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The Incidence of Tuberculosis in Adolescents in the Context of Proposed TB Vaccine Trials

Hassan Mahomed

**Thesis presented for the degree of
Doctor of Philosophy
in the School of Public Health and Family
Medicine**

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May 2013

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Verver

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town. The work on which this thesis is based is original research and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely the work of the candidate, or in the case of multi-authored published papers, constitutes work for which the candidate was the lead author. The contribution of the candidate to included multi-authored papers is further delineated in the introduction to each included paper as appropriate. Approval for inclusion of published papers in this thesis was obtained from the Doctoral Degrees

Board of the University of Cape Town.

Hassan Mahomed, May 2013

Abstract

Background

Tuberculosis (TB) is a significant global health problem and the development of new TB vaccines is one strategy proposed to address this scourge. Adolescents are a potential target group for new TB vaccines. Limited data are available in the scientific literature on the epidemiology of TB in adolescents. This thesis aimed to add substantial data on adolescent TB epidemiology through a cohort study of TB infection and disease in adolescents in a high burden setting. Such data will support clinical trials of new TB vaccines in adolescents but the knowledge gained will also be of value for TB Control Programmes.

Methods

Adolescents aged 12-18 years were recruited from 11 high schools in the rural town of Worcester and surrounding areas of the Western Cape Province of South Africa. They were screened at baseline for latent TB infection using both the tuberculin skin test (TST) and an interferon gamma release assay, the QuantiFERON® TB Gold (in-tube) assay (QFT). They were also screened for TB disease using an algorithm composed of a set of screening tests. They were followed up for at least two years for incident TB and the predictive value of the baseline TST and QFT for incident TB disease was compared. Demographic, socio-economic and clinical predictive factors for latent TB infection prevalence at enrolment and for incident TB disease during follow up were determined. A survey of attitudes to participation in TB vaccine clinical trials in a subset of

adolescents from these schools was also conducted. Both studies had ethics approval. Standard scientific statistical techniques were used to analyse the data.

Results

Fifty eight percent (6363) of the target population of 10,492 adolescents were recruited into the main cohort study. A prevalence of latent TB infection amongst the study participants at enrolment of 55% (TST) and 51% (QFT) was found. Predictive factors for latent infection were: being of black or mixed race origin compared to being of white or indian origin, older age (>15 years), previous household TB contact, low parental income and low education status of the parents. The TST and QFT were found to have good agreement (% agreement 84.8%, kappa [κ] = 0.70, 95%CI 0.68–0.71) in contrast to certain studies in other settings. A baseline prevalence of TB disease of 3/1000 was found in adolescents. While the TST and QFT were sensitive predictors of the presence of TB disease, none of the screening tests evaluated (TB related symptoms, recent household contact, TST or QFT) had high positive predictive values (all less than 2%) making these tests impractical for routine use. Given the imperative for screening in TB vaccine trials, these data are important for deciding on choice of screening tests in a clinical trial setting. Both the TST and QFT were found to be predictive of the onset of TB and were equally predictive. An incidence of bacteriologically confirmed active TB of 0.45/100 (95% confidence interval 0.29-0.72) person years (pyrs) was found in this cohort. Using different definitions of active TB, the rate varied from 0.31-0.59/ 100 pyrs. Risk factors gleaned at baseline that were predictive of the onset of TB disease were: being of black or mixed race origin, maternal education of primary school or less or

unknown, evidence of latent infection (positive TST or QFT) and absence of a BCG scar. Knowledge of TB was fair amongst adolescents but willingness to participate in TB vaccine trials varied depending on the procedures involved.

Limitations

Important limitations were as follows. The data presented are likely to underestimate the true prevalence and incidence of TB amongst adolescents in general since adolescents not at school are likely to have higher rates of TB than those attending school. On the other hand TB rates amongst those recruited are likely to have been higher than those not agreeing to participate since participation rates were higher in poorer schools than in more affluent schools. Chest radiographs as a screening tool for TB could not be evaluated because this method of screening was excluded for logistical reasons. The number of TB cases is likely to have been underestimated since smear screening was the main method of case detection and smear negative culture positive TB cases would have been missed.

Application of results

A range of data was obtained through these analyses which will be very useful for planning TB vaccine trials in adolescents and also for TB Control Programmes. Since data were collected in a high TB burden setting, the findings are mainly generalisable to such settings rather than low burden settings. Nevertheless, efficacy trials of new TB vaccines are likely to be carried out in high TB burden settings making these results highly relevant to TB vaccine efficacy trial planning. Policy with respect to the use of interferon gamma release assays will be informed by this data given that it is a relatively

new diagnostic modality. Knowledge of the baseline prevalence of TB disease and the utility of different screening tools amongst healthy adolescents would help the design and costing of screening approaches to be used in TB vaccine clinical trials which include adolescents. The data on the prevalence of latent TB infection will assist with the selection of TB vaccine candidates for this target group will be of value given that certain vaccines are designed to target those with latent infection. These data will also support planning where latent TB infection is an exclusion criterion such as in safety trials. TB incidence rates can be used to plan samples sizes for efficacy trials. The comparison of the TST and QFT with respect to prevalence of latent TB infection and predictive value for TB disease provide evidence for policies on the use of these tools in clinical trials and for TB Control Programmes in high burden settings. The fact that these measures showed good agreement and were equally predictive of the onset of TB disease, suggest that the QFT need not replace the TST in current routine practice. However, the two tests may be used interchangeably to equal effect. The knowledge and attitudes of adolescents towards participation in TB vaccine trials provides some guidance with respect to what to expect when approaching this group for recruitment purposes.

In summary, the prevalence of latent TB infection, the prevalence and incidence of TB disease and predictive factors for latent infection and disease as well as the knowledge and attitudes of adolescents towards participating in TB vaccine trials are described in this thesis. The application of these results to TB vaccines trials and potential value in TB Control Programmes is discussed.

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University of Cape Town

Dedication:

To Colette, Rosa, Rubin and Nadiya who endured my absences and distracted mind during this period.

To the staff at the South African TB Vaccine Initiative (SATVI) who conducted the study (and those who were not directly involved), all of whom have left me with fond memories of our work together.

Glossary of abbreviations and terms

Adolescent	– any child from 12 to 18 years of age.
Active TB	– the presence of active disease processes in the body usually associated with the presence of <i>Mycobacterium tuberculosis</i> or typical histological changes.
BCG	- Bacillus Calmette Guérin
Boost vaccine	- a vaccine given in addition to another vaccine for the same disease but usually at a later time point.
HIV	- Human Immunodeficiency Virus
IGRA	- interferon gamma release assay
Latent TB infection	– a state whereby <i>Mycobacterium tuberculosis</i> is present in the body in a contained way without the host experiencing any feelings of ill health. Usually measured through the presence of a positive TST or IGRA.
PPD	- Purified Protein Derivative (of tuberculin)
QFT	- QuantiFERON® TB Gold (in-tube) assay (Cellestis, Victoria, Australia)
TB	- tuberculosis.
TB prevalence	– the number of active TB cases at a point in time as a proportion of total number at risk.
TB incidence	– number of new cases of active TB occurring over a defined period
TST	- Tuberculin Skin Test
WHO	- World Health Organisation

Note on included publications:

Please note that the final accepted version of the manuscript of each paper is included in this thesis according to the formatting style of the journal concerned including the references. The referencing and references in all other sections are according to the Vancouver method. The tables and figures for the papers are shown after the references in the applicable chapter. For the other sections, figures and tables are shown in the text.

Note on references

Please note that each chapter has its own reference list.

Note on TST cut-offs used

A number of cut-offs for the tuberculin skin test (TST) are used in this thesis. During study design, 10 mm was used as a cut-off as a level above which an investigation for TB was performed since this was the cut-off commonly used in clinical practice and in the South African National TB Control Programme guidelines (see 2009 edition). A reviewer of the paper in reflected in chapter 3, however, argued that a cut-off of 5 mm be used for analysis as this made more sense given the distribution of TST indurations. Thus reference to both cut-offs are found throughout the thesis. In addition, the 15 mm cut-off was used for certain analyses as this is regarded by many in the literature as the most specific cut-off indicating latent TB infection given that such a strong response was unlikely to be caused by BCG or non tuberculous mycobacteria.

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Chapter 1: Introduction

Tuberculosis (TB) remains one of the major health challenges in the world today. The World Health Organisation (WHO) has estimated that 8.7 million people were diagnosed with TB and 1.4 million died of TB in 2011 (1). A global alliance of TB stakeholders, the STOP TB Partnership, has set out a strategy for combating TB globally (2). Included are strategies for better TB drugs, improved diagnostics and more effective vaccines. The current vaccine against TB, Bacillus Calmette Guérin (BCG)(currently administered at birth in mainly developing countries) is known to be at least partially effective against disseminated forms of TB in young children but has variable efficacy against pulmonary forms of TB (3, 4). Since the 1990s, there has been a groundswell of effort to develop new TB vaccines with 12 vaccines currently being actively tested in clinical trials (5). Among these new TB vaccines, some are classified as pre (latent TB) infection, others as post (latent TB) infection and still others as therapeutic (6). Review and modelling of interventions to tackle the TB epidemic show that new TB vaccines have a potentially powerful role in combating TB (7-9). Murray and Salomon of the Harvard School of Public Health developed an epidemiological model published in 1998 which showed that a new vaccine with 50% efficacy could lower TB incidence by 36 million and prevent 9 million deaths over a period of 32 years if introduced in 1998 (8). Abu-Raddad et al. have shown using a mathematical model that a combination of novel TB drugs, diagnostics and vaccines could reduce TB incidence by 71% (7). Dye and Williams in their review aver that a technology that could “remove or neutralize [TB] infection” such as a new TB vaccine “could radically alter the approach to TB Control”.

Age curves of TB show peaks in numbers and incidence in young children and adults (10-12) (Figure 1 and 2).

Figure 1: Age distribution of TB cases in Cape Town: Department of Health, Provincial Government of the Western Cape Province, 2004

