Immune biomarkers of pulmonary tuberculosis treatment response and disease severity among HIV-infected and uninfected individuals from Kwazulu-Natal, South Africa

Presented by

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209514963

A thesis by publications and a manuscript submitted in fulfilment of the requirements for the degree of ***Doctor of Philosophy (Medicine)*** in the School of Laboratory Medicine and Medical Science, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Supervisor: **Dr Aida Sivro**

Co-Supervisor: **Professor Kogieleum Naidoo**

2023

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PREFACE

The experimental work described in this thesis was conducted at the at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) Laboratory, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. This work was supervised by Dr Aida Sivro and Professor Kogieleum Naidoo.

S. Rambaran [redacted] Date October 13th, 2023

Dr A Sivro [redacted] Date September 23rd, 2023

Professor K Naidoo _____ Date October 13th, 2023

DECLARATION 1 - Author Contributions

I, Miss Santhuri Rambaran declare as follows:

1. This work has not been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

2. My contribution towards the project were as follows:

   I participated in the conception and design of the study.
   I optimised and performed all the laboratory assays including:
   - measurement of 24 cytokines/chemokines in plasma specimens by multiplex assays and conventional ELISAs.
   - flow cytometry analysis to determine phenotypic profiles of monocytes, dendritic cells and $CD4^+$ T cells in PBMC samples.

   I analysed and interpreted the data which was further validated by a designated CAPRISA statistician.

   The manuscripts were written by me, reviewed by my supervisors Dr Aida Sivro and Professor Kogieleum Naidoo and approved by all co-authors.

Signed: _[redacted]

Date: October 13th, 2023

DECLARATION 2 - Plagiarism Declaration

I, Miss Santhuri Rambaran declare as follows:

1. The research reported in this dissertation, except where otherwise indicated, is my original research. The experimental work described in this thesis was carried out by me and was conducted at the at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) Laboratory, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa.
2. This dissertation has not been submitted in any form for any degree or diploma for examination to any tertiary institution.

3. This dissertation does not contain other scientists' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists.

4. This dissertation does not contain other scientists' writing, unless specifically acknowledged as being sourced from other scientists. Where other written sources have been quoted, then their words have been re-written, but the general information attributed to them has been referenced.

5. This dissertation does not contain text, graphics or tables taken from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference section.

Signed: _[redacted]_____ Date: October 13th, 2023

Santhuri Rambaran (Candidate)

Supervisor's Declaration

I hereby declare that I have read and approved this thesis for submission and believe its contents to be the original work of the candidate, with other work adequately referenced

Signed: [redacted] Date: September 23rd, 2023

Dr Aida Sivro (Supervisor)

Signed: Date: October 13th, 2023

Professor Kogieleum Naidoo (Co-Supervisor)

DELCARATION 3 - Manuscripts and Publications Declaration

This thesis has been submitted to fulfil the requirements for thesis submission by manuscript, which may have at least three papers with the student as the prime author, and at least two of the papers that constitute original research. This thesis includes two published papers and one manuscript under review to meet the UKZN PhD requirements for submission by manuscript. The work presented here originated from the use of biological specimens from the CAPRISA 011 Improving Retreatment Success (IMPRESS) trial and the CAPRISA 002 Acute HIV Infection study. The study protocol for the CAPRISA 011 and 002 trials can be accessed online at www.caprisa.org/Pages/CAPRISAStudies.

Listed below are the manuscripts and publications emanating from this study.

Publication 1:

Santhuri Rambaran, Kogieleum Naidoo, Lara Lewis, Razia Hassan-Moosa, Dhineshree Govender, Natasha Samsunder, Thomas J Scriba, Nesri Padayatchi and Aida Sivro.

Effect of Inflammatory Cytokines/ Chemokines on Pulmonary Tuberculosis Culture Conversion and Disease Severity in HIV-Infected and -Uninfected Individuals From South Africa. Published in *Frontiers in Immunology* on 01 April 2021 (PMID: 33868272).

Publication 2:

Santhuri Rambaran, Thando Glory Maseko, Lara Lewis, Razia Hassan-Moosa, Derseree Archary, Sinaye Ngcapu, Nigel Garrett, Lyle R. McKinnon, Nesri Padayatchi, Kogieleum Naidoo and Aida Sivro. **Blood monocyte and dendritic cell profiles among people living with HIV with *Mycobacterium tuberculosis* co-infection**. Published in *BMC Immunology* on 21 July 2023. (PMID: 37480005)

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Santhuri Rambaran, Thando Glory Maseko, Lara Lewis, Derseree Archary, Sinaye Ngcapu, Lyle R. McKinnon, Nesri Padayatchi, Kogieleum Naidoo, Aida Sivro. **$CD4^+$ T cell profiles during TB/HIV co-infection and their associations with TB treatment response and cavitary disease.** Under internal review with CAPRISA Scientific Review Committee.

CONFERENCE PRESENTATIONS

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e-Poster Presentation

Santhuri Rambaran, Kogieleum Naidoo, Razia Hassan-Moosa, Dhineshree Govender, Lara Lewis, Natasha Samsunder, Nesri Padayatchi and Aida Sivro. **Plasma cytokines/chemokines as biomarkers of pulmonary tuberculosis culture conversion and disease severity in HIV infected and uninfected individuals in South Africa.** Virtual live Keystone Symposia eSymposia meeting eSymposia | Tuberculosis: Science Aimed at Ending the Epidemic from December 2-4, 2020.

Plasma cytokines/chemokines as biomarkers of pulmonary tuberculosis culture conversion and disease severity in HIV infected and uninfected individuals in South Africa

Santhuri Rambaran[1], Kogieleum Naidoo[1,2], Razia Hassan-Moosa[1,2], Dhineshree Govender[1], Lara Lewis[1], Natasha Samsunder[1], Nesri Padayatchi[1,2] and Aida Sivro[1,3,4]

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Introduction

- Prevention and control strategies in retreated TB individuals are urgently needed given the complex interaction between HIV and TB in hyperendemic settings such as South Africa.
- Sputum culture conversion at 2 months following TB treatment has been used as a surrogate biomarker of TB therapy response and long-term cure; however it has a low predictive value for predicting treatment failure and relapse [1].
- Identification of blood-based biomarkers that are non-sputum-based tests is imperative to accurately monitor treatment efficacy, treatment failure and predict relapse.
- Certain cytokines and chemokines could also mediate immune mediated pathology in TB disease by driving the enhanced infiltration of immune cells to the site of infection [2,3].
- HIV is known to cause dysregulation of the TB immune response [4,5].
- Here we aimed to determine if candidate plasma immune biomarkers detected in re-infected individuals with active TB disease, associated with 8-week TB culture conversion, time to culture conversion and disease severity in TB and TB-HIV coinfected individuals.

Methods and Materials

Study Design

- Nested un-matched case (n= 31) - control (n=101) study with cases defined as those participants who failed to culture convert within 8-weeks of treatment initiation from Improving Retreatment Success (IMPRESS, NCT02114684) trial

Patient Population and Setting

- Adults > 18 years, with a previous history of TB and the current diagnosis of rifampicin susceptible sputum smear-positive MTB by GeneXpert MTB/RIF® technology treated at an urban clinic (CAPRISA eThekwini Research Clinic)

Cytokine/Chemokine measurements

- Millipore Milliplex® assays (Map Human Cytokine / Chemokine Panel I and IV) were used and analysed on a BioPlex-200 system (Bio-Rad)
- Soluble CD14 (sCD14) levels were measured using the Human CD14 Quantikine® ELISA Kit and human Lipopolysaccharide-Binding Protein (LBP) plasma levels were measured using the LBP kit (R&D Systems Inc, USA).

Statistical Analysis

- Using multivariable models, randomization arm, HIV status, age, gender, BMI, lung cavitation, alcohol and smoking use (total cohort) were corrected for and additionally viral load and CD4 count (HIV infected subgroup)
- Logistic regression was used for the association between plasma cytokine/chemokine expression at baseline and 8-week culture conversion and disease severity measured by lung cavitation
- Cox proportional hazards model was used to determine the association between cytokine/chemokine expression at baseline and time to culture conversion

Results

- IP-10 expression significantly associated with an increased odds of culture conversion by 8 weeks among the total cohort (OR 4.255, 95% CI 1.025 – 17.544, p=0.046) and HIV infected subgroup(OR 10.204, 95% CI 1.247 – 83.333, p=0.030) (Fig.1).
- MCP-3 (adjusted hazards ratio (aHR) 1.723, 95% CI 1.040 – 2.855, p=0.035) and IL-6 (aHR 1.409, 95% CI 1.045 – 1.899, p=0.024) expression associated with shorter time to culture conversion among the total cohort (Fig.2).
- IL-6 (aHR 1.783, 95% CI 1.128 – 2.820, p=0.013), IL-1RA (aHR 2.595, 95% CI1.136 – 5.926, p=0.024), IP-10 (aHR 2.068, 95% CI 1.034 – 4.137, p=0.040) and IL-1α (aHR 2.008, 95% CI 1.053 – 3.831, p=0.035) significantly associated with shorter time to culture conversion among the HIV infected subgroup.
- IL-6 (OR 2.543, 95% CI 1.254 – 5.160, p=0.010) and IL-1RA (OR 4.639, 95% CI 1.203 – 21.031, p=0.047) expression significantly associated with lung cavitation among total cohort, and in the HIV infected subgroup (IL-6: OR 2.644, 95% CI 1.062 – 6.585, p=0.037; IL-1RA: OR 7.795, 95% CI 1.177 – 51.611, p=0.033) (Fig.3).

Table 1. Demographic and clinical characteristics of participants

Variables	Cases n=31	Controls n=101	p-value
Randomization arm n (%)			
HRZE -Control	18 (58)	45 (45)	.220
HRZM - Active	13 (42)	56 (55)	
Age (y), median (IQR)	34 (28 – 43)	36 (31 – 41)	.502
Gender, n (%)			
Male	25 (81)	70 (69)	.259
Female	6 (19)	31 (31)	
Body mass index (kg/m^2), median (IQR)	19.28 (17.96 – 19.98)	20.42 (18.64 – 22.95)	**.031**
HIV status n (%)			
Negative	11 (35)	25 (25)	.256
Positive	20 (65)	76 (75)	
CD4 cell count (cells/mm^3), median (IQR)	288 (214 – 410)	248 (127 – 413)	.311
Viral load (copies/ml), median (IQR)	3453 (20 – 18289)	5878 (20 – 111087)	.386
ARV status* n (%)			
Yes	10 (50)	31 (41)	.609
No	9 (45)	43 (57)	
Lung Cavities n (%)			
None	6 (19.3)	33 (32.7)	.317
One Lung	14 (45.2)	42 (41.6)	
Both Lungs	11 (35.5)	26 (25.7)	
Days to first negative solid culture, median (IQR)#	84 (82 – 91)	42 (28 -55)	**<.0001**
Alcohol Use in the past 3 months n (%)			
Yes	13 (42)	24 (24)	.067
Smoking in past 3months n (%)			
Yes	15 (48)	29 (29)	.051
Smear Grade n (%)			
1+	6 (19.4)	21 (20.8)	.124
2+	2 (6.4)	22 (21.8)	
3+	23 (74.2)	58 (57.4)	

* 3 missing ARV status
# Measures for all variables, except days to first negative culture are reported at baseline

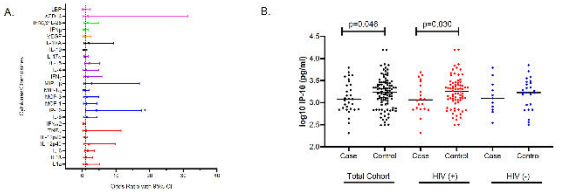

Figure 1. A) Association between plasma cytokine/chemokine expression and 8-week culture conversion. B) logged IP-10 expression in total cohort and separated by HIV status. P-values shown on the graphs are results of the multivariable analysis

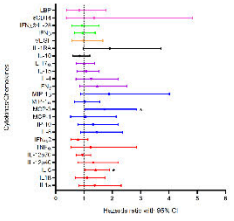

Figure 2. Association between cytokine/chemokine expression and time to culture conversion in the multivariable model. Individual associations are shown between time to culture conversion and each plasma biomarker (y-axis). Error bars depict 95% confidence intervals. The dotted line at **1** distinguishes the hazards ratio of higher than 1 (to the right) indicating shorter time to culture conversion and lower than 1 (to the left) indicating longer time to culture conversion. Asterisk depicts significant associations.

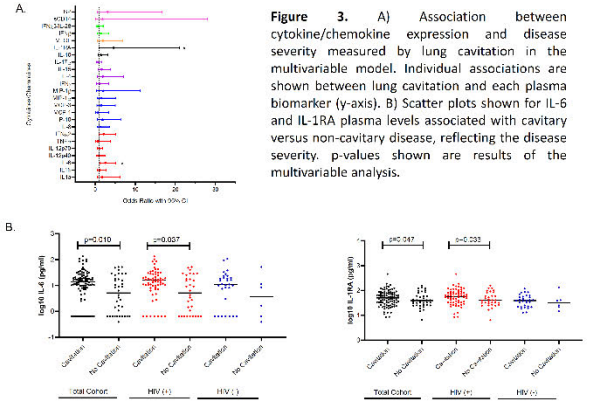

Figure 3. A) Association between cytokine/chemokine expression and disease severity measured by lung cavitation in the multivariable model. Individual associations are shown between lung cavitation and each plasma biomarker (y-axis). B) Scatter plots shown for IL-6 and IL-1RA plasma levels associated with cavitary versus non-cavitary disease, reflecting the disease severity. p-values shown are results of the multivariable analysis.

Discussion

- Identification and validation of TB diagnostic and surrogate markers in HIV-infected individuals are urgently required for patient management and development of new therapeutics and vaccines
- IP-10 significantly associated with an increased odds of 8-week culture conversion in multivariable models of both the total cohort and among HIV infected patients. A similar trend is visible among the HIV uninfected group.
- Our results suggest a dual role for IL-6; being associated with bacterial clearance, as well as the severity of the disease irrespective of HIV status.
- The data emanating from our study highlights the important potential of evaluating plasma biomarkers that can be used as an adjunct tool in TB screening and management of the disease in a high endemic setting with HIV co-infection.

Acknowledgments

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ETHICAL APPROVAL

All participants of the CAPRISA 011 trial (Clinicaltrials.gov, NCT02114684) and CAPRISA 002 study provided informed consent for the storage of and use of their specimens in future studies. University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) reviewed and approved the original studies [BFC029/13 (CAPRISA 011), E013/04 (CAPRISA 002) and BE432/12 (Healthy donors)]. The presented study was reviewed and approved by UKZN BREC under ethics number BREC/00000014/2019 (Appendix B).

DEDICATION

This thesis is dedicated to my beloved late dad, **Amrithkumar Harinam Rambaran.** You planted the seed of knowledge in my mind and nurtured it to the best you could. I have walked this somewhat tiresome, life-changing journey to achieve your wish with you and your words of wanting this title to your name always on my mind. I wish you were here to bask in this milestone achievement and to see this title attached to your name.

My mum, Shaveer & Santrika for your endless love and support - this last one is for you with all my love. Finally!

ACKNOWLEDGEMENTS

To my creator:

Yaa Devi Sarva-Bhutessu Buddhi-Ruupenna Samsthitaa | Namas-Tasyai Namas-Tasyai Namas-Tasyai Namo Namah

To that Devi Who in All Beings is Abiding in the Form of Intelligence,

Salutations to Her, Salutations to Her, Salutations to Her, Salutations again and again.

Dr Aida Sivro, my supervisor and mentor, thank you for your support and supervision during this very developmental phase of my life. I am appreciative for all your input and guidance that you have provided me with throughout my journey and giving me advice whenever I needed. No relationship is complete with the ups and downs, so thank you for allowing me to build character and grow as a person during this process. I have been blessed with you as my supervisor.

Professor Kogieleum Naidoo, my co-supervisor and mentor, thank you for your constant motivation and support towards me. Thank you for believing and trusting in me even when I would lose hope and my many discussions of calling it quits. I will always be grateful for the opportunities you have provided.

My parents, **Dad**: thank you for looking over me and guiding me to achieve this then a distant dream of yours, now a reality. **Mum**, you are the epitome of unconditional love and support. Your selflessness towards me is priceless and has never gone unnoticed. The sacrifices you have made and still make for me to achieve my goals and dreams has motivated me to this point. These qualities of yours have allowed me to be selfish to ultimately persevere to achieve my goals. There was not a parenting book in the world that could have prepared you for my eccentricity.

My siblings, Shaveer and Santrika, thank you for your unconditional support and love during these years, lending your ears to all my scientific jargon. You have stood beside me like two pillars (which is why I am the middle child) supporting me through this very long journey but never once asked me when it will end. I am grateful for you both. I hope that when you see this "end product" it will all come together like pieces of a puzzle that fit together perfectly just like the three of us.

Dr. Navisha Dookie, my dear bestie (more like my bigger sister), my best half and confidant thank you for being supportive and motivating always. I am extremely grateful to have someone like you in my life and you have played such a pivotal role throughout this fundamental journey of mine. Words will never be able to describe how much you mean to me.

Logan Pillay, a.k.a my personal chauffeur, my other dad in disguise. I always think of you as coming into my life as a guardian angel. Thank you for your support, motivation and kind words to complete this before you retire. I will always be indebted to you for just being the humble human being that you are and your wise words of wisdom and advice will forever live in my heart.

CAPRISA Laboratory Staff, a special thank you and appreciation to all that have assisted me in my time of need during my experiments and to all that have supported me and motivated me, "we waiting to see the result of hard work".

CAPRISA staff and colleagues thank you all for being the support structure that I needed through this journey.

The National Research Foundation (NRF) for providing me with a scholarship that has allowed me to pursue this degree.

College of Health Sciences (CHS) for running costs to purchase research reagents.

LIST OF ABBREVIATIONS

ACD	Acid citrate dextrose
ART	Antiretroviral Therapy
AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
APCs	Antigen presenting cells
ART	Antiretroviral Therapy
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette-Guérin
BMI	Body Mass Index
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CI	Confidence interval
CRP	C Reactive Protein
CFP	culture filtrate protein
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
DR	Drug Resistant
DS	Drug Sensitive
DST	Drug susceptibility testing
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESAT-6	early secreted antigenic target 6
HAART	Highly Active Antiretroviral Therapy
HDT	Host Directed Therapy
HIV	Human immunodeficiency virus
HRZE	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol
HRZM	Isoniazid, Rifampicin, Pyrazinamide, Moxifloxacin
IFN	Interferon
IFN-γ	Interferon gamma
IGRA	Interferon-gamma release assay

IP-10	Interferon gamma-induced protein-10
IL	Interleukin
IL-1RA	Interleukin-1 receptor antagonist
IQR	Interquartile range
IRIS	Immune Reconstitution Inflammatory Syndrome
IMPRESS	Improving Retreatment Success
KZN	KwaZulu-Natal
LAM	Lipoarabinomannan
LBP	Lipopolysaccharide Binding Protein
LDL	Low-Density Lipoprotein
LF - LAM	Lateral Flow lipoarabinomannan
LPS	Lipopolysaccharide
LTBI	Latent TB Infection
MCP	Monocyte chemotactic protein
MIP	Macrophage inflammatory protein
Mtb	*Mycobacterium tuberculosis*
MHC	Major Histocompatibility Complex
mDCs	myeloid Dendritic Cells
MDDCs	Monocyte Derived Dendritic Cells
MDR	Multi-drug resistant
MTCT	Mother-to-child transmission
NK	Natural killer cells
NRTI	Nucleoside Reverse Transcriptase Inhibitors
NNRTI	Non- Nucleoside Reverse Transcriptase Inhibitors
OR	Odds ratio
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
pDCs	Plasmacytoid dendritic cells
POC	Point Of Care
PPD	Purified protein derivative
PLWH	People living with HIV
PRRs	pattern recognition receptors

PrEP	Pre-exposure prophylaxis
RNA	Ribonucleic Acid
Treg	Regulatory T cell
SA	South Africa
sICAM	Soluble Intercellular adhesion molecule
STI	Sexually Transmitted Infection
SSA	Sub-Saharan Africa
TB	Tuberculosis
TNF	Tumor necrosis factor
Th	T helper
TST	tuberculin skin test
TLR	Toll like receptor
TNF-α	Tumour necrosis factor-α
TRuTH	TB Recurrence upon Treatment with HAART
UKZN	University of KwaZulu-Natal
VEGF	Vascular endothelial growth factor
VL	Viral load
WHO	World Health Organization
α	alpha
β	beta
γ	gamma
µg	microgram
µl	microlitre
ml	millilitre
%	Percentage

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Figure 1. (A) Representative gating strategy. The staining profile of PBMC sample from TB /HIV co-infected participant is shown. Differences in percentages of **(B)** Total $CD4^+$ T cells, **(C)** $CD38^+$ HLA-DR^+ on $CD4^+$ T cells **(D)** $CD25^+$ $CD4^+$ T cells **(E)** β7Hi $CD45RA^-$ between study groups (TB/HIV, TB, and HC).

THESIS ABSTRACT

Background: Tuberculosis is one of the major causes of morbidity and mortality worldwide. The COVID -19 pandemic has had a devastating impact on TB, contributing to increased incidence of both TB and drug-resistant TB. Identification of host immune biomarkers of TB risk, treatment outcome and disease severity are key to the development of more efficient diagnostics and treatment modalities. There is an urgent need for accurate and easily detectable non-sputum-based biomarkers that can correlate with the activity or burden of *Mycobacterium tuberculosis*. Here, we characterised soluble and cellular phenotypes during active TB and TB/HIV co-infection and assessed their associations with time to negative culture conversion and disease severity.

Methods: The study was performed utilizing stored plasma and peripheral blood mononuclear cells from the Improving Retreatment Success (IMPRESS) trial. Multiplex immunoassays and ELISAs were used to evaluate 24 cytokine and chemokine expression during active TB (n=132). Flow cytometry was used to evaluate phenotypic profiles of monocytes, dendritic cells (n=90) and $CD4^+$ T cells (n=75). A Cox proportional hazards and logistic regression models were used to assess the associations between the measured cytokines and chemokines, phenotypic profiles of monocytes, dendritic cells and $CD4^+$ T cells and time to negative culture conversion and lung cavitation in individuals with TB and TB/HIV co-infection.

Results: We identified soluble inflammatory signatures of treatment response and disease severity. IP-10 expression during active TB was associated with increased odds of sputum culture conversion by 8-weeks in the total cohort and among the HIV-infected individuals. Increased MCP-3 expression was associated with a shorter time to culture conversion in the total cohort. While among the HIV-infected individuals, higher expression of IL-1RA, IP-10 and IL-1α associated with a shorter time to culture conversion. Higher expression of IL-6 was significantly associated with shorter time to culture conversion and increased risk of lung cavitation in the overall cohort and among TB/HIV co-infected individuals. Additionally, higher IL-1RA expression was associated with the presence of lung cavitation in the total cohort and in HIV-infected individuals. We observed distinct monocyte and dendritic cell profiles in TB/HIV co-infection. Individuals with TB/HIV co-infection had a significantly

higher percentage of total monocytes and dendritic cells compared to healthy controls. Increase in CCR2, CD11b and CD40 was associated with active TB while decrease in CX3CR1 and increase in CD163 was associated with HIV infection. Expression of CX3CR1 on non-classical monocytes was associated with longer time to culture conversion while expression of CD86 on intermediate monocytes was associated with presence of lung cavitation. With respect to $CD4^+$ T cells HIV positive individuals with active TB had significantly lower percentage of $CD4^+$ T cells and significantly higher proportion of activated $CD4^+$ T cells compared to TB and healthy control groups. Percentage of $CD4^+$ T cells was significantly associated with increased risk, while the percentage of activated $CD4^+$ T cells was associated with decreased risk of lung cavitation. Integrin $\alpha4\beta7$ expressing $CD4^+$ T cells were increased in TB/HIV compared to TB group and was associated with longer time to TB culture conversion in co-infected individuals.

Conclusion: The data from this study provides valuable insight into the role that plasma immune biomarkers, monocytes, dendritic and $CD4^+$ T cells play in TB treatment response and disease severity in active TB and TB/HIV co-infection.

THESIS CONTEXT

There are five chapters presented in this thesis.

Chapter 1 provides the background and rationale for the research work presented in the subsequent chapters followed by the aims and objectives of this study and an overview of the study methods. The literature review included in this chapter provides a comprehensive insight into the TB and HIV epidemics and the host immune responses involved.

In **Chapter 2** we assessed the associations between plasma inflammatory markers and treatment response and disease severity to identify potentials non-sputum biomarkers of monitoring immune status among TB and TB/HIV coinfected individuals. The plasma expression of 24 cytokines/chemokines were measured using Multiplex Immunoassays and ELISAs in patients with active TB with and without concurrent HIV co-infection.

The resulting manuscript titled: "Effect of Inflammatory Cytokines/Chemokines on Pulmonary Tuberculosis Culture Conversion and Disease Severity in HIV-Infected and -Uninfected Individuals From South Africa" was published in *Frontiers in Immunology* on 01 April 2021 (PMID: 33868272).

In **Chapter 3** we characterised the associations between monocyte and dendritic cell subsets and time to culture conversion and cavitary disease in patients with recurrent active TB. Flow cytometry was used to characterize 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 and these were compared to samples from HIV positive individuals and healthy controls.

The resulting manuscript titled: "Blood monocyte and dendritic cell profiles among people living with HIV with *Mycobacterium tuberculosis* co-infection" was published in *BMC Immunology* on 21 July 2023 (PMID: 37480005).

In **Chapter 4** we characterised $CD4^+$ T cell phenotypes during active TB in patients with and without HIV co-infection and assessed their associations with TB treatment response and disease severity. Flow cytometry was used to characterize total $CD4^+$ T cell frequency and T cell activation as well as CD25 and β-integrin expression in individuals with active TB, TB/HIV co-infection and healthy controls.

The resulting manuscript titled: "$CD4^+$ T cell profiles during TB/HIV co-infection and their associations with TB treatment response and cavitary disease" is currently under internal review at CAPRISA Scientific Committee.

Chapter 5 summarizes and discusses the findings of this thesis. The final chapter also highlights the significance, strengths, limitations of the study and provides recommendations for future research.

CHAPTER 1

CHAPTER 1

INTRODUCTION - Literature Review

1.1.Tuberculosis background

Tuberculosis (TB) is an ancient infectious, communicable disease that has evolved into a superbug. It is believed that the genus *Mycobacterium* has been in existence for about 150 million years while *Mycobacterium tuberculosis* (Mtb) originated 15 000 years ago [1]. In 1882, Robert Koch, German microbiologist discovered that the bacillus, Mtb was the etiological agent responsible for TB [2]. Mtb is non-motile, intracellular microbe with a unique robust thick cell wall classified as an acid fast bacterium [3]. Mtb is known to affect the lungs most commonly, but it can infect any part of the body such as the spine and brain.

TB is a major global public health concern. Even though TB is both preventable and curable, following SARS-CoV-2/COVID-19, TB remains a leading cause of mortality for infectious diseases [4]. The continued high worldwide incidence of TB infections coupled with escalating resistance to previously and currently recommended antimicrobials, hampers the control of this airborne transmitted infection. Five to 10% of people infected with TB will develop symptomatic TB and the reason why some individuals develop active TB disease and others do not is not well understood [5]. Mtb is effective at subverting the host immune defences [6] and the interplay between Mtb and the host immune response plays a pivotal role in defining TB disease pathogenesis and disease progression [5]. The only vaccine licensed for prevention of TB disease is Bacille Calmette-Guerin (BCG), developed over 100 years ago by Albert Calmette and Camille Guérin using successfully attenuated *Mycobacterium bovis*. BCG prevents severe forms of TB in young children, however there is currently no vaccine that is effective in preventing TB disease in other populations. Development of effective vaccines, improved diagnostics, and novel, and shortened drug therapy are key in achieving control of TB globally [7].

1.2.Global TB Statistics and the TB Epidemic in South Africa

Globally, TB is currently the second leading infectious disease killer (following COVID-19) and the thirteenth (13^{th}) leading cause of overall mortality in 2021. An estimated quarter of the global population has been infected with TB. According to World Health Organization (WHO) Global TB Report 2022, in 2021, an estimated 10.6 million people developed active TB disease of whom 56.5 % were men (≥ 15 years), 32.5% were women and 11% children. Ninety percent of the TB cases occur among adults with the male: female ratio being 2:1. TB was responsible for an estimated 1.4 million deaths among HIV-negative people and an additional 187 000 among HIV-positive people globally in 2021 and this is almost double compared to HIV/AIDS deaths (0.68 million). Of the TB incident cases in 2021, 6.7% were among HIV-positive people [8]. Since 2007 and prior to COVID-19, TB has been the leading cause of death from a single infectious agent. About 23% of the world's population (1.7 billion people) have been estimated to have latent TB and are at risk of developing active disease [9].

Eighty seven percent (87%) of the estimated cases worldwide were from 30 high TB burden countries. In 2021, South Africa (SA) was among the 30 high TB burden countries. An estimated 304 000 people fell ill with TB and 56 000 died in 2021 in SA according to WHO Global TB Report 2022 [8]. SA was one of the seven high burden countries that had reached or passed the first milestone of a 20% reduction in the TB incidence rate by 2021 in comparison with 2015. SA has a high incidence of TB and HIV; in 2020, the country's TB incidence was estimated to be 554/100 000 [10]. Estimates of the percentage of new and previously treated TB cases with rifampicin resistant-TB were 3.4% and 7.1%, respectively [11].

In 2021, most people who developed TB were in the WHO regions of South-East Asia (45%), Africa (23%) and the Western Pacific (18%), with smaller proportions in the Eastern Mediterranean (8.1%), the Americas (2.9%) and Europe (2.2%). Although the global TB incidence rate was experiencing a steady decline of approximately 2% annually, it abruptly rebounded by 3.6% in 2020-2021, reversing most of the two decades' worth improvement progress. Between 2015 and 2021 the cumulative reduction was 10%, only halfway to the first milestone of the End TB Strategy.

The emergence of drug-resistant TB (DR-TB) remains a significant issue impacting public health. Globally in 2021, there was an estimated 450 000 incident cases of rifampicin resistant TB (RR-TB)/ multi-drug resistant TB MDR-TB and an estimated 191 000 deaths that occurred

due to MDR/RR-TB. The number of estimated incident cases of MDR/RR-TB in SA was 20 000.

SA is one of ten countries in 2021 that was able to reach TB testing coverage of more than 80%, and accounted for the 70% of the global gap between new MDR-TB cases identified and the number of people enrolled in treatment. SA was one of seven countries that collectively accounted for 82% of people who started treatment in 2021. KwaZulu-Natal (KZN), one of the nine provinces in SA, is burdened with the highest incidence of TB, followed by the Eastern Cape and Western Cape [12].

1.3.HIV background

The causative agent of AIDS, Human immunodeficiency virus (HIV), was not discovered until 1983 even though the initial cases of the disease made their appearances in 1981 [13, 14]. HIV is present in several body fluids of an infected individual, such as blood, semen, vaginal and rectal secretions and in breast milk. Sexual intercourse is the most common route of HIV transmission, the virus can also be transmitted from mother-to-child (MTCT) and through blood by needle sharing [15]. HIV type 1 (HIV-1) and HIV type 2 (HIV-2) are 2 lineages of HIV [16].

The HIV particle consists of an Envelope (Env), it is around 100 nm in diameter, and has an associated matrix which surrounds the capsid that protects the inner core. There are two copies of single-stranded RNA genome, polymerase and viral enzymes contained in the inner core [17]. One of the main reasons that the HIV is difficult to eradicate with current therapies is that HIV belongs to the genus *Lentivirus*, within the family of *Retroviridae* meaning it is a retrovirus and is therefore able to integrate its DNA into the host genome [18]. It has a small number of proteins and after entering a cell, single-strand RNA is reverse transcribed into HIV DNA that is integrated into the host DNA.

1.4.Global HIV statistics and the HIV Epidemic in South Africa

Since the start of the epidemic, an estimated 84.2 million people have been infected with HIV. According to the UNAIDS global HIV and AIDS statistics 2022 fact sheet, 1.5 million people were infected with HIV in 2021. In 2021, six in seven new HIV infections were among young adolescent girls aged 15-19 years and 63% of all new HIV infections are accounted for by girls and women aged 15-24 years in sub-Saharan Africa (SSA). Young women aged 15–24 years are twice as likely to be living with HIV than men of the same age. In 2021, there were around 650 000 people who died from AIDS-related illnesses globally [19].

SSA accounts for ~70% of the global HIV burden [20]. SA has the largest HIV epidemic in the world, with an estimated 7.5 million people living with HIV (PLWH) in 2021. SA accounts for a third of all new HIV infections occurring in Southern Africa. In 2021, there were 210,000 new HIV infections and 51,000 people died from AIDS-related illnesses in SA. SA has the world's largest antiretroviral treatment (ART) programme. The success of the ART programme is evident in the increase in national life expectancy from 56 years in 2010 to 63 years in 2018. However, HIV prevalence remains high, with 20.4% of people (one in five) living with HIV. Prevalence of HIV varies markedly between regions, ranging from 12.6% in Western Cape to 27% in KZN. KZN is the epicentre of SA's HIV epidemic with the highest HIV prevalence [21].

1.5.TB-HIV co-infection

TB and HIV form a deadly syndemic, with each pathogen worsening the pathogenesis of the other disease. The likelihood of TB infection is increased with HIV and the clinical course of HIV infection is worsened by TB [22]. HIV and TB both manipulate the host's immune responses by mechanisms that are poorly understood [23]. HIV co-infection is the most influential risk factor for the progression of Mtb to active disease and is associated with a 20-fold increase in the risk of latent TB reactivation [24, 25]. Untreated HIV-1 infection leads to immunosuppression characterized by severe depletion of $CD4^+$ T cells heightening the risk of TB infection [26]. In some HIV-1 patients, a proinflammatory state in the form of the immune reconstitution inflammatory syndrome (IRIS) occurs when the immune system is recovering following ART in the setting of Mtb infection [27].

TB remains the leading cause of hospital admissions and in-hospital mortality among people living with HIV [28]. Almost 50% of TB-related deaths are undiagnosed at autopsy as revealed by a meta-analysis of autopsy studies among PLWH [29]. TB accounts for 26% of AIDS-related deaths [24], with 99% of these deaths occurring in developing countries [30]. People living with HIV are 16 times more likely to develop active TB than HIV uninfected people [31]. People living with HIV with no evidence of TB require TB preventative therapy, which lessens the risk of developing TB and reduces TB/HIV death rates by around 40% [32]. South Africa is among the ten countries where there exists a disparity between the number of reported new and recurring cases of tuberculosis and the most accurate estimations of TB incidence [8]. In 2019, SA reported ~36, 000 HIV-associated TB mortalities [11]. Poor treatment outcomes are associated with co-infection of TB and HIV [33], whereby published data suggests that people living with HIV face a greater likelihood of drug-resistant TB compared to those without HIV [34, 35].

The use of ART has demonstrated a reduction in active TB disease incidence. However even if their $CD4^+$ T cell count is within the normal range HIV-positive individuals receiving ART remain at two-fold higher risk of developing active TB disease [26]. In 2021, 76% of notified TB patients had a documented HIV test result. The WHO African Region, has the highest burden of HIV-associated TB in 2021 and only 46% of TB patients known to be living with HIV were on ART [31].

Despite efforts to combat the disease, South Africa still ranks among countries with the highest rates of TB. The incidence rate was 615 per 100,000 population in 2019 [11]. In addition, a national survey conducted in 2017 revealed an overall prevalence rate of tuberculosis at 737/100,000 - indicating a significant burden on society. Furthermore, approximately one-third (28%) of all cases were co-infections with HIV [36]. Almost 60% of individuals diagnosed with TB disease in South Africa are co-infected with HIV, resulting in a dual burden of both diseases [37].

HIV-infected patients are typically severely immune suppressed with non-specific clinical symptoms making the diagnosis, pathogenesis, treatment, and prevention of TB a challenge. This population also often produce paucibacillary specimens [38] and are unable to produce sputum [39-41] of which is the mainstay of TB diagnostic tests. Additionally, PLWH are more likely to have extrapulmonary TB [42] and the accuracy of diagnostic tests is lower [43].

