King Dinuzulu Hospital (KDH) in KwaZulu-Natal (KZN), South Africa (SA). Study participants were ≥ 18 years old with pulmonary TB and Xpert MTB/RIF assay positive for rifampicin resistance. This study included both PLWH and HIV-negative men and women.

CAPRISA 011 study participants were recruited and treated at an urban clinic (CAPRISA eThekwini Research Clinic) adjoining the largest government outpatient TB facility, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KZN, SA. All enrolled participants were adults ≥ 18 years old, with a previous history of TB and a current diagnosis of rifampicin-susceptible sputum smear-positive MTB by GeneXpert MTB/RIF® technology. Both HIV-infected and uninfected participants were included in the study. Plasma samples collected at baseline prior to treatment initiation were used in this study, n= 128 (CAPRISA 020) and n= 78 (CAPRISA 011).

2.3. Laboratory Procedures:

2.3.1. Plasma sample collection and processing:

Peripheral blood was collected in acid citrate dextrose (ACD) tubes. Plasma was separated by centrifugation (1000xg for 10 minutes) and cryopreserved at −80 °C until use.

2.3.2 MMP measurements:

Cryopreserved plasma samples were thawed and mixed before performing the assays. MMP levels were measured using a Millipore Multiplex Human MMP Magnetic Bead Panel 2 kit following standardized laboratory protocols and analysed on a BioPlex-200 system (Bio-Rad). The Human MMP Panel included MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10.

2.3.2.1 Reagents preparation for Immunoassay: To prepare reagents for the immunoassay, each antibody-bead vial was sonicated for 30 seconds, vortexed for 1 minute, and 150 µL from each vial was added to a Mixing Bottle with Bead Diluent to reach a total volume of 3.0 mL. The beads were thoroughly vortexed, and any remaining mixture was stored at 2-8 °C for up to one month.

For Quality Controls 1 and 2, each was reconstituted with 250 µL deionized water, mixed, and allowed to sit for 5-10 minutes. They were then transferred to labelled polypropylene tubes, and unused portions were stored at ≤ -20 °C for up to one month.

To prepare 10X Wash Buffer, it was brought to room temperature, 60 mL was diluted with 540 mL deionized water.

The Human MMP Panel 2 Standard was reconstituted with 250 µL deionized water, mixed, and allowed to sit for 5-10 minutes before being transferred to a labelled polypropylene tube.

To create Working Standards, 1:3 serial dilutions were prepared starting with Standard 7, progressively diluting to generate Standards 6 through 1. Standard 0 was Assay Buffer (Figure 5).

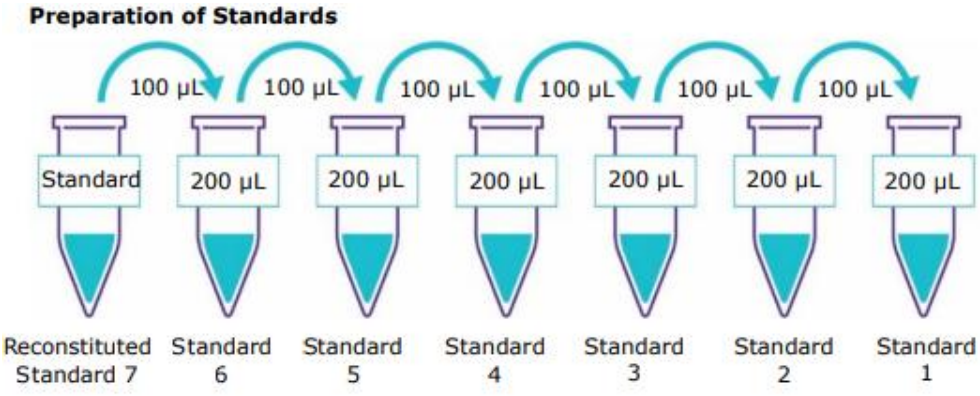

Figure 5: A graphical representation of the three-fold serial dilutions conducted for standard preparation. (Adapted from MILLIPLEX MAP Human MMP Magnetic Bead Panel 2 Assay protocol).

2.3.2.2 Immunoassay procedure: Before conducting the assay, all reagents were thawed to room temperature (20-25°C). The assay began by washing all 96-plate wells with 200 µl wash buffer at room temperature. Standards and controls were individually added to their designated wells, followed by the addition of 25 µl of assay buffer to the blank and sample wells. Phosphate-buffered saline (PBS) matrix solution was added to the blank, standards, and control wells, then 25 µl of the sample was added to the appropriate wells from the master plate.

Mixed beads were vortexed and added to each well of the plate, then sealed and incubated with agitation for 2 hours at room temperature. After incubation, the plate was washed twice with 200 µl wash buffer. 25 µl of detection antibodies was added to each well, and the plate was sealed. The plate was then agitated for 1 hour at room temperature, followed by the addition of 25 µl Streptavidin phycoerythrin. The plate was incubated for 30 minutes, washed with 200 µl of buffer, and filled with 100 µl sheath fluid per well for data acquisition on the Bio-Plex 200 Array reader system.

2.3.4 Cytokine measurements: Cytokine/Chemokine levels obtained from previous studies examining the effect of inflammation on TB disease in the same cohorts (Maseko *et al.*, 2023) and were provided by Dr. Santhuri Rambaran and Dr. Thando Maseko. Cytokines were measured using the Pro Human Cytokine Assay (Bio-Rad, USA). The following cytokines and chemokines were included in the analysis (based on the availability of data for both study cohorts): fibroblast growth factor (FGF) basic, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), interleukin (IL) -1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17 A, interferon γ–Inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP) -1α, MIP-1β, platelet-derived growth factor (PDGF) -BB, regulated on activation normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF) -α, Vascular endothelial growth factor (VEGF).

2.4. Statistical Analysis Plan: Statistical analyses were performed using IBM SPSS Statistics v29 (Armonk, NY), and R software v2.2.2, and the graphs were generated using GraphPad Prism v10. Linear regression was employed to analyse the difference in MMP expression between DS-TB and DR-TB. Logistic regression was used to examine the association between

measured MMPs and the presence of lung cavitation based on the assessment of a baseline postero-anterior chest radiograph by a certified pulmonologist and radiologist. Multivariable analyses adjusted for age, sex, body mass index (BMI), HIV status, smoking, alcohol use, and previous TB history. In the sub-analyses for the PLWH group, antiretroviral therapy (ART) status was adjusted for. Data distribution was assessed using the D'Agostino-Pearson omnibus normality test. Plasma MMP levels of DS-TB participants across different sputum smear grades (1+, 2+, and 3+) stratified by HIV status were compared using the Kruskal-Wallis test with Dunn's multiple comparisons adjustment. Spearman's rank correlation coefficients were used to assess correlations between cytokines/chemokines and MMPs. P values below 0.05 were considered significant.

CHAPTER 3:
MANUSCRIPT

In Chapter 3, we characterised plasma MMP expression profiles in participants with drug-susceptible and drug-resistant pulmonary TB, with and without HIV co-infection from CAPRISA 011 (IMPRESS) and CAPRISA 020 (INDEX). We evaluated associations between MMP levels and clinical outcomes, including lung cavitation, as well as correlations with demographic and immunological factors. In addition, we examined differences in MMP expression between cavitary and non-cavitary disease to better understand the role of host-mediated tissue damage in TB pathogenesis.

Prof. A. Sivro and I, Azande Sthoko Gazu, conceptualised and designed the study. I performed experimental work with contributions from Dr. T.G. Maseko. I conducted the primary data acquisition, cleaning, and statistical analyses, with contributions from Dr. T.G Maseko and Prof. A.Sivro, with statistical validation performed by Lateef B. Amusa**.** I wrote the first draft of the manuscript, which was reviewed by Prof. A. Sivro (supervisor), Dr. T.G. Maseko (co-supervisor), and the rest of the co-authors. The manuscript is submitted for publication to the journal *Tuberculosis.*

Role of matrix metalloproteinases in drug-resistant and drug-susceptible tuberculosis

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Keywords: Matrix metalloproteinases, HIV, Tuberculosis, DR-TB, DS-TB, lung cavitation

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Target journal: Tuberculosis

ABSTRACT

Tuberculosis (TB) remains a global health challenge, and lung damage is a major determinant of outcomes. Matrix metalloproteinases (MMPs) contribute to extracellular matrix degradation and inflammatory regulation, promoting tissue destruction and cavitation. We quantified plasma MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10 by multiplex immunoassay in 78 drug-susceptible (DS)-TB and 128 drug-resistant (DR)-TB participants. We assessed associations with baseline chest radiograph cavitation (DS-TB n=70; DR-TB n=125). In DS-TB, higher MMP-1 was associated with cavitation in univariable analysis (OR 9.86, 95% CI 1.45–84.5; p=0.025) but the effect was attenuated after adjustment for age, sex, HIV status, alcohol use, smoking, and BMI (p=0.097); among PLWH DS-TB participants (n=51), the univariable association persisted (OR 17.1, 95% CI 1.57–270; p=0.028) with an attenuated adjusted trend (p=0.065). In DR-TB, MMP-10 was associated with cavitation in univariable analysis (OR 8.16, 95% CI 1.32–59.2; p=0.029) but not after adjustment. No MMPs were associated with time to culture conversion or sputum smear grade. MMP-1 correlated positively with proinflammatory cytokines/chemokines, while other MMPs showed heterogeneous cytokine correlations. In multivariable linear regression, DR-TB was associated with higher MMP-1 (β=0.28, p=0.005) and lower MMP-7 (β=-0.235, p<0.001) and MMP-10 (β=-0.169, p=0.01) compared to DS-TB. Overall, these findings suggest differing involvement of MMPs in DS- versus DR-TB immunopathogenesis and lung pathology.