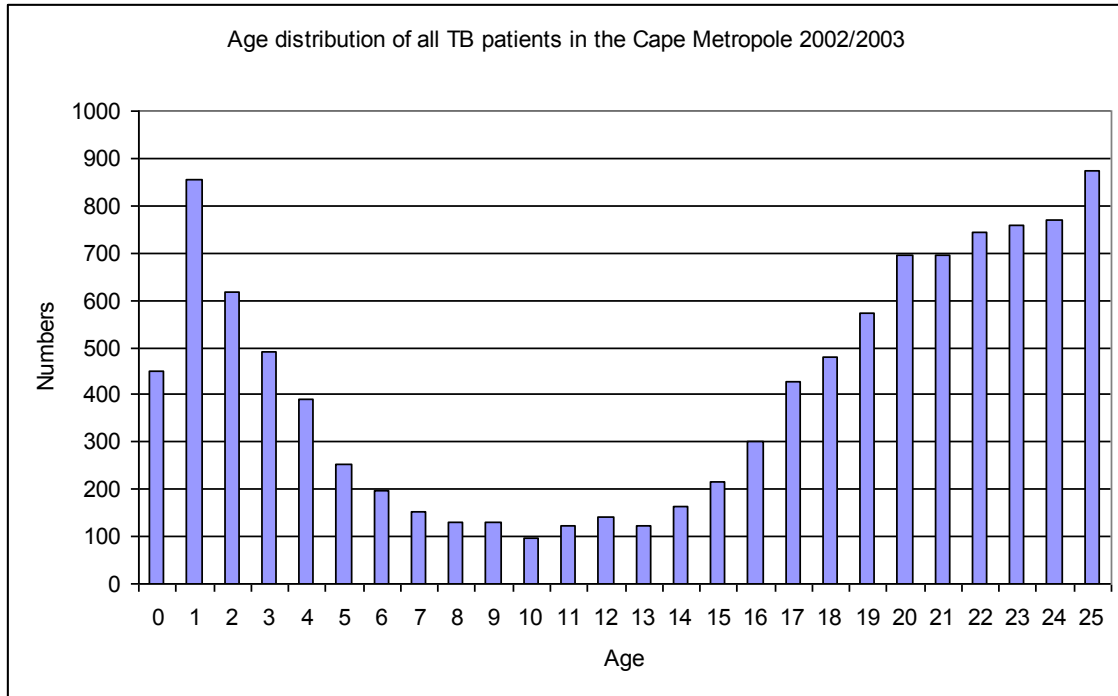
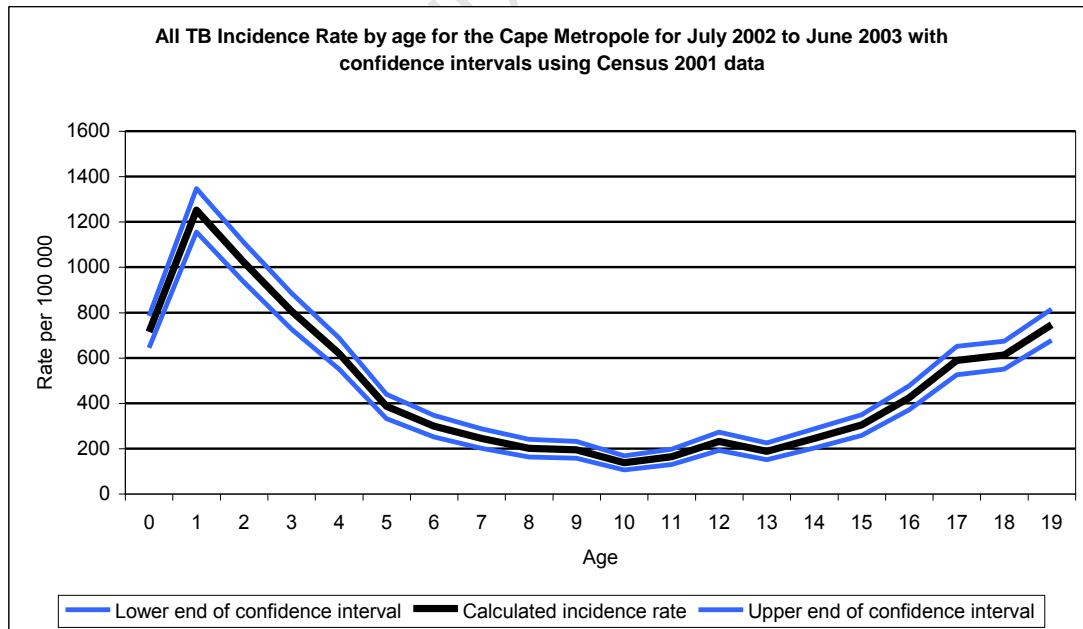


Figure 2: Incidence rate by age (0-19 years)



Given this distribution of TB cases, vaccine developers have focused on infancy and adolescence as age group targets for new TB vaccines. However, limited data are available on TB in adolescence. There was thus a keen interest in gathering more data on TB in adolescents so as to plan vaccine trials in this group. In addition, their potential for a pre-infection vaccine and their accessibility through schools as a vaccine target group were also areas requiring exploration. Specific information of interest on adolescent TB for stakeholders in the TB vaccine field has been:

- Incidence rates – to plan sample sizes for trials;
- Prevalence of latent TB infection (LTBI) – given that some TB vaccines are designed as pre latent TB infection vaccines while others are specifically designed as post latent TB infection vaccines;
- Morbidity and mortality, so that background severe adverse events could be anticipated;
- Screening tools for TB so that active TB could be excluded at baseline and detected during follow up; and
- Case-finding strategies and diagnostic algorithms for active TB.

Study setting

The South African TB Vaccine Initiative of the University of Cape Town, South Africa has an established research site in Worcester, about 110 kilometres from Cape Town. This area has a high reported all ages TB incidence rate based on routine data, of 1400/ 100 000 (13). The population of the Worcester and surrounding areas (Breede Valley Municipality) where the adolescent cohort study took place was estimated to be about 154 945 in 2010 (as provided by the Regional office of the Department of Health). Fruit, wine and chicken farming are

mainstays of the local economy. Based on the 2001 Census, only 26.6% of adults had completed a high school or higher level of education and 49.0% of employed persons earned R800 (~\$90) or less per month. The ethnic composition of the population is mainly “coloured or mixed race” (66%), 20% “black” and 14% “white”. Roughly 52% of adults were employed, 13% unemployed and 36% not economically active.

Figure 3: Map of location of SATVI trial site in Worcester relative to Cape Town.



A large trial comparing two methods of administering BCG was conducted at this site from 2001-2006 which prepared it for testing new TB vaccines (14). Six of the twelve vaccines being tested globally have been or are being tested at this site. It was in this context that a large adolescent cohort study was conducted. It started in 2005 and was completed in 2009.

Aims and Objectives

The author of this thesis was employed by SATVI during this period and was responsible for the conduct of this cohort study. The data generated on adolescents in this study form the basis of this PhD. Prior to the start of the cohort study, new interferon gamma release assays were starting to be used to measure latent TB infection as an alternative to the tuberculin skin test (TST) (15). There was thus interest in the role such tests could play in the TB vaccine trial arena. This was incorporated into the adolescent study.

While the main focus of the work was on relevance to TB vaccine trials, it was considered important to examine the application of the results obtained to TB care in general, to policies on diagnostic procedures and to the general health care of adolescents. Since data collection was to take place in the high TB burden setting described above, the results would be more applicable to other similar high TB burden contexts.

The main aims of the thesis thus were: 1) to describe key aspects of the epidemiology of TB in adolescents which would support the conduct of TB vaccine efficacy trials in this group and 2) to examine the public health implications of the epidemiological findings for TB Control Programmes in high TB burden settings. The thesis thus set the following objectives which form the main chapters of this thesis:

1. To determine the prevalence of and predictive factors for latent TB infection in adolescents.
2. To determine the prevalence of TB disease in adolescence and the utility of various screening methods.
3. To measure the incidence of TB disease and predictive factors for active TB in adolescence.

4. To compare the predictive value of the tuberculin skin test to that of an interferon gamma release assay in predicting TB disease onset.
5. To determine the knowledge and attitudes of adolescents towards participating in TB vaccine trials.

Ethics approval

Two protocols approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town were the basis for the collection of the data for these objectives:

- 1) A Knowledge, Attitudes and Beliefs Study of School-going Adolescents in the Worcester area with regard to TB Vaccine Research (UCT REC reference: 430/2004).
- 2) A Prospective Epidemiological Study of TB Disease and TB Infection in Adolescents in the Worcester and Surrounding Areas, Western Cape Province, South Africa (UCT REC ref: 045/2005). This study was known as the “Adolescent Cohort Study” and is referred to in some part of this thesis as such. A summary of the overall methods for this cohort study are set out in Appendix A and B since each chapter only describes the methods as it pertains to the particular objective addressed.

References

1. World Health Organisation. Global Tuberculosis Report 2012. World Health Organisation 2012. Available directly at http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf or by visiting http://www.who.int/tb/publications/global_report/en/. Accessed 24 May 2013.
2. Raviglione MC. The global plan to stop TB, 2006-2015. *Int J Tuberc Lung Dis*. 2006;10(3):238-9. Epub 2006/03/28.
3. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA*. 1994;271(9):698-702. Epub 1994/03/02.
4. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet*. 2006;367(9517):1173-80. Epub 2006/04/18.
5. Brennan MJ, Thole J. Tuberculosis vaccines: a strategic blueprint for the next decade. *Tuberculosis*. 2012;92 Suppl 1:S6-13. Epub 2012/03/28.
6. Hawkridge T, Mahomed H. Prospects for a new, safer and more effective TB vaccine. *Paediatr Respir Rev*. 2011;12(1):46-51. Epub 2010/12/22.
7. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proc Natl Acad Sci U S A*. 2009;106(33):13980-5. Epub 2009/08/12.
8. Murray CJ, Salomon JA. Expanding the WHO tuberculosis control strategy: rethinking the role of active case-finding. *Int J Tuberc Lung Dis*. 1998;2(9 Suppl 1):S9-15. Epub 1998/10/02.

9. Dye C, Williams BG. The population dynamics and control of tuberculosis. *Science*. 2010;328(5980):856-61. Epub 2010/05/15.
10. Donald PR. Childhood tuberculosis: the hidden epidemic. *Int J Tuberc Lung Dis*. 2004;8(5):627-9. Epub 2004/05/13.
11. Wood R, Lawn SD, Caldwell J, Kaplan R, Middelkoop K, Bekker LG. Burden of new and recurrent tuberculosis in a major South African city stratified by age and HIV-status. *PLoS One*. 2011;6(10):e25098. Epub 2011/10/22.
12. Mahomed H, Hawkridge T, Verver S, Geiter L, Hatherill M, Abrahams DA, et al. Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. *Int J Tuberc Lung Dis*. 2011;15(3):331-6. Epub 2011/02/22.
13. Groenewald P. Annual health status Report 2006. Worcester: Department of Health, Boland-Overberg Region, 2007.
14. Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahomed H, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. *BMJ*. 2008;337:a2052. Epub 2008/11/15.
15. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis*. 2004;4(12):761-76.

Chapter 2: Literature review

Introduction

Tuberculosis (TB) is a significant global public health problem with 8.7 million cases and 1.4 million deaths due to TB reported in 2011 (1). This has been compounded by the occurrence of increasingly resistant strains of *Mycobacterium tuberculosis* (Mtb) and the presence of HIV (Human Immunodeficiency Virus). The STOP TB Partnership has set out a “Global Plan to STOP TB” (2006-2015) (2, 3) and a new strategy for 2015 to 2025 is currently in development. In the area of technological improvements, the development of new drugs, new diagnostics and new vaccines for TB are all proposed as strategic components towards combating TB in the Global Plan. A model developed to illustrate the potential benefit of TB vaccines showed a 92% reduction in TB incidence by 2050 when a TB vaccine targeted at latent TB infected persons is combined with mass vaccinations of persons not infected with TB (4). Proposed target groups for new TB vaccines include infants, adolescents, HIV positive persons and HIV negative adults (5, 6). Adolescents are potentially an easily accessible target through the use of a school based vaccination programme. Epidemiological data are crucial for planning TB vaccine efficacy trials but limited data has been available on the epidemiology of TB in adolescents. Such epidemiological data will also assist TB Control Programmes with interventions relevant to adolescent TB, particularly in high TB burden settings.

In line with the stated objectives of this thesis, this literature review will describe the clinical features, prevalence and incidence of TB disease and TB infection in adolescents and the relevance of these measures to TB vaccine development and TB Control Programmes.

Search Strategy

Pubmed was searched on key words “adolescent” or “adolescence” AND “TB” or “tuberculosis”. Relevant articles (in English only) that were found were further reviewed for additional references on adolescent TB. Colleagues at collaborator institutions, the KNCV Tuberculosis Foundation, The Netherlands and Aeras, Rockville, Maryland, USA provided relevant literature on adolescent TB that they had access to. Studies which reported on adolescent TB clinical disease profiles, adolescent TB incidence and prevalence, and BCG vaccine trials which recruited adolescents were included. Studies excluded were individual case reports on adolescents, studies not reporting separate data for adolescents, outbreak investigations and studies reporting only TB programme outcome data for adolescents such as cure, adherence, etc. However, where there was a dearth of data in a specific area, other studies on adolescents e.g. in the HIV or Human Papilloma Virus (HPV) arena or adult studies on TB, were included. It is acknowledged that searching on “child or childhood TB” might have drawn more studies showing data on adolescent TB but the number of studies involved would have been too impractical to review. The search of reference lists of articles on adolescent TB would hopefully have drawn out the most important of these.

The clinical profile of TB in adolescents

Six adolescent case series found in the literature are reviewed in the section below. Two are from Cape Town, a high TB burden setting and the other four are from developed country settings. The purpose of focusing on this aspect of adolescent TB is to clarify the clinical nature of TB in adolescence and to specifically determine if it is closer to TB in young children (paucibacillary) or to adult type TB (cavitary). This distinction is important for defining endpoints in TB vaccine efficacy trials. Related to this was whether pulmonary or extra-pulmonary TB was

predominant. The age and gender distributions were important to help understand demographic risk factors. Any gaps in the current literature could also be determined.

