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Inflammation and cellular immune phenotypes in TB/HIV co-infection

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DECLARATION

The experimental work described in this dissertation was carried out at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), Nelson Mandela R University of KwaZulu-Natal, Durban, from April 2020 to April 2023, under the supervision of Dr. Aida Sivro and co-supervision of Professor Derseree Archary. The studies represent original work by the author and have not otherwise been submitted in any other form to another University. Where use has been made of the work of others, it has been duly acknowledged in the text.

Thando Glory Maseko (candidate)

25 April 2023

As the candidate's supervisor, I agree to the submission of this dissertation.

Dr. Aida Sivro

Prof. Derseree Archary

DECLARATION – PLAGIARISM

I, Thando Glory Maseko, declare that:

i. The research reported in this dissertation, except where otherwise indicated, is my original work.

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DECLARATION – MANUSCRIPTS

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CONFERENCE PRESENTATIONS

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1. **Thando Glory Maseko**, Santhuri Rambaran, Slindile Ngubane, Derseree Archary, Lara Lewis, Dhineshree Govender, Natasha Samsunder, Nesri Padayatchi, Kogieleum Naidoo and Aida Sivro. **Changes in natural killer cell phenotypes in TB/HIV co-infection.** 7th SA TB Conference 2022. 13 – 16 September 2022. Track 1: Pathogenesis of TB – pathogen and host. Awarded: **Best oral abstract award** for Track 1.

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DEDICATION

I would like to dedicate this thesis to my beloved mommy, **Patience Maseko**. Words can never be enough to express my heartfelt gratitude and appreciation to you. Thank you for your love, unwavering support, and constant encouragement. You have motivated me to reach greater heights in my academics. I appreciate your endless sacrifices to help me get to where I am today. I would also like to dedicate this thesis to my late great grandmother, Betty Thwala who forever lives in my heart.

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"I can do all things through Christ who strengthens me".
Philippians 4:13

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ETHICS APPROVAL

All study participants from the CAPRISA 011 Improving Retreatment Success Trial (BFC029/13) and the CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (BFC584/16) provided written consent for the use of stored biological specimens for future research.

This sub-study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (UKZN BREC) (BREC/00002197/2020) (Appendix C).

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LIST OF ABBREVIATIONS

ACD	Acid citrate dextrose
ADCC	Antibody-dependent cellular cytotoxicity
AGYW	Adolescent girls and young women
AIDS	Acquired immunodeficiency virus
AHI	Acute HIV infection
ART	Antiretroviral therapy
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette–Guérin
BMI	Body mass index
bNAb	Broadly neutralising antibody
BREC	Bioethics Research Committee
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CFU	Colony forming units
ELISA	Enzyme-Linked Immunosorbent Assay
Eotaxin	Eosinophil chemotactic protein
ESTAT-6	Early Secreted Antigenic Target 6 kDa
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
FGF-basic	Fibroblast growth factor basic
FMO	Fluorescent minus one
G-CSF	Granulocyte colony-stimulating factor
GALT	Gut-associated lymphoid tissue
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICAM	Intercellular adhesion molecule
IFN	Interferon
IL	Interleukin
Ig	Immunoglobulin
IMPRESS	Improving Retreatment Success
InDEX	Individualized M(X)drug-resistant TB Treatment Strategy Study
INSTI	Integrase strand transfer inhibitors
IP-10	Interferon γ–Inducible protein-10

IPT	Isoniazid preventive therapy
KIR	Killer-cell immunoglobulin-like receptor
LAM	Lipoarabinomannan
LBP	Lipopolysaccharide binding protein
LTBI	Latent TB infection
LC	Langerhans cells
LM	Lipomannans
LOD	Limit of detection
LPS	Lipopolysaccharide
MCP	Monocyte chemotactic protein
MDR-TB	Multidrug-resistant TB
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
MIG	Monokine induced by gamma interferon
Mtb	*Mycobacterium tuberculosis*
NCR	Natural cytotoxicity receptor
NHP	Non-human primate
NK cells	Natural killer cells
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside/nucleotide reverse transcriptase inhibitors
OR	Odds ratio
PAMP	Pathogen associated molecular pattern
PBMC	Peripheral blood mononuclear cell
pDC	Plasmacytoid DCs
PEP	Post-exposure prophylaxis
PGDF	Platelet-derived growth factor
PI	Protease inhibitors
PIM	Phosphatidylinositol mannoside
PLHIV	People living with HIV
PPD	Purified protein derivative
PrEP	Pre-exposure prophylaxis
PRR	Pattern-recognition receptors
RR	Risk ratio
RT	Room temperature
SA	South Africa
STI	Sexually transmitted infections

TB	Tuberculosis
TB-IRIS	TB-Immune Reconstitution Inflammatory Syndrome
TGF	Transforming growth factor
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
KZN	KwaZulu-Natal
UKZN	University of KwaZulu-Natal
UNAIDS	Joint United Nations Programme on HIV/AIDS
VEGF	Vascular endothelial growth factor
VLS	Viral load setpoint
WHO	World Health Organization
XDR-TB	Extensively drug resistant TB

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THESIS CONTEXT

This thesis contains six chapters which contain the following:

Chapter 1 provides a background of the epidemiology of TB, HIV and TB/HIV co-infection. The process of infection and subsequent innate and adaptive host immune responses to TB, HIV and TB/HIV co-infection are described. Additionally, chapter 1 provides a brief description of the study rationale, aims, and objectives.

Chapter 2 provides a brief overview of the study cohorts and methodology employed.

Chapter 3 describes natural killer (NK) cell phenotype in peripheral blood samples from the Improving Retreatment Success (IMPRESS) study participants during active TB and post TB treatment completion. The associations between NK phenotypes at active TB and time to culture conversion and cavitary disease was characterised in participants with TB and TB/HIV co-infection. Additionally, we assessed the effect of TB treatment completion on changes in NK cell phenotype. Briefly, we show that HIV infection skews NK cell subsets evidenced by higher percentages of functionally impaired $CD56^{neg}$ NK subset in TB/HIV co-infected participants. TB treatment completion resulted in redistribution of NK cell subsets and downregulation of several NK activating and inhibitory cell surface receptors. We identified several activating receptors (including NKG2D and NKp46) that were associated with time to culture conversion and presence of lung cavitation suggesting that NK cell responses play a key role in the pathogenesis of TB and TB/HIV co-infection. This work resulted in an original research article published by *Scientific Reports*.

Chapter 4 describes helper T cell subsets (Th1, Th2, Th9, Th17, Th17.1, $CCR6^{+}$DP and $CCR6^{+}$DN) and $\alpha_4\beta_7$, and $\alpha_4\beta_1$ integrin expression on memory $CD4^{+}$ T cells in TB and TB/HIV co-infected individuals from the IMPRESS cohort. We characterised changes in memory $CD4^{+}$ T cell subsets following TB treatment completion, as well as their effect on time to culture conversion and presence of cavitary disease. We observed higher percentages of Th2 cells and lower percentages of Th9 cells in TB/HIV co-infected participants compared to healthy controls. Percentages of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ expressing memory $CD4^{+}$ T cells were higher among TB/HIV co-infected participants compared to healthy controls suggesting that HIV effects tissue distribution of T helper cell subsets during active TB. This study identifies additional ways in which HIV induced changes in memory $CD4^{+}$ T cell phenotypes can affect Mtb control and disease pathology among TB/HIV co-infected participants. This work resulted in an original research article currently undergoing internal Centre for the AIDS Programme of Research in South Africa (CAPRISA) Scientific Committee review.

Chapter 5 investigates the effects of plasma cytokines/chemokines measured during active MDR/XDR TB on presence of cavitary disease. We identified increased IL-6 plasma levels as a biomarker of cavitary disease in multidrug-resistant/ extensively drug-resistant TB. This work resulted in an original research article published by *BMC Immunology*.

Chapter 6 discusses the major findings of this study and describes the study significance, limitations and future directions and recommendations.

ABSTRACT

Background: South Africa has the highest burdens of TB and HIV. HIV induced inflammatory and immune changes are known to increase the risk of TB recurrence and lead to poor disease outcome in co-infected patients. Lack of understanding of the protective and pathogenic host immune responses during TB and TB/HIV co-infection are hampering the development of novel diagnostic, preventative, and therapeutic modalities. Here we characterised soluble inflammatory, NK and $CD4^+$ T cell profiles in TB and TB/HIV disease.

Methods: NK and memory $CD4^+$ T helper cell phenotypes during active TB and post TB treatment completion in HIV positive and negative CAPRISA 011 Improving Retreatment Success study participants were characterised by flow cytometry. NK cell phenotypes were characterised using cell surface activating and inhibitory receptors (NKG2D, NKG2C, NKG2A, NKp30, NKp44, NKp46 CD158, and NKB1) while memory $CD4^+$ T cells were classified as T helper (Th) 1, Th2, Th17, Th17.1, $CCR6^+$DN, and $CCR6^+$DP based on the variable expression of CCR6, CCR4, CXCR3, and CCR5. Additionally, we also characterised $\alpha_4\beta_7$, $\alpha_4\beta_7^{hi}$ and $\alpha_4\beta_1$ integrin expressing memory $CD4^+$ T cells. Cox regression models were used to assess the association between NK and memory $CD4^+$ T cell phenotypes at active TB and time to culture conversion. Linear mixed models were used to assess the association between NK and memory $CD4^+$ T cell phenotypes at active TB and cavitary disease. We additionally characterised plasma cytokine/chemokine markers of cavitary disease in drug-resistant TB patients from the CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study using the 27-Plex Human Cytokine Immunoassay.

Results: We observed an expansion of functionally impaired $CD56^{neg}$ NK cells in the TB/HIV participants compared to TB (p=0.0176) and healthy control (p=0.0148) group. Consequently, the increase in percentage (%) of $CD56^{neg}$ NK cells was associated with shorter time to culture conversion in our study. TB treatment completion led to increase in % of total NK cells, a decrease in the $CD56^{bright}$ NK cell subset, and decreases in several activating and inhibitory NK cell receptors. Increased % of $CD56^{bright}$ NK cells was associated with longer time to culture conversion while increased expression of activating receptor NKG2D on $CD56^{dim}$ cells was associated with shorter time to culture conversion. Increased expression of NKp30 on $CD56^{bright}$ NK cells was associated with increased odds of cavitary disease while the increase in NKp46 on cytotoxic $CD56^{dim}$ cells was associated with reduced odds of cavitary disease in the overall CAPRISA 011 cohort. With regards to memory $CD4^+$ T cell responses, we observed higher percentages of Th2 cells, lower % of Th9 cells, and higher % of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin expressing memory $CD4^+$ T cells in TB/HIV co-infected participants compared to healthy controls. Increased IL-6 expression during active MDR/XDR TB was associated with higher risk of lung cavitation in CAPRISA 020 participants. Additionally smoking and previous history of TB were

associated with increased risk of cavitary disease while HIV and higher BMI were associated with reduced risk of cavitation during MDR/XDR TB.

Conclusions: We identified distinct changes in systemic inflammatory and NK cell and memory $CD4^+$ T cell populations with respect to active disease, treatment completion, bacterial clearance and disease severity in TB and TB-HIV co-infected individuals. The results of this study highlight biologically plausible and novel mechanisms by which concurrent HIV infection impairs the host immune control of Mtb infection.

CHAPTER 1

1. Introduction

1.1 Tuberculosis epidemiology

Tuberculosis (TB) is a communicable disease caused by the intracellular pathogen *Mycobacterium tuberculosis* (Mtb), which infected ~10 million people globally and caused ~1.6 million deaths as reported by the World Health Organization (WHO) in 2021 [1] (Figure 1). Primary Mtb infection occurs in the lungs and results in pulmonary TB while 15% of cases may clinically present a disseminated form of TB, called extrapulmonary TB [2,3]. In South Africa, a high TB burden country, 328,000 cases of TB and 25, 000 mortalities among HIV-negative individuals were reported in 2020 [1]. First line anti-TB drugs include isoniazid, rifampicin, pyrazinamide and ethambutol [4]. However, the acquisition of resistance to anti-TB drugs and transmission of drug-resistant (DR) Mtb strains poses a challenge for TB treatment programmes [5]. Multidrug-resistant (MDR) TB refers to infection with rifampicin and isoniazid-resistant Mtb strains, while extremely drug-resistant (XDR) TB refers to a type of MDR-TB further resistant to any fluoroquinolone and second-line injectable agents [6]. Globally, in 2019, the WHO reported 56% and 39% successful treatment rates among MDR-TB and XDR patients respectively [7]. Additionally, high mortality rates are reported for drug-resistant patients [8]. There were ~450,000 incident cases of MDR/ rifampicin resistant (RR)-TB and 191,000 mortalities reported globally in 2021 [9]. In South Africa, 6,784 cases of MDR-TB and 733 cases of XDR-TB were reported in 2020 [1]. In 2014, among South African XDR-TB patients receiving treatment, a mortality rate of 47% was reported; with these high mortality rates associated with HIV co-infection [10].

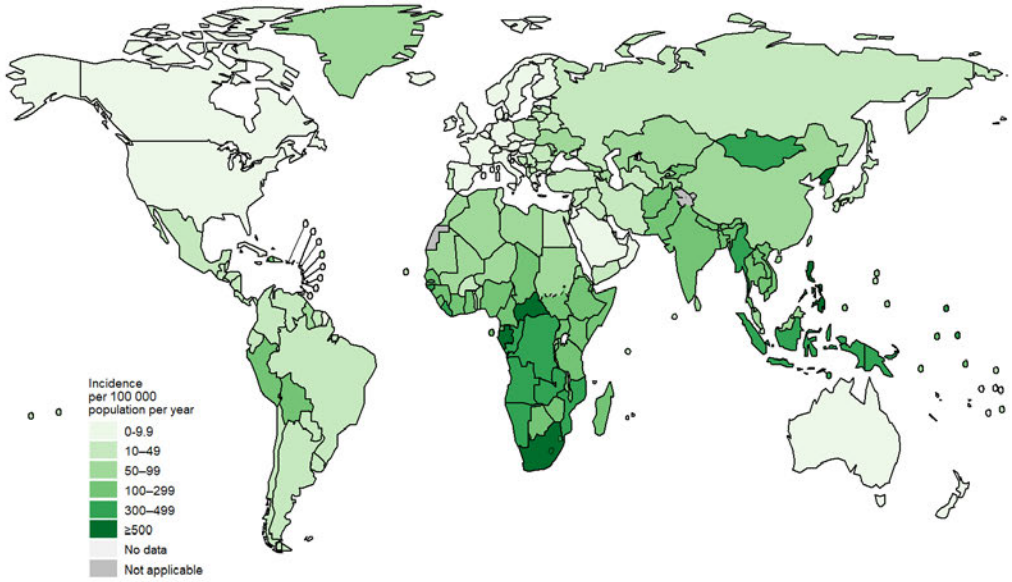


Figure 1. Global incident rates of TB in 2021. Approximately 10.6 million cases of new or reactivated TB were reported in 2021 with Africa accounting for 23% of these infections. An incident TB rate of 500 per 100, 000 population per year was reported in Central African Republic, Gabon, Lesotho, Philippines, and South Africa. Approximately 6.7% of total incident TB cases reported in 2021 globally were individuals co-infected with HIV. Adapted from the WHO, Global TB report 2022 [9].

1.2 HIV/AIDS epidemiology

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that 38.4 million [33.9 million-43.8 million] people were living with human immunodeficiency virus (HIV) in 2021 [11] with the eastern and southern African region accounting for 53.6% (20.6 million) of these infections [12]. Additionally, 680, 000 HIV/AIDS-related mortalities were reported in 2020 across the globe [1]. In South Africa , ~7.8 million individuals were living with HIV with 73% receiving antiretroviral therapy (ART) in 2020 [1]. In South Africa, a high TB and HIV burden country, ~7.5 million people were infected with HIV in 2018; with KwaZulu-Natal accounting for a staggering 41.1% of those infections [13,14]. Approximately 60% of South African adults acquire HIV infection by 25 years of age [15]. Adolescent girls and young women (AGYW) between 15–24 years are disproportionately affected by HIV and accounted for 26.5 % of all incident HIV infections in SA in 2020 [16]. HIV prevalence among AGYW in KwaZulu-Natal (KZN) was 1.76-fold greater than the national estimate in 2008 [16,17]. Poverty, financial and food insecurity, low education levels, intimate partner violence, substance, and sexual abuse are among some of the structural and psychosocial factors that contribute to increased risk of HIV among AGYW [18,19].

A study conducted on HIV risk and transmission networks among individuals aged 15-49 years in KZN reported that high HIV incidence rates among AGYW were attributed to sexual partnering between AGYW and males between 25–40 years who had contracted HIV from similar aged females [20]. A cross-sectional study on AGYW between 12-24 years reported inconsistent condom use among 79.4% of respondents participating in risky sexual behaviours, 56.1% had multiple sexual partners and 8.7% had self-reported STIs in the previous year [21]. Additional factors that make AGYW susceptible to HIV include transactional sex, condomless sex, early sexual debut and multiple concurrent sexual partners [21,22] with HIV risk increased during pregnancy [23]. The use of vaginal products by AGYW for the enhancement of sexual pleasure leads to genital inflammation and genital mucosal abrasions which increases HIV risk [24,25]. Intravaginal practices such as douching increases risk of pelvic inflammatory disease, bacterial vaginosis, sexually transmitted infection (STI), and HIV and impact the vaginal microbiome by reducing colonisation of *Lactobacilli* species [26,27]. Furthermore, vaginal microbiome was shown to affect the efficacy of topical tenofovir microbicide gels [28] with the presence of genital inflammation attenuating the protective efficacy of the gel [29]. High HIV incidence rates among AGYW in South Africa persist regardless of available HIV prevention measures in South Africa including male and female condoms, and pre- and post- exposure prophylaxis (PrEP and PEP) [15] suggesting a need for effective HIV prevention strategies specifically tailored for adolescent populations [30].

1.3 TB/HIV co-infection epidemiology

It is estimated that Mtb infects a third of the global population while less than 10% of those infected will likely develop active TB [31,32]. TB is the leading cause of death among people living with HIV (PLHIV), who are 18 times more likely to develop active TB [1] compared to HIV negative individuals. The WHO reported 187,000 HIV-associated TB mortalities globally in 2021 [9], 251, 000 in 2020 [1] while ~36, 000 were reported in South Africa in 2019 [33]. This figure grossly underestimates TB in South Africa as some cases of TB among PLHIV may not be diagnosed or notified. South Africa has been listed as one of the ten countries reported with a gap between the number of notified new and recurrent TB cases and best estimates of TB incidence [9]. Mtb and HIV-1 are synergistic infections that together accelerate immune dysfunction [32,34]. Infection with HIV-1 is not only a predisposing factor for TB but is known to exacerbate the infection while TB is reported to be one of the common causes of death among HIV/AIDS patients [35,36]. Co-infection with TB and HIV is associated with poor TB treatment outcomes [8] with PLHIV having a higher risk of drug-resistant TB in comparison to HIV-negative individuals [37,38].

Factors associated with increased drug-resistant outcome among TB/HIV co-infected individuals include malabsorption of TB treatment, poor treatment adherence or direct infection through exposure to drug-resistant TB cases [38].

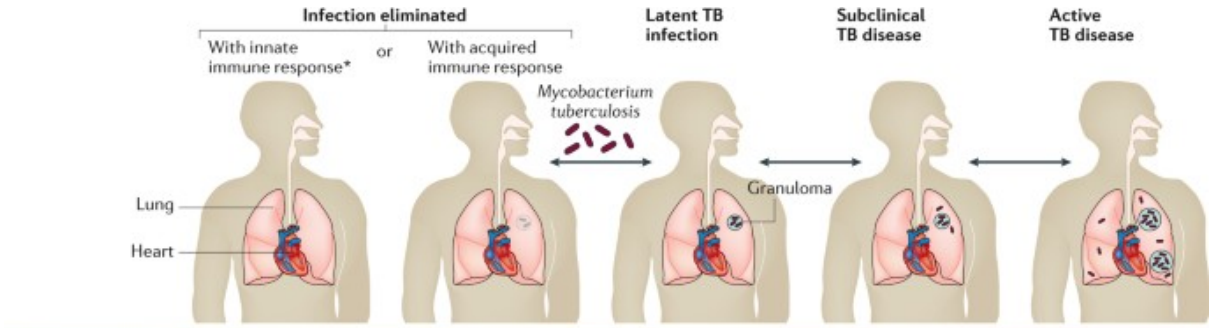
The use of highly active antiretroviral therapy (HAART) reduces the incidence of HIV-associated TB in areas that have high burdens for each disease [39]. A study investigating the timing of ART initiation during TB therapy reported a 56% decrease in mortality among sputum smear-positive patients following ART initiation and concurrent TB treatment at $CD4^+$ counts up to 500 cells/µl [40]. A major complication associated with the use of HAART or combination therapy in TB/HIV co-infected patients is TB-immune reconstitution inflammatory syndrome (TB-IRIS) experienced by 20-30% of TB/HIV co-infected patients [32]. TB-IRIS is secondary to exaggerated anti-TB inflammatory responses at the site of infection following immune recovery post ART initiation. Forms of TB-IRIS include paradoxical, unmasking, and central nervous system (CNS) TB-IRIS [41]. Paradoxical TB-IRIS presents clinically as recurrent TB and may occur in approximately 18% of co-infected patients [41]. Unmasking TB-IRIS refers to the inflammatory presentation of subclinical infection following the use of ART while CNS TB-IRIS presents as recurrent CNS-TB [41,42]. Further complications include drug toxicities among patients receiving HIV and TB treatment [43]. Screening and diagnosis of TB may be difficult among PLHIV. A study on the prevalence of subclinical TB among HIV-infected individuals reported 22% sputum smear-positive cases among PLHIV patients with asymptomatic subclinical TB in Cape Town, South Africa [44].

In PLHIV, the complicated clinical presentation during active TB, presence of paucibacillary disease, and lack of lung cavitation among pulmonary TB patients may lead to negative sputum smears while chest radiographs may appear as those of TB negative patients [32,45]. This suggests possible diagnostic delays among PLHIV which may negatively affect TB prevention and undermine TB management [46]. An accurate diagnosis of TB infection among PLHIV is necessary for timely treatment initiation and reduction in mortality rates [47]. Studies report that TB prevalence and deaths are higher among males than females [48] with males having poorer HIV/AIDS outcomes [49]. Men and women may have different TB outcomes due to suboptimal engagement with healthcare facilities [49]. Some studies report that males delayed accessing health facilities following symptom onset as it was experienced as stigmatising [49,50]. Among TB and or HIV positive men, social norms around masculinity and the fear of disclosing infection status are linked with poor treatment uptake [51].

1.4 *Mycobacterium tuberculosis*

1.4.1 Pathogen and the disease

The interaction between facultative intracellular Mtb bacilli, transmitted by aerosolized droplets, and the host immune system results in either bacterial clearance, latent or active infection [39,52]. Studies suggest that TB exists as a dynamic spectrum where hosts can progress from no TB to latent TB infection, subclinical TB infection or active TB or reverse to other positions in the spectrum depending on host immunity or comorbidities (Figure 2) [53].



TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

Nature Reviews | Disease Primers

Figure 2. TB Spectrum [53]. Hosts may eliminate Mtb through host immune mechanisms or have latent TB infection where the pathogen is contained in a granuloma and characterised by no symptoms. This may progress to subclinical TB characterised by actively replicating pathogens however presenting no symptoms. Subclinical TB may advance to active TB which leads to symptom presentation with actively replicating Mtb bacilli.

Innate and adaptive immune responses in defense against Mtb are multifaceted yet complementary [54,55]. Mtb infection causes inflammation in the lungs with the interaction between Mtb and alveolar macrophages and dendritic cells (DCs) resulting in the release of tumor necrosis factor (TNF)-α, interleukin (IL)-12, IL-23 and chemokines [56]. This inflammatory response leads to the formation of granulomas comprised of macrophages, dendritic cells, B cells, and T cells, which halt the logarithmic growth of Mtb leading to an immunological equilibrium stage referred to as latent TB infection (LTBI) [57,58]. This process aids in the recruitment of immune cells to the site of Mtb infection (Figure 3) [31]. Immune compromise due to risk factors such as comorbidities or HIV co-infection increases the

likelihood of reactivation of the latent infection [58,59]. HIV causes a depletion of antigen-specific $CD4^+$ T cells and a disruption in granulomas that causes an inability in the immune system to mount effective responses against Mtb [60]. Untreated or poorly treated TB leads to reactivation of pulmonary TB which is highly infectious and commonly characterised by the presence of lung cavitation [58,61,62]. Subsequently, transmission occurs when bacteria are released into the air through a productive cough and are inhaled by a new host [58]. Treatment of LTBI by isoniazid preventive therapy (IPT) was shown to reduce TB reactivation rates [63,64].

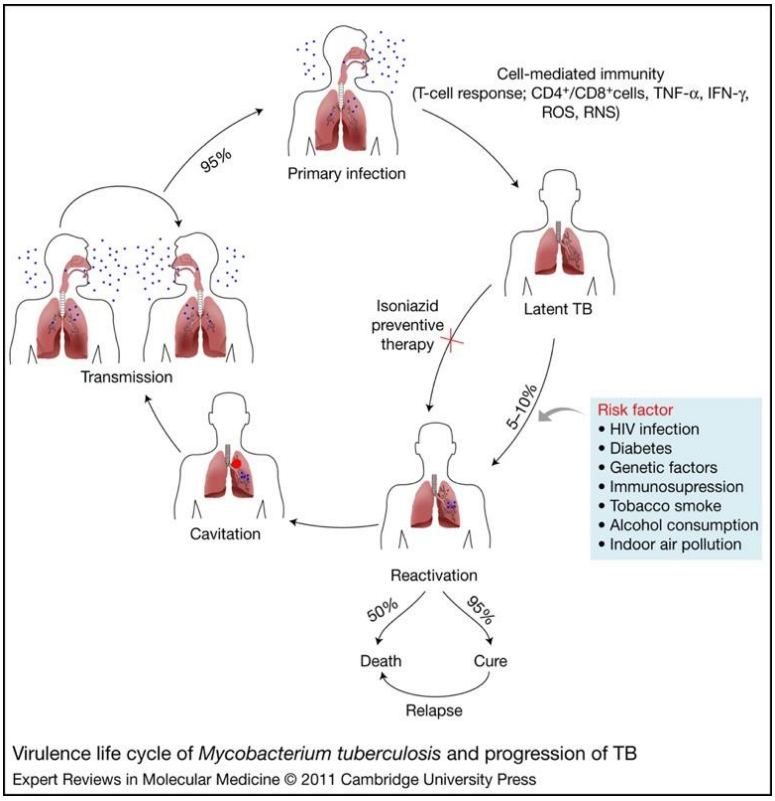


Figure 3. Life cycle of *Mycobacterium tuberculosis*. Primary Mtb infection induces cell-mediated immunity in efforts to clear or contain Mtb infection leading to latent TB infection. As depicted in the figure, without treatment, 5-10% of infected individuals will develop active TB due to several listed risk factors. Reactivated pulmonary TB is highly infectious and easily transmissible to a new host. Adapted from Kumar, *et al*. [65].

1.4.2 Immune responses to Mtb infection

1.4.2.1 Innate immunity

Innate immune cells recruited during Mtb infection include macrophages, DCs, neutrophils, and natural killer (NK) cells [52]. Following inhalation of aerosolized droplets containing Mtb, innate immune cells recognize Mtb pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs), nod-like and C-type lectin receptors, and elicit an inflammatory response [34,66]. These PAMPs include but are not limited to lipoarabinomannan (LAM), lipomannans (LMs), phosphatidylinositol mannosides (PIMs), mycolic acids, and lipoproteins [34,66,67]. The alveolar macrophages provide the first line of defense against Mtb by recognizing Mtb components which leads to activation, triggering of signaling pathways, and production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and recruitment of monocyte-derived macrophages [52,68]. Macrophages clear Mtb infection using various mechanisms including phagocytosis of Mtb bacilli, production of reactive oxygen and nitrogen intermediates such as nitric oxide [52], autophagy, and apoptosis which leads to phagosome maturation and mycobacterial killing [52,69]. Macrophages may in turn serve as niches for replicating Mtb pathogens following immune response evasion by the Mtb pathogens [66].

Phagocytic neutrophils are early responders during infection; they release proinflammatory cytokines, and oxidative species and undergo apoptosis following uptake of Mtb pathogens [54]. Mtb-infected neutrophils also serve as niches for Mtb [70] and are reported to be more abundant than macrophages in the bronchoalveolar lavage (BAL) fluid and sputum among patients with active pulmonary TB [71]. Additionally, neutrophils improve DC antigen presentation by promoting trafficking of Mtb pathogens to the lymph node [72]. Uptake of Mtb pathogens by immature DCs through phagocytosis and TLR signalling leads to DC maturation and trafficking to the lymph nodes; and subsequent antigen presentation and priming of naïve T cells via major histocompatibility complex (MHC) class I and II molecules [73,74]. Studies in mice report that infection of DCs by Mtb in the lungs can lead to impaired antigen presentation and $CD4^+$ T cell stimulation which results in persistent infection [75].

Macrophages, DCs, and neutrophils produce IL-12, IL-18 and IL-23 which promotes IFN-γ production by NK cells [76]. NK cells are innate lymphoid cells that mediate antimicrobial, antitumoral, and antiviral responses during infection [77]. The NK cell subsets are characterised by the variable expression of CD56 and FcγIII receptor (CD16) [78] including the highly cytotoxic $CD56^{dim}$ subset and the cytokine-producing $CD56^{bright}$ subset [79-81]. NK cell effector functions are regulated by germ-line encoded activating or inhibitory cell surface receptors [82,83]. The interaction of inhibitory receptors like killer cell immunoglobulin-like receptors (KIR) and NKG2A with MHC class I on target cells prevents damage to healthy cells however, the engagement of activating receptors such NKG2D, NKG2C, and natural cytotoxicity receptor (NCRs) (NKp30, NKp44, NKp46) leads to NK cell activation [79,84]. Subsequently, activated NK cells mediate contact-dependent cytotoxicity, cytolytic granule-mediated apoptosis of stressed or infected target cells, and initiate antibody-dependent cellular cytotoxicity (ADCC) [85]. NK

cells restrict the growth of Mtb using direct and indirect mechanisms [86]. Cytotoxic NK cells can recognize Mtb through cell surface receptors and mediate direct killing through the secretion of bactericidal molecules such as granulysin and perforin, and inhibit microbial growth by enhancing phagolysosomal fusion through IL-22 secretion [52,66].

