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Factors associated with latent tuberculosis infection among HIV positive adults ≥ 18 years in South Africa in 2016 - 2017: A cross-sectional study.

By

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Research report submitted to the Faculty of Health Sciences in partial fulfilment of the requirements for the degree of MSc Epidemiology in the field of Epidemiology and Biostatistics.

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DECLARATION

I declare that this report that I submit in partial fulfilment of the degree, Masters in Science in Epidemiology and Biostatistics at the School of Public Health at the University of Witwatersrand, Johannesburg is my independent work. This research report has not been submitted previously to any institution for examination or degree purposes.

Nagudi Galenda Jeniffer

Date: 15/04/2021

DEDICATION

I dedicate this work to two important females in my life, my mother Kyagamo Ketty Wakwale and daughter Bokang Pearl Kikine. I want to thank my mother for her relentless support, sacrifice and unconditional love in all my pursuits. To my daughter Pearl, you are my inspiration to keep working hard and soaring for greater heights.

ABSTRACT

Introduction: Latent tuberculosis infection (LTBI) is a major public health concern as it is one of the main sources of Tuberculosis (TB). Approximately 10% of individuals with LTBI will develop TB disease. HIV-infected individuals are among the populations at highest risk of LTBI and progressing to active TB disease. Understanding prevalence and risk factors of LTBI is important in implementation strategies such as TB preventive therapy (TPT) and vaccination. The main aim of this study was to estimate factors associated with LTBI among HIV-positive adults in South Africa.

Methods: This study was a cross-sectional study. Secondary data analyses were conducted on baseline data of HIV-positive adults enrolled for the $WHIP_3$ TB trial conducted in South Africa in 2016-2017. Participants' medical history, vital signs, CD4 count, and QuantiFERON-TB (QFT) results were collected through self-reporting, physical examination, medical records, and LTBI blood testing respectively. Descriptive statistics and logistic regression analysis using Stata 15® were used to describe the study population and determine factors associated with LTBI and $CD8^+$ T-cell response respectively.

Results: Of the 2511 enrolled participants, 1087 tested positive, giving a 43.3% LTBI prevalence. The study participants had an overall mean age of 41 years (9.9), 618 (24.6%) participants had a previous TB diagnosis and 1972(77.6%) had a CD4 count > 200cells/$mL^{3.}$ Higher LTBI prevalence was associated with increasing age (OR 1.01 [95%CI 1.0;1.03]), having a previous TB diagnosis (OR 1.34 [95%CI 1.10;1.63]) and having a CD4 count > 200cells/mL^3 (OR 1.60 [95%CI 1.25;2.05]). $CD8^+$ T-cell response was associated with having a previous TB diagnosis (OR 1.85 [95%CI 1.35;2.53]).

Conclusion: Given the high prevalence of LTBI among HIV-infected adults in South Africa, findings of this study should guide implementation and ramping of TPT. In addition, results should facilitate planning of the vaccine trials such as consideration of younger populations as they are less likely to be infected.

Key words: Tuberculosis, Latent Tuberculosis Infection, IGRA, $CD8^+$ T-cell response

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TABLE OF CONTENTS

LIST OF ACRONYMS

AOR	:	Adjusted Odds Ratio
HAART	:	Highly Active Antiretroviral Therapy
IGRA	:	Interferon -γ Release Assays
IPT	:	Izoniazid Preventive Therapy
IQR	:	Interquartile Range
LTBI	:	Latent Tuberculosis Infection
MTB	:	Mycobacterioum Tuberculosis Bacteria
OR	:	Odds Ratio
QFT-Plus	:	QuantiFERRON- TB Gold test
TB	:	Tuberculosis
TPT	:	TB Preventive Therapy
TNF	:	Tumor Necrosis Factor
TST	:	Tuberculin Skin Test
WHO	:	World Health Organization

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

This chapter provides a brief background on tuberculosis and latent tuberculosis infection, with descriptions and differentiation of the two medical terms. It also includes a background and current state of tuberculosis infections in both the global and South African contexts. This is followed by a brief insight into preventive treatment recommendations and available diagnostic tools for latent tuberculosis. The chapter ends with highlights on the importance of the research topic.

1.1 Background

1.1.1 Global epidemiology of TB

Tuberculosis (TB) is among the oldest diseases and is currently a global threat with up to 9 million new cases reported yearly (1). TB is an infectious airborne disease caused by mycobacterium tuberculosis bacteria (MTB) and can either be pulmonary TB which affects the lungs or extrapulmonary TB that affects other parts of the body like the brain, kidney, or spine (2). Globally, approximately 1.2 million deaths among HIV-negative individuals and 208,000 deaths among HIV-positive individuals were caused by TB in 2019 (3). According to the 2018 global TB report, the overall TB incidence rate was 132 cases per 100,000 population, and South Africa was classified as a high TB burden country with an estimated 322,000 new cases of TB (4). Over the years, TB management guidelines and recommendations have been updated on a regular basis as an effort to improve treatment outcomes among TB patients (5). In 2015, the World Health Organisation (WHO) set goals towards a combined effort from all countries worldwide, in ending the TB epidemic by 2030 (4).

1.1.2 Interventions to fight TB

In 2018, WHO recommended targeting implementation interventions to the following high-risk groups through latent tuberculosis infection (LTBI) treatment: HIV-infected individuals, pulmonary TB (PTB) case household contacts, and other at-risk groups like patients receiving dialysis (6). LTBI treatment or TB preventive treatment (TPT) is a known intervention for reducing TB incidence. Evidence for TPT indicates it is more effective in patients who already have LTBI (7). However, testing for LTBI is a huge impediment in the roll-out of TPT and has been seen as a rate-limiting step. Current recommendations do not advise that countries include testing for LTBI in their programs. However, studies where LTBI testing has been done, allow

us to determine the relative effectiveness of TPT in those who do and do not have LTBI (8–10).

Vaccination is an important way to prevent the development of TB disease and recent advances have made the prospect of a TB vaccine much more possible (11). The Bacilli Calmette-Guerin (BCG) vaccine administered at birth provides protection, particularly against extreme forms of TB during childhood (6). Re-vaccination has also been explored and found to be effective in reducing TB disease incidences, however, the benefits are limited to populations in which the first vaccination was effective (12). A randomized control trial carried out in Zambia, Kenya and South Africa found that the M72 vaccine prevented TB disease to up to 54% in HIV-negative adults (13).

1.1.3 Latent tuberculosis overview

Latent tuberculosis infection (LTBI) is a condition in which an individual is infected with MTB, but TB disease is absent. Individuals with LTBI are neither contagious nor symptomatic as they do not have classic TB disease signs and symptoms such as coughing, fever, night sweats, and unexplained weight loss, among others (2,14). Overall, 5-10% of individuals with LTBI will develop active TB at some point in their lifetime, and about half of these do so in the first two years of infection (2,15). The advancement of LTBI to TB disease is dependent on various factors such as bacterial, host, and environmental factors (16).

Immunocompromised individuals such as HIV- infected individuals are at a higher risk of LTBI infection progression to TB disease (16)(17). The effect of TB and HIV is two-way, as both HIV and TB disease continue to create a destructive synergism (18). HIV increases the risk of both TB infection and disease progression. On the other hand, TB slows down CD4 T-cell recouping resulting in HIV infection advancing towards AIDS and sometimes death (18). This causes a drawback in efforts being made in the management of both diseases. Therefore, it is imperative that LTBI is fully understood among HIV-infected individuals to curb one of the biggest reservoirs of TB disease.

1.1.4 Diagnosis of LTBI

The diagnosis of LTBI currently is by two methods; the tuberculin skin test (TST) and interferon-γ release assays (IGRA) test. The TST test was invented centuries ago and is cheaper, and the most used method (4,14). The TST is affected by the Bacilli Calmette-Guerin (BCG) vaccine and environmental non-tuberculosis mycobacterium. The TST has many

technical issues; for example, administration of the test and reading of test results needs experts or experienced staff (19,20). In addition, there has been a worldwide shortage of the purified protein derivative (PPD) which is the substance used for the TST, which has severely hampered TPT rollout in countries where it was included in guidelines (21). IGRA tests were introduced as alternatives to the TST, the two IGRA tests available are the QuantiFERON-TB Gold test (QFT) and the T-SPOT (22). The IGRA test has a higher sensitivity over the TST for TB disease diagnosis, superior specificity and negative predictive value for LTBI diagnosis mostly in individuals that received the BCG vaccine (23,24). In HIV-infected individuals, TST is less reliable compared to the IGRA tests, especially in persons with advanced HIV (25,26). Very few studies have reported prevalence of LTBI or risk factors of LTBI where the IGRA test, which is more reliable and accurate, has been used.

Understanding what proportion of patients currently in HIV care are positive for LTBI and what the risk factors are for LTBI will help guide the implementation of TPT programs. Furthermore, the planning and implementation of TB vaccine trials require measurement of the baseline LTBI infection and knowing the risk factors of LTBI (27,28).

1.2 Literature review

This section gives an in-depth overview of TB, LTBI, as well as the prevalence and current treatment options of both disease states. The section also provides a view on HIV and latent TB-coinfection, IGRA strengths and limitations as a diagnostic tool. Subsequent to this, the predictors of the $CD8^+$ T-cell response, and its effect on the diagnostic test are discussed. Lastly, the section concludes with the aim and study objectives of the research topic.

1.2.1 The End TB strategy: global achievements and challenges.

Globally the average incidence of TB is approximately 130 infections per 100,000 population (6). There is, however, marked variation with the range being from five to greater than 500 new cases per 100,000 population yearly. In 2015, the World Health Organisation set the global health strategy for TB prevention, care, and control (4). The strategy with targets set for 2034, consists of a 95% reduction in the number of TB deaths compared to 2015, a 90% reduction in TB incident rate compared to 2015, and 0% TB- affected families facing catastrophic costs (29). In 2020, the milestones expected to be achieved globally are a decrease in TB incidence by 20% and TB mortality rate by 35% (6). Currently, countries in the European region are on track in terms of achieving the 2020 milestones, however, high TB burden countries, especially in sub-Saharan Africa, are unfortunately unlikely to attain the set milestones (6). Some of the

challenges facing high TB burden countries such as South Africa in reaching the END TB strategy milestones include low treatment completion rate, malnutrition, silicosis, alcohol, tobacco use, HIV co-infection, and the most recent and detrimental being the COVID-19 pandemic (30–32)(33). Due to the COVID-19 pandemic, there has been resource diversion and health system focus from all other health programmes, TB inclusive (33). WHO has reported a 28% decrease in the number of people that have received TB care in 2020 as compared to 2019 in TB high-burden countries (34). Other factors that affect TB are "poverty, socio-economic and/ or gender inequality, food and/or job insecurity and weak health systems "(30).

A study done in South Africa to understand the TB care cascade in 2017 using an estimated overall TB burden of approximately 532,005 cases, estimated a 53% and 52% treatment completion success in general and among individuals co-infected with HIV respectively (34). A number of factors influence treatment success and some of these are the various existing difficulties concerning long periods of treatment regimens, drug administration frequency and patient adherence, and adverse events associated with TB drugs. A further challenge is the emergence of multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB; as individuals with either MDR/ XDR TB are infected with a TB strain that is resistant to the two or more potent TB drugs (5,35). In reference to treatment adherence, research priority has focused on developing regimens with shorter treatment durations (5). The global TB report indicated that by August 2019, a total of 23 drugs, several regimens, and 14 possible vaccines had been established from clinical trials (6). Other studies have indicated the importance of carrying out knowledge, attitudes, and practices (KAP) empowerment and training sessions for auxiliary primary health care staff in low and middle-income countries, as referring and identifying TB contacts heavily relies on them (36).