1.6.TB pathogenesis

Individual with active TB can disperses Mtb via aerosols into the air, these bacilli are then inhaled by individuals into the lung parenchyma of the respiratory tract and host alveoli [44]. The exact dose of Mtb bacilli in the droplets that may result in effective transmission remains unclear [45]. Exposure to Mtb does not indicate that an individual will become infected as there are some people that clear the bacteria, the exact mechanisms by which is unclear.

Latent tuberculosis is a term referred to bacteria that exist in a quiescent state for prolonged periods however clinically active tuberculosis may occur when the bacteria start replicating and escape immune control [7]. The host innate and adaptive immune responses play a vital role in containment of Mtb replication and control of Mtb infection and progression to active TB disease [46].

Following inhalation of Mtb via the respiratory tract, Mtb is translocated to the lower respiratory tract where the dominant cell type, alveolar macrophages are encountered and are infected by Mtb [47]. An influx of phagocytic cells to the site of infection is characteristic of early host response to Mtb infection [48]. The alveolar macrophages internalize the bacteria by receptor-mediated phagocytosis and often kill the bacteria [49]. However, if the alveolar macrophages fails to eliminate the organism, bacillary multiplication within the macrophage occurs and results in chemokine and cytokine release along with recruitment of other immune cells [46].

The hallmark of tuberculosis is the formation of granuloma structure which represents an organization of cells that play a role in Mtb containment. The typical granuloma is organized with a variety of immune cells such as activated macrophages, NK cells, dendritic cells (DCs) and neutrophils surrounded by T cells (lymphocytes). Granulomas can be divided into three types: solid granulomas that contain Mtb (considered as latency), necrotic granulomas during early stage of active TB disease and caseous granulomas occurring during severe TB. In the latter, there is bacterial dissemination with reactivation of TB [50]. The question of whether the granuloma favours the host or bacteria remains unanswered. Bacterial replication is suppressed through Mtb containment in granulomas; however bacteria adapt to this environment allowing it to persist for decades until bacterial replication resumes, granuloma raptures, and dissemination takes place. It is believed that lack of nutrients and oxygen in the

solid granulomas forces Mtb into this non-replicating state of dormancy. This makes treatment of Mtb difficult since most antibiotics target the replication machinery [51].

Incipient and subclinical TB are two transitional TB disease states between latent TB infection (LTBI) and active TB disease. Incipient TB is defined as viable Mtb that advances to active TB without further intervention and have no signs of active TB disease such as: clinical symptoms and radiographic abnormalities. Subclinical TB is defined by viable Mtb that does not cause clinical TB symptoms, but radiologic and microbiologic assays can detect abnormalities related to active TB [52, 53]. This form of TB is through to be prevalent in HIV positive individuals [54].

1.7.Tuberculosis diagnosis and treatment strategies

Diagnosis of TB has evolved over the past decade especially in low-income countries. Several rapid molecular diagnostic tests for DS-TB have been developed and recommended by WHO including Loopamp™ MTBC detection kit and FluoroType® MTB [both tests are used for the detection of Mycobacterium tuberculosis complex(MTBC)]. In addition to TB detection, drug resistance can also be detected using the following diagnostic tests: Xpert® MTB/RIF, Xpert MTB/RIF Ultra (detection of MTBC and Rifampin resistance-associated mutations), BD MAX™ MDR-TB (detection of MTBC and resistance towards isoniazid and rifampicin) and GenoType MTBDRplus (detection of MTBC and resistance towards first-line antimicrobials) [43, 55]. In most circumstances, a persistent cough for more than 2-weeks should be assessed for TB, as well as unexplained weight loss, fever or night sweats. Diagnosis of active TB includes: medical history of patient, clinical examination, bacteriological, radiological and laboratory test investigations. Symptoms alone are poorly predictive of TB.

Bacteriological diagnosis for TB includes microscopy, culture and molecular techniques. Currently 57% of the global TB patients access these diagnostic methods [56]. Sputum smear microscopy, which was developed more than 100 years ago, is the gold standard in most settings where TB is endemic even though it has many limitations. Sputum smear microscopy detects only 22 – 43% of active TB disease in HIV-infected individuals [57]. Light microscopy requires high bacterial load (3000 –5000 AFB/mL) to detect Mtb and has less sensitivity (50 -

70%) [58]. The diagnostic accuracy of sputum smear microscopy and routine chest radiology for HIV-associated tuberculosis is poor and culture-based diagnosis is slow [59].

The most widely used method to monitor treatment response is sputum culture since it allows differentiation between dead and live bacilli. Two (2) months culture conversion and time to culture conversion is used as a method for intermediate bacteriological endpoints.

Culture methods of Mtb from clinical specimens are used for definite diagnosis of TB and are the gold standard for drug susceptibility testing (DST) as well as identification of species. GeneXpert MTB/RIF assay has been recommended by WHO as a rapid diagnostic providing results within 2 hours while simultaneously detecting resistance to Rifampicin. SA was among the first countries to have adopted and implemented this assay [60]. The GeneXpert MTB/RIF test has shown good sensitivity of 98% (in smear-positive and culture positive cases), sensitivity of 67% (in smear negative and culture-positive cases), and specificity of 99% (smear negative and culture-negative cases) [61]. One important limitation of this test is the inability to distinguish between live and dead bacilli. Even after treatment completion, the assay may remain positive and should not be used to monitor response to treatment [62]. In SA, once a patient has been screened for TB, a sputum sample is taken to perform a GeneXpert MTB/RIF test and thereafter the appropriate treatment is initiated if positive for TB and or MDR-TB. Additionally, another specimen is taken and sent for culture, microscopy, and phenotypic DST.

TB can also be diagnosed using a chest X-ray to check for abnormalities that may be suggestive of active TB disease. These include lobe infiltrates (bi-lateral or uni-lateral), cavitation, and nodular shadows around the cavity; however, these findings particularly in HIV-positive patients are rarely conclusive and require history and clinical presentation to augment TB diagnosis [63]. Cavitation could be a marker for high bacillary burden from extensive disease [64]. Chest radiograph alone for the diagnosis of TB has a sensitivity of 96% but lacks specificity (46%) [65] hence, a combination of clinical parameters and further microbiological tests is required for diagnosis [47]. After completion of anti-TB treatment, between 20 – 50% of cavitary TB patients have persistent cavities [66]. If cavities are radiographically present during the first two months of therapy, there is a higher risk of treatment failure and relapse [64], and rates of cavitation are lower with poorly managed HIV co-infection [67], hence chest X-rays are not the ideal tool for treatment response monitoring.

Approximately 3.5 million people were provided with TB preventative treatment in 2021 [8]. Current treatment regimen consists of 4-6 month course of anti-TB drugs, with the cure rate of about 85% in drug-susceptible TB (DS-TB) , while treatment success rate in PLWH is 77% [8]. Cure rates in drug resistant TB (DR-TB) patients are significantly lower (60%). The TB treatment regimen for DS-TB consists of four first-line antimicrobials for 2 months (isoniazid, rifampicin, ethambutol, and pyrazinamide) followed by 4 months of continuation with isoniazid and rifampicin [68]. Despite completing anti-TB therapy some patients will experience recurrence of infection and may have an increased risk of M/XDR-TB. The death rate from TB disease without treatment is as high as 50% [69].

The tuberculin skin test (TST) is based on an injection of purified protein derivative (PPD) into the skin that results in a hypersensitive reaction with 48-72 hours in individuals with mycobacterium specific immunity. This test can diagnose latent TB infection but has a limited specificity since it is unable to distinguish between Mtb and other mycobacterial antigens, including BCG [70]. Interferon-Gamma Release Assays (IGRAs) that measures the interferon-gamma levels upon Mtb antigen stimulation [early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10)] of mononuclear cells from peripheral blood) [71] has been used to diagnose latent TB and has advantages over TST that include: sensitivity and specificity that is superior, only a single visit is required and there is no bias in reading of the test results [72].

Mycobacterial lipoarabinomannan (LAM) antigen is a potential marker of active TB and emerged as potential point-of-care tests for TB. Urine lateral flow lipoarabinomannan (LF-LAM) can also be used to identify TB by detecting the mycobacterial LAM antigen. It is a useful, inexpensive point of care test that can be used for TB diagnosis in HIV-positive, TB symptomatic patients. This test is easy to perform and provides results in 25 minutes [73].

Despite improvements in diagnosis, an estimated 4 million people with TB are missed every year. This highlights the need for more practical point of care (POC) tests for TB that will provide early diagnosis and reduce mortality and morbidity. There have been promising results from a new fingerstick blood test developed by Cepheid (the Xpert MTB Host Response [MTB-HR] prototype), that generates a “TB score” based on messenger RNA (mRNA) expression of 3 genes [74]. This test highlights the potential for use of host immune responses in improving TB diagnosis and outcome.

1.8. Summary of host immune responses to TB

1.8.1 Innate immune response to TB

Innate immune responses in the lung are important in the initial phase of Mtb infection, to restrict bacterial growth and prevent development of disease. A local inflammatory response occurs when innate cells detect Mtb by recognizing pathogen-associated molecular patterns (PAMPs) via toll-like receptors (TLRs) and nucleotide-binding oligomerization domain receptors (NLRs). Innate immune mechanisms are not sufficient to prevent progression of Mtb infection to active disease. Phagocytes infected with Mtb and or exposed to the Mtb antigens can prime $CD4^+$ and $CD8^+$ T cells by migrating to the regional lymph nodes.

Macrophages, neutrophils, DCs and natural killer (NK) cells are innate immune cells that play a major role in the primary response to TB. Although these cell types have distinct and overlapping roles to play, they are also easily manipulated by Mtb during infection [75]. The primary phagocytes involved in the uptake of Mtb in the lung are the alveolar macrophages, neutrophils and myeloid dendritic cells (mDCs) [27].

Alveolar macrophages that are the first cells to encounter Mtb and initiate phagocytosis through pattern recognition receptors (PRRs) resulting in effector functions and activation of other immune cells that are attracted by the production of inflammatory cytokines. Upon Mtb infection, the alveolar macrophages relocalize from the airways to the lung interstitium and thereafter initiate dissemination to monocyte-derived macrophages and neutrophils [76]. They can initiate an inflammatory response upon infection but their main function is to maintain steady-state in the alveolar microenvironment by removing debris and dead cells [77]. They are also able to become a niche for slow replication of Mtb and during latent infection phase they are a sanctuary for persistence of infection inside the phagosome [78].

Upon Mtb infection, neutrophils are the first cells to infiltrate the lungs [75]. Neutrophils can contribute to the early clearance of infection because they are rapidly recruited to sites of mycobacterial infection where they phagocytose bacilli; however they also can also exacerbate pathology by disseminating viable bacteria in established disease [79]. Neutrophils are a source of type I interferons in tuberculosis patients and active TB in humans is characterized by a neutrophil-driven blood transcriptional signature [80].

DCs are responsible for capturing, processing and presenting antigens to T cells and are considered one of the key cells during Mtb infection by initiating specific T cell responses in lymph nodes [27]. Due to antigen presentation, co-stimulating activity, and their large cytokine production capacity, DCs play a pivotal role in the immune defence system [81]. Inflammatory cytokines are produced by DCs upon stimulation which direct T cell responses (TNF and IFN-γ) [82] for the recruitment of cells to the site of infection by secreting proinflammatory cytokines (IL-1 and IL-6) [83]. At the onset of this inflammatory responses, DCs are highly represented at the site of Mtb infection [84, 85]. Immature DCs are highly specialized for antigen uptake and processing and are found in the lung mucosa [86]. Maturation only occurs post interaction of DCs with Mtb, resulting in the migration to lymphoid organs to prime T cells through cell surface expression of major histocompatibility complex (MHC) costimulatory molecules and the secretion of immunoregulatory cytokines such as IL-12 [87]. Studies have shown that there is an upregulation of MHC I and MHC II, CD40, CD54, CD58 and CD80 [88, 89] when infection of monocyte derived dendritic cells (MDDCs) with Mtb occurs. This upregulated phenotype is consistent with the activation of DCs, increased production of proinflammatory cytokines (IL-12, TNF-α, IL-1 and IL-6) that leads to maturation, possible migration and antigen processing and presentation [86].

NK cells are granular innate lymphocytes that possess cytolytic capacity. They are not MHC-restricted and therefore are able to act early during Mtb infection [90]. NK cells are activated by IFNs as well as cytokines derived from macrophages and by producing specific soluble mediators (GM-CSF, IL-12, TNF-α, IL-22, and IFN-γ) they can limit replication of Mtb [91]. Numerous *in vitro*, animal, clinical and epidemiological studies have shown that NK cells play a crucial role in controlling Mtb infection and disease progression [92, 93]. NK cells have been suggested to play a role in the early clearance of Mtb infection by identifying and eliminating both Mtb bacteria and infected host cells [94-96]. NK cells have the ability to lyse monocytes infected with Mtb, in which NK cell activating receptors such as NKp46 and NKG2D are recognized as crucial players [94, 95, 97]. The recognition of Mtb cell wall components by NKp44 [98] and TLR2 [96] allows for direct interaction between NK cells and Mtb, initiating a response that leads to the killing of the bacteria. Alterations in the levels and function of peripheral NK cells can indicate the activity of Mtb in the lungs. Specifically, during active TB, the frequency of peripheral NK cells is inversely correlated with the level of inflammation in the lungs [99].

Infected phagocytes become surrounded by a variety of immune cell types as a response to secreted cytokines and chemokines, resulting in granuloma formation [100]. Cytokines and chemokines are known as small proteins that are soluble and produced by cells acting largely in a paracrine manner to influence the activity of other cells. They regulate cell-mediated immune responses and are effector molecules with the ability to alter the quality of the immune response [101]. Knowledge on the function of cytokines has been drawn from studies of disruption in homeostasis caused by infection and the specific cytokine absence resulting in failure to control the process of the disease [102]. A cascade effect is also seen by some cytokines manipulating the production and actions of other cytokines, some may have an antagonistic action or synergistic effects [103]. Hence this complex interplay of cytokines and chemokines that are released during infection determine the outcome of Mtb infection [104, 105]. IL-18 promotes the recruitment of neutrophils, thereafter NK cells and T cells produce IFN-γ upon stimulation with IL-12 [106]. In response to Mtb, IL-12 responses have shown to play an important role since individuals who do not produce this cytokine were shown to be more susceptible to the infection [107]. IFN-γ is produced by T cells in response to mycobacterial antigens presented by macrophages [108, 109]. This results in the activation of macrophages to promote bacterial killing through reactive nitrogen, oxygen intermediates [110] and phagolysosome formation induction [111].

1.8.2. Adaptive immune response to TB

B and T lymphocytes are responsible for mediating adaptive immune responses and play a pivotal role in eliminating Mtb [82, 112-114]. The Mtb specific T cell responses play an important part in the outcome of the disease even though they are not detected until several weeks after infection [115]. The different subsets that naïve $CD4^+$ T cells can differentiate into is dependent on the immune environment and the cytokines produced by the APC during antigen presentation and T cell activation.

The adaptive immune response is initiated in the lymph nodes, where infected DCs traffic after the initial delay and persistence in the peripheral tissues [116, 117]. During Mtb infection, macrophages and DCs are the main APCs that capture, process and present antigens to naïve T cells through MHC to mount a T cell response. The process of activation and expansion of

the Mtb specific T lymphocytes occurs inside the lymph nodes [78]. TB-specific T cell responses are detectable only 2-8 weeks after Mtb infection [118].

Control of bacterial replication and protective immunity to Mtb requires adaptive immune responses that involve IFN-γ producing $CD4^+$ and $CD8^+$ T lymphocytes. $CD8^+$ T cells have an important role in the control of Mtb infection by secreting IFN-γ, TNF and producing cytolytic proteins (perforin and granulysin) to directly lyse the Mtb infected cells [119]. $CD4^+$ Th1 responses have been shown to play a key role in immune control of TB [120, 121] with IFN-γ production by $CD4^+$ T cells shown to be crucial for control of Mtb in mice models [122, 123].

Regulatory T cells (Tregs), a subset of $CD4^+$ T cells, play an important role in the control of the immune response and prevention of autoimmunity. During TB, Tregs may promote disease progression by dampening immunity and the function of effector T cells [124]. It has been found that there is an increased number of Tregs in both blood and at the site of infection during active disease. These cells reduce production of IFN-γ and IL-10 thereby suppressing the Mtb-specific immunity [124].

While the role of B cells and antibody responses in Mtb infection remains uncertain, they are known to enhance host immune responses against Mtb including T cell responses. Several passive transfer studies with antibodies against Mtb antigens have demonstrated improved outcomes including reduced dissemination, tissue pathology and bacterial burden[125-127] especially when administered in combination with IFN-γ [127]. BCG-induced antibodies were shown to enhance both the innate and cellular host immune responses against Mtb further supporting the important role of humoral immunity in Mtb response [128]. Furthermore, several serological studies have found lower antibody levels in children and adults with disseminated and extrapulmonary TB demonstrating a role of antibodies in Mtb containment [129-131].

1.9. HIV

1.9.1. Pathogenesis and Transmission

The primary cell targets for HIV-1 are the activated $CD4^+$ T cells. Following transmission, HIV replicates in the mucosal tissues and spreads to lymphoid organs within days (referred to as the eclipse phase). The virus replicates rapidly reaching a peak around day 30, with individuals being most infectious during this time. Following rapid viral replication, host immune system is able to achieve limited level of control resulting in a period of relatively stable viral replication (set point) which can last for years. During this time HIV causes progressive loss of $CD4^+$ T cells and general immune dysregulation ultimately resulting in AIDS. Resulting immunosuppression leads to increased risk of opportunistic infections and ultimately death [132].

HIV binds to the CD4 receptor on the surface of T cells, macrophages, monocytes and DCs. In addition, HIV requires a co-receptor including CC-chemokine receptor 5 (CCR5) or CXC-chemokine receptor 4 (CXCR4). CCR5 is mainly expressed on memory T cells, while CXCR4 expression can be found on both memory and naïve T cells [133]. Activated $CD4^+$ T cells are more permissive to viral replications and are the main targets of infection. Although DCs are not easily infected by HIV, they can capture the virus and lead to infection of surrounding T cells [134]. Furthermore, the interaction between the dendritic cell and $CD4^+$ T cell results in activation of the $CD4^+$ T cell, promoting HIV replication [135]. Increased infectivity has been linked to virus characteristics, which include higher envelope content, greater cell-free infectivity, stronger interaction with dendritic cells and resistance to IFNα [136].

Mucosal surfaces play a key role in HIV transmission and pathogenesis with the majority of early $CD4^+$ T cell depletion occurring in gastrointestinal tract independently of the viral transmission route [137]. HIV transmission is more efficient from male to female, therefore making women disproportionately more susceptible to HIV infection [138]. Paediatric HIV acquisition often occurs via MTCT and this occurs via three distinct routes: in *utero*, intrapartum, and in the postnatal period through breastfeeding [139].

Majority of HIV infections occur through heterosexual route. Mucosal and microbial environment of the female genital tract plays a key role in the transmission risk in women.

Presence of STIs and bacterial dysbiosis and the resulting genital inflammation are all factors that can increase the risk of HIV infection at the female genital tract [140-142].

After HIV crosses the mucosal epithelial barrier, it establishes a founder population of infected cells within hours of infection [143, 144]. During the first week of infection, this population expands locally and generates a viral pool that in the secondary lymphoid organs establishes a self-propagating systemic infection [143].

1.9.2. Acute Infection

The first period of infection from detection of the HIV RNA until formation of HIV-specific antibodies after infection (approximately 3-4 weeks later) is defined as the primary or acute HIV infection [145]. This is often associated with short symptomatic period involving fever, malaise, nonspecific rash and generalised lymphadenopathy, with the severity of symptoms highly correlated with peak viral load [146] . Once the HIV specific antibody and CD8+ T cell responses develop, the viral load decreases to a steady state referred to as a viral set point. The individuals with low/undetectable viral set point are called elite controllers.

During this period (as early as few days after infection [147]), HIV establishes latent infection in memory $CD4^+$ T cells. ART can prevent new infections, but it cannot eliminate viral reservoir allowing for low levels of replication [148, 149] and resurgence of virus in case of ART interruption.

Another important event during acute HIV infection is the upregulation of pro-inflammatory cytokines both in local tissues as well as systemically [150]. Increase in viral load is accompanied with increased levels of IP-10, IL-8, IL-10, IL-12 and CCL2 in the periphery [151]. Early trafficking of HIV to the gastrointestinal tissue [137] and the resulting destruction of the $CD4^+$ T cell population at this site is a major contributor to HIV associated chronic inflammation. The depletion of $CD4^+$ T cells at the gut mucosa damages the gut epithelial barrier and leads to microbial translocation and resulting inflammatory response [152, 153].

1.9.3. Chronic Infection

Immune activation and the progressive loss of $CD4^+$ T cell is a hallmark of chronic HIV infection. Breaks in the mucosal barrier due to the profound immunological damage to the gastrointestinal tract leads to the translocation of microbial products, including bacterial lipopolysaccharide (LPS), into the circulation. It was shown that during HIV infection bacterial translocation and plasma LPS levels correlated with immune activation [153]. Additionally, glycoprotein (gp)120 and nef (negative regulatory factor), or viral nucleic acids produced during viral replication themselves result in activation of proinflammatory cytokines and type I interferon (IFN), including IFN-α and IFN-β [145, 154, 155]. Increased expression of activation markers, such as CD38, HLA-DR and Ki67 are the result of basal immune hyper-activation in the infected host. The most reliable surrogate marker for immune activation, disease progression to AIDS, and death is CD38 [156]. Increased expression of HLA-DR and CD38 antigens on $CD8^+$ cells is one way that HIV infection activates the immune system. Due to its activation of the immune response, CD38 has prognostic utility. In addition to being a significant prognostic marker, CD38 actively participates in HIV infection [157]. Chronic immune activation also results in a decrease in $CD4^+$ and $CD8^+$ T cell half-life, abnormal T cell trafficking, clonal exhaustion of T cells, and drainage of memory T cell pools [158-160].

HIV can cause depletion of $CD4^+$ T cells both through direct cytopathogenic effects and indirectly. Inflammasome activation was shown to contribute to immune dysregulation and $CD4^+$ T cell depletion during HIV infection. Resting non-activated $CD4^+$ T cells are not permissive towards HIV infection, because HIV requires a cell to be activated in order to complete a full replication cycle. However, HIV can enter resting $CD4^+$ T cells and trigger cell death contributing to the massive depletion of $CD4^+$ T cells during HIV infection. It was recently shown that HIV entry into quiescent $CD4^+$ T cells leads to pyroptosis, a cell death triggered by inflammasome activation [161]. Pyroptotic death is characterized by the activation of caspase-1, resulting in swelling and bursting of the cell and release of mature IL-1β and other cellular contents that further fuel a strong inflammatory response. In addition to disruption of T cell responses, chronic HIV infection causes major dysregulation of other host innate and immune response.

During the initial weeks of HIV infection, there is a significant accumulation of $CD8^+$ T cells while also experiencing substantial depletion in $CD4^+$ T cells located within the gut. This leads to heightened permeability in the intestinal tract and eventual translocation of microbial

products into circulation [162, 163]. During HIV infection, microbial translocation plays a role in causing greater activation of monocytes. This is supported by the swift movement within 2 weeks from classical phagocytic monocytes ($CD14^{++}$ $CD16^{-}$) to an intermediate inflammatory subgroup ($CD14^{++}$ $CD16^{+}$) [164]. As a result, the various subsets of monocytes become impaired, ultimately resulting in less-than-ideal effector functions for phagocytosis, intracellular killing, chemotaxis and cytokine production [165].

The importance of NK cells in the initial control of viral replication and disease progression has been demonstrated in HIV infection. ART naïve individuals who experience heightened growth of cytotoxic NK cells at the onset of infection have shown an ability to control viral replication over a prolonged period [166]. The control of HIV is partially attributed to the effector functions exhibited by NK cells, which are linked with decreasing the viral setpoint in acute HIV patients [167]. In response to HIV infection, NK cells release β-chemokines and undergo degranulation as a means of hindering the binding of HIV to its intended target cells [168].

1.9.4. HIV diagnosis and treatment

WHO provides a comprehensive guide that consolidates existing and new clinical and programmatic recommendations on HIV prevention, testing, treatment, service delivery, and monitoring [169]. The WHO recommends immunological assays, particularly reactive enzyme immunoassay (EIA), as primary methods for diagnosing HIV. Over time, HIV testing algorithms have undergone changes due to improved test accuracy [18]. Detecting acute infection plays a crucial role in preventing further HIV transmission. It is hypothesized that acute infections contribute disproportionately to the number of new cases [170, 171].

The measurement of HIV antibodies, p24 antigen, viral load in plasma and CD4 count allows for the diagnosis and monitoring of HIV infection [132]. Rapid HIV testing can detect HIV-1 infection by using blood from a finger stick or collecting oral fluid. Test results are available in less than 30 minutes, making it highly advantageous for situations where providing follow-up care to patients is difficult [18]. Currently licensed rapid tests display limited sensitivity in identifying acute HIV infection; therefore, it is recommended to incorporate more-sensitive fourth-generation antigen-antibody tests and/or nucleic acid tests when risk factors indicate the possibility of an acute infection. Performing tests on oral fluid secretions results in reduced sensitivity, which leads to a delay of approximately 6 weeks when compared to the detection

time of equivalent blood specimens [172]. $CD4^+$ T cell counts serve as indicators for central immune defects in HIV/AIDS disease, and they have also been utilized jointly with viral load to monitor disease progression [173, 174]. Due to its precision, accuracy and reproducibility, flow cytometry is a technique utilized for enumerating $CD4^+$, $CD8^+$, $CD45^+$ (marker of immune cells) and $CD3^+$ (marker for circulating peripheral T cells) cells in the monitoring of immune response status [175, 176].

One of the most remarkable accomplishments in modern medicine is the advancement and implementation of ART, which plays a pivotal role in treating HIV infection. Initiating ART promptly results in a decrease in symptoms of acute HIV infection. This minimizes the immune damage and establishment and limits proliferation of viral reservoirs. Additionally, this approach significantly diminishes the chances of transmitting the virus to uninfected individuals [18]. To prevent viral replication, ART targets several steps of the HIV life cycle [177]. Standard regimen consists of 3 or more drugs in combination, that include: one nucleoside reverse transcriptase inhibitor (NRTI), one nucleoside reverse transcriptase translocation inhibitor (NRTI) and one integrase strand transfer inhibitor [169]. Several new ART drugs and drug classes are currently undergoing clinical development. It is recommended that patients with active TB start ART irrespective of CD4 cell count with the 'Test and Treat All' strategy [178]. As recommended by WHO guidelines, ART should begin as soon as possible within 2 weeks of anti-TB treatment. With the hope of preventing HIV, those individuals who are at high risk for HIV infection are given oral pre-exposure prophylaxis (PrEP) [18]. ART has transformed management and treatment of HIV infection, extended life expectancy and reduced MTCT. The risk of transmission is reduced by ART treatment through reduction of HIV viral load. If ART is not available, it is likely that HIV transmission rates from mother to newborn will occur frequently (15–25%) and increase significantly through breastfeeding (around 35–40%) [179].

Despite significant scientific advancements and the completion of multiple phase III studies, an HIV vaccine is still not available due to unsuccessful results. The most promising strategies for developing a vaccine are currently in the initial phases of clinical development (see www.avac.org and www.iavi.org). Vaccines based on adenovirus 5 as a platform were ineffective in providing protection and even elevated vulnerability to infection [180]. The use of a recombinant canarypox vector with an gp120 HIV envelope protein as part of the vaccine strategy resulted in moderate yet temporary protection observed in Thailand [181]. Many

broadly neutralizing antibodies have been discovered and are being formulated to target the conserved portions of HIV's envelope. Many of these antibodies have the ability to neutralize up to 90% of HIV strains individually, but when merged together they could attain an elevated percentage [182]. It is however important to note that interpretation of future clinical trials might become complicated with the use of long-acting PreP.

1.10. Immune complexities during TB-HIV co-infection

The function and homeostasis of host immune response are modulated by both Mtb and HIV infections. The TB-HIV co-infection has been termed “deadly symbiosis” since it accelerates the progression of both diseases; HIV can promote replication of Mtb and Mtb can also induce HIV replication and disease progression.

The main immunological feature of HIV infection is the massive depletion in $CD4^+$ T cells and in the setting of TB-HIV co-infection this has demonstrated the importance of $CD4^+$ T cell responses in TB pathogenesis. This is thought to be the main contributor as to why PLWH are at increased risk of TB infection, reactivation of latent infections and TB dissemination [183-185] . In addition to the general depletion of $CD4^+$ T cell, HIV was shown to preferentially target Mtb specific $CD4^+$ T cells further impairing Mtb control[183, 186] . Additionally, since $CD4^+$ T cell have been implicated in the development of cavitary disease, PLWH were found to have lower odds of cavitation than HIV negative individuals [187, 188]. HIV can also impact Mtb control through modulation of T helper cell (Th) responses, with a shift from Th1 to Th2 response [189]. In addition to the $CD4^+$ T cells numerical depletion, functional properties of the Mtb-specific $CD4^+$ T cells that remain are impaired by HIV-1 [27].

During Mtb infection, through the induction of proinflammatory cytokines and chemokines and upregulation of HIV co-receptors CCR5 and CXCR4, Mtb can fuel HIV replication and disease progression [190]. Studies have shown an increase in proinflammatory cytokines in the pleural fluid in comparison to plasma of co-infected individuals as well as higher viral replication in macrophages and lymphocytes in these Mtb located sites [191-193]. Immune activation is also a feature of both active and latent TB and this could increase HIV replication during co-infection [194, 195]. Additionally increased T cell activation was observed in TB infection, and this could also worsen HIV disease progression [194].

Studies conducted in KZN have shown specific differences in host responses to TB in PLWH. Thobakgale *et al* showed an association between the production of IL-1β from both monocytes and mDCs following BCG, TLR-2, TLR-4, and TLR-7/8 stimulation with TB recurrence outcomes [196]. Sivro *et al* measured cytokines in plasma samples from the TB Recurrence upon Treatment with HAART (TRuTH) cohort in SA. This study showed that inflammatory cytokines such as: IL-1β, IL-6 and interleukin-1 receptor antagonist (IL1-Ra) where associated with increased risk of TB reactivation [197]. Pillay *et al* measured microbial translocation markers and showed that sICAM and LBP associated with an increased risk of recurrence in HIV-positive individuals on ART [198]. These studies provide evidence of the impact that inflammatory cytokines as well as HIV mediated chronic immune activation due to translocation of microbial products, have on reactivation of TB.

To persist inside the host, both HIV and TB can inhibit antigen presentation. Through Nef, HIV can down-modulate the surface expression of CD80 and CD86 on APCs [199], and through gp120 inhibit up-regulation of CD40L on T cells and CD80 on APCs thereby inhibiting T cell activation [200]. This HIV-mediated impairment of pathogen uptake and recognition can have important consequences for early Mtb control. Macrophages are believed to be crucial for Mtb response[50]. Macrophages are a primary niche for Mtb and given their permissiveness to HIV-1 raises the possibility of these two pathogens co-infecting individual cells [4] [23].

In mice, Mtb has been shown to delay the activation of adaptive immune response by slowing bacterial transport to local lymph nodes [116]. There is a prolonged period of unchecked bacterial growth within the lungs that allows a 10,000–100,000-fold expansion of the Mtb pulmonary population. One driver of this delay is Mtb-mediated inhibition of MHC class II antigen presentation by DCs [201]. Antigen presentation is also manipulated by Mtb by diverting bacterial proteins through a vesicular export pathway, away from MHC class II presentation [202]. The viral protein Nef has been shown to promote the expression of immature, functionally incompetent MHC class II complexes in HIV [203, 204]. Data from recent studies have more directly linked these two by demonstrating that HIV-infected dendritic cells have reduced ability to upregulate key costimulatory molecules critical for antigen presentation (e.g. CD40, CD80, and CD86) in the presence of Mtb infection [205]. This would result in a combined delay in the onset of adaptive immunity.

The number of central and naïve $CD4^+$ T cells increases along with Mtb specific T cells upon ART treatment [206]. However, during co-infection, ART treatment may not always be

beneficial and can lead to exacerbation of TB, resulting in IRIS. TB-IRIS is an excessive immune response against Mtb that may occur in either HIV-positive or negative patients, during or after completion of anti-TB therapy [207-209]. Paradoxical and unmasking are two forms of TB-IRIS, paradoxical (TB treatment received before ART) is defined as new, recurrent or worsening symptoms of case that is being treated while unmasking (latent or undiagnosed TB that activates upon ART initiation) is an ART associated inflammatory manifestation of a subclinical infection with an accelerated presentation [207]. Anti-inflammatory treatment may be required as a result of severe inflammatory responses in TB-IRIS. Following ART, the dysregulated recovering immune responses with exaggerated inflammation and sustained Th1 responses against Mtb antigens enhances TB symptoms [210, 211]. This can result in granuloma disruption since they are dependent on the balance of pro- and anti-inflammatory cytokines for optimal Mtb control.

In patients with TB IRIS, many cytokines that were elevated have suggested an important role for innate cells of myeloid origin [212, 213]. In preliminary studies monocytes had been pointed as cells of interest in IRIS pathogenesis [214, 215]. Specific pro-inflammatory biomarkers of innate and myeloid cell activation appeared to be elevated in TB-IRIS patients and more importantly a clear activation of innate responses was seen in TB-HIV co-infected patients pre-ART who had received <4 weeks of anti-TB therapy. This and other studies highlighted the potential predictive value of $CD14^{++}$ $CD16^{-}$ monocyte frequency and plasma levels of CRP, TNF, IL-6, and soluble (s) CD14 for TB-IRIS [216-218].

1.11. Biomarkers and host directed strategies to improve TB diagnosis and outcome

The development of new antibiotics cannot keep up with the progression of drug resistance highlighting the need for host directed therapies (HDTs). Prolonged TB treatment, and the associated toxicity of the drugs results in poor compliance fulling the development of drug resistant Mtb. While treatment of drug-susceptible Mtb is generally effective, drug-resistant TB treatment efficacy is below 60% and can develop into progressive, untreatable disease. Amplification of host immunity, killing or containing Mtb and modulating inflammation to reduce destruction of lung tissue are the three main approaches being taken forward for HDT for TB [68].

The ultimate goal of biomarker research is to identify candidate molecules that can be used for the development of prognostic, diagnostic and therapeutic tools for patients [219]. TB biomarkers can generally be divided into: (i) Mtb components, (ii) antibody responses to Mtb antigens, (iii) cellular immune responses to Mtb antigens, and (iv) unbiased "omics" approach (i.e., transcriptomics, proteomics and metabolomics). Candidate biomarkers for TB should be able to differentiate people with active and latent TB in both healthy individuals as well as PLWH, and should predict clinical outcomes in diverse patient populations [220, 221].