The first series of 324 cases of TB in adolescents aged 10 to 18 years is the biggest and was drawn from primary care clinics and a dedicated TB hospital (1987-1992) in Cape Town (7). According to this series, most adolescents had evidence of intra thoracic TB (94%) and most had microbiological confirmation of disease by smear or culture (78%). Twelve percent had evidence of extra-thoracic TB (of whom 6% had both intra and extra-thoracic TB). Ten percent were described as having primary TB diagnosed mainly amongst those at the younger ages (<14 years) while the presence of cavitation increased with age. There was a slight predominance of females in the age range 13-17 years (55% female to 45% male). The authors comment on the potential effect of hormonal changes on the occurrence of TB given this predominance. HIV status was not reported but expected to be low given the low prevalence of HIV amongst pregnant women in Cape Town (<1%) at the time.

The next series of 52 adolescent TB cases who were aged 12-18 years was based on admissions to a hospital in France between 2000 and 2004 (8). The majority were foreign born (90%). Fifty two percent had isolated pulmonary disease, 31% had both pulmonary and extra-pulmonary disease and the remainder, 17%, had isolated extra-pulmonary disease. Sixty two percent had bacteriological culture confirmation of disease with 29% having had positive smears. The culture positive rate increased with age. Seven cases had evidence of mono-resistance. The gender balance amongst their cases was even - 48% female and 52% male. While a majority of cases were detected through the diagnosis of an ill child (67%) rather than through contact tracing (33%), a source case was identified in about half of cases. Seven (13%) were HIV positive of whom 4 were assessed as being due to perinatal transmission. Eighty two percent of cases had a

positive tuberculin skin test (TST) of >15 mm at diagnosis. Eighty three percent had abnormal chest x-rays – 70% had mediastinal lymphadenopathy, 54% parenchymal abnormalities and 26% had pleural effusions. Most commonly reported symptoms were fever (77%), weight loss (73%), weakness (72%) and cough (54%). Cavitory disease increased with age as in the Cape Town series reported above.

In a small case series of 8 patients (all pulmonary TB) aged 10-14 years seen in Cape Town at a primary care facility, 7 were smear positive with cavitory disease and one had a pleural effusion (9). All had symptoms which included cough, chest pain, night sweats, weight loss and fatigue. Seventy five percent had a known TB contact within 6-18 months of presentation. There was a strong female predominance (6 of the 8) and none were HIV positive. This was a highly selective study focusing on adolescents only with pulmonary TB to illustrate the fact that adolescents under 14 years of age can also be diagnosed using sputum smear examinations.

A Canadian series of 23 TB cases in adolescents aged 13-18 years seen at a hospital in Toronto between 1999 and 2004 has been reported (10). There was a female predominance (57% females to 43% males). Most had symptoms with cough (65%), weight loss (61%), fever (56%), nightsweats (39%), fatigue (34%) and bone pain (30%) being the most common. Most (21/23) had positive cultures, two of whom had resistant strains. All had positive TSTs. Only 5 had isolated pulmonary disease, 13 had extra-pulmonary disease and 5 had pulmonary and pleural TB. The majority were foreign born.

An older case series of 76 cases in adolescents aged 10 to 20 years published in 1986 was gleaned from 3 decades of records at a hospital in New York (11). Most (58%) had evidence of recent or primary infection. There were no differences in gender and most cases were seen in the 10-12 year age range in contrast to the other series. Only 16 (21%) had evidence of

cavitary disease and 7 (9%) had pleural effusions. Ten (13%) were diagnosed with extra-thoracic forms of TB. No resistant strains were detected.

A recent review by the Centers for Disease Control and Prevention, Atlanta, USA, of cases in children and adolescents reported to the National Surveillance System in the USA unfortunately does not report much data separately for adolescents but does mention that adolescents aged 13-17 years made up just over 50% of foreign born cases under 18 years diagnosed with TB and that there were more TB cases amongst foreign born adolescents than US born adolescents in the period 2008-2010 (12). Only one third of all cases reviewed were symptomatic at presentation but to what extent this applies to the adolescent cases can only be indirectly inferred. Cases in this series were identified either through contact investigations, investigation of symptoms and signs or through an incidental investigation of a presenting patient.

In summary, with the exception of the New York series, the picture that emerges is that adult type pulmonary TB (infiltrative with and without cavitation) which could be confirmed bacteriologically is common in adolescents. However, there is an age trend with cavitary disease increasing with age and the occurrence of primary TB occurring mainly at younger ages (The term "primary TB" remains confusing – evidence suggests that in adolescents, cavitary disease may follow soon after primary infection. Young children with "primary TB" may also develop cavities related to progressive disease and not consistent with a "post-primary" phenomenon). Where specified, the smear positive proportion in general was lower than what is the case for adults and this has implications for screening methods for TB to be used in adolescents. The data in the literature are based mainly on serious hospital level cases of TB in adolescents and consequently, a significant proportion of extra-pulmonary disease cases. There were limited data on community level cases. Cases identified through contact investigations

would have a different profile to cases identified through investigation of symptoms and signs in that the former would probably have an earlier or milder form of disease. Some series (3 out of 5) suggest a female predominance which is interesting given that there is a male predominance of 1.5-2.1:1 in adulthood (13). Most cases reported were symptomatic but this is not surprising since most were diagnosed through presentation at a health facility rather than through screening of contacts or a survey. Where reported, HIV prevalence was low. In developed countries, most adolescent cases were foreign born. Thus, while the literature provides a detailed profile of TB in adolescence, the data are biased due to being mainly from hospital based series. This leaves a gap in knowledge with respect to community level TB in adolescence. This is an important gap for TB vaccine efficacy trials since such trials will be community based. TB Control Programmes are also more often targeted at the community level.

TB prevalence

TB prevalence is an important indicator of TB burden and provides a guide for resource allocation for TB Control Programmes. Adolescents, a high proportion of whom can be found at school in South Africa, represent an accessible target group for TB Control Programmes should the burden of TB warrant it. Thus, TB prevalence studies in this group are needed. World Health Organisation surveys tend not to include children under the age of 15 years (14), an important subset of adolescents. In addition, while surveys include adolescents 15 years and older, data are often not presented in a way that TB prevalence can specifically be estimated in adolescence. The level of exposure to active TB in a school environment is an important public health issue given the risks this poses to others in an enclosed setting such as a classroom. Finally, TB vaccine clinical trials would need to exclude TB cases at enrolment. Therefore to plan such trials, data on TB prevalence are required.

Very little data are available on the specific population prevalence of active TB disease in adolescents. A BCG trial which started in 1950 in the United Kingdom (UK) screened 58,900 adolescents aged 14.5 to 15.5 years for TB prior to enrolment. A total of 156 were found to have active disease giving a prevalence of 3/1000 (15). Diagnosis was based on chest x-ray assessment and physician review. An older prevalence study (reported in 1936) in the pre-chemotherapeutic era of a convenience sample of 2,381 school-going and working adolescents aged 14-21 years in the UK found a prevalence of TB disease as follows: 6.5/1000 when the case definition was restricted to chest x-ray findings definitely compatible with TB; this increased to 11/1000 if chest x-rays probably compatible with TB were included (16). A prevalence study of a South Indian population in which 6,393 adolescents aged 10-19 years were x-rayed, found a prevalence of radiologically active TB disease of 4/1000 (17). This fell to 0.9/1000 when cases were required to have bacteriologically positive TB disease based on a smear and/ or culture done on a spot sputum but sputum tests were only done on those who had a positive chest x-ray or did not have a chest x-ray done. The true bacteriologically positive TB prevalence in this latter study is likely to have been underestimated since some may have had normal chest x-rays.

TB disease prevalence surveys which have included adolescents have been published but these have not separated out the prevalence among adolescents. A few of these studies on adults in developing countries are reported here to put the adolescent TB prevalence data into perspective. A TB prevalence study in Western Kenya which recruited 22,656 participants 15 years and older found an all TB prevalence of 6/1000 with a smear positive prevalence of 2.5/1000 (18). Half of the prevalent cases were HIV positive. A TB prevalence study done in Cape Town also in adults aged 15 years and older found a prevalence of bacteriologically confirmed TB of 10/1000 amongst 2,608 study participants from a known high TB burden area. The smear positive rate was 7/1000. The DETECTB study in Harare, Zimbabwe found a baseline smear

positive TB prevalence of 4/1000 amongst 9,000 adults >16 years, 21% of whom were HIV positive (19). A large prevalence survey of 38,000 persons in Eritrea found a smear positive prevalence of 0.9/1000 in adults 15 years and older (20). The global prevalence of TB in 2011 was estimated by the World Health Organisation as 2/1000 while in South Africa it was 8/1000 (1). Interestingly, the 22 high burden countries had a combined estimated prevalence also of 2/1000.

In summary, a few mainly old studies have reported active TB prevalence specific to adolescents varying from 0.9/1000 to 11/1000. Adult studies in developing countries reported similar prevalences varying from 0.9/1000 to 10/1000 using all TB or smear positive prevalence. One would have expected TB prevalence to be higher in adults. However, time and location of study varied between the adolescent and adult studies so comparisons between the two groups of studies should be viewed with caution. World Health Organisation and other survey data show that TB prevalence is higher in South Africa than elsewhere. Ideally, prevalence in adolescents should also be calculated as a proportion of overall prevalence when comparing countries since TB burden varies widely amongst countries; this was not possible given the dearth of data specific to adolescents.

Incidence of TB in adolescence

TB incidence data are critical to TB vaccine efficacy trial planning since these will form the basis of sample size estimations and therefore, the estimation of overall costs of such trials. TB incidence data are also essential to TB Control Programmes in that trends over time will indicate whether the problem is increasing or decreasing and whether further intervention is necessary.

Limited incidence data are available from BCG trials in adolescents. The British MRC trial which enrolled 14.5-15.5 year old adolescents, describe an annual incidence rate of 2.5/1000 for the

first 5 years of the study in the BCG unvaccinated group who were TST negative at baseline (15). A similar rate was found in those who were TST positive (induration diameter ≥ 15 mm) at baseline. Those who were vaccinated with BCG at study entry (in adolescence) had a rate of 0.4/1000. A second trial of BCG amongst Puerto Rican children aged 1-19 years, found an average annual TB incidence rate of 0.9/1000 amongst those aged 13-18 years at enrolment who had a positive TST at baseline during a mean 19 year follow up period (21). The trial of BCG revaccination conducted more recently in participants aged 7-14 years in Brazil found a rate of 0.3/1000 person years (22). De Pontual et al. reported a stable annual incidence of TB in adolescents in France of 0.036/1000 in adolescents of French origin while there was an increase from 0.3/1000 to 0.83/1000 amongst foreign born adolescents between 1991 and 2001 (8). They reported an increase in the annual TB case notification rate from 0.1/1000 in 1988 to 0.12/1000 in 1999 amongst adolescents in the poor area in Paris served by their hospital. The World Health Organisation reported a 1.25/1000 all ages global incidence rate for 2011 with 2.62/1000 in the Africa Region, 0.28 in the American Region and 0.42/1000 in the European Region (1). The estimated all ages incidence rate in South Africa was 9.93/1000.

In summary, limited specific data on TB incidence in adolescents are available. The overall incidence rate is higher in Africa than in Europe and America and it should be expected that rates of TB in adolescents in Africa will also be higher than those reported from studies in Europe and South America. Again, caution will be need to be exercised in comparing incidences in adolescents amongst countries since these will be related to the overall TB burden in each country.

Age distribution of active disease

TB vaccine efficacy trials in adolescents will need to be powered adequately in terms of number of cases to be accrued but would need to include the population at whom the vaccines will ultimately be targeted i.e. as early in age as possible prior to the upswing in incidence which occurs during adolescence. Thus age trends in incidence of TB in adolescents are important to determine. Since the risk of TB is age dependent, TB Control Programmes will need to know which age groups are at highest risk.

The age distribution of TB cases in Cape Town in HIV negative persons has been described based on routine data. These show peaks in young children and adulthood and a rising incidence in adolescence (23) as shown in the figure below (permission for the use of this figure was received from the first author of the paper referenced, R Wood).