1.2.2 TB preventive therapy: Priorities and recommendations

The populations considered to be at high risk of TB infection are HIV-infected individuals and a range of clinical groups; silicosis patients; patients on anti-necrosis factor (TNF) treatment; patients on dialysis; and those preparing for organ and hematological transplants (4,15,20,37). In 2018, the WHO set a target of extending LTBI treatment to 30 million individuals within 5 years in order to reach 6 million HIV-infected individuals globally (6). In 2018, South Africa accounted for 61% of the 1.8 million HIV-infected individuals that were initiated on TPT globally, which was an approximately 80% increase from 2017 (6). The WHO

recommendations for LTBI treatment vary among countries depending on the number of estimated TB cases and available resources(6). For countries like South Africa, due to the high TB burden and being resource-limited, WHO mainly recommends focusing on HIV-infected individuals, and those with household TB cases (5,6).

To date, the three main health interventions used to prevent TB are TB preventive therapy (TPT), transmission prevention of MTB using infection prevention and control, and BCG childhood vaccination (6). LTBI treatment options are categorised into short-term regimens where treatment can take less than six months and long-term regimens that are six months or longer. Approved LTBI treatment options comprise six months of isoniazid (6H) or longer, the two recently recommended regimens being three months of weekly rifapentine and isoniazid (3HP), and four months of daily rifampicin (4R) (6,38). However, short-duration rifamycin-based drugs are a preferred option to longer isoniazid regimens (39). In the effort to provide shorter duration regimen alternatives, studies have been done on these three treatment options. 3HP showed an equivalently safety and effectiveness profile as the other treatment regimens, and similar completion rates with 4R when administered both as directly observed therapy(DOT) and self-administered therapy (SAT) (40). Additionally, some evidence has shown an association between 3HP and decreased adverse drug reactions like hepatotoxicity, higher treatment completion rates because of fewer adverse drug reactions, and fewer deaths (40).

LTBI control is dependent on high rates of TPT initiation and completion to interrupt the process of TB disease development. Therefore, it is vital to understand factors influencing the initiation, adherence, and completion of TPT (41). Several studies have pointed out focus areas to improve LTBI control and these include; use of shorter duration TPT regimen options; social interventions tailored to the groups of individuals in which the program is being implemented; uptake increment through simplified guidelines, educating both health workers and patients on advantages of TPT, and political dedication (41,42). Furthermore, using accurate indicators to track progress and evaluate the success of the TPT programs is a necessity (42).

1.2.3. Latent Tuberculosis Infection (LTBI)

1.2.3.1 Prevalence of latent tuberculosis infection

LTBI is a major challenge in the public health sector and one of the main sources of TB disease cases(43). In 2014, an estimate of 1.7 million people (23%) of the global population was infected with LTBI: Africa, South-East Asia, and Western-Pacific were the highest prevalent

regions and accounted for 80% of the LTBI burden (4,43). In 2019, the estimate of individuals with LTBI was a quarter of the entire world population with an LTBI prevalence of up to 36%, 20%, and 5% in countries with a high, intermediate, and low incidence of active TB respectively (44).

Currently, the true burden of LTBI in Africa is unknown (45). However, some studies in various countries have estimated varying LTBI prevalence: 34.4% in Mozambique, 46% in Ethiopia, 48% in Zambia, and 49% in Uganda (46–50). Several studies in South Africa have been done in various groups of people providing an approximation of LTBI prevalence: 29.2% in pregnant women, 56% adolescents (12-18years), and 64% in Health Care workers (51–53). A community-based study carried out in one of the urban townships of Johannesburg observed an LTBI prevalence of 34.3% (54).

A systematic review and meta-analysis aimed at establishing the global prevalence of LTBI using studies between 2005-2018 that used either IGRA or TST as diagnostic tools, obtained a prevalence of 24.8% and 21.2% for IGRA and TST respectively, as geographical estimates ranged from 11-27% using IGRA and 12-33% for TST (44). Some of the reasons that could account for the difference in prevalence obtained from the various studies are; the difference in diagnostic tools used; methods used to sample and their quality; varying study populations and sizes; assumptions of trends for countries with missing data, and the effect of age (44).

1.2.3.2 Factors associated with latent tuberculosis infection

Studies have sought to determine factors associated with LTBI in different non-HIV-infected study populations. These studies found the following factors to be associated with LTBI: age, education level, social-economic status, alcohol use, household TB contact, no BCG vaccine, leaving home for school or work, nature of individual's occupation, smoking and or illicit drug use (40,47,48,51,55–57).

A higher prevalence of LTBI was related to increasing age and alcohol consumption, lower attained education level, and social-economic status (47,48,51,55). Individuals with the following characteristics were found to have a high prevalence of LTBI: residing in urban settings; that had a household TB contact; had no history of receiving the BCG vaccine; were smoking; used illicit drugs; and in a particular profession, for example, health workers (40,47,48,51,56). Other LTBI predictive factors were: ethnicity; male gender; married couples; and individuals' that had to leave home for school or work. It is believed that this was attributed

to the social networks they are involved in outside the homes that exposed them to LTBI (47,58).

Few studies have been done to assess the effectiveness of both TPT and active TB treatment regarding preventing LTBI occurrence or reoccurrence. The studies showed inconsistent findings, as some found a negative association between LTBI and TPT or active TB treatment while other studies found a positive association between LTBI and the respective treatments (59–66). In other words, TPT and active TB treatment decrease the possibility of LTBI activation or reactivation, therefore, a lower LTBI prevalence was observed in individuals that had received either treatment (59,61–63,65). In other studies, a high LTBI prevalence was related to the history of active TB or treatment and TPT (60,64,66,67).

1.2.4 HIV and latent TB coinfection

HIV co-infection with TB is of public health concern. Globally an estimate of 10 million people developed TB disease, 9% of these were HIV-positive, and 72% of the TB cases were from Africa in 2017(4). Between 2014-2016, the leading cause of mortality was HIV-associated TB in South Africa (68). Despite some studies seeking to determine factors associated with LTBI in the general population, little is known about the factors associated with LTBI, particularly in HIV-infected individuals. Studies done in low- TB burden countries found an association between LTBI and the following factors: higher CD4 level; not being on highly active antiretroviral therapy (HAART); shorter period since patient started HAART; and being a local resident (69–71). A study performed in Cape Town, South Africa established an association between LTBI and being a current TB contact, schooling, and/or employment, BCG vaccination, and smoking (69).

Factors that have been associated with a higher prevalence of LTBI among HIV-positive patients were an individual either not on HAART or having been on HAART for a short period. The theory being that the body has not been able to mount an adequate immunological response (70,71). Other factors related to a higher prevalence in HIV individuals were having a household TB case, and no history of receiving the BCG vaccine, as discussed earlier (69). The usage of crowded and inadequately ventilated public transport on the way to school and work explains how schooling and employment were related to a higher LTBI prevalence (69).

On the other hand, low LTBI prevalence among HIV infected individuals is related to having a higher CD4 count of above 200 cells/mL^3, and/or higher CD4/CD8 ratio, since they at a lower risk of infection (70,71). Contrary to other studies, a study done in South Africa found that HIV

positive smokers had a lower LTBI prevalence, as this was probably a result of reduced production of interferon-gamma, which in turn affects the performance of the diagnostic test (69). Studies done in low TB burden countries have found that being a local resident was associated with a low LTBI prevalence, therefore the prevalence of LTBI is dependent on an individual's country of origin and/or residence (70,71). A summary of factors associated with LTBI among the general population and HIV-infected individuals are presented in Table 1.

Table 1: Summary of factors associated with latent tuberculosis Infection in varying populations.

Population	General Population	HIV-infected individuals	Common factors between general and HIV-infected population
Risk factors	• Older age(51,55) • Male gender(51,60) • Married couples(72) • Lower education level (51,55) • Health care worker occupation(48,56) • Black/mixed race(51) • Alcohol use(55) • Illicit drug use(40) • Lower social economic status(51,55) • HIV/AIDS(40,48) • Previous TB treatment history (64)	• Lower CD4 count(67) • Lower CD4/CD8 ratio(71) • Shorter duration on Antiretroviral therapy(71) • History of IPT(49,57)	• Having a house hold TB contact(51,60) • History of BCG vaccination(48) • Leaving home for school or employment(47)

1.2.5 Latent tuberculosis diagnosis

The currently available diagnostic tools are the tuberculin skin test (TST) and the interferon-y release assays (IGRA)tests: QuantiFERON-TB Gold test (QFT) and the T-SPOT (22).
The IGRA test was introduced as an alternative method due to the TST limitations which include the nature of administration, result interpretation, and sensitivity being affected by BCG vaccine and environmental non-tuberculosis bacterium (14,19,20). On the other hand, IGRA tests have limitations as well as they are expensive, require fully established laboratory facilities and expertise to operate (73).

A retrospective cohort study done on frozen lymphocytes of Swiss HIV-infected patients suggested that TST and IGRA test (T-SPT.TB) had similar sensitivity in detecting LTBI (74). A longitudinal study among HIV-positive patients mentioned that TST expressed superiority in risk analysis over the IGRA test (75). However, some studies recommend IGRA tests for epidemiological studies as they are better at detecting LTBI prevalence since the TST results are affected by malnutrition, non-TB mycobacteria, parasitic infections, and HIV (24,76).

The QuantiFERON- TB Gold test (QFT-Plus) is one of the IGRA tests that use ELISA to identify interferon-γ responses in the blood sample (22). The QFT-Plus consists of 2 tubes: TB1 and TB2 that have MTB peptides designed to trigger both $CD8^+$ and $CD4^+$ T-cells. Since $CD8^+$ T-cell response is associated with the $CD4^+$ T-cell response, this provides evidence of the major role played by $CD4^+$ T-cell in MTB defence (77). The sensitivity and specificity of the test among HIV-positive patients are inadequate for ruling out the presence of MTB as indeterminate results are more common in individuals from high TB burden countries and with a $CD4^+$ T-cell count of < 200 cells/mL^3 (22). The possible reason for the difference in the performance of IGRAs between high and low TB burden countries is that $CD4^+$ T-cell response is affected by malnutrition (78). However, more studies are required to determine the effects of HIV, parasitic infections, non-TB mycobacterium on IGRAs performance (76).

1.2.5.1 $CD8^+$ T-cell Response

The $CD8^+$ T-cells are one of the main lymphocytes activated by the immune system as a protective mechanism in the presence of an infection (79). $CD8^+$ T-cells enhance IFN-γ release while using the QFT hence increasing the likelihood of obtaining a higher positivity rate during LTBI diagnosis (80). In addition, $CD8^+$ T-cell response has been found to be associated with active TB disease as elevated levels of $CD8^+$ T-cells have been observed among active TB

patients (77). The variation in immune response between TB infection to active TB highlight the importance of determining the association between LTBI, active TB, and the $CD8^+$ T-cell response. There is limited literature on factors affecting individuals' $CD8^+$ T-cell response, especially among those that are HIV-infected. However, the few studies done found an association between a decreased $CD8^+$ T-cell response and low CD4 cell count; having no previous TB diagnosis; increasing age; smoking; male gender, and not having a history of receiving TPT (25,79,81–85).

Smoking impairs immune response resulting in a reduced $CD8^+$ T- cell response (79,82). An increase in age comes with reduced system functionality, the immune system inclusive, therefore $CD8^+$ T-cell response decreases with an increase in age (83). Even after successful TPT, patients are likely to have a persistently elevated $CD8^+$ T-cell response (67). Overall females seem to be more "immune-privileged" than their male counterparts, nonetheless, the hormonal changes for example after menopause that come with aging lead to added risks to infection as the immune response is altered, likewise the $CD8^+$ T-cell response (83,84). HIV-infected patients with a low CD4 cell count (100 -200cells/mL^3) tend to have indeterminate QFT results and a reduced $CD8^+$ T-cell response (25).