Co-culture of NK cells with *M. bovis* Bacillus Calmette–Guérin (BCG) in the presence of IL-2 showed that $CD56^{bright}$ NK cells were a major source of IFN-γ in response to *M. bovis* [87] while unprimed $CD56^{dim}$ NK cells were shown to lyse Mtb-infected monocytes within 24 hours of co-culture experiments suggesting a role for NK cells in the early clearance of Mtb [88].
Changes in the expression of NK cell surface receptors, homing receptors, and subset distribution were reported at the onset of pulmonary TB [89]. Frequencies of $CD56^{bright}CD16^{+/-}$ NK cells are reduced in TB patients compared to healthy controls with a reduction in NKp30, NKp46, and DNAM-1 expression at the onset of TB [89].

1.4.2.2 Adaptive immunity

The adaptive immune responses in TB are facilitated by T and B cells and are initiated following priming of naïve cells by antigen presenting cells [90,91]. T cells, particularly the $CD4^+$ T cells play a crucial role in the immune response against Mtb, and this is particularly evident in PLHIV with $CD4^+$ T cell depletion resulting in increased susceptibility to TB. Mtb immune evasion strategies lead to delayed priming of T cells and trafficking to the site of infection resulting in further Mtb replication [92,93]. During TB infection primary antigen presenting cells activate naïve antigen-specific T cells [94,95]. The activated polyfunctional T cells proliferate and traffic to the site of infection where $CD4^+$ T cells polarize into T helper 1 (Th1) cells and secrete IFN-γ and TNF-α. These cytokines activate macrophages which induce phagosome formation and Th17 cells which aid in the recruitment of neutrophils, tissue repair, and proinflammatory cytokine production [34,95,96]. The macrophage activation leads to the formation of granulomas which are surrounded by B and T cells; and release of oxygen and nitrogen intermediates for pathogen lysis [34,97,98]. Activation of macrophages by IFN-γ leads to microbicidal functions such as reactive oxygen species production [68]. The macrophage and dendritic cell secreted IL-23 and IL-12 jointly induce the production of IL-17 by Th17 cells which attracts phagocytes such as neutrophils to the site of infection [68,93]. Neutralisation studies in mice showed that TNF-α was necessary for normal granuloma formation [99,100] and the recruitment of leukocytes into granulomas [101] while IFN-γ plays a role in maintaining the integrity of granulomas [102] and regulation of T cell responses [100]. Earlier Th1 responses in the lungs of vaccinated mice were linked with earlier pro-inflammatory and secondary responses and early Mtb control [103].

The cytotoxic $CD8^+$ T cells through perforin, and granulysin production also aid in pathogen killing. While data on the role of $CD8^+$ T cells in human TB is limited, animal studies have demonstrated that $CD8^+$ T-cell responses play an important role in protection against and long-term control of infection [104,105]. Induction of $CD8^+$ T cell response to Mtb antigens is also considered to be a key immune correlate for successful Mtb vaccine strategies [106,107]. $CXCR5^+HLA\text{-}DR^+$ activated B cells and plasma cells are present in lung granulomas of non-human primates (NHPs) with antigen-specific IgG secretion by plasma cells being reported within these structures. Additionally, plasma cells specific for mycobacterial antigens Early Secreted Antigenic Target 6 kDa (ESTAT-6) and Mtb culture filtrate protein (CFP) were observed in the lung and draining lymph nodes [108].

Plasma B cells produce TB-specific antibodies that regulate opsonization and ADCC and activate complement and formation of memory B cells [109,110]. Passive transfers of monoclonal antibodies (mAb) IgG and IgA in Mtb infected mice led to positive outcomes. Passive transfer of arabinomannan specific IgG3 mAb in neutrophil and polymorphonuclear leukocyte depleted mice prior to respiratory challenge increased survival although Mtb colony forming units (CFU) in the lung were comparable to control group [111]. Additionally, a study on paediatric populations suggested that decreased levels of mycobacterial antigen specific IgG in serum may lead to a predisposition to extrapulmonary infection [112]. IgA and IgG antibodies may reduce Mtb dissemination through neutralisation while IgG may also play a role in promoting phagocytosis [109]. B cells are also able to modulate immune cell function through pro- and anti-inflammatory cytokine production. Upregulation of IL-6, IL-10, TNF-α was observed in B cell culture supernatants following stimulation with lipopolysaccharide (LPS) while IL-1β responses were observed following stimulation with BCG with plasma B cells being the key cytokine producers [113]. Mtb-activated B cells also modulate Mtb specific $CD4^+$ T cell responses through antigen presentation to naïve T cells in the lung [114].

1.5 Human immunodeficiency virus

1.5.1 Pathogen and disease

HIV has two subtypes which include the prevalent HIV-1 which is responsible for 95% of AIDS cases and HIV-2 which is mainly confined to West Africa [115,116]. HIV may be transmitted from person-to-person through sharing of contaminated needles, blood products, and sexual intercourse. In addition, HIV can be transmitted from infected mother to child throughout pregnancy or breastfeeding [117,118]. The majority of HIV infections occur at mucosal surfaces with sexual transmission accounting for 80% of infections [119]. Co-infection with STIs leads to inflammation and recruitment of immune target cells such as activated $CD4^+$ T cells; while ulcerative STIs may cause epithelial damage that compromises mucosal integrity resulting in increased susceptibility to HIV infection [119,120]. The compromised genital mucosal

integrity may cause exposure of HIV target cells in the lamina propria [119]. Common ulcerative STIs include herpes simplex virus (HSV) type 2 [121] and syphilis caused by *Treponema pallidum* [122]. During sexual HIV transmission, the virus penetrates or bypasses the cervicovaginal, penile or rectal mucosal epithelial barriers [119].

Early targets for infection include dendritic cells, $CD4^+$ T cells, and macrophages [123,124]. Infected $CD4^+$ T cells and DCs increase viral spread by trafficking to draining lymph nodes where T cells can be *trans*-infected [125,126]. HIV is a retrovirus that utilises the CD4 receptor and CCR5, CXCR4 chemokine co-receptors on target cells as attachment sites, to fuse with the host cell and replicate [127,128].
Alternatively, HIV may infect a cell and remain inactive resulting in latent infection and viral reservoir formation [129]. HIV replication is initiated following binding of viral envelope protein (Env) gp120 to the CD4 receptor on host cells such as memory T cells resulting in subsequent binding of gp41 to CCR5 or CXCR4 coreceptors which leads to fusion of the viral and host membranes [130]. This allows entry of the viral capsid into the cell (Figure 4). The viral RNA genome is reverse transcribed into double-stranded deoxyribonucleic acid (DNA) by HIV reverse transcriptase. The HIV DNA traffics into the cell nucleus and integrates with the host's DNA using viral integrase [118]. The host's transcription machinery transcribes the HIV DNA into HIV messenger ribonucleic acid (mRNA) which becomes exported into the cytoplasm and used to make viral accessory and structural proteins through translation in an activated cell. Following splicing of gene products, the envelope proteins, core proteins, and RNA enzymes assemble within the host cell membranes and bud, releasing mature progeny virions which will initiate a new cycle of infection [118,120,131]. Reinfection may occur through cell-to-cell transmission or infection of susceptible cells by free virions [132].

Antiretroviral drugs approved for treatment of HIV comprise of classes of drugs such as non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), CXCR4 and CCR5 antagonists, integrase strand transfer inhibitors (INSTIs) and post attachment inhibitors which different target stages of the HIV life cycle [133,134]. The three stages of HIV infection include initial acute HIV infection (AHI), asymptomatic HIV infection or chronic HIV infection which progresses AIDS [135]. The use of antiretroviral therapy (ART) suppresses cell-to-cell HIV transmission, HIV replication and lengthens the period between HIV acquisition and the last stage of infection, AIDS [132,136]. The persistence of latent HIV reservoirs such as memory T cells, monocytes and macrophages presents a challenge for HIV cure. Potential strategies explored for HIV cure include gene therapy; ‘shock and kill’ method for latency reversal and infected cell killing; and ‘block and lock’ method which aims to silence proviruses and block viral reactivation [137,138].

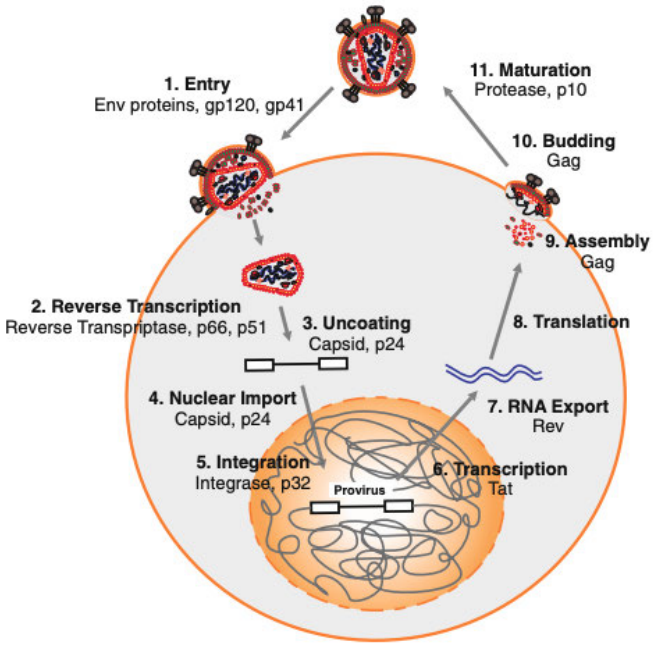


Figure 4. Life cycle of HIV. 1. Entry of HIV into the host cell following binding of HIV envelope proteins gp120 and gp41 to cell receptors and co-receptors; 2. Reverse transcription of HIV RNA into HIV DNA by HIV reverse transcriptase enzyme, a p66/p51 heterodimer; 3. Uncoating of viral capsid made of structural p24 protein, leading to release of HIV genome into cytoplasm; 4. Nuclear import of viral core; 5. Integration of HIV DNA into host cell chromosomes mediated by integrase, creating a provirus; 6. Transcription of integrated DNA mediated by HIV transactivating protein (Tat); 7. RNA transcripts exported out the cell nucleus mediated by regulator of expression of virion proteins (Rev); 8. translation; 9. Assembly of structural components to form HIV virions at host cell membrane; 10 and 11. Budding and maturation of HIV virions. Adapted from Kirchhoff [139].

1.5.1.2 Stages of HIV infection

Acute HIV infection is characterised by a rapid decline in the $CD4^+$ T cells and rapid increase in cell-associated and cell-free virions in circulating blood with peak viremia reaching ~1 million copies/mL [140,141]. Additionally, this stage is characterised by the establishment of latent HIV reservoirs such as $CD4^+$ memory T cells and macrophages [142,143]. Gut-associated lymphoid tissue (GALT) and lymph nodes represent a rich source of tissue HIV reservoirs [143]. Symptoms of AHI, associated with virus-specific immune responses, occur between 2-6 weeks post HIV-1 exposure, and include fever, rash, fatigue, headaches, diarrhoea, and malaise while signs include lymphadenopathy and tachycardia [129,144,145]. AHI is also marked by the presence of detectable antibodies against HIV viral protein p24 within 6-12 weeks post infection [146,147]. Within 3-6 months during AHI the HIV viral load setpoint (VLS) is reached which represents a balance between HIV viral turnover and host immune response [148].

Elite controllers are PLHIV that can suppress viral loads below the detectable threshold without ART (>50 copies/mL) [149] through T cell responses and protective human leukocyte antigen (HLA) alleles [150,151]. The hallmarks of HIV-1 infection are the depletion of $CD4^+$ T cells and the loss of cell-mediated immunity [145]. This results in increased susceptibility among PLHIV to opportunistic infections such as Mtb that lead to increased morbidity and mortality in this population [152,153]. Clinical latency or asymptomatic HIV infection, occurring between AHI and AIDS is characterised by a gradual increase in viremia due to continuous replication and decrease in $CD4^+$ T cells [154,155]. Persistent immune activation plays a key role in the pathogenesis of HIV and leads to T cell exhaustion characterised by loss of proliferative and effector functions and lack of responsiveness to antigenic stimulation [133,156]. Chronic immune activation is a driving force in the depletion of $CD4^+$ T cells [155]. Combination ART restores some immune function delaying the onset of AIDS [157]. The final stage of HIV infection, AIDS is characterised by a $CD4^+$ T cell count below 200 cells/mm^3 [133] and associated with opportunistic infections [158] such as candidiasis, *Pneumocystis* pneumonia, TB and opportunistic cancers such as Kaposi sarcoma [159-161].

1.5.2 Immune response to HIV infection

1.5.2.1 Innate immunity

Innate immune responses at mucosal surfaces hinder the establishment and spread of infection [162]. HIV sensing by TLR7 and TLR8 leads to DC activation and type I IFN and TNF-α production [163]. This leads to the induction of innate immune responses [164]. Langerhans cells (LCs), located in the mucosal epithelium, along with other subsets of DCs were shown to be susceptible to HIV infection [165]. LCs binding of HIV is mediated by a c-type lectin receptor langerin. Infected cells migrate from the epithelium to the draining lymph nodes to present the antigen to naïve $CD4^+$ and $CD8^+$ T cells [166] which leads to immune activation and development of immunological memory [167]. Conventional DCs located in the sub-epithelium or lamina propria mediate HIV uptake using the DC-specific intracellular adhesion molecule-grabbing non-integrin (DC-SIGN) which may lead to endocytosis and storage of non-degraded viral particles [168]. Plasmacytoid DCs (pDCs) endocytose HIV which leads to potent production of type 1 and type 2 interferons [164,168,169].

Type 1 interferons promote IFN-stimulated gene expression that mediate the inhibition of HIV replication [170] such as the induction of restriction factor APOBEC3G expression by IFN-α [161]. Myeloid DCs (mDCs), potent antigen presenting cells that are more restrictive to productive infection and capture HIV virions, process and present the antigens to T cells [171].

Myeloid DCs produce copious amounts of IL-12 which regulate innate immunity, induces IFN-γ production, and $CD4^+$ T cell differentiation into Th1 phenotype [168,172]. Tissue-resident macrophages in the mucosa phagocytose the virions, process, and present the antigens to $CD4^+$ T cells [173]. Type 1 activated macrophages secrete pro-inflammatory cytokines [174] and serve as reservoirs of HIV following infection. Without ART, peak viremia during AHI is associated with a cytokine storm with some cytokines remaining elevated even in the second stage of HIV infection [175]. Some of the cytokines/chemokines that are highly elevated and resolve following peak viremia include monocyte chemotactic protein (MCP)-1, IFN-γ, IL-1 receptor antagonist protein (IL-1Ra), and IFN-α. In addition, inducible protein (IP-10), monokine induced by gamma interferon (MIG), IL-12, and IL-8 are also elevated during AHI [175]. Later in the infection, pathological effects of HIV replication are associated with microbial translocation and resulting systemic inflammation characterised by upregulation of numerous inflammatory markers including soluble CD14 (sCD14) and IL-6 [176,177] . The level of immune activation in PLHIV was shown to be a better predictor of disease progression than the levels of viral replication [178,179].

The effector functions of NK cells play a role in the control of HIV and have been associated with a reduction of HIV viral setpoint in individuals with AHI [180]. In response to HIV infection, NK cells degranulate and produce β-chemokines that block HIV binding to target cells [181] (Figure 5). Co-culture studies of PBMCs from PLHIV with autologous CD16 cross-linked NK cells showed NK-mediated suppression of HIV however the addition of CC-chemokine MIP-1α and MIP-1β and RANTES neutralising antibodies led to reduced HIV suppression [182]. Increased NKG2D, NKp30 and NKp44 expression on $CD56^{dim}$ NK cells have previously been associated with lower viral load set point and viral load among long-term non-progressors and progressors [183] suggesting that NK cell responses are critical in the control of early HIV infection. In the analysis of correlates of risk of infection post the RV144 HIV-1 vaccine efficacy trial, NK cell mediated ADCC promoted by plasma IgG against variable regions 1 and 2 of the HIV envelope gp120 was inversely correlated with risk [183-185]. HIV infection induces changes in NK cell phenotype and function which are thought to hamper disease control [186]. While functional NK cell subsets comprise of $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets, a dysfunctional $CD56^{neg}$ NK cell subset is expanded during chronic HIV infection which has been correlated with HIV disease progression [187,188]. A significant decrease in the expression of activating receptors NKp46, NKp80, and NKG2D has been observed among individuals with chronic HIV infection compared to healthy controls resulting in HIV infected cells escaping lysis by NK cells [189].

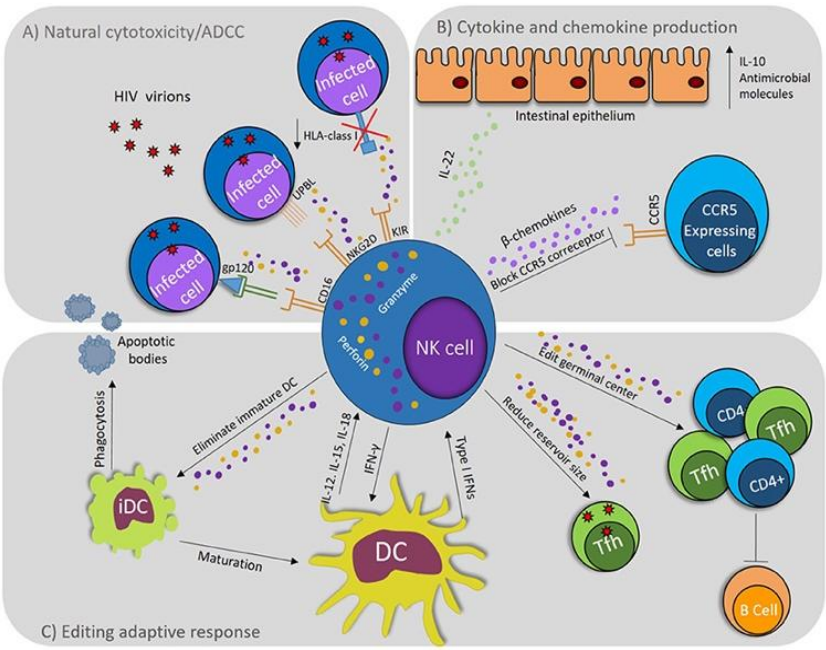


Figure 5. NK cell responses in HIV infection. NK cells mediate (A) cytotoxic responses including ADCC, perforin, and granulysin secretion following binding of NKG2D to UL16-binding proteins (UPBL) (B) cytokine and β-chemokines secretion to displaces HIV binding on target cells (C) DC maturation and editing which leads to subsequent NK cell activation and elimination of infected follicular helper T cells and editing of germinal centres which affects antibody production. Adapted from Flórez-Álvarez, *et al.* [181]**.**

1.5.2.2 Adaptive immunity

The progressive loss of $CD4^+$ T cells and the imbalance in $CD4^+$ T cell homeostasis is a distinctive feature of HIV infection, resulting in impaired host immunity and ultimately, if left untreated, death. $CD4^+$ T cells are key mediators of the host immune response, coordinating both cellular and humoral host responses against infections. Activated $CD4^+$ T cells are the main cellular targets for HIV [190]. HIV viral proteins including gp120, Vpr, Nef, Vpu and HIV protease have pro- and anti-apoptotic properties with the induction of apoptotic pathways being necessary for the pathogenesis of the virus [191,192] . HIV with defective Nef gene was observed in HIV long-term non-progressor, this was thought to explain the lack of disease progression and stable $CD4^+$ T cell counts [193]. Additionally, gp120 mediated cross-linking of CD4 receptors and chemokine co-receptors also promotes activation-induced apoptosis among $CD4^+$ T cells [194]. Pyroptosis of bystander T cells is also thought to contribute to T cell depletion and inflammation which leads to the recruitment of immune cells to infected lymph nodes [195]. In addition, $CD4^+$ T cell apoptosis was observed following infection with HIV without the envelope gene which was attributed to the Tat protein which promotes Fas-mediated apoptosis [196].

Among $CD4^+$ T cell subpopulations, Th17 cells, which play a role in epithelial barrier integrity, are preferential targets for HIV [197,198]. The selective destruction of Th17 $CD4^+$ T cells from the gastrointestinal mucosa results in loss of the intestinal barrier integrity and microbial translocation resulting in chronic immune activation [176,199].

AHI is associated with a Th1 profile with increased IL-2 and IFN-γ production and mediation of cell-mediated immunity. This profile shifts to a Th2 profile which is associated with increased IL-4 and IL-10 production and mediation of humoral immunity [172,200]. IL-4 mediated downregulation of IL-2 by cross-regulation may explain the observed reciprocal and time-dependent relationship between IL-2 and IL-4 production among PLHIV [201]. IL-2 plays a role in the homeostasis of T cells, development of T cell lineages, and regulates expression of cytokine receptors [202,203]. The loss of Th1 responses accompanied by an increase in Th2 response is hypothesised to be predictive of time to progression to AIDS and, lower CD4 counts [204] and thought to contribute to loss of HIV control [205]. Depletion of activated $CD4^+$ T cells and the associated chronic immune activation leads to impaired cytolytic activity among $CD8^+$ T cells [206].

$CD8^+$ T cells are activated following antigen presentation by APCs, IL-12, and type 1 interferon production and binding of co-stimulatory molecules which leads to clonal expansion [207,208]. The $CD8^+$ T cells play an important role in the control of HIV replication during AHI [209]. The frequency of HIV-specific cytotoxic $CD8^+$ T cells have been inversely correlated with plasma viral load [210]. In rhesus macaque studies, vaccine induced $CD8^+$ T cells against Vif and Nef HIV proteins controlled SIV replication [211]. Furthermore, HIV non-progressors have more highly functional $CD8^+$ T cells compared to HIV progressors [212]. The non-cytotoxic $CD8^+$ T cell responses in AHI have previously been associated with lower plasma viremia [213]. $CD8^+$ T cells lyse HIV-infected cells through the secretion of perforin and granzymes; and mediate the secretion of macrophage inflammatory protein (MIP)-1α, (MIP)-1β, regulated upon activation, normal T cell expressed secreted (RANTES) which bind co-receptors on cells to prevent binding by HIV [158]. In response to viral infection, the $CD8^+$ T cells also secrete cytokines such as IFN-γ and IL-2 and TNF-α [214,215]. Activated $CD8^+$ T cell MIP-1α and MIP-1β secretion is higher among individuals with chronic HIV in comparison to those with AIDS [216] In addition, HIV-specific $CD8^+$ T cell responses during chronic HIV infection are impaired and have less proliferative and cytolytic potential and polyfunctionality [209,214]. Although $CD8^+$ T cells control HIV through recognition of HIV epitope variants, mutation of HIV epitopes promotes escape from recognition [212,217]. HIV impairs humoral responses by inducing hypergammaglobulinemia, B cell hyper-activation, and skewed memory cell differentiation which leads to functional abnormalities [218]. HIV binds to B cells through complement receptor CD21 on mature B cells while complement protein binding to circulating virions leads to the production of TNF and IL-6 however, memory B cells and activated B cells of viremic HIV infected individuals were shown to produce less IL-6 and TNF-α in

response to TLR-9 agonist compared to aviremic individuals [219,220]. During early HIV infection, non-neutralising IgM antibodies undergo class switching to IgG and IgA antibodies against the HIV envelope proteins such as gp41 and gp120 [221,222]. In addition, the presence of IgG1 antibodies against HIV may induce ADCC by NK cells [223]. However, HIV depletion of $CD4^+$ T cells leads to impaired B cell CD40 and $CD4^+$ T cells CD40 ligand-mediated class switching. Subsequently, B cells develop low-affinity antibodies through T cell independent IgM production and class switching [224]. Therefore, HIV contributes to the development of poor antibody responses. B cell responses are also delayed in the presence of a rapidly mutating virus. Broadly neutralising antibodies (bNab), which are vaccine targets, develop in very few patients later during infection [220,225]. The delay in neutralising antibody responses may also be explained by the loss of T cells in the GALT [225].

1.6 Immune response in TB/HIV co-infection

Mtb and HIV jointly worsen the associated disease condition by gradual impairment of the host immune responses. Both HIV and Mtb cause immune activation reflected by the upregulation of pro-inflammatory cytokines [226,227]. Through increase in proinflammatory cytokines and upregulation of CCR5 and CXCR4 co-receptors for viral entry, Mtb infection creates an environment that facilitates increased HIV replication [228]. Additionally, secretion of CCL2 by Mtb-infected macrophages leads to recruitment of HIV targets $CCR2^+$ monocytes and $CD4^+$ T cells further fuelling HIV replication [229]. On the other side, HIV-induced inflammatory and immune changes increase the risk of TB recurrence and lead to poor disease outcomes in co-infected patients [39]. SIV-mediated chronic immune activation was shown to be the driving force in LTBI reactivation in non-human primates [230]. Our group has previously shown that TB recurrence in HIV co-infected individuals on ART is associated with markers of systemic inflammation including IL-6, IL-1β, and IL-1Ra [231]. We have subsequently shown that that increase in systemic inflammation and the risk of TB recurrence are associated with HIV-mediated translocation of microbial products and increased levels of lipopolysaccharide binding protein (LBP) and soluble intercellular adhesion molecule (sICAM) [232]. Furthermore, the increase in systemic inflammation was also associated with poor treatment outcome and cavitary disease in patients with recurrent TB with and without HIV co-infection [233].

HIV-mediated chronic immune activation is a driving factor in TB reactivation in macaque models and leads to severe impairment of cellular immune responses [59]. Furthermore, immune activation associated with active TB infection has been linked with faster HIV disease progression [234]. Both latent TB and active TB disease were shown to contribute to immune activation with elevated levels of soluble CD14 (sCD14), C-reactive protein (CRP), IL-6, and IFN-γ-induced protein 10 (IP-10) being observed among individuals with active TB co-infected with HIV. Increased levels of biomarkers of inflammation such

as soluble IL-6, CRP, and D-dimer prior to ART initiation were associated with risk of progression to AIDS [235] and therefore, TB/HIV co-infection could fuel HIV progression [234]. In addition, higher CD38 expression was observed on $CD4^+$ and $CD8^+$ T cells of PLHIV with latent TB compared to those with no TB while higher CD38 and HLA-DR co-expression was observed on $CD4^+$ and $CD8^+$ T cells of PLHIV with active TB. This study suggests that increased T cell activation is also observed among individuals with latent TB and could potentially contribute to increased HIV replication and disease progression [234].

Several key monocyte/macrophage functions including pathogen recognition and uptake and phagocytosis are impaired during HIV co-infection with important consequences for early Mtb control [236]. HIV infected DCs have diminished antigen presentation capacity due to inability to upregulate key co-stimulatory molecules (CD40, CD80 and CD86) delaying the onset of adaptive immune response and further increasing Mtb bacterial load [237]. HIV infection was shown to disrupt NK cell mediated elimination of Mtb infected cells as well as NK cell production of essential cytokines. In addition to suboptimal NK cell responses, PLHIV with LTBI had reduced IL-15, IFN-γ and granzyme B production [238]. While the effects of TB/HIV co-infection on NK cell homeostasis remain unexplored, both diseases individually cause changes in distribution of NK cell subsets and activating and inhibitory NK cell receptors that are key to NK cell function. HIV infection is known to cause a shift in NK cell surface marker expression [239] including HIV-mediated downregulation of NK cell activating receptors that are key for efficient recognition and killing of Mtb-infected cells including NKG2D, and NKp46 [189,238]. Considering that NK cell responses play a key role in control of both Mtb and HIV infections, changes in NK cell homeostasis could have important consequences for disease pathogenesis.

The importance of $CD4^+$ T cell responses in TB pathogenesis was clearly demonstrated in HIV co-infection with severe depletion in $CD4^+$ T cell numbers. HIV-specific and non-specific mechanisms of T cell apoptosis are largely responsible for $CD4^+$ T cell depletion and subsequent immunodeficiency in untreated patients which increase host susceptibility to opportunistic infections like Mtb [191,240]. HIV was shown to preferentially deplete Mtb-specific $CD4^+$ T cells [241] resulting in increased risk of TB reactivation, extrapulmonary infection, and increased Mtb bacillary burden among HIV-1 positive individuals [94]. Depletion of $CD4^+$ T cells in NHPs led to an exacerbation of primary TB and TB dissemination with reduced absolute $CD8^+$ T cell numbers suggesting a role for $CD4^+$ T cells in the proliferation and recruitment of $CD8^+$ T cells [242]. Th1 and Th17 cells are central in the mediation of protective Mtb responses as Th1-mediated IFN-γ production leads to activation of macrophages, stimulation of phagosome maturation and phagocytosis and restriction of Mtb growth while Th17 cells mediate antibacterial, and pro-inflammatory responses [96,243]. HIV infection is known to cause depletion of Th17 cells and a shift from Th1 to Th2 response and this could further hamper Mtb control, however,

helper T cell distribution and its effect on TB outcomes in TB/HIV co-infection has not been well characterised.

The dysregulated T cell and immune responses induced by HIV cause PLHIV to be susceptible to disseminated TB [244,245]. PLHIV have impaired inflammatory responses to Mtb infection [246] and develop high bacillary burden with poor containment due to poor granuloma formation [245] ultimately resulting in dissemination of TB from the lungs. Consequently, HIV co-infected TB patients have a lower chance of cavitary disease. Among TB patients with and without active TB infection, CD4 counts above 200 cells/µl were associated with a four-fold increase in the odds of lung cavitation while lung cavitation is rare in patients with advanced HIV infection [32].

