The Predictive Value of a QuantiFERON Conversion in the Development of Active Tuberculosis Disease in Adolescents.

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A mini-dissertation submitted to the Faculty of Health Sciences, University of Cape Town, in partial fulfilment of the requirements for the degree Master of Public Health (Epidemiology and Biostatistics).

DECLARATION

TITLE: MPH Mini-Dissertation

MPH COURSE CODE: PPH7015W

I, Shingai Machingaidze Student No. MCHSHI002 declare that the work that I have submitted is my own and where the work of others has been used (whether quoted verbatim, paraphrased or referred to) it has been attributed and acknowledged.

SIGNATURE:

DATE: 8 April 2011

Senate requires all students to make a declaration when submitting written work; they declare that the work submitted is their own and that where the work of others has been used (whether it has been quoted verbatim or paraphrased or referred to) it has been attributed and acknowledged using a standard referencing convention.

ACKNOWLEDGEMENTS

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ABSTRACT

Interferon gamma release assays **(**IGRAs) are regarded as an alternative measure to the tuberculin skin test (TST) of latent tuberculosis infection (LTBI) and have been recommended for serial testing in populations with a continued risk of tuberculosis exposure. However evidence is still lacking on several key questions that include defining IGRA conversion and its predictive value for the development of tuberculosis disease.

As an extension of a prospective epidemiological study of TB disease and infection in adolescents in the Worcester and surrounding areas in the Western Cape carried out from 2005 to 2009, a subset of adolescents who were identified to have converted their IGRA status were followed up and observed for the occurrence of active TB disease over a further period of two years and compared to non-converters. A known risk of developing active TB disease is associated with TST conversions. This risk in those who have converted their IGRA (QuantiFERON) status is unknown.

The Protocol (Part A) of this dissertation outlines the study design and methodology used in the conduct of the research study. The Literature Review (Part B) aims to contextualize this study by examining the available literature on the role of the TST in diagnosing TB and how IGRAs compare to the TST, the interpretation of serial IGRA data with a focus on IGRA conversion, the prognosis of an IGRA conversion and its potential use as a predictive test for the development of active TB disease. The Manuscript (Part C) presents the results of the study. The main objectives were to evaluate the incidence rate of active tuberculosis disease following QuantiFERON Gold In-Tube (QFT-IT) conversion and to assess its predictive value for the development of tuberculosis disease. The results show that QFT conversion is indicative of an approximately 7-fold higher risk of progression to TB disease (compared to non-converters). The predictive value of QFT conversion for the development of active TB disease is 2.4% within 2 years following QFT conversion. Further studies in high and low TB burden settings are required to validate these findings.

PART A: PROTOCOL

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1. PROTOCOL SYNOPSIS

Title: The Predictive Value of a QuantiFERON Conversion in the development of Active Tuberculosis Disease in Adolescents.

Rationale: With tuberculosis (TB) accounting for nine million new cases of TB and 1.8 million deaths reported in 2007, it is amongst the world's most lethal infectious agents causing more adult deaths than any other pathogen. TB has been declared a global health emergency by the World Health Organisation (WHO). *Mycobacterium tuberculosis* (*Mtb*) is the primary causative agent of TB and strains of *Mtb* that are resistant to the antibiotics currently used to treat TB are rapidly emerging worldwide.

The tuberculin skin test (TST) has traditionally been used to diagnose TB infection for almost a century. However, TST results are often confounded by non-tuberculous mycobacterium infection (NTMs), BCG, individual immune status, the method of test administration and the reading of results. The fairly recent identification of early secreted antigenic target 6 (ESAT-6) and the related culture filtrate protein (CFP-10), that are not present in BCG or most non-tuberculous mycobacteria, has been of major significance in the development of TB diagnostics. These proteins are coded within the region of difference 1 (RD-1) of the *Mtb* genome. In persons with latent TB infection (LTBI), memory T cells produce interferon-gamma (IFN-γ) in response to *Mtb* antigens. There are two commercially available Interferon-Gamma Release Assays (IGRAs) that are licensed for use in many countries: QuantiFERON TB Gold (Cellestis®) and T-Spot (Oxford Immunotec®).

IGRAs such as QuantiFERON (QFT) have been recommended for serial testing but data is very limited on the interpretation of repeat IGRA testing. Although existing studies are limited, they suggest that conversions, reversions and non-specific variations do occur with IGRAs as they do with TST, and to date there is no consensus on how to define and/or interpret these conversions and reversions. A known increased risk of developing active TB disease is associated with TST conversions. This risk in those who have converted their QFT status remains unknown.

Objectives:

1. To determine the risk of developing active TB disease in adolescents with a converted QuantiFERON status compared to a group whose QuantiFERON status remained negative throughout the study.

2. To determine relative risk using different definitions of conversion.

Study Design: This study is an extension of a prospective epidemiological study of TB disease and infection in adolescents in the Worcester and surrounding areas in the Western Cape carried out from 2005 to 2009, in which 6363 participants were enrolled from local public schools. In this follow-on study, a subset of adolescents who were identified to have converted their QFT status during the original study will be followed up and observed for the occurrence of active TB disease over a period of two years. A similar sized, random sample of participants identified to have a QFT status that remained negative throughout the original study will be used as the control group.

Evaluations and Follow-up: Participants that are successfully enrolled into this study will be screened for TB disease at baseline and every 6 months thereafter based on set criteria. A total of 5 study visits per participant (including baseline evaluation) will be carried out over the 2 years of follow-up. All participants suspected of having TB disease or otherwise identified to have TB disease will have all study tests carried out and referred for treatment if necessary. In addition, all participants will also be monitored for the development of active TB disease through the surveillance of TB registers at local TB clinics and hospitals for the duration of the study.

2. INTRODUCTION

With tuberculosis (TB) accounting for nine million new cases of TB and 1.8 million deaths reported in 2007[1], it is amongst the world's most lethal infectious agents causing more adult deaths than any other pathogen. TB has been declared a global health emergency by the World Health Organisation (WHO). *Mycobacterium tuberculosis* (*Mtb*) is the primary causative agent of TB and strains of *Mtb* that are resistant to the antibiotics currently used to treat TB are rapidly emerging worldwide. In developing countries where TB is endemic, extensive neonatal vaccination programs using attenuated strains of *Mycobacterium bovis*, collectively referred to as Bacillus Calmette-Guerin (BCG), have reportedly reduced the incidence of severe forms of childhood TB. To date, no vaccine has been shown to reliably prevent pulmonary tuberculosis in adults. Various studies show the protective efficacy of vaccination with BCG against the development of TB in adults has ranged from 80% in some studies to little or no efficacy in other studies where the TB burden is greatest.[2]

A key method of helping to reduce the global TB burden particularly in developing countries is the identification and treatment of latent tuberculosis infection (LTBI), traditionally diagnosed using the tuberculin skin test (TST) which is dependent on a delayed hypersensitivity response to purified protein derivative (PPD) of *Mtb*. TST is a relatively cheap test which requires no laboratory component and has been used as the standard test in the identification of *Mtb* infection for almost a century. It is important to note that TST results can however be confounded by non-tuberculous mycobacterium infection (NTMs), BCG, individual immune status, the method of test administration and the reading of results. In addition two patient visits are required, one for administration and one for reading, 48-96 hours apart. The fairly recent identification of early secreted antigenic target 6 (ESAT-6) and the related culture filtrate protein (CFP-10), that are not present in BCG or most non-tuberculous mycobacteria, has been of major significance in the development of TB diagnostics. These proteins are coded within the region of difference 1 (RD-1) of the *Mtb* genome. In persons with LTBI, memory T cells produce interferon-gamma (IFN-γ) in response to *Mtb* antigens.[3]

There are two commercially available Interferon-Gamma Release Assays (IGRAs) that are licensed for use in many countries: QuantiFERON TB Gold (Cellestis®) and T-Spot (Oxford Immunotec®). Operationally, the advantages of IGRAs over TST are that these assays require one visit with results available in 24 hours; they are able to avoid boosting due to repeat testing and have a standardized interpretation.[4] IGRAs have their limitations in that they require an equipped laboratory with trained personnel and like TST, they cannot distinguish between latent and active TB disease.

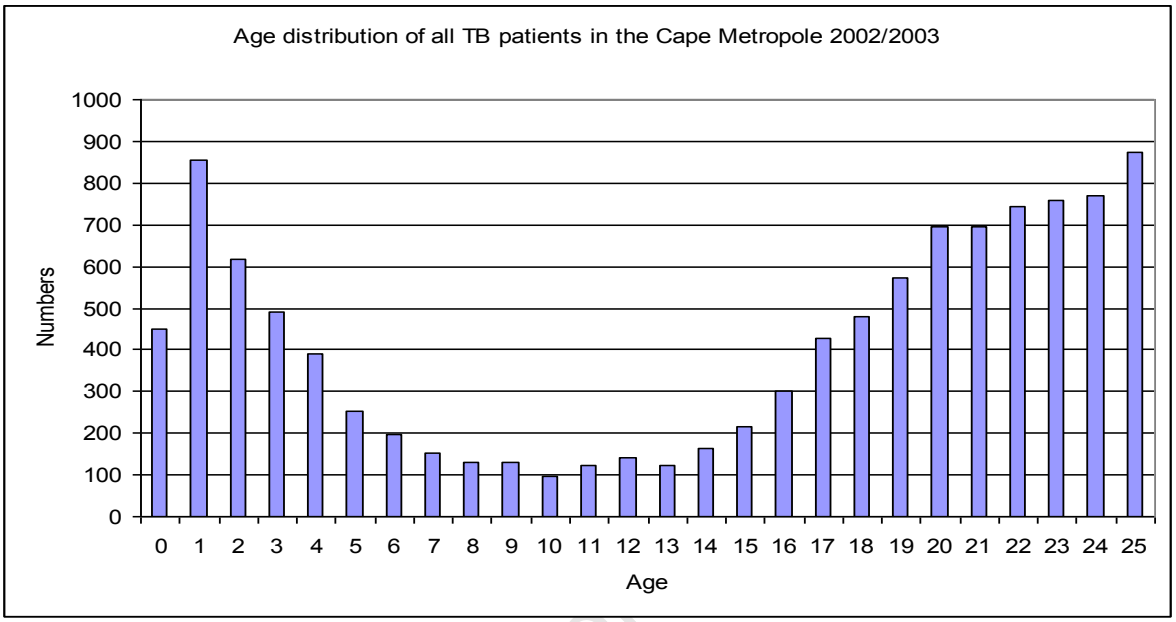
One such IGRA will be used as the basis of comparison in this study, the QuantiFERON Gold In-Tube (QFT-G IT) assay which was recently approved by the US Food and Drug administration (FDA). This is a modified version of the QuantiFERON Gold (QFT-G) assay.

When compared to QFT-G, the newer version QFT-G IT includes a third antigen called TB7.7 and requires only 3ml of blood compared to the 16ml required by QFT-G. This additional antigen is thought to enhance the sensitivity of the assay without reducing its specificity. Both versions of QuantiFERON (QFT) are based on the 16-24h whole blood enzyme-linked immunorsorebnt assay (ELISA).[5]

IGRAs such as QFT have been recommended for serial testing but data is very limited on the interpretation of repeat IGRA testing. Although existing studies are limited, they suggest that conversions, reversions and non-specific variations do occur with IGRAs as they do with TST, and to date there is no consensus on how to define and/or interpret these conversions and reversions.[6] If a simple negative to positive definition is used, then conversion rates may be higher with IGRAs than with TST. These higher conversion rates could indicate that these assays are more sensitive at identifying new infections but this has not been established.[7]

It has been shown that the risk of TB disease after a peak in early childhood begins to increase from about 12 years of age and has a second peak in early adulthood. For this reason the adolescent age group is often considered a priority group for vaccination against TB.[8] This is supported by data on the age distrubution of TB cases from the Cape Town Metropole in 2002 and 2003 (shown in the figure below). This period is often referred to as the "golden years" and

this pattern has been observed in many countries spanning a variety of geographic, cultural and economic settings where the annual risk of infection is high[9]. Studying the prevalence of infection, the annual risk of TB infection and the risk of TB disease given infection in adolescents, in areas where vaccine trials are under way is of high priority.



Reference: Provincial Authority Western Cape Province, Unpublished Data 2004

As an extension of a prospective epidemiological study of TB disease and infection in adolescents in the Worcester and surrounding areas in the Western Cape carried out from 2005 to 2009, a subset of adolescents who were identified to have converted their QFT status will be followed up and observed for the occurrence of active TB disease over a period of two years. A known risk of developing active TB disease is associated with TST conversions. This risk in those who have converted their QFT status remains unknown.

3. RESEARCH AIM AND OBJECTIVES

3.1. Research Question

Can QuantiFERON conversion be used as a predictor in the development of active TB disease in adolescents?

3.2. Hypothesis

QuantiFERON conversion in adolescents represents a higher risk of progression to TB disease when compared to adolescents who do not undergo QuantiFERON conversion.

3.3. Objectives

1. To determine the risk* of developing active TB disease in adolescents with a converted QuantiFERON status compared to a group whose QuantiFERON status remained negative.
2. To determine relative risk using different definitions of conversion.

**The risk as measured by the incidence rate of TB disease. This is the most likely end point to be used in a Phase III clinical trial of a new TB vaccine in adolescents.*

Case definition - A case of tuberculosis will be defined as any case that is diagnosed by a physician and confirmed by two or more smear positive for AFB and/or one positive culture for Mycobacterium tuberculosis.

Other case definitions (e.g., abnormalities on a chest radiograph, symptomatic response therapy, etc) may be used as alternate definitions which may increase sensitivity but may increase the number of false positives.