Finding the right balance between pro- and anti-inflammatory responses is crucial in determining what happens during initial Mtb infection. If there's an imbalance towards either excessively aggressive pro-inflammatory or anti-inflammatory cytokine response, it could result in ineffective bacterial control and the development of active disease would be more likely to occur [222, 223]. Cytokines reflect local or systemic inflammatory milieu and could aid as biomarkers for potential clinical effects of therapeutic interventions [101]. Cell phenotyping is a promising approach to aid diagnosis and monitor treatment outcomes. Point of care diagnostic test development has been hindered by the paucity of knowledge of definite/specific biomarkers of different states in TB. There is a need for biomarker discovery and validation that will define combination markers to assist case finding, increase treatment access and improve clinical prognosis. It is also pivotal to monitor immune responses in HIV and TB co-infected individuals and since they may require tailoring to specific demographic populations. Cytokines can theoretically serve as promising candidates for inclusion in adjunctive immunotherapies because of the crucial role they play in immune function [224]. Cytokines may be able to polarize the immune response to favour protecting the host by strengthening the immune and memory responses or by disrupting and penetrating the granuloma to expose Mtb bacilli to anti-TB treatment [224]. A promising HDT strategy has been shown for individuals with active DR- and DS-TB by reducing excessive cytokine responses in order to reduce immunopathology [225, 226]. Other approaches have looked at delivering cytokines in order to improve bacterial clearance. Specifically, a phase-II trial assessed the aerosol delivery of recombinant IFN-γ-1b as an adjunct to DOTS (Directly Observed Treatment, Short-course) for patients with cavity pulmonary TB. The findings showed favourable immunomodulation by accelerating the clearance of Mtb sputum and lowering inflammatory cytokines at the site of illness [227]. However, more recent preclinical findings have demonstrated the propensity for exacerbated lung infection and harmful consequences of increased IFN-γ production by CD4 T cells in mice models [228]. While TNF-

α activates monocytes/macrophages and maintains granuloma integrity, high amounts may exacerbate pathology. Therefore, because of their antagonistic and synergistic interactions, the use of IFN-γ and TNF-α modifying medications remains contentious despite some promising therapeutic effects. Furthermore, even though some cytokine-based treatments have shown promise, their use may be limited due to their high cost, possible toxicity, and role in immunopathology [225].

Mtb triggers the activation of lung epithelial cells and macrophages, leading to the production of multiple matrix metalloproteinases (MMPs) including MMP-1, MMP-3, MMP-7, MMP-9, and MMP-10 during its active infection phase [229]. MMPs play a role in deteriorating the pulmonary extracellular matrix, intensifying inflammation, and causing tissue damage as well as pulmonary cavitation in individuals with tuberculosis [230, 231]. Therefore, targeting MMP activity holds great potential as a HDT approach in alleviating inflammatory pathology among patients.

During Mtb infection, the adaptive immune response primarily relies on cell-mediated immunity [232]. For treating TB, a promising HDT strategy involves modifying the cellular immune response. Numerous molecules have been recognized for their capacity in regulating the cell-mediated immune response. Among them, statin is a widely acknowledged inhibitor of 3-hydroxy-3-methylglutaryl-CoA that effectively diminishes serum low-density lipoprotein (LDL) cholesterol level in humans [233]. In a study using mice as a model for tuberculosis, it was found that treatment with statins leads to notable reductions in both lung bacterial load and disease pathology. This improvement is primarily attributed to lower levels of membrane cholesterol which enhances the process of phagosomal maturation/acidification and autophagy [234].

Understanding host biomarkers play a significant role in moving towards non-sputum-based diagnostic tests for TB treatment monitoring. Good biomarkers for monitoring treatment progress are Mtb-specific $CD4^+$ T cells that correlate with Mtb antigen/bacilli burden [235]. Tests that measure T cell activation such as T cell activation marker (TAM)-TB have demonstrated potential for active TB in children (83% sensitivity vs culture) and is a promising diagnostic for TB treatment monitoring [236-238].

Developing a non-sputum, non-invasive POC test for TB is vital in aiding rapid diagnosis and reducing mortality and morbidity. Despite decades of investment in TB research, monitoring

of TB treatment is heavily reliant on sputum culture and smear microscopy. The evaluation of immunological parameters in biological fluids offers a potential surrogate marker for treatment response and assessing the progression of latent TB to active disease and may be particularly useful in settings with high rates of Mtb infection [239]. An association between 2-month sputum culture conversion on TB therapy and non-relapse cure has been established [240, 241], however, this is not always predictive of TB cure [64, 242-244].

A promising TB disease diagnostic and correlate of risk (ACS COR) is the host blood RNA profiling that can predict progression from latent to active disease [80, 245-248]. This method has a 66% sensitivity and 81% specificity within a year of TB diagnosis [249]. Cepheid has developed a new fingerstick blood test (the Xpert MTB Host Response [MTB-HR] prototype), that generates a "TB score" based on messenger RNA (mRNA) expression of a 3-gene signature. Preliminary data show that it could be used as a triage test for screening TB patients [74]. Biomarkers selected for further development as POC tests and field implementation studies in high-tuberculosis-risk groups should ideally perform well in people without HIV and in PLWH, before and during ART.

HDTs are an emerging and promising strategy to improve diagnosis and treatment of TB disease. In addition to hindering the Mtb replication, HDTs can be used to reduce the inflammation mediated lung destruction and therefore improve lung function following TB treatment. However, immune responses are often multifaceted, and their modulation requires careful dosing and timing and more importantly requires detailed knowledge of the host immune responses to Mtb, especially in the context of co-infections.

1.12. Study rationale

TB remains a significant public health concern and prior to the coronavirus (COVID-19) pandemic, TB was the leading cause of illness and death from a single infectious agent. The HIV/AIDS epidemic in South Africa has contributed to the drastic surge of TB cases hindering the control of TB infection in this hyper-endemic setting. Together these dual epidemics pose one of the most critical public health concerns. The lack of understanding of immune biomarkers that provide protection against TB, immunological mechanisms responsible for controlling bacterial growth, and immune biomarkers associated with TB outcomes poses a

barrier to achieving significant advancements in the development of vaccines and more effective treatment strategies.

In this study we assessed the phenotypic profiles of monocytes, dendritic and $CD4^+$ T cells as well as plasma cytokine/chemokine expression as markers of TB culture conversion and disease severity. Measurement of soluble plasma biomarkers could represent a cost-effective and practical method to assess individuals' risk of TB disease and response to treatment. Both Mtb and HIV co-infection can hinder innate immune function, altering tissue distribution and the activation of the adaptive immune response overall increasing the pathogenesis and hindering viral and bacterial clearance. $CD4^+$ T cell responses are one of the key determinants of disease pathology in both Mtb and HIV however their phenotypes during TB-HIV co-infection and impact on TB disease severity remains largely uncharacterized. The proposed study will provide crucial information on the nature of host immune responses during TB and TB-HIV co-infection and their impact on TB treatment outcome and disease severity.

Hypothesis: During active TB disease, pre-existing HIV infection will impair innate and adaptive immune responses resulting in increased severity and poor treatment outcome.

1.13. Overall study aim

a. To characterise host immune responses during TB-HIV co-infection and identify immune biomarkers of TB disease severity and time to culture conversion.

1.14. Specific study objectives

1. To identify soluble inflammatory markers of culture conversion and disease severity in TB and TB/HIV coinfected individuals.
2. To characterise innate immune cell phenotypes during active TB in HIV co-infected individuals and asses their impact on TB disease severity and treatment outcome.
3. To characterise $CD4^+$ T cell activation and phenotype in patients with active TB with HIV-coinfection and assess their impact on TB disease severity and treatment outcome.

1.15. Overview of study methods

1.15.1 Study cohorts

The studies presented here were performed using specimens from the following cohorts: CAPRISA 011 Improving Retreatment Success (IMPRESS), CAPRISA 002 Acute infection study and local healthy controls.

1.15.1.1. IMPRESS CAPRISA 011 study

CAPRISA 011 cohort, was an open-label, randomized controlled trial (NCT02114684) [250]. IMPRESS trial was designed to determine if a regimen containing moxifloxacin, substituted for ethambutol compared to a standard control regimen for 24 weeks would be superior at improving TB retreatment outcomes and shortening TB treatment duration. The study enrolment started in November 2013 in Durban, KwaZulu-Natal, South Africa and follow-up was completed in July 2017. Patients were recruited and treated at an urban clinic (CAPRISA eThekwini Research Clinic) adjoining the largest government outpatient TB facilities, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KZN, South Africa. All participants recruited into the trial were adults > 18 years, with a previous history of TB and the current diagnosis of rifampicin-susceptible *Mycobacterium tuberculosis* by either GeneXpert MTB/RIF technology, sputum smear microscopy or both. Both HIV infected and uninfected participants were included in the study as well as those on ARVs. Only participants on drugs known to be contra-indicated with drugs used in the study, were ineligible for study participation. The intensive phase of treatment included 2 weekly visits followed by monthly visits during the continuous phase. Participants underwent a digital chest x-ray every 6 months at the PCZCDC. Uni-Gold™ (Trinity Biotech, Ireland) and Alere Determine™ (Alere Scarborough, Inc) rapid HIV test was used to test the samples for HIV. Results positive for HIV were confirmed by a second licenced rapid HIV test and the discordant results were confirmed by an Enzyme-Linked Immunosorbent Assay (ELISA). All participants who were HIV positive had their CD4 counts measured using the TruCOUNT method (BD Biosciences, San Jose, US). A nested PCR with Expand Long Template PCR system (Roche Diagnostics) was used to determine viral loads in HIV positive samples. The assay yields a lower detection limit of 40 HIV RNA copies/ml with a range of 40-10^7 HIV RNA copies/ml.

1.15.1.2. Acute Infection CAPRISA 002 study

CAPRISA 002 Acute HIV Infection study, a prospective observational cohort study aimed to identify viral immune and host genetic factors that predict HIV disease progression during ART [251, 252]. The cohort was followed monthly to determine HIV infection rates and clinical presentation of early HIV infection. Recruitment of participants started in 2004 at two KwaZulu-Natal sites: Durban (urban site) and Vulindlela (rural site). To investigate the natural history of HIV-1 subtype C infection and to assess risk factors for disease progression, the study originally recruited a cohort of HIV-uninfected women ≥18 years at high risk of HIV infection (Phase 1). The women who acquired HIV were enrolled into Phase 2 (acute infection). At the time of screening, women who were pregnant and/or had planned travel from the study site for more than 3 months were excluded.

1.15.1.3. Healthy control cohort

Control samples were obtained from healthy donors within CAPRISA. Individuals in the study had no current or previous history of TB (self-reported) and were confirmed HIV negative.

1.15.2. Sample collection and processing

Plasma: Peripheral blood was collected in Acid citrate dextrose (ACD) tubes. The tubes were centrifuged for 7 minutes at 1400rpm and plasma was retrieved and cryopreserved at -80ºC for future analysis. Stored plasma were thawed on the day of the assay. All samples assayed went through one freeze thaw cycle. The plasma samples were used to quantify selected cytokine/chemokine expression using standard Enzyme-linked immunosorbent Assay (ELISA) and multiplex cytokine assays.

Peripheral blood mononuclear cells (PBMCs): PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO) in liquid nitrogen for long-term storage. Cryopreserved PBMCs were thawed by pre-warming R10 cell culture media [RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 1.7 nM sodium glutamate, and 5.5 mL HEPES buffer] to 37 ºC. The frozen cryovials with the cells were immediately

immersed into a preheated 37 ^{o}C water bath with the cap facing upwards. Once the cells were ~75% thawed, the vial was transferred to the biosafety cabinet and wiped down with 70% alcohol. Cell culture media was added to the vial in a drop wise manner. The cells were transferred to a new tube and washed with 8ml of R10 media. The cells were centrifuged at room temperature (RT) for 7 minutes at 1250rpm to remove residual DMSO. The supernatant was discarded, and the cells were resuspended in 5ml of R10. Cells were rested for 3 hours at 37 ^{o}C, 5% CO2. Cells were counted using the trypan blue method and prepared for flow cytometry staining.

1.15.3. Immunological assays

Cytokine/Chemokine analysis: Selected cytokines/chemokines were measured using either the multiplex cytokine assays or standard ELISAs. The Milliplex MAP Human Cytokine / Chemokine Panel I and IV were used to analyse 22 analytes: IFNα2, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL-10, IL-12p(40), IL-12p(70), IL-15, IL-17α, MCP-1, MCP-3, MIP1α, MIP1β, TNFα, VEGF-A. This is a bead-based immunoassay that allows for precise measure of multiple analytes in a single low volume sample. The 96-well plate was read on the Bio-Plex™ 200 plate reader.

For the measurements of plasma Lipopolysaccharide-Binding Protein (LBP) and soluble CD14 (sCD14) we used the Human Lipopolysaccharide-Binding Protein (LBP) (R&D systems, USA) and CD14 Quantikine® ELISA (R&D systems, USA) kits. The LBP and CD14 assays use a sandwich ELISA principle which measures the antigen between two sets of antibodies (capture and detection). Assay plates were read at a 450nm wavelength on an ELISA microplate reader.

All assays were performed as per manufacturer's instructions.

Flow cytometry: Following rest (3 hours), cells were stained with conjugated monoclonal antibodies for 20 minutes at RT. Antibody staining was performed in 96 well V-bottom plates in a total volume of 50 µl. Following incubation, cells were washed twice with 250 µl PBS-1 and resuspended in 400µl (1X) cell fix (BD) before acquisition on a BD FACSDiva™ software (v8.0.2) of the BD LSRFortessa™ flow cytometer. Two flow cytometry panels were optimised, one for characterisation of blood monocytes and dendritic cells and second for the characterisation of peripheral T cells. At least 200,000 events were acquired per sample for monocytes and dendritic cells and 100,000 events for T cell panels. Fluorescence minus one

(FMO) control tubes were used for defining gates during analysis. Flow cytometry data was analysed by hierarchal gating using FlowJo software (v10.7.1) and exported to Excel.

1.15.4. Data analysis

Statistical analyses were performed using IBM SPSS Statistics version 27, SAS version 9.4. Graphs were made using GraphPad Prism version 9.3.1.

D'Agostino-Pearson omnibus normality test was used to assess data distribution and based on the results either parametric or non-parametric analysis methods were employed.

All cytokines/chemokines with less than 60% detectability were analysed as binary variables. Continuous cytokines were log transformed to adjust for skewness. The analysis of baseline demographic and clinical characteristics, categorical variables of randomization arm, gender, HIV status, alcohol use and lung cavitation were analysed using the Pearson Chi-Square test or Fisher's Exact test. Continuous variables of age, BMI, viral load and CD4 counts were analysed using the non-parametric Mann-Whitney U test.

To determine the association between measured markers and cell population frequencies at baseline and time to culture conversion (first of two consecutive negative TB culture results), measured in days, a Cox proportional hazards model was used. A logistic regression model was used to determine the association between measured markers and 8-week culture conversion status as well as disease severity measured by presence of lung cavitation. Multivariable analysis, adjusted for randomization arm, age, sex, HIV status, lung cavitation, alcohol use, smoking and BMI. In addition, $CD4^+$ T cell counts, and HIV viral load were adjusted for in sub-analysis of HIV- positive sub-group. Randomization arm was excluded in the multivariable lung cavitation analysis as this was not relevant for the studied timepoint.

To assess differences in frequencies of monocytes and dendritic cells among TB/ HIV, TB, HIV and healthy donors, a one-way ANOVA with Tukey's post-test was performed on normally distributed markers, and non-parametric Kruskal Wallis test with Dunn's post-test was done on markers which were not normally distributed.

1.15.5. Study Ethics

The nested study presented in this thesis obtained ethics from UKZN Biomedical Research Ethics Committee (BREC/00000014/2019, Appendix B). CAPRISA 011 (Clinicaltrials.gov, NCT02114684), was approved by Medicines Control Council of South Africa (MCC Ref: 20130510). This study was reviewed and approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) [BFC029/13]. The CAPRISA 002 study was reviewed and approved by the research ethics committees of the UKZN BREC (E013/04), the University of Cape Town (025/2004), and the University of the Witwatersrand (MM040202). The study on healthy donors was reviewed and approved by UKZN BREC (BE432/12). Written informed consent for screening, enrolment and specimen storage was obtained from all participants.

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CHAPTER 2

CHAPTER 2 - Rationale

Effect of Inflammatory Cytokines/Chemokines on Pulmonary Tuberculosis Culture Conversion and Disease Severity in HIV-Infected and -Uninfected Individuals From South Africa

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Promising data from the recent study on Cepheid 3-gene host response fingerstick blood test (Sutherland et al., 2022), highlight the importance of characterising biomarkers of TB risk, protection and treatment response in order to improve diagnostics and optimise and shorten current treatment regiments. The development of fast and accurate, non-sputum based, diagnostics could have a major global impact on TB control. Similarly, the ability to identify individuals at high risk of treatment failure and to utilise host directed therapies to shorten and customise TB treatment could have an immense impact on reducing TB morbidity and mortality.

In **Chapter 2** we determined the associations between plasma inflammatory markers measured during active TB and treatment response and disease severity among both TB and TB/HIV co-infected individuals from the CAPRISA 011 IMPRESS trial. This study highlights the impact of soluble inflammatory responses in TB pathogenesis and treatment responses.

Dr Sivro, Prof Naidoo, and I conceptualized and designed the study. I prepared the specimens, optimised and performed the cytokine assays. I performed the statistical analysis and data interpretation. Ms. Lewis, the CAPRISA 011 study statistician, validated the analysis. I wrote initial draft of the manuscript that was reviewed and edited by my supervisors and other co-authors. The manuscript was published in *Frontiers in Immunology*, speciality section on *Microbial Immunology* on 1st of April 2021 (PMID: 33868272). The work from this publication was presented as an e-poster at the Virtual live Keystone Symposia eSymposia meeting eSymposia | Tuberculosis: Science Aimed at Ending the Epidemic from December 2-4, 2020.

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Effect of Inflammatory Cytokines/ Chemokines on Pulmonary Tuberculosis Culture Conversion and Disease Severity in HIV-Infected and -Uninfected Individuals From South Africa

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Novel tuberculosis (TB) prevention and control strategies are urgently required. Utilising specimens from the Improving Retreatment Success (NCT02114684) trial we assessed the associations between inflammatory markers, measured during active TB, with treatment response and disease severity in HIV-infected and uninfected individuals. Multiplex immunoassays and ELISA were used to measure plasma expression of 24 cytokines/chemokines. Cytokines were log transformed to adjust for skewness. We conducted a nested, un-matched, case (n= 31) - control (n=101) study with cases defined as those participants who failed to sputum culture convert within 8-weeks of TB treatment initiation. Additionally, we examined the association between the measured cytokines and time to culture conversion and presence of lung cavitation using cox proportional hazards and logistic regression models, respectively. Multivariable analyses adjusted for a wide range of baseline clinical and demographic variables. IP-10 expression during active TB was associated with increased odds of sputum culture conversion by 8-weeks overall (aOR 4.255, 95% CI 1.025 – 17.544, p=0.046)) and among HIV-infected individuals (OR 10.204, 95% CI 1.247 – 83.333, p=0.030). Increased MCP-3 (aHR 1.723, 95% CI 1.040 – 2.855, p=0.035) and IL-6 (aHR 1.409, 95% CI 1.045 – 1.899, p=0.024) expression was associated with a shorter time to culture conversion in the total cohort. Higher plasma expression of IL-6 (aHR 1.783, 95% CI 1.128 – 2.820, p=0.013), IL-1RA (aHR 2.595, 95% CI 1.136 – 5.926, p=0.024), IP-10 (aHR 2.068, 95% CI 1.034 – 4.137, p=0.040) and IL-1α (aHR 2.008, 95% CI 1.053 – 3.831, p=0.035) were significantly associated with shorter time to culture conversion among HIV-infected individuals. Increased IL-6 and IL-1RA expression was significantly associated with the presence of

lung cavitation during active TB in the total cohort (OR 2.543, 95% CI 1.254 – 5.160, p=0.010), (OR 4.639, 95% CI 1.203 – 21.031, p=0.047) and in HIV-infected individuals (OR 2.644, 95% CI 1.062 – 6.585, p=0.037), (OR 7.795, 95% CI 1.177 – 51.611, p=0.033) respectively. Our results indicate that inflammatory cytokines/chemokines play an important role in TB disease outcome. Importantly, the observed associations were stronger in multivariable models highlighting the impact of behavioural and clinical variables on the expression of immune markers as well as their potential effects on TB outcome.

Keywords: inflammation, biomarker, lung cavitation, HIV, Tuberculosis

INTRODUCTION

Tuberculosis is the leading cause of death from a single infectious agent with an estimated 10 million new infections reported in 2019 (1). Globally, among HIV-uninfected individuals, an estimated 1.2 million TB deaths occurred, and an additional 208, 000 deaths were recorded among HIV-infected individuals in 2019. Africa accounted for 25% of the TB cases in 2019 and South Africa (SA) is one of eight countries accounting for two thirds of the global burden of TB. SA has the largest HIV epidemic in the world with 7.5 million people living with HIV, 200,000 new HIV infections and 72,000 deaths from AIDS-related illnesses in 2019 (UNAIDS Data, 2020). HIV-infected individuals are 15-22 times more likely to develop TB than HIV-uninfected individuals and TB is a leading cause of HIV-related deaths (WHO, 2019). Furthermore, despite suppressive ART, people living with HIV/AIDS (PLWHA) remain at heightened risk of recurrent TB during their lifetime.

Despite use of TB therapy for many decades, clinical decision making and monitoring of TB treatment response is universally dependent on microbiologic assessment of sputum culture conversion at 2 months post TB-treatment start, despite available data showing low predictive value of two-month sputum tests for predicting treatment failure and relapse (2). Moving away from sputum to more sensitive blood-based biomarkers is imperative for efficient monitoring of treatment efficacy, and early detection of treatment failure.

Measurement of plasma 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. A number of studies have examined the effect of soluble plasma cytokine/chemokine responses on TB severity and response to treatment and several cytokines/chemokines such as TNFα, IFNγ, IL-1β, and IP-10 have been linked with disease outcome, presentation or severity (3–6). We have previously identified several inflammatory cytokines (including IL-1β, IL-6 and IL-1RA) associated with risk of TB recurrence in ART treated HIV co-infected cohort (7). Further characterisation of soluble biomarkers, especially in the context of TB/HIV co-infection, will provide valuable insight into the immunological pathways affected and provide new tools for TB screening and monitoring of treatment outcome.

Since HIV is known to cause dysregulation of the TB immune response (8, 9), here we aimed to determine if candidate plasma immune markers detected in individuals with recurrent, active TB disease, were associated with early and late culture conversion and disease severity in TB and TB-HIV co-infected individuals.

MATERIALS AND METHODS

Ethics Statement

Study participants were part of the CAPRISA 011 Improving Retreatment Success trial (IMPRESS, Clinicaltrials.gov, NCT02114684), approved by Medicines Control Council of South Africa (MCC Ref:20130510). Written informed consent was obtained from all study participants prior to enrolment. University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) reviewed and approved the study protocol (BFC029/13). The nested study protocol was reviewed and approved by UKZN BREC (BREC/00000014/2019).

Study Participants

Study was performed on stored plasma specimens from CAPRISA 011 study participants that were recruited and treated at an urban clinic (CAPRISA eThekwini Research Clinic) adjoining the largest government outpatient TB facility, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KwaZulu-Natal (KZN), South Africa (SA) (10). All enrolled participants were adults ≥ 18 years, with a previous history of TB and the current diagnosis of rifampicin susceptible sputum smear-positive *Mycobacterium tuberculosis* (MTB) by GeneXpert MTB/RIF® technology. Smear microscopy grading was done using a standardized grading scale: smear 1+ (10 to 99 AFB in 100 fields), smear 2+ (1 to 10 AFB per field in at least 50 fields) and smear 3+ (>10 AFB per field in at least 20 fields) (11). Both HIV-infected and uninfected participants were included in the study. Patients received 8 weeks of intensive phase TB treatment with 2 weekly clinical follow-up visits, and 16-weeks of continuous phase TB treatment with monthly clinical follow-up. We conducted a nested, un-matched case-control study. Cases were defined as those participants who failed to culture convert within 8-weeks of treatment initiation, where culture conversion was defined as the first of two negative sputum cultures at two consecutive visits without an intervening

culture positive result. Based on the definition and sample availability, 31 cases and 111 controls were selected for the study.

Sample Processing

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

Cytokine/Chemokine Measurement

Cryopreserved plasma samples were thawed and mixed by vortexing before assays were performed. Cytokine/Chemokine levels were measured using the Millipore Milliplex® assays (Map Human Cytokine/Chemokine Panel I and IV) and analysed on a BioPlex-200 system (Bio-Rad). The Human Cytokine/Chemokine Panel I included the following cytokines and chemokines: pro-inflammatory [IL-1α, IL-1β, IL-6, IL-12(p40), IL-12(p70), TNFα, IFNα2], chemokines (IL-8, IP-10, MCP-1, MCP-3, MIP1α, MIP1β), adaptive (IFNγ, IL-4, IL-15, IL-17α), anti-inflammatory (IL-10, IL-1RA), and growth factors (VEGF).

The Human Cytokine/Chemokine Panel IV included the following cytokines: IFNβ and IL-28B/IFNλ3. Soluble CD14 (sCD14) levels were measured using the Human CD14 Quantikine® ELISA Kit and human Lipopolysaccharide-Binding Protein (LBP) plasma levels were measured using the LBP kit (R&D Systems Inc, USA). All assays were performed following manufacturer's instructions. Samples with values outside the range of the standard curve were assigned the value half the limit of detection in pg/mL, LOD/2.

Statistical Analyses

Fisher's Exact, Chi-Square and Mann-Whitney U tests were used to compare baseline characteristics between cases and controls. All biomarkers with more than 60% of samples above the limit of detection were analysed as continuous variables and log-transformed to adjust for skewness [IFNγ, IL-1β, IL-1RA, IL-6, IL-8, IL-10, IL-12(p70), IL-17α, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and VEGF-A]; those with more than 40% of samples below detectability were analysed as binary variables [IFNα2, IL-1α, IL-4, IL-12(p40), IL-15, MCP-3, IFNβ and IFNλ/IL-28]. Logistic regression was used to measure the strength of association between plasma cytokine/chemokine expression at baseline and 8-week culture conversion status and discriminatory ability of the model was quantified using the area under the receiver operating curve (AUC). A Cox proportional hazards model was used to determine the association between cytokine/chemokine expression at baseline and time to culture conversion (first of two consecutive negative TB culture results), measured in days. To determine the association of plasma cytokine expression at baseline with disease severity measured by lung cavitation presence, a logistic regression model was used with presence of lung cavitation at baseline as the predictor outcome and AUC was measured. Multivariable analyses adjusted for a wide range of baseline clinical and demographic variables including study arm, age, sex, HIV status, lung cavitation, alcohol use, smoking and BMI. In addition, when analysing HIV-infected individuals CD4 counts, and viral load were adjusted for. Study arm was excluded in the multivariable lung cavitation analysis as this was not relevant for the studied timepoint. To determine the association between the systemic levels of cytokines/chemokines and bacterial burden at baseline, a one-way ANOVA with Tukey's multiple comparisons test was done on normally distributed cytokines and non-parametric Kruskal Wallis test with Dunn's multiple comparisons test was done on not normally distributed cytokines. Statistical analyses were performed using IBM SPSS Statistics version 25, SAS version 9.4 and graphs were made using GraphPad Prism (V8.1.2).

RESULTS

Participant Characteristics

A total of 31 cases and 101 control samples were included in the final analysis. Ten controls were excluded from the original analysis as they did not meet the control definition (7 were not TB culture positive at baseline; 1 patient died before the 2nd TB culture negative result; and 2 had inconsistent TB culture results). There were no significant differences between cases and controls for the following characteristics: study arm, age, sex, HIV status, lung cavitation, CD4 count and viral load (**Table 1**). Body mass index (BMI) was significantly higher in controls [cases: 19.28 (IQR 17.96 – 19.98) vs controls: 20.42 (18.64 – 22.95), (p=0.031)]. There was a trend towards higher alcohol (p=0.067) and cigarette use (p=0.051) in cases.

Effect of Plasma Cytokines/Chemokines Expression at Active TB on 8-Week TB Culture Conversion

We examined the association between plasma cytokine expression during active TB and early culture conversion (8 weeks). We observed no significant association between expression of measured cytokines/chemokines at active TB and TB culture conversion by 8 weeks in bivariable analysis adjusting for study arm (**Supplementary Table 1**, **Figure 1A**). Following multivariable analyses, adjusting for study arm, age, sex, BMI, HIV status, lung cavitation, alcohol use and smoking, we found that increased IP-10 expression was significantly associated with increased odds of early culture conversion [odds ratio (OR) 4.255, 95% CI 1.025 – 17.544, p=0.046] (**Figure 1B**, **Supplementary Table 1**).

We next examined the association between cytokine expression at active TB and 8-week TB culture conversion among HIV-infected individuals while additionally adjusting for CD4 count, and viral load in a multivariable model (**Supplementary Table 2**). An increase in IP-10 expression during active TB was associated with increase in the odds of culture conversion by 8 weeks (OR 10.204, 95% CI 1.247 – 83.333, p=0.030). The levels of IP-10 expression in all individuals and stratified by HIV status are shown in **Figure 1C**. While the sample size was too small to do a detailed analysis for the HIV-uninfected TB patients, IP-10 expression followed the same pattern across all subgroups irrespective of HIV status (**Figure 1C**). These results, in concordance with previously published data highlighting the importance of IP-10 in TB pathogenesis (12–17).

TABLE 1 | Demographic and clinical characteristics of study participants.

Variables	Cases n=31	Controls n=101	p-value
Study Arm n (%)			
HRZE -Control	18 (58)	45 (45)	.220
HRZM - Active	13 (42)	56 (55)	
Age (y), median (IQR)	34 (28 – 43)	36 (31 – 41)	.502
Sex, n (%)			
Male	25 (81)	70 (69)	.259
Female	6 (19)	31 (31)	
Body mass index (kg/m^2), median (IQR)	19.28 (17.96 – 19.98)	20.42 (18.64 – 22.95)	**.031**
HIV status n (%)			
Negative	11 (35)	25 (25)	.256
Positive	20 (65)	76 (75)	
CD4 cell count (cells/mm^3), median (IQR)	288 (214 – 410)	248 (127 – 413)	.311
Viral load (copies/ml), median (IQR)	3453 (20 – 18289)	5878 (20 – 111087)	.386
ARV status* n (%)			
Yes	10 (50)	31 (41)	.609
No	9 (45)	43 (57)	
Lung Cavities n (%)			
None	6 (19.3)	33 (32.7)	.317
One Lung	14 (45.2)	42 (41.6)	
Both Lungs	11 (35.5)	26 (25.7)	
Days to first negative solid culture, median (IQR)#	84 (82 – 91)	42 (28 -55)	**<.0001**
Alcohol Use in the past 3 months n (%)			
Yes	13 (42)	24 (24)	.067
Smoking in past 3months n (%)			
Yes	15 (48)	29 (29)	.051
Smear Grade n (%)			
1+	6 (19.4)	21 (20.8)	.124
2+	2 (6.4)	22 (21.8)	
3+	23 (74.2)	58 (57.4)	

**3 missing ARV status.*

#Measures for all variables, except days to first negative culture are reported at baseline. Significant p-values (<0.05) are bolded.

Effect of Plasma Cytokine/Chemokine Expression at Active TB on Overall Time to Culture Conversion

In order to further assess the impact of systemic inflammation during TB active disease on treatment response we used a Cox proportional hazards model to examine the association between cytokine expression during active TB and days to culture conversion (n=132). In the bivariable analysis, increased expression of IL-1RA (p=0.008) and MIP-1β (p=0.041) were significantly associated with shorter time to culture conversion (**Figure 2A**). In the multivariable analysis increased expression of MCP-3 [adjusted hazards ratio (aHR) 1.723, 95% CI 1.040 – 2.855, p=0.035] and of IL-6 (aHR 1.409, 95% CI 1.045 – 1.899, p=0.024) during active TB were significantly associated with shorter time to culture conversion (**Figure 2B**, **Supplementary Figure 1** and **Supplementary Table 3**).

A sub-analysis of HIV-infected individuals was performed adjusting for the effects of viral load and CD4 count (**Supplementary Table 4**). In the bivariable and multivariable models, higher IL-1RA (aHR 2.595, 95% CI1.136 – 5.926, p=0.024) and IL-1α (aHR 2.008, 95% CI 1.053 – 3.831, p=0.035) expression were significantly associated with shorter time to culture conversion. IL-6 (aHR 1.783, 95% CI 1.128 – 2.820, p=0.013) and IP-10 (aHR 2.068, 95% CI 1.034 – 4.137, p=0.040) were significantly associated with shorter time to culture conversion in the multivariable model. Observed increase in inflammatory markers during active TB likely contribute to enhanced cellular responses and faster bacterial clearance.

Effect of Plasma Cytokines/Chemokines Expression at Active TB on Disease Severity

To determine the association between systemic levels of cytokines/chemokines and disease severity measured by the presence of lung cavitation, we compared circulating levels of cytokines/chemokines in all individuals with cavitary and non-cavitary disease using logistic regression. In the bivariable analysis, increased expression of IL-6 (p=0.04) was significantly associated with cavitary disease. IL-6 (OR 2.543, 95% CI 1.254 – 5.160, p=0.010) and IL-1RA (OR 4.639, 95% CI 1.203 – 21.031, p=0.047) positively associated with the odds of lung cavitation in the multivariable model (**Figures 3A, B**, **Supplementary Table 5**).

We performed a sub-analysis of the HIV-infected individuals and adjusted for viral load and CD4 count (**Supplementary Table 6**). Increased expression of IL-6 (OR 2.644, 95% CI 1.062 – 6.585, p=0.037) and IL-1RA (OR 7.795, 95% CI 1.177 – 51.611, p=0.033) were associated with increased odds of cavitation. IL-6 and IL-1RA expression among total cohort and stratified by HIV status in those with and without cavitary disease show similar patterns irrespective of HIV status (**Figure 3C**). These results highlight the dual nature of the host immune response with similar responses being associated with bacterial clearance as well as disease severity and tissue damage.

Association Between Plasma Cytokine/Chemokine Expression and Bacterial Burden at Active TB

To determine the association between systemic levels of cytokines/chemokines and bacterial burden at active disease, we examined the differences in plasma cytokine/chemokine levels in patients with different smear grades (classified as 1+, 2+ and 3+). We observed no clear dose response with any of the measured cytokines/chemokines and smear grade (**Figure 4**). There was a trend towards higher IP-10 expression in Smear 2+, compared to the Smear 1+ group and trend for higher MIP-1α in Smear 3+ compared to Smear 1+ group (**Figure 4A**). In HIV-infected patients IP-10 levels were higher in Smear 2+ when compared to Smear 1+ group (**Figure 4B**). In the HIV-uninfected group,

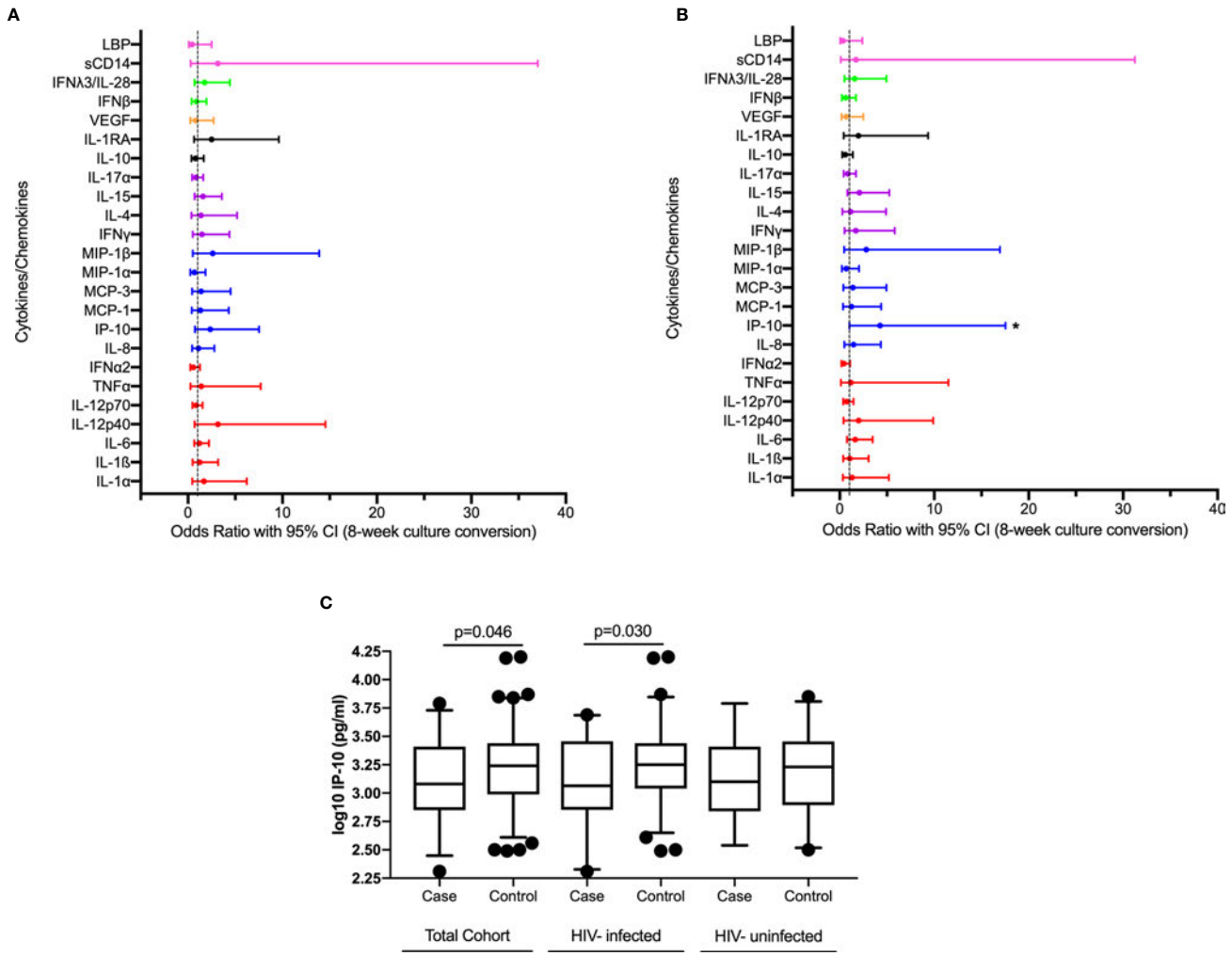

FIGURE 1 | Association between plasma cytokine/chemokine expression at active TB and 8-week culture conversion (n=132) in a **(A)** bivariable and **(B)** multivariable logistic regression model. Individual associations are shown between 8-week culture conversion and pro-inflammatory cytokines (red), chemokines (blue), adaptive response cytokines (purple), anti-inflammatory (black), growth factors (orange), interferons (green) and plasma biomarkers (pink) with error bars depicting 95% confidence intervals. Asterisk indicates significant association ($p<0.05$). **(C)** IP-10 expression among cases and controls in IMPRESS Cohort (cases n=31, controls n=101) and within the nested group of HIV-infected (cases n=20, controls n=76) and HIV-uninfected (cases n=11, controls n=25) individuals. Cytokines were plotted on log scale (Log 10), Box and Whiskers (5–95%). p-values shown on the graphs are results of the multivariable logistic regression.