T cell trafficking to the site of viral/bacterial replication is critical for clearance and control of both HIV and Mtb. The rapid homing of antigen-specific Th1 cells to the lung is critical for the containment of Mtb [247]. Very late antigen-4 ($\alpha_4\beta_1$) integrin expression was observed on 83% of antigen-specific Th1 cells in BAL 48 hours following bronchoscopic challenge with purified protein derivative (PPD) of Mtb [247]. Interestingly high $\alpha_4\beta_1$, and lymphocyte Peyer patch adhesion molecule ($\alpha_4\beta_7$), a gut homing integrin expressing $CD4^+$ T cells are targets for HIV infection [248]. Additionally, levels of circulating $\alpha_4\beta_7^+$ $CD4^+$ T cells are predictive of increased risk of HIV acquisition and progression [249]. HIV-induced inflammatory changes are known to affect activation and surface integrin expression on T cells [250] which could in turn affect the trafficking of these cells into the lungs and affect Mtb control and clearance. The changes in T cell trafficking during TB/HIV and its impact on TB pathogenesis remains unknown.

1.7 Study rationale

HIV and TB infections are frequently concomitant, suggesting the need for joint solutions to improve the health in this population. Both TB and HIV infections have profound effects on the host immune responses, which either directly or indirectly contribute to the morbidity and/or mortality associated with these diseases. Progress towards a successful vaccine and more effective treatment against TB is hindered by the lack of understanding of the immune biomarkers of protection and immune mechanisms that favour control of bacterial growth versus disease progression. Measurement of host immune biomarkers could represent a cost-effective, real-time method to determine and understand an individual's immune status and its effect on TB risk and the subsequent response to TB therapy.

Here we characterised innate and adaptive immune responses during active TB with and without HIV co-infection, focusing on the NK cell phenotype and T helper cell subset distribution. As highlighted previously, NK cells play a key role in control of both Mtb and HIV replication. However, HIV is known to cause redistribution in NK cell subsets and downregulate NK cell activating receptors that are key for efficient recognition and killing of Mtb infected cells. Similarly, Th1 and Th17 responses are key for the control of Mtb [251] and HIV infection is known to cause depletion of Th17 cells and a switch from Th1 to Th2 profile. Additionally, we examined the cell surface integrin expression on $CD4^+$ T cells to assess if HIV alters the trafficking of these cells to the tissues during active TB infection. Finally, we looked at inflammatory markers of MDR and XDR-TB since MDR/XDR-TB, being a more aggressive form of TB, is likely to produce stronger inflammatory response, exaggerating tissue damage and TB associated pathogenesis.

While previous studies have examined the nature of these responses during individual HIV and TB infections, data on the impact of these responses on TB severity and disease outcomes as well as the nature of these responses during TB-HIV co-infection is limited. The proposed study aims to fill important gaps in understanding TB-HIV coinfection dynamics and could provide new avenues for TB treatment and control.

Hypothesis: TB/HIV co-infection will have detrimental effects on host immune responses during active Mtb resulting in increased disease severity and poor treatment outcome.

1.8 Aim and objectives

Overall study aim: To determine the effect of HIV-induced immune changes on TB disease severity and treatment response in TB-HIV co-infected patients.

Specific objectives:

1. To characterise NK cell phenotype and T helper cell subset distribution during active TB in HIV infected and uninfected study participants from CAPRISA 011 IMPRESS study.
2. To determine the effect of TB treatment completion on NK cell phenotype and T helper cell subset distribution
3. To determine the role of NK cell phenotype and T helper cell subset distribution during active TB on TB disease severity and treatment response.
   To characterise the effect of systemic inflammation on disease severity in patients with active MDR/XDR TB

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CHAPTER 2

2. Methodology overview

2.1 Ethics approval

The CAPRISA 011 Improving Retreatment Success Trial (IMPRESS, BFC029/13; Clinical trial.gov; NCT02114684) and CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX, BFC584/16; Clinical trial.gov, NCT03237182) were approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) and registered with the South African Clinical Trials Register (SANCTR). The IMPRESS trial was further approved by the South African Health Products Regulatory Authority (Ref:20130510). The CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX) was approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC, BFC584/16) and registered with the South African Clinical Trials Register (NCT03237182). Healthy donors were also included in the study (UKZN BREC BE432/12). This sub-study was approved by the UKZN BREC (BREC/00002197/2020). All study participants provided written informed consent for the use of stored biological specimens.

2.2 Study cohorts

This study was performed using peripheral blood mononuclear cells from the CAPRISA 011 IMPRESS study cohort and healthy controls from KwaZulu-Natal, South Africa, as well as plasma samples from the CAPRISA 020 InDEX cohort.

CAPRISA 011 IMPRESS Study

The 70 CAPRISA 011 IMPRESS study participants were enrolled into an open-label randomised control trial conducted at the CAPRISA eThekwini HIV-tuberculosis clinic in Durban, South Africa. The IMPRESS trial compared the effectiveness of interventional Moxifloxacin-containing treatment to standard TB treatment for the improvement of culture conversation rates in smear-positive pulmonary TB [1]. The TB treatment in the trial consisted of a two-month intensive treatment phase which included standard TB drugs (rifampicin, isoniazid, pyrazinamide, and ethambutol) for the control arm and substitution of moxifloxacin for ethambutol in the intervention arm. The subsequent four-month continuous phase consisted of rifampicin and isoniazid for the control group and moxifloxacin, rifampicin, and isoniazid for the intervention group. Recruited participants were ≥ 18 years with a history of anti-TB treatment and a current TB diagnosis as confirmed by either a positive culture of Mtb sputum or rifampicin susceptibility as determined by GeneXpert MTB/RIF® technology (Cepheid, USA). The HIV status of participants was tested using rapid tests [Uni-Gold™ (Trinity Biotech, Ireland) and Alere Determine™ (Alere Scarborough, Inc)] with an HIV positive test result confirmed with a

second rapid HIV test. The Enzyme-Linked Immunosorbent Assay (ELISA) was used to confirm conflicting results following the rapid HIV tests. HIV positive males and females with any CD4 count measured using TruCOUNT method (BD Bioscience, USA) and those already on ART, provided the ART regimen was not contraindicated with the study drugs, were included along with HIV-negative adults. HIV viral loads were determined using a nested PCR with the Expand Long Template PCR System (Roche Diagnostics). Participants with resistance to the standard treatment drugs and those having received antibiotics against Mtb 14 days prior screening, and pregnant, and breastfeeding individuals were not eligible to participate in the study. The participants had a chest x-ray at the Prince Cyril Zulu Communicable Disease Centre in KZN, South Africa bi-annually.

CAPRISA 020 InDEX study

The 128 CAPRISA 020 InDEX study participants were enrolled in a randomised controlled clinical trial assessing if a gene-derived individualised treatment approach in patients with drug-resistant TB improved treatment success. Enrolled InDEX study participants were recruited to King Dinuzulu Hospital (KDH) in KwaZulu-Natal, South Africa. Study participants were ≥ 18 years with pulmonary TB and Xpert MTB/RIF assay positive for rifampicin resistance. The GenoType MTBDR*plus* assay was used to confirm MDR-TB while the GenoType MTBDR*sl* line probe assay was used to confirm pre-XDR/XDR using sputum samples. Only drug-resistant TB (MDR-TB, PRE-XDR/XDR-TB) participants were included in the study. HIV testing will be carried out using a licensed rapid HIV test. HIV-positive results will be confirmed by a second licensed rapid HIV test. Discordant results will be confirmed by ELISA. HIV-positive patients on ARVs with any CD4 count were included in the study. Participants with any serious acute condition, chronic or clinically significant medical condition as per the clinician's discretion were not eligible to participate in the study. The presence of lung cavitation was determined by postero-anterior chest radiograph unless a chest radiograph performed 14 days before the date of screening was available for review. In the intervention arm, all liquid media culture (MGIT) positive samples consistent with MDR-TB, pre-XDR, and/or XDR were sent to the National Institute for Communicable Diseases (NICD), the Centre for Tuberculosis (CTB) for Whole Genome Sequencing (WGS). Subsequently, the intervention arm received an individualised treatment regimen based on the WGS results and line probe assay results while the MDR-TB and XDR participants in the control arm received an appropriate MDR/XDR treatment regimen as per the South African National DR-TB guidelines. In the intensive phase, participants were followed up twice in the first month and every month thereafter and followed up every two months in the continuation phase of therapy.

Healthy controls

Thirteen healthy donors were recruited from Durban, KwaZulu Natal, South Africa. All participants were ≥ 18 years of age and were confirmed HIV-negative and self-reported no current or previous history of TB.

2.3 Collection and processing of plasma and peripheral blood mononuclear cell samples

Peripheral blood plasma was obtained following centrifugation [1600 rpm for 10 minutes at room temperature (RT)] of peripheral blood collected in acid citrate dextrose tubes and cryopreserved at -80 ºC until use. The peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved in freezing media [fetal bovine serum (FBS) containing 10% (v/v) dimethyl sulfoxide (DMSO)] and stored in liquid nitrogen until use. Upon use, the cryopreserved PBMCs were thawed using a 37 ºC water bath. Once 75% thawed, the PBMC vials were disinfected with 70% ethanol and placed into a biosafety cabinet. Approximately 1mL of cell culture media (RPMI 1640 medium containing 2 mM L-glutamine and 25 mM HEPES buffer supplemented with 10% v/v FBS, 100 U/ml penicillin, and 100 ug/ml streptomycin) was added in a drop-wise manner. The PBMCs were transferred into a new tube with 8 mL of prewarmed culture media and centrifuged (1250 rpm for 7 minutes at RT) and the supernatant discarded to remove residual DMSO. The cells were resuspended in 5 mL of cell culture media and rested for 3 hours (h) at 37 ºC, 5% CO_2. Subsequently, cells were counted using the trypan blue method.

2.4 Flow cytometry

Cellular phenotyping of NK cells and T helper cells using PBMCs was conducted using flow cytometry. Two flow cytometry panels were designed and optimised to characterise NK cell phenotypes and one flow cytometry panel was designed and optimised to assess T helper cell phenotype and integrin expression where we characterised $\alpha_4\beta_7$, $\alpha_4\beta_{7hi}$ and $\alpha_4\beta_1$ expressing memory $CD4^+$ T cells (Table 1). We characterised the expression of activating and inhibitory NK cell surface receptors (NKG2D, NKG2C, NKG2A, NKB1, CD158, NKp30, NKp44, NKp46) on two transcriptionally distinct NK cell subsets: $CD56^{dim}$ and $CD56^{bright}$ cells. The $CD4^+$ memory T cells were classified into Th1 ($CCR6^-CCR4^-CXCR3^+CCR5^+$), Th2 ($CCR4^+CCR6^-$), Th9 ($CCD6^+CCR4^{-)}$, Th17 ($CCR6^+CCR4^+$), Th17.1 ($CCR6^+CCR4^-CXCR3^+$), $CCR6^+$DN ($CCR6^+CCR4^-CXCR3^-$), and $CCR6^+$DP ($CCR6^+CCR4^-CXCR3^+$).

One million cells were surface stained with conjugated monoclonal antibodies for 20 minutes at RT in the dark in a 96-well V-bottom plate with a total reaction volume of 50 µl. Prior to surface staining, cells used for the NK cell panels were incubated with 10% Mouse IgG for 15 minutes in the dark at 4 ºC, centrifuged at 2100 rpm for 3 minutes and the supernatant discarded. Following surface staining, the cells were washed twice in 200 µl of phosphate buffered saline (PBS-1) [1x PBS, 2% fetal bovine serum (FBS)] and resuspended in 400 µl 1X CellFix™ (BD) and transferred to facs tubes. A minimum of 200,000 lymphocytes were acquired on a flow cytometer (BD LSR Fortessa™ Cell analyzer, USA) using BD FACSDiva software v8.0.2. The data analysed by hierarchical gating using FlowJo software

version 10.8.1. The fluorescent minus one (FMO) control tubes were used to define gates for select NK and T cell markers. Following gating, the data were exported to Excel for final analysis.

Table 1. Flow cytometry antibody panels for NK cell and T cell phenotypic characterization

Panels	Antigen	Fluorochrome	Clone	Manufacturer	Catalogue No.
NK cell Panel 1	CD3	AFluor700	SK	Biolegend	344822
	CD19	AFluor700	HIB19	BD Biosciences	557921
	CD14	AFluor700	MSE2	BD Biosciences	557923
	CD16	BV785	3G8	Biolegend	302046
	CD56	PECy7	B159	BD Biosciences	567747
	NKG2D	PerCPCy5.5	1D11	BD Biosciences	562364
	NKG2C	PE	134591	R&D Systems	FAB138P
	NKp30	BV711	P30–15	BD Biosciences	536383
	NKp44	APC	P44–8	BD Biosciences	558654
	Live/Dead	Amcyan	–	Thermofisher	L34957
NK cell Panel 2	CD3	AFluor700	SK	Biolegend	344822
	CD19	AFluor700	HIB19	BD Biosciences	557921
	CD14	AFluor700	MSE2	BD Biosciences	557923
	CD16	V450	3G8	BD Biosciences	560474
	CD56	PECy7	B159	BD Biosciences	567747
	CD335 (NKp46)	BV786	PE2	BD Biosciences	563329
	CD159a (NKG2A)	APC	Z199	Beckman Coulter	A60797
	CD158e	PE	Z27.3.7	Beckman Coulter	IM3292
	NKB1	FITC	Dx9	BD Biosciences	555966
	Live/Dead	Amcyan	–	Thermofisher	L34957
T cell Panel	CD3	APC-H7	SK7	BD	560176
	CD4	BV605	RPA-T4	BD	562658
	CD8	AF488	RPA-T8	BIOLEGEND	301021
	CD45RA	BV711	HI100	BIOLEGEND	304138
	CD49d (α)	PE	9F10	BD	555503
	CD29 (β1)	AF647	TS2/16	BIOLEGEND	303018
	β7	PE-Cy5	FIB504	BD	551059
	CD194/CCR4	PE-Cy7	L291H4	BIOLEGEND	359410
	CD195/CCR5	BV421	2D7	BD	562576
	CD196/CCR6	BV785	G034E3	BIOLEGEND	353422
	CD183/CXCR3	PECF	G025H7	BIOLEGEND	353736
	Live/dead	Amcyan	-	Thermofisher	L34957

2.5 Measurement of plasma inflammatory cytokines/ chemokines

The cryopreserved plasma samples were thawed at 4 ºC overnight and vortexed. Plasma levels of inflammatory cytokines/ chemokines were simultaneously quantified using the Pro Human Cytokine 27-Plex Assay (Bio-Rad, USA) on a 96-well plate and analysed on a BioPlex-200 system (Bio-Rad). The Human Cytokine/Chemokine Panel includes the following cytokines and chemokines: 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-17A, 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). All assays were performed following the manufacturers' instructions. Samples with values below the limit of detection (LOD) were assigned the value half of the LOD (LOD/2) in pg/mL.

2.6 Statistical analysis

All experiments were run blinded to the clinical status of the patients. Statistical analyses were performed using IBM SPSS Statistics version 25, R software version 4.2.2, and the graphs made using GraphPad Prism v9. Flow cytometry data was analysed by hierarchical gating using FlowJo software version 10.8.1 and exported to Excel. Cytokine/chemokine expression, cellular markers, and responses (as categorical and continuous variables) were correlated to demographic, clinical, and behavioural variables in linear mixed models, and time-to-event models. When analysed as continuous variables, cytokine/chemokine data were transformed to reduce variation, if detected in >60% of samples. Cytokine/chemokines with less than 60% of samples below detectability were analysed as binary variables. Multivariable models corrected for potentially relevant confounders or effect modifiers including age, sex, body mass index (BMI), previous TB history, lung cavities, smoking, and alcohol consumption; HIV viral load (VL), and CD4 count (if HIV positive). The distribution of the data was examined using the D'Agostino-Pearson omnibus normality test. Paired t-test and Wilcoxon signed-rank test were used to compare variables between paired samples depending on the distribution of the data while the Mann-Whitney test and Unpaired t-test were used to compare two groups of variables. When more than two groups were compared, either ANOVA with Tukey's multiple comparisons test or Kruskal Wallis test with Dunn's multiple comparisons test were used.

CHAPTER 3

Natural killer cell phenotypes in TB and TB/HIV co-infection

NK cells are cytotoxic innate lymphocytes key to recognition and clearance of tumour and infected cells. NK cell effector functions play a key role in the control of both Mtb and HIV replication, and both pathogens have developed ways of modifying NK cell responses to their benefit. This involves the redistribution of NK cell subsets, increase in inhibitory receptor expression, and decrease in natural cytotoxicity receptors [2-5]. These pathogen-induced changes in NK cell response could have important consequences for the cognate disease in the context of co-infection. While NK cell responses in TB and HIV have been well characterised, the nature and the impact of the NK cell responses on TB pathogenesis in the context of TB/HIV co-infection have not been explored.

In **Chapter 3** we characterised NK cell phenotypes at active TB and post TB treatment completion in 70 patients from the CAPRISA 011 IMPRESS study. We examined the differences in NK cell phenotypes between healthy controls and IMPRESS study participants with active TB and active TB and HIV co-infection. Additionally, we examined the changes in NK cell phenotypes following TB treatment completion as well as the association between NK cell phenotypes at active TB and time to culture conversion and the presence of cavitary disease.

Dr. A. Sivro and I, Thando Glory Maseko, conceptualised and designed the study. I retrieved and processed the peripheral blood mononuclear (PBMC) samples. I optimised two flow cytometry panels to characterise NK cell phenotypes and performed the flow cytometry experiments. I acquired, cleaned up, and analysed the data. The obtained results were validated by Lara Lewis, a designated CAPRISA 011 study biostatistician. I wrote the first draft of the manuscript which was reviewed by Dr. A. Sivro and the rest of the co-authors. The paper has been published by *Scientific Reports*. Results from this work were presented at the 7th South African TB Conference, IXth South African Immunology Conference and the 11th Infectious Disease Symposium in Africa.

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NK cell phenotypic profile during active TB in people living with HIV-evolution during TB treatment and implications for bacterial clearance and disease severity.

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NK cell phenotypic profile during active TB in people living with HIV-evolution during TB treatment and implications for bacterial clearance and disease severity

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Natural killer (NK) cells, key effector cells of the innate immune system, play an important role in the clearance and control of *Mycobacterium tuberculosis* and HIV infections. Here, we utilized peripheral blood specimens from the Improving Retreatment Success CAPRISA 011 study to characterize NK cell phenotypes during active TB in individuals with or without HIV co-infection. We further assessed the effects of TB treatment on NK cell phenotype, and characterized the effects of NK cell phenotypes during active TB on mycobacterial clearance and TB disease severity measured by the presence of lung cavitation. TB/HIV co-infection led to the expansion of functionally impaired CD56^{neg} NK cell subset. TB treatment completion resulted in restoration of total NK cells, NK cell subset redistribution and downregulation of several NK cell activating and inhibitory receptors. Higher percentage of peripheral CD56^{bright} cells was associated with longer time to culture conversion, while higher expression of NKp46 on CD56^{dim} NK cells was associated with lower odds of lung cavitation in the overall cohort and the TB/HIV co-infected participants. Together these results provide a detailed description of peripheral NK cells in TB and TB/HIV co-infection and yield insights into their role in TB disease pathology.

Natural killer (NK) cells are lymphocytes of the innate immune system that have both cytotoxic and cytokine-producing effector functions and play a critical role in the recognition and destruction of infected and cancerous cells[1]. NK cells are classified into two functional subsets based on CD56 and CD16 surface marker expression. Cytokine-producing CD56^{bright} NK cells have a mainly immunomodulatory function and CD56^{dim} NK cells are primarily cytotoxic effector cells[2]. NK cells distinguish healthy cells from 'stressed/infected/malignant' cells based on the expression of surface activating and inhibitory receptors, which engage with target cells to regulate NK cell activity[3]. These responses play a key role in the pathogenesis of intracellular pathogens such as *Mycobacterium tuberculosis* (Mtb) and human immunodeficiency virus (HIV)[4,5].

The importance and role of NK cell responses in Mtb infection and disease has been demonstrated by a number of in vitro, animal, clinical and epidemiological studies[6,7]. Early clearance of Mtb infection was proposed to be mediated by NK cells through recognition and lysis of Mtb and Mtb-infected cells[8–10].NK cells are known to lyse Mtb-infected monocytes with NK cell activating receptors NKp46 and NKG2D playing a key role in this process[8,9,11]. Additionally, NK cells can interact and respond to Mtb through direct recognition of Mtb cell wall

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components by NKp44[12] and TLR2 which results in Mtb killing[10]. Mtb infection is known to modify NK cell phenotype and function through downregulation of activating NK cell receptors (including NKp46) and expansion of anergic $CD56^{neg}$ NK cell subsets[13]. A longitudinal study from South Africa has linked peripheral NK cell profiles and frequencies with TB disease progression, treatment response, and lung pathology[14]. Decreased NK cell frequency was associated with active TB disease progression, while increased NK cell levels and function was reported following successful TB treatment completion[14–16]. Changes in peripheral NK cell levels and activity are thought to reflect Mtb activity in the lung, with peripheral NK cell frequency during active TB being inversely correlated to the inflammatory burden in the lung[14]. Increased NK cell-mediated cytotoxicity was also associated with increased presence of lung cavitation, further implicating NK cells in lung immunopathology[17].

In HIV infection, NK cells have been shown to play an important role in the early control of viral replication and disease progression. Increased proliferation of cytotoxic NK cells during the early stages of infection has been associated with long-term viral control in antiretroviral naïve individuals[18]. Inducible expression of natural cytotoxicity receptors NKp30 and NKp46 and increased Interferon-gamma (IFN-γ) production following NK cell activation correlates inversely with the size of the HIV-1 viral reservoir[19]. The activating killer cell immunoglobulin-like receptor (KIR) allele KIR3DS1, in combination with HLA-B alleles that encode molecules with isoleucine at position 80 (HLA-B Bw4-80Ile), was shown to be associated with delayed progression to AIDS[20]. HIV infection causes profound changes in the phenotypes and functions of NK cells and these changes are thought to hinder disease control[21]. This includes an HIV-mediated decrease in the expression of NKp44, NKp46, and NKp30 natural cytotoxicity receptors and the expansion of the anergic $CD56^{-}CD16^{+}$ NK cell subset[21].

The convergence of the TB and HIV epidemics in sub-Saharan Africa has devastating consequences. TB is the leading cause of death in people living with HIV (PLHIV)[22,23] and PLHIV are 18 times more likely to develop TB than people without HIV infection[24]. Despite the heightened need to elucidate the immunopathogenesis of TB/HIV, there remains limited data on NK cell phenotypes and immune responses in TB/HIV co-infection[25–27]. Here, we utilized specimens from the Improving Retreatment Success (IMPRESS, CAPRISA 011) study to characterize the effects of TB treatment on NK cell phenotype, and to characterize and assess the effects of NK cell phenotypes during active TB on mycobacterial clearance and TB disease severity in patients with or without HIV co-infection.

Methods

Study population. This sub-study included stored peripheral blood mononuclear cells (PBMCs) from 70 HIV-infected and uninfected adult participants from the CAPRISA 011 IMPRESS study at active TB (prior to treatment initiation) and at treatment completion time points based on sample availability. The CAPRISA 011 IMPRESS study was an open-label randomized control trial conducted at the CAPRISA eThekwini HIV-tuberculosis clinic in Durban, South Africa[28]. The IMPRESS trial compared the effectiveness of an interventional moxifloxacin-containing treatment to standard TB treatment for the improvement of culture conversion rates in smear-positive pulmonary TB[28]. TB treatment in the trial consisted of a two-month intensive treatment phase and four-month continuous phase. Recruited participants were ≥ 18 years with a history of anti-TB treatment and a current TB diagnosis as confirmed by either a positive culture of MTB in sputum or by GeneXpert MTB/RIF® technology (Cepheid, USA). Additionally, 13 PBMC samples from healthy controls from KwaZulu-Natal, South Africa were included in this study.

Ethics statement. Ethics approval for the CAPRISA 011 Improving Retreatment Success (IMPRESS) Trial was obtained from the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BFC029/13; Clinical trial.gov; NCT02114684) and the South African Medicines Control Council (Ref:20130510). The protocol for this sub-study was approved by the UKZN Biomedical Research Ethics Committee (BREC/00002197/2020). Ethical approval for the use of PBMC samples from healthy controls from KwaZulu-Natal, South Africa was obtained from University of KwaZulu-Natal Biomedical Research Ethics Committee (BE432/12). All study participants provided written informed consent prior to enrolment and approval for the use of stored biological specimens for future research. All the experiments were performed in accordance with the Helsinki Declaration.

Sample collection and processing. Peripheral blood was collected in acid citrate dextrose tubes and PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation[29]. Isolated PBMCs were cryopreserved in fetal bovine serum (FBS) with 10% (v/v) DMSO and stored in liquid nitrogen until use.

Flow cytometry experiments. Cryopreserved PBMC samples were thawed using a 37°C water bath, washed, and resuspended in 5 ml R10 media (RPMI 1640 medium containing 2 mM L-glutamine and 25 mM HEPES buffer supplemented with 10% v/v FBS, 100 U/ml penicillin and 100 ug/ml streptomycin (Lonza). The resuspended cells were rested at 37°C in 5% CO_2 for 3 h. One million cells per panel were resuspended in 10% mouse IgG in PBS-2 (PBS supplemented with 2% FBS) and incubated for 15 min in the dark at 4°C. The cells were pelleted by centrifugation, and surface stained per panel for 20 min at room temperature in the dark. Flow cytometry panels are described in Supplementary table 1. Panels included a LIVE/DEAD fixable dead cell stain (ThermoFisher Scientific, L34957) that was diluted with 50 µl of DMSO as per manufacturer's instructions. The stock was diluted 1/40 and 3 µl of the dilution was used for final staining (per 50 µl reaction). Following staining, cells were washed twice using PBS-2 and resuspended in 1X CellFix™ (BD). Minimum of 200,000 events were acquired on a flow cytometer (BD LSRFortessa™ Cell analyzer, USA) using BD FACSDiva software v8.0.2. with the data analyzed using FlowJo software version 10.8.1.

Flow cytometry gating strategy. The NK cell gating strategy used to identify total NK cells and NK cell subsets is presented in Supplementary Fig. 1. Total NK cells were identified as live $CD3^{-}CD14^{-}CD19^{-}$ cells expressing CD56 and/or CD16. Total NK cells were further separated into the following subsets based on CD56 and CD16 expression: $CD56^{bright}$ ($CD56^{++}CD16^{+/-}$), $CD56^{dim}$ ($CD56^{+}CD16^{++}$), $CD56^{neg}$ ($CD56^{-}CD16^{+}$) and $CD56^{dim}CD16^{-}$. We further characterized NK cell surface marker expression (Supplementary Fig. 2) on transcriptionally distinct $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets[30]. Surface markers included activating and inhibitory NK cell surface receptors (NKG2D, NKG2C, NKG2A, NKp30, NKp44, NKp46, NKB1, and CD158)[30].

Statistical analysis. The statistical analyses were conducted using IBM SPSS Statistics v27 (Armonk, NY) and figures were compiled using GraphPad Prism v9.3.1. The distribution of the data was assessed using the D'Agostino-Pearson omnibus normality test. To investigate differences in the percentage (%) of total NK cells, % NK cell subsets, and differential NK cell marker expression across participant subgroups, one-way ANOVA with Tukey's multiple comparisons test and Kruskal Wallis test with Dunns multiple comparisons test were used on normally distributed and non-normally distributed data, respectively. Depending on the distribution of the data, a paired t-test or Wilcoxon signed-rank test were used to assess the difference between the proportion of total NK cells, NK cell subsets, and surface marker expression during active TB (prior to treatment initiation) and following treatment completion. The relationship between NK cell frequencies and NK cell surface marker expression at active TB with days to culture conversion (defined as two consecutive negative TB sputum culture results) was modelled using Cox proportional hazards models. Those who died were excluded from the analysis while those that did not culture convert before leaving the study were censored on their study termination date. Logistic regression was used to determine the association between NK cell frequencies and NK cell surface marker expression during active TB with the presence of lung cavitation at baseline, an indicator of TB disease severity. The multivariable analyses adjusted for baseline clinical and demographic variables, including treatment randomization arm, age, gender, and HIV status. The sub-analysis of the TB/HIV co-infected group adjusted for treatment randomization arm, age, and gender.

Results

Cohort characteristics. The CAPRISA 011 IMPRESS cohort (n = 70) was comprised of 75.7% males with a median age of 35.5 years [interquartile range (IQR) 29–43)] and median BMI of 19.6 kg/m^2 [interquartile range (IQR) 18.2–22.4)], (Table 1). The study included 44 (62.9%) HIV positive and 26 (37.1%) HIV negative participants with active TB. The HIV positive participants had a median CD4 count of 285 $cells/mm^3$ (IQR 123–413) and 50% were on antiretroviral therapy (ART). Overall, 62.9% of the participants had lung cavitation in one or

Variables	Total cohort n = 70	HIV positive n = 44	HIV negative n = 26
Randomization arm n (%)			
HRZE—Control	29 (41.4)	17 (38.6)	12 (46.2)
HRZM—Active	41 (58.6)	27 (61.4)	14 (53.8)
Gender, n (%)			
Male	53 (75.7)	31 (70.5)	22 (84.6)
Age (y), median (IQR)	35.5 (29.0–43.0)	37.0 (31.0–41.0)	33.0 (24.8–50.5)
Body mass index (kg/m^2), median (IQR)	19.6 (18.2–22.4)	19.7 (18.1–22.5)	19.3 (18.4–22.2)
HIV status, n (%)			
Positive	44 (62.9)	44 (100.0)	–
Negative	26 (37.1)	–	26 (100.0)
HIV Viral load (copies/ml), median (IQR)	–	45,165.0 (2857.3–558,065.0)	–
CD4 cell count ($cells/mm^3$), median (IQR)	–	285 (123–413)	–
ARV status[a], n (%)			
Yes	–	22 (50.0)	
No	–	20 (45.5)	
Lung Cavities[b], n (%)			
None	18 (25.7)	13 (29.5)	5 (19.2)
One Lung	24 (34.3)	17 (38.6)	7 (26.9)
Both Lungs	20 (28.6)	11 (25.0)	9 (34.6)
Alcohol Use in the past 3 months n (%)			
Yes	17 (24.3)	14 (31.8)	3 (11.5)
Smoking in past 3 months n (%)			
Yes	25 (35.7)	15 (34.1)	10 (38.5)

Table 1. Demographic and clinical characteristics of CAPRISA 011 IMPRESS participants. [#]Values for clinical and demographic variables reported at baseline except days to first negative culture. [a]Two participants missing ARV status. [b]Eight participants with missing lung cavitation status.

both lungs. The healthy donors consisted of 54% females with a median age of 35 years (IQR 32–39) and median BMI of 23.29 kg/m2 (IQR 19.73–31.30), Supplementary table 2.