1.1 Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade and remodel extracellular matrix (ECM) components, including collagens and elastin[1]. MMPs are generally synthesized and secreted as inactive zymogens and are activated by proteolytic cleavage in the extracellular space or at the cell surface. The MMP family comprises more than 20 enzymes, commonly classified by substrate preference and domain structure, including collagenases (e.g., MMP-1), gelatinases (e.g., MMP-2 and MMP-9), stromelysins, and membrane-type MMPs[2]. Under physiological conditions, MMP expression and activity are tightly controlled and support processes such as wound healing, tissue repair, and remodelling; however, during infection and inflammation, dysregulated MMP activity can promote pathological tissue destruction. In chronic inflammatory diseases characterized by matrix degradation—including fibrosing interstitial lung diseases, cystic fibrosis, and erosive inflammatory arthropathies—excessive MMP activity contributes to tissue damage[3,4]. Beyond matrix remodelling, MMPs also regulate immune responses by modulating cytokines and chemokines, influencing leukocyte migration, and processing cell-surface receptors, thereby shaping the balance between host defence and immunopathology[5].

In tuberculosis (TB), MMPs are increasingly recognized as important mediators of tissue damage and disease progression. MMP-1 has been strongly associated with lung matrix destruction and cavitation in pulmonary TB[6]. MMP-9 is also implicated in TB immunopathology, including leukocyte recruitment and granuloma dynamics, and can facilitate cell migration by remodelling the ECM. Other MMPs, such as MMP-8 and MMP-10, have been linked to inflammatory regulation through effects on neutrophil activity, processing of inflammatory mediators, and remodelling of the tissue microenvironment, thereby influencing the balance between host defence and immunopathology[7,8]. Excessive or dysregulated MMP expression contributes to pulmonary pathology and may differ between drug-susceptible (DS)-TB and drug-resistant (DR)-TB due to differences in disease duration, immune activation, and bacillary persistence[9]. DR-TB is often associated with delayed effective treatment and prolonged antigenic stimulation, which may sustain inflammatory pathways and downstream mediators of tissue destruction, including MMPs. MMP-driven matrix degradation and aberrant repair contribute to cavitation and permanent structural lung damage, which can be associated with impaired local perfusion and reduced antibiotic penetration, potentially creating environments that support ongoing *M. tuberculosis* (MTB)

persistence(Sabir *et al.*, 2019). In this context, host-directed approaches that modulate MMP activity could help limit progressive lung damage and improve outcomes. This study aimed to explore the roles of MMPs in DS-TB and DR-TB to better understand how these host enzymes contribute to disease progression and pathology across different TB contexts.

1.2 Methods

1.2.1 Study Ethics. The CAPRISA 011 Improving Retreatment Success (IMPRESS) trial (Clinicaltrials.gov, NCT02114684) was approved by the Medicines Control Council of South Africa (MCC Ref: 20130510) and the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) [BFC029/13]. The CAPRISA 020 Individualized M(X) drug-resistant TB Treatment Strategy Study (InDEX) was approved by the UKZN BREC (BFC584/16) and registered with the South African Clinical Trials Register (NCT03237182). The nested study presented here was approved by the UKZN BREC (BREC/00007522/2024).

1.2.2 Study Cohorts. Plasma samples for this study were obtained from the CAPRISA 020 InDEX and CAPRISA 011 IMPRESS cohorts. The CAPRISA 020 InDEX study was a randomized controlled clinical trial that assessed whether a gene-derived individualized treatment approach in participants with DR-TB improved treatment success. Enrolled CAPRISA 020 InDEX study participants were recruited from King Dinuzulu Hospital (KDH) in KwaZulu-Natal (KZN), South Africa (SA). Study participants were ≥ 18 years old with pulmonary TB and Xpert MTB/RIF assay positive for rifampicin resistance. This study included both PLWH and HIV-negative men and women. CAPRISA 011 IMPRESS study participants were recruited and treated at the CAPRISA eThekwini Research Clinic, adjoining the largest government outpatient TB facility, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KZN, SA. All enrolled participants were adults ≥ 18 years old, with a previous history of TB and a current diagnosis of rifampicin-susceptible sputum smear-positive MTB by GeneXpert MTB/RIF® technology. Both HIV-infected and uninfected participants were included in the study. All study participants provided written informed consent before enrolment and approval for the use of stored biological specimens for future research. All of the experiments were performed in accordance with the Helsinki Declaration. Plasma samples from n= 128 (CAPRISA 020) and n= 78 (CAPRISA 011) collected at baseline prior to treatment initiation were used in this study.

1.2.3 Plasma sample collection and processing. Peripheral blood was collected in acid citrate dextrose (ACD) tubes. Plasma was separated by centrifugation (1000 x g for 10 minutes) and cryopreserved at −80 °C until use.

1.2.4 MMP measurements. Cryopreserved plasma samples were thawed and mixed before performing the assays. MMP levels were measured using the Millipore Milliplex ® assays (Map Human MMP Magnetic Bead Panel 2 kit, catalogue number HMMP2MAG-55K) following manufacturer's instructions and analysed on a BioPlex-200 system (Bio-Rad). The Human MMP Panel included MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10. Values falling below the limit of detection (LOD) were assigned a value of LOD/2.

Paired cytokine/chemokine data was obtained from the previous studies examining the same cohorts(Rambaran *et al.*, 2021).

1.2.5 Statistical Analysis. Statistical analyses were performed using IBM SPSS Statistics v29 (Armonk, NY), R software v2.2.2, and GraphPad Prism v10. Linear regression was employed to analyse the difference in MMP expression between DS-TB and DR-TB. Logistic regression was used to examine the association between measured MMPs and the presence of lung cavitation based on the assessment of a baseline postero-anterior chest radiograph by a certified pulmonologist and radiologist. Multivariable analyses adjusted for age, sex, body mass index (BMI), HIV status, smoking, and alcohol use. In the sub-analyses for the PLWH group, antiretroviral therapy (ART) status was adjusted for. Data distribution was assessed using the D'Agostino-Pearson omnibus normality test. Plasma MMP levels of DS-TB participants across different sputum smear grades (1+, 2+, and 3+) stratified by HIV status were compared using Kruskal-Wallis test with Dunn's multiple comparisons adjustment. Spearman's rank correlation coefficients were used to assess correlations between cytokines/chemokines and MMPs.

1.3 Results

1.3.1 Participants' characteristics

The demographic and clinical characteristics of study participants from the DS- and DR-TB cohorts are described in Tables 1 and 2. The DS-TB cohort enrolled 78 participants: 49 with lung cavitation, 21 without, and 8 excluded for missing lung cavitation status. The median age was 37 years [inter-quartile range (IQR) 31–43], with 61.5% (48/78) male and 38.5% (30/78) female. The median BMI was 33 kg/m² (IQR 31–39). HIV co-infection was present in 70.5% (55/78) of study participants. In the DS-TB cohort, BMI, $CD4^+$ count, and HIV viral load were higher in individuals without lung cavitation compared to those with lung cavitation **(Table 1)**.

The DR-TB cohort enrolled 128 participants, including 61 with lung cavitation and 64 without, and 3 participants were excluded due to missing lung cavitation status. The median age was 35 years (IQR 29–42), with 57.0% (73/128) being male and 43.0% (55/128) female. The median BMI was 20.2 kg/m² (IQR 18.5–22.7). Participants with rifampicin-resistant TB and MDR-TB accounted for 89.8% (115/128) of the cohort, while those with Pre-XDR/XDR-TB accounted for 10.2% (13/128). HIV co-infection was present in 72.7% (93/128) of study participants. In the DR-TB cohort, individuals without lung cavitation had significantly higher HIV viral load levels compared to those with lung cavitation **(Table 2)**.

Table 1. Demographic and clinical characteristics of participants with DS-TB (n=78).

Variables	Total Cohort (n=78)	Lung cavitation (n=49)	No lung cavitation (n=21)
Randomization arm, n (%)			
HRZE—Control	38(48.7%)	22(45.0%)	8(38.0%)
HRZM—Active	40(51.3%)	27(55.0%)	13(62.0%)
Age (y), median (IQR)	37(31-43)	37(30- 42)	38(32-44)
Gender, n (%)			
Male	48(61.5%)	41(63.3%)	7(33.0%)
Female	30(38.5%)	16(36.7%)	14(67.0%)
Body mass index (kg/m^2), median (IQR)	33(31- 39)	33(29.88- 36.53)	39.08(32.48- 45.20)
HIV status, n (%)			
Positive	55(70.5%)	34(69.4%)	17(80.9%)
Negative	23(29.5%)	15(30.6%)	4(19.1%)
ART status,[a] n (%)			
Yes	22(28.2%)	13(26.5%)	9(42.9%)
No	31(39.7%)	20(40.8%)	7(33.3%)
Smear grade[b], n (%)			
1+	18 (23.1%)	8 (38.0%)	10 (20.0%)
2+	6 (7.7%)	2 (9.5%)	5 (10.0%)
3+	41 (52.6%)	10 (48.0%)	34 (69.0%)
4+	1 (1.3%)	1 (4.8%)	0 (0.0%)
Smoking, n (%)			
Yes	23(29.5%)	17(35.0%)	3(14.3%)
No	55(70.1%)	32(65.0%)	18(85.7%)
Alcohol use, n (%)			
Yes	17(21.8%)	13(26.5%)	2(9.5%)
No	61(78.2%)	36(73.5%)	19(90.5%)

[a] – 2 participants with missing ART status

* 8 participants with missing lung cavitation status

[b] 11 participants with missing smear grades status

Table 2. Demographic and clinical characteristics of participants with DR-TB (n=128).