QuantiFERON conversion definition - A change in QFT status from negative at baseline (<0.35IU/ml) to positive (≥0.35IU/ml) at a subsequent time during the original study.

4. STUDY METHODS

4.1. Study Design

This is an extension of a prospective, observational cohort study conducted in adolescents 12-18 years old (at time of enrolment into original study) in Worcester and its surrounding areas, with no experimental interventions. Participants whose QFT-G IT test status converted from having been negative at baseline during the original cohort study to being positive at a subsequent timepoint will be followed up for at least a further two years after their 2-year study visit or for a maximum of 5 years post conversion and observed for the development of active TB disease. A similar sized, randomly sampled control group of participants who have remained QFT-G IT negative throughout the original cohort study will undergo similar follow-up. As with the original cohort study, hospital admission and mortality surveillance will be conducted for all participants.

4.2. Study Procedures

Inclusion and Exclusion Criteria

Inclusion Criteria

- Male and female adolescent volunteers who were enrolled into the original cohort study, living in the greater Worcester area and attending public schools

- Participants identified to have converted their QFT-G IT test status from negative at baseline to positive at a subsequent timepoint in the original cohort study

  OR

  Participants who were randomly selected from those whose QFT-G IT status remained negative throughout the original cohort study

- Informed consent from participants and/or participant's parent/legal guardian and informed assent from adoelscents still <18 years of age

Exclusion Criteria

- Participant and family planning to move from study area in the next 3 years

Subject Selection and Screening

It is estimated that approximately 600 out of the 800 participants who have been identified to have converted their QFT-G IT status will agree or be available to participate in this study. A similar sized random sample of participants identified from the existing database, whose QFT-G IT status remained negative throughout will be selected as a control group. Study purpose and procedures will be explained to participants and parents. Consent for this additional follow-up will be obtained using supplementary consent/assent forms to the existing consent/assent forms used for the original cohort study. Participants who are 18 years or older at the time they are approached will be asked to sign consent for this extended follow-up. Participants who are younger than 18 years of age will require parental consent and participant assent for the extended follow-up period.

Study Visits

Baseline Evaluation

Once consent and/or assent forms have been signed, participants will be considered enrolled, assigned a subject identification number and baseline (Study Day 0) evaluations must be completed as layed out in the data-capture form (DCF) (Appendix C). Participants will be asked questions pertaining to their medical history. This assessment will include details of current or past TB disease, history of close contact with an individual with confirmed TB disease, current symptoms of TB disease, and history of hospitalisations since last study visit as well as the diagnosis of acute or chronic diseases. Demographic characteristics (participant's gender, DOB, race/ethnicity, occupation and monthly income if out of school and employed) will be reviewed with the participant. If the participant has had a new household/close TB contact since the last study interview or the participant is symptomatic of TB disease or currently on TB treatment, they will be referred for TB investigation where all study tests must be completed described below. History of a diagnosis of TB since last contact through the original study will be elicited.

Subsequent 6-Monthly Study Visits

Participants will have follow-up visits every 6 months following their baseline evaluation (6, 12, 18, 24 months) where they will be repeatedly assessed for current or past TB disease, history of close contact with an individual with confirmed TB disease, current symptoms of TB disease, and history of hospitalisations as well as the diagnosis of acute or chronic diseases since last study visit. If necessary participants will be referred for TB investigation where all study tests as described below must be completed.

Two year Visit

This will be the final study visit and participants will be assessed as before. Study staff are to complete a checklist pertaining to participant's status at the end of the study (study completed, loss to follow-up (LTFU), withdrew, death).

In-Between Visits

All participants will be subject to passive surveillance for tuberculosis through ongoing checking of TB registers at local clinics and hospitals until all 2 year study visits have been completed. Tuberculosis cases picked up this way (or by parent/participant contacting study staff) will be referred for TB investigation and all study tests are to be completed. These will be captured as 'In-between Visits' on the data-capture form.

Specimen Collection

Study staff will follow SATVI standard operating procedures used in the original cohort study regarding sputum specimen collection, processing, storage and shipment.

TB Evaluation and Diagnostic Processes

Once referred for TB investigation, following an assessment of participant clinical history and household/close TB contact since the last study visit, all participants will be asked for two

sputum samples. If a participant fails to produce sputum, they will be referred for induced sputum procedures. TB culture will be performed on all sputum samples. A chest x-ray will be performed as well as an HIV test when permitted by the participant. Tests will only be carried out if outstanding from participant's medical records.

All participants will individually receive counseling in a confidential setting on the results of their tests and given a written summary of the test results.

All test results will be communicated to the study participant's medical care provider who will make all decisions related to treatment and will manage any treatment that is needed. If the sputum specimen is smear or culture positive the results will also be reported to the public sector tuberculosis control program. TB treatment is available at no cost to the public at all TB control programme clinics.

Medical care providers, especially clinicians in the public sector TB clinics, will be informed about the extension of the study and asked to continue referring all individuals enrolled in the study for the diagnostic process (only outstanding tests from medical records will be carried out).

Study staff will arrange to record whether treatment is started, any treatment prescribed, the date started, the date stopped and the treatment outcome. Outcome will be recorded using WHO DOTS program definitions for cured, completed, failed, interrupted, transferred and died.

Participant Withdrawal

As participation in this study is voluntary, all study participants will reserve the right to withdraw from the study for whatever reasons they see fit. It will be made clear that they can do so without facing any penalty or loss of benefits to which they are otherwise entiled to. All withdrawals must be fully documented on an addendum to the DCF.

5. COMPENSATION AND PARTICIPANT INCENTIVES

Particpants will receive no monetary compensation*. They will be given a packet of crisps and a coke at each study visit.

**In the original cohort study, participating schools were offered incentives (e.g., book vouchers or IT equipment) proportional to the number of learners per school, or cash for another purpose. An equal rate per learner was also paid to a maximum of $2,000 per school. An amount of R50 (at every occasion of blood draw) in the form of a non-cash payment such as a voucher was paid to learners who agreed to participate in the study in lieu of the time offered and the discomfort experienced.*

6. MORBIDITY AND MORTALITY SURVEILLANCE

Due to the fact that no experimental product is being administered in this study, there will be no formal adverse event surveillance. Identification of acute illness and/or injuries for all participants will be through passive surveillance of hospital records and documentation of all hospital visits by participants. This information will be recorded on the DCF.

An autopsy will be requested for participants who die during the study. Due sensitivity will be exercised in approaching respective families to whom it will be made clear that there will be no loss of benefits or any other negative consequences should they refuse for the autopsy to be carried out. Regarding autopsy results, counseling will be provided to families where necessary. Autopsies will be carried out by a district surgeon in Worcester working in consultation with a pathologist at the University of Cape Town.

When deaths are due to unnatural causes such as violence or motor vehicle accidents, autopsies are required by law and these will be conducted by the district surgeon.

Parents/guradians will be given the option of a verbal autopsy should they refuse for a full or targeted autopsy to be carried out.

Premature Study Termination

This study may be terminated prematurely if:

- If the ethics review board instructs premature termination
- If the sponsor decides to discontinue the study for financial or other reasons
- If in the best interest of the participants, continuation of the study is thought to be harmful

7. STATISTICAL CONSIDERATIONS

Sample Size Calculation and Estimates of TB Disease Incidence

The primary endpoint will be calculated as the cummulative incidence of new TB disease cases in participants who have converted their QFT status, relative to the cummulative incidence of new TB disease cases in participants in the randomly sampled control group of participants whose QFT status was shown to have remained negative throughout the study.

A new case of TB will be defined as any participant diagnosed with TB by a physician and confrimed by two smears positive for acid fast bacilli (AFB) and/or one positive culture.

It is estimated that of the 800 participants identified to have converted their QFT status, approximately 600 will agree to be part of this extended follow-up study. A similar sized randomly sampled control group will be selected from participants who were identified to have remained QFT negative throughout the study.

The predicted cumulative incidence rate of TB over the two years following QFT conversion is 3% based on preliminary results obtained from the original cohort study. The expected sample size of 600 will give a rate estimate [95% confidence interval (CI)] of 3% (1.6% to 4.4%) in the post-conversion group. The predicted cumulative incidence rate of TB over two years in the QFT negative group (control group) is 0.8% based on preliminary results from the original cohort study. Given a sample size of 600, the expected rate estimate [95% CI] is 0.8% (0.09% to 1.5%) and the rate ratio will be 3.75 (95% CI 1.5 – 10.6).

Statistical Summaries/Analyses and Computer Methods

A study report will be generated at the end of the study follow up, once all data queries have been identified and cleared. Following the locking of the databese, data will be imported from the database for statistical analysis in STATA 10 software under a Windows Operating System. All results will be reported as appropriate effects with a measure of precision [95% CI].

8. DATA MANAGEMENT

All information will be recorded on a data capture form (DCF). Where necessary, a specific addendum will be completed and attached to the DCF in cases such as withdrawal and/or hospitalisation. Data collected on DCF's will be entered into a relational Microsoft Access database. Data will be entered continuously as the study progresses. A Data Manager will be responsible for creating the database, as well as monitoring all entries. Only authorised personel will be granted authorisation to access the database.

DCF's will be periodically checked by the study investigator (or designee) to ensure all information is captured correctly and that any corrections made on the DCF adhere to the requirements of the Good Clinical Practice (GCP) guidelines. Data will be stored safely to ensure confidentiality of particpants is maintained. On completion of the study, the database will be checked, data cleaned and all outstanding queries will be resolved. The database will then be locked and no further changes can be made.

All study records (source documents, signed informed consent forms, copies of DCF's, IRB/IEC correspondence and approval letters etc) will be kept safely for a period of time of up to 2 years following the completeion of the study. On request, the Sponsor may inspect all study records and these should be made available.

The reliability of information collected for this study will be optimised by ensuring these measures are in place:

- Thorough training of study staff (clinical research workers, data capturers, study co-ordinator)

- Use of a clear and simple DCF
- Periodic checks of database by Data Manager and Study Manager
- Ensuring all participants enrolled into the study meet inclusion criteria

9. ETHICS AND COMMUNICATION

Ethics and Regulatory Considerations

All study procedures are in accordance with the Declaration of Helsinki. The study protocol and informed consent form will be reviewed and approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) of the Aeras Global TB Vaccine Foundation and the University of Cape Town prior to any protocol-specified procedures being conducted. Reports are to be made to the IRB as to the progression of the study at least once a year.

Written informed consent will be obtained from study participants over 18 years and parents/ legal representative of participants younger than 18 years prior to enrolment and the conducting of any protocol-specified procedures.

Each participant will be assigned a coded number and together with the parcipant's initials, will form the participant identification (ID) number which will be used to identify the participant on laboratory specimens, source documents, DCF, study reports etc. Participants will retain ID numbers previously assigned to them in the main study. This will ensure confidentiality is maintained. Written permission will be obtained from the participant/legal representative before any participant information is released except as required by monitoring and/or auditing of the study by the Sponsor or local regulatory authorities.

Institutional Review Board or Independent Ethics Committee

Prior to the start of the study, the investigator must obtain written approval for the study protocol and informed consent form from the IRB or IEC. All study documents requiring approval from the IRB/IEC must be submitted for approval. Modifications/ammendments to the protocol may not be implemented without prior written IRB/IEC approval except when

necessary to eliminate immediate harm to the particiant or when modifications only involve logistical/administrative aspects of the study.

An established community advisory board is available for consultation should the need arise.

Informed Consent

Informed consent will be documented by using a written document approved by the IRB/IEC (APPENDIX B). Informed consent must be obtained from all participants and/or the participant's legal representative who after a clear explanation by the investigator (or qualified designee) in their home language as to the purpose of the study, its goals, expected benefits and known/potential risks will sign on the designated page of the document.

All information must be provided in a way that is clear and understandable for the participant and participant's legal representative. The document will have translations in Afrikaans and isiXhosa to facilitate for better understanding. The participant/ participant's legal representative should be given information as to who to contact in the event of a study-related illness. It will be made clear to the participant/ participant's legal representative that no experimental intervention will be implemented in this study and no blood draws or TST's will be carried out. It will also be made clear to the participant/participant's legal representative that participation in this study is voluntary and that they are free to withdraw from the study at anytime for whatever reason they see fit without any penalty or loss of benefits to which they are otherwise entitled.

Relationship with Regional Health Services in the Study Area

The regional and local TB control program staffs are aware of the study and will be notified of its extension and requested to continue assisting and supporting the study. All participants will be informed of their results by the study team. The policy for the South African TB control program is that they do not offer treatment to person's with LTBI over the age of 5 years. Persons with microbiological or other evidence of TB disease will be prescribed a standard regimen as recommended by WHO.

Results of all TB investigations will be communicated to the participant and participant's medical care provider. If the sputum smear or culture are positive, and the participant does not have a medical care provider they will be given a referral letter to their nearest public sector TB treatment clinic. Free TB treatment is available to this community. Results of positive smears and cultures will also be reported to the tuberculosis control program.

Participants will be given a TB Fact sheet that describes TB signs and symptoms as well as the relationship between TB and HIV (APPENDIX F). Participants will be advised to visit their nearest clinic should any of them arise.

10. REPORTING AND PUBLICATION

The findings of the study will be reported back to the sponsor and relevant persons within the community. All manuscripts, abstracts and presentations using data from this study must be reported to the sponsor 30 days prior to their submission. A manuscript(s) will be published in an accredited medical/scientific journal.