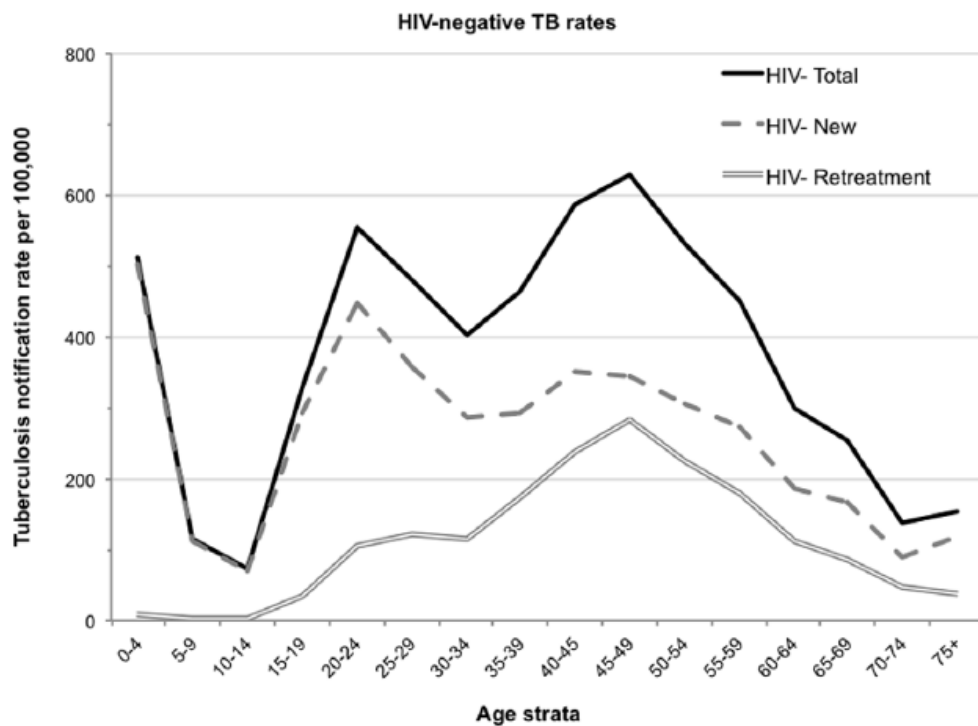


Figure 4. Notification rates for all, new and recurrent tuberculosis cases for HIV-negative residents aged 15 years to 49 years in Cape Town. HIV-uninfected population denominators derived from the product of Cape Town age population pyramid [10,11] and 1-HIV-prevalence [12].
doi:10.1371/journal.pone.0025098.g004

Donald et al. describe a hypothetical age and gender distribution in a high burden setting compared to a low burden setting; what is observed is that the age distribution seen in the Cape Town data shown above is typical of high burden settings (24). A review of TB notifications in 1980 in Finland showed incidence rates of 10-14 year olds and 15-19 year olds of 0.040/1000 and 0.066/1000 respectively (25). The Finnish authorities stopped the BCG revaccination programme in 1990 after a steady decline in overall TB incidence but this did not impact on the downward trend in overall TB incidence and in 1995, incidence rates were 0.006/1000 and 0.012/1000 in 10-14 year olds and 15-19 year olds respectively (25). These rates are much lower than those in developing countries as given in the WHO report mentioned above (1). A second report looking at data from Sweden and Finland showed the highest rates in those over 65 years in indigenous Swedes and Finns while foreigners living in Sweden and Finland had a peak in the adult age range of 15-64 years (26). The occurrence of disease in adolescents is likely to have been due to recent infection whereas the disease in those over the age of 65 was more likely due to reactivation disease.

In summary, adolescence is a period of rising TB incidence particularly in developing countries. Where a rising TB incidence in adolescence is seen in developed countries, this is due to foreign born persons.

Latent TB infection

Latent TB infection represents an important entity in that it provides evidence of exposure to *Mtb* and represents an increased risk of progressing to active TB disease. In TB vaccine trials, there has been a concern that giving new vaccines containing TB antigens may be harmful to individuals who are latently infected with TB. In addition, new TB vaccines are designed to be targeted at either latently infected or latently uninfected individuals. A closer examination of

the nature of this state and its prevalence is therefore warranted in the context of the epidemiology of TB in adolescents.

The pathogenesis of TB is described as follows: exposure to *Mtb* which may lead to latent infection which may then lead to active TB disease which in turn leads to recovery, chronic infection or death (27). The above (27) illustrates the evolution of TB well. Latent TB infection is a state which follows on exposure to *Mtb*. It is described as a quiescent state maintained by the immune response which prevents replication and tissue damage (28). Its significance lies in the fact that it represents evidence of exposure and that a proportion of those with latent TB infection progress to active TB disease through re-activation of this latent state. Evidence for the existence of this state comes from post mortem studies of persons dying of unrelated reasons in whom live *Mtb* bacilli were found. One third of the world's population is estimated to be infected with *Mtb* (28). It is acknowledged that a large literature on TST positivity from the last century exists even if not referenced in this thesis.

Historically, the TST (Mantoux) test was used to measure the presence of this latent state (29). The test was developed as a diagnostic tool by Robert Koch in the late 19th century and has gone through an evolution in its use. Essentially it involves the application of *Mtb* proteins to the skin or injection into the skin. Current recommended use involves the injection of a specific dose of an internationally standardized "purified protein derivative" or PPD of *Mycobacterium tuberculosis* intradermally into the volar surface of the forearm (30, 31). The size of the reaction to this injection is read 48 to 96 hours later by measuring the amount of induration at the site of injection in millimetres. Pre-defined cut-offs are used to interpret the result (30). Alternatively, the distribution of induration in a specific population is used to guide interpretation (27).

Lack of specificity has long been a problem with the TST in that the vaccine BCG and non tuberculous mycobacteria (NTMs) also induce a response to the TST injection (32). This has led to the development of interferon gamma release assays (IGRAs) which focus on the so-called “region of difference” which utilise antigens of *Mtb* which do not occur in BCG and most NTMs (33). Two commercial interferon gamma release assays are available, the QuantiFERON TB assays (Cellestis, Victoria, Australia) and the T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom). Besides the advocated increased specificity, the advantages of these tests are that: 1) they require a single visit while the TST requires two, 2) repeated measures do not result in boosting which does occur with the TST and 3) the result is objective as it is generated in vitro whereas the TST requires human measurement which is prone to error or inter and/or intra reader variation. However, these assays are more expensive and require a sophisticated laboratory infra-structure and skilled laboratory staff.

IGRAs have been evaluated extensively over the past decade or more to determine their comparability or superiority to the TST (34). One area of specific interest has been the predictive value of IGRAs for TB disease. The patient with a positive TST has an established risk of progression to active TB disease (35). Specifically in adolescents in the British MRC BCG trial, those with strong TST reactions at enrolment had a high attack rate within two years of entry (36). Furthermore, amongst those who converted from a negative TST to a positive TST during the trial, 5.2% developed TB disease (all types) during follow up with 80% of cases occurring within the first two years after conversion.

Whether IGRAs have the same predictive value as TST has been the subject of a number of investigations (37-42). All these studies found IGRAs to be predictive of an increased risk of progression to TB disease when positive. Diel et al., Leung et al. and Bakir et al. found IGRAs to

be superior to the TST in predicting TB disease onset (37, 38, 40) while Kik et al., Hill et al. and Lienhardt et al. did not (39, 41, 42). The reasons for the differences are not clear but may be due to poor reading technique of the TST, varying environmental prevalences of NTMs or use of different TST cut-offs. These results are relevant to the question of whether IGRAs should replace the TST in clinical trials of TB vaccines or in clinical practice. Most of these studies were based on household contact investigations while only one was based on a cohort study (40). More population based cohort data on IGRAs are needed to guide what should be used in clinical trials which are by their nature population based.

The relevance of latent TB infection to TB vaccine trials is twofold. In the first place, Robert Koch soon after he discovered *Mycobacterium tuberculosis* tried to administer a protein extract from the organism, tuberculin, as a form of treatment for active TB disease (43). It was found in certain instances that tuberculin aggravated the course of disease. Based on these outcomes, the concern with new TB vaccines is that the administration of TB antigens through these vaccines may result in negative consequences should they be administered to individuals with latent TB infection or active TB disease (Koch Phenomenon). New TB vaccines are first tested in individuals in whom latent TB infection is absent and are then usually tested in small groups of latent TB infected persons to establish their safety as part of product development. Screening for latent TB infection is thus an important part of pre vaccination evaluations in safety trials of new TB vaccines.

Secondly, latent TB infection is also important because latently infected individuals have a higher risk of progressing to TB (35). Latently infected individuals are thus an important target group for new TB vaccines. Also, any new TB vaccine targeted at adults or adolescents in a high

TB burden context will need to have been evaluated in latently infected persons since a high proportion of adults/ adolescents will already be latently infected at the time of vaccination.

Limited data are available on the presence of latent TB infection in adolescents. Middelkoop et al. conducted a TST survey in 831 children aged 5-17 years in 2006-2007 in a high TB and HIV burden township in Cape Town, South Africa (44). Using a 10 mm cut-off, they found a prevalence of latent TB infection of 45.1% in children aged 12-13 years and 52.5% in children aged 14-17 years, with increasing age being a significant predictor of latent TB infection. A follow up survey by the same research group at a secondary school in the same community found a latent TB prevalence of 53.9% in an age range of 13-22 years amongst 620 HIV negative participants (45). In this latter paper, the authors introduce the concept of “force of infection”. Force of infection is calculated by showing the change in latent TB infection prevalence between age groups. They show adolescence to be a period of high “force of infection” because of a change in this prevalence between age groups during adolescence of between 5 and 7%. This concept differs from annual risk of TB infection (ARTI) in that force of infection quantifies the change in infection prevalence from the prior year whereas ARTI describes average annual risk of infection since birth. In the adolescent group where force of infection was studied, a positive TST was associated with age and male gender.

The BCG trial in adolescents in the United Kingdom which recruited 56,700 adolescents from 1950-52 conducted TSTs at recruitment and found the following (after exclusions) (15): 60% were TST negative, 28% were TST positive based on the administration of 3 tuberculin units (TUs) and a further 12% who were initially TST negative after the administration 3 TUs, were then positive after the administration of 100 TUs. The 40% TST positive proportion assumed to be latent TB infected is likely to be an underestimate since adolescents who had been exposed

to TB through a household contact were excluded from study entry and did not reach the stage of TST testing. A South Indian prevalence survey amongst a population of 35,000 published in 1963 found a prevalence of latent TB infection amongst those aged 10-19 years of 33% (17). A study in New York, USA found a prevalence in 1991-1993 of 11.5% to 23.3% in school entrants aged 12-16 years (46). Another study from the same period found prevalences of 12.8% and 24.1% at two high schools in San Diego, USA (47). Being of foreign origin was a major predictor of a positive TST in these two USA studies. US guidelines on targeted tuberculin testing recommended middle and high schools as ideal places for such testing because prevalences were higher amongst adolescents than younger children (48). Prevalence in Rotterdam, The Netherlands in 1967 to 1968 was 0.03% in adolescents aged 10 -19 years as derived from data based on tuberculin surveys over a three year period (49).

In summary, latent TB infection is an important condition in the TB context and is relevant to both TB Control Programmes and TB vaccine trials. Both the TST and IGRAs are used to measure latent TB infection and both have advantages and disadvantages. Studies of the predictive value of IGRAs compared to TST for TB disease have shown varying results but these studies have been done mainly in contacts of TB cases. Prevalence of latent TB infection is lower in low burden settings such as the US, where being of foreign origin was a major risk factor, and The Netherlands. High prevalences were found in high burden settings as seen in the Cape Town and South Indian study. A relatively high prevalence was reported in the UK BCG trial but this dates back to a time when the TB burden was high there. In general, limited data on the prevalence of latent TB infection in adolescents are available in the literature particularly from high burden settings. Comparisons between countries and studies are limited by variations in time of study and location. Very few studies reported on risk factors for latent TB infection.