Variables	Total cohort n=128	Lung cavitation [a] n=61	No lung cavitation n= 64
TB diagnosis			
RR/MDR	115(89.8%)	57(93.0%)	56(88.0%)
Pre-XDR/XDR	13(10.2%)	4(7.0%)	8(12.0%)
Age (years), median (IQR)	35(29-42)	33(29- 43)	36(30- 41)
Gender, n (%)			
Male	73(57.0%)	33(54.0%)	39(61.0%)
Female	55(43.0%)	28(46.0%)	25(39.0%)
Body mass index (kg/m^2), median (IQR)	20.20(18.50- 22.75)	19.8(17.80- 21.65)	20.50(18.85- 23.73)
HIV status, n (%)			
Positive	93(72.7%)	37(61.0%)	53(82.8%)
Negative	35(27.3%)	24(39.0%)	11(17.2%)
ART status,[b] n (%)			
Yes	84(66.0%)	33 (54.0%)	49(76.0%)
No	9(7.0%)	4(7.0%)	4(6.0%)
Alcohol use, n (%)			
No	120(94.0%)	57(93.0%)	60(94.0%)
Yes	8(6.0%)	4(7.0%)	4(6.0%)
Smoking, n (%)			
No	118(92.0%)	53(87.0%)	62(97.0%)
Yes	10(8.0%)	8(13.0%)	2(3.0%)

* [a] 3 participants with missing lung cavitation status
[b] 35 participants with miss ART status

1.3.2 Association between plasma MMPs and the presence of lung cavitation

Logistic regression models were used to determine the association between plasma MMP levels and disease severity measured by the presence of lung cavitation. In univariable analyses, MMP-1 levels were significantly associated with increased odds of lung cavitation [odds ratio (OR) 9.86, 95% confidence interval (CI) 1.45–84.5, p=0.025] in the DS-TB cohort. We observed a similar trend following multivariable analyses adjusting for age, sex, HIV status, alcohol use, smoking, and BMI (adjusted OR (aOR) 8.56, 95% CI 0.73–128, p=0.097) **(Table 3)**.

In a sub-analysis of the PLWH individuals (n=51), MMP-1 remained significantly associated with lung cavitation in the univariable analysis (OR 17.1, 95% CI 1.57–270, p=0.028), with a trend observed after adjusting for ART use (OR 17.6, 95% CI 1.07–540, p=0.065) (**Table 4**).

Table 3. Association between plasma MMPs expression and lung cavitation in the total DS-TB cohort (n=70).

MMP	Univariable			Multivariable		
	OR	95% CI	p-value	aOR	95% CI	p-value
MMP1	9.86	1.45, 84.5	**0.025**	8.56	0.73, 128	0.097
MMP2	0.39	0.00, 21.6	0.657	0.24	0.00, 62.2	0.613
MMP7	1.84	0.14, 27.7	0.641	0.18	0.00, 9.35	0.386
MMP9	0.54	0.10, 2.77	0.456	0.38	0.04, 3.16	0.361
MMP10	4.94	0.54, 57.3	0.168	4.16	0.23, 98.8	0.348

*Eight participants were excluded due to missing lung cavitation status

Table 4. Association between plasma MMPs expression and lung cavitation among PLWH in the DS-TB cohort (n=51).

MMP	Univariable			Multivariable		
	OR	95% CI	p-value	aOR	95% CI	p-value
MMP1	17.1	1.57, 270	**0.028**	17.6	1.07, 540	0.065
MMP2	0.18	0.00, 13.2	0.457	0.10	0.00, 16.3	0.394
MMP7	2.10	0.10, 52.5	0.628	0.72	0.01, 50.7	0.871
MMP9	0.55	0.08, 3.50	0.520	0.21	0.02, 1.89	0.165
MMP10	6.90	0.39, 166	0.203	3.55	0.11, 171	0.489

Among DR-TB individuals (n=125), MMP-10 expression was significantly associated with lung cavitation in the univariable analysis (OR 8.16, 95% CI 1.32–59.2, p=0.029), however this association was not significant following adjustment for age, sex, HIV status, alcohol use, smoking, and BMI (aOR 4.06, 95% CI 0.44–40.2, p=0.219) **(Table 5).**

We observed no significant associations between MMP levels and the presence of lung cavitation in PLWH individuals **(Table 6)**, with a trend observed for MMP-10 in the multivariable analysis (aOR 9.37, 95% CI 0.84–125, p=0.076).

Table 5. Association between plasma MMPs expression and lung cavitation in the total DR-TB cohort (n=125).

MMP	Univariable			Multivariable		
	OR	95% CI	p-value	aOR	95% CI	p-value
MMP1	1.23	0.43, 3.56	0.698	0.97	0.28, 3.29	0.956
MMP2	0.31	0.01, 7.02	0.464	0.08	0.00, 3.55	0.196
MMP7	0.91	0.13, 6.30	0.924	0.55	0.05, 6.13	0.632
MMP9	2.52	0.88, 7.78	0.093	2.02	0.60, 7.06	0.257
MMP10	8.16	1.32, 59.2	**0.029**	4.06	0.44, 40.2	0.219

*Three participants were excluded due to missing lung cavitation status

Table 6. Association between plasma MMPs expression and lung cavitation in people living with HIV in the DR-TB cohort (n=90).

MMP	Univariable			Multivariable		
	OR	95% CI	p-value	aOR	95% CI	p-value
MMP1	0.85	0.22, 3.16	0.801	0.54	0.13, 2.23	0.401
MMP2	0.25	0.01, 10.6	0.473	0.07	0.00, 4.67	0.222
MMP7	1.32	0.13, 12.9	0.811	1.36	0.12, 15.7	0.804
MMP9	2.50	0.77, 8.91	0.137	2.49	0.71, 9.35	0.158
MMP10	5.45	0.63, 54.7	0.132	9.37	0.84, 125	0.076

1.3.3 Association between MMP expression and time to culture negativity

To assess the relationship between MMP levels and TB treatment response, we evaluated the association between plasma MMP expression and time to culture conversion using the Cox proportional hazards regression analyses across DS-TB and DR-TB cohorts. In the DS-TB cohort, no significant associations were observed between MMP levels and time to culture conversion, both in the total cohort and the PLWH sub-group **(Sup. Tables 1 and 2)**. Similarly, in the DR-TB cohort, measured MMPs were not significantly associated with time to culture conversion in the total cohort and the PLWH sub-group **(Sup. Tables 2 and 3)**.

1.3.4 Association between MMP expression and bacterial burden at baseline

To investigate the association between plasma MMP levels and bacterial burden, we compared plasma MMP levels across different sputum smear grades (1+, 2+, and 3+). The smear grades analysis was conducted only for participants in the DS-TB cohort, as corresponding data for the DR-TB cohort were not available at the time of analysis. In both the total DS-TB cohort and the PLWH subgroup, no statistically significant differences were observed in MMP-1, MMP-2, MMP-7, MMP-9, or MMP-10 levels across the different smear grades **(Sup. Figure 1a-b)**.

1.3.5 Correlation between plasma MMP and cytokine expression

To examine the correlation between plasma MMP levels and plasma cytokine/chemokine expression during active TB, we performed a Spearman's rank correlation analysis. In the total DS-TB cohort (**Figure 1A**) we observed a significant positive correlation between MMP-1 and PDGF-bb (r=0.367, p=0.001), G-CSF (r=0.275, p=0.015), FGT (r=0.243, p=0.031), IL-17 (r=0.337, p=0.003), IL-8 (r=0.588, p<0.05), IL-6 (r=0.344, p=0.002), IL-1Rα (r=0.412,

p=0.0002), and IL-1β (r=0.269, p=0.017). We observed a significant positive correlation between MMP-2 and IL-17 (r=0.263, p=0.020) and a negative correlation between MMP-2 and MIP-1β (r=-0.258, p=0.023) and IL-9 (r=-0.245, p=0.031). We also observed a significant positive correlations between MMP-7 and IL-8 (r=0.502, p<0.0001), IL-1Rα (r=0.359, p=0.001), IL-1β (r=0.313, p=0.005), IL-4 (r=0.245, p=0.031), IL-7 (r=0.225, p=0.0533), IL-17 (r=0.309, p=0.006), and MCP-1 (r=0.257, p=0.023) and a negative correlation with IL-9 (r=-0.236, p=0.037) and MIP-1β (r=-0.238, p=0.036). MMP-9 was positively correlated to with Eotaxin (r=0.284, p=0.0117) and had a negative correlation with IL-17 (r=-0.284, p=0.0117), IL-12 (r=-0.272, p=0.016), and IL-1b (-0.324, p=0.0038). MMP-10 correlated positively with IL-1Rα (r=0.369, p=0.001) and IL-8 (r=0.35, p=0.002).

We observed a similar trend in the PLWH sub-group for the DS-TB cohort **(Figure 1B)**. In addition to the above-described correlation, MMP-1 was significantly correlated with IL-7 (r=0.368, p=0.0062) and negatively correlated with Eotaxin (r=-0.219, p=0.05). We observed a significant positive correlation between MMP-2 and IL-4 (r=0.307, p=0.0228) and a negative correlation between MMP-2 and IP-10 (r=-0.299, p=0.0266) and RANTES (r=-0.312, p=0.0204). MMP-7 correlated positively with TNF-α (r=0.291, p=0.0311) and correlated negatively with RANTES (r=-0.314, p=0.0196). MMP-10 correlated negatively with RANTES (r=-0.3, p=0.026).