11. ACKNOWLEDGEMENTS

This protocol was adapted from an original cohort study protocol - A Prospective Epidemiological Study of TB Disease and TB Infection in Adolescents in the Worcester and Surrounding Areas, Western Cape Province, South Africa [Protocol Number EPI-002-ZA], 2005-2009. Prof. Gregory Hussey (Principal Investigator) and Dr Hassan Mahomed (Study Manager) were responsible for both the protocol development and study procedures of the original cohort study. I have received direct supervision from Dr Hassan Mahomed in developing this protocol.

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APPENDIX A: Timeline																																	
	Year 1(months)												Year 2(months)													Year 3(months)								
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10
Protocol Development																																		
Ethics Aprroval																																		
DCF & Database Creation																																		
Protocol Specific Training																																		
Enrollement & Follow-up of Participants					1st year follow-up													2nd year follow-up																
Data Cleaning						Continuous data-cleaning																												
Data Analysis & Study Report Preparation																		Preliminary analysis begins after 1 year follow-up																
Final Study Report																																		
Dissemination																																		

APPENDIX B: Participant Consent Form

Supplementary Participant Information and Consent Form for the extension of the Adolescent Cohort Study

UNIVERSITY OF CAPE TOWN / AERAS GLOBAL TB VACCINE FOUNDATION
Participant Information and Consent Form

Protocol Title: **A prospective Epidemiological Study of TB Disease and TB infection in Adolescents in the Worcester and Surrounding Areas, Western Cape Province, South Africa**

You have agreed before to take part in a study of TB in adolescents. To remind you:

This research study was done by the South African TB Vaccine Initiative (SATVI), Institute of Infectious Disease and Molecular Medicine, University of Cape Town Medical School, Anzio Rd, Observatory, Cape Town in collaboration with the Aeras Global TB Vaccine Foundation, USA. The person in charge of the study is Professor Gregory Hussey, Director of SATVI. Your child has completed follow up for that study. We would like to continue follow up for a further two years in some of the children who took part in that study. The children we will follow up are either those who became infected with TB during the study period or those children who showed no sign of becoming infected during the study period. This was shown by blood tests done during the study.

Voluntary Participation

You may choose not to continue in the study. As a result of that decision, no health care will be taken away from you if you say no. You will not pay a penalty or lose any benefits you have earned if you say no. You are free to leave the study at any time.

Reason for further follow up

We want to study a new blood test for diagnosing TB infection and TB disease. We want to know if the new blood test shows us those who will develop TB.

When somebody is not infected with TB, the new test gives a negative result. This uninfected individual may then become infected and when tested again, the test will give a positive result. This change with respect to the test results from negative to positive is called conversion. We want to see how many of the children who converted in the study develop TB disease. We want to compare the number of children with TB disease in those who have converted to those that tested negative during the study.

Procedures to be followed

If you agree to continue participating in the study we will ask you some questions about your health status since you were last seen by study staff.

Sputum Testing: If you have new TB-related symptoms or have lived with someone who has been diagnosed with TB disease since the last visit by a study team member, you will be asked to provide two sputum samples. You may be asked to produce a sputum sample immediately or will be given containers to take home and asked to produce sputum specimens early in the morning. A nurse will explain what sputum is and how to produce it. If you are still not able to produce sputum, a nurse will assess him/her. If needed, we will arrange for him/her to have more sputum tests, induced sputum, a chest x-ray and an assessment by a doctor. The induced sputum procedure will be explained in another consent form.

Follow-up:

Individual Follow-up

A study team member will visit you every six months. At this visit the study team member will interview you about TB signs and symptoms and TB in the household.

If you have new symptoms and have been in contact with a new TB case, you will be asked to produce sputum for tests to see if they have TB disease.

Hospital and Clinic Follow-up

Study staff will be reviewing records of local area hospitals and clinics. When they find records of children participating in the study they will record information about the visit. They will record the diagnosis at discharge. This information will help to identify those who have been TB diagnosed in hospitals or clinics. When you sign this form, you will be giving us permission to get these records.

Study Close Out: The study will last for up to two years from the start date. At the last visit you will be asked about TB signs and symptoms. We will also ask about TB cases who might live in the home. If you are suspected of TB disease, you will be asked to produce sputum for tests to see if you have TB disease. You may be asked to have a chest x-ray if the study staff feel that this is necessary.

Cause of Death Investigations: It is important to know the cause of death if someone passes away. It is very rare for a child in this age group to pass away. However, if this should happen, we would want to know if TB or a related condition has something to do with the

death. This will require further investigations. We will approach your parents or legal guardian to discuss these with them in more detail should you pass away.

Study Eligibility

You cannot participate in this further follow up part of the study if you plan to move from the study area or out of Cape Town in the next two years. There may be other reasons that the study doctor or his staff will talk to you about.

Risks and Benefits to You

You will have the benefit of early referral for treatment should TB or any other disease be detected. The screening procedures used will not always detect early cases of TB so if you develop symptoms of TB you should still seek advice from your normal doctor or clinic about this. You will be provided with a list of these symptoms.

Compensation

No compensation will be paid to you for taking part in this further follow up phase.

Privacy

You will receive a copy of this consent form. We will keep your study records private to the best of our ability. Nobody but the researchers in this study will see your records unless you say OK. Your name will not be in any publication written from this study.

Contact Information for Questions or Concerns

This study has been approved by the Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, and the Research in Human Subjects/Institutional Review Board of the Aeras Foundation, which approves all trials involving human subjects. The study has also been seen and approved by the Regional Director of the Department of Health in the Boland Overberg Region.

If you have any concerns about your rights as a research participant you should contact the secretary of the research committee at the Faculty of Health Sciences on (021) 406 6492.

If at anytime you have questions or concerns about this study or are injured as a result of participation in this study you should contact:

Principal Investigator:

Professor Gregory Hussey, South African TB Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town Medical School, Anzio Road,

Observatory, Cape Town, South Africa. Telephone (27) 21 406 6013/14 Fax (27) 21 406 6081 email: gregory.hussey@uct.ac.za

Program Manager:

Elmarie Simon. SATVI Project Office, Brewelskloof Hospital, Worcester, Western Cape Province, South Africa. Telephone 023-3465400 Fax 023-346 5406 email: elmarie.simon@uct.ac.za

Consent/Assent To Take Part In This Study

I have read and discussed this concent form and understand the contents. My questions have been answered. I freely agree to take part in the study and agree to have my blood stored for use in future research of the TB germ and germs related to the TB germ.

_____

Name of study participant/ child *(please print)*

☐ Study participant under 21 years of age ***(tick only if applicable and complete below)***

_____

Signature of participant Date

_____

Name of study personnel taking assent *(please print)*

_____

Signature of study personnel taking assent Date

☐ Interpreter used ***(tick only if applicable)***

☐ Witness used ***(tick only if applicable; tick one below to describe witness)***

☐ Study personnel ☐ Independent/impartial person

_____

Name of witness *(please print)*

_____

Signature of witness

Study enrolment number: _____

APPENDIX C: Parental Consent Form

Supplementary Parent/ Guardian Information and Consent Forms for the extension of the Adolescent Cohort Study

UNIVERSITY OF CAPE TOWN / AERAS GLOBAL TB VACCINE FOUNDATION
Parent/Guardian Information and Consent Form

Protocol Title: **A prospective Epidemiological Study of TB Disease and TB infection in Adolescents in the Worcester and Surrounding Areas, Western Cape Province, South Africa**

You have agreed before for your child to take part in a study of TB in adolescents. To remind you:

This research study was done by the South African TB Vaccine Initiative (SATVI), Institute of Infectious Disease and Molecular Medicine, University of Cape Town Medical School, Anzio Rd, Observatory, Cape Town in collaboration with the Aeras Global TB Vaccine Foundation, USA. The person in charge of the study is Professor Gregory Hussey, Director of SATVI. Your child has completed follow up for that study. We would like to continue follow up for a further two years in some of the children who took part in that study. The children we will follow up are either those who became infected with TB during the study period or those children who showed no sign of becoming infected during the study period. This was shown by blood tests done during the study.

Voluntary Participation

You may choose for your child not to continue in the study. As a result of that decision, no health care will be taken away from you or your child if you say no. Neither you nor your child will pay a penalty or lose any benefits you have earned if you say no. Your child is free to leave the study at any time.

Reason for further follow up

We want to study a new blood test for diagnosing TB infection and TB disease. We want to know if the new blood test shows us those who will develop TB.

When somebody is not infected with TB, the new test gives a negative result. This uninfected individual may then become infected and when tested again, the test will give a positive result. This change with respect to the test results from negative to positive is called conversion. We want to see how many of the children who converted in the study develop TB disease. We want to compare the number of children with TB disease in those who have converted to those that tested negative during the study.

Procedures to be followed

If you agree for your child to continue participating in the study we will ask you some questions about your child's healthstatus since your child was last seen by study staff. We will then ask your child to agree to continue being in the study. If he or she agrees, we will then ask your child the same questions to confirm what you have told us.

Sputum Testing: Children who have new TB-related symptoms or have lived with someone who has been diagnosed with TB disease since the last visit by a study team member will be asked to provide two sputum samples. Your child may be asked to produce a sputum sample immediately or he/she will be given containers to take home and asked to produce sputum specimens early in the morning. A nurse will explain what sputum is and how to produce it. If your child is still not able to produce sputum, a nurse will assess him/her. If needed, we will arrange for him/her to have more sputum tests, induced sputum, a chest x-ray and an assessment by a doctor. The induced sputum procedure will be explained in another consent form.

Follow-up:

Individual Follow-up

A study team member will visit your child every six months. At this visit the study team member will interview your child about TB signs and symptoms and TB in the household.

If your child has new symptoms and has been in contact with a new TB case, your child will be asked to produce sputum for tests to see if they have TB disease.

Hospital and Clinic Follow-up

Study staff will be reviewing records of local area hospitals and clinics. When they find records of children participating in the study they will record information about the visit. They will record the diagnosis at discharge. This information will help to identify those who have been TB diagnosed in hospitals or clinics. When you sign this form, you will be giving us permission to get these records.

Study Close Out: The study will last for up to two years from the start date. At the last visit your child will be asked about TB signs and symptoms. We will also ask about TB cases who might live in the home. If your child is suspected of TB disease, they will be asked to produce sputum for tests to see if they have TB disease. Your child may be asked to have a chest x-ray if the study staff feel that this is necessary.

Cause of Death Investigations: It is important to know the cause of death if someone passes away. It is very rare for a child in this age group to pass away. However, if this should happen, we would want to know if TB or a related condition has something to do with the death. This will require further investigations. We will approach you to discuss these with you in more detail should your child pass away.

Study Eligibility

Your child cannot participate in this further follow up part of the study if you plan to move from the study area or out of Cape Town in the next two years. There may be other reasons that the study doctor or his staff will talk to you about.

Risks and Benefits to You

Your child will have the benefit of early referral for treatment should TB or any other disease be detected. The screening procedures used will not always detect early cases of TB so if your child develops symptoms of TB you should still seek advice from your normal doctor or clinic about this. Your child will be provided with a list of these symptoms.

Compensation

No compensation will be paid to you or your child for taking part in this further follow up phase.

Privacy

You will receive a copy of this consent form. We will keep your child's study records private to the best of our ability. Nobody but the researchers in this study will see your records unless you say OK. Your name will not be in any publication written from this study.

Contact Information fro Questions or Concerns

This study has been approved by the Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, and the Research in Human Subjects/Institutional Review Board of the Aeras Foundation, which approves all trials involving human subjects. The study has also been seen and approved by the Regional Director of the Department of Health in the Boland Overberg Region.

If you have any concerns about your rights as a research participant you should contact the secretary of the research committee at the Faculty of Health Sciences on (021) 406 6492.

If at anytime you have questions or concerns about this study or are injured as a result of participation in this study you should contact:

Principal Investigator:

Professor Gregory Hussey, South African TB Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town Medical School, Anzio Road, Observatory, Cape Town, South Africa. Telephone (27) 21 406 6013/14 Fax (27) 21 406 6081 email: gregory.hussey@uct.ac.za

Program Manager:

Elmarie Simon. SATVI Project Office, Brewelskloof Hospital, Worcester, Western Cape Province, South Africa. Telephone 023-3465400 Fax 023-346 5406 email: elmarie.simon@uct.ac.za

Consent to Take Part in this Study

I have read and discussed this consent form and understand the contents. My questions have been answered. I voluntarily consent to have my child participate and agree to have my child's blood stored for use in future research of the TB germ and germs related to the TB germ.

_____

Name of study participant/ child *(please print)*

☐ Study participant under 21 years of age ***(tick only if applicable and complete below)***

_____

Signature of participant Date

_____

Name of parent / legal guardian *(please print)*

_____

Signature of parent/ legal guardian Date

_____

Name of study personnel taking assent *(please print)*

_____

Signature of study personnel taking assent Date

☐ Interpreter used ***(tick only if applicable)***

☐ Witness used ***(tick only if applicable; tick one below to describe witness)***

☐ Study personnel ☐ Independent/impartial person

_____

Name of witness *(please print)*

_____

Signature of witness

Study enrolment number: _____

APPENDIX D: Data Capture Form

A Prospective Epidemiological Study of TB Disease and TB Infection in Adolescents in the Worcester and Surrounding Areas, Western Cape Province, South Africa.

Protocol Number: EPI-002-ZA

Data Capture From

Participant Enrolment and Demographics (Study Day 0)	
Date of respondent interview	____/_____/200__ (DD/MON/YYYY)
Respondent *(please circle all that apply)*	Mother/ father/ guardian/ participant/ other
Date of participant enrolment interview	____/_____/200__ (DD/MON/YYYY)

Study group assigned *(please circle)*	QFT Converter	QFT non-Converter

Information obtained from parent and confirmed with participant if less than 18years. Participant and/or respondent should have completed Informed Consent.