IL-10, IFNγ and IL-1RA levels tended to be higher in Smear 3+ compared to Smear 2+ group (**Figure 4C**). Our data indicated that measured systemic plasma cytokines/chemokines are not reliable indicators of the bacterial load as measured by the smear grades.

DISCUSSION

Identification and characterisation of host biomarkers of TB disease severity and treatment response are important tools in progress towards TB elimination and control (18). Here we characterised the role of several plasma cytokines/chemokines, measured during active TB, on early and delayed culture conversion and disease severity in HIV-infected and -uninfected individuals.

Increased IP-10 levels during active TB were associated with early bacterial clearance after 2 months of intensive TB therapy in the total cohort as well as HIV-infected subgroup after adjusting for covariates. IP-10 (also known as CXCL10) is a chemokine that induces chemotaxis, apoptosis, cell growth inhibition and angiostasis (19). A number of published studies have highlighted the role of IP-10 in TB pathogenesis and the potential of using IP-10 as a biomarker of treatment response in TB patients (12–17). Additionally, IP-10 was shown to contribute to the inhibition of mycobacterial replication in the *ex vivo* model of human whole blood assay (20). Due to its stability and high expression, IP-10 has demonstrated potential to be developed into a simple point-of-care (POC) test (21–23). Our data supports these observations and highlights the role of

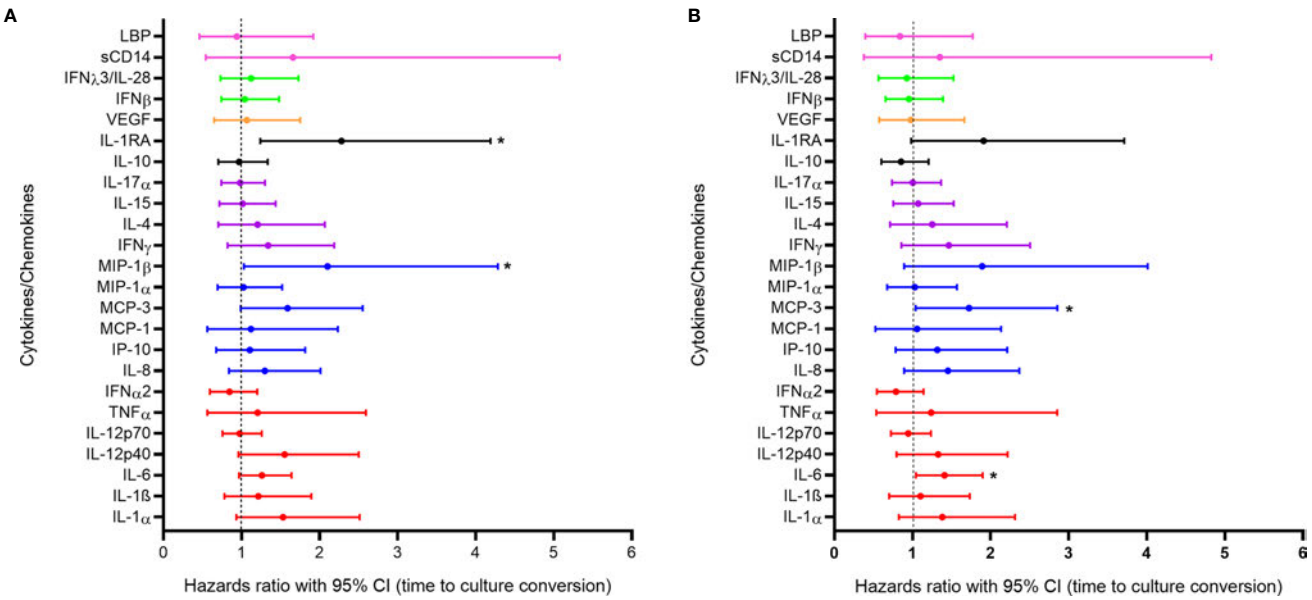

FIGURE 2 | Association between cytokine/chemokine expression measured at active TB and time to culture conversion (n=132) in **(A)** bivariable and **(B)** multivariable models. Individual associations are shown between time to first negative TB culture result and pro-inflammatory cytokines (red), chemokines (blue), adaptive response cytokines (purple), anti-inflammatory (black), growth factors (orange), interferons (green) and plasma biomarkers (pink) with error bars depicting 95% confidence intervals. The dotted line at 1 distinguishes the hazards ratio of higher than 1 (to the right) indicating shorter time to culture conversion and lower than 1 (to the left) indicating longer time to culture conversion. Asterisk indicates significant associations ($p < 0.05$).

IP-10 in TB clearance among HIV-infected and -uninfected patients with recurrent TB.

After adjusting for covariates, IL-6 and MCP-3 were significantly associated with shorter time to culture conversion in the total cohort. Chemokines, such as MCP-3 play an important role in host response to MTB, with MTB-exposed macrophages showing highly increased MCP-3 expression (24). Furthermore, a strain of BCG that secretes high levels of functional MCP-3 displayed improved immunogenicity and enhanced antigen-specific T cell responses (25). Similar to IP-10, increased MCP-3 levels during active TB likely contribute to enhanced cellular responses and faster bacterial clearance. In addition to its previously identified role as a biomarker of active pulmonary TB (26–28), we found that increased IL-6 expression at active disease is significantly associated with faster bacterial clearance. IL-6, a pleiotropic proinflammatory cytokine, plays an important role in generation of T and B cell responses. Importantly, IL-6 is known to play an important role in protective host immune responses to TB (29, 30) and is essential for generating Th1 cellular responses considered central for MTB control (31). In addition to IL-6 and IP-10, IL-1RA and IL-1α demonstrated an association with shorter time to culture conversion in HIV-infected individuals. IL-1α is an important immunoregulatory cytokine that depending on the magnitude of stress or damage caused by the infection can initiate an inflammatory response or reparative fibrosis (32). IL-1RA is a member of IL-1 family that binds to the IL-1 receptor but does not induce a response (33); its expression is upregulated by inflammatory cytokines including IL-1α and IL-6 as an anti-inflammatory control mechanism (34, 35). IL-1 and IL-1R were shown to be critical for host resistance to MTB (36, 37), while IL-1RA was shown to be a marker of TB disease activity (38, 39). While some of the markers seem to be affected by HIV infection, IL-6 levels are correlated with overall shorter time to culture conversion in both patient groups. Overall, our data shows that the magnitude and nature of inflammatory cytokine expression during active TB disease can be indicative of more efficient cellular response and host's ability to clear the infection.

Pulmonary cavitation, a hallmark of pulmonary TB, is associated with high bacterial burden and subsequent increase in inflammatory response. Additionally, the host immune response is thought to drive the development of TB cavities (40). Our results indicate that increased plasma IL-6 and IL-1RA levels are associated with cavitary disease in both HIV-infected and uninfected TB patients. Elevated concentrations of both IL-6 (41) and IL-1RA in bronchoalveolar lavage (BAL) fluid were previously found to be associated with tissue necrosis and resulting cavity formation in patients with active pulmonary TB (42). Associations of IL-6 and IL-1RA with bacterial clearance and disease severity (measured by lung cavitation) highlight the dual nature of the host immune response to infections; while immune activation is required for successful pathogen clearance and initiation of protective cellular responses it can also contribute to immune mediated lung pathology and worsened disease outcome.

We observed no clear dose response between, measured plasma cytokines/chemokines and bacterial burden as

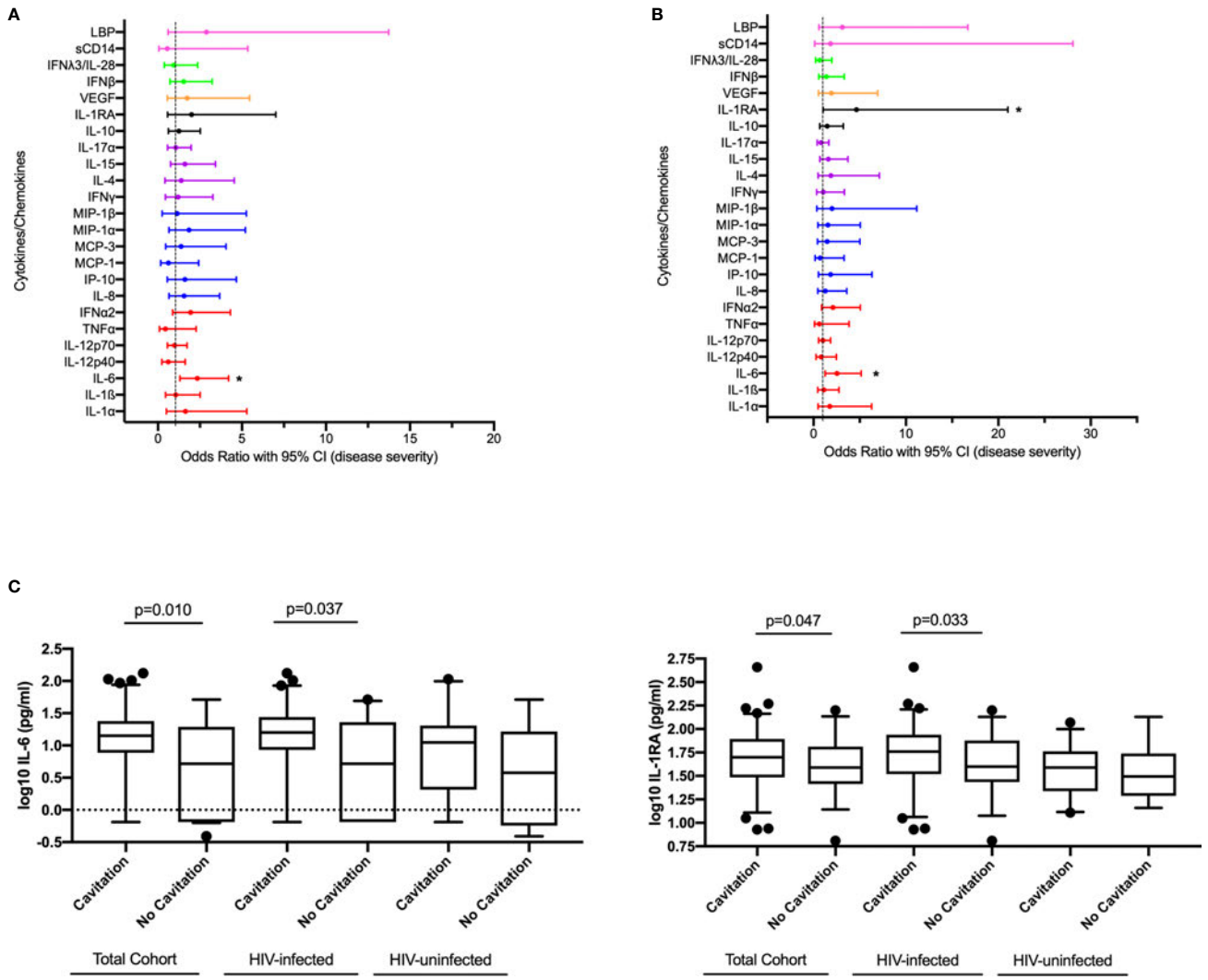

FIGURE 3 | Association between cytokine/chemokine expression measured at active TB and disease severity measured by lung cavitation (n=132) in both **(A)** univariable and **(B)** multivariable model. Individual associations are shown between 8-week culture conversion and pro-inflammatory cytokines (red), chemokines (blue), adaptive response cytokines (purple), anti-inflammatory (black), growth factors (orange), interferons (green) and plasma biomarkers (pink) with error bars depicting 95% confidence intervals. The dotted line at 1 is the interpretation of odds ratio. Asterisk in **(A, B)** indicates significant associations ($p < 0.05$). **(C)** IL-6 and IL-1RA plasma levels associated with cavitary versus non-cavitary disease in total cohort (Cavitation n=93, no cavitation n=39), HIV-infected participants (Cavitation n=63, no cavitation n=33) and HIV-uninfected individuals (Cavitation n=30, no cavitation n=6). Cytokines were plotted on log scale (Log 10), Box and Whiskers (5–95%). p-values shown on the graph **(C)** are the results of the multivariable logistic regression.

measured by smear grades. Sputum acid fast bacilli provides an indication of bacillary load and is most often used to monitor TB patients in resource limited settings; however, this method does not distinguish live and dead organisms, has low sensitivity and the specificity predicting treatment failure or relapse is modest (2, 43, 44).

Our study has several limitations, including a relatively small sample size and a clinically complex cohort of patients requiring correction for a wide range of covariates. Future studies should examine cellular phenotypes in order to link the observed inflammatory responses with protective cellular responses to MTB. Our study was focused on drug susceptible TB and future research should also assess immune biomarkers in drug-resistant TB as host immune responses are known to vary between different MTB isolates (45–49). Our study additionally demonstrates that the identified inflammatory markers of disease have low predicative power when considered on their own, with AUC values ranging from 0.56 – 0.65 (**Supplementary Figure 2**). The associations we observed were stronger in the multivariable models highlighting the important effect of other behavioural and clinical variables on the expression of immune markers and their potential confounding effects on TB outcome (50–55).

In summary, our study confirms the importance of inflammatory markers, including IP-10 and IL-6, in TB disease

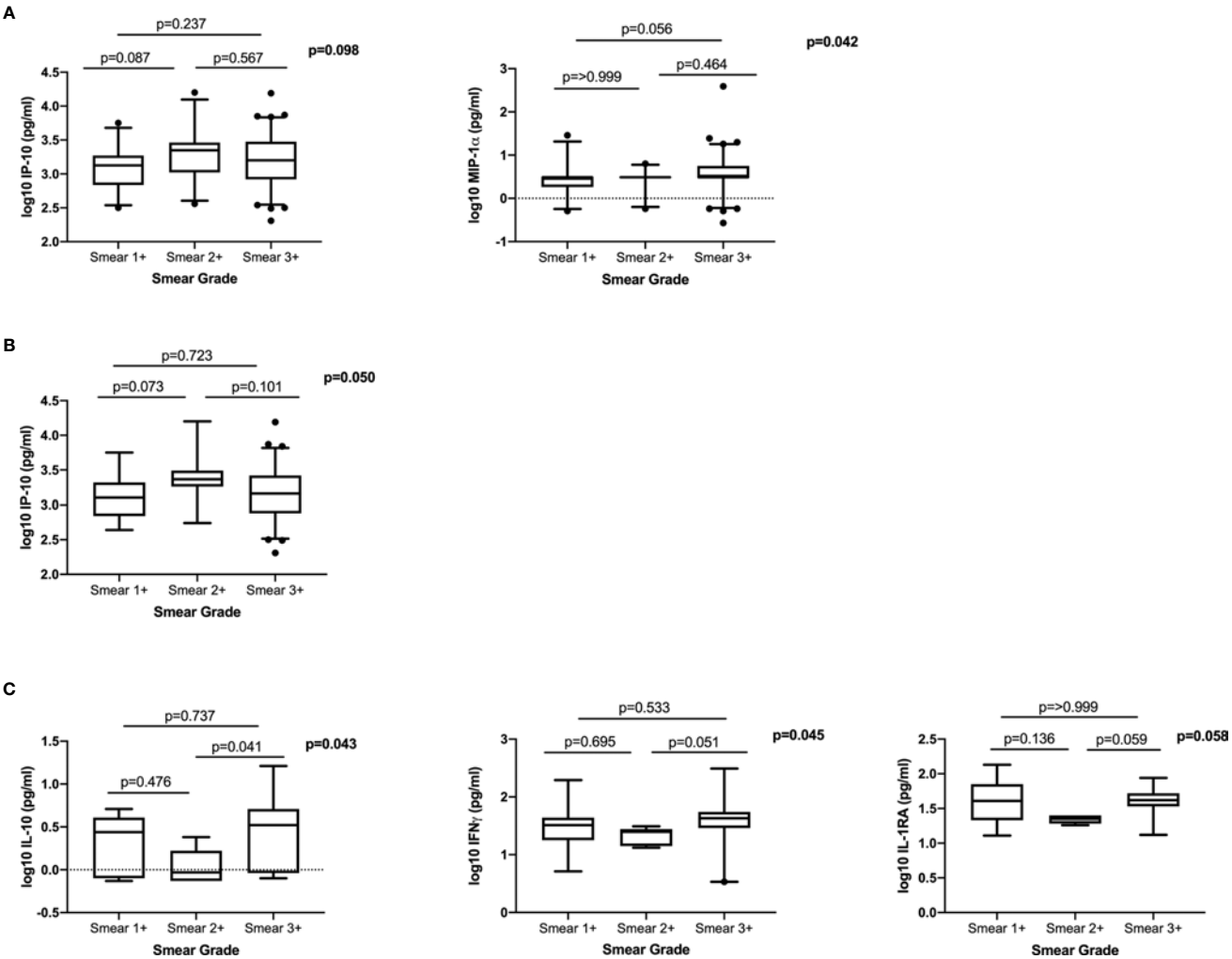

FIGURE 4 | The relationship between plasma cytokines/chemokines and bacterial burden measured by smear grades: **(A)** Total cohort; (Smear 1+ n= 27, Smear 2+ n=24, Smear 3+ n=81), **(B)** HIV-infected (Smear 1+ n=16, Smear 2+ n=18, Smear 3+ n=62) and **(C)** HIV-uninfected (Smear 1+ n=11, Smear 2+ n=6, Smear 3+ n=19). Only cytokines that were significantly different or trending towards significance are shown. Based on distribution IP-10 was analysed using a one-way ANOVA with Tukey's multiple comparisons test and MIP-1α, IFNγ, IL-10 and IL-1RA were analysed using non-parametric Kruskal Wallis test with Dunn's multiple comparisons test. Cytokines were plotted on log scale (Log 10), Box and Whiskers (5–95%).

pathogenesis. Further studies are needed to confirm the utility of identified inflammatory markers in TB management. The development and progression of TB disease are influenced by combined effects of various immune as well as behavioural and clinical variables that will have to be considered when utilising immune biomarkers as predictors of risk or protection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Biomedical Research Ethics Committee, University of KwaZulu-Natal. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS, KN, and SR conceptualized and designed the study. SR and AS performed the experiments. SR, AS, LL, and KN analyzed the data. SN, AS, and KN wrote the manuscript. AS, KN, NS, RH-M, DG, TS, and NP supervised clinical and/or experimental aspects

of the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.641065/full#supplementary-material

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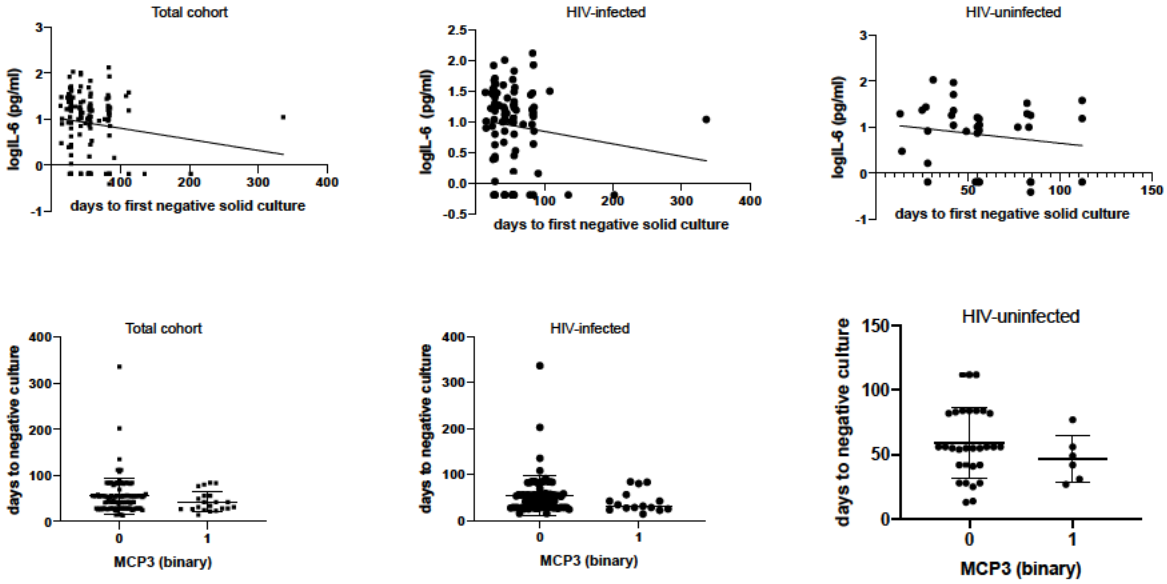
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

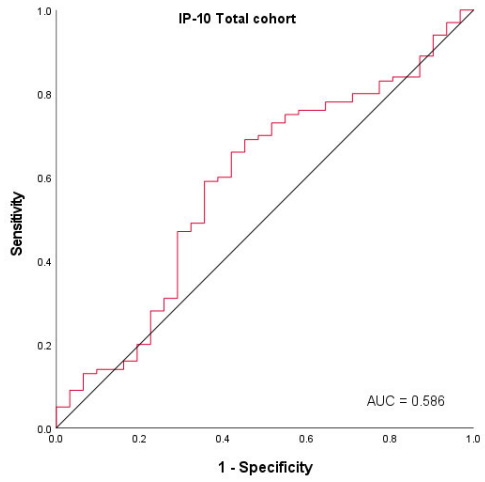
The handling editor declared a past co-authorship with one of the authors TS.

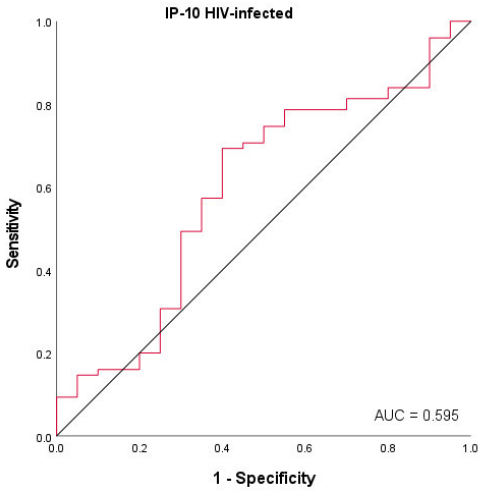
Supplemental Materials



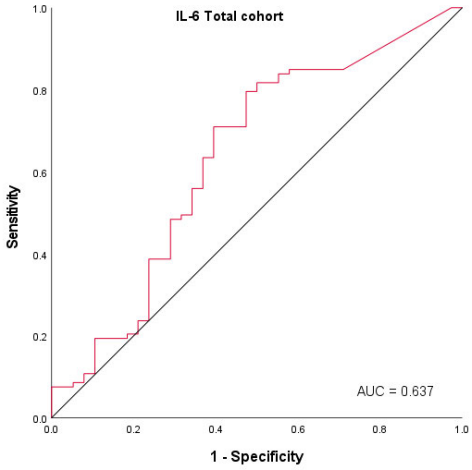
Supplemental Figure 1. Association between significant plasma cytokine/chemokine at active TB and days to negative culture result (A) Correlation between plasma IL-6 levels and days to negative culture in total cohort, HIV-infected and -uninfected study participants (B) comparison of days to negative culture between individuals with detectable and undetectable MCP3 plasma levels in total cohort, HIV infected and uninfected study participants.

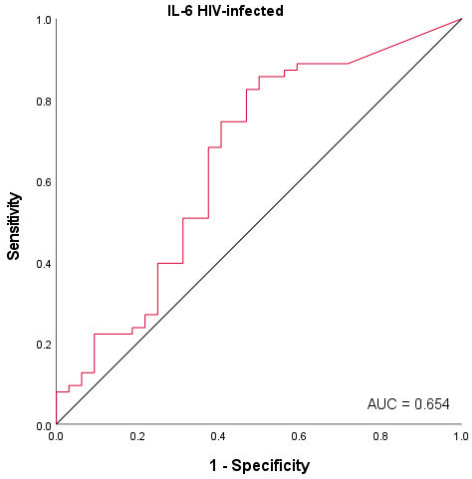
A.


IP-10 Total cohort
Sensitivity
1 - Specificity
AUC = 0.586

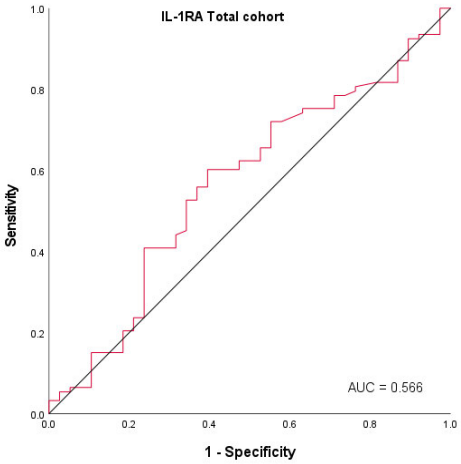

IP-10 HIV-infected
Sensitivity
1 - Specificity
AUC = 0.595

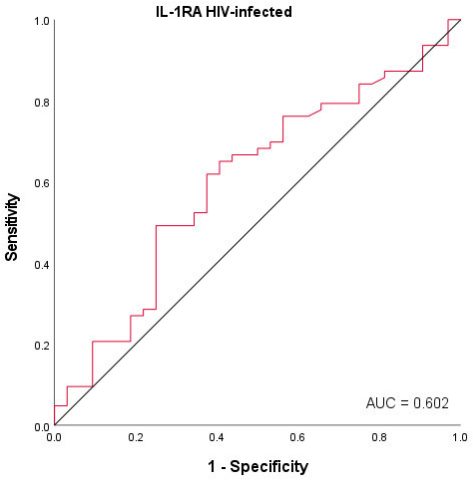
B.


IL-6 Total cohort
Sensitivity
1 - Specificity
AUC = 0.637


IL-6 HIV-infected
Sensitivity
1 - Specificity
AUC = 0.654

C.


IL-1RA Total cohort
Sensitivity
1 - Specificity
AUC = 0.566


IL-1RA HIV-infected
Sensitivity
1 - Specificity
AUC = 0.602

Supplemental Figure 2. Receiver-operating characteristic (ROC) analysis. ROC analysis was performed with cytokine/chemokine values as predictors and 8-week culture conversion and disease severity measured by presence of lung cavitation as response variables. (A) chemokine IP-10 as a predictor of 8-week culture conversion, AUC=0.586 (total cohort), AUC=0.595 (HIV infected); (B) IL-6 as a predictor of lung cavitation, AUC=0.637 (total cohort), AUC=0.654 (HIV infected) and (C) IL-1RA as a predictor of lung cavitation, AUC=0.566 (total cohort), AUC=0.602 (HIV infected).

Supplemental Table 1. Association between plasma cytokine/chemokine expression at active TB and 8-week culture conversion among total cohort

	Bivariable[b]			Multivariable[c]		
*Cytokine /chemokine	OR	CI	p-value	OR	CI	p-value
IFN-γ	1.475	0.496 – 4.386	.485	1.698	0.496 – 5.814	.399
IFN-α2[a]	0.555	0.244 – 1.262	.160	0.422	0.164 – 1.087	**.074**
IL-10	0.773	0.362 – 1.653	.508	0.594	0.255 – 1.385	.227
MCP-3[a]	1.384	0.426 – 4.499	.589	1.379	0.385 – 4.940	.622
IL-12p40[a]	3.162	0.688 – 14.539	.139	1.991	0.401 – 9.893	.400
IL-12p70	0.850	0.470 – 1.538	.591	0.718	0.355 – 1.453	.358
IL-15[a]	1.577	0.695 – 3.580	.276	2.070	0.818 – 5.238	.124
IL-17A	0.831	0.428 – 1.613	.584	0.847	0.419 – 1.709	.642
IL-1RA	2.494	0.646 – 9.615	.185	1.965	0.413 – 9.346	.396
IL-1α[a]	1.672	0.449 – 6.224	.443	1.303	0.327 – 5.188	.707
IL-1β	1.229	0.474 – 3.185	.672	1.047	0.360 – 3.049	.932
IL-4[a]	1.367	0.360 – 5.193	.647	1.147	0.269 – 4.892	.852
IL-6	1.200	0.653 – 2.208	.556	1.623	0.756 – 3.484	.214
IL-8	1.100	0.435 – 2.786	.840	1.447	0.482 – 4.348	.509
IP-10	2.347	0.73 – 7.519	.152	4.255	1.025 – 17.544	**.046**
MCP-1	1.316	0.403 – 4.31	.649	1.229	0.344 – 4.386	.751
MIP-1α	0.676	0.248 – 1.842	.444	0.683	0.229 – 2.037	.494
MIP-1β	2.618	0.491 – 13.889	.259	2.809	0.465 – 16.949	.260
TNF-α	1.397	0.253 – 7.692	.701	1.149	0.116 – 11.494	.905
VEGF	0.808	0.241 – 2.703	.729	0.685	0.189 – 2.488	.566
IFN-β[a]	0.892	0.379 – 1.962	.724	0.624	0.227 – 1.718	.362
IL-28[a]	1.752	0.692 – 4.436	.237	1.565	0.496 – 4.941	.445
sCD14	3.155	0.271 – 37.037	.358	1.718	0.094 – 31.25	.715
LBP	0.436	0.076 – 2.494	.351	0.329	0.046 – 2.381	.271

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumour necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; OR, odds ratio; CI, confidence interval.
* cytokine values were logged, if not binary

[a] Analysed as binary variables
[b] Bivariable analyses adjusted for randomization arm
[c] Multivariable analyses adjusted for randomization arm, age, gender, HIV status, lung cavitation, alcohol use, smoking and BMI.

Supplemental Table 2. Association between cytokine/chemokine expression at active TB and 8-week culture conversion among HIV-infected patients

	Bivariable[b]			Multivariable[c]		
*Cytokine/ chemokine	OR	CI	p-value	OR	CI	p-value
IFN-γ	0.769	0.192 – 3.077	.711	0.875	0.158 – 4.854	.878
IFN-α2[a]	0.645	0.234 – 1.783	.399	0.601	0.181 – 1.997	.406
IL-10	0.835	0.348 – 2.004	.686	0.519	0.181 – 1.490	.223
MCP-3[a]	1.084	0.271 – 4.342	.909	0.933	0.169 – 5.146	.936
IL-12p40[a]	2.372	0.492 – 11.440	.282	1.182	0.196 – 7.136	.855
IL-12p70	0.749	0.39 – 1.439	.386	0.68	0.305 – 1.515	.346
IL-15[a]	1.709	0.625 – 4.675	.296	2.656	0.764 – 9.231	.124
IL-17A	0.737	0.296 – 1.835	.512	0.776	0.277 – 2.174	.631
IL-1RA	3.030	0.595 – 15.385	.182	2.179	0.299 – 15.873	.442
IL-1α[a]	1.810	0.369 – 8.882	.465	1.596	0.266 – 9.571	.609
IL-1β	1.484	0.495 – 4.444	.482	1.131	0.311 – 4.132	.851
IL-4[a]	0.832	0.201 – 3.434	.799	0.675	0.122 – 3.720	.652
IL-6	1.124	0.525 – 2.404	.765	2.375	0.803 – 7.042	.118
IL-8	0.727	0.205 – 2.571	.620	1.159	0.216 – 6.211	.863
IP-10	3.049	0.717 – 12.987	.131	10.204	1.247 – 83.333	**.030**
MCP-1	2.994	0.445 – 20	.260	3.584	0.425 – 30.303	.240
MIP-1α	0.278	0.054 – 1.433	.126	0.21	0.029 – 1.534	.124
MIP-1β	1.096	0.145 – 8.333	.929	1.399	0.128 – 15.385	.784
TNF-α	0.786	0.089 – 6.944	.828	1.017	0.056 – 18.519	.991
VEGF	0.573	0.134 – 2.451	.453	0.387	0.076 – 1.969	.253
IFN-β[a]	0.782	0.286 – 2.136	.631	0.477	0.127 – 1.792	.273
IL-28[a]	2.401	0.854 – 6.752	.097	1.577	0.445 – 5.589	.481
sCD14	2.681	0.092 – 76.923	.567	4.484	0.114 – 166.667	.423
LBP	0.337	0.042 – 2.695	.306	0.178	0.016 – 1.946	.157

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumor necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; OR, odds ratio; CI, confidence interval.

[*] cytokine values were logged, if not binary
[a] Analysed as binary variables
[b] Bivariable analyses adjusted for randomization arm
[c] Multivariable analyses adjusted for randomization arm, age, gender, CD4 count, viral load, lung cavitation, alcohol use, smoking and BMI.

Supplemental Table 3. Association between plasma cytokine/chemokine expression and days to culture conversion

	Bivariable[b]			Multivariable[c]		
*Cytokine/ chemokine	HR	CI	p-value	HR	CI	p-value
IFN-γ	1.341	0.822 – 2.189	.240	1.466	0.858 – 2.506	.162
IFN-α2[a]	0.846	0.595 – 1.203	.351	0.788	0.544 – 1.142	.208
IL-10	0.969	0.703 – 1.335	.846	0.853	0.602 – 1.207	.369
MCP-3[a]	1.590	0.990 – 2.553	**.055**	1.723	1.040 – 2.855	**.035**
IL-12p40[a]	1.552	0.963 – 2.502	**.071**	1.328	0.795 – 2.218	.279
IL-12p70	0.976	0.757 – 1.259	.851	0.946	0.724 – 1.236	.685
IL-15[a]	1.016	0.718 – 1.438	.929	1.072	0.753 – 1.527	.698
IL-17A	0.983	0.743 – 1.302	.907	1.004	0.738 – 1.366	.981
IL-1RA	2.281	1.242 – 4.190	**.008**	1.910	0.982 – 3.713	**.056**
IL-1α[a]	1.532	0.934 – 2.514	**.091**	1.383	0.826 – 2.314	.217
IL-1β	1.217	0.782 – 1.894	.383	1.102	0.701 – 1.733	.673
IL-4[a]	1.206	0.703 – 2.069	.497	1.254	0.712 – 2.210	.433
IL-6	1.261	0.968 – 1.642	**.085**	1.409	1.045 – 1.899	**.024**
IL-8	1.300	0.839 – 2.013	.240	1.454	0.892 – 2.368	.133
IP-10	1.107	0.675 – 1.817	.687	1.317	0.784 – 2.213	.298
MCP-1	1.121	0.562 – 2.235	.746	1.060	0.526 – 2.135	.871
MIP-1α	1.027	0.693 – 1.521	.895	1.030	0.676 – 1.569	.892
MIP-1β	2.102	1.031 – 4.286	**.041**	1.892	0.892 – 4.013	**.096**
TNF-α	1.207	0.562 – 2.595	.630	1.238	0.537 – 2.854	.616
VEGF	1.068	0.651 – 1.752	.794	0.977	0.574 – 1.664	.932
IFN-β[a]	1.043	0.743 – 1.482	.815	0.954	0.654 – 1.392	.808
IL-28[a]	1.124	0.730 – 1.731	.597	0.928	0.564 – 1.526	.769
sCD14	1.659	0.542 – 5.078	.375	1.350	0.378 – 4.827	.644
LBP	0.941	0.461 – 1.921	.868	0.839	0.397 – 1.772	.646

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumour necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; HR, hazards ratio; CI, confidence interval.