QFT- Plus tests are prospective indicators for the categorization of various phases of MTB infection using the $CD8^+$ T-cell response that in return can be used as a surrogate marker for treatment response as well (78). $CD8^+$ T-cell can be used to characterize immune profiles into active, latent, and recently acquired and to detect disease advancement, relapse, and treatment failure (78,86). However, better diagnostic tests are required to establish better biomarkers like the inducible protein (IP-10) that is less affected by HIV infection and due to the fact that IGRA results are negatively affected by LTBI progression (75,87). Figures 1 and 2 show results obtained from the evaluation of $CD8^+$ T-cell antigens in active TB and LTBI participants (77). Figure 2 shows the individual profiles of the $CD8^+$ T-cell in HIV-negative patients from baseline to week 24 while on anti-TB therapy (78).

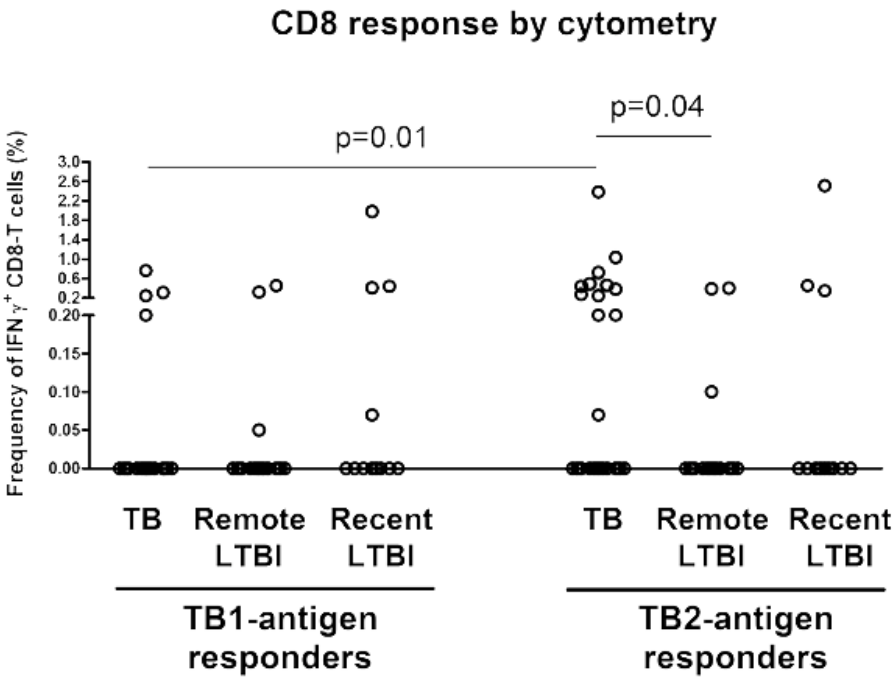


Figure 1: "Flow cytometric evaluation of CD4 and CD8+ T-cell antigens in active TB and LTBI participants with remote and recent infection. Remote infection; reported contact with a smear-positive pulmonary TB patient at least 3 years before the enrolment. Recent infection; reported contact with a smear-positive pulmonary TB patient no more than 3 months before the enrolment" (77)**.**

Figure 1 above shows that $CD8^+$ T-cell response from TB2 stimulation is higher among individuals with active TB disease as compared to those with LTBI. This is an indication that the MTB burden affects the $CD8^+$ T-cell response from TB2 stimulation (77).

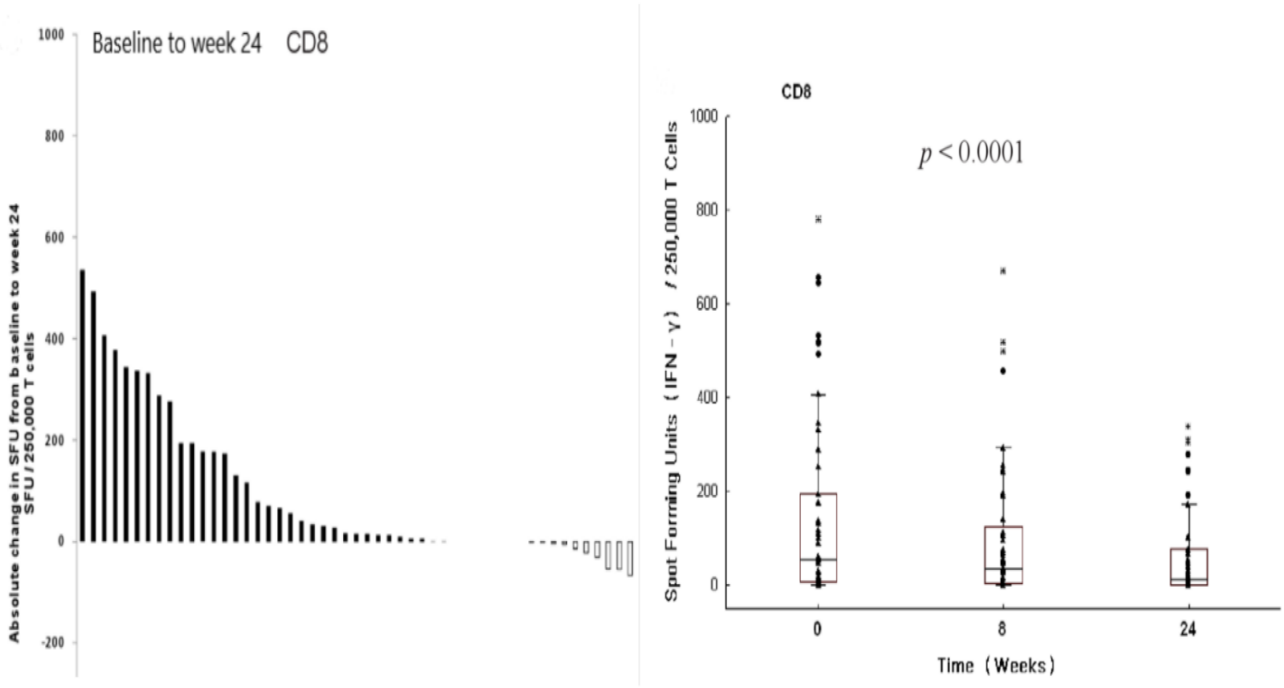


Figure 2: "Individual profiles of absolute change in the CD8$^+$ T-cell from baseline to week 24 in HIV-negative pulmonary TB patients on anti-TB treatment"(78)**.**

Figure 2 shows a steady decline of CD8$^+$ T-cell response over time among the patients on anti-TB treatment. This reinforces the suggestion to use CD8$^+$ T-cell response as an indicator of TB disease progression or reoccurrence and treatment success or failure (78).

1.2.6 Problem statement

Globally 1.7 (23%) billion people are infected with LTBI from whom active TB cases arise. HIV-infected individuals are among the populations at the highest risk of LTBI progression into active TB disease (43,88). A study done in South Africa estimated that the annual expenditure on treatment and prevention on HIV and TB would increase from US$ 2.3 billion in 2016 to US$2.9 billion in 2018 (89). Inadequate knowledge of factors associated with LTBI among HIV-positive patients in a high TB and HIV prevalent country like South Africa, poses a risk of increased TB incidence and increased annual expenditure on prevention and treatment of TB.

1.2.7 Justification

Limited literature is available on factors associated with LTBI in countries with high TB and HIV prevalence like South Africa (70). Currently, the diagnosis of LTBI using tools like IGRA tests is solely dependent on an individual's immune or T-cell response in the presence of infection (90). Knowledge of factors associated with LTBI is vital for the development of implementation strategies of the END-TB strategy, and ascertaining predictors of IGRA results will optimize the usage of more accurate diagnostic tools in clinical settings.

1.2.8 Research question

What factors are associated with latent tuberculosis infection (LTBI) among HIV-positive adults ≥ 18 years in South Africa in 2016 – 2017?

1.2.9 Aim of the study

To investigate factors associated with latent tuberculosis infection (LTBI) among HIV-positive adults ≥ 18 years in South Africa in 2016 - 2017.

1.2.9.1 Study objectives

1. To determine the prevalence of LTBI among HIV-positive adults.
2. To investigate factors associated with LTBI among HIV-positive adults.
3. To determine predictors of $CD8^+$ T-cell response among LTB infected HIV-positive adults.

CHAPTER 2: METHODS

This chapter begins with a description of the study design used and few details of the primary study, whose baseline data was used in the current study. This is followed by an explanation of the criterion used for selecting individuals used in both the primary and current study. The acquisition of the dataset and its management is discussed. In addition, the categorization of the various variables collected from the dataset is shown. The chapter ends with how the data was analyzed using selected statistical approaches.

2.1 Study design

The study was a cross-sectional study of baseline data nested within a parallel, two-part, open-label, individually randomized, pragmatic clinical trial evaluating the effect of 3 months of isoniazid and rifapentine in HIV-positive individuals ($WHIP_3TB$ clinical trial).

2.2 Study site

The primary study enrolled individuals in the study from November 2016 to November 2017. It was conducted in five facilities in South Africa at either a primary health clinic or a community health center that provides ambulatory care for HIV-positive patients. These five clinics and health centers were located in three districts: Thembisa, Rustenburg, and Klerksdorp.

Rustenburg and Klerksdorp are found in the North West province while Thembisa is found in Gauteng province. Both provinces have experienced an increase in net migration(91). The economy in Rustenburg is heavily dependant on the platinum mining industry (92). Similarly, Klerksdorp is known for the presence of the gold mining industry (93). Both districts experience a large number of diverse populations, immigrants inclusive that come to work in the mining industry. Tembisa is majorly driven by retail and property developments (94).

As of August 2019, the HIV prevalence in Gauteng was 13.05% and a similar prevalence of 13.59% in the North West province (95). The TB incidence rate in Gauteng and North West province was 330 and 528 per 100,000 respectively in 2015 (96).

2.3 Study population

The study population included HIV-positive adults (≥ 18 years) that were enrolled for the $WHIP_3$ TB trial in South Africa in 2016-2017. Inclusion criteria in the primary study included HIV-positive adults who were on ART for at least 3 months prior to study enrolment. Some of the exclusion criteria comprised: confirmed/suspected TB disease; isoniazid/rifampicin

resistance; TB treatment/ IPT within the past year; Protease Inhibitor (PI) based/ raltegravir containing ART regimens. The total number of patients enrolled in the study site are shown in Table 2.

2.4 Sampling

The primary study collected data from individuals at least two years of age and other criteria as earlier mentioned. A total of 2541 individuals were enrolled at the five South African sites. However, this study excluded individuals less than 18 years old and individuals with missing IGRA test results. The baseline data collected on the individuals at enrolment was used for the present study.

2.5 Data collection

The following case report forms (CFRs) were completed at baseline and used for the present study:

i. Baseline medical history – Self-reported
ii. Vital signs/ Physical exam – Readings and examinations were taken during enrolment.
iii. QFT results – Obtained from blood drawn from enrolled participants for testing.
iv. CD4 count – Abstracted from medical records.

The data was entered manually on Merge eClinicalOS data management system. Data for this study was obtained from the Aurum Institute in South Africa in an excel format. The data provided was anonymized.

2.6 Data management

Data obtained from the Aurum Institute was in a password protected excel format. Data was then imported into the statistical software, STATA version 15®. Data cleaning included checking variables for inconsistencies such as missing data and outliers in STATA. Cross tabulations were carried out on categorical variables to identify missing observations. Probability distribution curves were used to identify outliers among continuous variables. Categories of age group and CD4 count were created. There were no inconsistencies found in the dataset.