Effect of active TB and TB/HIV co-infection on NK cell percentage and phenotype. We compared the percentage of NK cell populations and NK cell surface receptor expression across the following participant groups: CAPRISA 011 TB/HIV co-infected participants (TB/HIV), CAPRISA 011 HIV-negative participants with active TB (TB), and healthy controls (Fig. 1). The TB/HIV group had a significantly higher % of $CD56^{neg}$ NK cells compared to the TB group ($p = 0.0176$) and healthy controls ($p = 0.0148$) (Fig. 1a).

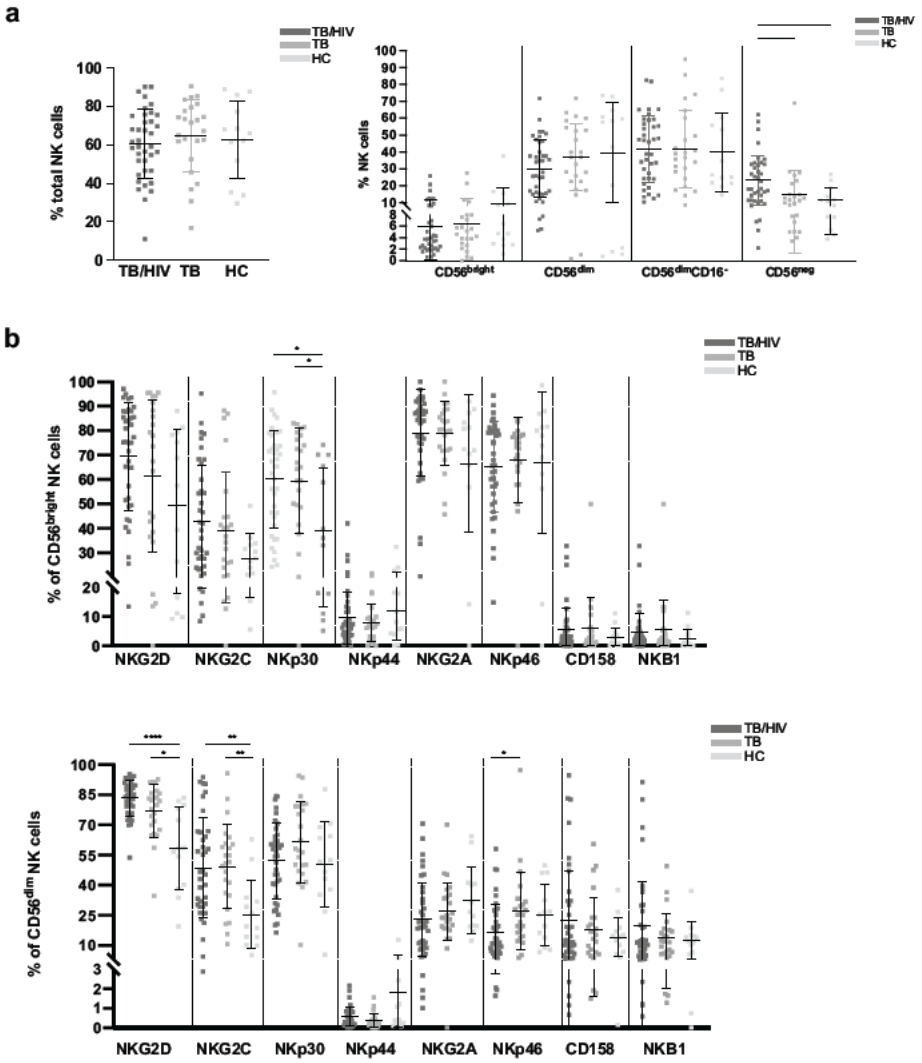

Figure 1. Differences in NK cell percentage and phenotype between TB/HIV co-infected and TB infected participants and healthy controls. (**a**) Differences in the % of total NK cells and NK cell subsets across participant groups (TB/HIV, TB, healthy controls). (**b**) Percentage of NK cell activating and inhibitory receptors on $CD56^{bright}$ and $CD56^{dim}$ NK cells across participant groups (TB/HIV, TB, healthy controls). *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

The expression of cytotoxicity receptor NKp30 on CD56^{bright} NK cells was significantly higher in the TB/HIV ($p = 0.0348$) and TB ($p = 0.0445$) groups in comparison to the healthy controls (Fig. 1b). NKG2D expression on CD56^{dim} NK cells was significantly higher in TB/HIV ($p = 0.0001$) and TB group ($p = 0.0308$) in comparison to healthy controls. Similarly, NKG2C expression on CD56^{dim} NK cells was significantly higher in TB/HIV ($p = 0.0055$) and TB group ($p = 0.0084$) in comparison to healthy controls. A decrease in NKp46 on CD56^{dim} NK cells in TB/HIV group compared to TB group was observed ($p = 0.0171$) (Fig. 1b).

Changes in NK cell percentage and phenotype following TB treatment completion. The differences in the percentage of NK cells and NK cell surface receptor expression between active TB and post-treatment completion were examined in 34 paired samples (Fig. 2). Following treatment completion, we observed a significant increase in the total NK cell population ($p = 0.024$) (Fig. 2a). With respect to NK cell subsets, we observed a decrease in CD56^{bright} NK cells following treatment completion ($p = 0.032$), in the total cohort.

Several NK cell surface markers were downregulated on CD56^{bright} NK cells following treatment completion, including NKG2A ($p = 0.0001$), NKp46 ($p = 0.003$), CD158 ($p = 0.026$), and NKB1 ($p = 0.006$). On CD56^{dim} NK cells, we observed a significant downregulation of NKG2D ($p = 0.0001$) and NKp30 ($p = 0.001$) following TB treatment completion in the total cohort (Fig. 2b).

Similar differences and trends were observed in HIV positive sub-group (Supplementary Fig. 3). In addition, NKp44 was significantly upregulated in HIV positive participants on CD56^{bright} NK cells following treatment completion ($p = 0.049$). On CD56^{dim} NK cells, there was an additional significant decrease in CD158 ($p = 0.003$) and an increase in NKG2C ($p = 0.013$) expression following treatment completion.

Association between NK cell percentage and phenotype during active TB and time to culture conversion. We utilized a cox regression model to assess the association between NK cell percentage and phenotype at active TB on time to negative culture conversion, (Table 2, Supplementary table 4, Supplementary Fig. 4). Higher % of CD56^{bright} NK cells was associated with longer time to culture conversion [adjusted hazards ratio (aHR) 0.893, 95% CI: 0.825–0.966, $p = 0.005$] while the higher % of CD56^{neg} NK cells was associated with shorter time to culture conversion (aHR 1.029, 95% CI: 1.006–1.052, $p = 0.013$) in the multivariable model controlling for the treatment randomization arm, age, gender, and HIV status. Increased expression of NKG2D on CD56^{dim} NK cells was associated with longer time to culture conversion (aHR 0.961, 95% CI: 0.932–0.990, $p = 0.0085$) (Table 2, Supplementary table 4).

We observed a similar trend in the sub-analysis of HIV co-infected participants (n = 39) with the higher % of CD56^{bright} NK cells being associated with longer time to culture conversion (aHR 0.858, 95% CI: 0.768–0.959, $p = 0.007$). Increased expression of NKp46 on CD56^{bright} NK cells was associated with longer time to culture conversion (aHR 0.975, 95% CI: 0.952–0.999, $p = 0.045$), (Supplementary table 5).

Association between NK cell percentage and phenotype during active TB and cavitary disease. Binary logistic regression models were used to assess the association of NK cell percentage and phenotype on TB disease severity measured by presence of lung cavitation (Table 3, Supplementary table 6). Higher expression of NKp30 on CD56^{bright} NK cells increased the odds of lung cavitation in the total cohort [adjusted odds ratio (aOR) 1.036, 95% CI: 1.002–1.071, $p = 0.039$)]. Higher expression of NKp46 on CD56^{dim} NK cells lowered the odds of lung cavitation in the total cohort (aOR 0.958, 95% CI: 0.921–0.997, $p = 0.035$) and among the TB/HIV co-infected participants (aOR 0.937, 95% CI: 0.883–0.994, $p = 0.032$), (Table 3, Supplementary tables 6 and 7).

Discussion

Both Mtb and HIV cause alterations in the NK cell repertoire with consequences for pathogen control and disease outcome. In this study, we assessed the effects of TB treatment on NK cell percentage and phenotype, and the characteristics and effects of NK cell phenotypes during active TB disease on bacterial clearance and disease severity in patients with or without HIV co-infection.

We observed an expansion of the CD56^{neg} NK cells in the TB/HIV group compared to TB group and healthy controls. This is consistent with previous reports showing an increased proportion of CD56^{neg} NK cell subset during both HIV and TB infections[31–34]. Expansion of the CD56^{neg} NK cell subset during HIV infection is associated with rapid and early progression to AIDS[35]. This NK cell subset was shown to have diminished cytolytic and cytokine-producing capacity, a likely sequela of NK cell exhaustion due to chronic inflammation resulting from prolonged viral and bacterial infections[31,36].

We observed an increase in activating receptor NKp30 expression on immunomodulatory, weakly cytotoxic CD56^{bright} NK cell subsets in the TB and TB/HIV groups compared to healthy controls, potentially increasing the recognition and response against Mtb and HIV-infected cells. The CD56^{dim} NK cell subset had increased expression of activating receptors NKG2D and NKG2C in TB and TB/HIV group compared to healthy controls. An increase in NKG2D NK cell expression was reported following exposure to Mtb-infected monocytes[9] pinpointing NKG2D as one of the principal receptors involved in the lysis of Mtb-infected mononuclear phagocytes. Expansion of NKG2C^{+} NK cells was previously reported in response to human cytomegalovirus (CMV) and HIV/Simian immunodeficiency virus (SIV) infections[37–39] and is thought to play an important role in viral control and slower disease progression [39]. We additionally observed a decrease in NK cell activating receptor NKp46 on CD56^{dim} NK cell subsets in the TB/HIV group compared to the TB group. Expression of natural cytotoxicity receptors including NKp46 is known to be decreased among viremic HIV positive individuals with a concomitant decrease in NK cytolytic activity[40]. As NKp46 is involved in the lysis of Mtb-infected monocytes[8], this HIV-mediated decrease in NKp46 could further impair NK cell-mediated Mtb control in TB/HIV co-infected patients.

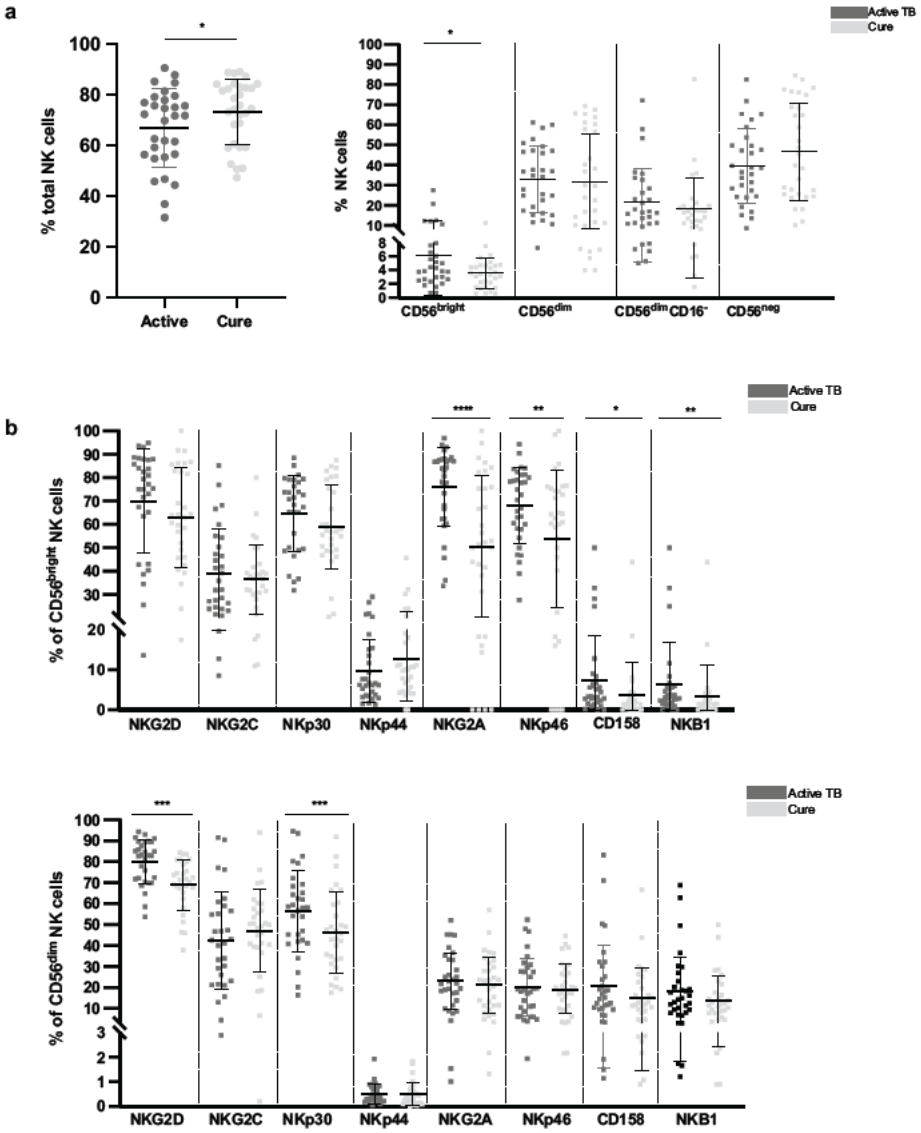

Figure 2. Effect of TB treatment completion on NK cell percentage and phenotypic marker expression in the CAPRISA 011 (n = 34). (a) Effect of TB treatment completion on the percentage of total NK cells and NK cell subsets. (b) Differences in activating and inhibitory receptor expression on $CD56^{bright}$ and $CD56^{dim}$ NK cells between active TB and post treatment completion. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

Following TB treatment, we observed an increase in the total NK cell proportion and a decrease in $CD56^{bright}$ NK cells. Decrease in total NK cells during active TB and their restoration following treatment have been reported previously[14,41]. Changes in peripheral NK cell proportions are thought to reflect bacterial burden and Mtb activity in the lungs, with an increase in NK cells being associated with successful treatment outcomes and a decrease

Cohort	Subset	Cell marker	Bivariable			Multivariable		
			HR	CI	*p*-value	aHR	CI	*p*-value
Total cohort	% of CD56^{bright} NK cells		0.939	0.877–1.007	0.077	0.893	0.825–0.966	**0.005**
	% CD56^{neg} NK cells		1.022	0.999–1.045	0.063	1.029	1.006–1.052	**0.013**
	% of CD56^{dim} NK cells	NKG2D	0.977	0.953–1.001	0.064	0.961	0.932–0.990	**0.009**
TB/HIV co-infected	% of CD56^{bright} NK cells		0.951	0.882–1.026	0.196	0.858	0.768–0.959	**0.007**
	% of CD56^{bright} NK cells	NKp46	0.997	0.977–1.018	0.794	0.975	0.952–0.999	**0.045**

Table 2. Significant association between NK cells and NK phenotypic markers with time to culture conversion (n = 63). Significant values are in [bold].

Cohort	Subset	Cell marker	Bivariable			Multivariable		
			OR	CI	*p*-value	aOR	CI	*p*-value
Total cohort	% of CD56^{bright} NK cells	NKp30	1.028	0.998–1.058	0.068	1.036	1.002–1.071	**0.039**
	% of CD56^{dim} NK cells	NKp46	0.971	0.937–1.006	0.103	0.958	0.921–0.997	**0.035**
TB/HIV co-infected	% of CD56^{dim} NK cells	NKp46	0.943	0.892–0.998	**0.044**	0.937	0.883–0.994	**0.032**

Table 3. Significant associations between frequencies of NK cells and NK phenotypic markers with disease severity measured by lung cavitation (n = 55). Significant values are in [bold].

in NK cells being associated with disease progression[14]. A decrease in CD56^{bright} NK cells during active TB has also been reported[13], and this could potentially be due to a redistribution of NK cells by trafficking to the site of infection[14] with an increase in CD56^{bright} NK cells observed in the pleural fluid of TB patients[42].

Successful treatment completion resulted in downregulation of several NK cell activating receptors including NKp46 levels on CD56^{bright} NK cells and NKG2D and NKp30 levels on CD56^{dim} NK cells. We additionally observed a decrease in inhibitory receptors NKG2A, CD158 (KIR), and NKB1 on CD56^{bright} NK cells following treatment completion. In addition to the above, HIV co-infected participants had a significant increase in NKp44 levels on CD56^{bright} NK cells and an increase in NKG2C levels on CD56^{dim} NK cells following TB treatment completion. The observed decrease in the expression of NK cell activating receptors following treatment completion likely reflects the accompanying decrease in Mtb burden, with both NKG2D and NKp46 shown to play a key role in NK cell recognition and clearance of Mtb infected cells[8,9].

Data on the role of inhibitory NK cell receptors in Mtb infection is limited. Inhibitory receptors like NKG2A function as immune checkpoints to prevent overactivation of the host immune system[43,44]. Viral infections, including HIV, are known to upregulate NKG2A expression on peripheral NK cells causing immunosuppression and reduction in NK cell cytotoxic activity[45–47]. Decreased NKG2A expression and increased KIR expression is also associated with NK cell maturation and differentiation[48,49]. Therefore, the observed decrease in inhibitory NK cell receptors following TB treatment completion likely corresponds to the decrease in Mtb burden in the lungs and reduction in NK cell activity.

Interestingly, in TB/HIV co-infected participants, a significant increase in NKp44 levels on CD56^{bright} NK cells and an increase in NKG2C levels on CD56^{dim} NK cells following TB treatment completion was found. Both NKp44 and NKG2C expression is associated with HIV and Mtb control. However, NKp44-expressing NK cells were also implicated in HIV disease progression, through lysis of NKp44 ligand expressing CD4^{+} T cells, a ligand that is specifically induced on CD4^{+} T cells from HIV-infected patients[50]. How TB treatment completion leads to increased expression of these activating NK receptors and their implications in HIV and TB pathogenesis remains to be determined.

An association between higher percentage of systemic anergic CD56^{neg} NK cells during active TB and shorter time to culture conversion was observed. Additionally, higher percentage of peripheral CD56^{bright} NK cells and higher expression of activating receptor NKG2D on CD56^{dim} NK cells were associated with longer time to culture conversion. One potential explanation for these associations could be the inverse relationship between percentage of these cells in the blood and in the lungs as they may be redistributed to the tissues of higher antigen concentration. This is supported by previous study showing an inverse correlation between peripheral NK cell frequencies and inflammatory burden in the lung[14]. Similarly, severe COVID-19 disease was associated with reduced frequencies of peripheral NK cells[51–53] and increased frequency of NK cells in the lungs[54,55]. However, better understanding of composition and function of NK cells in lung tissues and how these relate to peripheral NK cells and TB pathology is needed.

We further observed an association between activating receptors NKp30 and NKp46 and the presence of lung cavitation. Higher expression of NKp30 on systemic CD56^{bright} NK cells during active TB was associated with increased odds of lung cavitation while the higher expression of NKp46 on systemic CD56^{dim} NK cells was associated with lower odds of lung cavitation. NK cells can aid in pathogen clearance however, they can also be rather detrimental and contribute to lung immunopathology. NK cell related inflammation and injury to the

pulmonary structures was described in lung infection mouse model[56], and increased NK cell activity in the blood has been previously associated with cavitary disease in humans[57].

Our study has several limitations including the use of peripheral blood specimens to look at changes in lung immunopathology. Furthermore, our panels did not include an exhaustive list of activating and inhibitory receptors and due to limitations in cell numbers, we were unable to assess NK cell responsiveness. Despite these limitations, our data still shows distinct changes in systemic NK cell populations with respect to active disease, treatment completion, and disease severity in TB and TB-HIV co-infected individuals.

Data availability

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

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Author contributions
Designed the study: A.S., T.G.M.;Performed the experiments: T.G.M., S.R., S.N., A.S.;Analysed the data: T.G.M., A.S., L.L.;Wrote the first draft of the paper: T.G.M., A.S.;Collected specimens and clinical data: R.H.M., SN2;Supervised clinical and/or experimental aspects of the study: A.S.; N.P.; K.N.; D.A.; R.H.M.; R.P.;All authors contributed to the editing and finalisation of the manuscript.

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Competing interests
The authors declare no competing interests.

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Supplementary material

NK cell phenotypic profile during active TB in people living with HIV– evolution during TB treatment and implications for bacterial clearance and disease severity.

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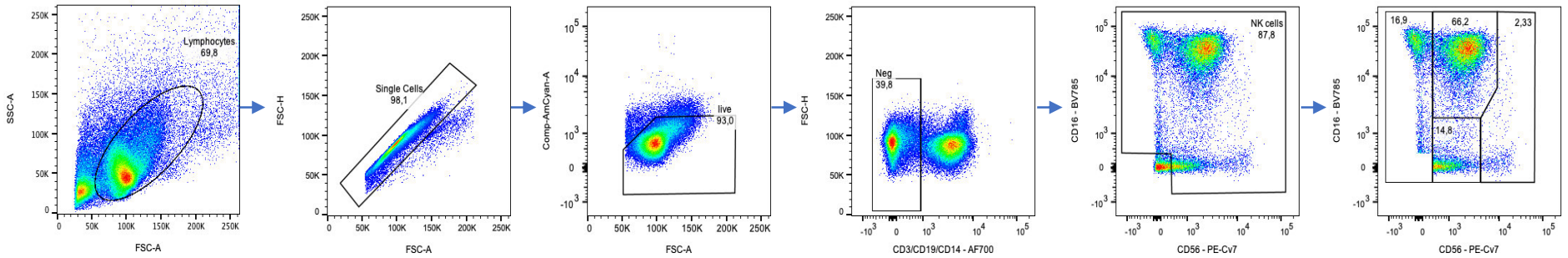
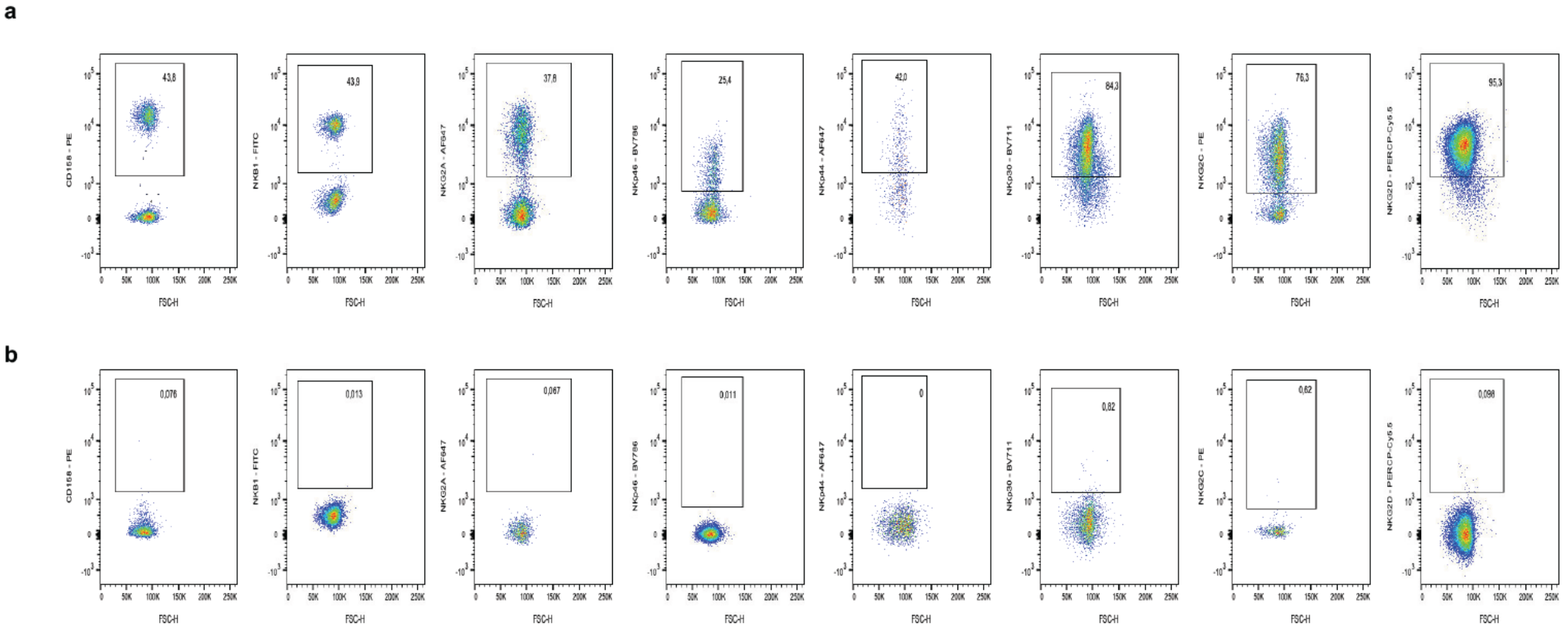
Supplementary table 1. Antibody panels for NK cell phenotypic characterization

Panels	Antigen	Fluorochrome	Clone	Manufacturer	Catalogue No.
Panel 1	CD3	AFluor700	SK	Biolegend	344822
	CD19	AFluor700	HIB19	BD Biosciences	557921
	CD14	AFluor700	MSE2	BD Biosciences	557923
	CD16	BV785	3G8	Biolegend	302046
	CD56	PECy7	B159	BD Biosciences	567747
	NKG2D	PerCPCy5.5	1D11	BD Biosciences	562364
	NKG2C	PE	134591	R&D Systems	FAB138P
	NKp30	BV711	P30–15	BD Biosciences	536383
	NKp44	APC	P44–8	BD Biosciences	558654
	Live/Dead	Amcyan	–	Thermofisher	L34957
Panel 2	CD3	AFluor700	SK	Biolegend	344822
	CD19	AFluor700	HIB19	BD Biosciences	557921
	CD14	AFluor700	MSE2	BD Biosciences	557923
	CD16	V450	3G8	BD Biosciences	560474
	CD56	PECy7	B159	BD Biosciences	567747
	CD335 (NKp46)	BV786	PE2	BD Biosciences	563329
	CD159a (NKG2A)	APC	Z199	Beckman Coulter	A60797
	CD158e	PE	Z27.3.7	Beckman Coulter	IM3292
	NKB1	FITC	Dx9	BD Biosciences	555966
	Live/Dead	Amcyan	–	Thermofisher	L34957



Supplementary figure 1. Representative flow cytometry plot illustrating parent gating. Lymphocytes were identified by plotting side scatter area (SCA) and forward scatter area (FCA). Single cells were identified by plotting forward scatter height (FCH) and area (FCA). Live cells were then identified by low expression of Aqua Dead cell stain. We then excluded cells with CD3, CD19 and CD14 surface markers by gating on the negative population. We identified the total NK cell population by gating on the CD56 and CD16 positive cells. These were further separated by gating on $CD56^{bright}$, $CD56^{dim}$, $CD56^{neg}$ and the $CD56^{dim}CD16^{-}$ subsets.



Supplementary figure 2. Representative flow cytometry plots showing expression of NKG2D, NKG2C, NKp30, NKp44, NKp46, NKG2A, NKB1 and CD158 on peripheral blood mononuclear cells. (a) Representative NK cell phenotypic marker expression from TB/HIV co–infected participant (b) Flourescence Minus One (FMO) controls.

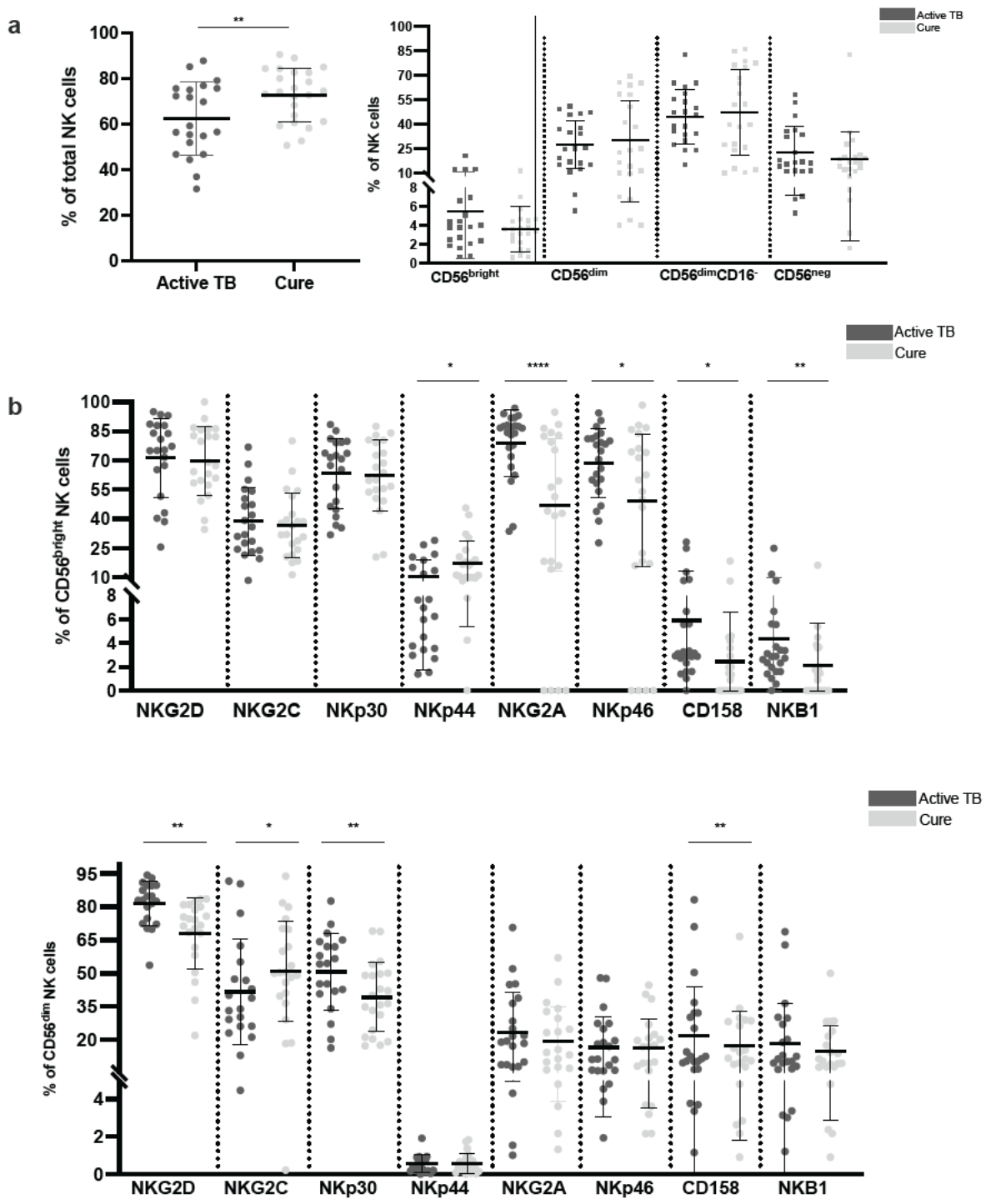
Supplementary table 2. Demographic and clinical characteristics of healthy controls.