Screening for TB

Screening for certain diseases is now a routine part of health care as a means of early detection leading to early treatment and a resultant reduction in morbidity and mortality for that disease (50, 51). Screening is also important for clinical trials where persons with certain illnesses or conditions need to be excluded or included in trial participation. For screening to be effective, screening tools need to be both sensitive and specific i.e. be able to detect as many cases of disease as possible but also to minimize positive screening test results not due to the condition being tested for (false positives).

As described by Hennekens and Buring, besides the sensitivity and specificity of screening tools, the diseases being screened for should meet certain criteria (50): 1) the disease should be a significant public health problem in respect of disease severity and risk of mortality, 2) treatment prior to disease onset should be beneficial, and 3) it should be a highly prevalent condition.

Wilson and Jungner summarised 10 criteria for screening to be worthwhile as a public health intervention (51) which are similar but more detailed. These are set out in Table 1.

Table 1: Prerequisites for successful screening

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1. The condition sought should be an important health problem.
 2. There should be an accepted treatment for patients with the disease.
 3. Facilities for diagnosis and treatment should be available.
 4. There should be a recognizable latent or early symptomatic phase.
 5. There should be a suitable test or examination.
 6. The test should be acceptable to the population.
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7. The natural history of the condition should be understood.
 8. There should be an agreed policy on whom to treat as patients.
 9. The cost should be economically balanced in relation to possible expenditure on medical care as a whole.
 10. Screening should be a continuing process and not a short lived project.
-

With respect to screening for TB disease specifically, this is important for both public health care services and for clinical trial investigators. Public health services would like to detect TB early to minimize morbidity and mortality due to TB and to prevent the spread of disease to others as it is an infectious condition. For those involved in TB vaccine clinical trials, screening for TB is important for exclusion at baseline should active TB be an exclusion criterion, but also to detect TB disease during follow up for efficacy trials of new TB vaccines. Since there is no correlate of protection for TB vaccines, the measurement of clinical outcomes such as TB disease is the only way in which vaccine efficacy can currently be measured (5).

The WHO has developed an approach to screening for TB in prevalence surveys and three algorithms are offered:

- 1) Symptom and chest x-ray screening on all. Those with symptoms or abnormal chest x-rays are referred for a sputum smear and culture.
- 2) Symptom and chest x-ray screening, and sputa taken for smears on all. Cultures are only done if any of the former are positive.
- 3) Sputa are taken for smears and cultures on all.

These range from the more affordable option, 1, to the more expensive option, 3. There is, however, a loss in sensitivity with the less expensive methods of screening. Options 2 and 3 also require substantial laboratory capacity because large numbers of cultures would need to be performed. Option 3 would not miss any cases except perhaps pauci-bacillary cases amongst HIV positive persons (and younger adolescents if used for adolescent screening, as they more often have pauci-bacillary disease compared to older adolescents) while option 1 would miss smear or culture positive cases without symptoms or chest x-ray abnormalities, and option 2 would miss smear negative, culture positive cases without symptoms or chest x-ray abnormalities (this may also be a factor in younger adolescents).

Data from surveys in different countries on varying target populations support differing screening strategies. A survey in which 1,170 adults in a high burden TB community setting in Cape Town were screened, found any abnormality on chest x-ray to be highly sensitive for bacteriologically positive TB (97%). However, a positive predictive value derived from the data provided was low at 6% (52). The authors found symptoms to have a low sensitivity (all less than 60%). A study in Kenya in 7,342 HIV positive and uninfected participants also found chest x-rays to be highly sensitive for bacteriologically confirmed TB (94%) but the derived positive predictive value was low at 2% (53). Individual symptoms had low sensitivities but “any TB symptom” (at least one) had a sensitivity of 90%. This later study compared their findings with other studies and found “any TB symptom” to have variable sensitivities, “cough” to have a low sensitivity but x-ray abnormalities to generally have high sensitivities. Differences in findings were reported as being due to cultural differences, differences in study design or population studied or due to differences in definitions use.

In summary, screening for TB disease in adolescents is important for TB vaccines trials and would be relevant to TB Control Programmes should they consider programme interventions specifically for adolescents. No data on screening for TB specifically in adolescents in a community setting was found. While most general surveys start at age 15 years, data specifically for the adolescent age component was not found. Two surveys in high burden settings in adults found chest x-rays to be sensitive tools for detecting TB while the sensitivity of symptoms varied. Positive predictive values were low for all screening methods in these surveys.

Readiness to participate in TB vaccine trials

Adolescence represents a unique development stage, physically and psychologically. Vaccination in adolescence provides an opportunity for prevention (54). The increasing incidence of TB in adolescence (23), the high force of TB infection in adolescence (45) and the fact that adolescents are an accessible population through schools make them a feasible target for new boost TB vaccines. Both safety and efficacy trials would need to be conducted in this group. It was not clear how willing they would be to participate in TB vaccine trials. Their level of knowledge of TB, vaccines and clinical trial research may influence such participation but this was unknown. Adolescents have been the subject of two very large clinical trials of BCG (15, 22). One starting in 1950 in the United Kingdom recruited 58,000 adolescents aged 14.5 to 15.5 years (15) and one in Brazil which recruited 240,000 school children aged 7-14 years (22). In the Brazilian trial where participation was on an “opt out” basis, one quarter of children were absent on the day of vaccination giving an indirect indication of participation rate but absence may have been due to other reasons. In the British trial, 60% agreed to participate but reasons for non-participation were not provided (15). BCG is however a registered vaccine and is known, and participation in such trials may not reflect attitudes to trials of experimental TB vaccines.

No data were found on knowledge and attitudes in general or specifically amongst adolescents towards TB and willingness to participate in TB vaccine trials. These questions have been examined in the HIV arena. An investigation into adolescents' willingness to participate in HIV vaccine trials in a peri-urban community in Cape Town found that 79% were definitely or probably willing to participate in such trials (55). Increasing age, being sexually active and length of residence in the community were significantly associated with willingness to participate. The main reasons given for willingness to participate were to help find a vaccine to protect their loved ones but also a hope that the vaccine would protect themselves from HIV. A study on community attitudes towards HIV vaccine trials in adolescents revealed concerns about consent and confidentiality of procedures (56). The ethical and legal challenges of including adolescents in research on HIV have been highlighted but it has been argued that it is important for this group to be included while ensuring that they are protected from harm (57). A review of studies on knowledge of HIV/AIDS amongst youth (aged 14-35 years) in South Africa found a high awareness of AIDS as a sexually transmitted disease which was fatal (58). The youth were less knowledgeable about HIV transmission and methods of prevention.

Since Human Papilloma Virus (HPV) vaccination is under consideration for adolescents, knowledge and attitudes surveys on this vaccine were reviewed. A survey of parental and adolescent knowledge and attitudes on HPV vaccination in Finland was published in 2007 (59). The postal survey of 1,990 adolescents and their parents had a low response rate of between 20% and 40%. Of those who responded, 83% of adolescents and 86% of parents were willing to accept HPV vaccination of adolescents. Low knowledge of HPV and general concerns about the safety of vaccinations were associated with unwillingness to undergo vaccination. A telephonic survey of women in Kentucky in the USA showed women to be interested in vaccination for themselves (85%) but less so for young girls aged 10-15 years (68%) although only 44% had

heard of HPV (60). Acceptability of young girls being vaccinated decreased with age.

Interestingly, higher education and higher income were associated with lower acceptability.

In summary, adolescent participation in 2 BCG trials have varied from 60 to 75% but these numbers may not be representative of future participation in trials of experimental TB vaccines. Knowledge and attitudes may influence participation in TB vaccine trials but no data was available on this in general or specifically for adolescents. Data from the HIV and HPV arenas suggest that adolescents are interested in HIV and HPV vaccine trials but there are legal and ethical obstacles, safety concerns and lack of knowledge that would need to be overcome.

Gaps in the literature linked to thesis objectives

This literature review has provided an overview of literature available on TB in adolescents relevant to this thesis but has revealed certain gaps. Limited data are available on the prevalence of latent TB infection specifically in adolescents. Very few studies have reported on TB disease population prevalence in adolescents. While the TB disease profile in adolescents has been examined in a number of studies, this has come mainly from hospital based series with limited data on adolescent TB as seen at a community level. Few studies have reported on TB incidence in adolescents. In general, most data on adolescence has come from developed countries rather than developing country settings. Many of the data are quite old with very few recent studies on adolescents. With respect to IGRAs, data are mainly from developed countries while it has been reported that these tests behave differently in high TB burden settings. No data on the use of IGRAs in adolescents have been reported. No data on screening approaches for TB in adolescents was found. No data on attitudes of adolescents towards participating in TB vaccines trials have been published although some data on adolescent attitudes towards HIV and HPV vaccine trials are available. Thus, the data to be produced on the basis of the

objectives set for this thesis will provide a useful contribution to the scientific literature on adolescent TB.

References

1. World Health Organisation. Global Tuberculosis Report 2012. World Health Organisation 2012. Available directly at http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf or by visiting http://www.who.int/tb/publications/global_report/en/. Accessed 24 May 2013.
2. Raviglione MC. The global plan to stop TB, 2006-2015. *Int J Tuberc Lung Dis*. 2006;10(3):238-9. Epub 2006/03/28.
3. Stop TB Partnership. The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis: World Health Organisation; 2010. Available at: http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf. Accessed 24 May 2013.
4. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proc Natl Acad Sci*. 2009;106(33):13980-5. Epub 2009/08/12.
5. Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, Hanekom W, et al. Predictive value of recent QuantiFERON conversion for tuberculosis disease in adolescents. *Am J Respir Crit Care Med*. 2012;186(10):1051-6. Epub 2012/09/08.
6. Hawkridge T, Mahomed H. Prospects for a new, safer and more effective TB vaccine. *Paediatr Respir Rev*. 2011;12(1):46-51. Epub 2010/12/22.
7. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR. The clinical and radiological features of tuberculosis in adolescents. *Ann Trop Paediatr*. 2000;20:5-10.

8. de Pontual L, Balu L, Ovetchkine P, Maury-Tisseron B, Lachassinne E, Cruaud P, et al. Tuberculosis in adolescents: A French retrospective study of 52 cases. *Pediatr Infect Dis J.* 2006;25(10):930-2. Epub 2006/09/29.
9. Marais BJ, Gie RP, Hesselning AH, Beyers N. Adult-type pulmonary tuberculosis in children 10-14 years of age. *Pediatr Infect Dis J.* 2005;24(8):743-4. Epub 2005/08/12.
10. Kam A, Ford-Jones L, Malloy P, Khan K, Kitai I. Active tuberculosis among adolescents in Toronto, Canada: clinical features and delays in diagnosis. *Pediatr Infect Dis J.* 2007;26(4):355-6. Epub 2007/04/07.
11. Nemir RL. Perspectives in adolescent tuberculosis: three decades of experience. *Pediatrics.* 1986;78(3):399-405. Epub 1986/09/01.
12. Winston CA, Menzies HJ. Pediatric and adolescent tuberculosis in the United States, 2008-2010. *Pediatrics.* 2012;130(6):e1425-32. Epub 2012/11/28.
13. Diwan VK, Thorson A. Sex, gender, and tuberculosis. *Lancet.* 1999;353(9157):1000-1.
14. World Health Organisation. Tuberculosis prevalence surveys: A handbook: World Health Organisation; 2011. Available at http://whqlibdoc.who.int/publications/2011/9789241548168_eng.pdf. Accessed on 24 May 2013.
15. B.C.G. and vole bacillus vaccines in the prevention of tuberculosis in adolescents; first (progress) report to the Medical Research Council by their Tuberculosis Vaccines Clinical Trials Committee. *BMJ.* 1956;1(4964):413-27. Epub 1956/02/25.
16. Wingfield RC, Macpherson AM. Latent adolescent pulmonary tuberculosis. *BMJ.* 1936;1(3927):741-3. Epub 1936/04/11.