Participant details and Socio-economic Information					
Participant's Name/ Surname					
Participant DOB (DD/MM/YYYY)					
School (if still at school)					
Class (if still at school)					
Occupation/ grant (if NOT at school)					
Participant 18 years and older Gross monthly income *(circle 1)*	None	1 - R1000	R1001 - R4000	>R4000	Other

Contact Information CONFIDENTIAL – for contact purposes only (Do not include in analysis database)		
Address (participant)		
Address		
Suburb/ Town		Postal code
Home telephone #		
Work telephone (participant)		
Work telephone # (mother)		
Mother's Cell #		
Work telephone # (father)		

Father's Cell #		
Work telephone # (guardian)		
Guardian's Cell #		
Participant's Cell #		
Name of usual doctor and Ph No		

Study Day 0			
TB Contact History			
Has anyone with whom the participant is living with been diagnosed with TB since Study Day 720 of the previous study? *(please circle one)*	Y	N	Unk
If so, who? (specify relationship to participant: father, mother, sibling, grandparent, aunt, uncle, cousin, friend, lodger)			
If yes, since when? (month, year)			
At what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)			

If the participant has a new household TB contact since the last study interview (Study Day720), ***refer for TB investigation.***

History of TB since Study Day 720		
Was participant investigated for TB since Study Day 720 of the previous study?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who? (specify the name of the clinic, or indicate 'Ukn' if not known)		
Was the participant put on TB treatment since Study Day 720 of the previous study?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained. If any of the study procedures are not yet done, or if results are not available these procedures must be arranged (smears, cultures, chest x-ray and HIV test).

Note: Two sputum samples for smear and culture must be taken for each TB investigation.

Study Day 0			
Clinical History since Study Day 720			
Symptoms	*Please circle one for each symptom*		Specify number of weeks
Unexplained cough	Y	N	
Unexplained fever	Y	N	
Haemoptysis	Y	N	
Unexplained weight loss	Y	N	
Unexplained night sweats	Y	N	
Other (specify)	Y	N	
Other (specify)	Y	N	

If the participant has at least one symptom for two weeks or more or haemoptysis which is new or worsening since last contact, ***refer for TB investigation****.*

Medical History / Hospitalisation since Study Day 720		
Has the participant had any significant illness or been hospitalized since Study Day 720 of the previous study?	Y	N
If yes, when? (month, year)		

If the participant reports significant illness or was hospitalized since Study Day720 of the previous study, fill in separate Hospitalisation DCF.

Confirmed information with participant	Yes	No

Parent/ Guardian interviewer	
Participant interviewer	

Study Day 0			
Is TB investigation needed for:			
Recent household contact since Study Day 720 of the previous study?	Yes		No
Symptoms?	Yes		No
Being investigated for TB or on TB treatment	Yes		No
If any of the above are answered "Yes", then issue 2 sputum jars with instructions.			
Sputum jars issued	Yes		No
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

Follow Up: 180 Days		
Was participant seen for a follow-up interview/evaluation?	Y	N
If no, reason participant not seen for follow-up visit. (specify reason for refusal: not contactable, out of study area, withdrew consent, died)		
Date of interview/evaluation (DD/MON/YYYY)	____/_____/200____	
Name of interviewer		

Clinical History since Study Day 0			
Symptoms	*Please circle one for each symptom*		Specify number of weeks
Unexplained cough	Y	N	
Unexplained fever	Y	N	
Haemoptysis	Y	N	
Unexplained weight loss	Y	N	
Unexplained night sweats	Y	N	
Other (specify)	Y	N	
Other (specify)	Y	N	

If the participant has at least one symptom for two weeks or more or haemoptysis which is new or worsening since last contact, ***refer for TB investigation****.*

Medical History / Hospitalisation since Study Day 0		
Has the participant had any significant illness or been hospitalized since the last study visit?	Y	N
If yes, when? (month, year)		

If the participant reports significant illness or was hospitalized since the last study visit, fill in separate Hospitalisation DCF.

Study Day 180			
TB Contact History			
Has anyone with whom the participant is living with been diagnosed with TB since the last study visit? *(please circle one)*	Y	N	Unk
If so, who? (specify relationship to participant: father, mother, sibling, grandparent, aunt, uncle, cousin, friend, lodger)			
If yes, since when? (month, year)			
At what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)			

If the participant has a new household TB contact since the last interview, ***refer for TB investigation.***

History of TB since Study Day 0		
Was participant investigated for TB since the last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who? (specify the name of the clinic, or indicate 'Ukn' if not known)		
Was the participant put on TB treatment since the last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained. If any of the study procedures are not yet done, or if results are not available these procedures must be arranged (smears, cultures, chest x-ray and HIV test).

Note: Two sputum samples for smear and culture must be taken for each TB investigation.

Study Day 180		
Study Examinations and Tests		
Is TB investigation needed for:		
New household TB contact since last visit?	Yes	No
New or worsening symptoms?	Yes	No

Being investigated for TB or on TB treatment	Yes		No
If any of the above are answered "Yes", then issue 2 sputum jars with instructions.			
Sputum jars issued	Yes		No
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

Follow Up: 360 Days (1 Year)		
Was participant seen for a follow-up interview/evaluation?	Y	N
If no, reason participant not seen for follow-up visit. (specify reason for refusal: not contactable, out of study area, withdrew consent, died)		
Date of interview/ evaluation (DD/MON/YYYY)	____/_____/200____	
Name of interviewer		

Clinical History since Study Day 180			
Symptoms	*Please circle one for each symptom*		Specify number of weeks
Unexplained cough	Y	N	
Unexplained fever	Y	N	
Haemoptysis	Y	N	
Unexplained weight loss	Y	N	
Unexplained night sweats	Y	N	
Other (specify)	Y	N	
Other (specify)	Y	N	

If the participant has at least one symptom for two weeks or more or haemoptysis which is new or worsening since last contact, ***refer for TB investigation****.*

Medical History / Hospitalisation since Study Day 180		
Has the participant had any significant illness or been hospitalized since the last study visit?	Y	N
If yes, when? (month, year)		

If the participant reports significant illness or was hospitalized since the last study visit, fill in separate Hospitalisation DCF.

Study Day 360			
TB Contact History			
Has anyone with whom the participant is living with been diagnosed with TB since the last study visit? *(please circle one)*	Y	N	Unk
If so, who? (specify relationship to participant: father, mother, sibling, grandparent, aunt, uncle, cousin, friend, lodger)			
If yes, since when? (month, year)			
At what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)			

If the participant has a new household TB contact since the last interview, ***refer for TB investigation.***

History of TB since Study Day180		
Was participant investigated for TB since last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who? (specify the name of the clinic, or indicate 'Ukn' if not known)		
Was the participant put on TB treatment since the last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained. If any of the study procedures are not yet done, or if results are not available these procedures must be arranged (smears, cultures, chest x-ray and HIV test).

Note: Two sputum samples for smear and culture must be taken for each TB investigation.

Study Day 360			
Study Examination and Tests			
Is TB investigation needed for:			
New household TB contact since last visit?	Yes		No
New or worsening symptoms since last visit?	Yes		No
Being investigated for TB or on TB treatment	Yes		No
If any of the above are answered "Yes", then issue 2 sputum jars with instructions.			
Sputum jars issued	Yes		No
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		

Follow Up: 540 Days		
Was participant seen for a follow-up interview/evaluation?	Y	N
If no, reason participant not seen for follow-up visit. (specify reason for refusal: not contactable, out of study area, withdrew consent, died)		
Date of interview/ evaluation (DD/MON/YYYY)	____/_____/200____	
Name of interviewer		

Clinical History since Study Day 360			
Symptoms	*Please circle one for each symptom*		Specify number of weeks
Unexplained cough	Y	N	
Unexplained fever	Y	N	
Haemoptysis	Y	N	
Unexplained weight loss	Y	N	
Unexplained night sweats	Y	N	
Other (specify)	Y	N	
Other (specify)	Y	N	

If the participant has at least one symptom for two weeks or more or haemoptysis which is new or worsening since last contact, ***refer for TB investigation***

Medical History / Hospitalisation since Study Day 360		
Has the participant had any significant illness or been hospitalized since the last study visit?	Y	N
If yes, when? (month, year)		

If the participant reports significant illness or was hospitalized since the last study visit, fill in separate Hospitalisation DCF.

Study Day 540			
TB Contact History			
Has anyone with whom the participant is living with been diagnosed with TB since the last study visit? *(please circle one)*	Y	N	Unk
If so, who? (specify relationship to participant: father, mother, sibling, grandparent, aunt, uncle, cousin, friend, lodger)			
If yes, since when? (month, year)			
At what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)			

If the participant has a new household TB contact since the last interview, ***refer for TB investigation.***

History of TB since Study Day 360		
Was participant investigated for TB since last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who? (specify the name of the clinic, or indicate 'Ukn' if not known)		
Was the participant put on TB treatment since the last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained. If any of the study procedures are not yet done, or if results are not available these procedures must be arranged (smears, cultures, chest x-ray and HIV test).

Note: Two sputum samples for smear and culture must be taken for each TB investigation.

Study Day 540			
Study Examination and Tests			
Is TB investigation needed for:			
New household TB contact since last visit?	Yes		No
New or worsening symptoms since last visit?	Yes		No
Being investigated for TB or on TB treatment	Yes		No
If any of the above are answered "Yes", then issue 2 sputum jars with instructions.			
Sputum jars issued	Yes		No
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		

Follow Up: 720 Days (2 Year)		
Was participant seen for a follow-up interview/evaluation?	Y	N
If no, reason participant not seen for follow-up visit. (specify reason for refusal: not contactable, out of study area, withdrew consent, died)		
Date of interview/ evaluation (DD/MON/YYYY)	____/_____/200____	
Name of interviewer		

Clinical History since Study Day 540			
Symptoms	*Please circle one for each symptom*		Specify number of weeks
Unexplained cough	Y	N	
Unexplained fever	Y	N	
Haemoptysis	Y	N	
Unexplained weight loss	Y	N	
Unexplained night sweats	Y	N	
Other (specify)	Y	N	
Other (specify)	Y	N	

If the participant has at least one symptom for two weeks or more or haemoptysis which is new or worsening since last contact, ***refer for TB investigation****.*

Medical History / Hospitalisation since Study Day 540		
Has the participant had any significant illness or been hospitalized since the last study visit?	Y	N
If yes, when? (month, year)		

If the participant reports significant illness or was hospitalized since the last study visit, fill in separate Hospitalisation DCF.

Study Day 720			
TB Contact History			
Has anyone with whom the participant is living with been diagnosed with TB since the last study visit? *(please circle one)*	Y	N	Unk
If so, who? (specify relationship to participant: father, mother, sibling, grandparent, aunt, uncle, cousin, friend, lodger)			
If yes, since when? (month, year)			
At what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)			

If the subject has a new household TB contact since the last interview, ***refer for TB investigation.***

History of TB since Study Day 540		
Was participant investigated for TB since last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who? (specify the name of the clinic, or indicate 'Ukn' if not known)		
Was the participant put on TB treatment since the last study visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic? (specify the name of the clinic, or indicate 'Ukn' if not known)		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained. If any of the study procedures are not yet done, or if results are not available these procedures must be arranged (smears, cultures, chest x-ray and HIV test).

Note: Two sputum samples for smear and culture must be taken for each TB investigation.

Study Day 720			
Study Examination and Tests			
Is TB investigation needed for:			
New household TB contact since last visit??	Yes		No
New or worsening symptoms?	Yes		No
Being investigated for TB or on TB treatment	Yes		No
If any of the above are answered "Yes", then issue 2 sputum jars with instructions.			
Sputum jars issued	Yes		No
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

TB Investigation in between Visits (1)

Reason for TB investigation between visits			
Referred by the surveillance team	Parent/ participant contact with study staff	Referred by health services	Other: specify:

History of TB		
Date of interview	____/_____/200____	
Was participant investigated for TB since last visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who?		
Was the participant put on TB treatment since last visit	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic?		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained and any of the study procedures not yet done, must be arranged (smears, cultures, chest x-ray and HIV test). Two sputum samples for smear and culture must be taken.

Study Examination and Tests			
Sputum jars issued	Yes	No	
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

TB Investigation in between Visits (2)

Reason for TB investigation between visits			
Referred by the surveillance team	Parent/ participant contact with study staff	Referred by health services	Other: specify:

History of TB		
Date of interview	____/_____/200____	
Was participant investigated for TB since last visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who?		
Was the participant put on TB treatment since last visit	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic?		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained and any of the study procedures not yet done, must be arranged (smears, cultures, chest x-ray and HIV test). Two sputum samples for smear and culture must be taken.

Study Examination and Tests			
Sputum jars issued	Yes	No	
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

TB Investigation in between Visits (3)

Reason for TB investigation between visits			
Referred by the surveillance team	Parent/ participant contact with study staff	Referred by health services	Other: specify:

History of TB		
Date of interview	____/_____/200____	
Was participant investigated for TB since last visit?	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, by who?		
Was the participant put on TB treatment since last visit	Y	N
If yes, when? (DD/MON/YYYY)	____/_____/200____	
If yes, at what clinic?		

If the participant is currently on TB treatment for TB or is under investigation for TB, the results of their investigations must be obtained and any of the study procedures not yet done, must be arranged (smears, cultures, chest x-ray and HIV test). Two sputum samples for smear and culture must be taken.