Variables	Healthy control n = 13
Gender, n (%)	
Male	7 (46)
Female	6 (54)
Age (y), median (IQR)	35 (32 – 39)
Body mass index (kg/m^2), median (IQR)	23.29 (19.73 – 31.30)
HIV status, n (%)	
Positive	-
Negative	13

Supplementary table 3. NK cell population frequencies among participant groups

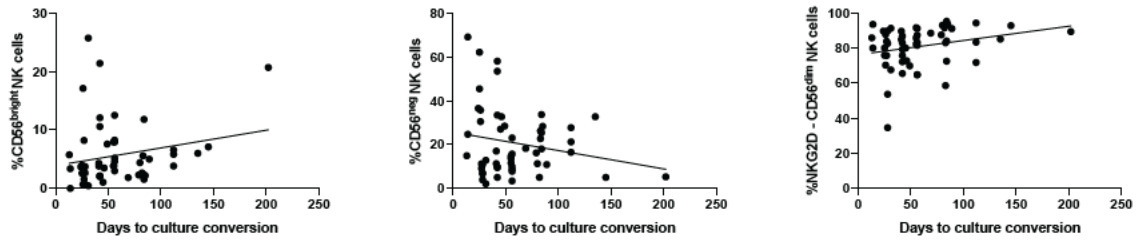
	TB/HIV	TB	HC	p-value
	Median (IQR)	Median (IQR)	Median (IQR)	
% Total NK cells	60.35	69.55	66.00	0.6841
	(46.70 - 75.15)	(54.85 - 79.05)	(43.60 - 81.65)	
% NK cells				
$CD56^{bright}$	3.730	4.895	4.720	0.4026
	(2.133 - 8.225)	(2.750 - 7.855)	(2.875 - 12.37)	
$CD56^{dim}$	26.80	36.85	57.70	0.1926
	(15.15 - 46.15)	(22.50 - 54.10)	(1.865 - 62.70)	
$CD56^{+}CD16^{-}$	42.45	37.85	26.50	0.9604
	(24.13 – 57.18)	(25.18 – 57.48)	(21.55 – 58.15)	
$CD56^{neg}$	18.10	11.40	10.70	**0.0028**
	(11.20 - 32.70)	(6.960 – 19.63)	(6.450 – 16.20)	
NK cell markers				
$CD56^{bright}$				
NKG2C	37.55	35.55	27.70	0.0927
	(23.75 - 61.60)	(21.65 - 44.75)	(21.30 - 33.60)	
NKG2D	75.30	70.50	57.50	0.1092
	(51.08 - 87.75)	(37.08 - 90.70)	(13.90 - 78.75)	
NKp30	65.35	64.70	36.00	**0.0264**
	(43.88 - 74.90)	(48.93 – 77.68)	(14.45 – 67.75)	
Nkp44	7.190	7.095	8.160	0.6916
	(3.683 - 13.05)	(2.960 - 9.895)	(4.150 - 20.50	
NKG2A	85.60	80.50	72.50	0.1619
	(74.60 - 91.00)	(72.15 - 88.18)	(61.35 - 85.30)	
NKp46	65.90	73.00	77.90	0.5296
	(54.20 - 80.00)	(63.85 - 77.78)	(59.30 - 84.25)	
CD158	2.905	2.805	1.130	0.3698
	(1.510 - 5.958)	(1.043 - 6.200)	(0.3100 - 4.280)	
NKB1	2.730	2.175	0.7200	0.1430
	(1.360 - 5.000)	(1.085 - 5.100)	(0.000 - 3.630)	
$CD56^{dim}$				
NKG2C	46.10	51.20	19.50	**0.0045**
	(29.18 - 66.50)	(35.28 - 60.68)	(12.25 - 32.90)	

NKG2D	84.85	81.00	58.30	**0.0001**
	(78.45 - 89.73)	(68.18 - 85.88)	(39.25 - 79.10)	
NKp30	55.60	57.75	51.80	0.1326
	(38.80 -64.50)	(47.83 - 79.40)	(36.50 - 66.70)	
NKp44	0.4400	0.2800	0.3900	0.1540
	(0.1800 - 0.8450)	(0.1550 -0.3950)	(0.2400 - 1.835)	
NKG2A	19.00	23.80	26.70	0.0958
	(8.770 - 33.10)	(18.53 - 35.00)	(19.55 - 41.00)	
NKp46	11.10	25.20	25.30	**0.0106**
	(6.230 - 25.00)	(15.05 - 31.43)	(11.33 - 39.30)	
CD158	11.60	14.30	14.20	0.9525
	(5.860 - 32.20)	(5.773 - 22.90)	(6.135 - 17.95)	
NKB1	10.80	11.80	12.20	0.9396
	(4.980 - 27.00)	(5.750 - 17.53)	(6.110 - 14.80)	

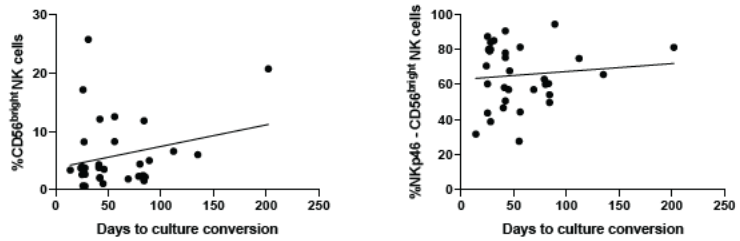


Supplementary figure 3. Effect of treatment completion on NK cell percentage and phenotypic marker expression in TB/HIV co–infected participants (n = 26). (a) Effect of treatment on the percentage of total NK cells and NK cell subsets. (b) Differences in activating and inhibitory receptor expression on $CD56^{bright}$ and $CD56^{dim}$ NK cells between active TB and post treatment completion. *p < 0.05; ** p < 0.01, *** p < 0.001 and ****p < 0.0001

A.



B.



Supplementary figure 4. Significant associations between peripheral NK cells and NK phenotypic markers and time to culture conversion. A) total cohort B) TB/HIV co-infected study participants.

Supplementary table 4. Association between NK cell phenotypes and time to culture conversion among the total cohort (n=63).

		Bivariable				Multivariable		
	Cell marker	**HR**	**CI**	**p–value**		**aHR**	**CI**	**p–value**
% Total NK cells		0.979	0.961 – 0.998	**0.027**		0.987	0.968 – 1.006	0.171
% $CD56^{bright}$		0.939	0.877 – 1.007	0.077		0.893	0.825 – 0.966	**0.005**
% $CD56^{dim}$		0.988	0.972 – 1.004	0.141		0.983	0.966 – 1.001	0.063
% $CD56^{dim}CD16^{–}$		1.006	0.991 – 1.021	0.443		1.008	0.992 – 1.025	0.319
% $CD56^{neg}$		1.022	0.999 – 1.045	0.063		1.029	1.006 – 1.052	**0.013**
% $CD56^{bright}$	NKG2C	0.988	0.975 – 1.002	0.090		0.992	0.978 – 1.005	0.232
	NKG2D	1.002	0.991 – 1.013	0.690		1.004	0.993 – 1.015	0.502
	NKp30	1.003	0.988 – 1.018	0.676		1.002	0.987 – 1.018	0.763
	NKp44	1.021	0.983 – 1.061	0.273		1.014	0.975 – 1.055	0.493
	NKB1	0.987	0.956 – 1.018	0.409		1.004	0.969 – 1.041	0.818
	NKG2A	1.006	0.987 – 1.025	0.528		0.998	0.979 – 1.017	0.802

	NKp46	0.995	0.978 – 1.012	0.530		0.986	0.97 – 1.003	0.099
	CD158	0.994	0.966 – 1.023	0.692		1.015	0.982 – 1.049	0.380
% $CD56^{dim}$	NKG2C	1.004	0.992 – 1.017	0.467		1.007	0.994 – 1.019	0.304
	NKG2D	0.977	0.953 – 1.001	0.064		0.961	0.932 – 0.990	**0.009**
	NKp30	0.993	0.979 – 1.008	0.376		0.994	0.978 – 1.01	0.462
	NKp44	1.024	0.513 – 2.046	0.946		0.769	0.37 – 1.598	0.481
	NKB1	0.997	0.983 – 1.011	0.691		0.999	0.985 – 1.015	0.943
	NKG2A	0.994	0.977 – 1.011	0.507		0.990	0.972 – 1.008	0.283
	NKp46	1.004	0.986 – 1.021	0.697		0.999	0.98 – 1.018	0.900
	CD158	0.999	0.988 – 1.011	0.915		1.001	0.988 – 1.013	0.924

Supplementary table 5. Association between NK cell phenotypes and time to culture conversion among TB/HIV co–infected participants (n=39)

		Bivariable				Multivariable		
	Cell marker	**HR**	**CI**	**p–value**		**aHR**	**CI**	**p–value**
% Total NK cells		0.982	0.958 – 1.005	0.128		0.993	0.968 – 1.020	0.621
% $CD56^{bright}$		0.951	0.882 – 1.026	0.196		0.858	0.768 – 0.959	**0.007**
% $CD56^{dim}$		0.99	0.969 – 1.012	0.372		0.987	0.962 – 1.013	0.326
% $CD56^{dim}CD16^{-}$		1.001	0.981 – 1.022	0.891		1.006	0.982 – 1.031	0.622
% $CD56^{neg}$		1.024	0.996 – 1.052	0.089		1.023	0.997 – 1.05	0.080
% $CD56^{bright}$	NKG2C	0.991	0.974 – 1.008	0.277		0.996	0.980 – 1.013	0.672
	NKG2D	1.009	0.993 – 1.024	0.272		1.009	0.994 – 1.024	0.251
	NKp30	1.011	0.993 – 1.029	0.25		1.007	0.987 – 1.026	0.505
	NKp44	1.014	0.970 – 1.06	0.532		1.002	0.956 – 1.051	0.932
	NKB1	0.965	0.920 – 1.012	0.142		0.990	0.940 – 1.043	0.706
	NKG2A	1.007	0.986 – 1.030	0.508		0.988	0.964 – 1.013	0.339
	NKp46	0.997	0.977 – 1.018	0.794		0.975	0.952 – 0.999	**0.045**
	CD158	0.977	0.938 – 1.019	0.282		1.007	0.961 – 1.056	0.760
% $CD56^{dim}$	NKG2C	1.006	0.992 – 1.021	0.411		1.013	0.997 – 1.03	0.119

	NKG2D	0.952	0.911 – 0.994	**0.026**		0.953	0.905 – 1.003	0.066
	NKp30	0.997	0.977 – 1.017	0.751		0.993	0.972 – 1.014	0.485
	NKp44	0.965	0.4 – 2.325	0.936		0.682	0.282 – 1.65	0.396
	NKB1	0.993	0.976 – 1.01	0.394		0.998	0.981 – 1.015	0.811
	NKG2A	0.993	0.972 – 1.015	0.540		0.983	0.96 – 1.005	0.133
	NKp46	0.997	0.969 – 1.026	0.851		0.981	0.951 – 1.011	0.213
	CD158	0.994	0.98 – 1.009	0.459		0.999	0.984 – 1.014	0.872

Supplementary table 6. Association between NK cell phenotypes and disease severity (lung cavitation) in the total cohort (n=55)

	Cell marker	Bivariable				Multivariable		
		OR	**CI**	**p–value**		**aOR**	**95% CI**	**p–value**
% Total NK cells		0.993	0.962 – 1.025	0.649		0.991	0.960 – 1.024	0.605
% $CD56^{bright}$		0.924	0.842 – 1.015	0.099		0.921	0.834 – 1.016	0.101
%$CD56^{dim}$		1.027	0.991 – 1.064	0.140		1.027	0.991 – 1.065	0.144
%$CD56^{dim}CD16^{-}$		0.974	0.945 – 1.003	0.083		0.971	0.942 – 1.001	0.059
% $CD56^{neg}$		1.032	0.985 – 1.082	0.190		1.041	0.989 – 1.095	0.124
% $CD56^{bright}$	NKG2C	0.980	0.954 – 1.006	0.128		0.979	0.953 – 1.006	0.127
	NK2D	1.011	0.988 – 1.034	0.348		1.014	0.989 – 1.040	0.274
	NKp30	1.028	0.998 – 1.058	0.068		1.036	1.002 – 1.071	**0.039**
	NKp44	1.039	0.956 – 1.128	0.371		1.050	0.957 – 1.152	0.302
	NKB1	1.053	0.940 – 1.178	0.372		1.052	0.938 – 1.179	0.388
	NKG2A	1.015	0.981 – 1.051	0.387		1.018	0.981 – 1.057	0.343
	NKp46	0.993	0.961 – 1.026	0.672		0.992	0.958 – 1.028	0.673
	CD158	1.068	0.956 – 1.194	0.246		1.068	0.955 – 1.194	0.248
% $CD56^{dim}$	NKG2C	0.997	0.973 – 1.022	0.840		0.997	0.972 –1.023	0.824
	NKG2D	1.015	0.965 – 1.067	0.557		1.020	0.966 – 1.077	0.470

	NKp30	1.005	0.976 – 1.034	0.760		1.005	0.974 – 1.034	0.755
	NKp44	0.552	0.174 – 1.745	0.311		0.558	0.158 – 1.971	0.365
	NKB1	1.041	0.987 – 1.098	0.135		1.040	0.988 – 1.094	0.132
	NKG2A	0.983	0.952 – 1.016	0.318		0.982	0.950 – 1.015	0.276
	NKp46	0.971	0.937 – 1.006	0.103		0.958	0.921 – 0.997	**0.035**
	CD158	1.026	0.988 – 1.066	0.177		1.025	0.988 – 1.064	0.184

Supplementary table 7. Association between NK cell phenotypes and disease severity (lung cavitation) among TB/HIV co–infected participants (n=35)

		Univariable				Multivariable		
	Cell marker	**OR**	**CI**	**p–value**		**aOR**	**CI**	**p–value**
% Total NK cells		0.996	0.958 – 1.036	0.838		0.994	0.952 – 1.038	0.778
% $CD56^{bright}$		0.913	0.806 – 1.035	0.154		0.840	0.685 – 1.031	0.096
%$CD56^{dim}$		1.009	0.966 – 1.053	0.700		1.009	0.965 – 1.055	0.697
%$CD56^{dim}CD16^{-}$		0.990	0.954 – 1.027	0.598		0.988	0.950 – 1.027	0.540
% $CD56^{neg}$		1.023	0.971 – 1.078	0.398		1.031	0.972 – 1.095	0.308
% $CD56^{bright}$	NKG2C	0.987	0.954 – 1.020	0.433		0.983	0.949 – 1.019	0.353
	NKG2D	1.003	0.969 – 1.038	0.876		1.004	0.969 – 1.040	0.825
	NKp30	1.029	0.990 – 1.069	0.142		1.035	0.993 – 1.079	0.108
	NKp44	1.028	0.941 – 1.123	0.538		1.037	0.939 – 1.144	0.474
	NKB1	1.068	0.916 – 1.244	0.402		1.069	0.911 – 1.254	0.416
	NKG2A	1.011	0.974 – 1.049	0.564		1.016	0.973 – 1.060	0.469
	NKp46	1.001	0.965 – 1.039	0.962		1.001	0.961 – 1.043	0.959
	CD158	1.099	0.938 – 1.287	0.244		1.109	0.939 –1.311	0.223
% $CD56^{dim}$	NKG2C	0.990	0.961 – 1.020	0.518		0.990	0.961 – 1.020	0.513
	NKG2D	0.956	0.870 – 1.049	0.342		0.960	0.870 – 1.058	0.407

	NKp30	1.002	0.966 – 1.040	0.895		0.999	0.962 – 1.038	0.976
	NKp44	0.591	0.152 – 2.297	0.447		0.571	0.133 – 2.462	0.453
	NKB1	1.058	0.987 – 1.134	0.114		1.063	0.986 – 1.146	0.112
	NKG2A	0.970	0.933 – 1.009	0.126		0.968	0.930 – 1.008	0.113
	NKp46	0.943	0.892 – 0.998	**0.044**		0.937	0.883 – 0.994	**0.032**
	CD158	1.046	0.990 – 1.105	0.108		1.050	0.991 – 1.113	0.100

CHAPTER 4

Memory $CD4^+$ T cell phenotypes in TB and TB/HIV co-infection

$CD4^+$ T cells are crucial for the control of Mtb infection with Th1 and Th17 cells being key effector cells for the control of Mtb [1,2]. Trafficking of these cells to the affected tissues plays a major role in the clearance and containment of Mtb.

HIV mediated depletion of $CD4^+$ T cells is associated with increased incidence of active TB and increased TB mortality. Additionally, HIV mediated changes in T helper cell distribution and cell surface integrin expression [3] could have important consequences on T cell trafficking into the lungs and the subsequent TB control.

In **Chapter 3** we characterised memory $CD4^+$ T cell subsets at active TB and post TB treatment completion in participants from the CAPRISA 011 IMPRESS study. We assessed the distribution of helper T cell subsets and $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrin expression on memory $CD4^+$ T cells in 45 participants with active TB with or without HIV co-infection. In addition, we characterised the effect of TB treatment completion on memory $CD4^+$ T cell subsets as well as the effects of measured $CD4^+$ memory T cell subsets at active TB on time to culture conversion and presence of cavitary disease.

Dr. A. Sivro and I, Thando Glory Maseko, conceptualised and designed the study. I retrieved and processed the peripheral blood mononuclear (PBMC) samples. I optimised and performed flow cytometry experiments to characterise memory $CD4^+$ T cell subsets. I acquired, cleaned up and analysed all the data. The obtained results were validated by Lara Lewis, a designated CAPRISA 011 study biostatistician. I wrote the first draft of the manuscript which was reviewed by Dr. A. Sivro and the rest of the co-authors. The manuscript is undergoing internal CAPRISA scientific committee review.

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T helper cell subsets and integrin $\alpha_4\beta_7$ and $\alpha_4\beta_1$ expression in TB-HIV co-infection

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Abstract:

Background: $CD4^+$ T cell responses are crucial for Mtb control and HIV mediated depletion of Mtb-specific $CD4^+$ T cells leads to increased susceptibility to TB, disease progression and TB reactivation.

Methods: Using flow cytometry**,** we assessed the differences in memory $CD4^+$ T cell phenotypes in TB infected participants, TB/HIV co-infected participants, and healthy controls. T helper cell memory populations included Th1, Th2, Th17, Th17.1, $CCR6^+$DN, $CCR6^+$DP. In addition, $\alpha_4\beta_7$, $\alpha_4\beta_7^{hi}$ and $\alpha_4\beta_1$ expressing memory $CD4^+$ T cells. Additionally, we characterised the effect of systemic $CD4^+$ T cell subsets on cavitary disease and Mtb clearance in TB-infected and TB/HIV co-infected participants from the CAPRISA 011 Improving Retreatment Success (IMPRESS) trial as well as the effect of anti-TB treatment on these subsets.

Results: A higher percentage of Th2 (p=0.0267) and lower percentage of Th9 cells (p=0.0001) were observed in TB/HIV co-infected participants compared to healthy controls. TB/HIV co-infected participants had a significantly lower percentage of Th17.1 (p=0.0263) and higher percentage of $CCR6^+$DN (p=0.0299) and $CCR6^+$DP (p=0.0144) cells compared to TB-infected participants. TB/HIV co-infected participants had a higher percentage of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ expressing memory $CD4^+$ T cells in comparison to healthy controls. Additionally, TB/HIV co-infected participants had higher percentage of $\alpha_4\beta_7$ (p=0.0011) and $\alpha_4\beta_7^{hi}$ (p=0.0425) expressing memory $CD4^+$ T cells compared to TB participants. Following TB treatment completion, we observed a significant increase in percentage of $CCR6^+$DP cells (p=0.0481) in the total cohort. Cox proportional hazards were used to assess the association between $CD4^+$ T subtypes with time to culture conversion while logistic regression models were used to assess the association between cavitary disease and $CD4^+$ T subtypes respectively. We observed no significant association between memory $CD4^+$ T subtypes with time to culture conversion and cavitary disease in the total cohort and among TB/HIV co-infected individuals, likely due to limited sample size.

Conclusion: During active TB, HIV induces changes in $CD4^+$ T cell subset distribution and lymphocyte trafficking marker expression that may have detrimental effects on Mtb control.

Keywords: Tuberculosis, HIV, $CD4^+$ T cells, T helper cells, integrins

Introduction

$CD4^+$ T cells play a crucial role in the host immune response against TB and HIV [1,2]. HIV induced immune suppression and depletion of $CD4^+$ T cells have further highlighted the role that $CD4^+$ T cells play in TB infection. People living with HIV (PLHIV) are 15 times more likely to get infected with Mtb compared to their HIV negative counterparts [3]. In addition to the general depletion of $CD4^+$ T cells, HIV was shown to specifically deplete *Mycobacterium tuberculosis* (Mtb) specific $CD4^+$ T cells resulting in an increased risk of TB infection, reactivation of latent TB infection, and TB dissemination [4,5]. Aside from their protective roles in Mtb clearance, $CD4^+$ T cell responses have also been implicated in the development of lung cavitation [6,7], with HIV positive individuals having a lower odds of cavitary TB disease compared to HIV negative individuals with TB [8-10].

HIV infection is also known to affect the balance in Th responses, with a shift from a Th1 to a Th2 response [11] and an extensive depletion of Th17 cells [12]. Th1 and Th17 cells are considered to be the main effector cells during TB infection, and disease. IFN-γ producing Th1 cells activate antimicrobial activity in macrophages while Th17 cells, through their proinflammatory action, have been implicated in TB pathology [13]. Rapid trafficking of antigen-specific $CD4^+$ T cells to the lung is crucial for the control of Mtb replication [14,15], and was shown to be mediated by integrin $\alpha_4\beta_1$ [16]. HIV was shown to preferentially target activated, central memory $CD4^+$ T cells [17,18] and $CD4^+$ T cells which express integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$ [19-21] .Therefore, HIV mediated changes in $CD4^+$ T cell subsets could have important effects on Mtb replication and TB disease outcome.

South Africa has a high burden of both TB and HIV. The diagnosis and treatment of Mtb among TB/HIV co-infected individuals is challenging due to atypical clinical presentations, including paucibacillary, smear negative disease [22,23] and complex drug interactions between concomitantly administered anti-TB treatment and antiretroviral therapy, respectively [23,24]. Utilising specimens from the CAPRISA 011 Improving Retreatment Success (IMPRESS) clinical trial, we assessed the differences in memory $CD4^+$ T cell phenotypes between TB and TB/HIV co-infected participants as well as the effect of TB treatment on changes in $CD4^+$ T cell memory phenotypes. Additionally, we assessed the associations between systemic memory $CD4^+$ T cell subsets and time to culture conversion and cavitary disease.

Materials and methods

Study cohort

Stored peripheral blood mononuclear cell (PBMC) samples included in this sub-study were obtained from 45 participants with active TB with or without HIV co-infection from the CAPRISA 011 Improving Retreatment Success (IMPRESS) trial (ClinicalTrials.gov: NCT02114684) and from 13 healthy controls from KwaZulu-Natal, South Africa (BE432/12). The IMPRESS study was an open-label randomised control trial comparing interventional moxifloxacin-containing treatment to standard TB treatment for the improvement of culture conversation rates in people with active pulmonary TB [25]. Trial inclusion criteria were age ≥ 18 years, history of TB treatment, and a TB diagnosis confirmed by GeneXpert MTB/RIF® (Cepheid, USA) or positive Mtb sputum culture. The IMPRESS study obtained ethical approval from the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BFC029/13) and the South African Medicines Control Council (Ref:20130510). All the participants provided written informed consent for study enrolment and approval for the use of stored biological specimens for research purposes. The protocol for this sub-study was approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC/00002197/2020).

Flow cytometry experiments and gating strategy

Thawed PBMCs were washed and resuspended in pre-warmed 5 ml complete media [RPMI 1640 medium containing 25mM HEPES buffer and 2mM L-glutamine supplemented with 10% v/v fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Lonza)] and rested at 37ºC in 5% CO_2 for 3 hours. The flow cytometry panel (Supplementary Table 1) was used to surface stain one million cells for 20 minutes in the dark. Cells were washed twice and resuspended in 1x CellFix™ (BD) and acquired on the flow cytometer (BD LSRFortessa™ Cell analyzer) using the FACSDiva software version 8.0.2 (BD, USA). The gating strategy used to identify T helper (Th) cell memory populations Th1, Th2, Th9, Th17, Th17.1, $CCR6^+$DP and $CCR6^+$DN expression is shown in Figure 1. $CD4^+$ memory T cells were identified as live $CD3^+CD4^+CD45RA^-$ cells, and further classified into Th1 ($CCR6^-CCR4^-CXCR3^+CCR5^+$), Th2 ($CCR4^+CCR6^-$), Th9 ($CCD6^+CCR4^-$), Th17 ($CCR6^+CCR4^+$), Th17.1 ($CCR6^+CCR4^-CXCR3^+$), $CCR6^+$DN ($CCR6^+CCR4^-CXCR3^-$), and $CCR6^+$DP ($CCR6^+CCR4^-CXCR3^+$). Additionally, we gated on $\alpha_4\beta_7$, $\alpha_4\beta_7^{hi}$ and $\alpha_4\beta_1$ expressing memory $CD4^+$ T cells (Figure 1).

Statistical analysis

Data were analysed using IBM Statistics version 27 and GraphPad Prism version 9.3.1. with data distribution determined using the D'Agostino-Pearson omnibus normality test. To assess differences in percentage of T helper cell memory populations and integrins across participant subgroups, one-way ANOVA with Tukey's multiple comparisons test was used on normally distributed data, and Kruskal

Wallis test with Dunns multiple comparisons test on non-normally distributed data. To compare T helper cell profiles between active TB and following treatment completion, we performed a paired t-test for parametric data and the Wilcoxon signed-rank test for non-parametric data. The association between $CD4^+$ T cell subtypes and days to culture conversion (two consecutive negative sputum culture results) was modelled using Cox proportional hazards models. In the Cox proportional hazards model, deceased participants were removed from the sample while those that did not culture convert before leaving the study were censored on their study termination date. The association between the $CD4^+$ T cell subtypes and cavitary disease at baseline was modelled using logistic regression models. The multivariable Cox and logistic regression models were adjusted for age, sex, and HIV status, treatment randomization arm. For the multivariable analysis of TB/HIV co-infected sub-group we adjusted for age, sex, and treatment randomization arm.

Results

Participant characteristics

The CAPRISA 011 IMPRESS study group (n=45) was made up of 66.7% (n=30) males with a median age of 33 years [interquartile range (IQR) 27 – 43] (Table 1). People living with HIV made up 57.8% of the study group (n=26). Lung cavitation was present in 28 (62.2%) participants in the total study group. Participants with HIV had a median HIV viral load of 26 520 copies/ml (IQR 26 520 – 668 236) and median CD4 count of 285 cells/mm^3 (IQR 127.5 – 382), with 50% receiving antiretroviral therapy.

Table 1. Clinical and demographic characteristics of CAPRISA 011 IMPRESS study population.

Variables	Total cohort n = 45	HIV positive n = 26	HIV negative n = 19
Randomization arm n (%)			
HRZE - Control	18 (40.0)	9 (34.6)	9 (47.4)
HRZM - Active	27 (60.0)	17 (65.4)	10 (52.6)
Gender, n (%)			
Male	30 (66.7)	14 (53.8)	16 (84.2)
Age (y), median (IQR)	33 (27 – 43)	33.5 (30.8 – 41.5)	29 (23 - 52)
Body mass index (kg/m^2), median (IQR)	20.4 (18.6 – 23.4)	20.8 (18.9 – 23.9)	20.0 (18.5 – 21.8)
HIV Viral load (copies/ml), median (IQR)	-	26, 520 (940 – 668236)	-
CD4 cell count (cells/mm^3), median (IQR)	-	285.5 (127.5 – 382)	-
ARV status[a], n (%)			
Receiving ART	-	13 (50.0)	-
Lung Cavities[b], n (%)			
None	11 (24.4)	7 (26.9)	4 (21.1)
Unilateral	15 (33.3)	9 (34.6)	6 (31.6)
Bilateral	13 (28.9)	8 (30.8)	5 (26.3)
Alcohol Use in the past 3 months n (%)			
Yes	8 (17.8)	7 (26.9)	1 (5.3)
Smoking in past 3months n (%)			
Yes	15 (33.3)	7 (26.9)	8 (42.1)

Clinical and demographic characteristics reported at baseline
HRZE: treatment comprised of isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E)
HRZM: isoniazid (H), rifampicin (R), pyrazinamide (Z), Moxifloxacin (M);
[a] One participant with missing ARV status
[b] Six participants with missing lung cavitation status

Effect of HIV co-infection on percentage of memory CD4$^+$ T cell subsets in patients with active TB

We compared the percentage (%) of memory CD4$^+$ T cell subsets (Th1, Th2, Th9, Th17, Th17.1, CCR6$^+$DN, CCR6$^+$DP, $\alpha_4\beta_7^+$, and $\alpha_4\beta_1^+$) across participant groups: CAPRISA 011 TB/HIV co-infected

participants (TB/HIV), CAPRISA 011 HIV-negative participants with active TB (TB), and healthy controls (HC), (Figures 1 and 2). We observed significantly higher percentage of Th2 cells in TB/HIV co-infected participants compared to healthy controls (p=0.0267) and a lower % of Th9 cells among the TB/HIV co-infected compared to healthy controls (p=0.0001) and TB infected participants (p=0.0001). The TB/HIV co-infected participants had lower % of Th17.1 (p=0.0263) cells compared to TB infected participants. Additionally, the % of CCR6^{+}DN and CCR6^{+}DP cells was higher among TB/HIV co-infected participants compared to TB-infected participants (p=0.0299 and p=0.0144 respectively). The % of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin expressing memory CD4^{+} T cells were significantly higher among TB/HIV co-infected participants in comparison to healthy controls (p=0.0037, p=0.0007 respectively). The % of $\alpha_4\beta_7$ and $\alpha_4\beta_7^{hi}$ expressing memory CD4^{+} T cells were higher among TB/HIV co-infected participants compared to TB-infected participants (p=0.0011, p=0.0425 respectively).