In the total DR-TB cohort (**Figure 2A**), we observed positive correlations between MMP-1 and RANTES (r=0.186, p=0.0355), PDGF-bb (r=0.335, p<0.05), IP-10 (r=0.2, p=0.023), IFN-γ (r=0.191, p=0.031), IL10 (r=0.182, p=0.0402), IL-8 (r=0.34, p<0.05), IL-6 (r=0.358, p<0.05), and IL-1Rα (r=0.377, p=0.00001). MMP-2 correlated negatively with RANTES (r=-0.269, p=0.002), MIP-1β (r=-0.214, p=0.0152), PDGF-bb (r=-0.279, p=0.0014), MIP-1α (r=-0.358, p=0.0003), IP-10 (r=-0.353, p=0.00004), IFN-γ (r=-0.218, p=0.0135), FGF (r=-0.198, p=0.0254), Eotaxin (r=-0.332, p=0.00034), IL-9 (r=-0.199, p=0.0242), IL-6 (r=-0.234, p=0.0077), IL-4 (r=-0.203, p= 0.0214), and IL-1ra (r=-0.317, p=0.00027). MMP-7 was positively correlated with MIP-1α (r=0.21, p=0.017), IP-10 (r=0.25, p=0.004), IL-8 (r=0.223, p=0.00009), IL-6 (r=0.187, p=0.035), and IL-1ra (r = 0.179, p=0.043). MMP-9 was negatively correlation with MCP-1 (r=-0.208, p=0.0182) while MMP-10 was negatively correlated with MCP-1 (r=-0.195, p=0.02722), GM-CSF (r=-0.187, p=0.03407) and IL-12 (r=-0.205, p=0.02028).

We observed a similar trend for MMP-1, MMP-9, and MMP-10 in the PLWH sub-group (**Figure 2B**). Additionally, we observed a significant negative correlation between MMP-2 and GM-CSF (r=-0.223, p=0.032), and G-CSF (r=-0.242, p=0.020), while MMP-7 correlated positively with IL-13 (r=0.206, p=0.032).

Panel A: Total DS-TB cohort **B: PLWH only**

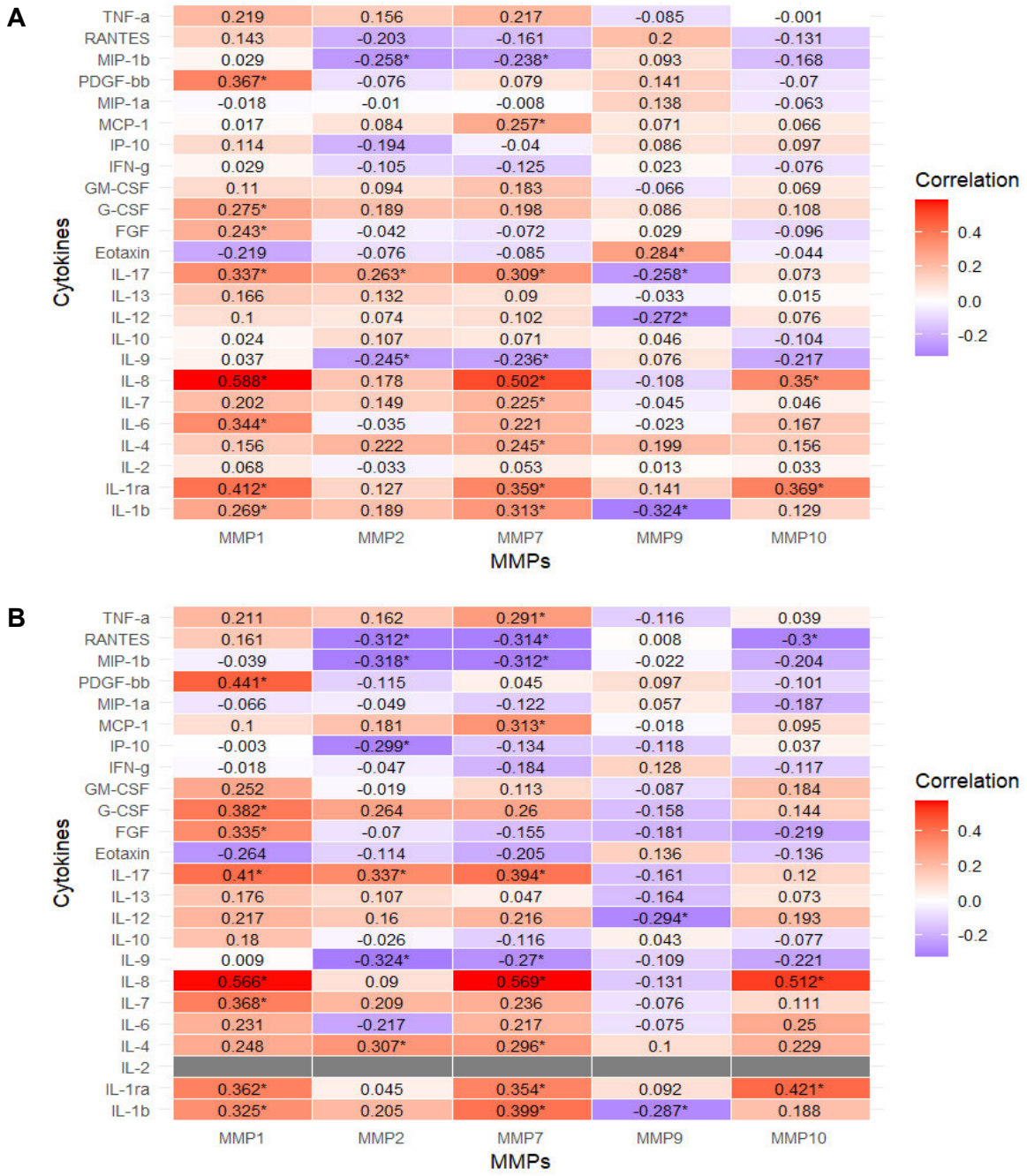


Figure 1. Heatmap of correlation between measured MMPs and cytokine/chemokine expression for the IMPRESS cohort: **(A)** total cohort, **(B)** PLWH only. The heatmap displays the pairwise Spearman correlation coefficients between MMPs, cytokines, and clinical variables. An asterisk (*) indicates a statistically significant correlation at the 5% level.

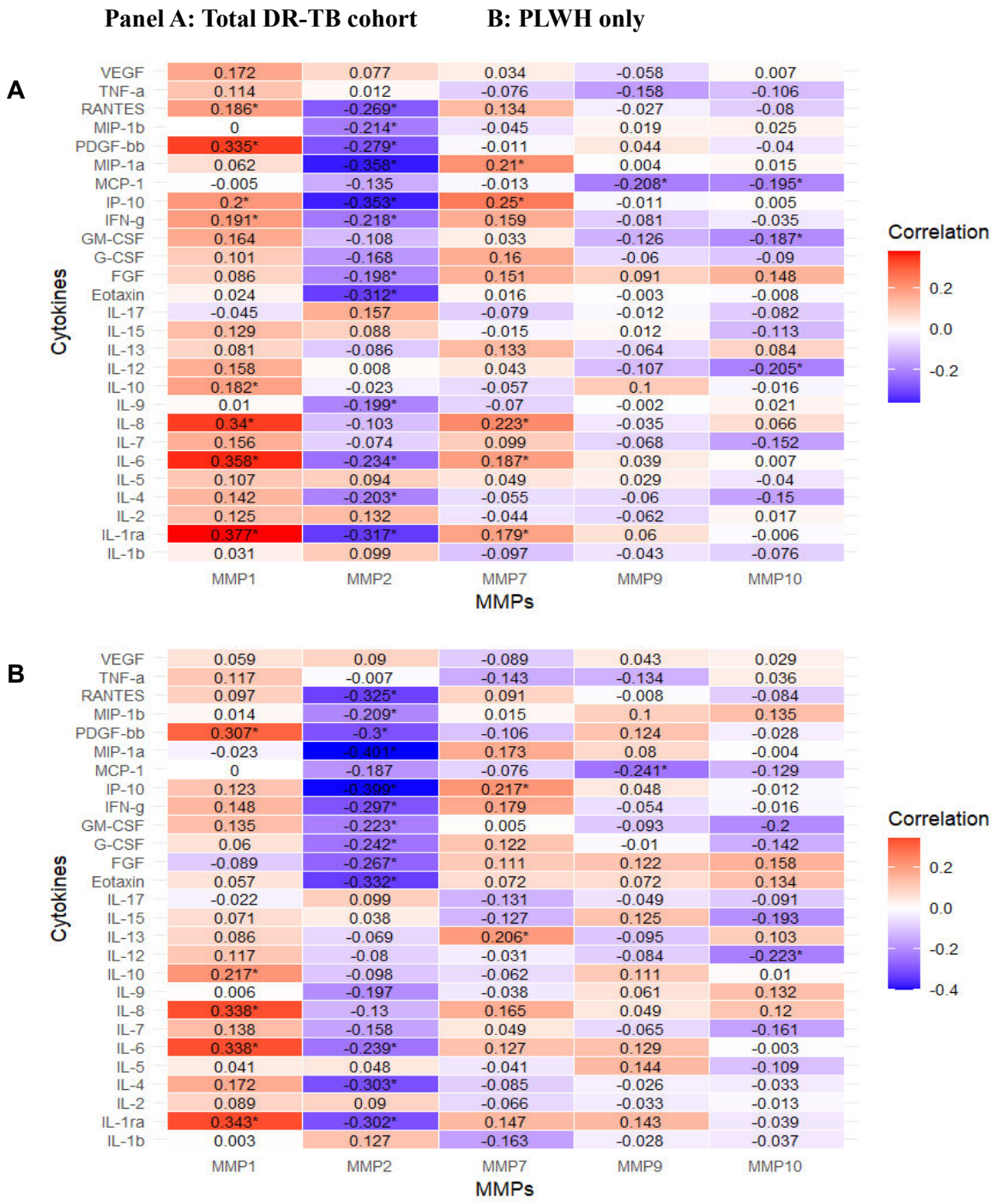


Figure 2. Heatmap of correlation between measured MMPs and cytokine/chemokine expression for the InDEX cohort: **(A)** Total cohort. **(B)** PLWH participants only. The heatmap displays the correlations among MMPs, cytokines, and clinical variables within the Index cohort. An asterisk (*) indicates a statistically significant correlation at the 5% level.