Study Examination and Tests			
Sputum jars issued	Yes	No	
Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date sputum jars issued (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Repeat Date of sputum jar collection (DD/MON/YYYY)	1.____/_____/____ 2.____/_____/____		
Clinical assessment if unable to produce sputum	Not needed	TB highly likely	TB highly unlikely
Referred for sputum induction	Yes		No
Sputum induction performed	Yes		No
Date of induced sputum (DD/MON/YYYY)	____/_____/200___		N/A

Investigation for TB Disease (1)			
		Date (DD/MON/YYYY)	Result
Sputum1	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Sputum2	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Chest x-ray		___/_____/____	Description:
			Abnormal, consistent with TB
			Abnormal but not TB
			Normal
			Other
HIV test		___/_____/____	Positive
			Negative
Comments			

TB Treatment						
Referred for TB treatment		Yes	No			
Clinic Name						
Rx started	Yes	No	Rx name			
Rx start date (DD/MON/YYYY)		___/_____/____	Rx stop date (DD/MON/YYYY)	___/_____/____		
Standard DOTS therapy started		Yes	No			
Treatment outcome *(please circle one)*	Cured	Completed	Failed	Transferred	Interrupted	Died

Other Referrals		
Referred for other investigations and/or treatment	Yes	No
If yes, specify other investigations and/or treatment		

Investigation for TB Disease (2)			
		Date (DD/MON/YYYY)	Result
Sputum1	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Sputum2	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Chest x-ray		___/_____/____	Description:
			Abnormal, consistent with TB
			Abnormal but not TB
			Normal
			Other
HIV test		___/_____/____	Positive
			Negative
Comments			

TB Treatment						
Referred for TB treatment		Yes		No		
Clinic Name						
Rx started	Yes	No	Rx name			
Rx start date (DD/MON/YYYY)		___/_____/____	Rx stop date (DD/MON/YYYY)	___/_____/____		
Standard DOTS therapy started		Yes		No		
Treatment outcome *(please circle one)*	Cured	Completed	Failed	Transferred	Interrupted	Died

Other Referrals		
Referred for other investigations and/or treatment	Yes	No
If yes, specify other investigations and/or treatment		

Investigation for TB Disease (3)			
		Date (DD/MON/YYYY)	Result
Sputum1	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Sputum2	Normal/Induced *(please circle one)*	___/_____/____	Direct
			Culture
			Sensitivity
			Speciation
Chest x-ray		___/_____/____	Description
			Abnormal, consistent with TB
			Abnormal but not TB
			Normal
			Other
HIV test		___/_____/____	Positive
			Negative
Comments			

TB Treatment						
Referred for TB treatment		Yes		No		
Clinic Name						
Rx started	Yes	No	Rx name			
Rx start date (DD/MON/YYYY)		___/_____/____	Rx stop date (DD/MON/YYYY)	___/_____/____		
Standard DOTS therapy started		Yes		No		
Treatment outcome *(please circle one)*	Cured	Completed	Failed	Transferred	Interrupted	Died

Other Referrals		
Referred for other investigations and/or treatment	Yes	No
If yes, specify other investigations and/or treatment		

Follow Up 720 Days Participant Status

Participant Status
(please check one box):

☐ Completed

☐ Lost to follow-up
Date of last contact: ___/_____/____
DD/MON/YYYY

☐ Withdrew consent
Date of withdrawal: ___/_____/____
DD/MON/YYYY
Reason for withdrawal:_____
_____

☐ Death
Date of death: ___/_____/____
DD/MON/YYYY
Please check one in each item
Death certificate obtained? Y ☐ N ☐
Cause of death per death certificate: _____
_____
_____
Verbal autopsy performed? Y ☐ N ☐
Verbal autopsy results: _____
_____
_____
_____
Was TB the cause of death? Y ☐ N ☐
Full autopsy performed? Y ☐ N ☐
Full autopsy results: _____
_____
_____
_____
Was TB the cause of death? Y ☐ N ☐
Directed limited autopsy performed? Y ☐ N ☐
Directed limited autopsy results: _____
_____
_____
_____
Was TB the cause of death? Y ☐ N ☐

APPENDIX E: Withdrawal/Refusals Addendum

Withdrawal/Refusals Record ACS Extension

*Participants and their parents must be assured of their right to refuse and their right to withdraw and that there will be **NO** consequences to them answering this question. The collection of this information is merely for us to find out how we can improve participation in the study.*

Assignment *(please circle one)*: QFT converter QFT non-converter

Name of participant:_____

School:_____

Grade:_____

Male/ Female:_____

Age:_____

Point of refusal/ withdrawal *(please tick one)*:

Parental consent	Adolescent assent	Study Day 0	Study Day 180	Study Day 360
Study Day 540	Study Day 720	Other:		

Date of refusal:	____/_____/20___

Reason for refusal/ withdrawal:

_____

_____

Name of Interviewer: _____

APPENDIX F: Medical History/Hospitalisations Addendum

Medical History / Hospitalisation			
Has the participant had any significant illness or been hospitalized since the last study visit, including hospitalization(s) for acute illness(es)?	Y	N	Unk
If YES, please specify since which study visit.			

Episode	**Diagnosis/Acute Illness**	**Please tick one**	**Month, Year**	**Length of stay*(in days)***	**Hospital name**
1.	Asthma				
	Pregnancy				
	TB				
	Injury/Burns				
	Other (specify):				
2.	Asthma				
	Pregnancy				
	TB				
	Injury/Burns				
	Other (specify):				
3.	Asthma				
	Pregnancy				
	TB				
	Injury/Burns				
	Other (specify):				
4.	Asthma				
	Pregnancy				
	TB				
	Injury/Burns				
	Other (specify):				

APPENDIX G: TB FACT SHEET

10 Facts About Tuberculosis

1. 1.8 million people died from TB in 2007 worldwide.
2. More than 9 million cases of TB were reported in 2007 worldwide.
   (1.37 million of these 9 million TB cases were HIV positive)
3. South Africa is ranked 5th in the world for the number of TB cases.
4. South Africa has the highest number of new TB cases in the world.
5. 73% of TB cases in South Africa are HIV positive.
6. TB kills more youths and adults than any other infectious disease.
7. One third of the world's population is currently infected with the TB Bacillus.
8. TB is a contagious disease but only people that are sick with TB in the lungs are infectious (Pulmonary TB).
9. TB spreads though the air when infectious people cough, spit, talk or sneeze.
10. Left untreated, a person with active TB can infect between 10 and 15 people every year.

These are the **signs and symptoms** you should look out for. If you experience any of them, you should consult the nursing sister or doctor at your nearest clinic:

- **Coughing for three weeks or more**
- **Blood in sputum (Haemoptysis)**
- **Unexplained night sweats for three weeks or more**
- **Unexplained weight loss**
- **Unexplained fever for more than three weeks**
- **Unexplained loss of appetite**
- **Unexplained shortness of breath**

Should you experience any of these symptoms please contact the SATVI office (023) 346 5400: Sr. F Kafaar or Nurse M van Wyk

The figures quoted above were taken from the latest WHO global TB report:
Global Tuberculosis Control 2009, Epidemiology Strategy and Financing, WHO 2009.
ACS: 10 Facts about TB, Version 3.0, 21 May 2009. (English)

PART B: STRUCTURED LITERATURE REVIEW

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1. OBJECTIVES OF LITERATURE REVIEW

The study to be reported on focuses on the predictive ability of a converted interferon gamma release assay (IGRA) for tuberculosis (TB) disease. To contextualize this study, this literature review aimed to examine the available literature on the problem of TB disease and latent TB infection (LTBI) globally, the role of the tuberculin skin test (TST) in diagnosing TB and how IGRAs equate to the TST. Specifically we looked at the diagnosis of TB disease using IGRAs, the interpretation of serial IGRA data with a focus on IGRA conversion, the prognosis of an IGRA conversion and its potential use as a predictive test for the development of active TB disease.

2. SEARCH STRATEGY

A search was conducted in PubMed for articles published in English, related to the detection and/or diagnosis of tuberculosis infection and disease, the use of IGRAs, as well as the occurrence, measurement and interpretation of IGRA conversion and how these compare to TSTs. The following search phrases were used and combined using the Boolean operator "AND": (1) interferon gamma release assay OR interferon-γ assay OR IGRA OR T-cell assay; (2) tuberculosis OR TB OR tuberculous; (3) tuberculin skin test OR TST; (4) quantiFERON OR QFT (5) conversion OR IGRA conversion OR QFT conversion OR TST conversion (6) predictive value OR predictive test. The electronic literature search was supplemented by articles received from subscribing to an online TB diagnostics workgroup as well as checking the reference lists of articles selected for inclusion in this review.

3. SUMMARY OF THE LITERATURE

Tuberculosis was declared a global health emergency over fifteen years ago by the World Health Organisation (WHO), and it remains amongst the world's most lethal infections causing more adult deaths than any other disease.[1] It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*), resulting in 9.4 million new cases of TB and 1.3 million deaths due to TB being reported in 2009.[1] Despite effective treatment regimens being available worldwide, the chronic nature of the disease as well as the increasing emergence of *Mtb* strains that are resistant to the currently available antibiotics, make TB control difficult. The Global Plan To Stop TB 2011-2015 Report

produced by the Stop TB Partnership (WHO) states that new technologies are required for optimal prevention, diagnosis and treatment for all forms of TB in people of all ages.[2] They outline three strategies to combat TB: new diagnostics, new drugs and new vaccines. In the last five years, much progress in TB vaccine research has resulted in novel TB vaccine development and strengthening of the TB vaccine pipeline. Significant progress has also been observed in the development of new TB drugs, with at least eleven new or repurposed TB drugs currently under clinical investigation. However, minimal progress has been made in the development of new, more sensitive, simpler and cost effective diagnostic tools that will ultimately improve TB control as well as improve the quality of patient care.[2]

The "gold standard" for TB disease identification is the growth by culture of *Mtb* from biological specimens. The WHO 2008 Global Tuberculosis Report highlights the lack of culture facilities in the government health sector in most developing countries. Sputum smear examination is the mainstay of diagnosis in such settings.[3] Latent Tuberculosis Infection (LTBI) is defined as a positive TST result in an asymptomatic individual who has been exposed to TB and is showing no clinical or radiographic signs indicative of active TB disease.[4] Five to 10% of those with LTBI will develop active TB disease during their lifetime (per year for HIV positive individuals) as a result of reactivation of the bacillus.[5] The identification and treatment of LTBI to prevent progression to active TB disease thus remains a key method of helping to reduce the global TB burden. In developing countries, children under age 5 and HIV positive persons receive prophylactic treatment if diagnosed with LTBI. However, in developed countries where greater resources are available, a significantly larger number of persons diagnosed with LTBI receive prophylactic treatment.

3.1. Tuberculin Skin Test

The tuberculin skin test (TST) has been the standard test used for diagnosing LTBI for over 100 years.[6] The TST is dependent on a delayed hypersensitivity response to purified protein derivative (PPD) of *Mtb*. It is a relatively cheap test that requires no laboratory component. However, its known limitations are that the TST is confounded by non-tuberculous mycobacterial infections (NTMs), individual immune status, Bacillus Calmette-Guerin

(BCG) vaccination, the method of test administration as well as the need for a second patient visit for the reading of results 48-96 hours post administration.[7]

Although the TST has been used for many years, there are still aspects regarding the administration and interpretation of results that remain controversial. The use of repeated tuberculin tests have shown that tuberculin reactions may increase in size due to one of three reasons: random variability from differences in administration, reading of results, or biologic response; immunologic recall of preexisting delayed type hypersensitivity to mycobacterial antigens (boosting); or new infection (conversion). It has also been shown that tuberculin reactions may decrease in size (reversion).[6]

Tuberculin conversion in general may be defined as 'the development of new delayed type hypersensitivity to mycobacterial antigens following new infection with *Mtb*, NTMs or BCG vaccination.'[6] Various studies report the occurrence of tuberculin conversion, as well as an association between conversion and the development of TB disease.[8,9] Recent conversion is a known factor representing a higher risk for progression to TB disease compared to more remote infection. In a study of contacts of index cases with TB disease with documented tuberculin conversion, 7% developed active TB disease in the first year and an additional 7.5% developed TB disease in the 3 year period that followed.[8] Among a total of 29 000 adolescents, in those randomised to not receive BCG vaccination in Britian, tuberculin conversion was observed in 2 170 (7.5%) during the 2 year follow up period. TB disease was observed in 5.2% of those who converted (80% of these within 2 years).[9]

The booster phenomenon of an increased tuberculin reaction when retested within a short period of time without the presence of new infection results from the recall of waned cell-mediated immunity. Boosting is associated with TB infection that is remote in time, NTM sensitization and BCG vaccination and thus appears to be a nonspecific response to any preceding mycobacterial exposure.[6] Maximal boosting is observed if the interval between the first and second test is between 1 and 5 weeks but boosting can still be detected one or more years after a first negative tuberculin test.[10] Boosting is best distinguished from conversion on clinical grounds. Boosting is said to have occured when an increase in reaction size is seen after an interval of 1 to 5 weeks in which the individual has not been

exposed to *Mtb*. In contrast, conversion is said to have occurred when a previously TST-negative individual tests positive after either receiving BCG vaccination or following exposure to *Mtb*.[6]

Serial tuberculin testing has shown that tuberculin reversion may occur (ie. a decrease in tuberculin reaction size upon retesting). Various tuberculin reversion rates are reported for different studies with an increased occurrence in those in whom the boosting phenomenon has been identified.[6,11]

3.2. Interferon-gamma Release Assays

The recent identification of *Mtb* antigens absent from BCG and most NTMs has allowed for the development of more specific diagnostic tests than the TST. The interferon-gamma release assays (IGRAs) provide a new tool for the diagnosis of LTBI as well as for surveillance for new TB infections.[12] These tests detect interferon-gamma (IFN – γ) released by T cells when exposed to *Mtb* antigens in vitro. The antigens used are the early secreted antigenic target 6 (ESAT-6) and the culture filtrate protein 10 (CFP-10). Operationally, IGRAs have several advantages over the TST: they are unaffected by prior BCG vaccination, the tests can be completed in one visit with the results available within 24 hours; they are able to avoid false positives due to repeat testing; and have a standardized interpretation.[13] Limitations of IGRAs include the high cost, need for equipped laboratories and trained personel, and like TST, they cannot distinguish between LTBI and active TB disease.