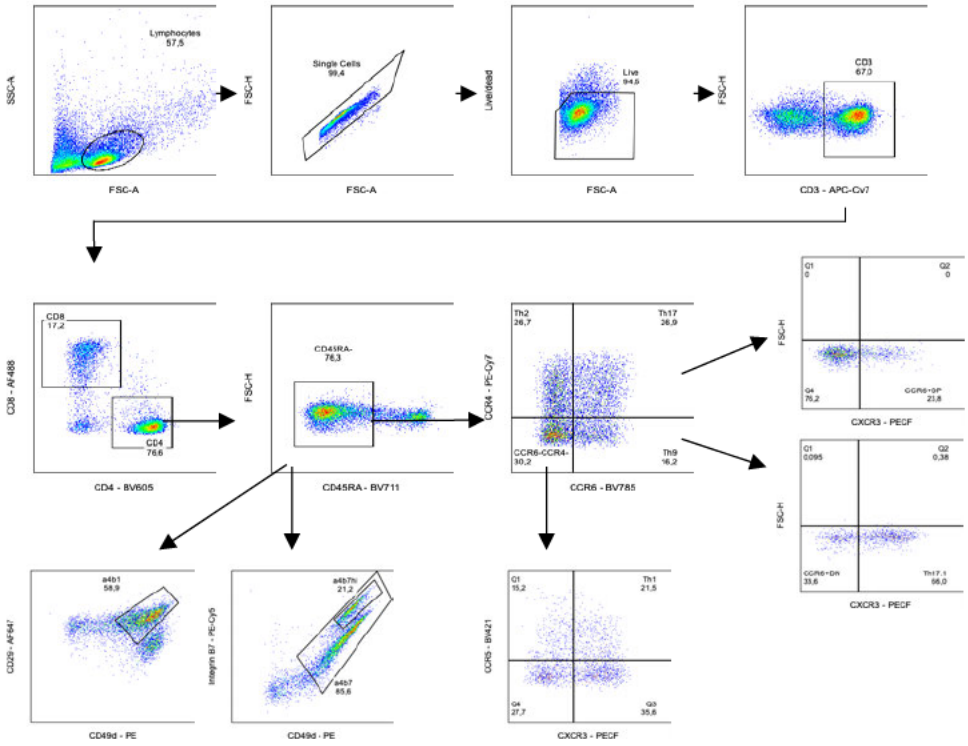

Figure 1. Representative flow cytometry gating from a TB/HIV co-infected participant. Following gating on lymphocytes and singlets, CD4^{+} memory T cells were identified as live CD3^{+}CD4^{+}CD45RA^{-} cells and further classified into Th1 (CCR6^{-}CCR4^{-}CXCR3^{+}CCR5^{+}), Th2 (CCR4^{+}CCR6^{-}), Th9 (CCD6^{+}CCR4^{-}), Th17 (CCR6^{+}CCR4^{+}), Th17.1 (CCR6^{+}CCR4^{-}CXCR3^{+}), CCR6^{+}DN (CCR6^{+}CCR4^{-}CXCR3^{-}), and CCR6^{+}DP (CCR6^{+}CCR4^{-}CXCR3^{+}). Additionally, we gated on α4β7, α4β7^{hi} and α4β1 expressing memory CD4^{+} T cells.

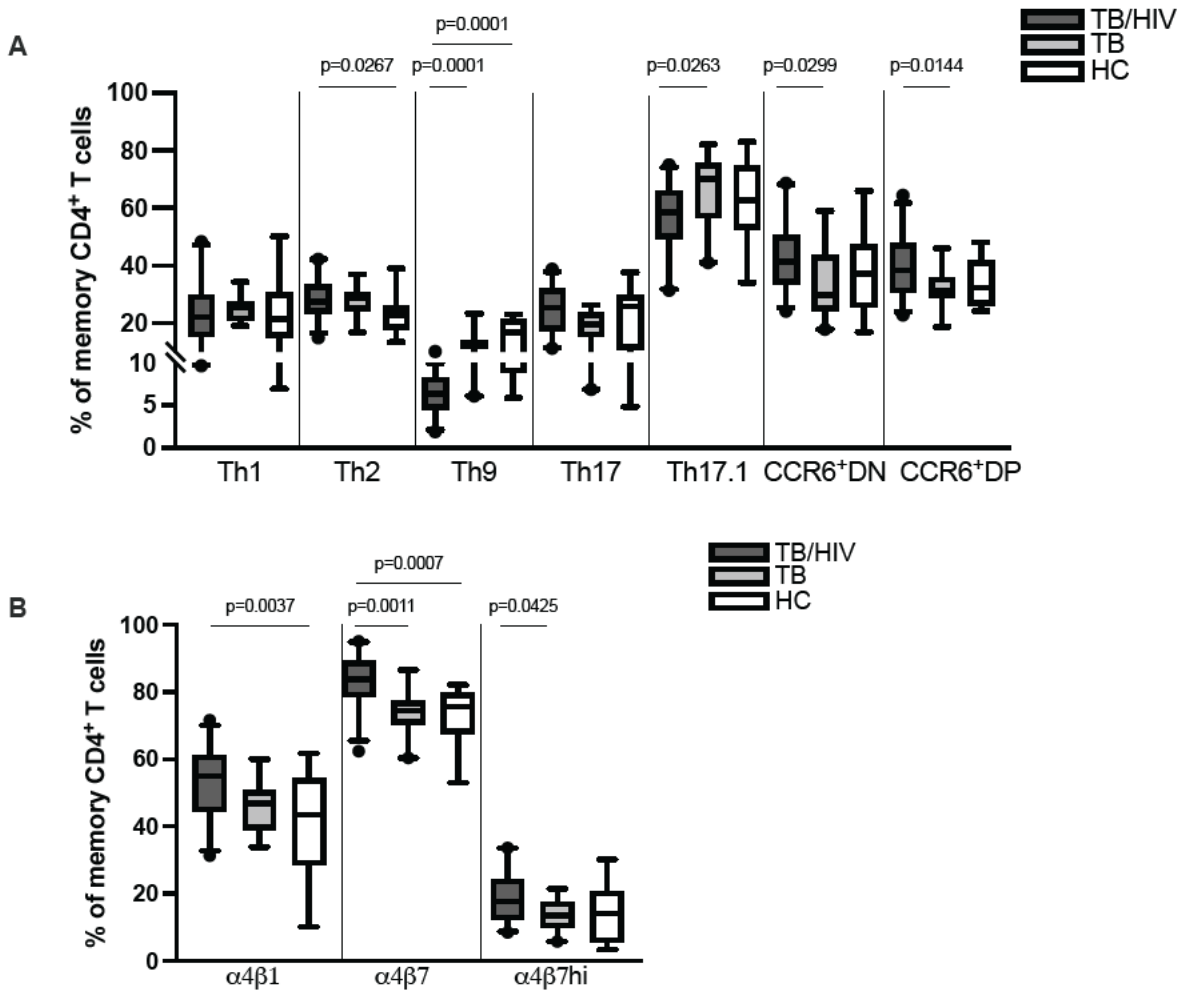

Figure 2. $CD4^+$ memory T cell subsets across study groups (TB/HIV, TB, HC). A) Th cell populations B) α4β1, α4β7 and $\alpha4\beta7^{hi}$ integrin expression. Boxes from box and whisker plot represent median and interquartile ranges while whiskers represent 5^{th} - 95^{th} percentiles.

Effect of TB treatment completion on memory $CD4^+$ T cell subsets.

To assess the effect of anti-TB treatment completion, we compared the percentages of memory $CD4^+$ T cell subsets during active TB and following TB treatment completion in paired samples (n=20) (Figure 3). We observed a significantly higher % of $CCR6^+$DP cells following treatment completion (p=0.0481) in the overall cohort. We observed no other significant changes in memory $CD4^+$ T cell profiles between active TB and after treatment completion in the overall cohort, including in TB/HIV co-infected participants (Supplementary Figure 1).

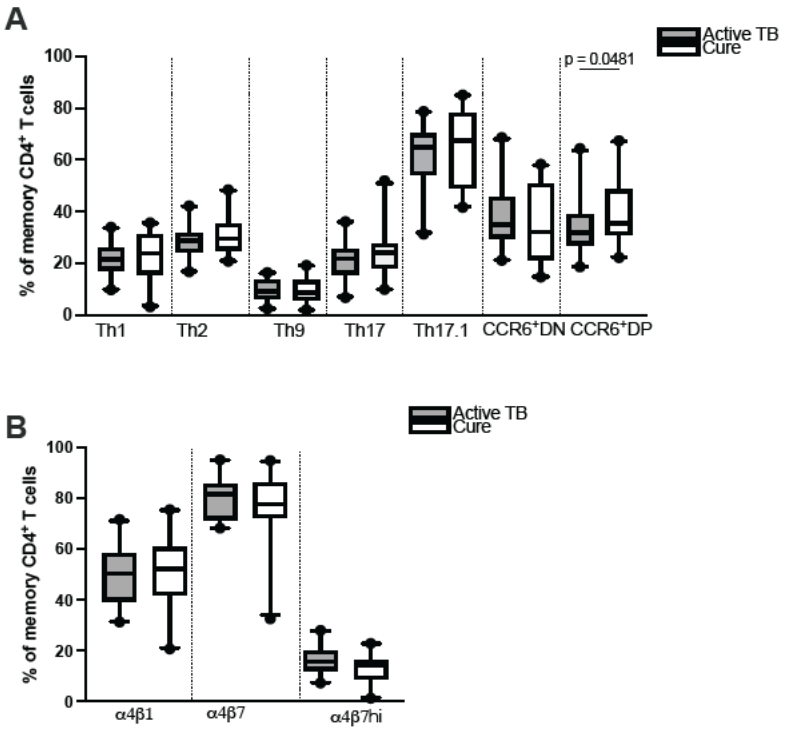

Figure 3. Effect of TB treatment completion on memory $CD4^+$ T cell subsets in TB and TB-HIV co-infected participants (n=20). (A) Differences between Th cell populations and (B) α4β1, α4β7 and $\alpha4\beta7^{hi}$ expression during active TB and post anti-TB treatment completion. Boxes from box and whisker plot represent median and interquartile ranges while whiskers represent 5th - 95th percentiles.

Association between memory $CD4^+$ T cell subsets and time to culture conversion and cavitary disease.

We analysed the association between memory $CD4^+$ T cell subsets and time to culture conversion as well as cavitary disease. We observed no significant association between measured memory $CD4^+$ T subtypes and time to culture conversion in the total cohort or the TB/HIV sub-group (Table 2, Supplementary Table 2). In addition, we observed no significant association between memory $CD4^+$ T cell subsets and cavitary disease in the total cohort or the TB/HIV sub-group (Table 3, Supplementary Table 3).

Table 2. Associations between memory CD4$^+$ T cell subsets and time to culture conversion in the total cohort (n=37)

Marker	Bivariable			Multivariable		
	HR	95% CI	p-value	aHR	95% CI	p-value
Th1	0.973	0.927 – 1.022	0.276	0.973	0.931 – 1.018	0.235
Th2	1.035	0.981 – 1.091	0.214	1.018	0.959 – 1.081	0.559
Th9	0.974	0.893 – 1.062	0.550	0.931	0.792 – 1.094	0.384
Th17	0.989	0.943 – 1.037	0.651	0.971	0.921 – 1.024	0.282
Th17.1	0.991	0.962 – 1.022	0.584	0.983	0.950 – 1.017	0.332
CCR6$^+$DP	1.002	0.968 – 1.037	0.915	0.995	0.956 – 1.036	0.822
CCR6$^+$DN	1.008	0.978 – 1.040	0.596	1.017	0.983 – 1.052	0.339
α4β1	0.992	0.958 – 1.028	0.671	0.992	0.956 – 1.029	0.664
α4β7	1.012	0.973 – 1.053	0.553	1.003	0.962 – 1.046	0.875
α4β7^{hi}	1.016	0.964 – 1.071	0.551	0.999	0.943 – 1.060	0.986

Table 3. Associations between memory CD4$^+$ T cell subsets and cavitary disease in the total cohort (n=39)

Marker	Bivariable			Multivariable		
	OR	95% CI	p-value	OR	95% CI	p-value
Th1	1.003	0.923 – 1.089	0.951	1.003	0.925 – 1.088	0.938
Th2	0.933	0.836 – 1.041	0.216	0.924	0.822 – 1.039	0.187
Th9	1.070	0.895 – 1.279	0.458	1.150	0.861 – 1.536	0.344
Th17	1.002	0.917 – 1.096	0.960	1.004	0.910 – 1.108	0.934
Th17.1	0.997	0.941 – 1.057	0.928	0.994	0.936 – 1.057	0.855
CCR6$^+$DP	1.007	0.940 – 1.078	0.848	1.013	0.941 – 1.091	0.730
CCR6$^+$DN	1.004	0.947 – 1.063	0.903	1.007	0.947 – 1.070	0.829
α4β1	1.043	0.972 – 1.120	0.240	1.061	0.980 – 1.149	0.143
α4β7	1.040	0.957 – 1.131	0.354	1.058	0.961 – 1.165	0.252
α4β7^{hi}	0.963	0.874 – 1.062	0.451	0.946	0.846 – 1.058	0.331

Discussion

The TB and HIV syndemic remains a public health challenge. $CD4^+$ T cells responses are crucial for host defense against Mtb; however, HIV-mediated depletion of Mtb-specific T cells results in an increased risk of TB infection, TB reactivation, and disease progression [6,26]. Here, we identified several differences in $CD4^+$ memory T cell phenotype in the context of TB/HIV coinfection.

We observed significantly higher % of Th2 cells in TB/HIV co-infected participants compared to healthy controls. While Th1 responses are key to Mtb control, Th2 responses result in loss of TB control and are associated with more severe disease [27]. Additionally, the progression of clinical TB is associated with a Th2 immune response signature, alongside elevated levels of the suppressor of cytokine signalling 3 (SOCS3) [27]. Th2-type cytokine responses with an increase in IL-4 expression was observed in HIV seronegative patients with cavitary TB [28] with IL-4 being thought downregulate Th1 responses [29]. Patients with higher $CD4^+$ T cell percentage and predominant IFN-γ expression characteristic of Th1 responses had non-cavitary TB while Th2 responses were correlated with cavitary TB in patients with active TB regardless of HIV status [30]. HIV is known to cause a switch from Th1 to Th2 response [11] and this could be another mechanism by which HIV impairs Mtb control in co-infected patients.

The percentage of Th9 cells (IL-9 producing $CD4^+$ T helper cells) was significantly lower in TB/HIV co-infected participants compared to both TB and healthy control groups. Plasma Th9 cytokine production was shown to be significantly decreased with progression from acute to chronic HIV infection [31]. While data on Th9 responses in TB is limited, higher levels of Th9 cells were reported in tuberculous pleural effusion compared to blood, with IL-9 produced by Th9 cells associated with wound healing [32].

We additionally observed a lower % of Th17.1 cells in TB/HIV co-infected participants compared to TB participants. $CCR6^+$ Th cell populations are distinguished by chemokine receptor and cytokine expression with Th17.1 cells producing IL-17 and IFN-γ [33]. Th17.1 ($CCR6^+CXCR3^+CCR4^-$) cells were shown to be important in control of TB infection [34] and inversely correlate with Mtb burden [35]. The % of $CCR6^+$DN ($CCR4^-CXCR3^-$) and $CCR6^+$DP ($CCR4^+CXCR3^+$) cells was higher among TB/HIV co-infected participants compared to TB-infected participants. Peripheral $CCR4^+CCR6^+$ and $CXCR3^+CCR6^+$ T cells were shown to be highly permissive to HIV infection due to co-expression of CCR5 [36] while $CCR6^+$ DN cells have been implicated in HIV persistence during ART [37]. The role of these cells in TB infection is hard to define as most studies do not distinguish different subclasses of the $CCR6^+$ subset.

We observed an increase in both $\alpha_4\beta_7$ and $\alpha_4\beta_1$ integrins in TB/HIV co-infection compared to healthy controls. Additionally, we observed increased expression of $\alpha_4\beta_7$ and $\alpha_4\beta_7^{hi}$ in TB/HIV co-infection

compared to active TB alone. Integrin $\alpha_4\beta_1$ has been implicated in lung homing of $CD4^+$ T cells several lung diseases including asthma [38] and TB [16]. Integrin $\alpha_4\beta_7$, a gut homing marker, was shown to play an important role in HIV pathogenesis [39,40], with increased expression of the $\alpha_4\beta_7$ integrin on circulating memory $CD4^+$ T cells associated with increased risk of HIV acquisition and disease progression [41]. The alternations in integrin expression during TB/HIV co-infection could have consequences for $CD4^+$ T cell distribution and trafficking during Mtb infection.

This study has several study limitations including a small sample size precluding the adjusting for the effects of other clinical and demographic variables that may impact the observed differences in $CD4^+$ T cell phenotypes. Additionally, we did not observe any significant effect of measured memory $CD4^+$ T cell phenotypes on time to culture conversion and the presence of cavitary disease at baseline and this is likely impacted by the small sample size and lack of statistical power. Another limitation of the study is the lack of cytokine production measurement to identify Th cell subsets more accurately. Characterising immunological TB and TB-HIV biomarkers is crucial for a better understanding of the TB and TB/HIV pathogenesis and development of vaccines and host-targeted therapeutics.

Competing interest

The authors declare no conflict of interests.

Availability of data and materials

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

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Authors contributions

Designed the study: AS, TGM;

Performed the experiments: TGM, SR, SN, AS;

Analysed the data: TGM, AS, LL;

Wrote the first draft of the paper: TGM, AS;

Collected specimens and clinical data: RHM;

Supervised clinical and/or experimental aspects of the study: AS; NP; KN; DA; RHM; RP;

All authors contributed to the editing and finalisation of the manuscript.

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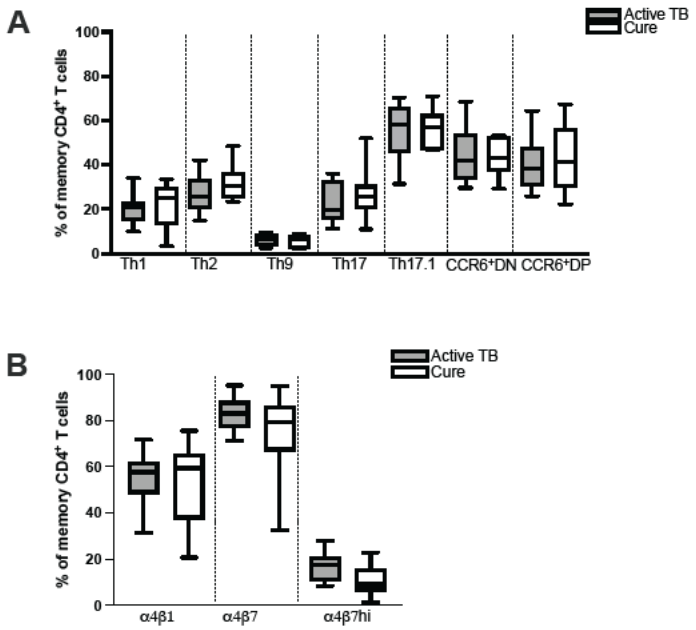
Supplementary material

Supplementary Table 1. Antibody panels for memory $CD4^+$ T cell subset flow cytometric characterisation.

Antigen	Flourochrome	Clone	Manufacturer	Catalogue No.
CD3	APC-H7	SK7	BD	560176
CD4	BV605	RPA-T4	BD	562658
CD8	AF488	RPA-T8	BIOLEGEND	301021
CD45RA	BV711	HI100	BIOLEGEND	304138
CD49d (α)	PE	9F10	BD	555503
CD29 (β1)	AF647	TS2/16	BIOLEGEND	303018
β7	PE-Cy5	FIB504	BD	551059
CD194/CCR4	PE-Cy7	L291H4	BIOLEGEND	359410
CD195/CCR5	BV421	2D7	BD	562576
CD196/CCR6	BV785	G034E3	BIOLEGEND	353422
CD183/CXCR3	PECF	G025H7	BIOLEGEND	353736
Live/dead	Amcyan	-	Thermofisher	L34957



Supplementary Figure 1. Effect of TB treatment completion on memory $CD4^+$ T cell subsets in TB-HIV co-infected participants (n=10). Differences between Th cell population and (B) α4β1 and α4β7 integrin expression during active TB and post anti-TB treatment completion. Boxes from box and whisker plot represent median and interquartile ranges while whiskers represent 5th - 95th percentiles.

Supplementary Table 2. Associations between memory $CD4^+$ T cell subsets and time to culture conversion among TB/HIV co-infected participants (n=21)

Marker	Bivariable			Multivariable		
	OR	95% CI	p-value	OR	95% CI	p-value
Th1	0.987	0.938 – 1.040	0.929	0.980	0.937 – 1.025	0.383
Th2	1.019	0.956 – 1.088	0.558	1.014	0.945 – 1.087	0.703
Th9	1.001	0.804 – 1.246	0.992	0.937	0.733 – 1.198	0.604
Th17	0.996	0.936 – 1.061	0.912	0.999	0.938 – 1.065	0.979
Th17.1	0.984	0.948 – 1.022	0.414	0.985	0.947 – 1.025	0.459
$CCR6^+DP$	0.972	0.920 -1.028	0.324	0.972	0.920 – 1.027	0.305
$CCR6^+DN$	1.016	0.978 – 1.055	0.422	1.015	0.975 – 1.055	0.472
α4β1	1.016	0.971 – 1.063	0.500	1.019	0.972 – 1.068	0.437
α4β7	1.036	0.980 – 1.094	0.213	1.025	0.965 – 1.089	0.420
$α4β7^{hi}$	1.066	0.973 – 1.167	0.170	1.038	0.934 – 1.153	0.490

Supplementary Table 3. Associations between memory $CD4^+$ T cell subsets and cavitary disease in the among TB/HIV co-infected participants (n=17)

Marker	Univariable			Multivariable		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Th1	1.000	0.917 – 1.090	0.996	0.992	0.907 – 1.085	0.858
Th2	0.931	0.817 – 1.060	0.281	0.942	0.821 – 1.082	0.399
Th9	1.314	0.865 – 1.995	0.201	1.375	0.866 – 2.182	0.177
Th17	0.996	0.893 – 1.111	0.941	0.991	0.886 – 1.108	0.873
Th17.1	1.003	0.933 – 1.078	0.933	0.996	0.921 – 1.078	0.930
$CCR6^+DP$	1.014	0.932 – 1.103	0.750	0.996	0.905 – 1.095	0.933
$CCR6^+DN$	0.998	0.929 – 1.073	0.965	1.006	0.929 – 1.088	0.889
$\alpha4\beta1$	1.089	0.994 – 1.194	0.068	1.081	0.975 – 1.199	0.140
$\alpha4\beta7$	1.072	0.956 – 1.201	0.233	1.057	0.939 – 1.189	0.359
$\alpha4\beta7^{hi}$	0.929	0.822 – 1.050	0.239	0.929	0.818 – 1.055	0.256

CHAPTER 5

Plasma biomarkers of cavitary disease in MDR/XDR tuberculosis

Amplified by the HIV/AIDS epidemic, MDR/XDR TB, with its high mortality rates, poses a dire threat to public health. This is particularly relevant in South Africa where the burden of the two diseases is most severe. Drug resistance mainly occurs when patients are exposed to inadequate treatment due to long waits for drug susceptibility testing results, highlighting the need for more efficient diagnostic and treatment options for DR-TB. Lung cavitation, a defining pathologic feature of Mtb infection, is associated with poor outcomes, high transmission rates, and development of drug resistance [1]. Identifying blood-based biomarkers of disease severity could represent a cost-effective method to customise treatment and improve health outcomes.

Chapter 4 investigates the association between systemic inflammatory markers measured at active disease and presence of cavitary disease in 128 patients with MDR/XDR TB enrolled in the CAPRISA 020 InDEX study. We additionally assessed the association between relevant demographic and clinical variables on presence of cavitary disease in this population.

Dr. A. Sivro and I, Thando Glory Maseko, conceptualised and designed the study. I optimised and performed the experiments. I cleaned up, formatted, and analysed the data. The obtained results were validated by Marothi Letsoalo, a designated CAPRISA 020 study biostatistician. I wrote the first draft of the manuscript which was reviewed by Dr. A. Sivro and the rest of the co-authors. The manuscript was submitted on 9 January 2023 and has been published by *BMC Immunology*.

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Higher plasma interleukin – 6 levels are associated with lung cavitation in drug-resistant tuberculosis

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Abstract

Background Lung cavitation is associated with heightened TB transmission and poor treatment outcomes. This study aimed to determine the relationship between systemic inflammation and lung cavitation in drug-resistant TB patients with and without HIV co-infection.

Methods Plasma samples were obtained from 128 participants from the CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX) prior to treatment initiation. Lung cavitation was present in 61 of the 128 drug-resistant TB patients with 93 being co-infected with HIV. The plasma cytokine and chemokine levels were measured using the 27-Plex Human Cytokine immunoassay. Modified Poisson regression models were used to determine the association between plasma cytokine/chemokine expression and lung cavitation in individuals with drug-resistant TB.

Results Higher Interleukin-6 plasma levels (adjusted risk ratio [aRR] 1.405, 95% confidence interval [CI] 1.079–1.829, p = 0.011) were associated with a higher risk of lung cavitation in the multivariable model adjusting for age, sex, body mass index, HIV status, smoking and previous history of TB. Smoking was associated with an increased risk of lung cavitation (aRR 1.784, 95% CI 1.167–2.729, p = 0.008). An HIV positive status and a higher body mass index, were associated with reduced risk of lung cavitation (aRR 0.537, 95% CI 0.371–0.775, p = 0.001 and aRR 0.927, 95% CI 0.874–0.983, p = 0.012 respectively).

Conclusion High plasma interleukin-6 levels are associated with an increased risk of cavitary TB highlighting the role of interleukin-6 in the immunopathology of drug-resistant TB.

Keywords Tuberculosis, HIV, MDR, XDR, Biomarker, Lung cavitation, Inflammation

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Background

Lung cavitation is a key pathological feature of *Mycobacterium tuberculosis* (Mtb) infection that results in extensive caseous necrosis. Lung cavities are pathologic gas-filled spaces in lung parenchyma associated with a high bacterial burden resulting in increased transmission, poor drug penetration, and increased morbidity and mortality in people with tuberculosis (TB) [1].

While the mechanism by which cavities are formed is unknown, they are thought to be a consequence of a complex host-pathogen interaction, involving various biochemical, biophysical, microbiological and immunological processes [1]. Data on immunological drivers remain limited, although the inflammatory processes that drive cell necrosis rather than apoptosis during Mtb infection are thought to play a key role in cavity formation [2, 3]. Lung cavitation has several negative consequences for the patient including increased symptom burden, poor drug penetration, longer time to culture conversion, poor treatment outcomes, increased risk of relapse, long-term respiratory impairment, reduced quality of life, and an increased risk for the development of drug resistance [1, 4–6]. Cavitation is shown to promote development of drug resistance in multiple ways, including increasing the frequency of replication-induced mutations [7, 8] through high rates of bacterial proliferation as well as through poor penetration of antimicrobial drugs resulting in pharmacokinetic-pharmacodynamic mismatch [9]. Drug-resistant TB is a major driver of TB related mortality in sub-Saharan Africa, where the incidence of DR-TB is rising. Within sub-Saharan Africa, South Africa has the highest prevalence of multidrug-resistant (MDR)-TB and has recorded the largest outbreak of extensively drug-resistant (XDR)-TB ever reported [10]. Treatment outcomes for DR-TB remain poor, with reports from SA revealing a 70% mortality rate and treatment cure/completion of only 11% after 60 months of follow-up in XDR-TB patients [11]. The presence of lung cavities leads to an increased risk of developing XDR-TB, even among MDR-TB patients on directly observed therapy [12].

The current TB treatment regimens have multiple challenges, including high pill burdens, drug toxicities, prolonged treatment duration and adherence challenges [13]. New approaches to control TB are needed for both diagnosis and treatment, with host-directed therapies being a promising treatment strategy for TB [14]. Identifying blood biomarkers of disease severity and treatment outcome could represent a cost-effective method to personalise treatment and improve treatment outcome. We have previously identified several inflammatory markers associated with TB risk in HIV positive, antiretroviral therapy (ART) experienced individuals (specifically lipopolysaccharide binding protein (LBP), intercellular adhesion molecule (ICAM) -1, interleukin (IL) -1β, IL-6 and IL-1Ra) [15, 16] as well as inflammatory markers associated with disease severity (measured by presence of lung cavitation) in drug-susceptible pulmonary TB (including IL-6 and IL-1Ra) [17]. Here we utilised biological specimens from a well characterised cohort with comprehensive clinical and demographic data to characterise immune markers of lung cavitation in MDR/XDR-TB patients.