1.3.6 Differences in plasma MMP expression between DS and DR TB

We ran a multivariable linear regression model adjusting for age, sex, HIV status, smoking, alcohol use, and BMI to analyse the differences in MMP expression between DS-TB and DR-TB. We observed significantly higher plasma MMP-1 levels in DR-TB participants compared to DS-TB (aβ=0.28, 95% CI 0.084–0.476, *p*=0.005), and lower levels of MMP-7 (aβ = -0.235, 95% CI -0.344 to -0.126, *p*<0.001) and MMP-10 (aβ = -0.169, 95% CI -0.298 to -0.04, p=0.01) (**Table 3**).

Table 7. Multivariable linear regression results assessing the association between TB type (DS-TB =0, DR-TB =1), and MMP expression levels (MMP-1, -2, -7, -9, and -10).

	TB type	aCoefficient (aβ)	95% CI	p-value
MMP1	DR-TB	0.28	0.084, 0.476	**0.005**
	DS-TB	Ref		
MMP2	DR-TB	-0.004	-0.076, 0.069	0.917
	DS-TB	Ref		
MMP7	DR-TB	-0.235	-0.344, 0.126	**<0.001**
	DS-TB	Ref		
MMP9	DR-TB	0.077	-0.119, 0.273	0.439
	DS-TB	Ref		
MMP10	DR-TB	-0.169	-0.298, -0.04	**0.01**
	DS-TB	Ref		

1.4 Discussion

In this study, we investigated the association between plasma MMPs and key clinical outcomes in TB, including lung cavitation and drug resistance, as well as their associations with host immune responses. Given the high prevalence of HIV co-infection in our cohorts, we also assessed whether HIV status influenced these associations. Our findings provide new insights into how circulating MMPs may contribute to/reflect TB immunopathology.

In DS-TB, higher plasma MMP-1 was associated with lung cavitation in univariable analyses in both the overall cohort and among PLWH participants, although these associations were attenuated after multivariable adjustment. Additionally, its expression positively correlated with expression of several pro-inflammatory markers previously associated with lung cavitation, including IL-6 and IL1Rα (Rambaran *et al.*, 2021, Elkington *et al.*, 2011). Previous studies have similarly reported that elevated MMP-1 levels are associated with active pulmonary TB and the presence of lung cavitation(Kathamuthu *et al.*, 2020, Kumar *et al.*, 2018) and reduced lung function(Soemarwoto *et al.*). MMP-1 has been identified as an important enzyme contributing to the destruction of the lung in human disease, such as TB(Imai *et al.*, 2001). It significantly contributes to lung cavitation by directly degrading essential ECM components, including fibrillar collagens, through its potent collagenolytic activity(Elkington *et al.*, 2011). This dysregulated proteolytic activity, often resulting from an imbalance with tissue inhibitors, drives the breakdown of the collagen matrix and other structural proteins(Tiwari and Martineau, 2023). MTB was shown to up-regulate epithelial cell MMP-1 secretion (up to a 100-fold) via a monocyte-dependent network(Elkington *et al.*, 2005). This effect is amplified in airway epithelial cells adjacent to granulomas, promoting the breakdown of the ECM, which ultimately contributes to tissue damage and the formation of lung cavities. We did not observe associations between MMP-1 and bacterial burden, which could be influenced by factors such as the limited sample size, differences in study population, or disease stage. This is consistent with previous studies, which also reported poor correlation between systemic inflammatory markers and bacterial load [11]. MMP-1 was proposed as a therapeutic target to limit immunopathology in TB, with R032-3555 (a compound used in phase III clinical trials for arthritis) shown to suppress MTB-driven MMP-1 activity(Elkington *et al.*, 2011, Hemmings *et al.*, 2001). Interestingly, despite significantly higher MMP-1 levels in DR-TB compared to DS-TB, we observed no significant association between plasma MMP-1 and the presence of lung cavitation in DR-TB. Several factors could contribute to this discrepancy. The

immunological responses in DR-TB may differ significantly from those in DS-TB, potentially leading to varied expression or activity of MMP-1 or alternative mechanisms of cavitation. The immunological responses in DR-TB may differ from those in DS-TB due to prolonged antigenic stimulation, higher bacillary burden, and repeated or ineffective treatment exposure, which can lead to sustained immune activation and immune exhaustion. These conditions may alter cytokine profiles, such as skewing towards chronic type I interferon or dysregulated TNF-α and IL-1 signaling, thereby modifying the regulation and activity of MMP-1 (Cox and Overall, 2008). In this context, cavitation in DR-TB may be driven less by MMP-1–mediated matrix degradation and more by alternative mechanisms, including altered macrophage activation states, neutrophil-driven tissue damage, or differential regulation of other proteases. There was a weak association with elevated MMP-10 levels and the presence of lung cavitation in the DR-TB. Similarly, Brilha *et al.* (2017) directly examined MMP-10 in TB and reported large MTB-driven increases in MMP-10 secretion, inhibition of MMP-10 reduced collagen breakdown, and elevated MMP-10 levels in patient respiratory samples. However, while MMP-10 upregulation has been documented in various lung diseases, direct in vivo evidence demonstrating that MMP-10 causes cavity formation is still lacking(Brilha *et al.*, 2017).

Despite the observed associations with lung cavitation, we found no significant relationship between plasma MMP levels at baseline and time to culture conversion in either cohort. This suggests that MMP expression during active disease is not predictive of treatment response and bacterial clearance in our setting. Some studies report similar findings, whereas others have observed associations depending on specimen type and study endpoint. For example, Ugarte-Gil *et al.* reported that elevated sputum levels of MMP-1, -2, -8, and -9 at diagnosis were associated with delayed early culture conversion (2 weeks) in TB patients(Ugarte-Gil *et al.*, 2013). In HIV-associated TB, elevated baseline plasma MMP-3, -7, -8, -10, and PIIINP were associated with MTB bloodstream infection and 12-week mortality, supporting the view that circulating MMPs reflect disease severity and systemic involvement rather than the speed of bacterial clearance(Walker *et al.*, 2025)

While MMP-1 was primarily positively associated with pro-inflammatory cytokines, other MMPs displayed a more complex and heterogeneous relationship with the inflammatory response, showing both positive and negative associations across different cytokines. For example, MMP-2 demonstrated weaker or inverse correlations with several pro-inflammatory

cytokines, suggesting a potential regulatory role rather than a purely pro-inflammatory function. This complexity may be partly explained by the regulation of MMP-2 activity by tissue inhibitors of metalloproteinases (TIMPs), which can counteract excessive MMP-driven inflammation. In particular, TIMP-2 has been shown to exert anti-inflammatory effects by suppressing microglial activation in response to inflammatory stimuli, thereby limiting the overall inflammatory response (Lee and Kim, 2014).

Our study has several limitations. First, MMPs were measured only at baseline, preventing us from assessing their dynamic changes during treatment. Second, the relatively small sample sizes in stratified analyses, may have limited statistical power. Third, plasma measurements may not fully capture local pulmonary MMP activity, which could differ from systemic expression. Notably, a further limitation, is that we did not measure TIMPs; this data may provide added insight into whether the ratios of the MMPs to TIMPs serve as a marker for cavitatory versus non-cavitatory TB. Finally, although we adjusted for key confounders, residual confounding may remain, particularly due to HIV disease stage and ART timing.

In conclusion, our findings suggest potential differences in MMP involvement in lung pathogenesis between DS- and DR-TB. MMP-1 showed associations with markers of lung pathology and pro-inflammatory cytokine responses in DS-TB, particularly among PLWH individuals; however, these associations were strongest in univariable analyses and were attenuated in multivariable models. While MMPs were not predictive of bacterial clearance, they may reflect underlying immunopathology and tissue remodelling during active TB disease. Future studies should investigate longitudinal changes in MMPs during treatment and validate these observations in larger, multi-centre cohorts, including tissue-based analyses such as bronchoalveolar lavage fluid and, where feasible, lung tissue biopsies, to better define their role in TB pathogenesis.

1.5 Acknowledgments: The authors would like to thank the study participants and the CAPRISA clinical and laboratory staff for their contributions to the project.

1.6 Author contributions: Designed the study: A.S., A.S.G.; Performed the experiments: A.S.G, T.G.M.; Analysed the data: A.S.G, T.G.M., L.M., L.L, A.S.; Wrote the first draft of the paper: A.S.G., A.S.; Collected specimens and clinical data: S.E., D.G.; Supervised clinical

and/or experimental aspects of the study: A.S., D.A., S.N.,K.N., R.P.; All authors contributed to the editing and finalisation of the manuscript.

1.7 Funding: TGM was supported by the DST-NRF Centre of Excellence (CoE) in HIV Prevention (CoE UID number: 96354). DA was supported through the SAMRC Self-Initiated Grant and the NRF of South Africa Thuthuka (grant no. TTK160517165310), the NRF Research Career Advancement Fellowship (grant no. RCA13101656388), the Poliomyelitis Research Foundation of South Africa (PRF 17/02) and an EDCTP fellowship (grant no. TMA2017SF-1960). NP and the InDEX study is supported by EDCTP Senior Fellowship (grant no. TMA2018SF-2467 Research reported in this publication was supported by the Strategic Health Innovation Partnerships (SHIP) Unit of the South African Medical Research Council with funds received from the South African Department of Science and Technology and by the European and Developing Countries Clinical Trials Partnership (EDCTP) (TMA2018CDF-2372 and TMA2018SF-2476). The funding sources listed here did not have any role in the analysis or preparation of the data in this manuscript, nor was any payment received by these or other funding sources for this manuscript.