2.7 Measurement of variables

The study variables used in the present study were grouped into two categories, namely: explanatory variables which include: demographic variables and medical history and outcome variables which include: LTBI outcome and CD8 response.

2.7.1 Explanatory variables

a) Demographics

- **Age** – A continuous numerical variable recorded in years.
- **Gender** – A binary variable recorded as "Male" or "Female"
- **Ethnic group**- A Categorical nominal variable recorded as "Black /African", "Coloured", "Indian/Asian", "White/European" or "Other".
- **Highest Level of Education**- A Categorical ordinal variable recorded as "No School/Pre-school only" "Grade 1-3" "Grade 4-7" "Grade 8-11" "Grade 12" "Above matric".
- **Marital status**- A Categorical nominal variable recorded as "Single, never married" "Cohabitating" "Married" "Divorced" "Widowed".
- **Employment status**- A Categorical nominal variable recorded as "Employed full-time" "Informal employment/piece jobs" "Employed part-time" "Not applicable (child)" "Student/scholar/learner" "Retired/Pensioner" "unemployed" or "Other".
- **Type of job**- A categorical nominal variable that will be created and recorded as "health care worker" "prison warder/ correctional facility" or "miner"
- **Site of enrolment**- A categorical variable recorded according to clinic of enrolment.

b) Medical history

- **Previous TB diagnosis/ treatment-** A binary variable recorded as "Yes" or "No".
- **Taken IPT before -** A binary variable recorded as "Yes" or "No".
- **CD4 count**- A continuous variable recorded in cells/mL^3 categorised into two groups : "$\leq$ 200 cells/mL^3" " > 200 cells/mL^3"

c) Others

- **Ever smoked**- A binary variable recorded as "Yes" or "No".
- **Recreational drug usage**- **A** binary variable recorded as "Yes" or "No".

2.7.2 Outcome variables

- **Latent Tuberculosis Infection (LTBI) - A** binary variable created depending on the QFT results and recorded as "Positive" or "Negative".
- **CD8 response** - **A** binary variable created based on the QFT results. The CD8 response was obtained by subtracting the TB1 Ag (CD4 response) from the TB2 Ag (CD4 response + CD8 response). The cut-off value of 0.6IU/mL was used as individuals with greater or equal to 0.6IU/mL were considered to have a CD8 response and those with less than 0.6IU/mL were considered not to have a CD8 response (97).

2.8 Data Analysis

2.8.1 Objective 1- To determine the prevalence of LTBI among HIV-positive adults.

The prevalence of LTBI was calculated as a proportion (i.e. total number of participants with LTBI as the numerator and the total number of individuals with a positive or negative LTBI test result as the denominator) *100% as shown in Table 3. Descriptive statistics of the demographic characteristics and medical history of the study participants were computed. For categorical variables, proportions and frequencies were calculated and represented in tables and graphs. For continuous variables, the Shapiro Wilk test for normality was used to assess the distribution of the continuous variables and standard deviations test (sdtest) assessed equal variance(98,99). Means and standard deviations (SD), medians and interquartile ranges (IQR) were calculated and presented as appropriate. Overall descriptive statistics of enrolled individuals are shown in Table 4. Table 5 shows descriptive statistics of study participants stratified by LTBI status that explains that a test of comparison was computed to determine the difference in proportions of the outcome based on demographic characteristics.

2.8.2 Objective 2- To investigate factors associated with LTBI among HIV-positive adults.

Factors associated with LTBI among HIV-positive adults were investigated and the results obtained are presented in Table 6 of the results section.

For categorical variables, Pearson's chi-squared test was used to compare demographic characteristics and medical history proportions between HIV positive adults with LTBI and those without (100,101). A trend analysis was also carried out on the ordered categorical variables to test for presence of an ordered relationship across the outcome (102). For

continuous variables, a Wilcoxon rank-sum test was used to compare the median scores between HIV positive adults with LTBI and those without (103).

Univariable and multivariable logistic regression models were fitted to determine factors associated with latent tuberculosis among the individuals enrolled in the study (104)

2.8.2.1 Univariable analysis

To understand and determine independent associations between each explanatory variable and the outcome i.e. latent tuberculosis. Point estimates (Odds Ratio, Confidence intervals) for the univariable analysis were calculated and are presented in Table 6.

2.8.2.2 Multivariable analysis

The multivariable analysis was carried out to determine the most parsimonious model that explains the LTBI outcome. A multivariable logistic regression model was run and adjusted odds ratios (AOR) were obtained to understand the association between latent tuberculosis and chosen explanatory variables as shown in Table 6.

2.8.2.3 Selection of variables and final logistic model

All variables that did not meet the criteria of having ≥ 10 number of events per variable(EPV) were excluded (105). This criteria is also referred to as the "rule of thumb". Excluded variables included being employed as a prison or working at a correctional facility, being employed as a miner and ethnicity due to the Indian/Asian race. An additional exclusion criterion was evidence of multicollinearity in which variables exhibited linear functionality with others (106). Backward stepwise regression also known as backward selection procedure with a cut off of a p-value < 0.2 was run. Variables selected from the backward stepwise regression model were: Age, CD4 count, and previous TB diagnosis. Some of the limitations that may arise from using stepwise regression include the selection of independent variables based on chance, choosing of variables that contribute a large portion at each step, and the removal of variables whose relationship becomes insignificant on the addition of other variables (107). Due to these limitations, additional variables were included in the final model based on literature: gender, level of education, employment status, marital status, history of IPT, smoking status, and or history and recreational drug usage (40,47,55,72). The final model's fit was then examined using the likelihood ratio chi-square test by running the goodness of fit test as shown in Table 7.

2.8.2.4 Sensitivity analysis

According to the QFT manual, outcomes among HIV infected individuals indicate indeterminate results because of low $CD4^+$ T-cell count and generally test negative using the TST, therefore, indeterminate results may be considered as negative in sensitivity estimates (108). A sensitivity analysis was carried out to measure the predictive ability of the chosen logistic model with regards to classifying individuals' latent tuberculosis status. Individuals with an indeterminate test result were classified as having a negative latent tuberculosis test result and a multivariable logistic model was run. The adjusted odds ratio (AOR) of selected explanatory variables remained very similar if not the same. Therefore, the model with LTBI as a binary outcome was chosen since excluding individuals that had an indeterminate result for LTBI did not affect obtained results. Table 8 shows the results obtained from the sensitivity analysis.

2.8.3 Objective 3- To determine the predictors of $CD8^+$ T-cell response among LTB infected HIV-positive adults.

The obtained results are shown in Table 10 in the results chapter. The analysis was restricted to individuals that had tested LTBI positive as these individuals are most likely to have a $CD8^+$ T-cell response. However, four individuals that tested LTBI negative had a $CD8^+$ T-cell response and this could be attributed to the fact that a positive LTBI positive test is not solely dependent on $CD8^+$ T-cell response but other immunological responses such as the mitogen level. Continuous and categorical variables were analyzed as in objective 2 to determine the comparison of demographic and clinical characteristics among individuals with a $CD8^+$ T-cell response and those without a $CD8^+$ T-cell response as shown in Table 9.

2.8.3.1 Univariable analysis

To understand and determine independent associations between each variable and the $CD8^+$ T-cell response the odds ratio of each variable was obtained by running a univariable logistic model. The results obtained from the analysis are shown in Table 10.

2.8.3.2 Multivariable analysis

The multivariable analysis was carried out to investigate whether $CD8^+$ T-cell response was dependent on multiple explanatory variables. A multivariable logistic regression model was run and adjusted odds ratios (AOR) were obtained to understand the association between latent tuberculosis and chosen explanatory variables as shown in Table 10.

2.8.3.3 Selection of variables and final logistic model

A similar approach as in objective 2 was executed by first excluding variables that do not meet the "rule of thumb" the running a backward stepwise regression model with a cut off of a P-value < 0.2. The stepwise model selected the following variable: Age, CD4 count, whether or not the individual had ever smoked, and previous TB history. Variables were added into the final model based on previous literature: the history of receiving IPT, gender, and recreational drug usage (40,57,81,83). The goodness of fit of the final model to predict $CD8^+$ T-cell response was examined by running the goodness of fit test, and results are reported in Table 11.

For both objectives 2 and 3, the Pearson Chi-Square and Wilcoxon tests' significance were based on the P-value < 0.05. For logistic regressions, odds ratios, P-values, and 95% confidence intervals (CIs) were recorded. The significance of variables was based on all the above; Odds ratio $\neq 1$, P-value < 0.05, and 95% CI excluding 1.

2.8.4 Centering of continuous variables

Centering involves the process of getting the mean of a variable and subtracting it from all other observations on that particular variable in the dataset, making the created variable's mean zero (109). This procedure improves model fitting as it reduces multicollinearity (109)(110).

Both age and CD4 count were centered and the final chosen logistic model ran. Centering of both age and CD4 count was carried out in the effort to meaningfully interpret the odds of LTBI with age and CD4 count since neither 0 years nor 0 cells/mL^3 provide meaningful values (110). In addition, results showed that the most infected age group was the 40-49 years, therefore we were interested in examining the effect of age when an individual is within this age group and above regarding getting infected with latent tuberculosis.

Higher prevalence was associated with a unit increase above the average CD4 count however, CD4 count was categorised into two categories: "$\leq$ 200 cells/mL^3" "> 200 cells/mL^3" based on the scientific backing that HIV-infected individuals with CD4 count < 200 cells/mL^3 are more immunocompromised and this was investigated in the study (111).

2.8.5 Missing values

Overall, the explanatory variables chosen for the final model in both objectives 2 and 3 had a total of two missing values accounting for 0.08% of the total number of observations of the variables. No imputation was carried out for the missing values as there was no scientific justification to do so.

2.9 Ethical Considerations

Ethical clearance was granted by the University of the Witwatersrand Human Research Ethics Committee, Johannesburg, South Africa (Certificate number: M191061). The primary study obtained ethics approval from Wits HREC (Ref. #151110) and the London School of Hygiene and tropical medicine interventions research ethics committee (Ref. #10502).

CHAPTER THREE: RESULTS

This chapter focuses on results obtained from using chosen statistical approaches for analysis. Enrolment of individuals at the different study sites during the primary study and a flow chart of individuals included in the current study is shown. The LTBI prevalence among the study population is shown, and outcomes obtained as per the objectives of the study are explained.

3.1 Overview

A total of 2541 individuals were tested for latent tuberculosis from five sites in South Africa in the primary study. All the patients tested were HIV-positive and were on ART treatment. The total patients enrolled in the study per site are shown in Table 1 below.

Table 2: Total patients enrolled in the WHIP$_3$TB clinical trial per study site.