Materials and methods

Study participants

The study aimed to characterise biomarkers of lung cavitation in MDR/XDR-TB patients and was performed utilising stored plasma specimens from the CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX). The InDEX study was a randomized controlled clinical trial assessing if a gene-derived individualised treatment approach in patients with drug-resistant TB improved treatment success. Enrolled InDEX study participants were recruited to King Dinuzulu Hospital (KDH) in KwaZulu-Natal, South Africa following a confirmed diagnosis of drug-resistant TB. The presence of lung cavitation was determined by postero-anterior chest radiograph unless a chest radiograph performed 14 days prior to the date of screening was available for review. Study participants were >18 years with pulmonary TB and Xpert MTB/RIF evidence of rifampicin resistance. For this sub-study, 128 available plasma samples at baseline prior to treatment initiation were evaluated.

Sample collection and processing

Peripheral blood was collected in acid citrate dextrose (ACD) tubes. Plasma was separated by centrifugation (1600 rpm for 10') and cryopreserved at −80 °C until use.

Cytokine/Chemokine measurement

Cryopreserved plasma samples were thawed and mixed before performing the assays. Cytokine/Chemokine levels were be measured using the Pro Human Cytokine 27-Plex Assay (Bio-Rad, USA) that covers major inflammatory cytokines and analysed on a BioPlex-200 system (Bio-Rad). The Human Cytokine/Chemokine Panel includes the following cytokines and chemokines: 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). All assays were performed following manufacturers' instructions. Samples with values below the limit of detection (LOD) were assigned the value half of the LOD (LOD/2) in pg/mL.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 25, R software version 4.2.2 and the graphs made using GraphPad Prism (V9). All cytokines/chemokines with more than 60% of samples above the limit of detection were log-transformed to reduce variance (IL-1Ra, IL-4, IL-6, IL-7, IL-8, IL-9, IL-13, G-CSF, MCP-1, FGF basic, MIP-1α, MIP-1β, TNF-α, PDGF-BB, Eotaxin, IP-10, RANTES). Cytokines/chemokines with less than 60% detectability were analysed as binary variables (IL-1β, IL-10, IL-12, IL-17, VEGF, GM-CSF, IFN-γ) while cytokines with less than 10% of samples detectable (IL-5, IL-15, IL-2) were excluded from the multivariable analysis to avoid quasi-complete separation. To determine the association between plasma cytokine expression at baseline with disease severity measured by lung cavitation, univariable and multivariable modified Poisson regression models were used. The presence of lung cavitation at baseline was the outcome. Multivariable analyses adjusted for clinical and demographic variables which included age, sex, HIV status, smoking, BMI and history of previous TB. In addition, the use of antiretroviral therapy (ART) was adjusted for when analysing HIV-infected individuals. The association between covariates adjusted for in the multivariable models with lung cavitation was also assessed using modified Poisson regression models.

Table 1 Demographic and clinical characteristics of participants

Variables	Total cohort n = 128	Lung cavitation n = 61	No lung cavitation n = 64
Age (years), median (IQR)	35 (29–41.2)	33.0 (29–43)	36 (30–41)
Female, n (%)	55 (43)	28 (45.9)	25 (39.1)
Body mass index (kg/m^2), median (IQR)	20.2 (18.5–22.7)	19.8 (17.9–21.5)	20.5 (19.0–23.6)
HIV Positive, n (%)	93 (72.7)	37 (60.7)	53 (82.8)
ARV status[a], n (%)			
No	9 (7)	4 (6.6)	4 (6.2)
Yes	84 (65.6)	33 (54.1)	49 (76.6)
Previous TB History, n (%)			
No	77 (60.2)	37 (60.7)	38 (59.4)
Yes	51 (39.8)	24 (39.3)	26 (40.6)
TB diagnosis			
RR/MDR	115 (89.8)	57 (93.4)	56 (87.5)
Pre-XDR/XDR	13 (10.2)	4 (6.6)	8 (12.5)
Alcohol use, n (%)			
No	118 (92.2)	53 (86.9)	62 (96.9)
Yes	10 (7.8)	8 (13.1)	2 (3.1)
Smoking, n (%)			
No	120 (93.8)	57 (93.4)	60 (93.8)
Yes	8 (6.2)	4 (6.6)	4 (6.2)
Lung cavities*, n (%)			
None	64 (50)	-	64 (100)
Unilateral	46 (35.9)	46 (75.41)	-
Bilateral	15 (11.7)	15 (24.59)	-

[#] Values for clinical and demographic variables reported at baseline

*Three participants with missing lung cavitation status

[a] 34 participants with missing ARV status

Results

Participant characteristics

The demographic and clinical characteristics of study participants are described in Table 1. The median age was 35 years (IQR 29–41.2), with 57% (73/128) being males. The median body mass index (BMI) was 20.2 kg/m^2 (IQR 18.5–22.7). Participants with rifampicin-resistant TB and MDR-TB accounted for 89.84% (115/128) of the total cohort while participants with Pre-XDR/XDR-TB accounted for 10.16% (13/128). HIV co-infection was present in 73% (93/128) of study participants with 90.32% (84/93) receiving antiretroviral therapy (ART). The presence of lung cavitation was observed among 47.67% (61/128) of study participants.

Effect of plasma cytokine/chemokine expression on presence of lung cavitation

Modified Poisson regression models were used to assess the association between plasma cytokine/chemokine expression and lung cavitation in individuals with DR-TB. Following univariable and multivariable analyses adjusting for age, sex, BMI, HIV status, smoking and previous history of TB; increased plasma expression of IL-6 [aRR 1.405, 95% CI 1.079–1.829, p = 0.011] was associated with an increased risk of lung cavitation in the total cohort. A unit increase in the log expression of IL-6 led to a 41% increase in the risk of lung cavitation (Table 2). Similarly, a trend was observed in the sub-analysis of HIV positive participants controlling for ART use [aRR 1.424, 95% CI 0.991–2.046, p = 0.056 (Additional File 1).

Multivariable models that fitted lung cavitation against the adjusted variables (age, sex, BMI, HIV status, smoking and previous history of TB) in the total cohort, showed that HIV positive participants had a significant 46% reduction in the risk of lung cavitation in comparison to HIV negative participants [aRR 0.536, 95% CI 0.371–0.775, p = 0.001]. The risk of lung cavitation was 7% lower for each unit increase in BMI [aRR 0.927, 95% CI 9.874–0.983, p = 0.012] while males had a 44% lower risk of lung cavitation [aRR 0.559, 95% CI 0.381–0.821, p = 0.003]. Smoking was associated with an increased

Table 2 Association between plasma cytokine/chemokine expression with lung cavitation among HIV positive and negative participants with DR-TB (n = 125)

Cytokine/ Chemokine	Univariable			Multivariable		
	RR	95% CI	p-value	aRR	95% CI	p-value
IL-1Ra	1.158	0.761–1.760	0.494	1.115	0.736–1.689	0.607
IL-4	0.941	0.620–1.426	0.773	1.127	0.742–1.713	0.575
IL-6	1.347	1.006–1.803	**0.045**	1.405	1.079–1.829	**0.011**
IL-7	0.871	0.713–1.064	0.176	0.922	0.756–1.125	0.425
IL-8	1.019	0.557–1.863	0.951	0.967	0.583–1.603	0.896
IL-9	1.025	0.535–1.964	0.940	1.112	0.601–2.059	0.735
IL-13	0.853	0.628–1.157	0.307	0.851	0.624–1.161	0.309
G-CSF	0.967	0.821–1.139	0.689	0.987	0.850–1.147	0.868
MCP-1	0.951	0.749–1.208	0.682	1.072	0.828–1.387	0.597
FGF	1.393	0.659–2.944	0.386	1.324	0.614–2.859	0.474
MIP-1α	1.183	0.959–1.458	0.116	1.139	0.924–1.403	0.222
MIP-1β	1.084	0.530–2.218	0.825	1.199	0.609–2.361	0.600
TNF-α	0.934	0.635–1.375	0.731	1.097	0.766–1.572	0.614
PGDF-BB	1.075	0.802–1.441	0.628	1.175	0.891–1.550	0.253
Eotaxin	1.044	0.688–1.586	0.839	1.206	0.795–1.832	0.378
IP-10	1.039	0.797–1.354	0.778	1.116	0.880–1.414	0.366
GM-CSF	0.926	0.574–1.492	0.752	0.902	0.591–1.376	0.631
IFN-γ	0.751	0.502–1.124	0.164	0.808	0.552–1.185	0.275
IL-10	1.177	0.796–1.740	0.414	1.131	0.761–1.681	0.544
IL-12	0.868	0.607–1.241	0.438	0.887	0.627–1.256	0.499
IL-17	1.115	0.779–1.596	0.553	1.108	0.788–1.557	0.556
IL-1b	1.045	0.730–1.498	0.809	0.958	0.678–1.354	0.809
VEGF	1.155	0.741–1.801	0.525	1.176	0.792–1.744	0.378
RANTES	0.767	0.542–1.085	0.134	0.853	0.608–1.198	0.359

*Three participants were excluded due to missing lung cavitation status

risk of lung cavitation [aRR 1.784, 95% CI 1.167–2.729, p = 0.008] (Additional File 1).

Discussion

Cavitary TB is a serious consequence of pulmonary TB, and is associated with increased morbidity and mortality, the development of drug resistance and higher transmission rates [1]. In this study, we assessed the association between plasma cytokine/chemokine expression and lung cavitation among individuals with DR-TB. Increased plasma expression of IL-6 was associated with higher risk of lung cavitation among individuals with DR-TB. HIV positive participants, males and participants with higher BMI had a reduced risk of lung cavitation in participants with DR-TB. Additionally, Individuals who smoke had an increased risk of lung cavitation.

IL-6 is a pleiotropic cytokine with dual pro- and anti-inflammatory properties. IL-6 activates target cells either via membrane–bound IL-6R (classic signalling) or through soluble forms of IL-6R (sIL-6R, trans-signalling), the latter of which was shown to account for its proinflammatory properties [18]. IL-6 modulates innate and adaptive immunity [19]. It is secreted by various immune cells including macrophages, neutrophils and lung epithelial cells at sites of inflammation. IL-6, is induced by Mtb infection, [20] and has both positive and negative effects on host immune control of Mtb [21]. We have previously shown that plasma IL-6 is associated with both shorter time to culture conversion as well as increased risk of TB recurrence and presence of lung cavitation in patients with drug susceptible TB, highlighting its dual role in TB [15, 16]. Increased IL-6 levels measured in bronchoalveolar lavage (BAL) of patients with pulmonary TB were shown to correlate with lung cavitation [22]. Additionally, BAL IL-6 expression has previously been correlated with a chest high-resolution computed tomography score which factored cavities, miliary nodules and bronchial wall thickening among HIV negative patients with active TB [22]. IL-6 has also been implicated in promoting fibroblast development and subsequently, pulmonary fibrosis [23, 24]. Elevated IL-6 expression was also reported in pleural fluid of TB patients as well as in cerebrospinal fluid (CSF) in patients with TB meningitis [25–27]. While IL-6 is predominantly associated with disease severity in TB, knockout studies have shown that IL-6 plays an important role in controlling bacterial load early in the infection through induction of protective interferon gamma-producing T cell responses [28].

The COVID-19 pandemic has highlighted the use of host directed therapies targeting IL-6. This includes

biological agents that target the IL-6 itself (clazakizumab, olokizumab, sirukumab and siltuximab), its receptor (tocilizumab and sarilumab) and drugs that inhibit IL-6 *trans*-signalling by blocking the soluble IL-6 receptor (sIL-6R) (olamkicept) [29, 30]. These IL-6 inhibitors vary in potency, have distinct pharmacodynamics and result in distinct downstream effects. While cytokine and receptor blockers are known to inhibit the classic IL-6 pathway and result in significant immunosuppression, the sIL-6R inhibitor, olamkicept was shown to exclusively block IL-6 proinflammatory trans-signalling without affecting IL-6 classic signalling and causing immunosuppression [30]. As seen with the use of IL-6 targeting in COVID-19, the efficacy of any IL-6 therapy would depend on the severity of the disease and the timing of the intervention; as well as the ability to distinguish between IL-6 as a cause or a consequence of the disease.

Smoking was found to increase the risk of lung cavitation among DR-TB patients. Smoking is known to be associated with more extensive lung disease and cavitation as well as poor treatment outcome in pulmonary TB [31, 32]. HIV status, increased BMI and male sex were associated with reduced risk of lung cavitation. Previous studies have reported lower odds of lung cavitation in HIV positive individuals with pulmonary TB in comparison to HIV negative individuals and this was related to immunosuppression and the reduction in $CD4^+$ T cell counts that are thought to play a key role in lung immunopathology during Mtb infection [33–36]. In addition to lower prevalence of cavitary disease HIV positive individuals are more likely to have acid-fast smear negative disease, as well as to have extrapulmonary TB, all of which are associated with delays in TB diagnosis and poor treatment outcomes [37–39]. Higher odds of lung cavitation were reported among TB cases with severe malnutrition (BMI<than 16 kg/m^2) in comparison to individuals with a BMI greater than or equal than 18.5 kg/m^2 [40]. Weight loss is a clinical manifestation of TB and is known to increase following TB treatment [41] however, a lack of adherence to MDR-TB treatment leads to slower increases in BMI [42]. Contrasting results have been reported on the relationship between gender and TB disease severity. Greater lung involvement was reported in males in comparison to females with pulmonary TB using Ralph scoring [43] with pulmonary fibrosis also being more frequent among males in comparison to females [44]. The relationship between gender and lung cavitation among drug-resistant TB patients remains to be further characterised.

Previous studies have highlighted the role of IL-6 in drug-susceptible TB, however studies on cytokine signatures and their role in DR-TB are limited. Here we highlight the association between plasma IL-6 levels and presence of lung cavitation in a well-characterised cohort of individuals with active DR-TB. This study has several limitations, including a lack of access to viral load and CD4 counts for HIV positive individuals with DR-TB at sampling timepoint. Additionally, we were not able to determine whether the lung cavitation was related to previous TB disease or the current episode of TB. While our results show an association between plasma IL-6 and presence of cavitary disease in DR-TB, we are unable to distinguish between IL-6 as a consequence or a cause of the disease. Further studies are needed to determine the relationship between lung cavitation and IL-6 signalling and the potential of IL-6 blocking agents in TB treatment.

Abbreviations

ACD	Acid citrate dextrose
aRR	Adjusted risk ratio
ART	Antiretroviral therapy
BAL	Bronchoalveolar lavage
BMI	Body mass index
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CI	Confidence interval
CSF	Cerebrospinal fluid
DR-TB	Drug resistant tuberculosis
FGF	Fibroblast growth factor
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HIV	Human immunodeficiency virus
ICAM	Intercellular adhesion molecule-1
IFN-γ	Interferon gamma
IL	Interleukin
InDEX	The Individualized M(X) drug-resistant TB treatment Strategy Study
IP-10	Interferon gamma-induced protein 10
IQR	Interquartile range
KDH	King Dinuzulu hospital
LBP	lypopolysaccharide binding protein
LOD	Limit od detection
MCP-1	Monocyte chemoattractant protein-1
MDR	Multidrug-resistant
MIP	Macrophage inflammatory protein
Mtb	Mycobacterium tuberculosis
PDGF-BB	Platelet-derived growth factor-BB
RANTES	Regulated on activation, normal T cell expressed and secreted
RR	Risk ratio
TB	Tuberculosis
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
XDR	Extensively drug-resistant TB

Supplementary Information

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Additional File 1: Supplementary Table 1 and Supplementary Figure 1

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Authors' contributions

Designed the study: AS, TGM;Performed the experiments: TGM, SN, SR, AS;Analysed the data: TGM, AS, ML;Wrote the first draft of the paper: TGM, AS;Collected specimens and clinical data: SC, KN; Supervised clinical and/or experimental aspects of the study: AS, NS, NP, KN, SC, RP, DA; All authors contributed to the editing and finalisation of the manuscript.

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

Declarations

Ethics approval
The CAPRISA 020 Individualized M(X)drug-resistant TB Treatment Strategy Study (InDEX) was approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC, BFC584/16) and registered with the South African Clinical Trials Register (NCT03237182). This sub-study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC/00002197/2020). All the experiments were performed in accordance with the Helsinki Declaration. All study participants provided written informed consent for the use of stored biological specimens.

Consent for publication
Not Applicable.

Competing interests
The authors declare no conflict of interests.

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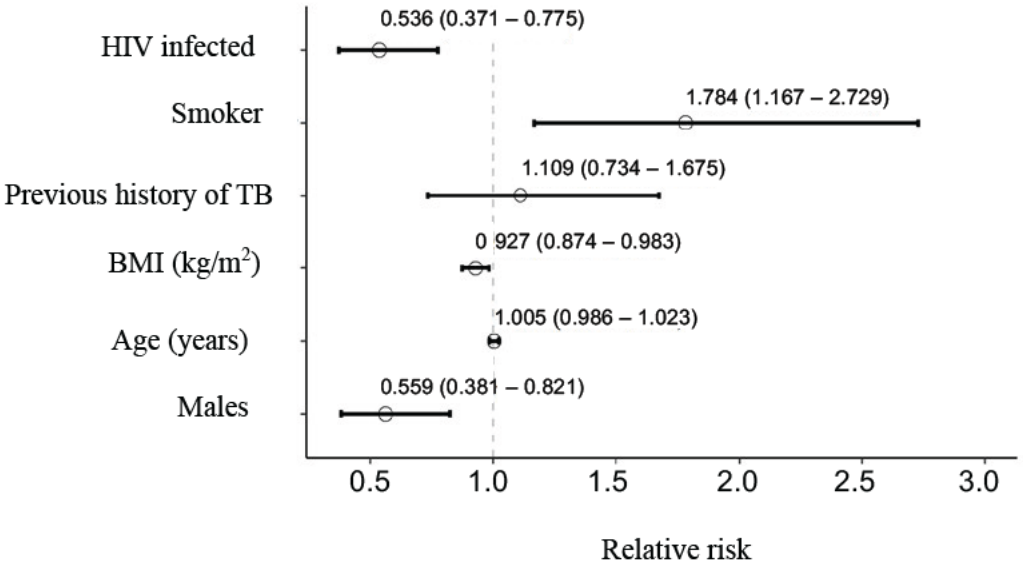
Publisher's Note

Supplementary material

Supplementary Table 1. Association between plasma cytokine/chemokine expression with lung cavitation among HIV positive participants with DR-TB

Cytokine/ Chemokine	Univariable			Multivariable		
	RR	95% CI	p-value	aRR	95% CI	p-value
IL-1Ra	1.180	0.693 – 2.009	0.541	1.098	0.653 – 1.848	0.724
IL-4	0.904	0.498 – 1.643	0.742	0.968	0.533 – 1.757	0.914
IL-6	1.471	0.997 – 2.171	0.052	1.424	0.991 – 2.046	0.056
IL-7	0.814	0.619 – 1.071	0.142	0.840	0.639 – 1.103	0.210
IL-8	0.805	0.328 – 1.977	0.637	0.704	0.305 – 1.622	0.410
IL-9	1.255	0.506 – 3.112	0.624	1.413	0.556 – 3.591	0.467
IL-13	0.952	0.621 – 1.460	0.823	0.926	0.590 – 1.453	0.739
G-CSF	0.903	0.713 – 1.144	0.398	0.877	0.706 – 1.090	0.238
MCP1	1.008	0.721 – 1.411	0.961	1.112	0.792 – 1.561	0.541
FGF	1.802	0.549 – 5.914	0.332	1.783	0.500 – 6.359	0.373
MIP-1α	1.158	0.844 – 1.588	0.363	1.080	0.792 – 1.474	0.626
MIP-1β	1.397	0.515 – 3.791	0.512	1.561	0.557 – 4.375	0.397
TNF-α	0.836	0.461 – 1.515	0.555	0.896	0.531 – 1.510	0.679
PGDF-BB	1.022	0.652 – 1.603	0.924	1.157	0.768 – 1.743	0.486
Eotaxin	1.201	0.699 – 2.061	0.507	1.339	0.771 – 2.324	0.300
IP-10	1.144	0.820 – 1.594	0.429	1.149	0.848 – 1.557	0.369
GM-CSF	0.872	0.456 – 1.668	0.679	0.814	0.448 – 1.479	0.500
IFN-γ	0.677	0.398 – 1.152	0.151	0.687	0.407 – 1.160	0.160
IL-10	1.145	0.665 – 1.971	0.626	1.073	0.627 – 1.834	0.797
IL-12	0.906	0.547 – 1.502	0.703	0.869	0.533 – 1.415	0.571
IL-17	1.062	0.648 – 1.743	0.810	1.062	0.646 – 1.747	0.813
IL-1β	0.892	0.537 – 1.480	0.657	0.791	0.474 – 1.320	0.370
VEGF	1.185	0.665 – 2.111	0.566	1.126	0.675 – 1.880	0.649
RANTES	0.826	0.505 – 1.350	0.445	0.897	0.557 – 1.444	0.654

*Three participants were excluded due to missing lung cavitation status



Supplementary Figure 1. Relative risk for covariates in a multivariable Poisson model prior correcting for cytokines/chemokines (n=125)

CHAPTER 6

6.1 Summary and discussion of the main findings

The TB and HIV syndemic remains a public health challenge. TB is currently second to COVID-19 as a leading cause of death from an infectious agent. HIV induced inflammation and immune malfunction lead to increased risk of TB reactivation and TB progression while PLHIV are 15 times more likely to develop active TB. HIV and TB infections are frequently concomitant, and potentiate each other, suggesting the need for joint solutions to improve the health in this population. The global burden of TB remains high, with increase in the global estimated number of deaths due to TB between 2019 and 2021[1]. In 2020 and 2021, the COVID-19 pandemic had a devastating impact on TB testing services and care. This resulted in an increase in missing cases due to reduced case detection suggesting that the estimated number of incident cases of TB and TB related deaths may have been underestimated as SA continues to be a high TB and HIV burden country. An estimated 4 million cases of TB are missed each year due to lack of adequate diagnostics highlighting the need for more practical, non-invasive, and non-sputum-based point-of care tests for TB. This calls for detailed characterisation of biomarkers of TB and different TB states.

There is a great interest in developing blood-based tests for TB with recent tests such as Cepheid 3-gene host response fingerstick blood test showing promising preliminary data [2]. The identification of host biomarkers is crucial for the validation of correlates of risk and protection in Mtb which are critical for the development of effective vaccines against TB as well as host immune based methods of diagnosis and treatment [3]. Additionally, with the rapid increase in drug-resistant TB cases, host directed therapies provide a promising approach for the modulation of inflammation and immunopathology [4] without the risk of development of further bacterial resistance.

Here we utilised samples from well described clinical cohorts to characterise host inflammatory, NK and $CD4^+$ T cell responses during active TB with and without HIV-coinfection and assess their impact on TB disease severity and treatment response. While previous studies have provided information on the nature of these responses during TB, their impact on TB outcomes and pathogenesis as well as the nature and impact of these responses during TB-HIV co-infection remains unknown.

NK cells are effector cells of the innate immune system and play a key role in early host defence roles against pathogens and cancer, [5] and clearance of Mtb and HIV. In **Chapter 3** we investigated the effect of TB/HIV co-infection on NK cell homeostasis by characterising differences in NK cell activating and inhibitory receptors, and subset distribution in samples from CAPRISA 011 study participants with active TB with and without HIV co-infection and in healthy controls. In addition, we associated the

measured NK cell phenotypes during active TB with TB treatment outcome and cavitary disease as well as evaluated the effect of TB treatment completion on changes in NK cell phenotype.

Here we demonstrate a redistribution of NK cell subsets among individuals with TB/HIV co-infection evidenced by an expansion of a functionally impaired $CD56^{neg}$ NK cell subset among TB/HIV co-infected participants in comparison to TB infected participants and healthy controls. The $CD56^{neg}$ NK cell subset rarely exists among healthy individuals and expands during untreated chronic viral infections such as HIV, CMV, Epstein-Barr virus, and Hepatitis C [6-9]. The increase in the anergic $CD56^{neg}$ NK cell subset has previously been correlated with HIV viral load, highly expanding in viremic PLHIV compared to PLHIV on HAART for more than two years [8,10]. In addition, expansion of the $CD56^{neg}$ NK cell subset has been associated with HIV disease progression [11] due to reduced cytotoxic function and cytokine production [8,11]. Levels of HIV viremia, associated with chronic immune activation, may lead to NK cell exhaustion which is also characterised by decreased cytokine production and cytolytic activity; and this in turn is likely to have a negative impact on Mtb control and clearance as evidenced by our data.

We then characterised the percentage of activating and inhibitory receptor expression on functional $CD56^{bright}$ and $CD56^{dim}$ subsets across participants groups and their impact on TB severity and treatment outcome. We observed an increase in NKp30 expression on the immunomodulatory $CD56^{bright}$ NK cells and an increase in NKG2D and NKG2C on cytotoxic $CD56^{dim}$ NK cells in the TB infected and TB/HIV co-infected participant groups compared to healthy controls, with this likely related to increase in NK cell activity due to associated viral and bacterial burden. We additionally observed a decrease in NK cell activating receptor NKp46 on $CD56^{dim}$ NK cell subsets in the TB/HIV group compared to the TB group, with this decrease likely associated with impaired Mtb control and outcome as NKp46 is involved in lysis of Mtb-infected cells [12] and is associated with lower risk of lung cavitation in our study. We additionally observed a significant association between increased NKp30 expression on cytokine producing $CD56^{bright}$ NK cells and increased risk of cavitary disease likely occurring due to inflammation driven tissue damage and associated immunopathology.

Successful TB treatment completion led to an increase in the % of total NK cells and decrease in % of $CD56^{bright}$ NK cells among TB infected and TB/HIV co-infected participants. Decrease in total NK cell numbers during progression from latent to active disease and their restoration following treatment was reported previously [13]. This is likely due to bacterial clearance and reduced bacillary load resulting in redistribution of NK cells previously recruited to sites of infection. Reduction in bacterial load following treatment completion also led to decrease in expression of several activating and inhibitory receptors likely resulting in reduced NK cell activity. Interestingly, in samples from TB/HIV co-

infected participants, an increase in NKp44 on $CD56^{bright}$ and NKG2C on $CD56^{dim}$ cells was observed; what are the impacts of this on TB and HIV pathogenesis remains to be determined.

One of the main mechanisms by which HIV affects TB disease is through HIV mediated depletion in $CD4^+$ T cell numbers [14,15] . However, in addition to the generalized $CD4^+$ T cell depletion, HIV is known to cause major changes in $CD4^+$ T cell phenotype distribution and cell trafficking markers, and these changes could have important impact on Mtb control and movement of cells to the site of infection. In **Chapter 4** we assessed the differences in memory $CD4^+$ T cell phenotypes between participant with active TB infected, participants with TB/HIV co-infection and healthy controls. As expected, we observed higher percentages of Th2 cells and lower percentages of Th9 cells in TB/HIV co-infected participants compared to healthy controls. HIV is known to cause a switch from Th1 to Th2 response profiles [16] with progressing HIV infection. As Th1 responses are key for Mtb clearance and control this HIV induced Th1->Th2 switch could be an important contributor to loss of Mtb control in TB/HIV co-infection. We additionally assessed differences in $\alpha_4\beta_7$ and $\alpha_4\beta_1$ lymphocyte trafficking marker expression in order to assess if concurrent HIV infection could be impacting immune cell distribution to the affected tissues. TB/HIV co-infected participants had higher percentages of systemic $\alpha_4\beta_1$ and $\alpha_4\beta_7$ expressing memory $CD4^+$ T cells compared to heathy controls. Rapid migration of $CD4^+$ T cells to the site of infection is necessary for early Mtb bacterial clearance however, altered lymphocyte trafficking marker expression among TB/HIV co-infected participants may have consequences on Mtb control. We observed no significant effect of HIV induced changes on $CD4^+$ T cell subset distribution and trafficking marker expression and time to culture conversion and the presence of cavitary disease at baseline, however this is likely due to small sample size and lack of statistical power, highlighting the need to further explore these findings in larger cohorts.

One of the main drivers of high TB related morality is the continuing increase in DR-TB. With the co-existing HIV/AIDS epidemic, the devastating impact of DR-TB is particularly evident in SA. Drug-resistance in Mtb mainly occurs due to exposure to inadequate treatment. The gold standard for drug-resistance testing is culture based and can take weeks to months. In the absence of drug susceptibility results, patients are often started on empirical treatment based on clinical discretion that can be associated with adverse effects. Furthermore, the ongoing COVID-19 pandemic has had a negative impact on TB diagnosis and treatment initiation. South Africa was listed as one of ten contributing countries accounting for an estimated 70% of the global gap between the estimated global MDR/XDR-TB incidence and treatment enrolment in 2020 [17].

There is an urgent need to increase treatment coverage, improve testing and diagnosis and develop novel immune-based treatment options for drug-resistant TB in the affected countries. Cavitary TB has previously been associated with poor treatment outcomes and high transmission rates and development

of drug resistance [18]. In **Chapter 5** we characterised immune markers of lung cavitation in MDR/XDR-TB patients. We found that higher IL-6 plasma expression was associated with higher risk of lung cavitation in drug resistant TB patients which suggests the role of IL-6 in the immunopathology of DR-TB. In previous CAPRISA studies, increased IL-6 levels were associated with increased risk of TB recurrence in HIV positive individuals on ART [19]. Additionally in drug susceptible TB, increased plasma IL-6 levels were associated with increased lung cavitation as well as faster bacterial clearance [20]. This highlights the dual nature of the host immune responses with the same responses that are necessary for bacterial clearance and control being responsible for tissue damage and associated immunopathology. Additionally, our study confirmed the role of smoking in increasing risk of lung cavitation [1,21], as well as the association between HIV positive status and increased BMI with lower risk of cavitary disease [22]. The potential use of IL-6 immunotherapy in TB would require careful consideration of the timing during the infection, dosage and specific pathways being inhibited in ordered to reduce immunopathology without effecting Mtb control and clearance.

6.2 Study limitations

Our study has several limitations including a relatively small sample size and the inability to control for additional clinical and demographic characteristics that could interfere with the observed results. For NK and T cell characterization, due to limited cell number, we were unable to examine Mtb specific NK and T cell responses. Additionally, as CAPRISA 020 Index is an ongoing study, we were unable to examine the effects of host immune responses on disease and treatment outcomes. It is also important to note that we are using systemic immune responses to infer what is happening at the site of infection in the lungs. Finally, the cohorts are clinically complex and other unmeasured characteristics and conditions could have confounded the data.