1.8 Data availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

1.9 Competing interests: The authors declare no competing interests.

Supplementary information

Sup. Table 1. Association between plasma MMPs expression and days to culture conversion in the DS-TB cohort (n=70).

MMP	Univariable			Multivariable		
	HR	95% CI	p-value	Ahr	95% CI	p-value
MMP1	0.78	0.31, 1.93	0.589	1.00	0.38, 2.61	0.994
MMP2	2.39	0.34, 16.7	0.381	0.87	0.07, 10.2	0.914
MMP7	0.48	0.10, 2.17	0.338	0.76	0.15, 3.90	0.738
MMP9	1.46	0.62, 3.45	0.390	1.56	0.56, 4.35	0.395
MMP10	0.78	0.31, 1.93	0.589	0.82	0.25, 2.69	0.747

Sup. Table 2. Association between plasma MMPs expression and days to culture conversion in people living with HIV in the DS-TB cohort (n=51).

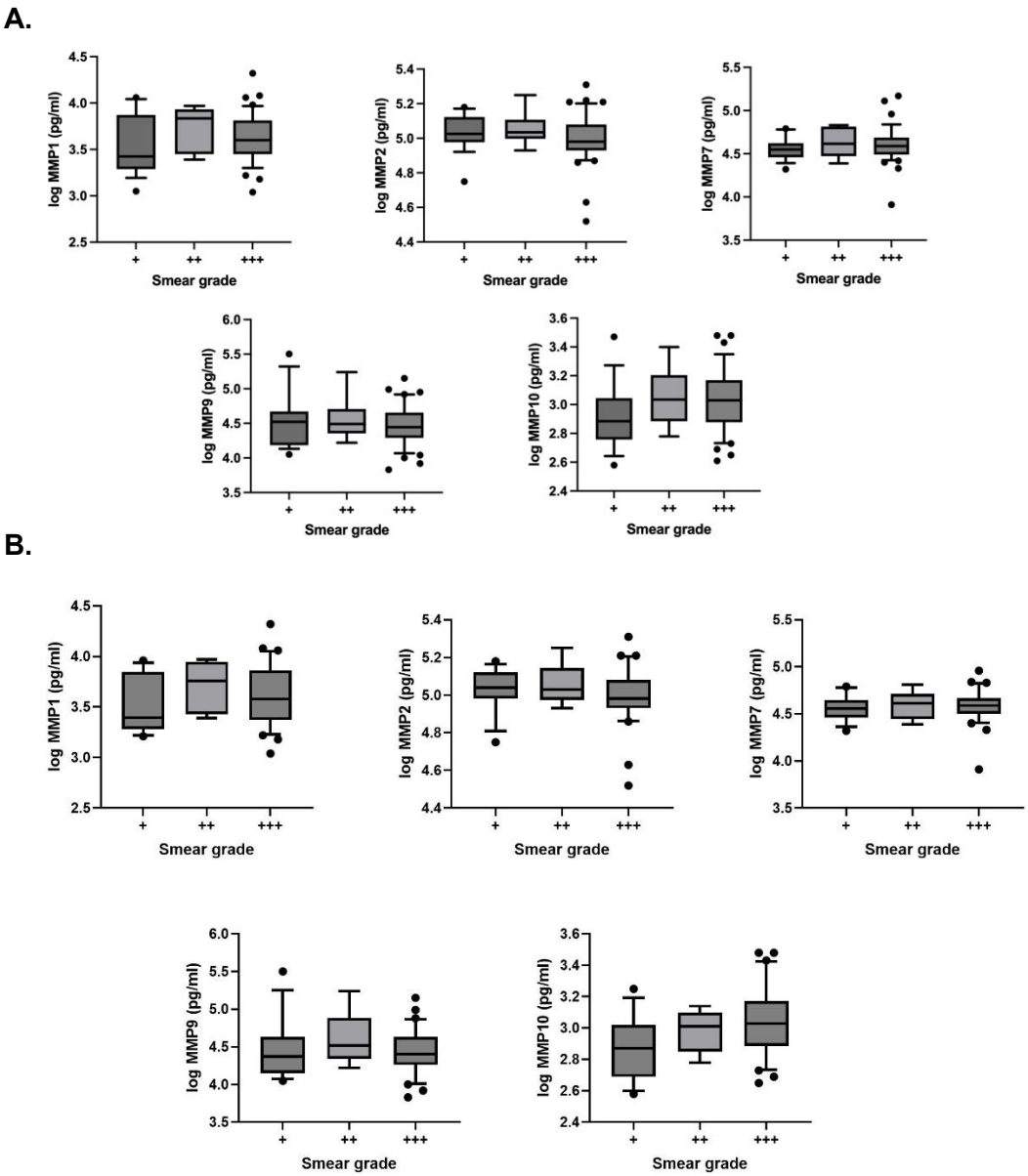
MMP	Univariable			Multivariable		
	HR	95% CI	p-value	aHR	95% CI	p-value
MMP1	1.00	0.32, 3.11	0.999	1.0	0.31, 3.21	0.993
MMP2	2.88	0.39, 21.5	0.302	3.62	0.39, 34.0	0.261
MMP7	0.39	0.07, 2.08	0.270	0.34	0.06, 1.91	0.219
MMP9	1.64	0.54, 4.97	0.385	1.55	0.50, 4.83	0.446
MMP10	1.40	0.32, 6.12	0.655	1.52	0.34, 6.89	0.587

Sup. Table 3. Association between plasma MMPs expression and days to culture conversion in the DR-TB cohort with (n=125).

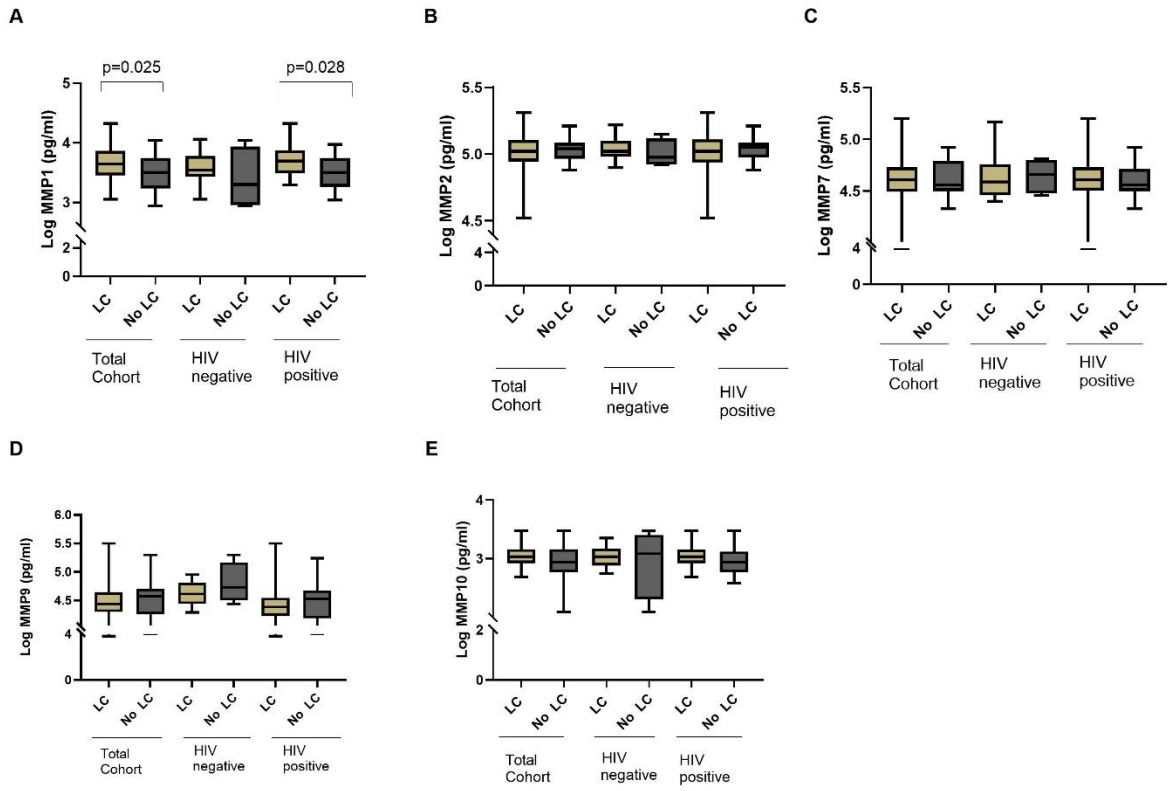
MMP	Univariable			Multivariable		
	HR	95% CI	p-value	aHR	95% CI	p-value
MMP1	0.65	0.36, 1.17	0.151	0.61	0.34, 1.12	0.114
MMP2	2.42	0.42, 13.9	0.323	2.16	0.37, 12.5	0.390
MMP7	0.84	0.26, 2.71	0.767	0.77	0.22, 2.70	0.685
MMP9	0.87	0.50, 1.50	0.607	0.83	0.45, 1.50	0.530
MMP10	1.58	0.61, 4.11	0.348	1.46	0.53, 4.03	0.464

Sup. Table 4. Association between plasma MMPs expression and days to culture conversion in people living with HIV in the DR-TB cohort (n=90).