[*] cytokine values were logged, if not binary
[a] Analysed as binary variables
[b] Bivariable analyses adjusted for randomization arm
[c] Multivariable analyses adjusted for randomization arm, age, gender, HIV status, lung cavitation, alcohol use, smoking and BMI.

Supplemental Table 4. Association between plasma cytokine/chemokine expression and days to culture conversion among HIV-infected patients

	Bivariable[b]			Multivariable[c]		
*Cytokine/ chemokine	HR	CI	p-value	HR	CI	p-value
IFN-γ	1.225	0.667 – 2.248	.513	1.704	0.831 – 3.498	.146
IFN-α2[a]	0.884	0.585 – 1.338	.561	0.949	0.594 – 1.515	.876
IL-10	0.947	0.667 – 1.346	.763	0.894	0.609 – 1.313	.568
MCP-3[a]	1.546	0.885 – 2.700	.126	1.620	0.847 – 3.097	.145
IL-12p40[a]	1.418	0.845 – 2.380	.186	1.278	0.701 – 2.328	.423
IL-12p70	0.899	0.684 – 1.182	.447	0.954	0.704 – 1.293	.763
IL-15[a]	1.045	0.693 – 1.575	.833	1.283	0.816 – 2.018	.281
IL-17A	0.961	0.647 – 1.427	.843	0.924	0.588 – 1.452	.732
IL-1RA	2.453	1.219 – 4.934	**.012**	2.595	1.136 – 5.926	**.024**
IL-1α[a]	1.828	1.043 – 3.205	**.035**	2.008	1.053 – 3.831	**.035**
IL-1β	1.357	0.832 – 2.212	.221	1.29	0.760 – 2.190	.346
IL-4[a]	0.972	0.531 – 1.778	.927	1.188	0.605 – 2.331	.617
IL-6	1.255	0.906 – 1.738	.172	1.783	1.128 – 2.820	**.013**
IL-8	1.161	0.675 – 1.998	.590	1.853	0.896 – 3.834	.096
IP-10	1.261	0.698 – 2.276	.442	2.068	1.034 – 4.137	**.040**
MCP-1	1.305	0.606 – 2.811	.497	1.322	0.564 – 3.099	.520
MIP-1α	0.893	0.466 – 1.714	.735	0.908	0.457 – 1.803	.783
MIP-1β	1.768	0.760 – 4.113	.186	1.926	0.739 – 5.019	.180
TNF-α	0.950	0.379 – 2.381	.912	1.54	0.619 – 3.828	.353
VEGF	1.040	0.599 – 1.808	.888	0.804	0.419 – 1.541	.511
IFN-β[a]	1.097	0.726 – 1.658	.660	0.911	0.565 – 1.471	.704
IL-28[a]	1.229	0.775 – 1.947	.380	1.015	0.585 – 1.760	.959
sCD14	1.957	0.472 – 8.118	.355	2.553	0.534 – 12.217	.241
LBP	0.914	0.401 – 2.081	.830	1.013	0.419 – 2.449	.978

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumor necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; HR, hazards ratio; CI, confidence interval.

[*] cytokine values were logged, if not binary
[a] Analysed as binary variables
[b] Bivariable analyses adjusted for randomization arm
[c] Multivariable analyses adjusted for randomization arm, age, gender, CD4 count, viral load, lung cavitation, alcohol use, smoking and BMI.

Supplemental Table 5. Association between plasma cytokine/chemokine expression at active TB and disease severity

	Univariable			Multivariable[b]		
*Cytokine/ chemokine	OR	CI	p-value	OR	CI	p-value
IFN-γ	1.208	0.446 – 3.273	.710	1.061	0.337 – 3.338	.919
IFN-α2[a]	1.946	0.878 – 4.313	.101	2.098	0.868 – 5.068	.100
IL-10	1.248	0.618 – 2.519	.536	1.477	0.674 – 3.236	.330
MCP-3[a]	1.371	0.464 – 4.054	.568	1.469	0.430 – 5.011	.539
IL-12p40[a]	0.609	0.230 – 1.616	.320	0.829	0.278 – 2.469	.736
IL-12p70	0.986	0.562 – 1.731	.962	1.007	0.549 – 1.846	.983
IL-15[a]	1.599	0.746 – 3.426	.228	1.600	0.689 – 3.716	.274
IL-17A	1.059	0.568 – 1.975	.857	0.805	0.391 – 1.654	.554
IL-1RA	2.005	0.573 – 7.016	.276	4.639	1.023 – 21.031	**.047**
IL-1α[a]	1.634	0.505 – 5.286	.412	1.744	0.483 – 6.301	.396
IL-1β	1.059	0.448 – 2.506	.896	1.115	0.452 – 2.749	.813
IL-4[a]	1.381	0.420 – 4.541	.595	1.874	0.493 – 7.122	.357
IL-6	2.343	1.305 – 4.207	**.004**	2.543	1.254 – 5.160	**.010**
IL-8	1.549	0.654 – 3.671	.320	1.277	0.454 – 3.595	.643
IP-10	1.604	0.551 – 4.670	.386	1.838	0.534 – 6.321	.334
MCP-1	0.624	0.160 – 2.427	.496	0.724	0.158 – 3.314	.678
MIP-1α	1.847	0.656 – 5.202	.245	1.548	0.474 – 5.054	.469
MIP-1β	1.143	0.248 – 5.265	.863	1.997	0.357 – 11.167	.431
TNF-α	0.446	0.088 – 2.270	.331	0.627	0.102 – 3.842	.614
VEGF	1.735	0.551 – 5.458	.346	1.936	0.540 – 6.943	.311
IFN-β[a]	1.524	0.718 – 3.231	.272	1.377	0.572 – 3.316	.476
IL-28[a]	0.942	0.375 – 2.367	.899	0.674	0.229 – 1.981	.473
sCD14	0.554	0.057 – 5.350	.610	1.839	0.120 – 28.084	.661
LBP	2.880	0.604 – 13.731	.184	3.122	0.584 – 16.704	.183

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumour necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; OR, odds ratio; CI, confidence interval.

[*] cytokine values were logged, if not binary
[a] Analysed as binary variables
[b] Multivariable analyses adjusted for age, gender, HIV status, alcohol use, smoking and BMI.

Supplemental Table 6. Association between cytokine/chemokine expression at active TB and disease severity among HIV-infected patients

	Univariable			Multivariable[b]		
*Cytokine/ chemokine	OR	CI	p-value	OR	CI	p-value
IFN-γ	1.402	0.435 – 4.517	.571	0.78	0.183 – 3.324	.737
IFN-α2[a]	2.271	0.927 – 5.563	**.073**	2.249	0.808 – 6.258	.120
IL-10	1.450	0.688 – 3.057	.329	1.727	0.716 – 4.165	.224
MCP-3[a]	1.481	0.431 – 5.082	.533	1.828	0.439 – 7.609	.488
IL-12p40[a]	0.840	0.295 – 2.396	.745	1.426	0.413 – 4.929	.641
IL-12p70	0.983	0.552 – 1.750	.954	0.983	0.497 – 1.947	.961
IL-15[a]	1.954	0.826 – 4.626	.128	1.826	0.666 – 5.008	.242
IL-17A	0.886	0.411 – 1.911	.758	0.602	0.236 – 1.538	.289
IL-1RA	2.815	0.697 – 11.371	.146	7.795	1.177 – 51.611	**.033**
IL-1α[a]	1.481	0.431 – 5.082	.533	1.654	0.422 – 6.480	.545
IL-1β	1.168	0.480 – 2.841	.733	1.376	0.527 – 3.591	.514
IL-4[a]	1.321	0.380 – 4.595	.662	1.587	0.391 – 6.442	.541
IL-6	2.780	1.378 – 5.608	**.004**	2.644	1.062 – 6.585	**.037**
IL-8	1.702	0.605 – 4.789	.313	0.971	0.243 – 3.880	.966
IP-10	1.615	0.485 – 5.370	.435	1.351	0.269 – 6.791	.715
MCP-1	0.782	0.143 – 4.270	.776	0.638	0.092 – 4.430	.650
MIP-1α	2.111	0.531 – 8.391	.289	2.179	0.445 – 10.664	.336
MIP-1β	2.160	0.385 – 12.128	.382	2.745	0.360 – 20.903	.330
TNF-α	1.014	0.163 – 6.296	.988	0.493	0.060 – 4.019	.509
VEGF	1.969	0.549 – 7.061	.299	1.935	0.429 – 8.726	.390
IFN-β[a]	1.315	0.563 – 3.072	.528	1.006	0.345 – 2.928	.813
IL-28[a]	0.688	0.264 – 1.791	.444	0.451	0.136 – 1.499	.233
sCD14	2.057	0.126 – 33.652	.613	1.971	0.077 – 50.757	.682
LBP	4.861	0.847 – 27.895	**.076**	5.43	0.768 – 38.392	**.090**

Abbreviations: IL, interleukin; IFN, interferon; IP, interferon gamma induced protein; sCD, soluble CD; TNF, tumor necrosis factor; VEFG, vascular endothelial growth factor; LBP, lipopolysaccharide binding protein; OR, odds ratio; CI, confidence interval.

[*] cytokine values were logged, if not binary
[a] Analysed as binary variables
[b] Multivariable analyses adjusted for age, gender, CD4 count, viral load, alcohol use, smoking and BMI.

CHAPTER 3

CHAPTER 3 - Rationale

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

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Host innate immune system plays an important role in both pathogenesis and clearance of *Mycobacterium tuberculosis* (Mtb). The major innate immune cells involved in Mtb response include monocytes/macrophages, DCs, neutrophils and NK cells. Monocytes and DCs specifically play an important role in pathogen recognition and clearance, as well as activation of the adaptive immune response. Alteration of the blood monocyte and dendritic cell profiles have been demonstrated during both Mtb and HIV infections and are known to play a role in disease pathogenesis.

In Chapter 3 we utilised samples from the CAPRISA 011 IMPRESS trial, the CAPRISA 002 Acute Infection Study and healthy controls to characterise the proportions and phenotypic profiles of peripheral blood monocytes and dendritic cells in TB and TB/HIV co-infection. Additionally, we determined the associations between the proportions and phenotypes of monocyte and dendritic cell subsets and time to culture conversion and lung cavitation as a measure of treatment response and disease severity, respectively.

Dr Sivro, Prof Naidoo, and I conceptualized and designed this study. I prepared the specimens, optimised, and performed the flow cytometry assays. I performed the statistical analysis and data interpretation. Mrs. Lewis, the CAPRISA 011 and CAPRISA 002 study statistician, validated the analysis. I wrote the initial draft of the manuscript that was reviewed and edited by my supervisors and other co-authors. This manuscript was published in *BMC Immunology* on 21 July 2023 (PMID: 37480005).

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

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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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Introduction

Despite being a treatable and preventable disease, tuberculosis (TB) continues to claim millions of lives each year and remains the leading cause of mortality among HIV positive individuals, causing approximately a third of HIV-1 associated deaths. At the same time HIV infection is the strongest risk factor for TB infection progressing to TB disease, with people living with HIV (PLWH) being approximately 18 times more likely to develop TB disease compared to people without HIV [1]. There continues to be an urgent need for novel approaches for diagnosis, treatment and prevention of TB in PLWH, especially in sub-Saharan Africa, where the syndemic interaction between TB and HIV is felt most acutely. Identification of immunological correlates of protection and risk for TB can aid in evaluation and development of novel vaccine candidates, new diagnostic methods and the development of shorter treatment regiments [2]. While significant work has been done in characterizing host immune response in TB and HIV infections individually, the innate and adaptive immune responses in TB/HIV co-infection remain poorly understood. HIV mediated chronic inflammation and the impairments in the innate and adaptive immunity are known to contribute to increased risk of TB in PLWH [3]. In our previous studies we have shown that increase in systemic inflammatory markers are a strong predictor of TB reactivation in HIV positive individuals [4, 5]. Immune activation and dysregulation of monocyte/macrophages and dendritic cell phenotype and function is known to contribute to both HIV and TB pathogenesis [6–9]. During TB and HIV infection, monocytes, and dendritic cells (DCs) can be activated due to increased bacterial/viral load, microbial translocation and gastrointestinal damage, and increased production of pro-inflammatory cytokines. Changes in their phenotype can affect cell migration and tissue distribution, activation of the adaptive immune response and lead to further increase in the inflammatory response, all of which can impact pathogen clearance and immunopathogenesis [10]. Phenotypic and functional alterations in circulating monocytes and dendritic cells have been reported from patients with pulmonary TB [11] and these changes are thought to play a role in bacterial persistence [12–14]. Enhanced monocyte activation was observed in PLWH with latent TB infection or prior active TB and could contribute to the pathogenesis of non-communicable disease in HIV [15].

A better understanding of the intricate and dynamic interactions between the host immune response and *Mycobacterium tuberculosis* (Mtb) in the context of HIV co-infection is crucial to the development of better drugs and vaccines for the prevention and treatment of TB in HIV co-infected individuals. In this study, we set out to characterize the phenotypic differences in monocyte and DC subsets during active pulmonary TB in HIV-positive and HIV-negative individuals from South Africa.

Materials and methods

Study population

Stored peripheral blood mononuclear cells (PBMCs) used for this study were from the CAPRISA 011 Improving Retreatment Success (IMPRESS) trial. This was an open label, randomized clinical trial to determine if treatment outcomes would be improved with the substitution of moxifloxacin [HRZM (isoniazid, rifampicin, pyrazinamide, and moxifloxacin), active arm] for ethambutol [HRZE (isoniazid, rifampicin, pyrazinamide and ethambutol), control arm] in patients with recurrent TB. CAPRISA 011 study participants were recruited and treated at an urban clinic operated by the Centre for the AIDS Programme of Research in South Africa (CAPRISA) eThekwini Research Clinic that adjoins the largest government outpatient HIV-TB facility, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KwaZulu-Natal (KZN), South Africa (SA) [16]. Participants enrolled in the study were adults ≥ 18 years who had a previous history of TB treatment completion, with rifampicin-susceptible Mtb and were sputum smear-positive by GeneXpert MTB/RIF® technology. Patients were monitored with 2-weekly clinical follow-up visits during the 8 weeks of intensive phase of TB treatment, and a monthly clinical follow-up during the 16 weeks of continuous phase of TB treatment. Patient enrolment started in November 2013 and follow-up ended in July 2017.

We conducted a cohort analysis that included 90 individuals with available PBMC samples taken during active TB (baseline), 60 were HIV positive and 30 HIV negative. As controls, we also analysed PBMCs from 19 HIV positive individuals without TB from the CAPRISA 002 Acute HIV Infection study, an observational cohort study aiming to identify viral immune and host genetic factors that predict disease progression [17], and from PBMC samples from 11 healthy donors.

Sample collection and processing

Peripheral blood was collected in acid citrate dextrose (ACD) tubes. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO) in liquid nitrogen for long-term storage.

Flow cytometry experiments

Thawed PBMCs were washed and resuspended in 5 ml R10 (RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin 1.7 nM sodium

glutamate and 5.5 mL HEPES buffer). Cells were rested for 3 h at 37 °C, 5% CO_2 before staining.

A total of 1×10^6 cells were surfaced stained in the dark at room temperature for 20 min with a panel of conjugated antibodies: anti-CD3 Alexa Fluor 700, anti-CD56 Alexa Fluor 700, anti-CD19 Alexa Fluor 700, anti-CD11c PE-Cy5, anti-HLA-DR PE CF594, anti-CD14 APC Cy7, anti-CD123 BV 421, anti-CD16 BV 785, anti-CD86 BV 650, anti-CD40 BV 711, anti-CD163 PE, anti-CD11b PE-Cy7, anti-CX3CR1 PerCP Cy5.5, anti-CCR2 Alexa Fluor 647 and Live/Dead™ fixable aqua dead cell stain (Sup. Table 1). Following staining, cells were washed twice with PBS-1 and fixed with 1 X CellFix™ (BD, 340,181). Cells were acquired on the BD Fortessa flow cytometer with BD FACSDiva software (v8.0.2). At least 200 000 events were collected. Flow cytometry data was analysed by hierarchal gating using FlowJo software and exported to Excel.

Flow cytometry gating

Representative gating is shown in Sup. Fig. 1. Fluorescence minus one (FMO) control tubes were used to define gates for select markers (Sup. Fig. 2). Total monocytes expression was classified as HLA-DR^+CD14^+. Based on the phenotypic dichotomous expression of surface markers CD14 and CD16 human monocyte subpopulations were classified into three major subsets: classical ($CD14^{++}CD16^-$, CM), intermediate ($CD14^{++}CD16^+$, IM] and non-classical ($CD14^+CD16^{++}$, NCM) [18]. A fourth monocyte subset was observed, described as transitional monocytes ($CD14^+CD16^-$, TM) [19, 20].

Total dendritic cells expression was classified as HLA-DR^+CD14^-. Circulating DCs were differentiated into cells of 2 lineages: myeloid ($CD123^-CD11c^+$, mDC) or plasmacytoid ($CD123^+CD11c^-$, pDC) [21]. Additionally, we observed and gated on an additional subset: $CD123^{dim}CD11c^{++}$ previously described as early percussors of mDCs [22, 23].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27, SAS version 9.4 and graphs were made using GraphPad Prism (V9.3.1).

D'Agostino-Pearson omnibus normality test was used to assess data distribution. To assess differences in frequencies of monocytes and dendritic cells among TB/HIV, TB, HIV and healthy donors, a one-way ANOVA with Tukey's post-test was performed on normally distributed markers, and non-parametric Kruskal Wallis test with Dunn's post-test was done on markers which were not normally distributed.

In the IMPRESS trial, a Cox proportional hazards model was used to determine the association between measured cell population frequencies at baseline and time to culture conversion (first of two consecutive negative TB culture results), measured in days. To determine the association between measured cell population frequencies at baseline with cavitary disease, a logistic regression model was used with presence of lung cavitation at baseline as the outcome. Multivariable analyses adjusted for a wide range of baseline clinical and demographic variables including randomization arm, age, sex, HIV status, lung cavitation, alcohol use, smoking and BMI. In addition, when analysing HIV positive individuals CD4 T cell counts, viral load and ART duration were adjusted for. Randomization arm was excluded in the multivariable lung cavitation analysis as this was not relevant for the studied timepoint. The *p*-values from cox proportional hazards and logistic regression model are reported and discussed as is [24]. The q-value resulting from the original FDR method of Benjamini-Hochberg (FDR, Q = 5%) is included in the supplemental tables.

Results

Cohort characteristics

Samples from 90 CAPRISA 011 (IMPRESS) study participants were included in the analysis (Table 1). The median age of the study participants was 34.5 [interquartile range (IQR) 29.0 – 41.5] and 76% were male. The median body mass index (BMI) was 19.4 (IQR 18.2 – 21.7). Of the 90 participants, 60 were HIV positive with a median CD4 cell count of 248 cells/mm^3 (IQR 150 – 446).

The median age of HIV positive participants from the CAPRISA 002 cohort was 33 (29 – 36). All participants were female with a median BMI of 32.7 (IQR 30.4 – 35.9) and a median CD4 cell count of 583 (IQR 361 – 717). Median age of healthy donors was 34 (32 – 40) and 45% were male.

TB and HIV related differences in monocyte and dendritic cell frequencies and phenotypes

To determine if frequency and phenotypes of the systemic monocytes and dendritic cells are modified by TB and TB/HIV co-infection we used flow cytometry to measure and characterize monocytes and dendritic cells ex vivo in PBMCs the four groups: TB/HIV, TB, HIV and heathy controls (HC), (Figs. 1, and 2, Sup. Table 2). The frequency of total monocytes (HLA-DR^+CD14^+) was significantly higher among participants with TB/HIV co-infection ($p = 0.018$) and TB ($p = 0.021$) compared to healthy controls (Fig. 1b). When characterizing different monocyte subsets, the frequency of non-classical monocytes in the HIV group was higher compared to both the TB/HIV ($p = 0.009$) and TB ($p = 0.005$) groups. Similarly, the frequency of intermediate monocytes was higher in the HIV group compared to TB/HIV group ($p = 0.042$).

Table 1 Demographic and clinical characteristics of the study participants

Variables	IMPRESS Total participants *n* = 90	IMPRESS TB/HIV positive *n* = 60	IMPRESS TB positive and HIV negative *n* = 30	CAPRISA 002 HIV positive *n* = 19	Healthy Donors *n* = 11
Randomization arm n (%)					
HRZE—Control	43 (48.0)	28 (47.0)	15 (50.0)	-	-
HRZM—Active	47 (52.0)	32 (53.0)	15 (50.0)	-	-
Age (y), median (IQR)	34.5 (29.0 – 41.5)	35.0 (31 – 40.7)	33.0 (24.0 – 50.5)	33.0 (29.0 – 36.0)	34.0 (32.0 – 40.0)
Gender, n (%)					
Male	68 (76.0)	42 (70.0)	26 (87.0)	-	5 (45.0)
Female	22 (24.0)	18 (30.0)	4 (13.0)	19 (100.0)	6 (55.0)
Body mass index (kg/m^2), mean (IQR)	20.6 (18.2 – 21.8)	20.5 (17.9 – 22.3)	20.9 (18.4 – 21.5)	32.9 (30.5 – 35.9)	24.7 (19.7 – 31.3)[a]
CD4 cell count (cells/mm^3), mean (IQR)	303.0 (150.0 – 446.0)	303.0 (150.0 – 446.0)	-	551.0 (361.0 – 717.0)	-
HIV viral load (copies/ml), mean (IQR)	94,907.0 (20.0 – 60,744.0)	94,907.0 (20.0 – 60,744.0)	-	56,399.0 (998.0 – 95,212.0)[c]	-
ART status n (%)					
Yes	33 (50.0)	30 (50.0)	-	11 (58.0)	-
No	27 (45.0)	27 (45.0)	-	8 (42.0)	-
Time on ART (months), median (IQR)	1.0 (0.0 – 36.0)	1.0 (0.0 – 36.0)	-	5 .0(0.0 – 46.0)	-
Lung Cavities n (%)					
None	25 (28.0)	21 (35.0)	4 (13.0)	-	-
One Lung	38 (42.0)	25 (42.0)	13 (43.0)	-	-
Both Lungs	27 (30.0)	14 (23.0)	13 (43.0)	-	-
Days to first negative solid culture, median (IQR)[b]	54.0 (28.0 – 79.8)	44.0 (28.0 – 79.8)	56.0 (42.0 – 82.3)	-	-
Alcohol Use in the past 3 months n (%)					
Yes	29 (32.0)	22 (37.0)	7 (23.0)	-	-
Smoking in past 3 months n (%)					
Yes	35 (39.0)	21 (35.0)	14 (47.0)	-	-

[a] 1 participant missing BMI

[b] Measures for all variables, except days to first negative culture are reported at baseline

[c] 7 missing viral load

The frequency of total dendritic cells (HLA-DR$^+$CD14$^-$) was higher in the TB/HIV ($p = 0.020$) and HIV ($p = 0.013$) groups compared to healthy controls (Fig. 1c). The frequency of mDCs was higher in the TB/HIV ($p = < 0.0001$) and TB ($p = 0.010$) groups compared to healthy controls, while the frequency of pDCs was lower in the TB/HIV ($p = 0.003$) and TB ($p = 0.023$) groups compared to healthy controls.

Given that the monocyte and dendritic cell function and phenotype are affected by TB and HIV infection [13, 25, 26] we further evaluated the expression of six phenotypic markers expressed by monocyte and dendritic cell subsets in the four study groups. We observed significant variation in cell surface marker expression across different subsets (Fig. 2, Sup. Table 2): monocytes (Fig. 2a) and DCs. We observed an increase in % CCR2 expression in the TB group compared to both the TB/HIV and HIV groups on classical and transitional monocytes and mDCs. The opposite trend was observed for CD123^{dim} CD11c^{++} cells with lower % CCR2 expression in the TB/HIV group than the other 3 groups. Frequency of CD11b was higher in the presence of active TB infection in both the TB/HIV and TB groups compared to healthy controls and the HIV group on non-classical and transitional monocytes and all three dendritic cell subsets. Frequency of CD40 on classical and transitional monocytes and CD123^{dim} CD11c^{++} cells was highest in the TB/HIV group, gradually decreasing in TB, HIV and healthy controls. Frequency of CD86 was significantly lower in the TB/HIV group compared to healthy controls on non-classical, transitional monocytes and mDCs. There was a decrease in % CX3CR1 on all monocyte and

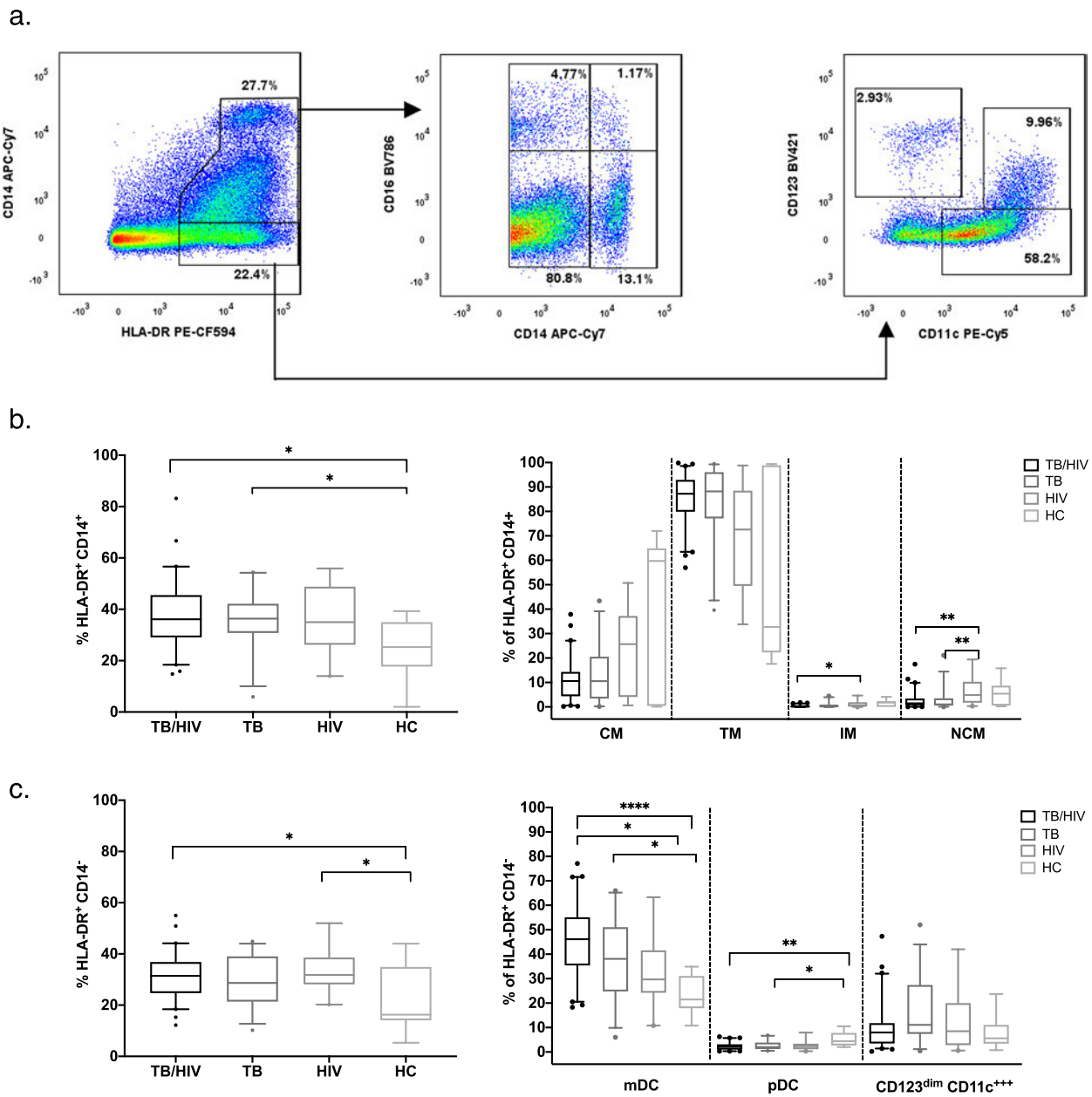

Fig. 1 **a** Representative gating of HLA-DR$^+$ CD14$^+$ (monocyte) and HLA-DR$^+$ CD14$^-$ (dendritic) cell populations. The staining profile of PBMCs from a representative TB/HIV co-infected participant is shown. **b** Differences in frequencies of total monocytes and monocyte subsets (classical, non-classical, intermediate, and transitional) between study groups (TB/HIV, TB, HIV and HC). **c** Differences in frequencies of dendritic cells and their subsets (mDC, pDC, and CD123^{dim} CD11c.$^{++}$) between study groups. **b** and **c** Boxes represent median and interquartile ranges; whiskers represent 5–95 percentiles. Differences in cell population frequencies among the groups were assessed using a Kruskal–Wallis test with Dunn's post-test for data that were not normally distributed and one-way ANOVA test with Tukey's post-test for normally-distributed data. *$p < 0.05$; ** $p < 0.01$ and ****$p < 0.0001$

DC subsets in the presence of HIV infection, with lowest expression in the TB/HIV and HIV groups compared to the TB group and healthy controls. HIV infection was associated with an increase in frequency of CD163 compared to the TB group in transitional monocytes, mDCs and CD123^{dim} CD11c^{++}.

Associations between monocyte and dendritic cell frequencies and phenotypes at active TB and time to culture conversion

We used a Cox proportional hazards model to assess the association between frequencies of monocyte and dendritic cell subsets and their phenotypes during active TB

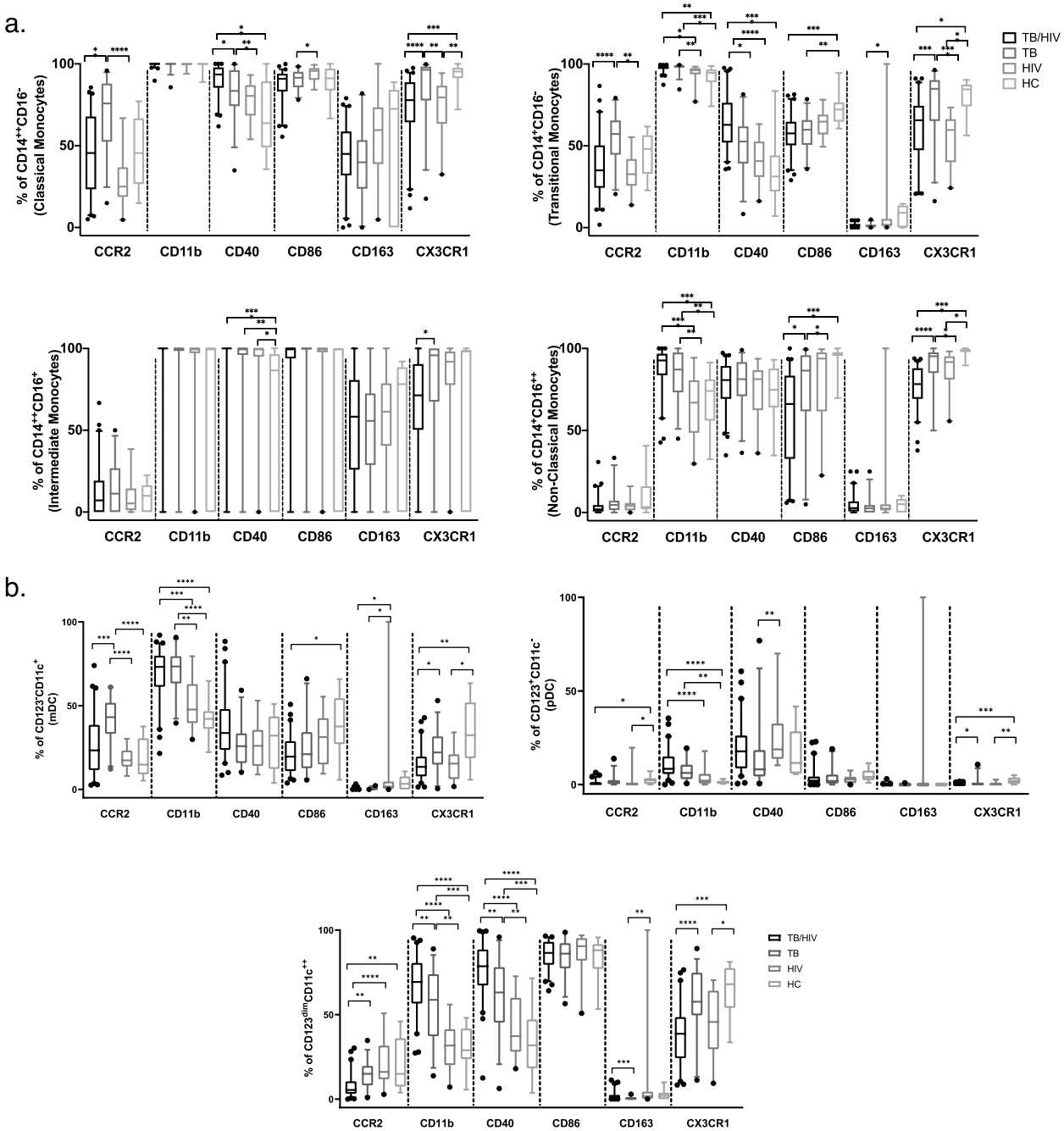

Fig. 2 Cell surface expression of CCR2, CD11b, CD40, CD86, CD163 and CXC3R1 on monocyte (**a**) and DC (**b**) cell subsets across study groups (TB/HIV, TB, HIV and HC). Boxes in **a** and **b** represent median and interquartile ranges; whiskers represent 5–95 percentiles. Differences among the groups were assessed using a Kruskal–Wallis test with Dunn's post-test for non-parametric data and one-way ANOVA with Tukey's post-test for parametric data. *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$

disease on time to negative culture conversion ($n = 90$, Table 2, Sup. Table 3).

In the bivariable model, increased % of CD11b expression on non-classical monocytes was associated with shorter time to culture conversion (aHR 1.02, 95%CI: 1.002–1.0437, $p = 0.019$). Increased % of CX3CR1 expression on non-classical monocytes was associated with the longer time to culture conversion in the multivariable model (aHR: 0.987, 95%CI: 0.963–0.997, $p = 0.019$).