17. Narain R, Geser A, Jambunathan MV, Subramanian M. Some aspects of a tuberculosis prevalence survey in a South Indian district. *Bull World Health Organ.* 1963;29:641-64. Epub 1963/01/01.
18. van't Hoog AH, Laserson KF, Githui WA, Meme HK, Agaya JA, Odeny LO, et al. High prevalence of pulmonary tuberculosis and inadequate case finding in rural western Kenya. *Am J Respir Crit Care Med.* 2011;183(9):1245-53. Epub 2011/01/18.
19. Corbett EL, Bandason T, Duong T, Dauya E, Makamure B, Churchyard GJ, et al. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster-randomised trial. *Lancet.* 2010;376(9748):1244-53. Epub 2010/10/07.
20. Sebhatu M, Kiflom B, Seyoum M, Kassim N, Negash T, Tesfazion A, et al. Determining the burden of tuberculosis in Eritrea: a new approach. *Bull World Health Organ.* 2007;85(8):593-9. Epub 2007/09/05.
21. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol.* 1974;99(2):131-8. Epub 1974/02/01.
22. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brita SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet.* 2005;366(October 8):1290-95.
23. Wood R, Lawn SD, Caldwell J, Kaplan R, Middelkoop K, Bekker LG. Burden of new and recurrent tuberculosis in a major South African city stratified by age and HIV-status. *PLoS One.* 2011;6(10):e25098. Epub 2011/10/22.
24. Donald PR. Childhood tuberculosis: the hidden epidemic. *Int J Tuberc Lung Dis.* 2004;8(5):627-9. Epub 2004/05/13.

25. Tala-Heikkila M, Tala, E. Tuberculosis trends amongst Finnish adolescents before and after discontinuation of the BCG revaccination programme - A TSRU Progress Report. Departments of Paediatrics and Diseases of the Chest, Turku University Central Hospital, Turku, Finland, 1997.
26. Tala-Heikkila M, Romanus, V, Tala, E. Two decades progress in childhood and adolescent tuberculosis in Finland and Sweden - A TSRU Progress Report. University Central Hospital of Turku, Finland and The National bacteriological Laboratory, Stockholm, Sweden, 1992.
27. Rieder H. Epidemiological basis of tuberculosis control: International Union Against Tuberculosis and Lung Disease; 1999.
28. Zumla A, Atun R, Maeurer M, Mwaba P, Ma Z, O'Grady J, et al. Viewpoint: Scientific dogmas, paradoxes and mysteries of latent Mycobacterium tuberculosis infection. Trop Med Int Health. 2011;16(1):79-83. Epub 2011/02/24.
29. Lee E, Holzman RS. Evolution and current use of the tuberculin test. Clin Infect Dis. 2002;34(3):365-70. Epub 2002/01/05.
30. Arnadottir T, Rieder HL, Trebucq A, Waaler HT. Guidelines for conducting tuberculin skin test surveys in high prevalence countries. Tuberc Lung Dis. 1996;77 Suppl 1:1-19. Epub 1996/06/01.
31. Rieder HL, Chadha VK, Nagelkerke NJ, van Leth F, van der Werf MJ, Foundation KT. Guidelines for conducting tuberculin skin test surveys in high-prevalence countries. Int J Tuberc Lung Dis. 2011;15 Suppl 1:S1-25. Epub 2011/02/10.
32. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis. 2006;10(11):1192-204.

33. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis.* 2004;4(12):761-76.
34. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med.* 2008;149(3):177-84.
35. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc.* 1970;26:28-106.
36. Sutherland I. The evolution of clinical tuberculosis in adolescents. *Tubercle.* 1966;47:308.
37. Bakir M, Millington KA, Soysal A, Deeks JJ, Efee S, Aslan Y, et al. Prognostic value of a T-cell-based, interferon-gamma biomarker in children with tuberculosis contact. *Ann Intern Med.* 2008;149(11):777-87.
38. Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood IGRA for developing active TB - an update. *Am J Respir Crit Care Med.* 2011;183(1):88-95.
39. Kik SV, Franken WP, Mensen M, Cobelens FG, Kamphorst M, Arend SM, et al. Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. *Eur Respir J.* 2010;35(6):1346-53.
40. Leung CC, Yam WC, Yew WW, Ho PL, Tam CM, Law WS, et al. T-Spot.TB outperforms tuberculin skin test in predicting tuberculosis disease. *Am J Respir Crit Care Med.* 2010;182(6):834-40.
41. Hill PC, Jackson-Sillah DJ, Fox A, Brookes RH, de Jong BC, Lugos MD, et al. Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts. *PLoS One.* 2008;3(1):e1379. Epub 2008/01/03.

42. Lienhardt C, Fielding K, Hane AA, Niang A, Ndao CT, Karam F, et al. Evaluation of the prognostic value of IFN-gamma release assay and tuberculin skin test in household contacts of infectious tuberculosis cases in Senegal. *PLoS One*. 2010;5(5):e10508. Epub 2010/05/14.
43. Burke DS. Of postulates and peccadilloes: Robert Koch and vaccine (tuberculin) therapy for tuberculosis. *Vaccine*. 1993;11(8):795-804. Epub 1993/01/01.
44. Middelkoop K, Bekker LG, Myer L, Dawson R, Wood R. Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults. *Clin Infect Dis*. 2008;47(3):349-55.
45. Middelkoop K, Bekker LG, Liang H, Aquino LD, Sebastian E, Myer L, et al. Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study. *BMC Infect Dis*. 2011;11:156. Epub 2011/06/03.
46. Scholten JN, Fujiwara PI, Frieden TR. Prevalence and factors associated with tuberculosis infection among new school entrants, New York City, 1991-1993. *Int J Tuberc Lung Dis*. 1999;3(1):31-41. Epub 1999/03/27.
47. Pong AL, Anders BJ, Moser KS, Starkey M, Gassmann A, Besser RE. Tuberculosis screening at 2 San Diego high schools with high-risk populations. *Arch Pediatr Adolesc Med*. 1998;152(7):646-50. Epub 1998/07/17.
48. Pediatrics Tuberculosis Collaborative Group. Targeted tuberculin skin testing and treatment of latent tuberculosis infection in children and adolescents. *Pediatrics*. 2004;114:1175-201.
49. van Geuns HA MJ, Styblo K. The yield from mass tuberculin testing of unvaccinated children and adolescents. *Bull Int Union Tuberc*. 1975;50(1):82-9.

50. Hennekens C, Buring JE. Chapter 13 Screening in Epidemiology in Medicine. First ed. Mayrent SL, editor. Little, Brown and Company, New York, USA; 1987.
51. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. Boletín de la Oficina Sanitaria Panamericana Pan American Sanitary Bureau. 1968;65(4):281-393. Epub 1968/10/01. Principios y metodos del examen colectivo para identificar enfermedades.
52. den Boon S, White NW, van Lill SW, Borgdorff MW, Verver S, Lombard CJ, et al. An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *Int J Tuberc Lung Dis*. 2006;10(8):876-82. Epub 2006/08/11.
53. Van't Hoog AH, Meme HK, Laserson KF, Agaya JA, Muchiri BG, Githui WA, et al. Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. *PloS One*. 2012;7(7):e38691. Epub 2012/07/14.
54. Rupp R, Rosenthal SL, Middleman AB. Vaccination: an opportunity to enhance early adolescent preventative services. *J Adolesc Health*. 2006;39(4):461-4. Epub 2006/09/20.
55. Jaspan HB, Berwick JR, Myer L, Mathews C, Flisher AJ, Wood R, et al. Adolescent HIV prevalence, sexual risk, and willingness to participate in HIV vaccine trials. *J Adolesc Health*. 2006;39(5):642-8. Epub 2006/10/19.
56. Jaspan HB, Soka NF, Strode AE, Mathews C, Mark D, Flisher AJ, et al. Community perspectives on the ethical issues surrounding adolescent HIV vaccine trials in South Africa. *Vaccine*. 2008;26(45):5679-83. Epub 2008/09/11.
57. Singh JA, Karim SS, Karim QA, Mlisana K, Williamson C, Gray C, et al. Enrolling adolescents in research on HIV and other sensitive issues: lessons from South Africa. *PLoS Medicine*. 2006;3(7):e180. Epub 2006/04/13.

58. Eaton L, Flisher AJ. HIV/AIDS knowledge among South African youth. *S A J Child Adolesc Ment Health*. 2000(12):97-124.
59. Woodhall SC, Lehtinen M, Verho T, Huhtala H, Hokkanen M, Kosunen E. Anticipated acceptance of HPV vaccination at the baseline of implementation: a survey of parental and adolescent knowledge and attitudes in Finland. *J Adolesc Health*. 2007;40(5):466-9. Epub 2007/04/24.
60. Hopenhayn C, Christian A, Christian WJ, Schoenberg NE. Human papillomavirus vaccine: knowledge and attitudes in two Appalachian Kentucky counties. *Cancer Causes Control*. 2007;18(6):627-34. Epub 2007/05/15.

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Chapter 3: Predictive factors for latent tuberculosis infection among adolescents in a high burden area of South Africa.

Paper overview

This analysis reports on the baseline prevalence of latent tuberculosis (TB) infection in the adolescent cohort that was studied as measured by both the tuberculin skin test and an interferon gamma release assay. The predictive factors for a positive test by either measure is shown as evaluated through a multivariate analysis.

Contribution to the thesis and novelty

This paper addresses the first objective of the thesis. As shown in the literature review, little data on the prevalence of latent TB infection are available for adolescents with none using the interferon gamma release assay. Little data on predictive factors for latent TB infection in adolescents were found. Such data will be of enormous value for those planning trials of TB vaccines in adolescents and for TB Control Programmes.

Contributions of candidate

I was the first author on the publication and was primarily responsible for overseeing the conduct of the study on which the publication is based. I was also primarily responsible for the data analysis and write-up of the study and this is clear from the roles of co-authors as described below.

H Mahomed: Involved in study conceptualisation, oversaw the conduct of the study, analysed the data and wrote the manuscript.

T Hawkrige: Involved in study conceptualisation and reviewed the manuscript.

S Verver: Involved in study conceptualisation, reviewed the analysis and reviewed the manuscript.

L Geiter: Involved in study conceptualisation and reviewed the manuscript.

M Hatherill: Reviewed the manuscript.

D Abrahams: Responsible for processing of QuantiFERON specimens and reviewed the manuscript.

R Ehrlich: Reviewed the analysis and manuscript.

WA Hanekom: Involved in study conceptualisation and reviewed the manuscript.

GD Hussey: Involved in study conceptualisation and reviewed the manuscript.

The SATVI Adolescent Team referred to in the article reference included the following:

F Kafaar: Was the study co-ordinator responsible for day to day management of the study and reviewed the manuscript.

M Tameris: Approved microbiology results and reviewed the manuscript.

H Geldenhuys: Approved microbiology results and reviewed the manuscript.

L Matzirofa: Extracted preliminary data tables and reviewed the manuscript.

L Workman: Designed the study database and reviewed the manuscript.

S Gelderbloem: Oversaw the laboratory where the QuantiFERON assays were done and reviewed the manuscript.

S Moyo: Reviewed the manuscript.

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A few further amendments have been made to this paper subsequent to publication.

University of Cape Town

Predictive factors for latent tuberculosis infection among adolescents in a high burden area in South Africa

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Abstract

Setting: A high tuberculosis (TB) burden area in South Africa (notification rate for all TB cases 1400/ 100 000).

Objective: To determine the prevalence of and predictive factors associated with latent TB infection in adolescents.

Design: Adolescents aged 12-18 years were recruited from high schools, clinical and demographic data were collected, and a tuberculin skin test (TST) and a QuantiFERON® TB GOLD In-Tube (Cellestis) (QFT) assay performed.

Results: 6,363 (58.2%) of 10,942 adolescents at the schools were enrolled. After exclusions, of 5,244 participants, 55.2% (95% confidence interval 53.8 -56.5) had TST \geq 5 mm, while 50.9% (49.5 - 52.2) were QFT positive. On multivariate analysis, Black/ Mixed Race racial groups, male gender, older age, household TB contact, low income and low education level were predictive factors for both TST and QFT positive results.

Conclusion: About half of adolescents were found to be latently infected with TB in a high TB burden area with demographic and poverty related socio-economic factors predicting the risk of tuberculosis infection. Adolescents from deprived communities should be considered an important target group for educational interventions by TB Control Programmes in high burden settings.