There are two commercially available IGRAs that have been approved by the US Food and Drug Administration (FDA) that are in clinical use: the QuantiFERON-TB Gold (Cellestis®, Carnegie, Australia) and T-Spot.*TB* (Oxford Immunotec®, Oxford, United Kingdom).[13] There are also in-house developed versions in clinical use as well. The QuantiFERON Gold In-Tube (QFT-G IT) is a newer version of the QuantiFERON Gold (QFT-G). The QFT-G IT includes a third antigen, TB7.7 which is thought to enhance the sensitivity of the assay without reducing its specificity. In addition, QFT-G IT requires only 3mL of blood compared to the 16mL of the QFT-G assay. Both versions of QuantiFERON (QFT) are based on a 16-24hour whole blood enzyme-linked immunosorbent assay (ELISA); while the T-Spot.*TB* is

based on an enzyme-linked immunospot assay (ELISPOT) that utilises peripheral blood mononuclear cells (PBMCs).[14]

In theory, IGRAs have operational advantages that make them ideal for serial testing.[15] IGRAs may also have a potential use in providing a more accurate estimate of the annual risk of TB infection (ARTI) in specific populations.[16] IGRAs have been recommended for serial testing in some countries but data is limited on the interpretation of repeat IGRA testing. There is evidence from existing studies[17], although limited, that suggests conversions, reversions and non-specific variations occur with IGRA serial testing, as they do with the TST. However, there is still no consensus on how to define and/or interpret these conversions and reversions.[13]

In order to interpret and utilise IGRAs in serial testing, data is required to answer several key questions[15,16]: (1) What is the reproducibility of T cell responses over time (within-subject variations over time)? (2) What is an IGRA "conversion," and what threshold (cut-off) should be used to define conversion? How can IGRA conversions be distinguished from non-specific (random) variations in T cell responses over time? (3) What is the prognosis of an IGRA conversion? Are individuals with strong conversions (i.e large increases in IFN-γ responses over time) at higher risk of progression to active disease than individuals with weak conversions or negative results? (4) What is an IGRA "reversion," and what threshold should be used to define reversion? What is the clinical significance and prognosis of an IGRA reversion? (5) Will treatment of individuals with IGRA conversions reduce their risk of progression to active TB disease?

3.2.1. Within-Subject Variability of IGRAs

According to a systematic review conducted by van Zyl-Smit *et al.* 2009, there is a lack of published, peer-reviewed IGRA reproducibility data. Although several studies have reported assessment of IGRA reproducibility, these have been done following tuberculin skin testing or in the context of contact screening and thus cannot be considered to be reproducibility studies.[18] In a study carried out in India, in 14 health care workers (HCW), all BCG vaccinated, over a two week period, quantitative results showed a 16% increase of IFN-γ response to be within the 'normal' within subject variability.[19] In another study

carried out in South Africa, in 26 HCW and low risk volunteers, all BCG vaccinated, over a period of three weeks; quantitative results showed that a change of +/-80% of any given IFN-γ response (QFT-G IT) or +/-3 spots (T-SPOT.TB) was considered to fall within the 'normal' expected within subject variability.[20] Although these sample sizes are small, they show that within-subject variability in IGRA results does occur and is not inconsequential in high TB burden settings. Variability is also seen to be more frequent in individuals with baseline positive IGRA results, and in those results that are around the cut-off points.[18]

3.2.2. IGRA Reversion

In general, a reversion may be defined as an IGRA result that becomes negative upon repeat testing (ie. a decrease in IFN-γ responses). However, it remains unclear what threshold should be used to define reversion.[15] Studies show that IGRA reversions are more likely when the baseline test results are discordant – positive IGRA but negative TST.[17,21] There are several possible reasons why reversion occurs. Reversion might reflect clearing of TB infection, might be due to biological variations within IGRA positive individuals, or due to variability in laboratory and test procedures.[15] Hill *et al.* 2007 propose that IGRA responses are inherently transient and need continued exposure to TB antigens to maintain high frequencies. Thus, reversion could reflect a dormant stage in the *Mtb* life cycle where antigens such as ESAT-6 and CFP-10 are not reliably secreted.[17]

The prognosis of an IGRA reversion remains unclear. Friedman *et al.* 2007 suggest that reversions are an indication of a lack of immunity to TB and that individuals with IGRA reversions should be retested when exposed again.[22]

3.2.3. *IGRA Conversion*

In general, a conversion may be defined as an IGRA result that becomes positive upon repeat testing (ie. an increase in IFN-γ responses) following a new infection with *Mtb*.[15] Despite high IGRA conversion rates having been reported in high-risk populations in TB high burden settings[13,23], there is still no consensus on how to define and interpret these conversions. Uncertainty remains on how much increase in IFN-γ response is an indication of a true new infection and how much is due to biological or test variability.[15] There is

evidence to support the view that if a simplistic negative-to-positive cut-off is used to define conversions, IGRA conversion rates may be higher than those observed with TST.[13,23]

In a cohort study conducted in rural India, where the occurrence of conversions and reversions were investigated among 250 TB contacts; the effect of using various definitions and criteria for conversion was evaluated. They evaluated two definitions for TST conversion and four definitions for QFT conversion. Among 11 contacts with baseline TST-positive/QFT-negative results, four (36%) were reported to have converted using a simple negative-to-positive definition. Among the 85 contacts reported to have had baseline TST-negative/QFT-negative results, the estimated rates of QFT conversions (using four different definitions) ranged between 11.8% and 21.2%. The highest conversion rate of 21.2% (95% CI 13-31) was estimated using the least stringent definition of negative to positive [baseline IFN-γ <0.35 IU/ml and follow-up ≥0.35 IU/ml]; while the lowest conversion rate of 11.8% (95% CI 6-20) was estimated using the most stringent definition of an increase from baseline IFN-γ <0.35 IU/ml to ≥0.70 IU/ml. Although not statistically significant, there was nearly a two-fold difference between the most and least stringent definition for QFT conversion. In contrast, the estimated TST conversion rates ranged between 7.5% and 13.8%. With the most stringent definition for TST conversion [baseline TST <10mm and follow-up TST ≥10mm, with 10mm increment], the conversion rate was 7.5% (95% CI 3-16); while a conversion rate of 13.8% was observed with the least stringent definition [baseline TST <10mm and follow-up TST ≥10mm, with 6mm increment]. Although not statistically significant, the TST conversion rate with the most stringent definition is about three-fold lower than the QFT conversion rate estimated with the least stringent definition.[13]

The observed higher conversion rate of IGRAs when compared to TST could possibly indicate an increased sensitivity for conversions (not necessarily for the diagnosis of LTBI). A proportion of this higher sensitivity is probably a result of minor non-specific variations around the diagnostic cut-off; where a more stringent cut-off for conversion may reduce misclassification of minor variations as conversions.[15, 13] With regard to TST, Menzies (2000) states that variability in biologic response and differences in test administration and reading will result in increases of less than 6mm in 95% of subjects. Thus, an increase of 6mm or more (above a baseline induration of at least 10mm) should be considered to represent true

biological change (conversion or boosting).[6] Using a stringent cut-off to define IGRA conversion would be comparable to the TST threshold for conversion, which requires a 6mm increase in size of induration above the baseline value to an induration of at least 10mm.[15,6]

In a previous study, Harada *et al.* 2004 recommended the use of a 'grey zone' when analysing QFT results (0.10-0.35 IU/ml), suggesting that results in this grey zone should be excluded from conversion rate calculations.[24] Pai *et al.* 2009 also explored the use of a 'zone of uncertainty' on either side of the existing QFT cut-off (0.35 IU/ml). They chose 0.20-0.50 IU/ml as the uncertainty zone; where any value <0.20 IU/ml was considered 'definitely negative' and any value > 0.50 IU/ml was considered 'definitely positive'. A person was considered to have a 'true conversion' if their IFN-γ result increased from <0.20 IU/ml at baseline to >0.50 IU/ml on the repeat test. Among the 18 contacts who had QFT conversion using the least stringent definition [baseline IFN-γ <0.35 IU/ml and follow-up ≥0.35 IU/ml], the uncertainty zone analysis suggested that 'true conversions' occurred in 9/18 (50%).[13]

3.2.4. What is the predictive value of an IGRA conversion?

An established risk of developing active TB disease is associated with TST conversions.[25] Clinical trials have confirmed that the treatment of people who are TST positive reduces their risk of progression to active TB disease, and as a result guidelines for targeted TST testing and treatment have been established.[26] If IGRAs were found to have the same (or better) sensitivity of predicting the development of active TB disease as TST, it would minimize the rates of false-positive responses in contacts of TB cases meaning fewer people being assessed for TB disease and put on chemoprophylactic treatment. In the developing world, this could aid in focusing scarce TB-control and treatment resources only on those individuals identified to have the highest risk of developing active TB disease.[27]

There seems to be a growing consensus that a positive IGRA is a more specific indicator of the presence of LTBI than TST, and that it might be a better predictor of those individuals who will rapidly progress to TB disease. Several studies support a higher rate of progression to TB disease in those who are QFT and T.SPOT.*TB* positive than in those who are TST

positive[27-30]; while several other studies report the predictive values of QFT and T.SPOT.*TB* for the progression to TB disease to be comparable to that of TST.[31-34]

With limited data available, much uncertainty remains with regards to defining IGRA conversion and ultimately the risk of developing active TB disease that is associated with IGRA conversion. Conversion is indicative of recent infection, and the prognosis of new (recent) infection is expected to be different from that of more remote infection. In addition, the risk of developing active TB disease following a 'strong conversion' might differ from that of a 'weak conversion'.[15] Although there is no data to directly assess the prognosis of IGRA conversions, recent studies provide evidence that suggests that responses to ESAT-6 and CFP-10 are closely associated with bacterial replication in vivo as well as with the progression from TB infection to TB disease.[16,35] Andersen *et al.* 2007 hypothesized that high and/or rising levels of IFN-γ produced in response to ESAT-6 by T cells from recently TB-infected people may signal early disease and could possibly be used as a prognostic marker for the development of clinical TB disease.[35] If confirmed in large prospective cohort studies, the quantitative IFN-γ level could allow for the identification of a subgroup of individuals who may be in the process of developing TB disease[15]; providing a means by which to establish which individuals with LTBI should be treated first to prevent the development of TB disease.

4. NEED FOR FURTHER RESEARCH

In order to obtain data that will directly answer the questions of defining IGRA conversion as well as the prognosis of an IGRA conversion, prospective cohort studies need to be conducted that explore varying definitions for conversion and follow individuals from the time of conversion to document the incidence of TB disease in the years that follow, as well as to allow for the calculation of the time taken to develop TB disease following an IGRA conversion. Such studies would also allow for the assessment of the effect of using varying definitions for IGRA conversion on the calculation of the annual incidence of conversion as well as the annual incidence rate of TB disease following IGRA conversion.

5. CONTRIBUTION OF DISSERTATION TO AVAILABLE LITERATURE

The proposed study was an extension of a prospective epidemiological study of TB disease and infection in adolescents in the Worcester and surrounding areas in the Western Cape carried out from 2005 to 2009, from which a subset of adolescents who were identified to have converted their IGRA (QFT) status were followed up and observed for the occurrence of active TB disease. The results of this study will contribute to the very limited available literature on IGRA conversion by providing the first measure (to our knowledge) of the incidence rate of active TB disease following an IGRA conversion. In addition, the results will also provide insight on whether IGRA conversion is predictive for the development of active TB disease (in comparison to individuals without IGRA conversion); as well as assess the effect of varying the definition of conversion on the incidence rates of active TB disease following IGRA conversion.

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PART C: MANUSCRIPT

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ABSTRACT

Rationale: Conversions and reversions occur with interferon-gamma release assay (IGRA) serial testing, as with the tuberculin skin test (TST). An established risk of developing tuberculosis disease (TB) is associated with TST conversions. This risk in those with a converted IGRA status is unknown.

Objectives: To evaluate the incidence rate of active tuberculosis disease following QuantiFERON TB Gold In-Tube (QFT-IT) conversion compared to non converters.

Methods: From a parent cohort study, adolescents identified as having a converted IGRA status (QFT Converters [n=534]) were followed up for 2 to 4 years and observed for incident tuberculosis disease and compared to adolescents whose IGRA status remained negative throughout (QFT Non-Converters [n=629]).

Measurements and Main Results: For QFT Converters, the tuberculosis incidence rate (all cases) was 1.2 cases per 100 person years (95% CI 0.66-1.94); cumulative incidence 2.8% (95% CI 1.58-4.59) mean 2.4 years of follow up. 13/15(87%) incident cases developed tuberculosis disease within 2 years following QFT conversion. A significantly lower tuberculosis incidence rate (0.17 cases per 100 person years [95% CI 0.02-0.62]) and cumulative incidence (0.32% [95% CI 0.03-1.14]) was observed for QFT Non-Converters. The incidence rate ratio was 6.9 (95% CI 1.9-24.6) for all cases TB and 7.4 (95% CI 1.3-43.3) for per protocol TB.