Table 2 Significant associations between monocyte and dendritic cell phenotypes and their surface expression markers with time to culture conversion among the total cohort

	Bivariable			Multivariable		
	HR	**CI**	***p*-value**	**HR**	**CI**	***p*-value**
%CD11b on NCM	1.020	1.003—1.037	**0.019**	1.017	0.999 -1.035	0.059
%CX3CR1 on NCM	0.987	0.972—1.002	0.097	0.980	0.963—0.997	**0.019**

NCM Non-classical monocytes

Next, we performed a sub-analysis of TB/HIV co-infected individuals adjusting for the effects of HIV viral load, CD4 count and ART duration ($n=60$, Sup. Table 4). In the bivariable model, higher % CD163 (aHR 0.983, 95% CI: 0.967 – 0.999, $p=0.043$) expression on classical monocytes was significantly associated with longer time to culture conversion, however this was not significant in the multivariable model (aHR 0.985, 95%CI: 0.967 – 1.003, $p=0.106$). As seen in the overall cohort, increased expression of CX3CR1 (aHR 0.971, 95% CI: 0.949 – 0.994, $p=0.013$) on non-classical monocytes significantly associated with longer time to culture conversion in the multivariable model.

Associations between monocyte and dendritic cell frequencies and phenotypes at active TB and cavitary disease

Next, we assessed the effect of monocyte and dendritic cell frequencies and phenotypes at active TB on cavitary disease as defined by the presence of lung cavitation using logistic regression models ($n=90$, Table 3, Sup. Table 5).

In the univariable model among the total cohort, higher frequency of the following cell populations was associated with presence of lung cavitation: % classical monocytes ($p=0.037$), %CD86 on classical monocytes ($p=0.049$), %CX3CR1 on classical monocytes ($p=0.036$), % CXCR31 on non-classical monocytes ($p=0.022$), % intermediate monocytes ($p=0.048$) and %CD86 on intermediate monocytes ($p=0.028$). Following multivariable analysis, %CD86 on intermediate monocytes remained significantly associated with presence of lung cavitation (aOR 1.017, 95% CI: 1.001–1.032), with a trend observed for % classical monocytes (aOR 1.066, 95%CI 0.996 – 1.142, $p=0.066$).

Frequency of transitional monocytes was associated with absence of lung cavitation in the univariable ($p=0.033$) and multivariable analysis (aOR 0.944, 95% CI: 0.892–0.999, $p=0.047$). Increase in total dendritic cells frequency was associated with the absence of lung cavitation [univariable model (aOR 0.946, 95%CI 0.894 – 1.000, $p=0.048$); multivariable model (aOR 0.945, 95%CI 0.890 – 1.005, $p=0.071$)].

Among TB/HIV co-infected individuals (Sup. Table 6), increase in %CD11b on non-classical monocytes was significantly associated with cavitary disease in both univariable ($p=0.017$) and multivariable (aOR 1.072, 95% CI: 1.013–1.135, $p=0.017$) models. Increase in %CD86 on intermediate monocytes was significantly associated with cavitary disease in the univariable model ($p=0.030$), with a trend observed in the multivariable model (aOR 1.020, 95% CI: 0.999–1.040, $p=0.062$).

Table 3 Significant associations between monocyte and dendritic cell phenotypes and their surface expression markers with cavitary disease among the total cohort

	Univariable			Multivariable		
	OR	**CI**	***p*-value**	**OR**	**CI**	***p*-value**
% Classical Monocytes (CM)	1.069	1.004—1.138	**0.037**	1.066	0.996—1.142	0.066
%CD86 on CM	1.053	1.000—1.109	**0.049**	1.054	0.994—1.117	0.079
%CX3CR1 on CM	1.022	1.001—1.043	**0.036**	1.017	0.995—1.039	0.138
%CX3CR1 on NCM	1.039	1.006—1.073	**0.022**	1.031	0.993—1.070	0.107
% Intermediate Monocytes (IM)	4.612	1.011—21.041	**0.048**	4.693	0.912—24.156	0.064
% CD86 on IM	1.015	1.002—1.029	**0.028**	1.017	1.001—1.032	**0.033**
% Transitional Monocytes (TM)	0.946	0.899—0.995	**0.033**	0.944	0.892—0.999	**0.047**
% Dendritic cells (%HLA-DR$^+$ CD14$^-$)	0.946	0.894—1.000	**0.048**	0.945	0.890—1.005	0.071

Discussion

Both TB and HIV infections have profound effects on the host immune system. Here we described differences in monocyte and dendritic cell frequencies and phenotypes in TB and HIV infection/co-infection.

The overall increase in frequency of circulating monocytes in TB and TB/HIV coinfected patients is consistent with previous studies that demonstrated an increase in circulating monocytes in TB patients [9, 27]. We observed an increase in the frequency of non-classical and intermediate monocytes in the HIV group compared to TB and TB/HIV participant groups. Non-classical and intermediate monocytes are known to be expanded in HIV positive individuals irrespective of ART treatment [28–30]. These two monocyte subsets, characterized by high CD16 expression, are generally considered pro-inflammatory, and are known to release large amounts of inflammatory cytokines and have high expression of CX3CR1 [31, 32].

HIV infection was associated with increased frequency of total dendritic cells in both TB/HIV and HIV groups compared to HCs. TB/HIV and TB participant groups had increased frequency of circulating mDCs compared to healthy controls and this was observed previously in patients with pulmonary TB [33]. In contrast, we observed lower frequency of pDCs in TB/HIV and TB groups compared to healthy controls. This decrease in peripheral pDCs is likely observed due to migration of the pDCs to the site of infection, as influx of pDCs into the lungs has been described as one of the defining features of active pulmonary TB in the macaque model [34].

We observed significant heterogeneity in measured cell surface marker expression across different cell subsets in the four study groups. We observed an increase in frequency of CCR2 on classical and transitional monocytes and mDCs in the TB group compared to TB/HIV and HIV groups. CCR2 is a chemokine receptor that drives the recruitment of cells to the inflammatory sites. Increase in CCR2 was linked with the high level of immune activation and inflammation in HIV infection [35] as well as pathogenesis of several immune-mediated diseases [36, 37]. In Mtb infection, CCR2 expression was shown to play an important role in the control of infection through changes in the recruitment of monocyte, dendritic cells and T cells into the lung [38, 39].

Mtb infection also increased CD11b expression in both TB/HIV and TB groups compared to healthy controls and HIV positive participants on non-classical, transitional monocytes and dendritic cells subsets. Integrin CD11b, a receptor for soluble intercellular adhesion molecule-1 (ICAM-1), plays an important role in inflammation and macrophage polarization [40]. We have previously identified CD11b ligand, sICAM-1 plasma levels as one of the predictors of TB recurrence in TB/HIV co-infected individuals [5]. Additionally, increased frequency of CD11b on non-classical monocytes was associated with shorter time to culture conversion among the total TB and TB/HIV IMPRESS cohort as well as presence of lung cavitation in the TB/HIV co-infected group, suggesting a role in both pathogen clearance and associated immunopathogenesis.

An increase in CD40 expression on classical and transitional monocytes and $CD123^{dim}$ $CD11c^{++}$ dendritic cells was observed in TB/HIV co-infected participants, gradually decreasing in TB group, followed by HIV and HCs. CD40 is a costimulatory receptor expressed on a variety of cells including monocytes, DCs and B cells, and plays an important role in T cell and macrophage activation through interaction with its ligand CD40L [41]. Upregulation of CD40 on monocyte and dendritic cells has been reported in HIV infection and is likely a consequence of increasing levels of immune activation and pro-inflammatory cytokines [42]. Mtb infection was shown to impair the CD40-CD40L interaction resulting in suboptimal antigen-specific $CD4^{+}$ T cell immune response [43].

Expression of co-stimulatory receptor CD86 was downregulated in the TB/HIV group compared to healthy controls in transitional and non-classical monocytes as well as mDCs. Both TB and HIV infections can lead to downregulation of CD86 [11, 44, 45] which is likely to result in impaired antigen presentation and T cell activation. Additionally, we observed a significant association between increased expression of CD86 on classical and intermediate monocytes and presence of lung cavitation in the overall cohort implicating it in TB immunopathogenesis.

Presence of HIV infection resulted in decreased frequency of CX3CR1 in the HIV and TB/HIV groups compared to TB group and healthy controls on all monocyte and dendritic cell subsets. HIV Tat was previously shown to supress CX3CR1 expression at both mRNA and protein level with subsequent induction of proinflammatory cytokines [46]. Decreased expression of CX3CR1 on circulating monocytes was also identified as a feature of sepsis-induced immunosuppression [47], and this decrease in expression in highly inflammatory conditions could result in impairment of cell migration into the tissues and negatively impact pathogen clearance. This is supported by the observed association between CX3CR1 expression on non-classical monocytes and longer time to culture conversion and presence of lung cavitation.

HIV infection was associated with increased frequency of CD163 on transitional monocytes, mDCs and $CD123^{dim}$ $CD11c^{++}$ cells and increase in % CD163 on classical monocytes was associated with longer time to culture conversion in the HIV co-infected group.

CD163 is a scavenger receptor expressed on cells of monocyte and macrophages lineage [48, 49]. HIV infection was previously associated with increased frequency of CD163+monocytes and this increase was associated with decrease in $CD4^+$ T cells and increase in viral loads [50, 51]. Additionally, CD163 has been previously proposed as a marker to monitor disease progression and treatment efficacy in TB disease [52].

There are several limitations to our study, including a relatively small sample size especially in the heathy control group and subsequent inability to control for clinical and demographic variables that could contribute to the observed differences in innate immune cell phenotypes. Information on the presence of latent TB in the HIV cohort was not available. Additionally, cryopreservation can affect cell phenotype and function [53]. Here we are analyzing systemic cell populations that may differ significantly from cell populations observed in the lungs, at the site of Mtb replication. Nevertheless, our data contributes to a deeper understanding of the host immune changes during TB/HIV co-infection and its impact on TB disease pathogenesis. A better understanding of the HIV and TB mediated immune changes and their effects on disease outcome could lead to the discovery of diagnostic biomarkers and novel disease targets for host-directed therapies to reduce the burden of both diseases in vulnerable populations. Modulation of host immune pathways that impact disease pathology could limit Mtb replication and improve disease outcomes without concerns for antimicrobial resistance.

Abbreviations

CAPRISA	Centre for the AIDS Programme of Research in South Africa
IMPRESS	Improving Retreatment Success
TB	Tuberculosis
Mtb	*Mycobacterium tuberculosis*
HIV	Human Immunodeficiency Virus
DCs	Dendritic cells
mDCs	Myeloid dendritic cells
pDCs	Plasmacytoid cells
HC	Healthy controls
PBMCs	Peripheral blood mononuclear cells
CM	Classical monocytes
IM	Intermediate monocytes
NCM	Non-classical monocytes
TM	Transitional monocytes
ICAM	Intercellular adhesion molecule
ART	Antiretroviral therapy
OR	Odds ratio
aOR	Adjusted odds ratio
HR	Hazard ratio
aHR	Adjusted hazard ratio
BMI	Body mass index
IQR	Interquartile range
KZN	KwaZulu-Natal
SA	South Africa
PCZCDC	Prince Cyril Zulu Communicable Disease Centre
PLWH	People living with HIV
ACD	Acid citrate dextrose
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-023-00558-z.

Additional file 1: Supplementary Table 1. Monocyte and Dendritic cell Phenotyping Antibodies. **Supplementary Figure 1.** Representative parent gating. The staining profile of PBMC sample from a TB/HIV participant is shown. **Supplementary Figure 2.** Expression of CD11b, CD163, CD86, CD40, CCR2 and CXCR31 on classical monocytes (A) from a representative TB/HIV participant B) FMO controls. **Supplementary Table 2.** Cell population frequencies among different study groups. **Supplementary Table 3.** Association between monocyte and dendritic cell phenotypes and their surface expression markers with overall time to culture conversion among the total cohort. **Supplementary Table 4.** Association between monocyte and dendritic cell phenotypes and their surface expression markers with overall time to culture conversion among the HIV positive individuals. **Supplementary Table 5.** Association between monocyte and dendritic cell phenotypes and their surface expression markers with cavitary disease among the total cohort. **Supplementary Table 6.** Association between monocyte and dendritic cell phenotypes and their surface expression markers with cavitary disease among the HIV positive individuals.

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Authors' contributions
SR: conceptualized and designed the study, performed the experiments, analysed the data, wrote the manuscript. AS: conceptualized and designed the study, performed the experiments, analysed the data, wrote the manuscript, and supervised clinical and/or experimental aspects of the study. KN: conceptualized and designed the study and supervised clinical and/or experimental aspects of the study. TGM: performed the experiments. LL: analysed the data and reviewed and edited. DA, SN, LRM, NG, RHM, NP: supervised clinical and/or experimental aspects of the study, reviewed and edited. All authors have read and approved the final manuscript.

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Availability of data and materials
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participation
All methods were carried out in accordance with relevant guidelines and regulations. Written informed consent was obtained from all study participants prior to enrolment. CAPRISA 011 (29/01/2014, Clinicaltrials.gov, NCT02114684), was approved by Medicines Control Council of South Africa (MCC Ref: 20130510). University of KwaZulu-Natal (UKZN) Biomedical Research Ethics

Committee (BREC) reviewed and approved the original studies [BFC029/13 (CAPRISA 011), E013/04 (CAPRISA 002) and BE432/12 (Healthy donors)]. The nested study presented was reviewed and approved by University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC/00000014/2019).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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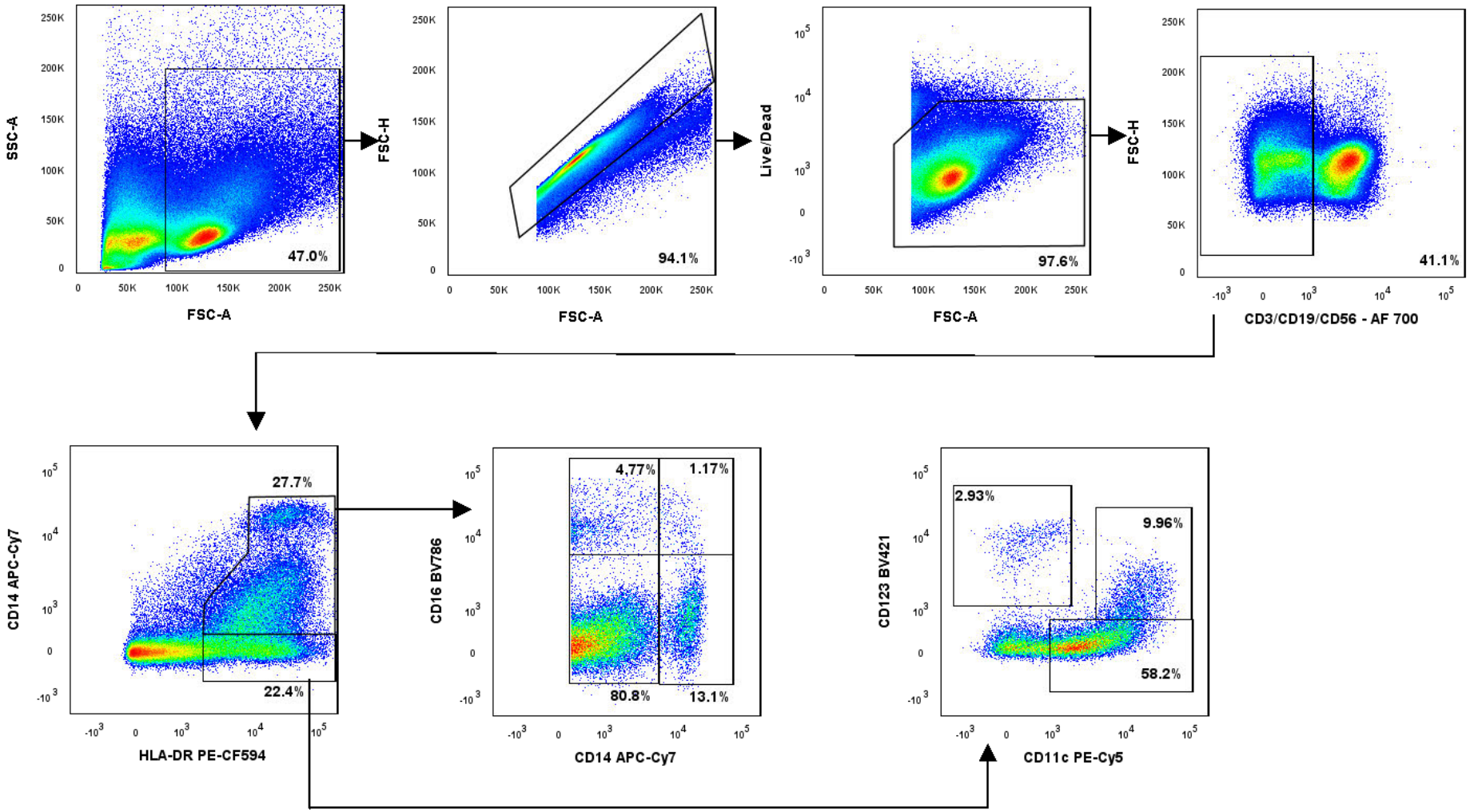
Publisher's Note

Supplementary material

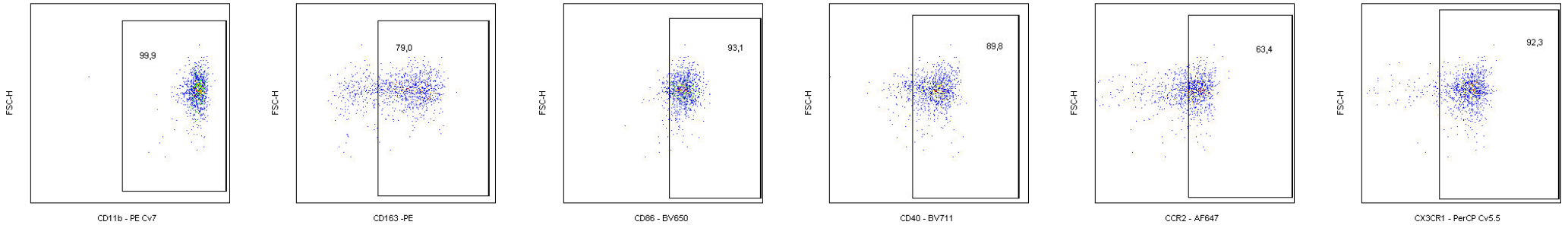
Supplementary Table 1. Monocyte and Dendritic cell Phenotyping Antibodies

Antibody	Fluorochrome	Clone	Cat #	Manufacturer	Volume (µl) per test (50µl)
CD11c	PECy5	3.9	301610	BioLegend	1
HLA-DR	PE CF594	G46-6	562304	BD	1
CD3	Alexa Fluor 700	SK7	344822	BioLegend	1
CD56	Alexa Fluor 700	5.1 H11	362522	BioLegend	2
CD19	Alexa Fluor 700	HIB1	302226	BioLegend	2
CD14	APC Cy7	HCD14	325620	BioLegend	1
CD123	BV421	6H6	48-1239-42	eBioscience	2
CD16	BV786	3G8	302046	BioLegend	1
CD86	BV650	IT2.2	305428	BioLegend	2.5
CD40	BV711	5C3	334334	BioLegend	2
CD163	PE	GHI/61	333606	BioLegend	1
CD11b	PECy7	ICRF44	301322	BioLegend	1
CX3CR1	PerCP Cy5.5	2A9-1	341614	BioLegend	2
CCR2 - CD192	Alex Fluor 647	48607	558406	BD	10
Live/Dead	AmCyan		L34957	ThermoFisher	3



Supplementary Figure 1. Representative parent gating. The staining profile of PBMC sample from a TB infected/HIV-infected participant is shown.

A.



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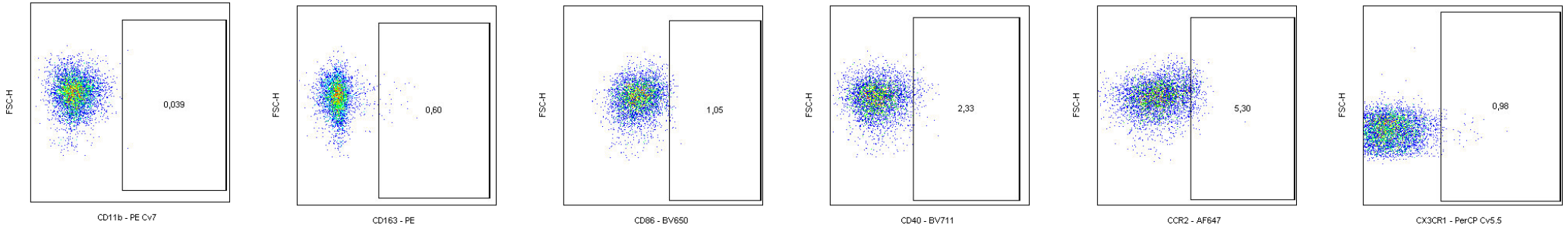

Supplementary Figure 2. Expression of CD11b, CD163, CD86, CD40, CCR2 and CXCR31 on classical monocytes (A) from a representative TB+/HIV+ participant B) FMO controls

Supplementary Table 2. Cell population frequencies among different study groups

	TB/HIV		TB		HIV		HC		p-value
	N	**Median (IQR)**	**N**	**Median (IQR)**	**N**	**Median (IQR)**	**N**	**Median (IQR)**	
% CD14 Positive (HLA-DR+ CD14+)	60	36.10 (29.08 – 45.53)	30	36.35 (30.78 – 42.18)	19	35.00 (26.20 – 48.80)	11	25.30 (17.70 – 35.00)	**0.0213**
% Classical Monocytes (CM)		10.60 (4.38 – 14.35)		10.55 (3.44 – 10.58)		25.70 (4.06 – 37.20)		59.70 (0.40 – 64.90)	**0.0447**
% CCR2		45.50 (23.40 – 57.80)		75.85 (52.38 – 87.98)		25.00 (18.70 – 36.80)		45.50 (26.60 – 66.70)	**<0.0001**
% CD11b		100.00 (99.90 – 100.00)		100.00 (99.90 – 100.00)		100.00 (99.60 – 100.00)		100.00 (99.80 – 100.00)	0.6012
% CD40		93.55 (85.40 – 97.83)		83.50 (74.23 – 96.15)		80.40 (68.40 – 87.00)		63.80 (49.00 – 89.40)	**<0.0001**
% CD86		90.90 (83.13 – 93.98)		91.50 (85.70 – 95.15)		96.70 (90.20 – 97.40)		91.20 (83.70 – 97.10)	**0.0285**
% CD163		44.95 (31.85 – 58.70)		39.85 (23.68 – 53.40)		59.50 (38.80 – 73.40)		72.50 (0 – 84.10)	0.0927
% CX3CR1		77.85 (64.30 – 88.75)		96.35 (77.60 – 98.33)		79.50 (64.30 – 86.70)		95.30 (91.30 – 97.60)	**<0.0001**
% Non - Classical monocytes (NCM)		1.45 (0.67 – 3.43)		1.05 (0.53 – 3.52)		4.85 (1.72 – 10.30)		5.42 (0.55 – 8.69)	**0.0019**
% CCR2		1.92 (0.65 – 4.51)		4.65 (1.52 – 7.21)		3.96 (1.37 – 5.74)		3.38 (2.10 – 16.00)	0.1062
% CD11b		92.70 (83.70 – 96.78)		87.15 (73.23 – 97.58)		67.00 (48.60 – 80.60)		74.10 (56.00 – 81.20)	**<0.0001**
% CD40		80.75 (69.30 – 89.38)		81.30 (70.95 – 91.70)		81.30 (62.40 – 86.80)		74.80 (64.00 – 87.80)	0.6320

% CD86		66.15 (32.70 – 83.43)			86.50 (61.75 – 95.90)			93.90 (61.70 – 97.60)			96.90 (95.50 – 97.70)	**<0.0001**
% CD163		2.68 (0.66 – 6.76)			2.55 (0.00 – 4.59)			2.37 (1.36 – 5.00)			5.07 (0.00 – 8.25)	0.7701
% CX3CR1		78.30 (69.30 – 87.93)			95.25 (85.03 – 97.63)			91.70 (81.10 – 95.20)			99.00 (97.80 – 99.60)	**<0.0001**
% Intermediate monocytes (IM)		0.08 (0.03 – 0.53)			0.19 (0.02 – 0.82)			0.51 (0.18 – 1.94)			1.78 (0.00 – 2.36)	**0.0216**
% CCR2		7.14 (0.00 – 19.18)			11.25 (0.00 – 26.80)			5.26 (1.120 – 14.40)			9.93 (0.00 – 16.40)	0.6318
% CD11b		100.00 (100.00 – 100.00)			100.00 (98.48 – 100.00)			100.00 (97.10 – 100.00)			100.00 (0.00 – 100.00)	**0.0282**
% CD40		100.00 (100.00 – 100.00)			100.00 (95.98 – 100.00)			99.80 (95.00 – 100.00)			86.60 (0.00 – 96.50)	**<0.0001**
% CD86		100.00 (93.90 – 100.00)			100.00 (99.75 – 100.00)			100.00 (97.70 – 100.00)			99.60 (0.00 – 100.00)	0.4167
% CD163		58.30 (25.90 – 80.68)			55.70 (28.75 – 72.53)			61.30 (40.50 – 78.70)			78.20 (0 – 88.50)	0.7277
% CX3CR1		71.35 (50.25 – 90.43)			95.85 (67.45 – 100)			91.90 (77.70 – 98.10)			98.20 (0 – 99.00)	**0.0087**
%Transitional monocytes (TM)		87.25 (79.85 – 92.90)			88.20 (77.13 – 96.08)			72.60 (49.50 – 88.50)			32.70 (22.30 – 98.90)	**0.0253**
% CCR2		35.05 (24.33 – 50.30)			57.25 (44.58 – 65.50)			32.70 (25.60 – 41.90)			48.10 (32.90 – 56.50)	**<0.0001**
% CD11b		98.15 (97.00 – 98.70)			98.55 (97.90 – 99.03)			96.20 (93.70 – 97.50)			94.60 (88.90 – 96.60)	**<0.0001**
% CD40		62.80 (52.03 – 76.35)			52.60 (39.20 – 61.95)			40.70 (31.00 – 52.60)			31.30 (22.10 – 44.10)	**<0.0001**
% CD86		57.60 (50.35 – 64.75)			59.85 (50.60 – 65.73)			64.80 (57.00 – 69.10)			72.20 (64.80 – 76.50)	**0.0002**

% CD163		1.50 (0.82 – 2.18)			1.01 (0.33 – 1.68)			1.70 (0.95 – 5.31)			9.08 (0.13 – 13.50)	**0.0134**
% CX3CR1		65.60 (47.38 – 74.48)			84.85 (65.20 – 90.15)			59.70 (40.00 – 66.30)			84.50 (74.10 – 87.40)	**<0.0001**
% CD14 Negative (HLA-DR+ CD14-)	60	31.45 (24.70 – 36.83)			28.65 (21.38 – 39.05)			31.80 (28.10 – 38.60)			16.30 (14.10 – 34.90)	**0.0147**
% mDC		46.15 (35.40 – 55.08)			38.10 (24.78 – 51.05)			29.70 (24.20 – 41.60)			21.50 (17.90 – 31.10)	**<0.0001**
% CCR2		23.30 (11.55 – 38.50)			43.15 (33.25 – 51.70)			17.30 (13.70 – 23.30)			14.80 (9.09 – 30.50)	**<0.0001**
% CD11b		73.20 (61.33 – 79.78)			73.40 (63.35 – 79.53)			47.70 (39.70 – 62.90)			42.10 (36.20 – 46.70)	**<0.0001**
% CD40		33.70 (23.65 – 47.93)			25.80 (15.80 – 33.40)			26.00 (14.10 – 35.50)			32.10 (12.30 – 43.50)	**0.0244**
% CD86		19.55 (10.98 – 28.75)			21.05 (12.75 – 34.20)			31.20 (15.00 – 42.50)			37.60 (27.10 – 54.40)	**0.0033**
% CD163		0.37 (0.16 – 0.67)			0.24 (0.13 – 0.92)			1.67 (0.42 – 4.67)			3.31 (0.03 – 7.69)	**0.0064**
% CX3CR1		13.45 (7.88 – 19.55)			22.10 (14.85 – 31.60)			15.40 (6.22– 20.90)			32.40 (18.70 – 51.90)	**0.0003**
% $CD123^{dim}CD11c^{++}$		8.00 (3.48 – 11.83)			11.10 (7.41 – 27.43)			8.50 (2.80 – 20.00)			5.58 (3.38 – 11.10)	0.0865
% CCR2		5.39 (3.230 – 10.48)			15.00 (8.21 – 19.60)			16.10 (11.80 – 31.60)			14.90 (7.48 – 36.00)	**<0.0001**
% CD11b		69.30 (56.60 – 80.55)			58.75 (37.28 – 73.88)			31.70 (20.20 – 41.30)			28.90 (23.70 – 41.70)	**<0.0001**
% CD40		78.65 (67.33 – 88.50)			63.10 (45.35 – 78.05)			37.30 (28.10 – 59.90)			31.80 (18.30 – 47.10)	**<0.0001**
% CD86		86.50 (79.53 – 93.00)			86.10 (77.48 – 92.25)			90.50 (82.00 – 95.20)			88.10 (77.10 – 91.70)	0.6115

% CD163		1.10 (0.43 – 2.46)			0.24 (0.05 – 0.81)			1.75 (0.28 – 4.37)			1.92 (0.00 – 3.74)	**0.0002**
% CX3CR1		38.70 (24.28 – 48.50)			57.60 (49.58 – 74.78)			45.70 (29.60 – 64.10)			68.00 (54.00 – 77.50)	**<0.0001**
% pDC	60	2.13 (0.97 – 3.14)			2.01 (1.21 – 3.86)			2.40 (1.12 – 3.40)			4.39 (2.69 – 7.80)	**0.0067**
% CCR2		0.36 (0.00 – 1.21)			1.05 (0.31 – 2.35)			0.25 (0.00 – 0.88)			2.37 (0.36 – 3.35)	**0.0024**
% CD11b		8.40 (5.66 – 15.00)			6.21 (2.96 – 10.58)			2.05 (0.57 – 5.73)			1.12 (0.47 – 1.94)	**<0.0001**
% CD40		17.75 (8.65 – 26.18)			8.10 (4.39 – 18.43)			18.80 (13.70 – 32.60)			11.50 (5.91 – 28.40)	**0.0117**
% CD86		2.07 (0.96 – 4.23)			1.86 (0.55 – 5.29)			2.75 (0.78 – 4.13)			4.04 (2.38 – 7.43)	0.2319
% CD163		0.00 (0.00-0.00)			0.00 (0.00-0.00)			0.00 (0.00-0.00)			0.00 (0.00-0.00)	0.6551
% CX3CR1		0.00 (0.00 – 0.29)			0.30 (0.00 – 0.92)			0.00 (0.00 – 0.78)			1.90 (0.45 – 3.77)	**<0.0001**

Supplementary Table 3. Association between monocyte and dendritic cell phenotypes and their surface expression markers with overall time to culture conversion among the total cohort.

	Bivariable				Multivariable			
	HR	**CI**	**p-value**		**HR**	**CI**	**p-value**	
% CD14 Positive (HLA-DR+ CD14+)	0.992	0.974 - 1.010	0.359		0.981	0.961 - 1.002	0.078	0.898
% Classical Monocytes (CM)	0.989	0.966 - 1.013	0.376		1.002	0.977 - 1.027	0.903	0.968
% CCR2	0.994	0.986 - 1.003	0.184		0.996	0.987 - 1.006	0.441	0.898
% CD11b	0.951	0.850 - 1.063	0.377		0.965	0.860 - 1.082	0.537	0.898
% CD40	0.999	0.980 - 1.018	0.891		1.001	0.978 - 1.024	0.958	0.968
% CD86	0.989	0.964 - 1.013	0.361		1.013	0.984 - 1.043	0.396	0.898
% CD163	0.995	0.984 - 1.006	0.358		0.995	0.983 - 1.007	0.416	0.898
% CX3CR1	0.996	0.987 - 1.005	0.400		0.998	0.987 - 1.009	0.717	0.914
% Non-Classical Monocytes (NCM)	0.976	0.916 - 1.040	0.460		0.986	0.928 - 1.047	0.646	0.914
% CCR2	0.993	0.964 - 1.023	0.643		0.988	0.952 - 1.025	0.518	0.898
% CD11b	1.020	1.003 - 1.037	**0.019**		1.017	0.999 - 1.035	0.059	0.898
% CD40	1.000	0.985 - 1.016	0.970		1.007	0.989 - 1.026	0.427	0.898
% CD86	0.999	0.992 - 1.006	0.755		1.001	0.994 - 1.009	0.712	0.914
% CD163	1.014	0.975 - 1.054	0.491		1.032	0.990 - 1.077	0.141	0.898
% CX3CR1	0.987	0.972 - 1.002	0.097		0.980	0.963 - 0.997	**0.019**	0.898
% Intermediate Monocytes (IM)	0.881	0.662 - 1.171	0.382		0.897	0.678 - 1.188	0.449	0.898
% CCR2	1.006	0.993 - 1.013	0.361		1.008	0.994 - 1.023	0.240	0.898
% CD11b	1.001	0.994 - 1.008	0.789		1.003	0.995 - 1.011	0.448	0.898
% CD40	1.001	0.994 - 1.009	0.723		1.004	0.995 - 1.012	0.374	0.898
% CD86	1.001	0.995 - 1.008	0.729		1.003	0.996 - 1.010	0.406	0.898
% CD163	0.997	0.990 - 1.003	0.313		0.998	0.991 - 1.005	0.581	0.898
% CX3CR1	1.002	0.996 - 1.009	0.512		1.004	0.997 - 1.001	0.229	0.898
% Transitional Monocytes (TM)	1.009	0.991 - 1.027	0.348		1.001	0.983 - 1.020	0.902	0.968
% CCR2	0.993	0.981 - 1.004	0.218		0.99	0.977 - 1.004	0.158	0.898
% CD11b	0.955	0.867 - 1.052	0.353		0.943	0.856 - 1.040	0.241	0.898
% CD40	1.000	0.987 - 1.013	0.977		1.003	0.988 - 1.017	0.716	0.914
% CD86	1.004	0.984 - 1.025	0.668		1.014	0.994 - 1.035	0.169	0.898
% CD163	0.958	0.787 - 1.166	0.669		1.079	0.872 - 1.334	0.485	0.898
% CX3CR1	0.995	0.984 - 1.006	0.392		0.994	0.981 - 1.007	0.371	0.898
% CD14 Negative (HLA-DR+ CD14-)	1.009	0.984 - 1.036	0.483		1.004	0.978 - 1.030	0.787	0.968
% mDC	1.004	0.991 - 1.017	0.565		1.001	0.985 - 1.017	0.908	0.968
% CCR2	0.994	0.982 - 1.006	0.300		0.987	0.973 - 1.002	0.091	0.898
% CD11b	1.001	0.987 - 1.015	0.894		0.991	0.975 - 1.008	0.314	0.898

% CD40	1.000	0.987 - 1.012	0.945		1.000	0.986 - 1.015	0.968	0.968
% CD86	0.997	0.981 - 1.013	0.691		1.001	0.983 - 1.020	0.905	0.968
% CD163	1.078	0.683 - 1.703	0.746		1.089	0.715 - 1.659	0.692	0.914
% CX3CR1	0.999	0.976 - 1.023	0.932		1.007	0.981 - 1.033	0.609	0.914
% CD123^{dim} CD11c^{++}	0.997	0.978 - 1.015	0.715		0.994	0.972 - 1.016	0.581	0.898
% CCR2	1.000	0.972 - 1.028	0.992		0.989	0.957 - 1.022	0.510	0.898
% CD11b	1.003	0.992 - 1.014	0.569		1.000	0.987 - 1.012	0.940	0.968
% CD40	1.000	0.989 - 1.011	0.931		0.999	0.986 - 1.013	0.934	0.968
% CD86	1.000	0.977 - 1.023	0.991		1.009	0.982 - 1.037	0.498	0.898
% CD163	1.068	0.968 - 1.177	0.188		1.038	0.936 - 1.152	0.477	0.898
% CX3CR1	0.995	0.983 - 1.006	0.358		0.994	0.980 - 1.008	0.430	0.898
% pDC	1.064	0.939 - 1.206	0.328		1.090	0.947 - 1.256	0.231	0.898
% CCR2	0.967	0.890 - 1.051	0.432		0.972	0.881 - 1.072	0.568	0.898
% CD11b	1.012	0.983 - 1.041	0.421		1.012	0.980 - 1.045	0.476	0.898
% CD40	1.000	0.986 - 1.015	0.967		0.997	0.982 - 1.013	0.713	0.914
% CD86	0.992	0.951 - 1.034	0.692		1.002	0.960 - 1.047	0.915	0.968
% CD163	1.247	0.484 - 3.215	0.648		1.716	0.744 - 3.960	0.206	0.898
% CX3CR1	0.972	0.837 - 1.129	0.713		0.992	0.845 - 1.164	0.918	0.968

Supplementary Table 4. Association between monocyte and dendritic cell phenotypes and their surface expression markers with overall time to culture conversion among the HIV-positive individuals.