Abstract word count (186)

Introduction

It was estimated that 9.3 million people developed tuberculosis (TB) disease and 1.8 million died from the disease in 2007¹. Despite this huge burden, very little has been published on adolescent TB²⁻⁵. Compared to younger children, this group has a rising incidence of TB disease with age, as shown in unpublished data from Cape Town drawn from routine TB notifications (figure 1). The increasing incidence with age may reflect an increasing susceptibility to TB disease due to a higher risk of TB infection. In order to prepare for future phase III trials of new vaccines in adolescents, our aim was to determine the epidemiology of latent TB infection in adolescence in the Worcester region, outside Cape Town, South Africa. This area has a high notified TB incidence rate among all ages of approximately 1400 per 100 000⁶. This agricultural area has an unemployment rate of 20% with 50% of those employed earning < \$100 per month (South African National Census 2001). New smear positive TB rates ranged between 454 – 631/100 000 between 2000 and 2004. The main objective of this study was to determine the prevalence of and predictive factors for latent TB infection among adolescents from this region.

Traditionally, the tuberculin skin test (TST) has been used to detect latent TB infection. Unlike the TST, which uses purified protein derivative (PPD) of *Mycobacterium tuberculosis* as antigen which is widely cross-reactive, antigens used in the newer interferon gamma release assays (IGRAs) are mostly specific to *M. tuberculosis*. These tests have been promoted as an alternative to the TST, as results should not be affected by prior exposure to BCG nor other non-tuberculous mycobacteria (NTMs)⁷⁻¹¹. It has been proposed that IGRAs would be most useful in settings

where BCG vaccination coverage is high¹⁰. This is the first study that we are aware of that examines predictive factors for TB infection using both the TST and an IGRA in adolescents.

Study Population and Methods

Study setting: The study took place in the town of Worcester (and surrounding villages), approximately 100 kilometres from Cape Town, South Africa.

Study participants: All adolescents aged 12-18 years attending all 11 public high schools in the study area were approached to participate.

Study procedures: At enrolment, a questionnaire was administered to study participants and their parents to collect demographic and socio-economic data. These included parental income and parental education. The racial classification of participants “White”, “Mixed race”, “Indian” and “Black” was based on a system used in the apartheid era, but still used today as a proxy for socio-economic status and to measure social change. History of BCG vaccination (reported), history of current and past TB disease, current and past TB household contacts, hospitalization in the past 6 months and information on chronic diseases were obtained. Participants were examined for the presence of a BCG scar.

Blood was taken for QuantiFERON[®] TB Gold In-tube (Cellestis, Victoria, Australia). A TST was then immediately administered using the Mantoux method on either forearm, using 2 tuberculin units of RT23 (Statens Serum Institut, Denmark). Induration at the TST site was read 48 - 96 hours later with a ruler or a caliper, by trained study personnel. Those with previous or current TB did not have a TST performed, in order to prevent severe allergic reactions. The QuantiFERON test (QFT) was performed as recommended by the manufacturers.

Data analysis and statistical considerations:

The sample size required to find a predictive factor for infection present in at least 25% of the population, with an odds ratio of 1.2, a power of 80% and alpha of 0.05, was estimated to be 5,200.

Data were captured in a Microsoft Access database, and analysed with Stata version 11.0 software (Quantec, Pretoria, South Africa). To ensure the integrity, validity and quality of data in the database, the following measures were put in place: Only trained data entry personnel were used with controlled access to the database. Normalization, referential integrity and data validation rules were implemented during database design. Data was verified and validated prior, during and after data entry according to a data entry standard operating procedure (SOP). An internal data monitor periodically reviewed a random 10% sample of case report forms (CRFs) entered on the database according to SOPs. Additionally, she reviewed all the CRFs entered on the database at the end of the study and corrections made accordingly.

Univariate and multivariate analyses were performed. Statistical significance was determined to be present at $p < 0.05$, or if confidence intervals did not cross one. A TST cut-off point of 5 mm was the default threshold used for analysis based on the frequency distribution of TST indurations which was not bimodal, although cut-off points of 10mm and 15 mm were used for certain analyses. For the purposes of analysis, Indian and White participants were grouped because they shared similar socio-economic profiles. For analysis of income, a cut-off was set at R4000 (~\$500) per month with the parent or guardian with the highest income determining

which category the adolescent was placed in. Education level was divided by primary school or less and secondary school or more.

Ethics: This study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town. Written informed consent was obtained from the parents of adolescents and assent obtained from adolescents.

Results

Study participants

6,363 adolescents aged 12-18 years (58.2%) were enrolled into the study, from a total of 10,942 adolescents attending the schools at the time of enrolment (2006 data supplied by the Department of Education). Enrolment rates ranged between 22.2% and 74.2% among schools, with those within communities with lower socio-economic status having higher enrolments. Reasons for non-enrolment included parental refusal, adolescent refusal, out of age range, inability to locate the participant, lack of availability of the parent or guardian for consent. Fear of needles was the reason most commonly provided when a reason was given for refusal. The proportion of females enrolled (54%) was greater than in the source population where the proportion of females was about 50% suggesting that proportionately less males enrolled than females. Not enough data was collected on “fear of needles” to specifically indicate if this was the reason why proportionately less males enrolled in the study.

The focus of analysis was on participants with both a TST and QFT result. Those with an indeterminate QFT result (n=13) were excluded. Since we did not give a TST to those with past (n=639) or current TB (n=22), we also excluded those diagnosed with active TB disease through

study procedures (n=21). As n=702 did not have a TST result, n=309 no QFT result, and some of these categories overlapped, this left n=5,244 (82.4% of total enrolled) participants for analysis. We did an additional analysis which included those with current or past TB (n=673) where the sample analysed was n=5,917.

The demographic profile of the enrolled participants is shown in Table 1. There was a small female predominance amongst enrolled participants (54.2%), compared with the source population (49.7%).

The prevalence of latent TB infection

Figure 2 shows the distribution of TST induration diameters (excluding 0 mm), which had a peak (mode) at 12 mm. There is no obvious sign of digit preference.

A total of n=2,894 out of n=5,244 participants, or 55.2% (95% confidence interval [CI] 53.8 - 56.5%), had a TST ≥ 5 mm, while n=2,669 or 50.9% (49.5 – 52.2%) of participants had a positive QFT. A total of n=2,214 out of n=5,244 participants, or 42.2% (95% CI 40.9 -43.6%), had a TST ≥ 10 mm and when 15 mm was used as the cut-off, n=981, or 18.7% (95% CI 17.7-19.8%) were TST positive. There was good agreement at 5mm (% agreement 84.8%, kappa 0.70 [95% CI 0.68-0.71]) and 10 mm TST cut-off (% agreement 81.4%, kappa 0.63 [95% CI 0.61-0.65]) but not the 15 mm cut-off (% agreement 64.3%, kappa 0.30 [95% CI 0.27-0.32]).

An additional analysis where we included those with current or prior TB gave a prevalence of latent TB infection with TST ≥ 5 mm of 60.3% (n=3,567/5,917 [95% CI 59.0 - 61.5]) and QFT of 53.7% (n=3,174/5,917 [95% CI 52.4 - 54.9]).

Predictive factors for latent TB infection

Significant predictive factors for latent TB infection as measured by both TST and QFT on univariate analysis were older age, Black and Mixed racial groups compared to White/ Indian racial groups, low parental income, maternal and paternal education at primary school level or less, current or prior household TB exposure and absence of chronic allergic disease (Table 1).

A multivariate model was developed including only the statistically significant associations from univariate analysis (Table 2) except that gender was added since this is a known risk factor for TB in adulthood. The following predictive factors were predictive of TB infection for both TST and QFT: Black and Mixed racial groups, age >15 years, household TB contact, parental monthly income <R4,000 (~\$500), maternal education primary school or less and male gender. In addition, absence of chronic allergic related diseases was significantly predictive of a positive QFT test only.

% QFT positive by age and gender

% QFT positive is shown by TST induration status by age and gender (Table 3). Interestingly, there are proportionally more QFT positive responses amongst females than males in the

>15mm TST group. A further elaboration of the relationship with age is shown in Figure 3 which shows a significant trend in increasing TB infection with age as demonstrated by both TST and QFT.

Discussion

We found more than half of adolescents to be latently infected with TB in a predominantly low income, high TB prevalence population using two different measures of latent TB infection.

Many demographic and socio-economic variables were predictive of latent TB infection, even on multivariate analysis. Predictive factors were similar using both measures. QuantiFERON did not show any significant advantage over TST in this study.

A survey amongst grade 9 (age 14-15) Norwegian students found a TST positive proportion of only 1.6% of whom only 9% were QFT positive¹². A study of the children of immigrants aged 4-18 in Canada found 21% to be TST positive⁵. In contrast, a study of a community with a high prevalence of HIV amongst adults in Cape Town showed a 52.5% TST positive level amongst adolescents aged 14-17¹³. These differences probably reflect differences in community exposure to infectious TB.

A higher latent TB infection prevalence was found when we included those with prior or current TB. This may be more representative of the total population because those with current or prior TB are likely to be a higher risk group for infection. (The inclusion of those with current TB in this analysis, means that this group cannot be defined as only latently infected. Strictly speaking, they should simply be regarded as having TB infection.)

The absence of an association between TST and BCG is in line with the conclusions of Farhat et al. who found that the BCG effect on TST is minimal in a situation where BCG is given at birth (as in South Africa) and the TST is done more than 10 years thereafter⁹. It is surprising nevertheless that no impact of BCG is seen in these data given that IGRAs are promoted as being more specific and not being affected by BCG and most NTMs. The study in Cape Town in a high HIV prevalence community also found no association between the presence of a BCG scar and TST positivity¹³.

Since QFT is considered a more specific test than TST, a lower proportion of QFT positives than TST positives is expected¹⁰ and this has been found in other studies^{12, 14, 15}. However, different TST cut-offs give different prevalences for latent TB infection. An examination of the TST distribution should guide determination of the cut-off as well as the use of methods such as mixture analysis. 25% of children deemed positive by virtue of the 5 mm cut-off were in the 5-9 mm range. Of these 64% were QFT positive. The fact that the majority were QFT positive supports the use of this cut-off as an indication of genuine latent TB infection. The 36% who were QFT negative is however a substantial proportion – either this is an indication that the QFT was not sensitive enough to detect latent TB infection in this group or that the TST cut-off misclassified these children as latently infected. Further exploration of this is needed to determine which explanation is correct.

History of TB contact and older age were expected to be predictive factors for latent TB infection. Socio-economic markers (lower parental income, fewer years of parent education) were demonstrated to be significant predictive factors, confirming that TB is rooted in poorer communities¹. Origin outside of a Western country and history of exposure to TB were reported as predictive factors for a positive QFT in Norwegian adolescents¹².

The strongest predictive factor for TB infection was racial group, even in a multivariate analysis including income and education. However, it is likely that socio-economic status is not fully captured by the income and education variables. Another study in South Africa showed that when a number of socio-economic factors were taken into account using a multivariate model, racial group disappeared as an independent predictive factor for TB disease¹⁶.

Male gender was shown to be a significant although weak predictive factor for a positive TST and QuantiFERON test on multivariate analysis. This was shown also for QFT in another study in adults in this area¹⁷. Males have higher rates of TB disease in adulthood¹⁸⁻²⁰. There appears to be a difference in risk of TB infection by gender with age, with adolescence representing a transition phase between childhood and adulthood²¹.

The reporting of allergy related conditions was associated with a lower prevalence of TB infection as measured by QuantiFERON. There is some evidence of an inverse relationship between atopy and mycobacterial disease although a recent meta analysis on this subject was inconclusive²².

The relevance of this study for TB control managers is that it shows that more than half of adolescents have latent TB infection in a high burden setting linked to socio-economic status and household exposure. This may be an important target group for educational programmes on TB to enhance early detection.