Conclusion: QFT conversion is indicative of an approximately 7-fold higher risk of progression to TB disease (compared to non-converters) in a cohort of adolescents in a high TB burden population. Further studies in high and low TB burden settings are required to validate these findings.

(Word Count = 250)

INTRODUCTION

Interferon-gamma release assays (IGRAs) provide a new tool for the diagnosis of latent tuberculosis infection (LTBI).[1] IGRAs are blood tests that detect interferon-gamma (IFN-γ) released by T-cells on exposure to *Mycobacterium tuberculosis* (*Mtb)* antigens. These antigens are more specific to *Mtb* than the purified protein derivative (PPD) used in the tuberculin skin test (TST).[2,3,4]

IGRAs have been recommended for serial testing for tuberculosis (TB) infection in populations with continued risk of TB exposure.[5] In low TB incidence countries initial testing with TST followed by IGRAs to confirm positive TST results or use of IGRAs as an alternative to TST has recently been recommended.[2,6,7] IGRAs have features that make them ideal for serial testing: they are unaffected by prior Bacilli Calmette Guérin (BCG) vaccination, they are *ex-vivo* assays that can be completed in one visit, they can be repeated without sensitization or boosting, they have a standardized interpretation, and there is evidence that suggests IGRAs may be better than TST at detecting recent rather than remote TB infection.[8-10] Although limited, data from existing studies shows that conversions, reversions and non-specific variations occur with IGRA serial testing as they do with TST serial testing.[10-13]

Several reviews indicate that in order to utilize IGRAs in serial testing, evidence is still required to answer several key questions that include[8,9,3]: (a) What is an IGRA "conversion" and what threshold should be used to define conversion? (b) What is the prognosis of an IGRA conversion? (c) What is an IGRA "reversion," and what threshold should be used to define reversion?

An established risk of developing active TB disease is associated with TST conversions.[14] The risk in those with a converted IGRA status remains unknown. IGRA conversion is indicative of recent infection, and the prognosis of new (recent) infection is expected to be different from more remote infection.[8]

In the present study we report the first measure (to our knowledge), of the TB incidence rate following IGRA conversion. The objective of our study was to evaluate the incidence

rate of active TB disease following QuantiFERON Gold In-Tube (QFT-IT) conversion and to assess the predictive value of QFT-IT conversion for the development of active TB disease. We also assessed the effect of varying the definition of QFT-IT conversion on the TB incidence rate. The study was conducted in Worcester, a small town in the Western Cape Province of South Africa with an estimated population of 151 806[15] and a TB incidence rate of 1400/100 000 reported in 2006.[16]

METHODS

Study Design

The study reported here is a prospective observational cohort study with no experimental interventions. It was conducted as an extension of a prospective epidemiological cohort study of TB disease and infection in adolescents (aged 12 to 18 at time of enrolment into parent cohort study) in Worcester and its surrounding areas in the Western Cape, South Africa.[17] Participants from both the active and passive follow up strategies of the parent cohort study[17] whose QFT-G IT test status converted from having been negative at baseline to being positive at a subsequent time point (QFT Converters at 6, 12, 18 or 24 months) were followed up for a further two years after their 2-year study visit of the parent study and observed for the development of active TB disease. This led to a maximum of up to 4 years post conversion follow up. A similar sized, randomly sampled control group of participants who remained QFT-G IT negative throughout the parent cohort study (QFT Non-Converters) underwent similar follow-up. The cohort resulting from these two subgroups (QFT Converters and QFT Non-Converters) was observed further from March 2009 until September 2010. Thus, observation time for all participants is measured beginning from 2-year visit of the parent cohort study, and continued until the last study visit in the subsequent study reported here. Participants from both study groups were seen at six monthly intervals and interviewed about: new TB symptoms; new household contacts; being screened for TB and/or diagnosed with TB since the last study visit. As with the parent cohort study[17], hospital admission and mortality surveillance were conducted for all participants. The study was approved by the Faculty of Health Sciences Human Ethics Committee at the University of Cape Town, South Africa. Informed consent was

obtained from participants >18 years; while participant assent and parental consent were obtained for those <18 years.

TB Diagnosis and Diagnostic Processes

A case of TB was defined as any case that was diagnosed by a physician and confirmed by two or more sputum smears positive for acid fast bacilli (AFB) and/or one positive sputum culture for *Mtb*. Participants suspected of having TB were investigated through sputum tests and/ or chest x-rays. In the case of negative sputum smears and cultures, or in the absence of these tests, TB diagnosis by physicians was based on chest x-rays and clinical symptoms.

Definitions of Conversion and Reversion

We explored four definitions for QFT conversion based on the definitions reported by Pai *et al.* (2009)[10]:

(1) baseline IFN-γ <0.35 IU/ml and follow up IFN-γ ≥ 0.35 IU/ml;[5]

(2) baseline IFN-γ <0.35 IU/ml and follow up IFN-γ ≥ 0.35 IU/ml, plus a 30% increase in IFN-γ over the baseline value;[18]

(3) baseline IFN-γ <0.35 IU/ml and follow up IFN-γ ≥ 0.35 IU/ml, plus an absolute increase of 0.35 IU/ml over the baseline value;[11] and

(4) baseline IFN-γ <0.35 IU/ml and follow up IFN-γ ≥ 0.70 IU/ml.[11]

Only one definition was explored for QFT reversions: baseline IFN-γ ≥ 0.35 IU/ml and follow up IFN-γ < 0.35 IU/ml.[10]

Uncertainty Zone Analysis

Some studies have reported a 'zone of uncertainty' regarding QFT conversion, where values falling in this zone might not be 'true conversions'.[19,10] We explored an uncertainty zone of 0.20 UL/ml-0.50 UL/ml as described by Pai *et al.* (2009) and any QFT results falling into this zone were said to be 'doubtful conversions'.[10]

Statistical Analyses

All data was captured in a Microsoft Access database. Categorical data was compared between the two study groups using the Pearson's χ^2 test. The two sample t-test was performed to assess whether there was a difference in the distribution of continuous variables between the two study groups. The cumulative incidence for TB disease for each study group is reported with the 95% confidence interval (CI). TB incidence rates were calculated for the two study groups and the different QFT conversion definitions. TB incidence rates are reported per 100 person time years of observation and the 95% CI are given for each rate. Observation time was calculated from the participants 2-year (24 month) study visit in the parent cohort study for both QFT Converters and QFT Non-Converters. Survival analysis was conducted to compare Kaplan-Meier survival estimates for the time taken to develop TB disease for the two study groups. Cox proportional hazards regression was conducted in order to assess if there was an association between baseline characteristics and the risk of developing TB disease. Statistical analyses were performed using STATA/IC 10 (Stata Corp, College Station, TX, USA) software.

RESULTS

Study Participants

The selection of study participants is set out in Figure 1. From May 2005 to February 2009, a total of 6363 adolescent participants were enrolled into the parent cohort study and followed up using either active or passive follow up strategies[17]; with participants in either follow up strategy receiving at least a baseline QFT and a follow up QFT at 24 months. From these participants, a total of 798 participants were identified to have a converted QFT status (baseline negative IFN-γ <0.35 IU/ml and follow up positive IFN-$\gamma \geq 0.35$ IU/ml at 6, 12, 18 or 24 months) on repeat testing. All contactable QFT converter participants were approached and consent for further follow up was obtained if they were willing to participate. Consent for continued follow up from March 2009 was obtained from 675 participants. An additional 8 participants were identified to have developed TB disease already by March 2009 and although these participants did not require further follow up;

they were included in the analysis as cases. From these 683 QFT Converters (675 + 8), only 534 (baseline negative IFN-γ <0.35 IU/ml and follow up positive IFN-γ ≥ 0.35 IU/ml at 24 months) were selected for inclusion in the analysis presented here. This was done in order to standardize the conversion point used by restricting the analysis only to those who converted at 24 months. From the parent cohort, a total of 1993 QFT Non-Converters were identified. These were participants whose QFT status remained negative throughout the study (baseline negative IFN-γ <0.35 IU/ml and follow up negative IFN-γ <0.35 IU/ml at 6, 12, 18 and 24 months). A random sample of these was selected for continued follow up from March 2009 and 629 QFT Non-Converters were subsequently consented and included in the analysis presented here. The mean follow up time for the QFT Converter group was 2.4 years (range 0.3 months to 48 months). The mean follow up time for the QFT Non-Converter group was 1.9 years (range 10 months to 37 months).

The baseline characteristics of the selected participants are shown per study group in Table 1. There was no significant difference between the two study groups regarding sex and employment status. Although the mean age at consent for continued follow up in this study for the two groups were similar - 18.71 years for QFT Converters and 18.48 years for QFT Non-Converters; the difference was found to be significant (p=0.01). There were also small but significant differences between the two study groups with respect to school attendance, BCG scar, ethnicity and income.

TB Incidence Rates and Cumulative Incidence by QFT group

15 participants in the QFT Converter group were diagnosed with TB, of whom 8 met the protocol case definition and 7/8 being culture positive. Two participants in the QFT Non-Converter group were diagnosed with TB, with one meeting the protocol case definition and being culture positive. The overall TB incidence rates and cumulative incidence by QFT group are shown in Table 2a. The TB incidence rate (all cases) for the QFT Converter group was 1.2 cases per 100 person years (95% CI 0.66-1.94) and the cumulative incidence was 2.8% (95% CI 1.58-4.59) over the 2.4 mean years of follow up. 13/15(87%) of the QFT Converter group incident cases developed TB within 2 years following QFT conversion.

The positive predictive value of QFT conversion for development of active TB disease within 2 years following conversion is 2.4% (13/534). The incidence rate (all cases) for the QFT Non-Converter group was 0.17 cases per 100 person years (95% CI 0.02-0.62) and the cumulative incidence was 0.32% (95% CI 0.03-1.14) over the 1.9 median years of follow up. Both QFT Non-Converter incident cases occurred within 2 years of follow up. The incidence rate ratio (IRR) was 6.9 (95% CI 1.9-24.6).

Per protocol analysis of TB Incidence Rates and Cumulative Incidence by QFT group

Using only the incident cases meeting the protocol TB case definition, both the incidence rates and cumulative incidence for both study groups were halved (Table 2b). The incidence rate ratio was 7.4 (95% CI 1.3-43.3).

Survival Analysis

Survival analysis showed a significant difference in Kaplan-Meier survival estimates for the time taken to develop TB disease for QFT Converters when compared to QFT Non-Converters (Log Rank test: χ^2=9.45; p=0.002). Cox proportional hazards regression revealed that other than the QFT conversion status [univariate analysis: hazard ratio (hr)=7.35; p=0.009], none of the variables assessed at baseline (Table 1) had a significant effect on the risk of developing TB disease.

Annual Incidence Rates in years following QFT conversion

A further analysis was conducted to assess the annual TB incidence rates in the years following QFT conversion. These incidence rates are shown in Table 3. In the first to the fourth year following QFT conversion, TB incidence rates were 1.7, 0.84, 0 and 4.8 per 100 person years respectively, with no significant difference being observed. Most cases, 9/15 (60%) occurred within the first year following QFT conversion. A much smaller number of participants were observed in the fourth year compared to years 1, 2 and 3, with 2/15 (13%) cases occurring.

The effect of varying the definition of QFT Conversion on the TB Incidence Rate

Among the 534 QFT Converters, the TB incidence rate using the four different QFT conversion definitions ranged between 1.20 and 1.34 per 100 person years. The number of participants, the number of incident cases that met the criteria for each QFT conversion definition as well as the incidence rate for each are shown in Table 4. The TB incidence rates were very similar across the four QFT conversion definitions with no significant differences observed.

Uncertainty Zone Analysis

Among the QFT Converters defined using the least stringent definition, 116/534 (21.7%) fell into the uncertainty zone [0.20 UL/ml-0.50 UL/ml]. These included 4/15(26.7%) of the incident cases. This analysis suggests that 'true conversion' [a change from <0.20 UL/ml to > 0.50 UL/ml] occurred in 418/534 (78.3%) QFT Converters and this included 11/15 (73.3%) of the incident cases. This results in a cumulative incidence of 2.6% (11/418).

Incidence of QFT Reversions

Participants with QFT reversions were not included in the analysis presented here. However, using a simple positive to negative definition for reversion [baseline IFN-$\gamma \geq 0.35$ IU/ml and follow up IFN-$\gamma < 0.35$ IU/ml] we were able to identify 241/6363 (4.1%) QFT reversions in the parent cohort study. There were no incident cases amongst the QFT reverters.

DISCUSSION

This study provides the first measure (to our knowledge) of the incidence rate of active TB disease following an IGRA (QFT) conversion as well as its predictive value for the development of active TB disease. QFT Converters are shown to have a higher risk of developing TB disease when compared to QFT Non-Converters. Survival analysis revealed a significant difference in the time taken to develop TB disease between the QFT Converters and QFT Non-Converters. QFT Converters were shown to have a 7-fold increased relative hazard of developing TB disease when compared to QFT Non-

Converters [hr=7.35; p=0.009]. The positive predictive value of QFT conversion for the development of active TB disease is 2.4% within 2 years following QFT conversion. Varying the definition of QFT conversion did not significantly affect the TB incidence rate.