	Bivariable				Multivariable			
	HR	**CI**	**p-value**		**HR**	**CI**	**p-value**	
% CD14 Positive (HLA-DR+ CD14+)	0.992	0.973 - 1.012	0.433		0.998	0.960 – 1.018	0.435	0.967
% Classical Monocytes (CM)	0.986	0.952 - 1.021	0.420		1.000	0.961 – 1.041	0.995	0.995
% CCR2	0.997	0.986 - 1.009	0.636		0.998	0.985 - 1.010	0.709	0.967
% CD11b	0.962	0.791 - 1.170	0.699		0.970	0.800 - 1.176	0.756	0.967
% CD40	0.995	0.965 - 1.025	0.733		1.018	0.981 - 1.056	0.349	0.967
% CD86	0.993	0.966 - 1.020	0.602		1.014	0.975 - 1.053	0.493	0.967
% CD163	0.983	0.967 - 0.999	**0.043**		0.985	0.967 - 1.003	0.106	0.967
% CX3CR1	1.002	0.990 - 1.014	0.729		0.997	0.982 - 1.012	0.647	0.967
% Non-Classical Monocytes (NCM)	0.974	0.891 - 1.064	0.557		0.948	0.868 - 1.036	0.239	0.967
% CCR2	1.013	0.962 - 1.066	0.636		0.991	0.928 - 1.059	0.796	0.967
% CD11b	1.022	0.999 - 1.045	0.059		1.016	0.992 - 1.040	0.196	0.967
% CD40	1.000	0.981 - 1.020	0.966		0.999	0.978 - 1.022	0.960	0.992
% CD86	0.999	0.991 - 1.008	0.881		0.996	0.986 - 1.006	0.409	0.967
% CD163	1.003	0.957 - 1.052	0.891		1.006	0.945 - 1.071	0.851	0.967
% CX3CR1	0.981	0.960 - 1.003	0.084		0.971	0.949 - 0.994	**0.013**	0.663
% Intermediate Monocytes (IM)	0.835	0.452 - 1.540	0.563		0.687	0.352 - 1.343	0.271	0.967
% CCR2	1.003	0.988 - 1.019	0.689		0.999	0.979 - 1.018	0.891	0.967
% CD11b	0.992	0.981 - 1.003	0.147		0.997	0.985 - 1.010	0.682	0.967
% CD40	0.992	0.981 - 1.003	0.147		0.998	0.985 - 1.011	0.781	0.967
% CD86	0.997	0.989 - 1.006	0.530		0.998	0.989 - 1.008	0.707	0.967
% CD163	0.992	0.984 - 1.001	0.066		0.993	0.983 - 1.003	0.179	0.967
% CX3CR1	1.002	0.993 - 1.010	0.728		1.000	0.990 - 1.009	0.939	0.992
% Transitional Monocytes (TM)	1.013	0.984 - 1.041	0.386		1.008	0.977 - 1.039	0.620	0.967
% CCR2	0.996	0.981 - 1.011	0.574		0.994	0.977 - 1.012	0.539	0.967
% CD11b	0.964	0.841 - 1.105	0.598		0.941	0.806 - 1.099	0.444	0.967
% CD40	0.998	0.980 - 1.016	0.795		1.014	0.993 - 1.034	0.185	0.967
% CD86	1.008	0.984 - 1.033	0.502		1.025	0.994 - 1.058	0.120	0.967
% CD163	0.899	0.694 - 1.165	0.421		0.973	0.731 - 1.296	0.852	0.967
% CX3CR1	1.004	0.987 - 1.021	0.636		0.996	0.976 - 1.016	0.671	0.967
% CD14 Negative (HLA-DR+ CD14-)	1.010	0.976 - 1.046	0.565		0.997	0.960 - 1.035	0.876	0.967
% mDC	0.994	0.977 - 1.012	0.510		0.987	0.965 - 1.010	0.274	0.967
% CCR2	0.997	0.982 - 1.011	0.655		0.993	0.974 - 1.013	0.493	0.967
% CD11b	1.002	0.986 - 1.020	0.777		1.002	0.977 - 1.028	0.871	0.967

% CD40	0.995	0.980 - 1.011	0.562		1.002	0.984 - 1.021	0.808	0.967
% CD86	0.984	0.962 - 1.006	0.144		0.977	0.950 - 1.005	0.111	0.967
% CD163	0.972	0.553 - 1.710	0.922		0.874	0.503 - 1.519	0.633	0.967
% CX3CR1	0.998	0.964 - 1.033	0.914		0.986	0.950 - 1.024	0.468	0.967
% CD123^{dim} CD11c^{++}	1.014	0.983 - 1.046	0.391		1.019	0.982 - 1.059	0.321	0.967
% CCR2	0.989	0.952 - 1.027	0.552		0.9653	0.917 - 1.011	0.130	0.967
% CD11b	1.003	0.988 - 1.019	0.656		1.000	0.981 - 1.020	0.973	0.992
% CD40	0.994	0.978 - 1.010	0.465		1.004	0.982 - 1.026	0.734	0.967
% CD86	0.996	0.966 - 1.026	0.780		0.992	0.953 - 1.032	0.685	0.967
% CD163	1.054	0.946 - 1.175	0.340		1.018	0.904 - 1.146	0.773	0.967
% CX3CR1	0.999	0.983 - 1.016	0.939		0.988	0.970 - 1.006	0.200	0.967
% pDC	1.045	0.883 - 1.237	0.609		1.080	0.870 - 1.339	0.486	0.967
% CCR2	1.127	0.924 - 1.374	0.237		1.168	0.892 - 1.529	0.259	0.967
% CD11b	1.020	0.988 - 1.053	0.226		1.020	0.978 - 1.064	0.357	0.967
% CD40	1.004	0.984 - 1.024	0.710		0.998	0.976 - 1.021	0.881	0.967
% CD86	1.002	0.953 - 1.054	0.935		1.019	0.956 - 1.085	0.569	0.967
% CD163	0.977	0.298 - 3.199	0.969		1.493	0.579 - 3.850	0.407	0.967
% CX3CR1	0.718	0.344 - 1.500	0.378		0.401	0.153 - 1.048	0.062	0.967

Supplementary Table 5. Association between monocyte and dendritic cell phenotypes and their surface expression markers with cavitary disease among the total cohort.

	Univariable				Multivariable			
	OR	**CI**	**p-value**		**OR**	**CI**	**p-value**	**q-value**
% CD14 Positive (HLA-DR+ CD14+)	1.015	0.976 - 1.055	0.453		1.013	0.968 - 1.060	0.567	0.741
% Classical Monocytes (CM)	1.069	1.004 - 1.138	**0.037**		1.066	0.996 - 1.142	0.066	0.574
% CCR2	1.017	0.999 - 1.036	0.066		1.009	0.989 - 1.029	0.400	0.660
% CD11b	1.059	0.846 - 1.327	0.615		1.066	0.828 - 1.372	0.619	0.753
% CD40	0.973	0.930 - 1.019	0.246		0.985	0.936 - 1.037	0.565	0.741
% CD86	1.053	1.000 - 1.109	**0.049**		1.054	0.994 - 1.117	0.079	0.574
% CD163	1.009	0.986 - 1.033	0.456		1.012	0.989 - 1.038	0.385	0.660
% CX3CR1	1.022	1.001 - 1.043	**0.036**		1.017	0.995 - 1.039	0.138	0.574
% Non-Classical Monocytes (NCM)	1.176	0.948 - 1.457	0.140		1.196	0.955 - 1.497	0.118	0.574
% CCR2	0.988	0.921 - 1.061	0.745		0.964	0.891 - 1.043	0.368	0.660
% CD11b	1.019	0.988- 1.052	0.231		1.028	0.992 - 1.065	0.128	0.574
% CD40	1.021	0.989 - 1.054	0.197		1.027	0.989 - 1.066	0.171	0.574
% CD86	1.009	0.993 - 1.024	0.285		1.003	0.986 - 1.020	0.705	0.782
% CD163	1.016	0.935 - 1.103	0.715		1.013	0.927 - 1.106	0.782	0.814
% CX3CR1	1.039	1.006 - 1.073	**0.022**		1.031	0.993 - 1.070	0.107	0.574
% Intermediate Monocytes (IM)	4.612	1.011 - 21.041	**0.048**		4.693	0.912 - 24.156	0.064	0.574
% CCR2	1.010	0.978 - 1.043	0.552		1.001	0.967 - 1.036	0.949	0.952
% CD11b	1.010	0.995 - 1.025	0.197		1.013	0.995 - 1.030	0.160	0.574
% CD40	1.011	0.996 - 1.026	0.157		1.014	0.996 - 1.032	0.121	0.574
% CD86	1.015	1.002 - 1.029	**0.028**		1.017	1.001 - 1.032	**0.033**	0.574
% CD163	1.008	0.994 - 1.023	0.270		1.009	0.993 - 1.024	0.276	0.604
% CX3CR1	1.011	0.998 - 1.026	0.106		1.009	0.993 - 1.024	0.272	0.604
% Transitional Monocytes (TM)	0.946	0.899 - 0.995	**0.033**		0.944	0.892 - 0.999	**0.047**	0.574
% CCR2	1.025	0.999 - 1.052	0.059		1.014	0.986 - 1.043	0.332	0.650
% CD11b	1.078	0.893 - 1.302	0.434		1.058	0.855 - 1.309	0.607	0.753
% CD40	0.979	0.952 - 1.006	0.130		0.987	0.957 - 1.018	0.411	0.660
% CD86	1.009	0.969 - 1.051	0.667		1.008	0.964 - 1.053	0.729	0.791
% CD163	1.253	0.817 - 1.922	0.301		1.353	0.840 - 2.180	0.213	0.574
% CX3CR1	1.019	0.997 - 1.043	0.096		1.013	0.988 - 1.039	0.309	0.630
% CD14 Negative (HLA-DR+ CD14-)	0.946	0.894 - 1.000	**0.048**		0.945	0.890 - 1.005	0.071	0.574
% mDC	0.999	0.969 - 1.030	0.955		1.007	0.974 - 1.042	0.668	0.782
% CCR2	1.026	0.998 - 1.056	0.073		1.015	0.984 - 1.048	0.344	0.650
% CD11b	1.025	0.994 - 1.057	0.118		1.024	0.989 - 1.059	0.179	0.574

% CD40	0.976	0.950 - 1.002	0.070		0.983	0.954 - 1.012	0.251	0.604
% CD86	0.986	0.952 - 1.022	0.446		0.983	0.942 - 1.026	0.427	0.660
% CD163	0.845	0.371 - 1.923	0.688		0.800	0.332 - 1.930	0.620	0.753
% CX3CR1	1.038	0.990 - 1.088	0.125		1.028	0.977 - 1.082	0.284	0.604
% CD123^{dim} CD11c^{++}	1.054	0.995 - 1.118	0.074		1.048	0.984 - 1.115	0.145	0.574
% CCR2	1.006	0.946 - 1.070	0.842		0.974	0.907 - 1.045	0.460	0.688
% CD11b	0.987	0.963 - 1.012	0.318		0.994	0.966 - 1.022	0.675	0.782
% CD40	0.990	0.965 - 1.015	0.433		0.999	0.971 - 1.028	0.952	0.952
% CD86	1.003	0.954 - 1.055	0.901		1.022	0.964 - 1.083	0.472	0.688
% CD163	0.822	0.670 - 1.009	0.061		0.869	0.697 - 1.083	0.211	0.574
% CX3CR1	1.019	0.995 - 1.044	0.126		1.012	0.984 - 1.040	0.417	0.660
% pDC	1.139	0.846 - 1.535	0.391		1.067	0.771 - 1.478	0.694	0.782
% CCR2	1.136	0.837 - 1.541	0.414		1.126	0.790 - 1.604	0.513	0.727
% CD11b	0.990	0.928 - 1.056	0.754		1.021	0.951 - 1.095	0.565	0.741
% CD40	1.008	0.975 - 1.042	0.639		1.027	0.985 - 1.070	0.214	0.574
% CD86	1.084	0.955 - 1.230	0.211		1.092	0.956 - 1.247	0.194	0.574
% CD163	0.421	0.087 - 2.046	0.284		0.353	0.057 - 2.167	0.261	0.604
% CX3CR1	1.029	0.743 - 1.424	0.864		0.948	0.659 - 1.364	0.773	0.814

Supplementary Table 6. Association between monocyte and dendritic cell phenotypes and their surface expression markers with cavitary disease among the HIV-positive individuals.

	Univariable				Multivariable			
	OR	**CI**	**p-value**		**OR**	**CI**	**p-value**	**q-value**
% CD14 Positive (HLA-DR+ CD14+)	1.019	0.976 - 1.065	0.395		1.016	0.959 – 1.077	0.588	0.780
% Classical Monocytes (CM)	1.051	0.977 - 1.131	0.182		1.054	0.971 – 1.144	0.211	0.644
% CCR2	1.016	0.994 - 1.039	0.158		1.016	0.990 – 1.042	0.237	0.644
% CD11b	1.344	0.762 - 2.373	0.307		1.322	0.677 – 2.579	0.413	0.739
% CD40	1.006	0.948 - 1.068	0.836		1.008	0.945 – 1.076	0.808	0.877
% CD86	1.047	0.991 - 1.107	0.099		1.052	0.985 – 1.123	0.131	0.644
% CD163	1.024	0.993 - 1.056	0.129		1.023	0.987 – 1.061	0.209	0.644
% CX3CR1	1.015	0.991 - 1.041	0.225		1.015	0.987 – 1.043	0.302	0.644
% Non-Classical Monocytes (NCM)	1.134	0.911 - 1.413	0.261		1.181	0.921 – 1.514	0.190	0.644
% CCR2	0.993	0.901 - 1.094	0.889		1.002	0.897 – 1.119	0.971	0.978
% CD11b	1.056	1.010 - 1.104	**0.017**		1.073	1.013 – 1.135	**0.016**	0.644
% CD40	1.023	0.984 - 1.063	0.254		1.023	0.980 – 1.067	0.303	0.644
% CD86	1.003	0.985 - 1.022	0.738		1.003	0.983 – 1.024	0.778	0.864
% CD163	1.09	0.971 - 1.225	0.144		1.127	0.985 – 1.290	0.081	0.644
% CX3CR1	1.024	0.982 - 1.068	0.265		1.019	0.971 – 1.070	0.434	0.739
% Intermediate Monocytes (IM)	3.506	0.729 - 16.868	0.118		5.389	0.810 – 35.829	0.081	0.644
% CCR2	1.010	0.973 - 1.041	0.594		1.010	0.968 – 1.053	0.649	0.780
% CD11b	1.018	0.995 - 1.041	0.133		1.013	0.989 – 1.038	0.297	0.644
% CD40	1.020	0.996 - 1.044	0.110		1.015	0.989 – 1.041	0.252	0.644
% CD86	1.021	1.002 - 1.040	**0.030**		1.020	0.999 – 1.041	0.058	0.644
% CD163	1.013	0.996 - 1.030	0.135		1.012	0.994 – 1.032	0.194	0.644
% CX3CR1	1.011	0.993 - 1.029	0.226		1.011	0.992 – 1.030	0.270	0.644
% Transitional Monocytes (TM)	0.954	0.898 - 1.014	0.132		0.947	0.883 – 1.016	0.131	0.644
% CCR2	1.015	0.985 - 1.046	0.330		1.015	0.977 – 1.054	0.444	0.739
% CD11b	1.165	0.895 - 1.516	0.257		1.202	0.857 – 1.684	0.286	0.644
% CD40	0.999	0.965 - 1.034	0.947		1.003	0.963 – 1.043	0.900	0.937
% CD86	1.014	0.967 - 1.062	0.572		1.029	0.970 – 1.092	0.336	0.661
% CD163	1.400	0.834 - 2.349	0.203		1.490	0.841 – 2.640	0.171	0.644
% CX3CR1	1.011	0.983 - 1.040	0.451		1.010	0.976 – 1.045	0.572	0.780
% CD14 Negative (HLA-DR+ CD14-)	0.942	0.880 - 1.008	0.086		0.942	0.873 – 1.015	0.117	0.644
mDC	1.010	0.974 - 1.048	0.578		1.027	0.979 – 1.077	0.280	0.644
% CCR2	1.012	0.980 - 1.045	0.473		1.010	0.971 – 1.051	0.615	0.780
% CD11b	1.035	0.999 - 1.072	0.059		1.039	0.991 – 1.090	0.115	0.644

% CD40	0.986	0.958 - 1.016	0.358		0.986	0.950 – 1.024	0.473	0.754
% CD86	0.982	0.935 - 1.031	0.463		0.988	0.930 – 1.049	0.693	0.803
% CD163	0.883	0.333 - 2.343	0.803		0.985	0.334 – 2.904	0.978	0.978
% CX3CR1	1.029	0.967 - 1.095	0.365		1.041	0.969 – 1.118	0.270	0.644
% CD123^{dim} CD11c^{++}	1.026	0.961 - 1.096	0.445		1.022	0.942 – 1.109	0.600	0.780
% CCR2	0.970	0.896 - 1.050	0.456		0.977	0.894 – 1.068	0.614	0.780
% CD11b	1.008	0.977 - 1.040	0.625		1.010	0.972 – 1.049	0.617	0.780
% CD40	1.009	0.977 - 1.043	0.569		1.008	0.972 – 1.047	0.658	0.780
% CD86	1.020	0.957 - 1.086	0.548		1.039	0.961 – 1.124	0.337	0.661
% CD163	0.871	0.704 - 1.078	0.204		0.874	0.684 – 1.118	0.285	0.644
% CX3CR1	1.005	0.975 - 1.037	0.732		1.008	0.973 – 1.044	0.652	0.780
% pDC	1.142	0.798 - 1.634	0.469		1.061	0.701 – 1.607	0.779	0.864
% CCR2	0.975	0.646 - 1.473	0.905		0.879	0.531 – 1.455	0.616	0.780
% CD11b	1.026	0.953 - 1.105	0.492		1.034	0.948 – 1.127	0.449	0.739
% CD40	1.019	0.976 - 1.063	0.397		1.013	0.964 – 1.065	0.604	0.780
% CD86	1.170	0.960 - 1.427	0.119		1.173	0.919 – 1.498	0.201	0.644
% CD163	0.374	0.048 - 2.931	0.349		0.325	0.018 – 5.836	0.445	0.739
% CX3CR1	0.925	0.233 - 3.680	0.912		1.125	0.244 – 5.178	0.880	0.935

CHAPTER 4

CHAPTER 4 – Rationale

$CD4^+$ T cell profiles during TB/HIV co-infection and their associations with TB treatment response and cavitary disease

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The profound rapid depletion of $CD4^+$ T cells during HIV-1 infection results in a compromised host immune response, leaving the individual vulnerable to Mtb infection. HIV-1 infection has also been associated with Mtb-specific T cell impairment, altering Mtb-specific host immune response, and increasing TB morbidity and mortality. Mtb infection itself is known to cause dysregulation of $CD4^+$ T cell responses, with decrease in overall $CD4^+$ T cell numbers and increase in T cell activation. The impact of Mtb and HIV on $CD4^+$ T cell responses during co-infection and the impact of these changes on TB disease severity and treatment response remains largely uncharacterized.

In **Chapter 4** we utilised stored samples from the CAPRISA 011 Improving Retreatment Success (IMPRESS) trial and healthy control cohort to characterise the $CD4^+$ T cell proportion, activation and CD25 and β7 integrin expression in patients with recurrent active TB with and without HIV-coinfection. We additionally assessed the associations between the measured markers and time to culture conversion and lung cavitation in the CAPRISA 011 cohort.

Dr Sivro, Prof Naidoo, and I conceptualized and designed this study. I prepared the specimens, optimised, and performed the flow cytometry assays. I performed the statistical analysis and data interpretation. Ms. Lewis, the CAPRISA 011 study statistician, validated the analysis. I wrote the initial draft of the manuscript that was reviewed and edited by my supervisors and other co-authors.

CD4$^+$ T cell profiles during TB/HIV co-infection and their associations with TB treatment response and cavitary disease

Short title: CD4$^+$ T cells in TB/HIV co-infection

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Abstract

$CD4^+$ T cell responses play a key role in the pathogenesis of TB and HIV. Utilizing peripheral blood mononuclear cells from the Improving retreatment Success (IMPRESS) trial we evaluated $CD4^+$ T cell phenotypes during active TB in patients with recurrent TB with and without HIV co-infection and assessed their associations with TB treatment response and cavitary disease using cox proportional hazards and logistic regression models, respectively. Flow cytometry was used to characterize total $CD4^+$ T cell percentage, CD4+ T cell activation as well as CD25 and β-integrin expression in individuals with active TB (n=25), TB/HIV co-infection (n=50) and healthy controls, (n=11).

Percent total $CD4^+$ T cells was significantly lower in TB/HIV compared to TB and healthy control groups ($p<0.0001$) with the % of activated $CD38^+HLA\text{-}DR^+$ $CD4^+$ T cells significantly higher among TB/HIV compared to the TB and healthy control groups ($p<0.0001$). Percent $CD25^+CD4^+$ T cells were higher among TB individuals in comparison to TB/HIV and healthy controls ($p<0.0001$). $CD4^+$ T cells associated with increased risk (OR 1.044, 95% CI 1.006 – 0.023, p=0.023) while T cell activation was associated with decreased risk (OR 0.910, 95% CI 0.839 – 0.988, p=0.025) of lung cavitation. Integrin α4β7 expressing cells ($\beta7^{Hi}$ $CD45RA^-$ $CD4^+$ T cells) were increased in TB/HIV compared to TB group (p=0.007) and associated with longer time to TB negative culture conversion in co-infected individuals (aHR 0.929, 95% CI 0.866 – 0.997, p=0.041). The observed HIV-related changes in $CD4^+$ T cell phenotypes during TB/HIV co-infection may play an important role in TB disease pathogenesis.

Introduction

The overlap between tuberculosis (TB) and HIV epidemics in sub-Saharan Africa represents one of the greatest public health challenges facing this region. Following COVID-19, TB remained the leading cause of death from a single infectious agent in 2021. Globally, 10.6 million people were infected with TB in 2021 with 187 000 deaths among HIV-positive people [1]. People living with HIV (PLWH) are at greater risk of latent tuberculosis infection (LTBI) reactivation [2] and have poorer treatment outcomes in comparison to the HIV-negative population [3, 4]. In PLWH, TB presentation is often subclinical, with the risk of dissemination being high [5]. Active TB infection in PLWH has been associated with higher HIV viral load, faster disease progression [6, 7] as well as increased mortality and morbidity [8]. The changes in the host immune response resulting from *Mycobacterium tuberculosis* (Mtb) and HIV co-infections fuel the pathology of both diseases.

$CD4^+$ T cells, and specifically $CD4^+$ Th1 responses, play a central role in the immune control of Mtb [9, 10] with IFNγ production by $CD4^+$ T cells shown to be essential for control of Mtb infection in animal models [11, 12] . TB clinical presentation and disease outcomes among the PLWH with $CD4^+$ T cell depletion have further demonstrated the critical role these cells play in TB pathogenesis [13-15]. Depletion of $CD4^+$ T cells by simian immunodeficiency virus (SIV) in non-human primates resulted in Mtb reactivation and disease progression highlighting the role of $CD4^+$ T cell responses in immune control of Mtb [16, 17]. In addition to the general depletion of $CD4^+$ T cells during HIV, studies have shown that HIV preferentially targets and depletes Mtb antigen specific $CD4^+$ T cells more so than cells that are specific to other antigens [18, 19], further impairing Mtb control. Both active TB and HIV are known to independently result in decreases in $CD4^+$ T cell numbers [20, 21] and increases in $CD4^+$ T cell activation [22-25]. In addition to their protective roles in controlling and clearing Mtb, several studies have implicated $CD4^+$ T cell responses in accelerating the lung tissue destruction that underlies cavitary TB, and therefore increased risk of transmission [26]. A positive correlation between circulating $CD4^+$ T cells and the risk of cavitary TB has been reported [27], with HIV positive individuals shown to have lower frequency of lung cavitation [28-30]. While both HIV and TB are known to deplete and impair $CD4^+$ T cell responses, the changes in $CD4^+$ T cell phenotype and function during co-infection, and their relationships with disease progression and clinical outcomes remain understudied. Here we utilised biological specimens from a well

characterised South African cohort with comprehensive clinical and demographic data to study the clinical implications of changes in $CD4^+$ T cell phenotype during active TB and TB/HIV co-infection.

Materials and Methods

Study population and design

This study utilized stored peripheral blood mononuclear cells (PBMCs) from the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 011 Improving Retreatment Success (IMPRESS) trial. CAPRISA 011 was an open label randomized clinical trial that compared treatment outcomes among re-treated TB patients receiving standard of care treatment versus a moxifloxacin containing regimen [31]. The IMPRESS cohort was based at an urban clinic operated by the CAPRISA eThekwini Research Clinic that adjoins the largest government outpatient HIV-TB facility, the Prince Cyril Zulu Communicable Disease Centre (PCZCDC) in KwaZulu-Natal (KZN), South Africa (SA). The study included adults ≥ 18 years of age with previous history of TB treatment completion, rifampicin-susceptible Mtb who were sputum smear-positive by GeneXpert MTB/RIF® technology. Patients were monitored during the 8 weeks intense phase of TB treatment with 2-weekly clinical follow-up visits and during the 16 weeks of continuous phase of TB treatment with a monthly clinical follow-up. A chest X-Ray was done at baseline and lung cavitation was graded as the presence of cavitation on either side of the lung.

We conducted a cohort analysis that included 75 individuals with available PBMC samples taken during active TB prior to TB treatment initiation (baseline) from the CAPRISA 011 study. As controls, we analysed PBMCs from 11 local healthy donors.

Ethics statement

CAPRISA 011 (Clinicaltrials.gov, NCT02114684), was approved by Medicines Control Council of South Africa (MCC Ref: 20130510). Written informed consent was obtained from all study participants prior to enrolment. University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (BREC) reviewed and approved the original studies [BFC029/13 (CAPRISA 011), and BE432/12 (Healthy donors)]. The nested study presented here was reviewed and approved by UKZN BREC (BREC/00000014/2019).

Sample collection and processing

Peripheral blood was collected in acid citrate dextrose (ACD) tubes. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO) in liquid nitrogen for long-term storage.

Flow cytometry

PBMCs were thawed, washed and resuspended in 5ml R10 (RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin 1.7nM sodium glutamate and 5.5 mL HEPES buffer). Prior to staining, cells were rested for 3 hours at 37^{o}C, 5% CO_2. A total of 1 x 10^6 cells were surfaced stained in the dark at room temperature for 20 minutes with a panel of premixed conjugated antibodies: anti-CD3 (APC-H7, clone SK7, BD Biosciences), anti-CD4 (ECD, clone SFCI12T4D11, Beckman Coulter), anti-HLA-DR (Alexa Fluor 700, clone L243, BioLegend), anti-CD38 (FITC, clone HIT2, BD Biosciences), anti-CD25 (BV421, clone 2A3, BD Biosciences), anti-CD45RA (BV605, clone HI 100, BD Biosciences), anti-β7 Integrin (PeCy5, FIB504, BD Biosciences) and Live/Dead™ fixable aqua dead cell stain (ThermoFisher). Following staining, cells were washed twice and fixed with 1 X CellFix™ (BD). Cells were acquired on the BD Fortessa™ Flow Cytometer (BD Biosciences, USA) and analysed with BD FACSDiva™ software (v8.0.2). At least 100,000 lymphocytes were collected. Flow cytometry data was analysed by hierarchal gating using FlowJo™ software and exported to Excel.

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. $CD4^+$ T cells were identified as cells expressing CD4 and CD3. Cell activation was assessed by co-expression of CD38 and HLA-DR. T regulatory-like cells were defined as $CD25^+$ $CD4^+$ T cells. Based on β7 and CD45RA expression $CD4^+$ T cells were classified as $\beta7^{Hi}$ $CD45RA^-$. $\beta7^{Hi}$ $CD45RA^-$ cells in the blood are shown to be >99% α4β7 integrin expressing $CD4^+$ T cells [32, 33]. Representative gating is shown in Figure 1A.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27 and SAS version 9.4, and graphs were made using GraphPad Prism (V9.3.1). D'Agostino-Pearson omnibus normality test was used to assess data distribution of each cell population. To assess differences in $CD4^+$ T cell percentages (%) and phenotype between study groups (TB/HIV, TB, and local healthy donors), a one-way ANOVA with Tukey's multiple comparisons post-test was performed on normally distributed markers, and non-parametric Kruskal Wallis test with Dunn's multiple comparisons post-test was done on markers with a non-normal distribution.

To determine the association between T-cell subset proportions at baseline and time to negative culture conversion (first of two consecutive negative TB culture results), measured in days, a Cox proportional hazards model was used. Additionally, a logistic regression model was used to determine the association between T-cell subset proportions at baseline and disease severity measured by presence of lung cavitation. Multivariable analysis, adjusted for randomization arm, age, sex, HIV status, alcohol use, smoking, BMI and lung cavitation (for the time to negative culture conversion analysis). In addition, $CD4^+$ T cell counts, and HIV viral load were adjusted for in sub-analysis of the PLWH group. Randomization arm was excluded in the multivariable lung cavitation analysis as this analysis was carried out at baseline, prior to randomization.

Results

Cohort characteristics

Samples from 75 CAPRISA 011 (IMPRESS) study participants were included in the analysis (**Table 1**). Approximately two-thirds (n= 50) were HIV-infected with a median CD4 cell count of 273 cells/mm^3 [interquartile range (IQR) 157 to 469] and HIV viral load of 2.10 log_{10} copies/ml (IQR 1.30 to 4.71). The median age was 35 (IQR 30 to 41) years in TB/HIV co-infection group, 29 (IQR 23.50 to 49) years in TB group and 34 (IQR 32 to 40) years in healthy controls. Among the IMPRESS cohort 76% of participants were male, compared to 45% in the healthy control group. The median body mass index (BMI) was 19.4 kg/m^2 (IQR 18.2 to 22.6) among TB/HIV co-infected group, 18.8 kg/m^2 (IQR 18.3 to 21.4) among the TB group and 23.3 kg/m^2 (IQR 19.7 to 31.3) among the healthy controls. In the total CAPRISA 011 cohort, 73% of study participants had lung cavitation on one or both lungs.

Table 1. Demographic and clinical characteristics of the study participants

Variables	IMPRESS Total participants n=75	IMPRESS TB positive and HIV-infected n=50	IMPRESS TB positive and HIV-uninfected n=25	Healthy Donors n=11
Randomization arm n (%)				
HRZE - Control	35 (47)	23 (46)	12 (48)	-
HRZM - Active	40 (53)	27 (54)	13 (52)	-
Age (y), median (IQR)	34 (29 – 41)	35 (30 – 41)	29 (23.50 – 49)	34 (32 – 40)
Gender, n (%)				
Male	57 (76)	35 (70)	22 (88)	5 (45)
Female	18 (24)	15 (30)	3 (12)	6 (55)
Body mass index (kg/m^2), median (IQR)	19.32 (18.32 – 21.74)	19.41 (18.21 – 22.64)	18.76 (18.33 – 21.36)	23.28 (19.73 – 31.30)
CD4 cell count ($cells/mm^3$), median (IQR)	273 (157 – 469.50)	273 (157 – 469.50)	-	-
HIV viral load (log_{10} copies/ml), median (IQR)	2.10 (1.30 – 4.71)	2.10 (1.30 – 4.71)	-	-
ART status* n (%)				
Yes	26 (52)	26 (52)	-	-
No	23 (46)	23 (46)	-	-
ART duration months, median (IQR)	1 (0 – 36)	1 (0 – 36)	-	-
Lung Cavities n (%)				
None	20 (27)	18 (36)	2 (8)	-
One Lung	33 (44)	20 (40)	13 (52)	-
Both Lungs	22 (29)	12 (24)	10 (40)	-
Days to first negative solid culture, median (IQR)[#]	54 (28 – 79)	42.5 (28 – 60.75)	56 (41.5 – 82.5)	-
Alcohol Use in the past 3 months n (%)				
Yes	23 (31)	19 (38)	4 (16)	-
Smoking in past 3months n (%)				
Yes	29 (39)	18 (36)	11 (44)	-

* 1 missing ARV status

[#] Measures for all variables, except days to first negative culture are reported at baseline

Comparison of baseline $CD4^+$ T cell phenotypes by study group

The proportion of $CD4^+$ T cells was significantly lower in the TB/HIV group compared to those in the TB and healthy control (HC) groups (both $p<0.0001$, Figure 1B, Supplemental Table 1). The proportion of activated $CD38^+HLA\text{-}DR^+$ $CD4^+$ T cells was significantly higher among individuals with TB/HIV co-infection compared to those with TB and healthy controls (both $p<0.0001$, Figure 1C). The proportion of $CD25^+CD4^+$ T cells was significantly higher in TB group compared to TB/HIV and healthy controls ($p<0.0001$), with $\%CD25^+CD4^+$ T cells significantly higher in TB/HIV group compared to healthy controls ($p=0.028$, Figure 1D). The proportion of $\beta7^{Hi}$ cells was significantly higher TB/HIV group compared to TB group ($p=0.007$, Figure 1E).

A.

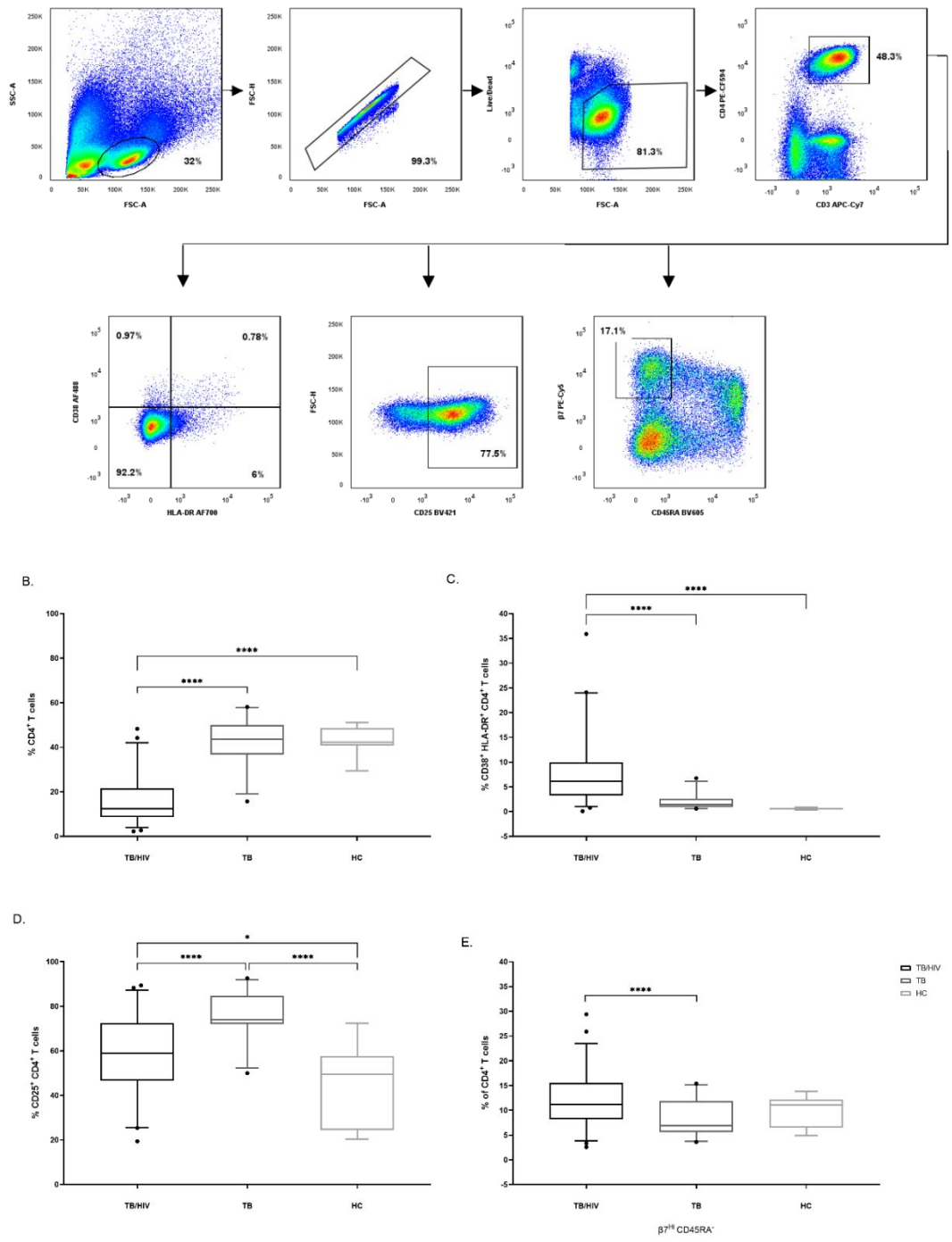

Figure 1. (A) Representative gating strategy. The staining profile of PBMC sample from TB /HIV co-infected participant is shown. Differences in percentages of **(B)** Total $CD4^+$ T cells, **(C)** $CD38^+$ $HLA\text{-}DR^+$ on $CD4^+$ T cells **(D)** $CD25^+CD4^+$ T cells **(E)** $\beta7^{Hi}$ $CD45RA^-$ between study groups (TB/HIV, TB, and HC). Boxes represent median and interquartile ranges; whiskers represent 5-95 percentiles. Differences in cell population percentages among the groups were assessed using a Kruskal-Wallis test with p-values adjusted for multiple comparisons using Dunn's post-test for data that were not normally distributed and one-way ANOVA test with p-values adjusted for multiple comparisons using Tukey's post-test for normally-distributed data. *$p < 0.05$; ** $p < 0.01$ and ****$p < 0.0001$.