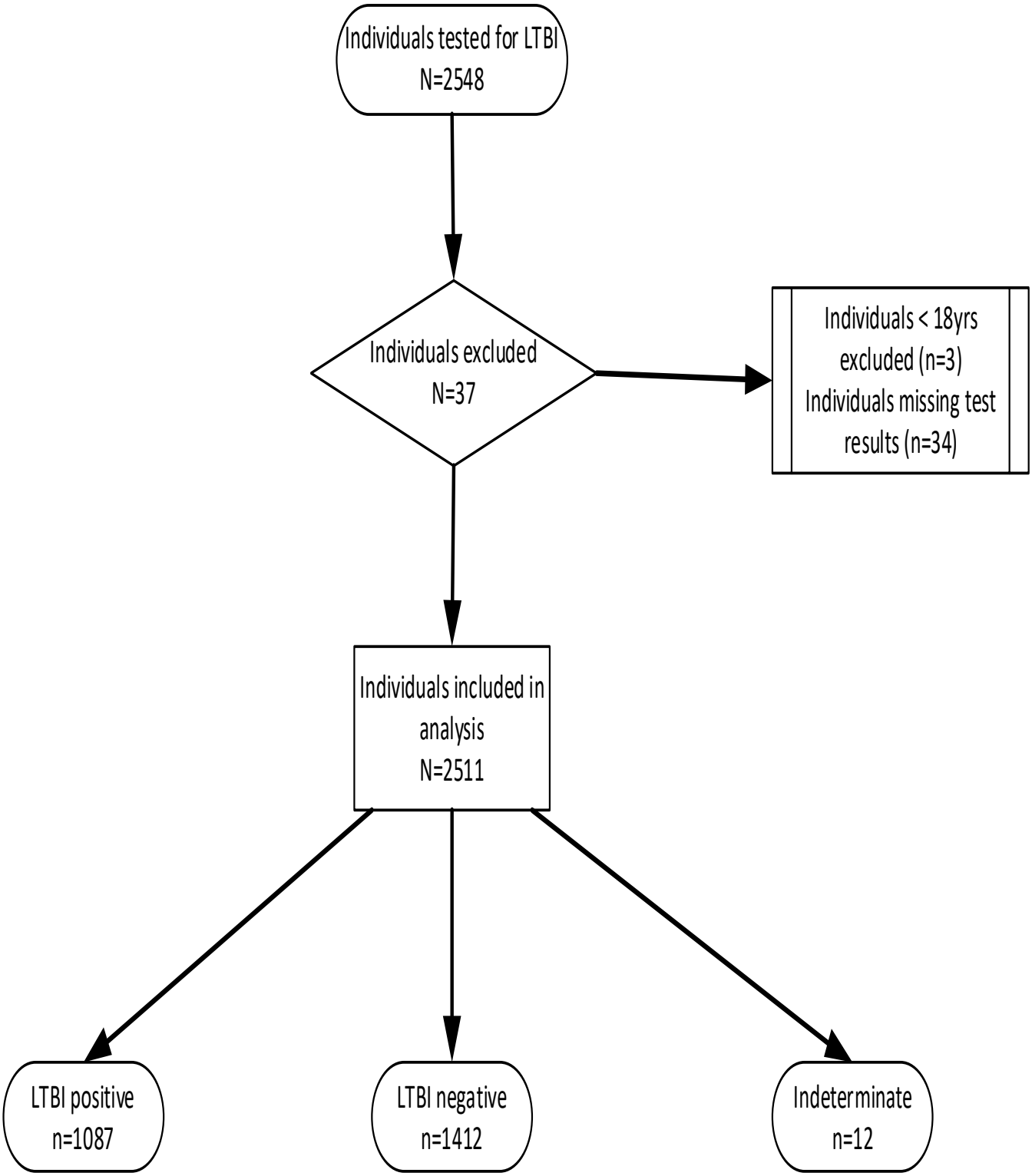
Site	**Male** N (%)	**Female** N (%)	**Total** N
1	250 (28.00)	643 (72.00)	893
2	50 (21.46)	183 (78.54)	233
3	225 (33.78)	441 (66.22)	666
4	71 (20.58)	274 (79.42)	345
5	112 (27.72)	292 (72.28)	404
Total	708 (27.86)	1833 (72.14)	2541

Overall, the latent tuberculosis prevalence was 43.50% as shown in Table 2 below.

Table 3: LTBI positives amongst participants selected for analysis in the study.

	LTBI positive		
Total of LTBI positive and negative results	**N**	**%**	**95% Confidence interval**
2499	1087	43.50	41.54 – 45.47

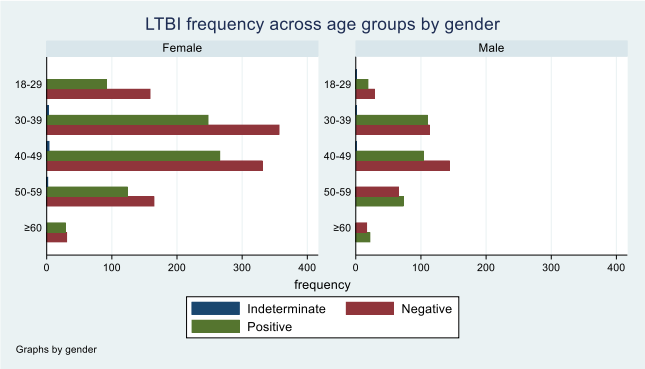
Figure 3: Flow chart of patients enrolled in the study and included in the analysis.



3.2 Distribution of individuals tested for LTBI

Figure 4 shows the overall distribution of individuals tested across age groups by gender. Overall, there were more females than males tested. Most of the females tested were in the 30-39 years age group while most males fell in the 40-49 years age group.

Figure 4: Bar graph showing the distribution of individuals tested for LTBI



3.3 Socio-demographic and clinical characteristics of enrolled individuals.

Table 4 shows the overall distribution of individuals tested. The majority of the individuals were; females (n=1811, 72.12%) ; aged 40-49 years (n=850, 33.85%, overall mean age of 41 years(SD=9.9) and median age of 41years(IQR=34-48); attained a grade 8-11 education level (n=1356, 54.00%); single/ never married (n=1449, 57.71%); were of black/African ethnicity (n=2487, 99.04%); unemployed (n= 1298, 51.69%); had not taken IPT before (n=2150, 85.62%); had no previous TB diagnosis (n=1891, 75.31%); had a CD4 count of above 200 cells/mL^3 (n=1972, 77.58%, overall mean CD4 count of 475.69 cells/mL^3 (SD=273.10) and median CD4 count of median 440 cells/mL^3(IQR=280-630.50 cells/mL^3).

Table 4: Characteristics of the HIV-positive adults analysed in the study (N=2511).

Variable	Category	Total (n)	Percentage (%)
Gender	Male	700	27.88
	Female	1811	72.12
Age groups(years)	18-29	300	11.95
	30-39	832	33.13
	40-49	850	33.85
	50-59	430	17.12
	60+	100	3.93
	Mean age (SD)	41.30 (9.92)	
	Median age (IQR)	41 (34-48)	
Highest level of education	No School/Pre-school only	50	1.99
	Grade 1-3	89	3.54
	Grade 4-7	383	15.25
	Grade 8-11	1356	54.00
	Grade 12	574	22.86
	Matric with Technical Qualification or Diploma	45	1.79
	Above matric	12	0.48
	Missing	2	0.08
Ethnic group	Black /African	2487	99.04
	Coloured	18	0.72
	Indian/Asian	4	0.16
	Missing	2	0.08
Marital status	Single, never married	1449	57.71
	Cohabitating	211	8.40
	Married	606	24.13
	Divorced	95	3.78
	Widowed	148	5.89
	Missing	2	0.08
Employment status	Employed full-time	596	23.74
	Informal employment/piece jobs	203	8.08
	Employed part-time	258	10.27
	Retired/Pensioner	75	2.99
	Unemployed	1298	51.69
	Other	79	3.15
	Missing	2	0.08
Taken IPT before	No	2150	85.62
	Yes	359	14.30
	Missing	2	0.08
Previous TB diagnosis	No	1891	75.31
	Yes	618	24.61
	Missing	2	0.08

Table 4 continued

Variable	Category	Total (n)	Percentage (%)
CD4 count	≤ 200 cells/mL[3]	336	13.38
	> 200 cells/mL[3]	1948	77.58
	Missing	227	9.04
	Mean age (SD)	475.7 (273.10)	
	Median age (IQR)	439 (280-633)	
Site	Thembisa	1939	77.22
	Rustenburg	232	9.24
	Klerksdorp	340	13.54
Health care worker	No	1015	40.42
	Yes	42	1.67
	Missing	1454	57.91
Prison warder/ correctional facility	No	1055	42.02
	Yes	2	0.08
	Missing	1454	57.91
Miner	No	1051	41.86
	Yes	6	0.24
	Missing	1454	57.91

1

[1] Individuals that were students, retired or unemployed were not further asked if they were employed as health workers, prison wardens or miners hence these have equal number of missing variables.

IPT- Izoniazid Preventive Therapy.

3.3.1 Demographic characteristics of HIV-positive adults by latent tuberculosis status

Of the 2511 individuals included in the analysis, a total of 1087 (43.29%) tested positive, 1412 (56.23%) tested negative, and 12 (0.48%) had indeterminate test results.

Table 5 shows demographic characteristics stratified by LTBI status. There was a significant difference in proportions of LTBI positive individuals based on their level of education, previous TB diagnosis, CD4 count, and site in which an individual was enrolled. From the trend analysis, there was statistically significant evidence of a trend between increase in age and being LTBI positive. There was no trend between level of education and LTBI as LTBI positivity seemed to decrease with education but later increase.

Table 5: Characteristics of the HIV-positive adults stratified by LTBI status (N=2499).

Variable	Category	LTBI -ve n (%) N= 1412	LTBI +ve n (%) N= 1087	P-value
Gender	Male	369 (52.94)	328 (47.06)	0.03
	Female	1043 (57.88)	759 (42.1)	
Age groups(years)	18-29	188 (62.88)	111 (37.12)	0.07
	30-39	470 (56.76)	358 (43.24)	
	40-49	475 (56.21)	370 (43.79)	P-trend =0.01
	50-59	231 (53.97)	197 (46.03)	
	60+	48 (48.48)	51 (51.52)	
	Mean age(SD)	40.84 (9.93)	41.79 (9.87)	
	Median age (IQR)	40 (34-48)	41 (34-48)	
Highest level of education	No School/Pre-school only	34 (68.00)	16 (32.00)	0.04
	Grade 1-3	44 (50.00)	44 (50.00)	
	Grade 4-7	218 (57.22)	163 (42.78)	P-trend =0.75
	Grade 8-11	738 (54.67)	612 (45.33)	
	Grade 12	349 (60.91)	224 (39.09)	
	Above matric	27 (49.09)	28 (50.91)	
Ethnic group	Black /African	1395 (56.36)	1080 (43.64)	
	Coloured	13 (72.22)	5 (27.78)	0.39
	Indian/Asian	2 (50.00)	2 (50.00)	
Marital status	Single, never married	807 (56.00)	634 (44.00)	0.51
	Cohabitating	111 (52.86)	99 (47.14)	
	Married	355 (58.77)	249 (41.23)	
	Divorced	55 (58.51)	39 (41.49)	
	Widowed	82 (55.41)	66 (44.59)	
	Missing	2 (100.00)	0 (0.00)	

Table 5 Continued

Employment status	Employed full-time	340 (57.53)	251(42.47)	0.80
	Informal employment/piece jobs	114 (56.16)	89 (43.84)	
	Employed part-time	137 (53.10)	121(46.90)	
	Retired/Pensioner	40 (54.05)	34 (45.95)	
	Unemployed	731 (56.58)	561(43.42)	
	Other	30 (61.22)	19 (38.78)	
	Missing	2 (100.00)	0 (0.00)	
Site	Thembisa	1084 (56.14)	847 (43.86)	0.25
	Rustenburg	141 (61.57)	88 (38.43)	
	Klerksdorp	187 (55.16)	152 (44.84)	
Taken IPT before	No	1191 (55.71)	947 (44.29)	0.08
	Yes	219 (61.00)	140 (39.00)	
	Missing	2 (100.00)	0 (0.00)	
Previous TB diagnosis	No	1103 (58.64)	778 (41.36)	<0.001
	Yes	307 (49.84)	309 (50.16)	
	Missing	2 (100.00)	0 (0.00)	
CD4 count	≤ 200 cells/mL^3	213 (63.39)	120 (35.71)	<0.01
	> 200 cells/mL^3	1064 (54.62)	877 (45.02)	
	Missing	133 (59.11)	90 (40.00)	
	Mean (SD)	456.84 (264.84)	499.52 (280.63)	
	Median (IQR)	426 (262-614)	461 (308-652)	
Health care worker	No	564 (55.84)	446 (44.16)	0.54
	Yes	27 (64.29)	15 (35.71)	
	Missing	821 (56.74)	626 (43.26)	
Prison warder/ correctional facility	No	590 (56.19)	460 (43.81)	0.95
	Yes	1 (50.00)	1 (50.00)	
	Missing	821 (56.74)	626 (43.26)	
Miner	No	588 (56.21)	458 (43.79)	0.94
	Yes	3 (50.00)	3 (50.00)	
	Missing	821 (56.74)	626 (43.26)	

3.4 Factors associated with latent tuberculosis

Out of the 2511 individuals tested, 12(0.48%) had indeterminate results. Individuals with indeterminate results were excluded since the sensitivity analysis showed no difference in point estimates after the removal of these individuals versus including them as negatives. LTBI was considered a binary outcome with LTBI positive and negative individuals.