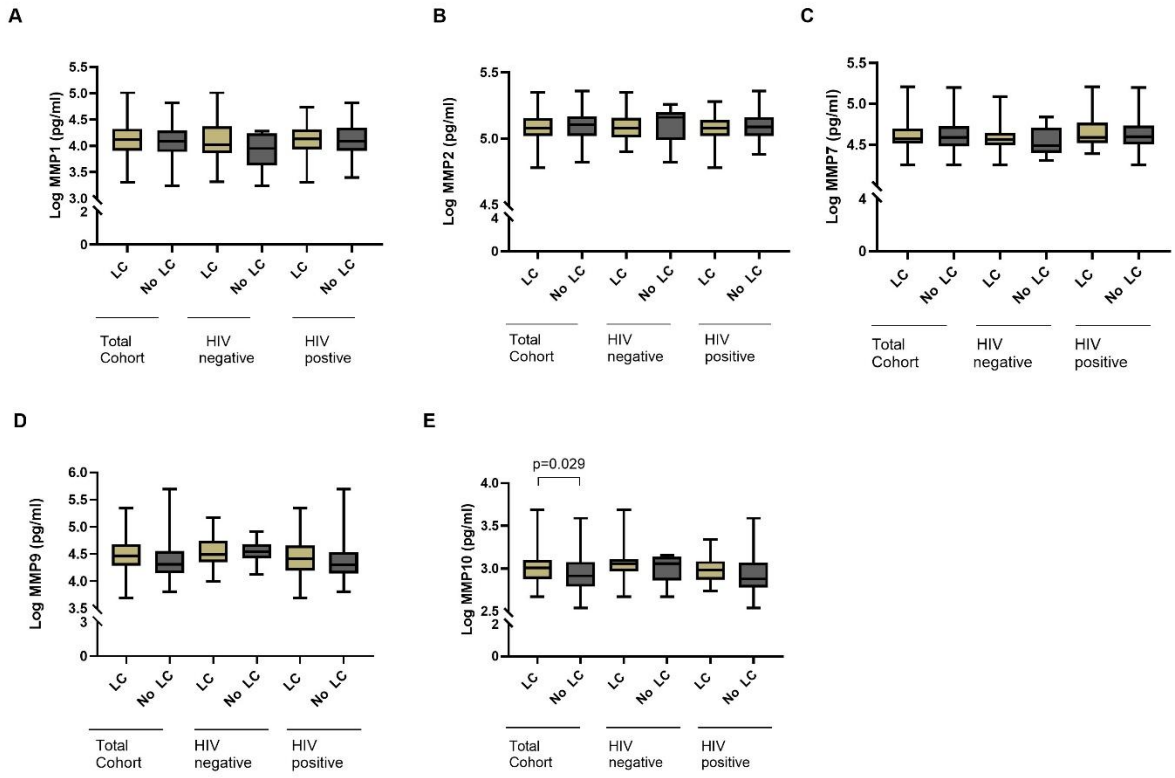
MMP	Univariable			Multivariable		
	HR	95% CI	p-value	aHR	95% CI	p-value
MMP1	0.86	0.40, 1.84	0.703	0.85	0.40, 1.83	0.680
MMP2	3.51	0.44, 28.1	0.236	3.69	0.45, 30.4	0.226
MMP7	1.62	0.44, 5.92	0.469	1.59	0.43, 5.85	0.489
MMP9	0.90	0.48, 1.70	0.747	0.91	0.48, 1.72	0.771
MMP10	1.39	0.45, 4.24	0.566	1.32	0.41, 4.25	0.639



Sup. Figure 1. The relationship between log MMP expression and bacterial burden in DS-TB cohort measured by smear grades: (**A)** the total cohort (Smear +1, n= 18; smear 2+, n= 6; smear 3+, n= 42), (**B) PLWH** subgroup (smear 1+, n= 12; smear 2+, n= 5; smear 3+ n= 30). Kruskal-Wallis test with Dunn's multiple comparisons test was used to analyze differences between groups. MMPs were plotted on a log scale, with box and whisker plots (10-90%).



Sup. Figure 2. Plasma levels of MMPs in DS-TB patients with and without lung cavities, stratified by HIV status. Box and whisker plots (10-90%) show the log-transformed plasma concentrations of (a) MMP-1, (b) MMP-2, (c) MMP-7, (d) MMP-9, and (e) MMP-10 in individuals with lung cavities (LC) and without lung cavities (No LC), analyzed across the total cohort, HIV-negative subgroup, and PLWH subgroup. Indicated p-values are the result of a univariable logistic regression analysis (Table 3 and Table 4).



Sup. Figure 3. Plasma levels of MMPs in DR-TB patients with and without lung cavities, stratified by HIV status. Box and whisker plots (10-90%) show the log-transformed plasma concentrations of (a) MMP-1, (b) MMP-2, (c) MMP-7, (d) MMP-9, and (e) MMP-10 in individuals with lung cavities (LC) and without lung cavities (No LC), across the total cohort, people without HIV , and the PLWH. . Indicated p-values are the result of a univariable logistic regression analysis (Table 5 and Table 6).

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CHAPTER 4:
DISCUSION

CHAPTER 4

4. DISCUSSION

4.1 Summary of Key Findings

This study investigated the role of circulating MMPs in pulmonary TB, with a particular focus on differences between DS-TB and DR-TB, and on associations with lung cavitation, bacterial burden, treatment response, and host inflammatory responses. Given the high prevalence of HIV co-infection within both study cohorts, analyses were additionally stratified by HIV status to explore its effects on MMP expression and associations with disease outcomes.

Both cohorts had a high burden of HIV co-infection (>70%), reflecting the epidemiological situation of TB in KwaZulu-Natal, South Africa. Lung cavitation was common across both cohorts, affecting approximately two-thirds of DS-TB participants and nearly half of DR-TB participants with available radiographic data. These cohorts, therefore, provided an appropriate clinical context in which to explore host mediators of lung pathology and inflammation in both DS-TB and DR-TB.

Association between MMPs and lung cavitation

In the DS-TB cohort, higher plasma MMP-1 levels were significantly associated with the presence of lung cavitation in univariable analyses. This association was observed in the overall DS-TB cohort and was stronger among individuals living with HIV. Although the association was attenuated after adjustment for key clinical covariates, including age, sex, BMI, HIV status, smoking, alcohol use, and ART use, the consistent trend suggests a relationship between circulating MMP-1 levels and cavitary lung disease in DS-TB. Similarly, previous studies have reported that elevated MMP-1 levels are associated with active pulmonary TB and the presence of lung cavitation(Kathamuthu *et al.*, 2020, Kumar *et al.*, 2018, Kübler *et al.*, 2015). MMP-1 has been identified as an important enzyme contributing to the destruction of the lung in human diseases such as TB (Imai *et al.*, 2001). It significantly contributes to lung cavitation by directly degrading essential ECM components, including fibrillar collagens, through its potent collagenolytic activity(Elkington *et al.*, 2011). This dysregulated proteolytic activity, often resulting from an imbalance with tissue inhibitors, drives the breakdown of the collagen matrix and other structural proteins(Tiwari and Martineau, 2023).

In contrast, MMP-1 was not associated with lung cavitation in the DR-TB cohort, despite higher overall MMP-1 levels in DR-TB compared to DS-TB. Instead, MMP-10 emerged as the only MMP associated with lung cavitation in DR-TB in univariable analyses, although this association did not persist after multivariable adjustment. No significant associations were identified between MMPs and cavitation among PLWH individuals with DR-TB, although a trend for MMP-10 was observed. Together, these findings suggest that the pathways contributing to lung cavitation may differ between DS-TB and DR-TB and may involve distinct MMPs or alternative immune mechanisms.

MMPs and bacterial burden

To assess whether circulating MMPs reflect bacterial burden, plasma MMP levels were compared across sputum smear grades in the DS-TB cohort. No significant associations were observed between MMP-1, MMP-2, MMP-7, MMP-9, or MMP-10 and baseline smear grade in either the total cohort or the PLWH subgroup. These findings indicate that systemic MMP expression is not directly associated with mycobacterial burden as assessed by smear microscopy and may instead reflect host-driven inflammatory and tissue remodeling processes rather than bacterial load. This contrasts with some previous reports linking MMP expression to mycobacterial burden: Kathamuthu *et al.* (2020) reported that plasma MMP and TIMP concentrations varied with bacterial burden and disease site in pulmonary and extrapulmonary TB, Kumar *et al.* (2018) found that elevated MMP levels reflected the severity and extent of disease in TB-diabetes comorbidity and declined following successful anti-tuberculosis treatment, and Walker *et al*. (2022) reported that elevated plasma MMP-8 was associated with sputum culture positivity in pulmonary TB(Kathamuthu *et al.*, 2020, Kumar *et al.*, 2018, Walker *et al.*, 2022) . However, other studies report more compartment-specific findings that align more closely with our results: Ugarte-Gil *et al*. (2013) found that induced sputum, rather than plasma, concentrations of MMP-1, -3, and -8 were associated with delayed early culture conversion, suggesting that local airway MMP activity may track more closely with bacterial burden and clearance than circulating plasma levels (Ugarte-Gil *et al.*, 2013). Together, these comparisons suggest that the relationship between MMPs and mycobacterial burden may be compartment- and MMP-specific, with sputum- or tissue-based measurements potentially offering a more sensitive readout of local host-pathogen interactions than plasma sampled systemically. Smear grading is also a relatively insensitive, semi-quantitative measure of

bacillary load, which may have further limited our ability to detect an association even if one exists at the tissue level. Future studies incorporating quantitative measures of bacterial burden (e.g., time-to-positivity or sputum bacillary load) alongside paired sputum and plasma MMP measurements would help clarify whether circulating MMPs reflect local or systemic disease processes.