Association between $CD4^+$ T cell proportion and phenotype at active TB and time to negative culture conversion

We used a Cox proportional hazards model to assess the association between measured $CD4^+$ T cell markers and time to negative culture conversion measured in days. There were no significant results in the total cohort (Supplemental Table 2).

Additionally, we performed a sub-analysis among the TB/HIV-co-infected cohort adjusting for CD4 count and viral load. The proportion of the $\beta7^{Hi}$ cells (aHR 0.929, 95% CI 0.866 – 0.997, p=0.041) associated with longer time to negative culture conversion in the multivariable model (Supplemental Table 3).

Association between $CD4^+$ T cell proportion and phenotype at active TB on disease severity

We used a logistic regression model (n=75, Supplemental Table 4) to assess the association between $CD4^+$ T cell subsets during active TB, with disease severity defined by the presence of lung cavitation. Among the total IMPRESS cohort in the univariable model, the proportion of $CD4^+$ T cells associated with an increased odds of lung cavitation (OR 1.044, 95% CI 1.006 – 1.084, p=0.023), while the proportion of $CD38^+$ HLA-DR^+ $CD4^+$ T cells associated with a decreased odds of lung cavitation (OR 0.910, 95% CI 0.839 – 0.988, p=0.025). While not significant, similar trend was observed in the multivariable models (% total $CD4^+$ T cells: aOR 1.020, 95% CI 0.969 – 1.075, %$CD38^+$ HLA-DR^+ $CD4^+$ T cells: aOR 0.938, 95% CI 0.858 – 1.026). We observed no significant associations in the sub-analysis of TB/HIV co-infected study group (Supplemental Table 5).

Discussion

The overlap between TB and HIV is a major barrier to achieving TB control, especially in endemic areas like sub-Saharan Africa. Both HIV and TB pathogens weaken host immunologic functions, impairing viral/bacterial control, and fueling disease progression. Loss and changes in $CD4^+$ T cell functional response is notably a hallmark of both diseases. Here we examined the changes in $CD4^+$ T cell phenotype during active TB with and without HIV co-infection and assessed their impact on TB treatment response and disease severity.

As expected, we observed a significant decrease in the percentage of total $CD4^+$ T cells as well as an increase in percentage of activated ($CD38^+HLADR^+$) $CD4^+$ T cells in individuals with TB/HIV co-infection compared to TB and HC study groups. As previously reported [26], we found that the percentage of $CD4^+$ T cells is positively associated with increased risk of lung cavitation in the IMPRESS cohort. In contrast we found that increased frequency of activated $CD4^+$ T cells associated significantly with lower odds of lung cavitation. The overall depletion of $CD4^+$ T cells and the increased percentage of $CD4^+$ activated cells in the TB/HIV co-infected patients likely contributes to the decreased risk of lung cavitation previously reported in HIV co-infected individuals [28-30].

In agreement with previous studies, we observed an increase in peripheral blood % of $CD25^+$ $CD4^+$ T cells (T regulatory-like cells) [34-36] in patients with active TB. This increase was evident in both TB and TB-HIV co-infected individuals compared to healthy controls. However, this increase was suppressed in TB-HIV co-infection compared to TB alone. T regulatory cells are known to impair and delay the protective immune responses in the early stages of TB infection, however in chronic disease they play an important role in regulating excessive inflammation and the associated tissue damage [37].

Integrin $\alpha4\beta7$ expressing $CD4^+$ T cells are early targets of HIV replication [38, 39] and were shown to play an important role in HIV/SIV acquisition and disease progression in both human [38] and animal studies [40-42]. Here we show an increased proportion of these cells in individuals with TB/HIV co-infection compared to individuals with active TB. While further studies are needed to assess the impact of this increase on HIV, this may contribute to the

increase in HIV viral load and accelerated disease progression previously reported in co-infected individuals [6, 7, 43]. Our data also shows that increased frequency of integrin $\beta7^{Hi}$ expressing cells associates with longer time to TB culture conversion in co-infected individuals, implicating these cells in TB pathogenesis.

This study has several limitations including a small sample size for the healthy controls and the disease groups. Additionally, we cannot exclude the influence of other unmeasured clinical and demographic variables. It is also important to note that this cohort of TB patients had a previous history of TB infection and we do not know if the assessed disease was a reinfection or relapse. Finally, the systemic compartment may not be reflective of the immune environment in the lungs as a site of Mtb replication. Despite these limitations, this study highlights the importance of $CD4^+$ T cell responses in TB pathogenesis and demonstrates a novel role of $\alpha4\beta7$ integrin expressing $CD4^+$ T cells in the pathogenesis of TB-HIV co-infection.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supporting Data Availability

Data generated will be made available to any investigator upon request by contacting the corresponding author.

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Authors contributions

Designed the study: AS, SR;

Performed the experiments: SR, TGM, AS;

Analysed the data: SR, AS, LL;

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

Collected specimens and clinical data: SN; NP; KN;

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

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

Supplemental Materials

Supplemental Table 1. CD4 T-cell population frequencies among different study groups

Supplemental Table 2. Association between T cell activation and phenotypes with overall time to negative culture conversion among the total cohort.

Supplemental Table 3. Association between T cell activation and phenotypes with overall time to negative culture conversion in HIV-infected subgroup

Supplemental Table 4. Association between T cell activation and phenotypes with cavitary disease in the total cohort

Supplemental Table 5. Association between T cell activation and phenotypes with cavitary disease among the HIV-infected subgroup

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Supplemental Material

Supplemental Table 1. $CD4^+$ T-cell population frequencies among different study groups

	TB/HIV N=50	**TB N=25**	**HC N=11**	
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**p-value**
% Total $CD4^+$	12.30 (8.61 – 21.50)	43.70 (36.80 – 49.95)	42.20 (40.70 – 48.60)	**<0.0001**
% Total $CD4^+CD38^+HLA\text{-}DR^+$	6.16 (3.27 – 9.97)	1.45 (0.94 – 2.64)	0.65 (0.42 – 0.75)	**<0.0001**
% Total $CD4^+$ $CD25^+$	58.95 (46.60 – 72.40)	73.90 (72.05 – 84.65)	49.50 (24.50 -57.70)	**<0.0001**
% $\beta7^{Hi}$ $CD45RA^-$	11.15 (8.18 – 15.53)	6.88 (5.62 – 11.85)	11.10 (6.48 – 12.20)	**0.0140**

Supplemental Table 2. Association between $CD4^+$ T cell frequencies and phenotype at active TB and time to negative culture conversion in total cohort

	Bivariable				Multivariable		
	HR	**CI**	**p-value**		**HR**	**CI**	**p-value**
% Total $CD4^+$	0.994	0.980 – 1.008	0.402		1.004	0.980 – 1.028	0.750
% Total $CD4^+$ $CD38^+$HLA-DR^+	1.014	0.980 – 1.049	0.418		0.997	0.959 – 1.037	0.894
% Total $CD4^+$ $CD25^+$	1.003	0.989 – 1.017	0.696		1.007	0.988 – 1.027	0.459
% $\beta7^{Hi}$ $CD45RA^-$	0.990	0.943 – 1.039	0.684		0.950	0.899 – 1.004	0.068

Supplemental Table 3. Association between $CD4^+$ T cell frequencies and phenotype at active TB and time to negative culture conversion in HIV co-infected group

	Bivariable				Multivariable		
	HR	**CI**	**p-value**		**HR**	**CI**	**p-value**
% Total $CD4^+$	1.004	0.977 – 1.031	0.792		1.002	0.967 – 1.037	0.919
% Total $CD4^+$ $CD38^+$HLA-DR^+	1.008	0.970 – 1.048	0.671		0.999	0.954 – 1.045	0.949
% Total $CD4^+$ $CD25^+$	1.008	0.989 – 1.028	0.398		1.006	0.984 – 1.029	0.585
% $\beta7^{Hi}$ $CD45RA^-$	0.972	0.914 – 1.033	0.362		0.929	0.866 – 0.997	**0.041**

Supplemental Table 4. Association between $CD4^+$ T cell frequencies and phenotype at active TB with cavitary disease in the total cohort

	Univariable				Multivariable		
	OR	**CI**	**p-value**		**OR**	**CI**	**p-value**
% Total $CD4^+$	1.044	1.006 – 1.084	**0.023**		1.020	0.969 – 1.075	0.449
% Total $CD4^+$ $CD38^+$HLA-DR^+	0.910	0.839 – 0.988	**0.025**		0.938	0.858 – 1.026	0.162
% Total $CD4^+$ $CD25^+$	1.017	0.988 – 1.048	0.253		0.996	0.962 – 1.031	0.819
% $\beta7^{Hi}$ $CD45RA^-$	0.946	0.863 – 1.038	0.243		0.992	0.897 – 1.097	0.874

Supplemental Table 5. Association between $CD4^+$ T cell frequencies and phenotype at active TB with cavitary disease among the HIV-infected subgroup

	Univariable				Multivariable		
	OR	**CI**	**p-value**		**OR**	**CI**	**p-value**
% Total $CD4^+$	1.001	0.951 – 1.055	0.956		1.005	0.938 – 1.076	0.891
% Total $CD4^+$ $CD38^+$HLA-DR^+	0.949	0.873 – 1.031	0.218		0.931	0.835 – 1.038	0.195
% Total $CD4^+$ $CD25^+$	0.997	0.963 – 1.031	0.847		0.999	0.951 – 1.030	0.605
% $\beta7^{Hi}$ $CD45RA^-$	0.968	0.874 – 1.072	0.530		0.949	0.845 – 1.065	0.375

CHAPTER 5

CHAPTER 5

5.1. Summary and Discussion

Prior to COVID-19 pandemic, TB was the leading cause of death from an infectious agent worldwide [1]. TB is estimated to have infected a quarter of global population, with most infected people able to clear the infection without development of disease. While TB treatment is effective in clearing infection in about 85% of people, an estimated 4 million infected people go undiagnosed every year; with TB death rate in untreated individuals being about 50% [2]. HIV infection has detrimental effects on both TB diagnosis as well as treatment success. TB accounts for a third of AIDS related deaths globally and is the leading cause of death in HIV positive people [3]. This highlights the need for more effective diagnosis and treatment modalities in TB and TB/HIV disease.

The search for new strategies to improve TB management and prevent further spread of the infection lies in research and understanding of the complex host immune responses. Host biomarkers and host directed therapies have long been proposed as a promising new avenue for TB diagnosis, treatment and prevention; however these efforts are hampered by the lack of understanding of the host immune processes that govern TB pathogenesis in both HIV positive and HIV negative individuals.

Here we utilised biological specimens from a well characterised clinical cohort of individuals with active, recurrent TB, from a region with highest TB and HIV incidence in the world, to characterise soluble and cellular innate and adaptive host immune responses during TB and TB/HIV co-infection and asses their impact on TB disease severity and treatment success.

Measurement of soluble markers represents a patient friendly and cost -effective method to assess individuals immune response and presence of infections and response to treatment. Point-of – care diagnostic tools measuring cytokines as means of detecting ongoing infections are being developed for various conditions, including diagnosis of sexually transmitted infections (STIs) and bacterial vaginosis (BV) in young women. The genital inflammation test (GIFT) is a new inexpensive diagnostic tool being developed in South Africa for detection of inflammation associated with STIs/BV, based on levels of IL1α, ILβ and IP-10

(www.gift.org.za). In context of TB, assessing plasma biomarkers could serve as an economical approach to assess individuals immune status, risk of TB reactivation, presence of active disease and the subsequent response to TB treatment. In **Chapter 2**, we measured a range of cytokines and chemokines and analysed their association with TB clinical aspects. The clinical outcomes included: 8-week culture status, time to negative culture conversion as well as lung cavitation and bacterial smear grade as a measure of disease severity and bacterial burden, respectively. IP-10 has previously been identified as a biomarker for TB diagnosis and monitoring response to treatment with the potential to be transformed into a straightforward point-of-care test [4-6]. This is supported by our data that showed that among the total cohort and HIV-positive subgroup, higher levels of IP-10 were associated with early clearance of bacteria after two months of intensive TB treatment highlighting the role IP-10 plays in TB clearance among recurrent infected individuals. Among HIV-positive individuals, IL-6, IL-1RA and IL-α associated with shorter time to culture conversion. Additionally, our data showed that elevated levels of plasma IL-6 and IL-1RA were linked with the presence of cavitary disease in TB patients, irrespective of HIV status. The host's immune response to infections is a complex phenomenon. On one hand, inflammation is necessary for effective elimination of pathogens and commencement of defensive cellular reactions; however, that same inflammatory response can also lead to lung damage and exacerbate disease progression. This is highlighted by associations between IL-6 and IL-1RA levels during active TB with both bacterial clearance and disease severity. Overall, our study confirmed the importance of IP-10 and IL-6 inflammatory markers in TB disease pathogenesis in both HIV positive and negative study participants. Importantly our study highlights the impact of other behavioural and clinical variables on the expression of the identified biomarkers, highlighting the need to verify these observations in other cohorts and with more diverse group of patients.

HIV mediated impairment of the innate and adaptive immune responses are known to contribute to the increased TB risk and faster disease progression in PLWH. The nature of the innate and adaptive immune responses during TB/HIV co-infection remains poorly characterised. Detailed knowledge of HIV and TB mediated immune changes during co-infection and the impact of these on TB and HIV disease outcomes are necessary for the development of host directed therapies and more efficient vaccine strategies in high-risk populations. In this study, we evaluated both the innate (**Chapter 3**) and adaptive (**Chapter 4**) cellular host responses in the context of TB/HIV co-infection and assessed their impact on TB disease outcomes.

Monocytes are the primary targets and major component of the early immune response to Mtb infection [7]. In **Chapter 3,** we characterised frequencies and phenotypic differences in monocyte and dendritic cell populations among four study subgroups: TB/HIV, TB, HIV, and healthy donors and assessed their associations with time to negative TB culture conversion and disease severity in patients with active TB. Consistent with prior research [8, 9], we noted an increase in percentage of circulating monocytes among individuals with active TB with and without HIV coinfection, while HIV infection was associated with increased frequency of total DCs.

Overall, we observed significant variation in the surface expression of CCR2, CD40, CD11b, CD86, CD163 and CX3CR1 across various cell subsets in all four study groups. Active TB infection was linked to higher levels of CCR2, CD11b and CD40; while lower levels of CX3CR1 and higher levels of CD163 were associated with HIV infection. Similarly, to previous IL-6 observations, increased frequency of CD11b on non-classical monocytes was associated with shorter time to culture conversion as well as lung cavitation in TB/HIV co-infected individuals implicating these responses in both Mtb clearance and disease immunopathology. HIV mediated downregulation of CX3CR1 and upregulation of CD163 likely results in impaired cell migration into the affected tissues, resulting in delayed/decreased adaptive response initiation and impaired Mtb clearance. The data presented offers significant understanding of the varied roles played by monocyte and dendritic cell subsets among individuals with recurrent TB and HIV co-infection.

HIV mediated $CD4^+$ T cell depletion is considered to be the main cause of increased TB morbidity and mortality in PLWH. $CD4^+$ T cell responses were shown to be key to Mtb control [10-14] and HIV infection was shown to specifically deplete Mtb specific $CD4^+$ T cells [15, 16]. In **Chapter 4,** we characterised $CD4^+$ T cells, including their activation, as well as CD25 and β-integrin expression among three study groups: individuals with active TB, those with TB/HIV co-infection, and healthy controls. As expected, TB/HIV group exhibited a significant reduction in percentage of total $CD4^+$ T cells, while the percentage of activated $CD4^+$ T cells expressing both CD38 and HLA-DR was significantly greater when compared to both the TB only and healthy control groups. Our data also showed an increased risk of lung cavitation associated with % of $CD4^+$ T cells, while a decreased risk was associated with increased T cell activation. This data likely explains the previously documented decrease in the risk of cavitary disease in PLWH [17-19]. Interestingly we observed an increased % of $\alpha4\beta7$ expressing $CD4^+$

T cells in peripheral blood of TB/HIV co-infected patients and this increase was associated with longer time to culture conversion in this subgroup. While the later observation implicates this subset in TB disease pathogenesis, the overall increase in these cells during active TB could lead to increased HIV viral load and faster disease progression as these cells were identified as early and preferential HIV target cells [20, 21]. Vedolizumab, an α4β7 blocker is used as an anti-inflammatory treatment to manage ulcerative colitis and Chron's disease [22, 23]and has been explored for HIV treatment and prevention[24, 25] . Whether integrin blockers have a role to play in TB treatment will require further studies to elucidate their exact role in TB and TB/HIV pathogenesis.

5.2. Strengths and significance

These studies provide detailed characterisation of soluble and cellular host immune responses among a clinical cohorts with active, recurrent TB and TB/HIV co-infection. Major strength of the study is the access to unique, well characterised biological specimens from the CAPRISA 011 IMPRESS study based in the region of highest TB/HIV co-infection rates in the world. Findings from these studies can provide information on immunological biomarkers for diagnostic and prognostic tools for monitoring of TB treatment response and disease severity among individuals that have recurrent TB.

5.3. Limitations

Our study has several limitations. We had a relatively small sample size from a single cohort of recurrent active TB individuals. Additionally due to limited cell numbers were unable to examine Mtb-specific T cell responses and other cellular phenotypes. For cellular phenotyping we used stored PBMCs and cryopreservation could have affected cell phenotype and function [26]. We analysed systemic cell populations and immune responses, and these may differ significantly from cell populations observed in the lungs, at the site of Mtb replication. Finally, all individuals from the IMPRESS cohort had a previous history of TB and we were unable to distinguish between re-infections and relapses.

5.4. Future recommendations

Future studies should validate the observed results in larger clinical cohorts in different regions of the world. These inflammatory and cellular phenotypes should also be investigated in other TB states including latent, subclinical, and drug-resistant TB due to typically diverse host immune reactions [27-31]. Longitudinal studies should assess changes in the measured phenotypes during and following TB treatment. The nature of the evaluated host responses should be evaluated concurrently at the site of Mtb replication, potentially using bronchoalveolar lavage (BAL) specimens, as well as systemically. While flow cytometry analysis might not be feasible due to low number of cells in these specimens, single-cell RNAseq could potentially be performed to gain a broad picture of the immune phenotypes and pathways in the lungs.

5.5. Conclusion

Our study provides detailed characterization of soluble inflammatory and innate and adaptive cellular host responses during active TB infection as well as the implication these responses have on TB disease severity and treatment outcome. Additionally, we have characterised the effects of HIV co-infection on the nature of the measured responses identifying new ways in which HIV mediated immune changes can hamper Mtb control. IP-10 and IL-6 were identified as important markers of Mtb activity and the associated immunopathogenesis in both HIV positive and negative individuals. We have observed high degree of variation with regards to monocyte and dendritic cell subsets and phenotypes during TB and TB/HIV infections with potential effects on cell migration, tissue distribution and activation of adaptive immune responses resulting in altered Mtb clearance rates and immunopathogenesis. Finally, we identified novel role for integrin α4β7 $CD4^+$ T cells in TB pathogenesis.

COVID-19 pandemic has had detrimental effects on both TB and HIV control efforts. The number of undiagnosed and untreated people with TB has grown significantly resulting in increased number of TB deaths and increased transmission rates. Globally, there were a total of 1.6 million deaths due to TB in 2021, with 187,000 of those deaths in PLWH [2]. With regards to HIV, COVID-19 had a negative impact on diagnosis and treatment initiation as well as increased HIV rates due to increased poverty [32] especially among the high-risk groups. Together, these combined effects in countries such as South Africa with high prevalence of

both diseases can have long-term devastating consequences. The failure to reach set out goals for TB and HIV control further highlights the need for novel treatment and prevention strategies. Increased understanding of host immune responses during TB and TB/HIV infection is crucial for the development of better diagnostic methods as well as vaccines and host directed therapies for TB prevention and treatment.

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APPENDICES

Appendix A: Contribution to other research projects and resulting co-authored publications.

In addition to my own research project, I assisted on several other projects during my time at CAPRISA and this work has resulted in 5 co-author publications and 1 submitted manuscript. The details of the projects and manuscripts are summarised below.

Evaluation of rapid antigen tests for SARS-CoV-2 / COVID-19

During COVID-19 pandemic, CAPRISA laboratory provided key services to the National Health Laboratory Service (NHLS) for SARS-CoV-2 testing. All staff and student resources were redirected towards SARS-CoV-2/ COVID-19 related projects. In collaboration with the Foundation for Innovative New Diagnostics (FIND), CAPRISA Laboratory evaluated the performance of 8 rapid antigen tests. As the result of this work, I am a co-author on 1 publication and 1 submitted manuscript reporting on the evaluations of rapid antigen tests for SARS-CoV-2 testing in South Africa. My role in these projects was to assist in performing of the evaluations and subsequent data analysis. The publication and manuscript details are listed below.

Natasha Samsunder, Gila Lustig, Slindile Ngubane, Thando Glory Maseko, **Santhuri Rambaran**, Sinaye Ngcapu, Stanley Nzuzo Magini, Lara Lewis, Cherie Cawood, Ayesha BM Kharsany, Quarraisha Abdool Karim, Salim Abdool Karim, Kogieleum Naidoo, Aida Sivro. **Field evaluations of four SARS-CoV-2 rapid antigen tests during SARS-CoV-2 Delta variant wave in South Africa**. *BMC Prognostic and Diagnostic Research*. July 25, 2023. PMID:37491317

Natasha Samsunder, Thando Gloria 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. **Evaluations of two SARS-CoV-2 rapid antigen tests during the first and second wave of COVID-19 infections in South Africa.** Submitted. *Journal of Clinical Virology Plus*. December 2022

Drug resistance and host immune responses in drug susceptible and drug resistant TB

Throughout my PhD I have assisted postdoctoral fellows and other junior students in the laboratory with their projects. My role was to assist in performing literature searches, optimising flow cytometry and cytokine panels and assisting with data acquisition and analysis. This work has resulted in 4 published articles:

Navisha Dookie, **Santhuri Rambaran**, Nesri Padayatchi, Sharana Mahomed, Kogieleum Naidoo. **Evolution of Drug Resistance in Mycobacterium tuberculosis: A Review on the Molecular Determinants of Resistance and Implications for Personalised Care**. *Journal of Antimicrobial Chemotherapy*, January 19, 2018. PMID: 29360989

Kimesha Pillay, Lara Lewis, **Santhuri Rambaran**, Nonhlanhla Yende-Zuma, Derseree Archary, Santhana Gengiah, Dhineshree Govender, Razia Hassan-Moosa, Natasha Samsunder, Salim S. Abdool Karim, Lyle R. McKinnon, Nesri Padayatchi, Kogieleum Naidoo, Aida Sivro. **Plasma biomarkers of Tuberculosis risk in HIV co-infected patients from South Africa.** *Frontiers in Immunology*, March 25, 2021. PMID: 33841412

Thando Glory Maseko, **Santhuri Rambaran**, Slindile Ngubane, Lara Lewis, Sinaye Ngcapu, Razia-Hassan-Moosa, Derseree Archary, Rubeshan Perumal, Nesri Padayatchi, Kogieleum Naidoo, Aida Sivro. **NK cell phenotypic profile during active TB in people living with HIV-evolution during TB treatment and implication for bacterial clearance and disease severity.** *Scientific Reports*. July 20, 2023. PMID: 37474556

Thando Glory Maseko, Slindile Ngubane, Marothi Letsoalo, **Santhuri Rambaran**, Derseree Archary, Natasha Samsunder, Rubeshan Perumal, Surie Chinappa, Nesri Padayatchi, Kogieleum Naidoo, Aida Sivro. **Higher plasma interleukin -6 levels are associated with lung cavitation in drug-resistant tuberculosis.** *BMC Immunology*. August 31, 2023. PMID:37653422

Appendix B: Ethical Approval Letters

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

07 October 2019

Miss Santhuri Rambaran (209514963)
School of Lab Med & Medical Science
Medical School Campus

Dear Miss Santhuri Rambaran,

Protocol reference number: BREC/00000014/2019
Project title: Innate cell phenotypes and predictors of late culture converters in TB retreatment individuals
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 **07 October 2019. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **07 October 2019.** 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 **12 November 2019.**

Yours sincerely

[redacted]

Prof V Rambiritch (Chair)

Biomedical Research Ethics Committee
Prof V Rambiritch (Chair)
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Website: http://research.ukzn.ac.za/Research-Ethics/

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

16 September 2020

Miss Santhuri Rambaran (209514963)
School of Lab Med & Medical Science
Medical School Campus

Dear Miss Rambaran,

Protocol reference number: BREC/00000014/2019
Project title: Innate cell phenotypes and predictors of late culture converters in TB retreatment individuals
Degree Purposes: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**07 October 2020**
Expiration of Ethical Approval:	**06 October 2021**

I wish to advise you that your application for recertification received on 09 September 2020 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee (BREC). 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 13 October 2020.

Yours sincerely

[redacted]

.....
Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Appendix C: Informed consent form for specimen storage and possible future research

APPENDIX I: IMPRESS PATIENT INFORMED CONSENT

STUDY INFORMED CONSENT FORM

TITLE OF STUDY: IMPROVING RETREATMENT SUCCESS (IMPRESS): An open label randomized controlled clinical trial comparing a 24 week oral regimen containing Moxifloxacin with a 24 week standard tuberculosis (TB) drug regimen for the treatment of smear-positive pulmonary TB in patients previously treated for TB

INFORMATION FOR PATIENTS

Short Title:	IMPRESS
Principle Investigator:	Dr Nesri Padayatchi
Address	719 Umbilo Road
	Doris Duke Medical Research Institute (2nd Floor)
	Nelson R Mandela School of Medicine,
	University of Kwa-Zulu Natal,
	Durban, 4013
Telephone	031 260 4555
Regulatory Authority:	Medicines Control Council (MCC)
Ethics Committee:	University of KwaZulu Natal Biomedical Research Ethics Committee (UKZN BREC)

WHAT IS THE DURATION OF THE STUDY?

If you are allocated to Intervention Arm the total study duration is 12 months

If you are allocated to Control Arm the total study duration is 12months.

It is possible that your time in the study may be extended if there is a problem with completing all the required doses of your TB medication.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

A total of 330 patients are expected to participate in the study.

USE OF STORED SAMPLES

Samples will be collected and stored for future research use. The research team may use these samples to confirm test results or to do an additional new test if required. Your samples will not be sold or used in other products that make money for researches. Should you decide not to have your samples stored this will not affect your ability to take part in the study. Your decision will not affect the quality of care you receive at the clinic

To protect your identity your sample container will not have your name or any information that may identify you. Only your patient number will be used on sample containers. If you do not agree, then samples for storage will not be collected. If you agree now and later change your mind, your sample will not be used for future testing. No matter what you decide, it will not affect your participation in the study and this will not affect the quality of care you receive from study staff.

_____ YES, I agree to have my samples stored

_____ NO, I do not agree to my samples being stored

The stored samples may be used for future research, to confirm test results, or to do additional testing. Your samples will not be sold or used in products that make money for the researchers. Any studies that use your samples will be reviewed by the Biomedical Research Ethics Committee of the University of KwaZulu Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed.

This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide

6. CAPRISA 002: VIRAL SET POINT AND CLINICAL DISEASE PROGRESSION: THE ROLE OF IMMUNOLOGICAL, GENETIC AND VIRAL FACTORS OVER THE COURSE OF DISEASE AND DURING ANTIRETROVIRAL THERAPY

ADOLESCENT CONSENT FOR SPECIMEN STORAGE FOR POSSIBLE FUTURE RESEARCH (PHASE II-IV)
VERSION 1.0, 13 OCTOBER 2017

Principal Investigator: Professor Salim Abdool Karim
University of KwaZulu-Natal
King George V Avenue
Durban 4001

Telephone No: 031 260 1611 (Fathima Sayed)
(Office hours 8am-5pm) 033 260 6863 (Duduzile Nkosi)
031 260 4453 (Nigel Garrett)

INTRODUCTION

You have been enrolled into a study named above. While you are taking part in this study, blood and other biological samples will be taken from you for testing. Some of these samples may be kept for future research relating to the study of HIV. This assent form gives you information about this storage and use of samples. You are being asked to consent to the storage of your blood and other samples. The study staff will discuss this with you. Please ask if you have any questions. If you agree to the storage of your blood and other samples, you will be asked to note this on the consent form. You will be given a copy of this form to keep. The results of tests on your stored samples will not usually be made known to you, and the researchers do not intend sharing this information with anyone else. If the researchers believe that information from tests on your stored material is important, they will make this available to you through your regular doctor. Please make sure that you update your contact information with the study staff so that they can contact you if the need arises.

Please note that since you are younger than 18 years of age, we would like your parent or guardian to agree to the storage of your specimens. Your parent or guardian has been given a similar consent form to read and sign. Even if your parent/legal guardian agrees to the storage of your specimens we will still require you to agree to the storage of your specimens.

BLOOD AND BIOLOGICAL SAMPLES

At your clinic visits, blood and other biological samples (vaginal swabs and urine) will be taken from you by study staff. Some of the blood and biological samples obtained during the study will be stored. As with your other samples, only a confidential Participant Identification (PID) number and not your name will be used to identify these samples. These samples may provide valuable information in the future when different immune system and HIV tests become available.

USE OF STORED SAMPLES

The stored samples may be used for future research, to confirm test results, or to make sure that the samples tested have all come from the same person. If new methods of testing become available, the samples may be used to see if these new tests give the same results. We may test your cells, proteins, other chemicals in your body and your genes (DNA). Some of the samples will also be tested to see how your nutritional status may be interacting with HIV infection. Your samples may be analyzed in laboratories outside of South Africa. Your samples will not be sold or used in products that make money for the researchers. Virus obtained from your sample may be used in vaccine research and development. Any studies that use your samples will be reviewed by the Ethics Committee of the University of KwaZulu-Natal.

The researchers do not plan to contact you or your regular doctor with any results that are done on the stored samples after the study has been completed. This is because research tests are often done with experimental procedures so the results from one study are generally not useful for making decisions on managing your health. Should a rare situation come up where the researchers decide that a specific test result would provide important information for your health, the researchers will notify the study doctor who will try to contact you or your regular

doctor. If you wish to be notified of this type of test result, you need to make sure that you contact the study nurse or doctor with any changes to your phone number or address. If you want your regular doctor to be told about this kind of test result, you need to provide the study team with the contact details of your regular doctor.

STORAGE OF SAMPLES

If you agree to have your samples stored they will be stored with your confidential PID number at laboratories that are specially designed to keep stored samples safely. Only approved researchers working on this project and related projects will be able to access your samples. The people who work at these laboratories will have access to your samples when they store them and keep track of them, but they will not know who you are as your samples will be stored by number. There is a possibility that your stored samples may be shipped and analysed overseas at specialized laboratories if a test is unavailable locally. There is no time limit on how long your samples may be stored.

BENEFITS

There is no direct benefit to you through having your samples stored. The benefit is to the researchers as they will be able to make sure that the procedures they are using are accurate and they may learn more about the HIV virus from your samples.

RISKS

There is very little risk to you when you have your samples stored. There is a small risk that others may find out information about your HIV status from stored samples. This risk is reduced as your sample is stored under a confidential PID number, and not your name. You are entitled to the same protections of confidentiality and privacy for stored samples as you are for the samples that are drawn and used during the study. It is possible that tests that have yet to be developed may tell us things about your health we cannot currently test for. Results from these tests may cause distress to you.

Some genetic testing may be done on your stored samples. The greatest risk is to your privacy. It is possible that if others found out information about you that is learned from these tests (such as information about your genes), it could cause you problems with family members (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems with getting a job or insurance. This risk is extremely small, because the test results do not identify you by name and they do not become part of your medical records.

CONFIDENTIALITY

The results of future tests of your samples will not go onto your medical record. Although every effort is made to make sure that your samples are stored confidentially (by number and not name), we cannot guarantee absolute confidentiality. If required to do so by law, your personal information may be disclosed. Medical records that identify you by name may be inspected by representatives from the agency that is funding this study and by the research team who is responsible for keeping information for this study.

PARTICIPANT RIGHTS

The decision to allow your samples to be stored is completely voluntary. You may decide not to allow your samples to be stored after the tests that are needed for this study have been done or you decide to stop participating in the study. If you do decide to allow your samples to be stored, you can change your mind at any time. You must contact the study doctor or nurse and let them know that you do not want your samples to be stored any more. If you decide to do this, your samples will no longer be stored. You will then be asked if you wish all stored samples to be destroyed. In this case, any samples that have been stored will be destroyed. You will then be asked to sign this form again under the section, "Withdrawal of Conent" so that there is a record of your decision. At the end of the study you will also be given the opportunity to review your consent for the storage of your samples.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS

If you have any questions about the storage of samples for this study, or would like to know more about the storage of blood, please call either of the following during office hours (8am-5pm):

eThekwini Site:	Fathima Sayed	031 260 1611
	Hlengi Shozi	031 260 1943
Vulindlela Site:	Duduzile Nkosi	033 260 6863
Project Director:	Nigel Garrett	031 260 4453

You are not giving up your legal rights by signing the informed consent document. If you have any questions about your rights as a research participant, you may contact during office hours (8am-4pm):

The Biomedical Research Ethics Administration Office
Room N40, Govan Mbeki Building, University Road, Westville Campus, Westville
Telephone: +27 (0)-31-260-4769 / 260-4553
Fax: +27 (0)-31-260-4609
Email: brec@ukzn.ac.za

SIGNATURES

Please read the statement below and think about your choice. No matter what you decide, it will not affect your care or participation in the CAPRISA 002 Study.

I agree to allow some of my biological samples to be stored and used for testing and future research in HIV studies (please tick only one).

_____ Yes

_____ No

I am aware that I may withdraw my consent at any time without prejudice to further care.

Name: _____
Volunteer

Signed: _____ Date:_____
Volunteer

Name: _____
Researcher

Signed: _____ Date:_____
Researcher

Name: _____ Date: _____
Witness

Signed: _____ Date: _____
Witness