From the univariate analysis, the following variables were found to have a significant association with greater chance of latent tuberculosis infection: male gender (OR=1.22, 95%CI:1.02-1.46), increasing age (OR=1.01, 95%CI:1.00-1.02), individuals that had previously diagnosed or treated for TB (OR=1.43, 95%CI:1.19-1.71), and CD4 count greater than 200cells/mL^3 (OR=1.46, 95%CI:1.15-1.86). Factors not associated with LTBI included: whether the individual was a health worker; prison warden or miner; individuals' highest level of education; ethnic group; marital status; employment status; whether the individual had taken IPT before or not; and recreational drug usage.

The multivariate analysis controlled for age, gender, education level, marital status, employment status, history of IPT, previous TB diagnosis, CD4 count, history of smoking and recreational drug use. LTBI was considered to be associated with: increase in age (OR=1.02,95%CI 1.00-1.03), individuals that had been previously diagnosed or treated for TB (OR=1.34, 95%CI:1.10-1.63), and individuals with a CD4 count greater than 200cells/mL^3 (OR=1.59, 95% CI:1.24-2.03).

Table 6: Factors associated with Latent Tuberculosis Infection (LTBI) (N=2274).

Variables	Categories	Univariable Analysis		Multivariable Analysis	
		OR (95% CI)	P-value	AOR (95% CI)	P-value
Age	Centered Age	1.01(1.0-1.02)	0.02	1.02 (1.0-1.03)	0.01
Gender	Female	1.00		1.00	
	Male	1.22 (1.02-1.46)	0.03	1.17 (0.94-1.46)	0.17
Health care worker	No	1.00		-	-
	Yes	0.70 (0.37-1.34)	0.28	-	-
Prison warder/ correctional facility	No	1.00		-	-
	Yes	1.28 (0.08-20.56)	0.86	-	-
Miner	No	1.00		-	-
	Yes	1.28 (0.26-6.39)	0.76	-	-
Highest level of education	No School/Pre-school only	1.00		1.00	
	Grade 1-3	2.13 (1.03-4.39)	0.04	2.23 (1.02-4.89)	0.05
	Grade 4-7	1.59 (0.85-2.98)	0.15	1.35 (0.81-3.09)	0.18
	Grade 8-11	1.76 (0.96-3.22)	0.07	1.91 (0.10-3.66)	0.05
	Grade 12	1.36 (0.74-2.53)	0.33	1.59 (0.82-3.11)	0.17
	Matric with Technical Qualification or Diploma	1.85 (0.79-4.30)	0.15	2.38 (0.98-5.81)	0.04
	Above matric	4.25 (1.11-16.21)	0.03	8.99 (1.59-50.68)	0.01
Ethnic group	Black /African	1.00		-	-
	Coloured	0.50 (0.18-1.40)	0.19	-	-
	Indian/Asian	1.29 (1.82-9.18)	0.80	-	-
Marital status	Cohabitating	1.00		1.00	
	Single, never married	0.88 (0.66-1.18)	0.39	0.86 (0.63-1.17)	0.35
	Married	0.79 (0.57-1.08)	0.14	0.68 (0.49-0.96)	0.03
	Divorced	0.80 (0.49-1.30)	0.36	0.56 (0.33-0.95)	0.03
	Widowed	0.90 (0.59-1.38)	0.63	0.73 (0.45-1.16)	0.18

Table 6 Continued

Variables	Categories	Univariable Analysis		Multivariable Analysis	
		OR (95% CI)	P-value	AOR (95% CI)	P-value
Employment status	Employed full-time	1.00		1.00	
	Informal employment/piece jobs	1.06 (0.77-1.46)	0.73	0.99 (0.70-1.39)	0.94
	Employed part-time	1.20 (0.89-1.61)	0.23	1.15 (0.84-1.58)	0.37
	Retired/Pensioner	1.15 (0.71-1.87)	0.57	0.83 (0.47-1.48)	0.54
	unemployed	1.04 (0.85-1.27)	0.70	1.05 (0.85-1.31)	0.63
	Other	0.87 (0.54-1.41)	0.59	0.81 (0.48-1.36)	0.42
Site	Rustenburg	1.00		-	-
	Thembisa	1.25 (0.95-1.66)	0.12	-	-
	Klerksdrop	1.30 (0.93-1.83)	0.13	-	-
Taken IPT before	No	1.00		1.00	
	Yes	0.80 (0.64-1.01)	0.06	0.80 (0.63-1.03)	0.08
Previous TB diagnosis	No	1.00		1.00	
	Yes	1.43(1.19-1.71)	<0.001	1.34(1.10-1.63)	<0.01
Ever smoked	No	1.00		1.00	
	Yes	1.27(1.03-1.57)	0.03	1.09(0.83-1.43)	0.53
CD4 Count	$\leq$ 200 cells/mL^3	1.00		1.00	
	> 200 cells/mL^3	1.46(1.15-1.86)	<0.01	1.60(1.25-2.05)	<0.001
Recreational drug usage	No	1.00		1.00	
	Yes	1.15(0.78-1.69)	0.48	1.13(0.72-1.80)	0.59

3.4.2 Goodness of fit of the selected model

The null hypothesis of the test is that the model is of good fit and the alternate hypothesis is the model is not of a good fit. From the results shown in Table 7, a P-value of > 0.05 indicates that our model is of a good fit.

Table 7: Goodness of fit test results.

Number of observations	Number of covariate patterns	Pearson chi2	P-value
2274	1765	1748.30	0.45

2

[2] Variables included in the final model were age, gender, education level, marital status, employment status, history of IPT, previous TB diagnosis, CD4 count, history of smoking and recreational drug use.

Variables excluded in the final model are represented by (-). Working as a prisoner or a miner and ethnicity particularly the Indian/Asian race were excluded as they didn't fulfil the criteria of at least 10 events per variable. Site and working as a health worker was not selected in the stepwise model and their addition to the model didnt not improve it.

IPT- Izoniazid Preventive Therapy

Results from the Hosmer-Lemeshow statistic were: Hosmer-Lemeshow chi2: 8.95, P-value: 0.34

3.5 Sensitivity analysis

As earlier discussed, the sensitivity analysis was carried out to determine whether the exclusion of individuals that had an indeterminate QFT test result changes obtained point estimates in the final model. Individuals with indeterminate results are considered to have obtained negative results in the analysis.

Excluding individuals with indeterminate results and including them into the analysis as negative results yields similar significant and insignificant variables with LTBI, as shown in Table 8 below.

Table 8: Table showing results from sensitivity analysis (N=2284).

Outcome - LTBI +ve/ -ve & Indeterminate			
Variables	**Categories**	**AOR (95%CI)**	**P-value**
Age	Centered Age	1.02 (1.0-1.03)	0.01
Gender	Female	1.00	
	Male	1.17 (0.94-1.46)	0.17
Highest level of education	No School/Pre-school only	1.00	
	Grade 1-3	2.17 (0.99-4.73)	0.05
	Grade 4-7	1.57 (0.80-3.07)	0.19
	Grade 8-11	1.90 (0.99-3.64)	0.05
	Grade 12	1.60 (0.82-3.12)	0.17
	Above matric	2.71 (1.16-6.32)	0.02
Marital status	Cohabitating	1.00	
	Single, never married	0.86 (0.63-1.18)	0.35
	Married	0.69 (0.49-0.97)	0.03
	Divorced	0.55 (0.32-0.95)	0.03
	Widowed	0.74 (0.46-1.18)	0.20
Taken IPT before	No	1.00	
	Yes	0.81 (0.63-1.04)	0.10
Previous TB diagnosis	No	1.00	
	Yes	1.34 (1.10-1.64)	<0.01
Ever smoked	No	1.00	
	Yes	1.09 (0.83-1.43)	0.52
Recreational drug usage	No	1.00	
	Yes	1.14 (0.722-1.81)	0.57
CD4 Count	≤ 200 cells/mL^3	1.00	
	> 200 cells/mL^3	1.61 (1.26-2.07)	<0.001

3.6. Individual characteristics by CD8$^+$ T-cell status.

Overall a total of 211(19.41%) individuals tested for LTBI had a CD8 response. Table 10 shows the characteristics of the individuals tested for LTBI by CD8 status. There was a significant difference in the proportion of CD8 response based on previous TB diagnosis. A greater proportion of participants with no history of previous TB diagnosis had no CD8 response compared to those that had a response. More individuals with a negative QFT result had no CD8 response in comparison to those that had a response.

Table 9: Characteristics of the HIV-positive adults stratified by CD8$^+$ T-cell status (N=1087).

Variables	Categories	CD8$^+$ T-cell Status		P-value
		No response n (%)	Response n (%)	
Age groups	18-29	99 (89.19)	12 (10.81)	0.09
	30-39	277 (77.37)	81 (22.63)	
	40-49	302 (81.62)	68 (18.38)	
	50-59	157 (79.70)	40 (20.30)	
	60+	41 (80.39)	10 (19.61)	
Gender	Male	263 (80.18)	65 (19.82)	0.82
	Female	613 (80.76)	146(19.24)	
CD4 Count	≤ 200 cells/mL[3]	97 (80.83)	23 (19.17)	0.82
	> 200 cells/mL[3]	701 (79.93)	176 (20.07)	
Recreational drug usage	No	832 (80.31)	204 (19.69)	0.29
	Yes	44 (86.27)	7 (13.73)	
Taken IPT before	No	764 (80.68)	183 (19.32)	0.85
	Yes	112 (80.00)	28 (20.00)	
Previous TB diagnosis	No	646 (83.03)	132 (16.97)	<0.01
	Yes	230 (74.43)	79 (25.57)	
Ever smoked	No	720 (80.90)	170 (19.10)	0.58
	Yes	156 (79.19)	41 (20.81)	

3

[3] IPT- Izoniazid Preventive Therapy

3.7. Predictors of $CD8^+$ T-cell response among LTBI positive individuals.

[4]In the univariate analysis, the CD8 response was significantly associated with an individual having a previous TB diagnosis or treated for TB (OR=1.93, 95% CI:1.44-2.59) diagnosis. Risk factors that were not associated with $CD8^+$ T-cell response were age; gender; CD4 count; recreational drug usage; a history of IPT; and if an individual had smoked.

The multivariate analysis controlled for age, gender, CD4 count, recreational drug usage, history of IPT, previous TB diagnosis and history of smoking. The risk factor associated with $CD8^+$ T-cell response was individual that had ever been diagnosed or treated for TB was most likely to have a CD8 response (OR=1.85, 95%CI:1.35-2.53).

Table 10: Predictors of the $CD8^+$ T-cell response (N=997).