6.3 Future directions and recommendations

Associations reported here should be validated in other relevant clinical cohorts and different settings to demonstrate generalizability of the results. Future studies should characterise changes in Mtb-specific NK and $CD4^+$ T cell responses during TB/HIV co-infection and their impact on TB disease progression and severity. Similarly, these host immune responses should be examined in different TB states, including latent, subclinical, and drug-resistant TB. Ideally, studies should link systemic immune responses with immune responses in the mucosal lung tissues. As it is difficult to obtain sufficient cell numbers from bronchoalveolar lavage samples to do extensive cell phenotyping, potential single cell transcriptomic approaches could be used. While we and others have focused on the negative effects of HIV on TB disease progression and outcome, the effects of TB on HIV progression also require attention. Several studies have correlated active TB with increased HIV viral load and faster disease progression [23,24], and the exact immune mechanisms of this effect are currently unknown.

6.4 Conclusion

In addition to characterising protective and detrimental host immune responses in TB pathogenesis, our study identified several new immune mechanisms by which HIV-mediated immunomodulation could impact TB disease outcomes during co-infection. Through NK cell subset redistribution and modulation of activating and inhibitory receptors HIV can impact both susceptibility to new infections and TB reactivation as well as disease severity and treatment response. In addition to severe depletion of $CD4^+$ T cells that is known to impact TB reactivation and disease outcomes, HIV can negatively modulate $CD4^+$ T cell responses by inducing a switch from protective Th1 to Th2 response further hampering Mtb control and clearance. Similarly, HIV mediated changes in cell surface integrin expression could have negative impact on T-cell trafficking to the site of infection and Mtb control in the lungs. Our results showing association between cavitary disease and systemic IL-6 levels during DR-TB highlight the proinflammatory nature of this cytokine as well as its role in both immunopathology and pathogen control. As demonstrated during the recent COVID-19 pandemic, host directed therapies targeting inflammatory responses pathways require careful consideration of the dosage, timing of treatment with respect to infection as well as in-depth understating of the immune pathways being affected. In addition to providing new avenues for immunotherapy, the reported findings can potentially be used for improved diagnostics. Several immune based tests are being developed for diagnosis of various diseases including TB (Cepheid 3-gene host-response test) and bacterial vaginosis [The Genital inflammation Test (GIFT) a cytokine-based point of care diagnostics test]. In addition to diagnosis, measurement of immune responses could be done to predict the risk of severe disease in patients.

The latest global and SA figures on TB incidence and mortality are concerning. A global increase of 4.5% in estimated number of people falling ill with TB between 2020 and 2021 was recorded, with more than 1.6 million TB deaths reported in 2021 alone. In South Africa specifically, 304, 000 new cases of TB (with more than half of those in PLWH) and 56,000 new TB- related deaths were recorded in 2021. The latest numbers and the set-backs due to COVID-19 pandemic make it highly unlikely that we will meet the goal of 90% reduction in TB deaths by 2023. There has been great emphasis placed on the importance of the development of new vaccines against Mtb, early TB diagnosis, and effective treatment intake and monitoring.

There remains an urgent need for the evaluation of TB host responses for the identification of biomarkers for TB risk, TB disease, treatment response, and TB drug resistance, particularly among PLHIV where co-infection presents a unique challenge for the host immune response [25,26]. Our study contributes to increased understanding of the role of host immune responses in Mtb control and pathogenesis in the presence of HIV co-infection and the results presented here could potentially contribute to development of future diagnostic, treatment, and prevention modalities against TB.

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APPENDICES

Appendix A: Poster presentations

Changes in natural killer cell phenotypes in TB/HIV co-infection

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Introduction

South Africa has the highest burdens of both TB and HIV infections globally. Overlap of the two epidemics has devastating consequences, with TB being the leading cause of death in PLWHIV [1,2]. Additionally, HIV is the driving factor for TB reactivation with PLWHIV being 18 times more likely to develop TB than people without HIV infection [3]. This suggests an urgent need to identify immunological markers of TB risk and treatment outcome in this setting. While the precise immune correlates of protection to TB infection have not been well-defined. Natural killer (NK) cells are increasingly recognized as a key component of the innate immune response to TB [4] and as a link between innate and adaptive immunity. The understanding of TB/HIV coinfection dynamics could fill important gaps and provide new avenues for TB treatment and control.

Methods

Study cohorts

Utilising peripheral blood mononuclear cells (PBMCs) from the Improving Retreatment Success (IMPRESS, NCT02114684) trial we assessed the NK cell phenotypes, measured during active TB and post TB treatment, in 70 TB and TB-HIV co-infected individuals. Participants were ≥ 18 years with a history of anti-TB treatment and a current TB diagnosis as confirmed by either a positive culture of MTB sputum or rifampicin resistance as determined by GeneXpert MTB/RIF® technology. Additionally, we included 13 healthy controls, and 17 HIV infected controls from the Acute HIV infection CAPRISA 002 study.

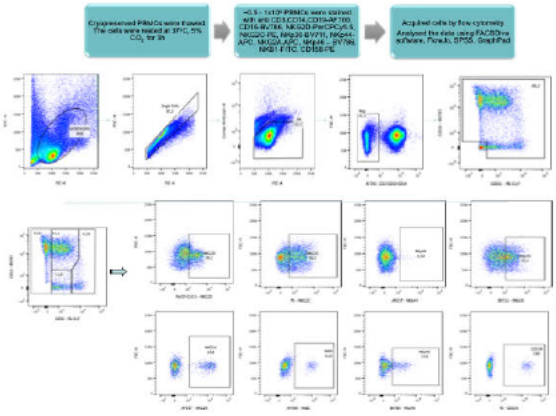

Figure 1. Representative gating strategy used for natural killer cells

Results

Table 1. Clinical and demographic characteristics of CAPRISA 011, CAPRISA 002 study cohorts and healthy donors

Variables	CAPRISA 011 (TB/HIV co-infected) N = 44	CAPRISA 011 (TB infected) N = 26	CAPRISA 002 (HIV infected) N = 17	Healthy donor N = 13
Gender, n (%)				
Male	31 (70)	22 (85)	-	7 (46)
Female	13 (30)	4 (15)	17 (100)	6 (54)
Age (y), median (IQR)	37 (31 – 41)	33 (25 – 50)	33 (28 – 35)	35 (32 – 39)
Body mass index (kg/m²), median (IQR)	19.70 (18.13 – 22.52)	19.25 (18.44 – 22.17)	32.75 (29.75 – 36.83)	23.29 (19.73 – 31.30)
HIV status, n (%)				
Positive	44 (100)	-	17 (100)	-
Negative	-	26 (100)	-	13 (100)
HIV Viral load[a] (copies/ml), median (IQR)	45165 (2857.25 – 558065)	-	17422 (1262.75 -133266.25)	-
CD4 cell count (cells/mm³), median (IQR)	285 (123 – 413)	-	583 (358 – 713)	-
ARV status[b], n (%)				
Yes	22 (50)	-	10 (59)	-
No	20 (45)	-	7 (41)	-

Values for clinical and demographic variables reported at baseline
[a] 7 participants with viral load not detected
[b] 2 participants missing ARV status

Effect of TB and HIV infection/co-infection on NK cell frequency and phenotype

Natural cytotoxicity receptor NKp30 was upregulated in CD56^{bright} NK cells while NKG2D and NKG2C were upregulated in CD56^{dim} NK cells in TB and HIV infection compared to healthy donors.

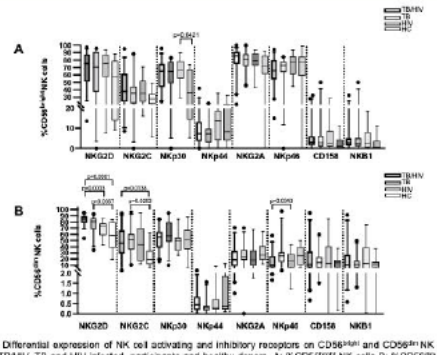

Figure 2. Differential expression of NK cell activating and inhibitory receptors on CD56^{bright} and CD56^{dim} NK cell subsets amongst TB/HIV, TB and HIV infected participants and healthy donors. A: %CD56^{bright} NK cells B: %CD56^{dim} NK cells; p-values shown on the graph are results of a Kruskal-Wallis test with Dunns multiple comparisons adjustment.

Effect of TB treatment completion on NK cell phenotypes

Upregulation of NKp44 and downregulation of NKG2A, CD158, NKp46 and NKB1 was observed following treatment on CD56^{bright} NK cells. TB treatment resulted in significant downregulation of NKG2D and NKp30 receptor expression on CD56^{dim} NK cells and upregulation of NKG2C.

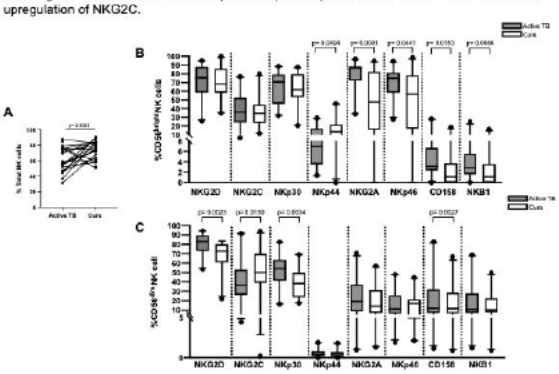

Figure 3. Changes in NK cell frequency and phenotype amongst TB/HIV co-infected participants during active TB and following anti-TB treatment completion. A: p-value results from paired t test B, C: p-values are results of a paired t test for parametric data and Wilcoxon test for non-parametric data analysis.

Effect of NK cell phenotype on disease severity measured by lung cavitation

Increased frequency of NKp46 on CD56^{dim} NK cells associated with lower odds of lung cavitation in TB and TB/HIV infected participants.

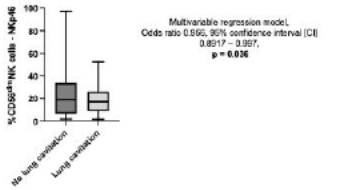

Figure 4. Effect of NK cell phenotype on disease severity measured by lung cavitation in TB and TB/HIV infected participants. p-value from multivariable regression model adjusting for study arm, gender, BMI, alcohol use, smoking and HIV status.

Discussion & conclusion

The effect of HIV infection on NK cell phenotype and function when co-infected with TB remains to be fully elucidated. Our data shows distinct changes in systemic NK cell populations with respect to active disease, treatment completion and disease severity in TB and TB-HIV co-infected individuals. NKp46 receptor expression on CD56^{dim} cells may have key implications in TB/HIV pathogenesis.

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2. Manosuthi, W., Chottanapund, S., Thongyen, S., Chaowanachan, T. & Sungkanuparph, S. Survival rate and risk factors of mortality among HIV/tuberculosis-coinfected patients with and without antiretroviral therapy. JAIDS Journal of Acquired Immune Deficiency Syndromes 43, 42-46 (2006).
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Higher plasma IL-6 levels are associated with lung cavitation in drug-resistant TB

Thando Maseko[1,2], Slindile Ngubane[1], Marothi Letsoalo[1], Santhuri Rambaran[1], Derseree Archary[1,3], Natasha Samsunder[1], Rubeshan Perumal[1,2], Surie Chinappa[1], Nesri Padayatchi[1,2], Kogieleum Naidoo[1,2] and Aida Sivro[1,2,3,4*]

[1]Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa; [2]South African Medical Research Council (SAMRC)-CAPRISA-TB-HIV Pathogenesis and Treatment Research Unit, University of KwaZulu-Natal Nelson R Mandela School of Medicine, Durban, South Africa; [3]JC Wilt Infectious Disease Research Centre, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada; [4]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada

Introduction

- Lung cavitation is a key pathological feature of *Mycobacterium tuberculosis* (Mtb) infection that results in extensive caseous necrosis.
- Lung cavitation is associated with heightened TB transmission and poor treatment outcomes.
- While the mechanism by which cavities are formed is unknown, they are thought to be a consequence of a complex host-pathogen interaction, involving various biochemical, biophysical, microbiological and immunological processes.

Methods

- Aim: To characterise immune markers of lung cavitation in MDR/XDR-TB participants from the CAPRISA 020 Individualized M(X) drug-resistant TB Treatment Strategy Study (InDEX).
- Cytokine/chemokine levels were measured in plasma samples from 128 MDR/XDR-TB patients from the InDEX study using the 27-Plex Bio-Plex assay.
- Multivariable Poisson regression models adjusting for age, sex, HIV status, smoking, BMI and previous history of TB were used to determine association between plasma cytokine expression and cavitary disease.

Results

Study cohort

- Lung cavitation was present in 61 of the 128 drug-resistant TB participants with 93 being co-infected with HIV **(Table 1)**

Table1. Clinical and demographic characteristics of InDEX cohort

Variables	Total cohort n=128	Lung cavitation n=61	No lung cavitation n=64
Age (years), median (IQR)	35 (29 – 41.2)	33.0 (29 – 43)	36 (30 - 41)
Female, n (%)	55 (43)	28 (45.9)	25 (39.1)
Body mass index (kg/m²), median (IQR)	20.2 (18.5 - 22.7)	19.8 (17.9 - 21.5)	20.5 (19.0 - 23.6)
HIV Positive, n (%)	93 (72.7)	37 (60.7)	53 (82.8)
ARV status, n (%)			
No	9 (7)	4 (6.6)	4 (6.2)
Yes	84 (65.6)	33 (54.1)	49 (76.6)
Previous TB History, n (%)			
No	77 (60.2)	37 (60.7)	38 (59.4)
Yes	51 (39.8)	24 (39.3)	26 (40.6)
TB diagnosis			
RR/MDR	115 (89.8)	57 (93.4)	56 (87.5)
Pre-XDR/XDR	13 (10.2)	4 (6.6)	8 (12.5)
Alcohol use, n (%)			
No	118 (92.2)	53 (86.9)	62 (96.9)
Yes	10 (7.8)	8 (13.1)	2 (3.1)
Smoking, n (%)			
No	120 (93.8)	57 (93.4)	60 (93.8)
Yes	8 (6.2)	4 (6.6)	4 (6.2)
Lung cavities*, n (%)			
None	64 (50)	-	64 (100)
Unilateral	46 (35.9)	46 (75.41)	-
Bilateral	15 (11.7)	15 (24.59)	-

[#] Values for clinical and demographic variables reported at baseline
*Three participants with missing lung cavitation status
[a] 34 participants with missing ARV status

⬆Plasma expression of IL-6 associated with increased risk of lung cavitation

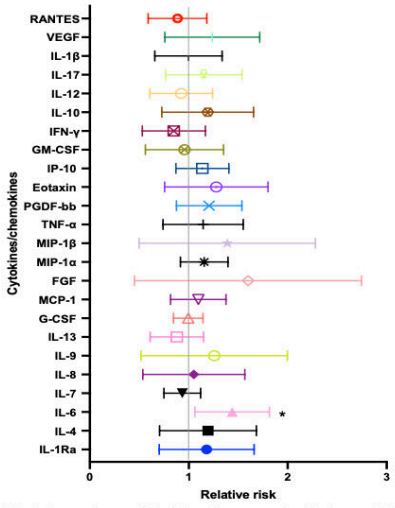

Figure 1. Association between plasma cytokine/chemokine expression with lung cavitation among HIV positive and negative participants with DR-TB (n=125). Asterix represents p-values<0.05.

PLHIV, males & participants with higher BMI had a reduced risk of lung cavitation

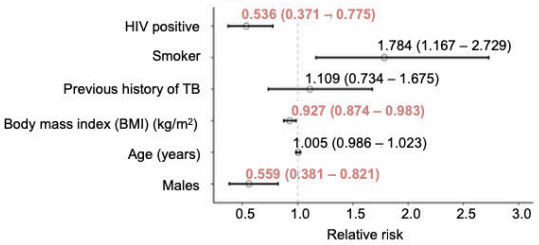

Figure 2. Relative risk for covariates in a multivariable Poisson model prior correcting for cytokines /chemokines (n=125)

Discussion

- IL-6 is a pleiotropic cytokine with dual pro- and anti-inflammatory properties.
- Increased IL-6 levels measured in bronchoalveolar lavage (BAL) of patients with pulmonary TB were previously shown to correlate with lung cavitation
- As seen with the use of IL-6 targeting in COVID-19, the efficacy of any IL-6 therapy would depend on the severity of the disease and the timing of the intervention; as well as the ability to distinguish between IL-6 as a cause or a consequence of the disease.

The original CAPRISA 020 InDEX study was supported by the by EDCTP Senior Fellowship (grant no. TMA2018SF-2467). TM was supported by the DST-NRF Centre of Excellence (CoE) in HIV Prevention (CoE UID number: 96354) and the Poliomyelitis Research Foundation (Grant no. 21/58). AS was supported by EDCTP Career Development Fellowship (TMA2016CDF-1582).

Appendix B: Co-authored manuscripts

ADDITIONAL MANUSCRIPTS AND CONTRIBUTION TO OTHER RESEARCH PROJECTS

In addition to my own research project, I assisted on several other projects during my PhD and this work has resulted in 2 published papers and 1 submitted manuscript that I am a co-author on. The details of the projects and manuscripts are summarised below.

MANUSCRIPT 1:

Samsunder *et al.*
Diagnostic and Prognostic Research (2023) 7:14
https://doi.org/10.1186/s41512-023-00151-3

Diagnostic and Prognostic Research

RESEARCH Open Access

Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa

Natasha Samsunder[1], Gila Lustig[1], Slindile Ngubane[1], Thando Glory Maseko[1], Santhuri Rambaran[1], Sinaye Ngcapu[1,2], Stanley Nzuzo Magini[1], Lara Lewis[1], Cherie Cawood[3], Ayesha B. M. Kharsany[1,2], Quarraisha Abdool Karim[1,4], Salim Abdool Karim[1,4], Kogieleum Naidoo[1,5] and Aida Sivro[1,2,5,6,7*]

Abstract

Background Rapid antigen tests detecting SARS-CoV-2 were shown to be a useful tool in managing the COVID-19 pandemic. Here, we report on the results of a prospective diagnostic accuracy study of four SARS-CoV-2 rapid antigen tests in a South African setting.

Methods Rapid antigen test evaluations were performed through drive-through testing centres in Durban, South Africa, from July to December 2021. Two evaluation studies were performed: nasal Panbio COVID-19 Ag Rapid Test Device (Abbott) was evaluated in parallel with the nasopharyngeal Espline SARS-CoV-2 Ag test (Fujirebio), followed by the evaluation of nasal RightSign COVID-19 Antigen Rapid test Cassette (Hangzhou Biotest Biotech) in parallel with the nasopharyngeal STANDARD Q COVID-19 Ag test (SD Biosensor). The Abbott RealTime SARS-CoV-2 assay was used as a reference test.

Results Evaluation of Panbio and Espline Ag tests was performed on 494 samples (31% positivity), while the evaluation of Standard Q and RightTest Ag tests was performed on 539 samples (13.17% positivity). The overall sensitivity for all four tests ranged between 60 and 72% with excellent specificity values (> 98%). Sensitivity increased to > 80% in all tests in samples with cycle number value < 20. All four tests performed best in samples from patients presenting within the first week of symptom onset.

Conclusions All four evaluated tests detected a majority of the cases within the first week of symptom onset with high viral load.

Keywords COVID-19, SARS-CoV-2, Antigen rapid diagnostic test, Performance evaluation

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MANUSCRIPT 2:

Rambaran *et al. BMC Immunology* (2023) 24:21
https://doi.org/10.1186/s12865-023-00558-z

BMC Immunology

RESEARCH Open Access

Blood monocyte and dendritic cell profiles among people living with HIV with *Mycobacterium tuberculosis* co-infection

Santhuri Rambaran[1,2,3], Thando Glory Maseko[1,2], Lara Lewis[1], Razia Hassan-Moosa[1,2], Derseree Archary[1,4], Sinaye Ngcapu[1,4], Nigel Garrett[1,5], Lyle R. McKinnon[1,6,7], Nesri Padayatchi[1,2], Kogieleum Naidoo[1,2] and Aida Sivro[1,2,4,6,8*]

Abstract

Background Understanding the complex interactions of the immune response mediated by *Mycobacterium tuberculosis* and HIV co-infection is fundamental to disease biomarker discovery, vaccine, and drug development. Using flow cytometry, we characterized the frequencies and phenotypic differences in monocytes and dendritic cell populations using peripheral blood mononuclear cells from individuals with recurrent, active pulmonary tuberculosis with and without coexisting HIV infection (CAPRISA 011, Clinicaltrials.gov, NCT02114684, 29/01/2014) and compared them to samples from HIV positive individuals and healthy controls. Additionally, we assessed the associations between the frequency of monocyte and dendritic cell subsets and time to culture conversion and cavitary disease in patients with active TB using a cox proportional hazards and logistic regression models.

Results Compared to healthy controls, the frequency of total monocytes (HLA-DR + CD14 +) was significantly higher in the TB/HIV and TB groups and the frequency of dendritic cells (HLA-DR + CD14-) was significantly higher in TB/HIV and HIV groups. We observed significant variation in the expression of CCR2, CD40, CD11b, CD86, CD163, CX3CR1 across different cell subsets in the four study groups. Increase in CCR2, CD11b and CD40 was associated with active TB infection, while decrease in CX3CR1 and increase in CD163 was associated with HIV infection. Expression of CX3CR1 (aHR 0.98, 95% CI 0.963 – 0.997, $p = 0.019$) on non-classical monocytes associated with longer time to TB culture conversion in the multivariable model correcting for randomization arm, age, sex, HIV status, lung cavitation, alcohol use, smoking and BMI. Higher surface expression of CD86 (aOR 1.017, 95% CI 1.001 – 1.032, $p = 0.033$) on intermediate monocytes associated with the presence of lung cavitation, while higher expression of transitional monocytes (aOR 0.944, 95% CI 0.892 – 0.999, $p = 0.047$) associated with the absence of lung cavitation in the multivariable model.

Conclusion These data provide valuable insight into the heterogenous role of monocyte and dendritic cells in TB and HIV infections.

Keywords Pulmonary-tuberculosis, Inflammation, HIV, Monocytes, Dendritic cells

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MANUSCRIPT 3:

STATUS:

Submitted: *Journal of Clinical Virology Plus* (JCVPLUS-D-22-00177)

Performance of SARS-CoV-2 rapid antigen tests during first and second wave of COVID-19 infections in South Africa

Natasha Samsunder, **Thando Glory Maseko**, Santhuri Rambaran, Slindile Ngubane, Sinaye Ngcapu, Stanley Nzuzo Magini, Razia Hassan-Moosa, Lara Lewis, Ayesha BM Kharsany, Quarraisha Abdool Karim, Salim Abdool Karim, Kogieleum Naidoo, Aida Sivro

Abstract

Background: Efficient testing strategies are key for diagnosis, clinical management, and outbreak control of SARS-CoV-2/COVID-19.

Methods: Here we evaluated two widely used rapid antigen tests, the SD Biosensor, STANDARD Q COVID-19 Ag Test (n=604) and the PanBio COVID-19 Ag Rapid Test (n=692) during the first (Ancestral variant with D614G mutation) and second (Beta variant) waves in South Africa. Two nasopharyngeal swabs were collected from different nostrils and shipped at room temperature to the central laboratory for processing. Abbott RealTime SARS-CoV-2 assay was used as a reference assay, with 58/604 samples testing positive in the Standard Q Ag test evaluation and 197/692 samples testing positive in the Panbio Ag Test evaluation.

Results: We observed overall low sensitivity for both tests: 53.45% for the SD Biosensor and 52.79% for the PanBio test. The specificity was 100% for SD Biosensor Ag test and 99.39% for Panbio Ag test. For samples with cycle threshold values <20, the test sensitivity rose to 88.46% for SD Biosensor Ag test and 83.65% for Panbio Ag test. Sensitivity was highest within the first week post symptom onset: 68.75% for SD Biosensor Ag test and 63.96% for Panbio Ag test.

Conclusions: As previously reported, tests performed best in symptomatic patients within the first week post symptom onset. The delay between sample collection and antigen test processing may have impacted the sensitivity of the assays.

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Appendix C: UKZN BREC approval letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 January 2021

Miss Thando Glory Maseko (214552192)
School of Lab Med & Medical Sc
Medical School

Dear Miss Maseko,

Protocol reference number: BREC/00002197/2020
Project title: Natural killer cell responses and lymphocyte trafficking in TB/HIV co-infection.
Degree Purposes: PhD

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 05 January 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations and (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_1_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 05 January 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC 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 (2015), South African National Good Clinical Practice Guidelines (2006) (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 09 February 2021.

Yours sincerely,

[redacted]
Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

18 January 2022

Miss Thando Glory Maseko (214552192)
School of Laboratory Medicine & Medical Science
Medical School

Dear Miss Maseko,

Protocol reference number: BREC/00002197/2020
Project title: Natural killer cell responses and lymphocyte trafficking in TB/HIV co-infection.
Degree Purposes: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**05 January 2022**
Expiration of Ethical Approval:	**04 January 2023**

I wish to advise you that your application for Recertification received on 11 January 2022 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 08 February 2022.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof S Singh
Deputy Chair: Biomedical Research Ethics Committee

Appendix D: Community outreach

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

UKZNDABA online
Your Campus Electronic Newsletter

17 November 2022 Volume: 10 Issue: 52

UKZN postgraduate students who provided educational outreach for high school learners.

Taking Education to Youngsters in Rural Areas

Click here for isiZulu version

UKZN postgraduate students from the College of Health Sciences who are also fellows at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) visited Sibanesihle High and Ngcedomhlophe High in the Mafakatini area of the uMgungundlovu Municipality to conduct a community outreach programme.

The students involved were Ms Noluthando Mazibuko-Motau, Ms Nomusa Zondo, Ms Thando Maseko, Ms Senamile Ngema, Mr Lungelo Ntuli, Ms Nonsikelelo Ndlela, Ms Sibongiseni Masondo, Ms Bongeka Mabaso and Ms Silindile Ngubane.

The aim of the outreach project was to inform and raise awareness among learners about the human immunodeficiency virus (HIV), tuberculosis (TB), sexually transmitted infections (STIs), the risks associated with an early sexual debut, and teenage pregnancy.

Speakers at the event were all positive about the outcome:

Ms Noluthando Mazibuko-Motau said: 'Our programme was a huge success with about 600 students from Ncedomhlophe High and 200 from Sibanesihle High attending. My talk focused on teenage pregnancy, where I emphasised the importance of abstaining from sex and the consequences of being a teenage/adolescent mother. I also spoke to the learners about the research conducted by postgraduates at CAPRISA.

Ms Nomusa Zondo said: 'My talk informed learners about my research at UKZN/CAPRISA, which is on understanding how biological factors affect pre-exposure prophylaxis (PrEP) effectiveness in African women. We hope to continue with this outreach programme, as it is essential to share our research findings and continually educate the public about scientific advances in diseases that directly affect them.'

Mr Lungelo Ntulispoke to scholars about Human Papillomavirus(HPV) infection - a common STI among young adolescent girls - and HPV prevention in young boys and girls.

Ms Thando Maseko said, 'The overlap and the devastating effect of TB and the HIV epidemic in sub-Saharan Africa represents one of the most significant public health challenges. My talk aimed to inform the learners about TB, cellular immune responses in TB/HIV co-infection and the importance of adhering to treatment.'

Ms Senamile Ngema said: 'As someone interested in TB, drug-resistant TB and treatment, my talk focused on the knowledge of the disease and crucial steps one needs to take if/when symptomatic and exposed, and the importance of TB treatment completion. I think it is about time that the scientific community goes out to various communities to share the knowledge they have generated and gained so that the next generation has a better understanding of health issues such as HIV and TB.'

Ms Sibongiseni Masondo told young girls about behavioural factors contributing to HIV and the risks that intravaginal products may have on genital inflammation. 'Girls must know the risks of douching and intravaginal products because of the harm they pose to genital health.'

Ms Nonsikelelo Ndlela covered information regarding the common causes and symptoms of bacterial vaginosis (BV). 'I believe that awareness about BV is very critical, considering that it increases the risk of HIV acquisition in young women.'

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- "Tireless Champion of Health in Africa" Honoured by UKZN
- TE Madiba Colorectal Unit Launched at Inkosi Albert Luthuli Hospital in Durban
- Leading Mycologist Delivers Guest Lecture at UKZN
- Education Academic Receives Prestigious Research Award
- Alumnus Nominated for Community and Human Rights Africa Award
- Eminent Physicists Deliver Lectures in Durban
- AORC Commemorates Ombuds Day
- Taking Education to Youngsters in Rural Areas
- University Community Rallies Behind Computer Literary Course for Campus Management Services Staff
- Humanities Academic Delivers Lecture Series at US University
- Aerotropolis Institute Africa Delivered Route Development Masterclass at UKZN
- Challenges Faced by African Arts, Humanities and Social Sciences Scholarship Investigated
- Good Support for 2022 UKZN Soccer Championship League

The students were accompanied by Mr Patrick Mdletshe, CAPRISA's Head of the Community Programme. 'CAPRISA remains committed to fighting HIV/AIDS, TB and STIs in communities and seeks to empower young women to protect themselves against HIV infections. The School-based programme, led by the fellows, was a robust, friendly dialogue about pertinent issues as well as encouraging learners to work hard in school,' said Mdletshe.

'Progress in HIV prevention is only possible when there are platforms for dialogue like this to facilitate learners' knowledge in making informed decisions,' said Sibanesihle Principal, Mr A Ngobese.

Words: Noluthando Mazibuko-Motau and Thando Maseko

Photograph: Lwazikazi Sibisi

- Finals
- Ellie Newman Memorial Moot Court Competition Celebrates 50th Anniversary
- Preservation Needs Symposium and Workshop
- UKZN Hosts Quaternary Research Conference in St Lucia's Wetlands
- Small-Scale Farmers' Adaptation to Climate Change Focus of Agricultural Symposium
- UKZN Hosts National Development Plan State Capacity Conference
- Training Workshop on Developing Teaching Portfolios and Teaching Files

Appendix E: Plagiarism report