The identification and treatment of latent tuberculosis infection (LTBI) to prevent progression to active TB disease remains a key method of helping to reduce the global TB burden (mostly in developed countries at this stage). The rationale for this being that treating LTBI will decrease the incidence of TB disease and consequently reduce the transmission of *Mtb*. However, the question remains as to which individuals with LTBI present the highest risk of progression to TB disease. The predictive value of positive IGRAs compared to TST for the development of TB disease has been evaluated in several studies, with inconsistent results of progression to TB disease in those who are QFT positive compared to those who are TST positive.[20-24] In contrast, several other studies (including the parent cohort study[17]) report the predictive values of QFT and T.SPOT.TB for the progression to TB disease to be comparable to that of TST.[11,25-27] A known risk of developing active TB disease is associated with TST conversions.[28] Clinical trials have confirmed that the treatment of people who are TST positive reduces their risk of progression to active TB disease, and as a result guidelines for targeted TST testing and treatment have been established.[29] If IGRAs were found to have the same (or better) sensitivity of predicting the development of active TB disease as TST, it would minimize the rates of false-positive responses in contacts of TB cases meaning fewer people being assessed for TB disease and put on preventative treatment. In the developing world, this could aid in focusing limited TB-control and treatment resources only on those individuals identified to have the highest risk of developing active TB disease.[20]

The results of this study show that the risk of developing TB disease is significantly higher in QFT Converters when compared to QFT Non-Converters. At baseline, some small but significant differences in demographic characteristics were observed between the two study groups and these differences could be said to have influenced the difference in 'survival' between the two study groups. However, following survival analysis, cox proportional hazards regression revealed that the only significant determinant for the

development of TB disease was the QFT group. A lack of significant risk factors other than QFT conversion may have been due to the small sample size.

The results of this study regarding the predictive value of IGRA (QFT) conversion for the development of TB disease are in line with those reported for tuberculin conversion and its association with the development of TB disease.[30] Recent conversion is a known factor representing a higher risk for progression to TB disease compared to more remote infection. Among a total of 29 000 adolescents, in those randomised to not receive BCG vaccination in Britain in 1966, tuberculin conversion was observed in 2 170 (7.5%) during the 2 year follow up period. TB disease was observed in 5.2% of those who converted (80% of these within 2 years).[30] While our sample size was much smaller, the results of our study show that from a total of 6363 adolescents followed up in a cohort study, QFT conversion was observed in 798 (12.5%) during the two years of follow up. In this analysis 534/798(67%) QFT Converters were assessed further for the development of TB disease. Of these, 15(2.8%) were diagnosed with TB disease (87% of these within 2 years following QFT conversion). Although our study reports a higher conversion rate (which is probably due to the higher TB incidence in the area), we report a lower TB disease rate. This may be due to our selection method: we only included converters who had a negative baseline QFT and a positive QFT at 24 months. Thus, some of the TB cases may have occurred between 0 (baseline)-24 months of the parent cohort study, but we could not include them in the analysis for correct comparison with the QFT Non-Converter group.

Our results suggest that 100 people identified to have converted their QFT status would require treatment to prevent 2-3 TB cases in a period of 2 years. However, the numbers based on these results are too low to warrant preventive therapy. The cost effectiveness of such preventive measures within a high TB burden setting such as ours, where resources are very limited, and re-infection is common, would have to be evaluated. Thus, at this stage the findings of this study might be of more relevance to low TB burden countries where many/most infected individuals receive preventive therapy.

We explored the effect of varying the definition of QFT conversion (as described elsewhere[10]) on the TB incidence rate for the QFT Converters. An interesting finding in our

study was that varying the definition of QFT conversion had very little impact on the TB incidence rate with the least stringent and most stringent definitions reporting very similar incidence rates. In addition, 14/15(93%) incident cases met the criteria for the most stringent definition suggesting that a considerable change in the quantitative measure of IFN-γ is observed for QFT converters who subsequently develop TB disease. Application of an 'uncertainty zone' showed 78% (with 11/15 (73%) incident cases) of QFT conversions to be 'true conversions'; resulting in a very similar cumulative incidence. Our results suggest that application of this zone results in fewer converters and fewer TB cases, but hardly affects the TB incidence rate. The use of such a zone would have to be explored further.

There were several limitations to the analysis presented here. The absolute number of QFT Converters identified from the parent cohort study was relatively small. Due to the structure of the parent cohort, the number of QFT Converters subsequently used in the analysis was even smaller suggesting that the TB incidence rate in the QFT Converter group was an underestimate. The intended extended follow up period was at least 2 years post QFT conversion, but a considerable number 175/534 (33%) of QFT Converters had an extended follow up period of < 2years post conversion due to premature termination of the study because of financial constraints. Several incident cases might have been missed as a result of this shortened observation time. Investigation and diagnosis of participants were partly dependent on public health services and not all test results were made available to study staff. This could possibly have had a negative impact on the per protocol analysis of the TB incidence rates. HIV status was not determined in this study except that all those diagnosed with TB were offered an HIV test. However, background HIV prevalence is relatively low in this community and HIV status was not expected to influence the results of this study.

We conclude that QFT conversion is indicative of an approximately 7-fold higher risk of progression to TB disease (compared to non-converters), in a cohort of adolescents in a high TB burden population. The predictive value of QFT conversion for the development of active TB disease is 2.4% within 2 years following QFT conversion. Varying the definition of QFT conversion seems to have minimal effect on the TB incidence rate. More prospective cohort studies in both high and low TB burden settings, with larger sample

sizes and longer follow up periods are required to further validate our findings so as to establish an optimum definition for QFT conversion, the clinical outcome for those identified to have converted and the predictive value of a QFT conversion for the development of TB disease.

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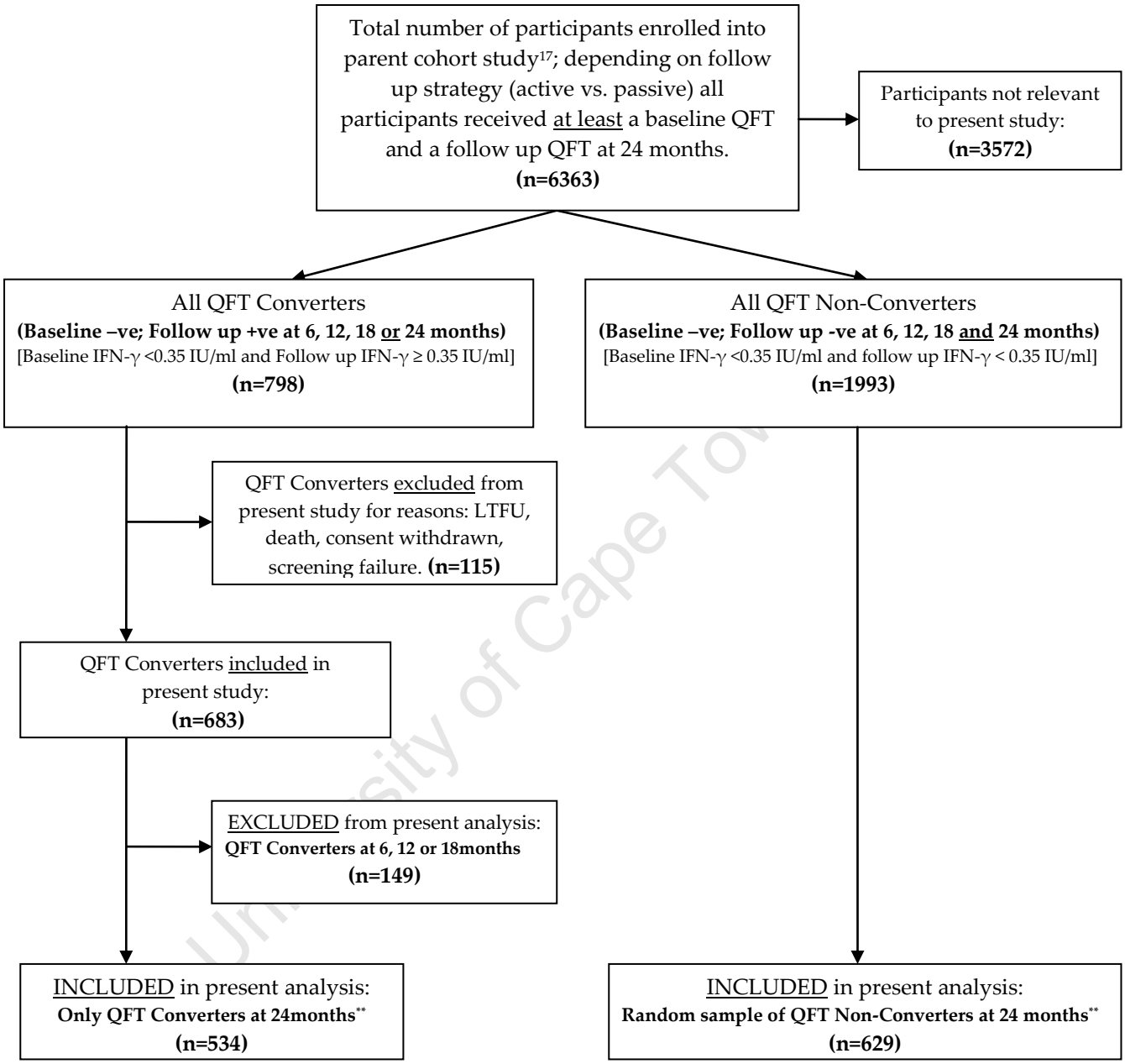
Figure 1. Flow diagram showing selection of participants



*QFT=QuantiFERON; IFN-γ=interferon gamma; LTFU=loss to follow up

**** Observation time was calculated from the participants 2-year (24 months) study visit for both QFT Converters and QFT Non-Converters.**

Table 1. Baseline Demographic Profile

Variable	n	QFT Converter n (column %)	QFT Non-Converter n (column %)	*p*
No. of participants	1163	534	629	
Sex				
Male	514	233 (44)	281 (45)	0.72
Female	649	301 (56)	348 (55)	
School				
In	638	274 (51)	364 (58)	0.03
Out	525	260 (49)	265 (42)	
Ethnicity				
Black	188	98 (18)	90 (14)	<0.001
Coloured	878	427 (80)	451 (72)	
White/Indian	97	8 (2)	86 (14)	
Employment status				
Employed	277	138 (26)	139 (22)	0.14
Unemployed	886	396 (74)	490 (78)	
BCG scar				
Present	494	257 (48)	237 (38)	0.001
Absent	299	119 (22)	180 (29)	
Not sure	370	158 (30)	212 (33)	
Income				
R1-R1000	89	56 (11)	33 (5)	0.004
R1001-R4000	104	53 (10)	51 (8)	
None	1000	425 (79)	545 (87)	
Average Age (SD)		18.7(1.4) yrs	18.5(1.5) yrs	0.01

*QFT=QuantiFERON; BCG=Bacille Calmette Guerin; yrs=years

**Age calculated for each participant at the point which they were re-consented for continued follow-up for the present study.

Table 2a. Overall TB Incidence and Cumulative Incidence by QFT group (all cases TB)

Study Group	N	TB Incident Cases	Observation Time (pyrs)	Incidence Rate per 100pyrs (95% CI)	Cummulative Incidence (95% CI)
QFT +ve	534	15	1268	1.2 (0.66-1.94)	2.8% (1.58-4.59)
QFT -ve	629	2	1169	0.17 (0.02-0.62)	0.32% (0.03-1.14)

*QFT=QuantiFERON; TB=tuberculosis; +ve=positive; -ve=negative; pyrs=person time years; CI=confidence interval

Table 2b. Overall TB Incidence and Cumulative Incidence by QFT group (*PP* TB cases)

Study Group	N	TB Incident Cases	Observation Time (pyrs)	Incidence Rate per 100pyrs (95% CI)	Cummulative Incidence (95% CI)
QFT +ve	534	8	1268	0.6 (0.27-1.24)	1.4% (0.65-2.93)
QFT -ve	629	1	1169	0.08 (0.002-0.48)	0.16% (0.004-0.88)

**PP*=per protocol; TB=tuberculosis; QFT=QuantiFERON; +ve=positive; -ve=negative; pyrs=person time years; CI=confidence interval

Table 3. Annual Incidence Rates in years following QFT conversion.

Time from QFT Conversion Date	N	Observation Time (pyrs)	TB Incident Cases (n)	Annual TB Incidence Rate (per 100 pyrs)	95% CI
Year 1	534	528	9	1.70	0.78-3.20
Year 2	524	475	4	0.84	0.23-2.14
Year 3	359	222	0	-	-
Year 4	113	41	2	4.8	0.58-16.16

*QFT=QuantiFERON; TB=tuberculosis; CI=confidence interval; pyrs= person time years

Table 4. The Effect of varying the definition of QFT conversion on the TB Incidence Rate.

Criteria for Defining QFT Conversion	Number of participants	TB Incident Cases (n)	Incidence Rate (per 100 pyrs)	95% CI
1. Baseline IFN-γ <0.35, repeat IFN-γ ≥0.35 IU/ml	534 (100%)	15	1.20	0.66-1.94
2. Baseline IFN-γ <0.35, repeat IFN-γ ≥0.35 IU/ml, plus 30% increase over baseline value	514 (90.3%)	15	1.22	0.68-2.01
3. Baseline IFN-γ <0.35, repeat IFN-γ ≥0.35 IU/ml, plus absolute increase of 0.35 IU/ml over baseline value	461 (80.7%)	15	1.34	0.75-2.20
4. Baseline IFN-γ <0.35, repeat IFN-γ ≥0.70 IU/ml	461 (80.5%)	14	1.25	0.68-2.08

*QFT=QuantiFERON; IFN-γ=interferon-gamma; TB=tuberculosis; CI=confidence interval; pyrs=person time years