Variables	Category	Univariable Analysis		Multivariable Analysis	
		OR (95% CI)	P-value	AOR (95% CI)	P-value
Age	Centered Age	1.01 (1.0-1.02)	0.27	1.00 (0.99-1.02)	0.75
Gender	Female	1.00		1.00	
	Male	1.04 (0.75-1.44)	0.82	1.05 (0.73-1.55)	0.80
CD4 Count	≤ 200 cells/mL^3	1.00		1.00	
	>200 cells/mL^3	1.06 (0.65-1.72)	0.82	1.08 (0.66-1.76)	0.77
Recreational drug usage	No	1.00		1.00	
	Yes	0.65(0.29-1.46)	0.30	0.56 (0.23-1.35)	0.20
Taken IPT before	No	1.00		1.00	
	Yes	1.04 (0.67-1.62)	0.85	1.10 (0.69-1.77)	0.68
Previous TB diagnosis	No	1.00		1.00	
	Yes	1.68 (1.22-2.31)	<0.01	1.68 (1.20-2.35)	<0.01
Ever smoked	No	1.00		1.00	
	Yes	1.11 (0.76-1.63)	0.58	1.19 (0.75-1.89)	0.46

3.7.1 Goodness of fit of the selected model

The null hypothesis of the test is that the model is of good fit and the alternate hypothesis is the model is not of a good fit. From the results shown in Table 11, a P-value of > 0.05 indicates that our model is of a good fit.

Table 11: Goodness of fit test results.

Number of observations	Number of covariate patterns	Pearson chi2	P-value
997	402	353.92	0.93

[4] IPT- Izoniad Preventive Therapy

Results from the Hosmer-Lemeshow statistic were: Hosmer-Lemeshow chi2: 2.83, P-value: 0.94

In this study, we set out to determine the LTBI prevalence, factors associated with LTBI, and predictors of $CD8^+$ T-cell response among HIV-positive adults. The prevalence of LTBI infection was estimated to be 43.50% (95%CI; 43.54 - 45.47). Increasing age, previous TB diagnosis or treatment, and higher CD4 cell count were associated with increased LTBI. An individual having a previous TB diagnosis or treatment was the only significant predictor of $CD8^+$ T-cell response.

4.1 Prevalence of Latent Tuberculosis Infection among HIV-positive adults.

The burden of LTBI among the HIV-positive adults in this study was high, with an overall prevalence of 43.50%. A similarly high prevalence was obtained from a study in Ethiopia among people living with HIV (PLWH) that observed an LTBI prevalence of 44% (49). Studies done in Zambia in a population-based study and an urban settlement in Uganda observed a slightly higher LTBI prevalence with nearly half the study population being infected with latent tuberculosis (48% in Zambia and 49% in Uganda) (47,50). On the other hand, a lower LTBI prevalence was observed in a study carried out in a commercial city in Mozambique that observed a prevalence of approximately 34% (48). The lower prevalence reported in Mozambique may be due to the small study sample size.

The 43.50% prevalence obtained in this study lies within the range obtained from the number of other studies conducted in South Africa that sought the LTBI prevalence, whose results ranged from 29.2% to 64% (51–54). These studies were carried out in different HIV-infected and/ or HIV-uninfected populations comprising adolescents, pregnant women, health care workers, and residents of an informal urban settlement. The studies with a lower prevalence than obtained in our study included a community-based survey study among residents of an informal urban township that included both HIV-infected and uninfected individuals (34%)(54). On the other hand, studies that observed a higher prevalence were: a systematic and metaanalysis study among health workers (47%) (53), a cross-sectional study among HIV-positive pregnant women enrolled in a randomised clinical trial (QFT-50%, TST- 70%) (52) and, a community survey study among adolescents aged 12-18 years (QFT- 50.9%, TST-55.2%) (51). The observed range of LTBI prevalence shows that LTBI prevalence is highly variable among study populations in South Africa. Prevalence variation could be attributed to the different study designs, study population, sample size, and LTBI diagnostic tools as some studies used LTBI results obtained from the TST.

The high prevalence obtained from this study confirms that South Africa is among the high TB endemic countries according to WHO (6). Studies done in low TB burden countries observed that study participants that were from high TB endemic countries were most likely to have LTBI (43,58). Since HIV-positive individuals are immunocompromised, this makes them more prone to LTBI and studies have identified being HIV positive as one of the LTBI risk factors (6,38,70). Results from our study indicate that a high proportion of individuals qualify for TB preventive therapy, and this may provide evidence to the policy that testing for LTBI is not necessary to determine whom to put on TPT.

4.2 Factors associated with LTBI among HIV-positive adults

A higher LTBI prevalence was significantly associated with increasing age, having had previous TB diagnosis or treatment, and having a CD4 count greater than 200cells/mL^3 from the multivariable analysis. The increase of LTBI prevalence with increasing age indicates a cumulative risk of infection as an individual gets older (47). This finding is consistent with studies done incomparably high endemic TB settings in an urban setting in Uganda and Western Cape, South Africa (47,51). Similarly, an increase in age was a risk factor of LTBI in studies done in Singapore, South Korea, and Canada; countries with a relatively lower TB burden compared to South Africa (55,57,60). These results show that regardless of whether an individual resides in a low or high TB endemic country, as one gets older, there is an increased possibility of LTBI. Since the infection is airborne, an increase in age translates into increased exposure to infectious air particles.

Individuals with a CD4 count greater than 200cells/mL^3 have an improved immunological status (71). Therefore, such individuals produce an adequate immune response in the presence of infection hence having a higher prevalence than those with a lower CD4 count (71). Due to the inadequate immune response among individuals with a lower CD4 count, the weaker IFN-γ response often results in indeterminate QFT results (25). This study alongside other studies observed similar findings, with individuals with a CD4 count of greater than 200cells/mL^3 being more likely to be LTBI positive (70,71). This provides some difficulty with the interpretation of QFT results, as it is likely that results are heavily dependent on the immune response rather than exposure to TB. This adds further evidence to disregarding the LTBI status when administering TB preventive therapy, that is known to have benefit by reducing TB incidence in patients with HIV.

Inconsistent findings have been observed in reference to the association between previous TB diagnosis or treatment and LTBI prevalence. Similar to our study, a study done in China with an aim of measuring IFN-γ response to active TB treatment, reported a higher LTBI prevalence in individuals with a previous TB diagnosis or treatment (64). The study observed an increased IFN-γ response after individuals completed TB treatment and attributed it to the continuous presence of T-cells arising from the primary TB infection (64). On the other hand, several studies have shown that reduced bacterial load due to the anti-TB treatment resulted in a decline in IFN-γ response hence a lower LTBI prevalence among individuals with a history of previous TB treatment (61–63). These conflicting findings have similarly been observed in the association between LTBI prevalence and an individual receiving IPT (65,66). In our study, an averagely high LTBI positivity (39%) was observed among individuals with a history of IPT. Despite this proportion being insignificant according to the Pearson-Chi test, it is likely that these individuals had lasting IFN-γ responses after IPT resulting in LTBI positive results (67). Another possibility is individuals could have been infected with LTBI after receiving IPT since individuals who had received IPT within the last year were excluded from the study.

Additionally, an individual having a history of IPT showed lower odds of LTBI in our study. However, this finding was not significant. These inconsistencies in findings could be attributed to the difference in the time between TB or IPT treatment completion and the time when individuals' IFN-γ response is investigated. Therefore, other studies need to be done to understand the IFN-γ response before, during, and after LTBI treatment to better inform future TPT roll-outs.

An individual having ever smoked and being a male were associated with a higher LTBI prevalence but this association was not shown when controlled for other factors. Smoking alters immune responses; making smokers more prone to latent tuberculosis infection hence a higher LTBI prevalence (82). Nonetheless, a study conducted in South Africa among HIV-infected individuals reported a lower LTBI prevalence among smokers due to the reduced production of interferon (69). Due to the social, cultural, and biological determinants of MTB transmission, LTBI prevalence disparities between males and females can be used to understand the higher prevalence seen among the males (83,84).

4.3 Predictors of $CD8^+$ T-cell response among HIV-positive adults.

In our study, $CD8^+$ T-cell response was an almost two-fold increase in individuals having been previously diagnosed or treated for TB. These findings are similar to those carried out in a cohort study in China, whose observation was despite anti-TB treatment completion, the survival of T-cells, $CD8^+$ T-cells inclusive was observed (64). In addition, $CD8^+$ T-cell response has been found to be associated with $CD4^+$ T-cell response (77). Therefore, individuals that have been on previous TB treatment may have recouped their $CD4^+$ T-cell count resulting into an increased $CD8^+$ T-cell response. On the contrary, other studies observed a decline in MTB specific T-cells starting from two months into anti-TB treatment; they reported that the decline was due to the decreased bacterial load from treatment leading to a reduction in the immune response in form of low $CD8^+$ T-cells (62,65,78).

Similar contradicting results have been observed in other studies in reference to the association between an individual having an IPT history and having a $CD8^+$ T-cell response (67,85). Having a history of IPT made an individual more likely to have a CD8 response in this study, however, this association was not statistically significant. These inconsistencies in findings may be a result of the variation in time between when individuals complete TB or IPT treatment and when their $CD8^+$ T-cell response is examined. In addition, since a $CD8^+$ T-cell response is a form of an immune response, studies done in different study populations are likely to report contradicting results as a population that is immune-compromised for instance HIV-infected patients would have a varying immune response compared to their non-infected counterparts. The study findings in our study are inconclusive and could benefit from findings from other studies to draw conclusions on the T-cell kinetics among individuals before, during, and after TB or LTBI treatment.

4.4 Study strengths and limitations.

Our study had a number of strengths which included firstly, it being among the first studies in South Africa using results obtained from using IGRA tests for LTBI diagnosis among HIV-infected individuals. Secondly, the data used in our study was collected from five sites in South Africa, allowing for more representation of results obtained from the study. Furthermore, our study provides some insight on predictors of $CD8^+$ T-cell response in HIV-positive adults; a population considered to be immune-compromised therefore having an increased likelihood of immune response impairment.

Some of the limitations of this study are as follows. Firstly, because of the cross-sectional nature of the study, causality between factors associated with LTBI and predictors of $CD8^+$ T-cell response cannot be established. In addition, the limitation of carrying out secondary data analysis from a selected sample of the main study whose aim was not to address the questions of this sub-study. Some information such as a history of BCG vaccination and its association with LTBI prevalence could have been of importance in the interpretation of results in our study however, it was not collected by the primary study. Nonetheless, most factors described elsewhere in other studies were available.

Additionally, there was a limitation in how smoking and illicit drug use were measured. As individuals were asked if they had ever smoked or used illicit drugs. These may not be providing an appropriate measure of the present risk of LTBI. Furthermore, such questions are prone to both recall and social desirability bias. These factors could have led to the underestimation of the association between smoking or illicit drug use with LTBI and $CD8^+$ T-cell response.

There is also a limitation of having used a single IGRA measurement for determining the LTBI prevalence or prognosis. As earlier discussed, the test is reliant on an individual's immune response. Therefore, individuals with an immune system that was heavily compromised could have gone undetected leading to the underestimation of LTBI prevalence and progression to TB disease among these individuals.