MMPs and treatment response

Plasma MMP levels at baseline were also evaluated as potential predictors of treatment response, measured by time to culture conversion. Across both DS-TB and DR-TB cohorts, no MMPs were associated with time to culture negativity in either univariable or multivariable Cox regression models. This lack of association was consistent in analyses stratified by HIV status. These results suggest that baseline circulating MMPs do not predict microbiological treatment response, reinforcing the interpretation that MMPs are more reflective of immunopathology and tissue remodeling than of bacterial clearance dynamics. Similar findings have been reported in some studies, although others have shown associations between MMP expression and culture positivity. For instance, *Ugarte-Gil et al*. (2013) demonstrated that elevated sputum levels of MMP-1, -2, -8, and -9 at diagnosis were associated with delayed early culture conversion (2 weeks) in TB patients(Ugarte-Gil *et al.*, 2013). In a later study, plasma MMP-8 concentrations were higher in culture-positive TB patients both at baseline and at six months of treatment(Walker *et al.*, 2025, Walker *et al.*, 2022).

Relationships between MMPs and inflammatory mediators

In both DS-TB and DR-TB cohorts, MMPs showed extensive and differential correlations with inflammatory mediators, highlighting their roles within broader immune networks.

MMP-1 demonstrated the strongest and most consistent positive correlations with pro-inflammatory cytokines and chemokines, including IL-6, IL-8, IL-1β, IL-1Rα, G-CSF, and PDGF-bb. These correlations were observed in both DS-TB and DR-TB and were largely preserved among PLWH participants. This pattern supports the role of MMP-1 as a marker of inflammatory activation and tissue-destructive immune responses during active TB.

In contrast, other MMPs showed more heterogeneous and sometimes inverse relationships with inflammatory mediators. MMP-2 frequently demonstrated negative correlations with inflammatory cytokines and chemokines, particularly in DR-TB, suggesting a potentially regulatory or counter-inflammatory role. MMP-7 and MMP-10 exhibited mixed correlation patterns, with associations varying by TB drug-resistance status and HIV infection. These findings underscore that individual MMPs are embedded in distinct immunological pathways rather than functioning as a uniform group.

Differences in MMP expression between DS-TB and DR-TB

Multivariable linear regression analyses revealed significant differences in circulating MMP expression between DS-TB and DR-TB. After adjustment for demographic and clinical variables, DR-TB was independently associated with higher MMP-1 levels and lower MMP-7 and MMP-10 levels compared to DS-TB, while no differences were observed for MMP-2 or MMP-9. These findings indicate that TB drug-resistance status is associated with distinct systemic MMP expression profiles, potentially reflecting differences in immune activation, or host-pathogen interactions in DR-TB.

Overall, the findings of this study demonstrate that circulating MMPs are differentially associated with lung pathology, inflammatory responses, and TB drug-resistance status. MMP-1 appears to be most closely linked to inflammatory lung damage in DS-TB, particularly among people living with HIV, while MMP-10 may play a role in DR-TB-associated cavitation. However, circulating MMPs were not associated with bacterial burden or treatment response, emphasizing their role as markers of immunopathology rather than microbial clearance. These results highlight the complexity of MMP biology in TB and support further investigation into MMP-mediated pathways as potential targets for host-directed therapies aimed at limiting lung damage and long-term pulmonary sequelae.

4.2 Study limitations

Our study has several limitations that should be acknowledged. First, MMPs were measured only at baseline, preventing us from assessing their dynamic changes during treatment.

Longitudinal measurements may have provided more insight into how MMP expression evolves alongside inflammation, tissue repair, and microbiological clearance. Second, the relatively small sample sizes in stratified analyses, especially in HIV-negative and non-cavitary subgroups, may have limited statistical power. Third, plasma measurements may not fully capture local pulmonary MMP activity, which could differ from systemic expression. MMP production is often much higher within lung tissue and granulomas than in systemic circulation, and plasma measurements may therefore underestimate the intensity of matrix degradation occurring within the lung. Finally, although we adjusted for key confounders, residual confounding, particularly due to ART status and HIV disease progression, may have influenced some associations.

4.3 Future directions

Future research should prioritise longitudinal studies that track MMP levels throughout the course of TB treatment. Such studies could clarify whether reductions in MMPs accompany clinical improvement, cavity closure, or decreased inflammation. Tissue-based approaches, including bronchoalveolar lavage or lung biopsy studies, may also be valuable for more accurately characterising the local immune environment and determining how MMPs contribute to cavity formation within the lung. In addition, validating these findings in larger, multi-centre cohorts will help improve generalisability across diverse patient populations and varying HIV and drug-resistance profiles. Investigating the therapeutic potential of targeting MMPs, particularly MMP-1 and MMP-10, may provide new strategies for reducing immunopathology and preventing long-term lung damage. Further research into how MMPs interact with regulatory pathways, such as TIMPs, may also help distinguish harmful from protective inflammatory responses and identify new intervention points for reducing tissue destruction.

4.4 Conclusion

In summary, this study highlights the significant roles of MMP-1 and MMP-10 in TB-associated immunopathology, particularly in the context of HIV co-infection. MMP-1 was strongly associated with lung cavitation in DS-TB and correlated with pro-inflammatory cytokines, reinforcing its importance in extracellular matrix degradation and tissue destruction.

Although MMP-10 showed a weaker association with cavitation in DR-TB, its involvement in MTB-driven collagenolysis suggests it may also contribute to pathological processes in severe disease. Despite their associations with disease severity, baseline MMP levels did not predict time to culture conversion, indicating that MMPs reflect immunopathology rather than bacterial clearance. Future work incorporating longitudinal sampling, larger cohorts, and tissue-based analyses is needed to validate these findings and further elucidate the mechanistic roles of MMPs in TB pathogenesis.

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Appendix A: UKZN BREC Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

10 October 2024

Miss Azande Sthoko Gazu (219013287)
School of Laboratory Medicine & Medical Science
Medical School

Dear Miss Gazu,

Protocol reference number: BREC/00007522/2024
Project title: Role of matrix metalloproteinases (MMPs) in drug-resistant tuberculosis (DR-TB) and drug-susceptible tuberculosis
Degree: MMedSci

EXPEDITED APPLICATION
APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 10 October 2024. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 10 October 2024. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2024), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 November 2024.

Yours sincerely,

[redacted]
Prof S Singh
Chair: Biomedical Research Ethics Committee

Appendix B: Oral Presentations

During my degree, I had the opportunity to present my research in the form of an oral presentation at a national conference:

1. **Azande Gazu**, Thando Maseko, Lateef Amusa, Lara Lewis, Surie Chinappa, Dhineshree Govender, Kogieleum Naidoo, Rubeshan Perumal, Aida Sivro. **Role of Matrix metalloproteinases in TB pathogenesis.** Track 3: **HIV Coinfection and Comorbidities.**

12th SA AIDS Conference, Johannesburg, 08 - 11 September 2025

Photos (Left to Right): Thando Maseko, Sanele Mbeje. Top right: Lisanthini Naidu. Bottom right: Thando and Azandu Gazu with other delegates.

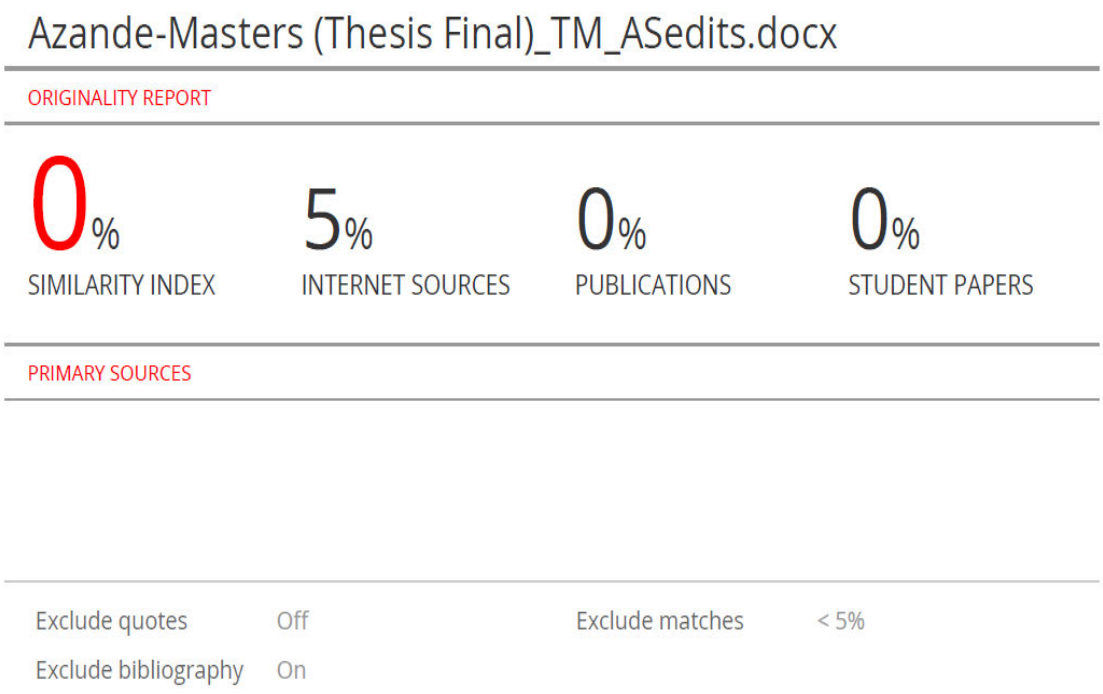


Appendix C: Community outreach

The CAPRISA research fellows, including myself, visited Asibemunye High School and Ngcedomhlophe High School (July 2024) in the Mafakatini area of uMgungundlovu Municipality, as well as Makhumbuza High School in Umlazi and Vulindlela Secondary School (August 2025). Here, we informed and raised awareness among the learners about HIV, TB, STIs, the risks associated with early sexual debut, and teenage pregnancy. Additionally, we answered questions and briefed the learners about our respective projects.



Appendix D: Turnitin Report



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