Limitations arising from using stepwise regression during analysis include the selection of variables by chance, variables chosen based on the portion they contribute at each step, and insignificance of certain variables in the presence of other variables. There were missing values since some of the data was obtained from medical records. Also, the study was prone to recall bias as some demographic data and HIV medical history were self-reported. Lastly results from the study may not be used as inference to other HIV-positive groups like pediatrics and pregnant women.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

Prevention of TB through LTBI treatment is an essential measure to reduce TB mortality and morbidity in countries with high TB and HIV burden like South Africa (14). Our study found that almost half of the participants had LTBI (43.50%). This is a clear indication of the need for more awareness programmes, especially among HIV patients and among health care professionals in facilities that offer HIV services. These programmes will encourage medical-seeking behavior, ensure timely diagnosis, and address any perspectives that hinder uptake and provision of TPT in an effort to decrease the TB disease reservoir. In addition since approximately half of the HIV-infected individuals would be LTBI positive and it is unclear whether those that are LTBI negative have not been exposed since advanced HIV is highly correlated with LTBI negativity. This supports the current guidelines that all HIV-infected individuals should be started on LTBI treatment. Furthermore, the reason for this recommendation was to reduce the multiple visits, costs and obstacles regarding LTBI testing (38). Additionally, such a high prevalence shows the necessity of ramping up TPT in order to achieve the set goals of putting an end to TB.

WHO recommends a country-specific selection of populations at high risk of LTBI according to the TB epidemiology and transmission as such prioritization would enable the health systems to cost-effectively and efficiently use resources (38). Our study involved HIV-infected individuals that are among the high-risk populations. The results showed that LTBI seemed to have reduced among individuals with a low CD4 count indicating that lower immunity is more likely to cause a negative LTBI test result. The inability of individuals with a low CD4 count to produce an optimal immunological response necessary for LTBI diagnosis shows that usage of QFT in individuals in the HIV advanced stage is unreliable. In addition, research should be intensified on how to improve QFT as a diagnostic tool or invention of alternative tools.

LTBI prevalence increased with increasing age in our study. This finding may be used to inform vaccine trials as the investigators should consider younger populations since they are less likely to be infected. Our study found a small proportion of those who tested positive that had a positive $CD8^+$ T-cell response. This illustrates that a positive $CD8^+$ T-cell response may be an additional tool during LTBI screening and testing by using it as a biomarker to identify individuals at highest risk of developing TB disease. Therefore, the revision of clinical algorithms is recommended as this will guide health care workers on the next steps to follow

to ensure close monitoring of the individuals at highest risk of disease progression. Furthermore, patients should be educated to be on the lookout for any early signs and symptoms of TB disease for timely intervention.

Current and future research is vital in informing and framing of TPT and attainment of End-TB set goals as this will result in the development of clear, feasible, and evidence-based policies. Therefore, results obtained from this study add to the limited literature available on LTBI and QFT in countries with high TB and HIV prevalence. Furthermore, results should guide the implementation of TPT and facilitate the planning of TB vaccine trials.

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APPENDICES

Appendix 1: Senate Plagiarism Policy

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Nagudi Galenda Jeniffer (Student number: 2245405) am a student registered for the degree of Epidemiology and Biostatistics in the academic year 2019.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 15/04/2021

Appendix 2: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Galenda Jeniffer Nagudi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191061

NAME: (Principal Investigator)	Galenda Jeniffer Nagudi
DEPARTMENT:	Public Health
PROJECT TITLE:	Factors associated with latent tuberculosis among HIV positive adults>=18 years in South Africa in 2016-2017
DATE CONSIDERED:	25/10/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Salome Charalambous and Leeanne Masilela
APPROVED BY:	Dr. C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/11/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

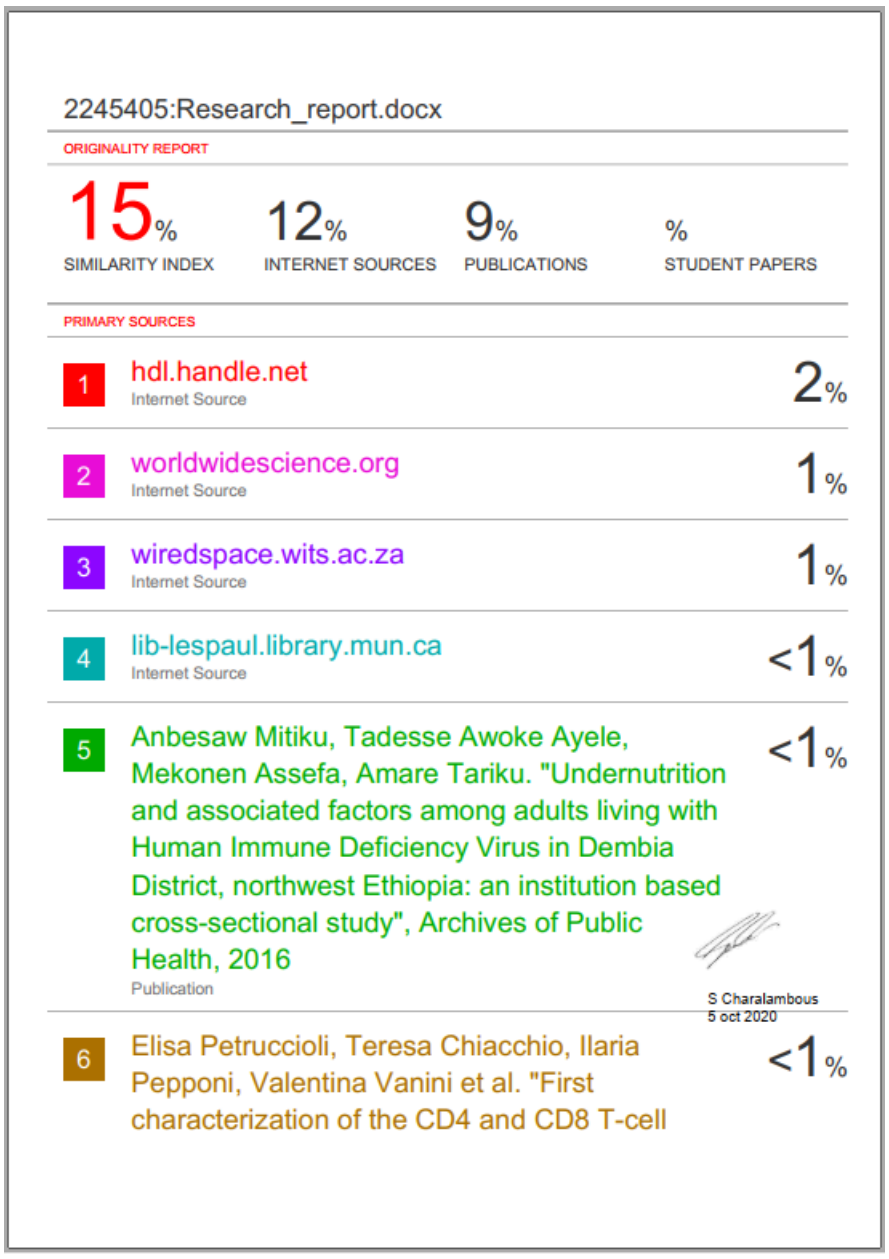
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

05/11/2019
Date

Appendix 3: Turnitin Report



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S Charalambous
5 oct 2020

Appendix 4: Summary of CD4 count among LTBI indeterminate individuals

A summary of CD4 count among the individuals that had an indeterminate test results is shown in Table 13 below.

Table 12: CD4 count among LTBI indeterminate individuals (N=12).

QFT results	CD4 count n (%)		Total n (%)
	≤ 200 cells	> 200 cells	
Indeterminate	3(30.00)	7(70.00)	10(100.00)

Appendix 5: Summary of the $CD8^+$ T-cell response among individuals tested for LTBI.

Table 13: $CD8^+$ T-cell response status by LTBI test results (N=2511).

QFT results	$CD8^+$ T-cell response		Total n (%)
	No response n (%)	Response n (%)	
Positive	876(80.59)	211(19.41)	1087(100.00)
Negative	1408(99.72)	4(0.28)	1412(100.00)
Indeterminate	12(100.00)	0(0.00)	12(100.00)
Total n (%)	2296(91.44)	215(8.56)	2511(100.00)

Appendix 6: Investigation of interactions

Three possibilities of interactions were investigated. These included: age and gender; history of IPT and previous TB diagnosis; and lastly history of smoking and recreational drug use. The basis of running these interactions included; females are immunologically superior to men however, with increasing age this superiority is lost (83,84); IPT and anti-TB treatments affect individuals' immunological response when exposed to latent tuberculosis infectious particles (60,64,66,67); Smoking and recreational drug use have both been found to impair an individuals' immune response towards infections, LTBI inclusive (40,79,82). It is on these foundations that interactions were investigated using the final models of both objectives 2 and 3 to examine their significance.

Both Table 14 and Table 15 show the results of the interaction pairs investigated. These interactions having a P-value greater than 0.05 showed that the interactions were insignificant indicating no evidence of interactions.

Table 14: Interactions among factors associated with LTBI (N=2274).

Variables		Multivariable analysis	
	Categories	**AOR(95%CI)**	**P-value**
Age	Age	1.02(1.0-1.03)	0.01
Gender	Female	1.	
	Male	1.68(0.71-3.98)	0.24
Highest level of education	No School/Pre-school only	1.	
	Grade 1-3	2.23(1.01-4.89)	0.05
	Grade 4-7	1.59(0.81-3.11)	0.17
	Grade 8-11	1.92(1.00-3.68)	0.05
	Grade 12	1.60(0.82-3.13)	0.17
	Above matric	2.96(1.26-6.955)	0.01
Marital status	Cohabitating	1.	
	Single, never married	0.86(0.63-1.17)	0.35
	Married	0.68(0.49-0.96)	0.03
	Divorced	0.56(0.33-0.95)	0.03
	Widowed	0.73(0.45-1.16)	0.18
Employment status	Employed full-time	1.	
	Informal employment/piece jobs	0.98(0.70-1.39)	0.93
	Employed part-time	1.15(0.85-1.59)	0.36
	Retired/Pensioner	0.84(0.47-1.50)	0.57
	unemployed	1.07(0.86-1.33)	0.55
	Other	0.83(0.50-1.39)	0.48
Taken IPT before	No	1.	
	Yes	0.85(0.65-1.12)	0.25
Previous TB diagnosis	No	1.	
	Yes	1.39(1.13-1.72)	<0.01

Table 14 continued

Ever smoked	No	1.	
	Yes	1.12(0.85-1.48)	0.43
CD4 Count	< 200	1.	
	Above 200	1.60(1.25-2.05)	<0.001
Recreational drug usage	No	1.	
	Yes	1.28(0.55-2.95)	0.56
Gender#Age	Female	1.	
	Male	0.99(0.97-1.01)	0.39
Ever smoked #Recreational drug usage	No#No	1.	
	No#Yes	1.	
	Yes#No	1.	
	Yes#Yes	0.82(0.30-2.22)	0.70
Taken IPT before # Previous TB diagnosis	No#No	1.	
	No#Yes	1.	
	Yes#No	1.	
	Yes#Yes	0.72(0.38-1.35)	0.31

Table 15: Interactions among predictors of $CD8^+$ T-cell response (N=997).

		Multivariable analysis	
Variables	**Category**	**AOR(95%CI)**	**P-value**
Age	Age	1.01(0.99-1.02)	0.75
Gender	Female	1.	
	Male	1.27(0.27-6.05)	0.76
CD4 Count	< 200	1.	
	Above 200	1.08(0.66-1.76)	0.77
Recreational drug usage	No	1.	
	Yes	0.57(0.24-1.36)	0.21
Taken IPT before	No	1.	
	Yes	0.99(0.57-1.72)	0.97
Previous TB diagnosis	No	1.	
	Yes	1.68(1.20-2.35)	<0.01
Ever smoked	No	1.	
	Yes	1.18(0.75-1.89)	0.46
Gender#Age	Female	1.	
	Male	1.0(0.96-1.03)	0.81
Taken IPT before # Previous TB diagnosis	No#No	1.	
	No#Yes	1.	
	Yes#No	1.	
	Yes#Yes	1.54(0.53-4.45)	0.43