Representivity of this sample may be limited because of non-agreement to participate by 42% of school children. As the main reason given for refusal to participate related to “fear of needles”, the impact on these analyses may be unbiased with respect to latent TB infection. According to the South African Census held in 2001, 80% of children aged 12-18 years were at school in this community. As school dropouts are likely to represent a higher risk group for TB, the infection proportion may be underestimated. On the other hand, there were higher study enrolment rates at lower socio economic status schools, and this is likely to have caused an over estimation of the infection risk. Nevertheless, we feel that the prevalences measured in this study are likely to be close to the true population prevalence in this age group in predominantly low income high TB incidence communities in South Africa. HIV testing was not done for this sub-study. This area of South Africa has much lower HIV sero-prevalence than the overall national prevalence according to annually conducted HIV prevalence surveys in South Africa (5-10% amongst pregnant women compared to 30% at national level). HIV infection is thus expected to be low in adolescents in the study area and unlikely to have a large influence on the prevalence of latent TB infection.

Conclusion

The prevalence of latent TB infection in this adolescent population as measured by TST and QFT was 55.2% and 50.9% respectively. Racial group is a strong predictor of TB infection in this community and this is likely to represent a proxy for socio-economic factors other than those measured. The predictive factor profile for both measures was similar.

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References

1. WHO. Global tuberculosis control 2009 : Epidemiology, Strategy and Financing : WHO 2009.; 2009.
2. Rodrigues L C, Pereira S M, Cunha S S, et al. B Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet* 2005;366(October 8):1290-95.
3. de Pontual L, Balu L, Ovetchkine P, et al. Tuberculosis in adolescents: A French retrospective study of 52 cases. *Pediatr Infect Dis J* 2006;25(10):930-2.
4. Weber HC, Beyers N, Gie R P, Schaaf H S, Fish T, Donald P R. The Clinical and radiological features of tuberculosis in adolescents. *Ann Trop Paediatr* 2000;20:5-10.
5. Brassard P, Steensma C, Cadieux L, Lands LC. Evaluation of a school-based tuberculosis-screening program and associate investigation targeting recently immigrated children in a low-burden country. *Pediatrics* 2006;117(2):e148-56.
6. Groenewald P. Annual Health Status Report 2006. Worcester: Department of Health, Boland-Overberg Region; 2004.
7. Richeldi L. An update on the diagnosis of tuberculosis infection. *Am J Respir Crit Care Med* 2006;174(7):736-42.
8. Dheda K, Smit RZ, Badri M, Pai M. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. *Curr Opin Pulm Med* 2009;15(3):188-200.

9. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006;10(11):1192-204.
10. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008;149(3):177-84.
11. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004;4(12):761-76.
12. Winje BA, Oftung F, Korsvold GE, et al. School based screening for tuberculosis infection in Norway: comparison of positive tuberculin skin test with interferon-gamma release assay. *BMC Infect Dis* 2008;8:140.
13. Middelkoop K, Bekker LG, Myer L, Dawson R, Wood R. Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults. *Clin Infect Dis* 2008;47(3):349-55.
14. Carvalho AC, Pezzoli MC, El-Hamad I, et al. QuantiFERON-TB Gold test in the identification of latent tuberculosis infection in immigrants. *J Infect* 2007;55(2):164-8.
15. Machado A, Jr., Emodi K, Takenami I, et al. Analysis of discordance between the tuberculin skin test and the interferon-gamma release assay. *Int J Tuberc Lung Dis* 2009;13(4):446-53.
16. Harling G, Ehrlich R, Myer L. The social epidemiology of tuberculosis in South Africa: a multilevel analysis. *Soc Sci Med* 2008;66(2):492-505.

17. Mahomed H, Hughes EJ, Hawkrigde T, et al. Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. *Int J Tuberc Lung Dis* 2006;10(3):310-6.
18. Christopoulos AI, Diamantopoulos AA, Dimopoulos PA, Goumenos DS, Barbalias GA. Male-female differences in the risk of tuberculosis in dialysis patients. *Int Urol Nephrol* 2009;41(3):671-7.
19. Lawson L, Lawson JO, Olajide I, et al. Sex differences in the clinical presentation of urban Nigerian patients with pulmonary tuberculosis. *West Afr J Med* 2008;27(2):82-6.
20. Crampin AC, Glynn JR, Floyd S, et al. Tuberculosis and gender: exploring the patterns in a case control study in Malawi. *Int J Tuberc Lung Dis* 2004;8(2):194-203.
21. Diwan VK, Thorson A. Sex, gender, and tuberculosis. *Lancet* 1999;353(9157):1000-1.
22. Obihara CC, Bollen CW, Beyers N, Kimpen JL. Mycobacterial infection and atopy in childhood: a systematic review. *Pediatr Allergy Immunol* 2007;18(7):551-9.

Table 1: Predictive factors for TB infection on univariate analysis

N:5244	Numbers (column %)	TST+ ¹ (row %)	OR ^{2,3} (95% CI)	QFT+ ⁴ (row %)	OR ^{2,3} (95% CI)
Gender					
Male	2,402 (45.8%)	56.1	1.07	51.8	1.07
Female (ref ⁵)	2,842 (54.2%)	54.4	(0.96; 1.20)	50.1	(0.96;1.20)
Age (years)					
>15	2,261 (43.1%)	60.0	1.39	55.1	1.34
≤15 (ref)	2,983 (56.9%)	51.7	(1.25; 1.56)	47.7	(1.20;1.50)
Racial group					
Black	995 (19.0%)	60.2	8.65 (6.35; 11.82)	52.7	8.8 (6.3; 12.4)
Mixed race	3,839 (73.2%)	58.2	7.96 (5.97; 10.64)	54.7	9.6 (6.9; 13.2)
Indian/ white (ref)	410 (7.8%)	5.1		11.2	
Parent income: classified on at least one parent's income					
≤R4000 /month	4,243 (80.9%)	60.4	3.40	56.1	3.37
>R4000 /month (ref)	921 (17.6%)	30.9	(2.91; 3.97)	27.5	(2.88; 3.94)
Unknown	80 (1.5%)				

Maternal highest education level					
≤ Primary school	1,510 (28.8%)	68.0	2.49	61.5	2.14
≥ High school (ref)	2,890 (55.1%)	48.0	(2.15; 2.84)	42.7	(1.88; 2.43)
Unknown:	844 (16.1%)				
Paternal highest education level					
≤ Primary school	686 (13.1%)	63.9.6	2.38	58.0	2.01
≥ High school (ref)	1,720 (32.8%)	42.6	(1.97; 2.87)	39.6	(1.69; 2.40)
Unknown:	2,838 (54.1%)				
BCG reported as being given					
No	46 (0.9%)	67.4	1.72	50.6	1.39
Yes (ref)	4,917 (93.8%)	54.6	(0.89; 3.34)	58.7	(0.74; 2.60)
Unknown	281 (5.4%)				
BCG scar					
Absent	1,490 (28.4%)	53.6	0.86	51.0	1.01
Present (ref)	2,064 (39.4%)	57.3	(0.75; 0.99)	50.8	(0.89; 1.16)
Unknown (Not sure)	1,690 (32.2%)				
Current or prior TB household contact					

Yes	1,332 (25.4%)	71.3	2.52	66.7	2.40
No (ref)	3,911 (74.6%)	49.7	(2.20; 2.88)	45.5	(2.11; 2.74)
Unknown	1 (0.02%)				

Chronic allergy-related conditions e.g. asthma, hay fever, eczema

Yes	53 (1.0%)	32.1	0.38	24.5	0.31
No (ref)	5,191 (99.0%)	55.4	(0.20; 0.70)	51.2	(0.16; 0.60)

History of hospitalization within the 6 months prior to enrolment

Yes	46 (0.9%)	45.7	0.68	43.5	0.74
No (ref)	5,198 (99.1%)	55.3	(0.37; 1.26)	51.0	(0.40; 1.38)

¹Tuberculin Skin Test positive (≥ 5 mm induration)

²OR=odds ratio

³"Unknown" excluded from OR calculations

⁴ QuantiFERON TB Gold positive ≥ 0.35 international units

⁵"ref" reference category for odds ratio calculation

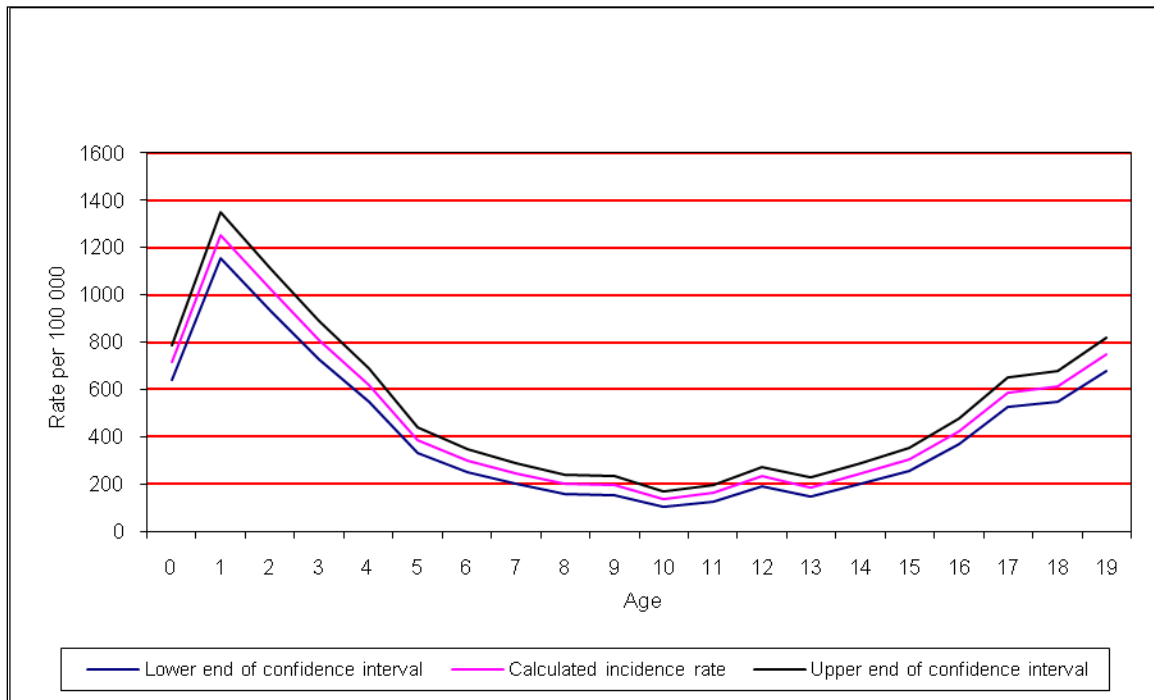
Table 2: Predictive factors for positive TST and positive QFT on multivariate analysis (N=5,163)

Variable	Odds	95%	Odds	95%
	Ratio	Confidence interval	Ratio	Confidence interval
	<i>TST</i>		<i>QFT</i>	
Racial group (mixed race)	4.2	3.1 – 5.7	4.9	3.5 – 6.9
Racial group (black)	3.9	2.8 - 5.4	3.8	2.7 – 5.5
Current or prior TB household contact	2.0	1.7 – 2.3	1.9	1.7 – 2.2
Income \leq R4000/ month	1.5	1.2 – 1.8	1.6	1.3 – 1.9
Maternal highest education level – \leq Primary school	1.7	1.5 – 1.9	1.5	1.3 – 1.7
Age (years) >15	1.4	1.3 – 1.6	1.4	1.2 – 1.5
Paternal highest education level – \leq Primary school	1.2	1.0 – 1.5	1.1	0.9 – 1.4
Gender - Male	1.12	1.0 – 1.3	1.2	1.0 – 1.3
Chronic allergy- related conditions			0.4	0.2 – 0.9

Table 3: % QFT positive by TST induration group by age and gender

Age	Gender	TST induration group			
		< 5mm	5 - 9 mm	10 -14 mm	>15 mm
13	Female	11.9	13.7	38.7	35.7
	Male	13.9	16.5	44.3	25.2
14	Female	10.8	14.8	40.1	34.3
	Male	13.0	13.4	44.6	29.0
15	Female	10.4	17.6	38.1	33.9
	Male	10.0	16.0	40.3	33.7
16	Female	9.8	12.5	39.4	38.2
	Male	9.2	19.2	40.2	31.4
17	Female	12.5	14.8	35.2	37.5
	Male	11.9	13.4	40.6	34.2
18	Female	8.1	25.2	34.2	32.4
	Male	4.7	24.2	46.1	25.0

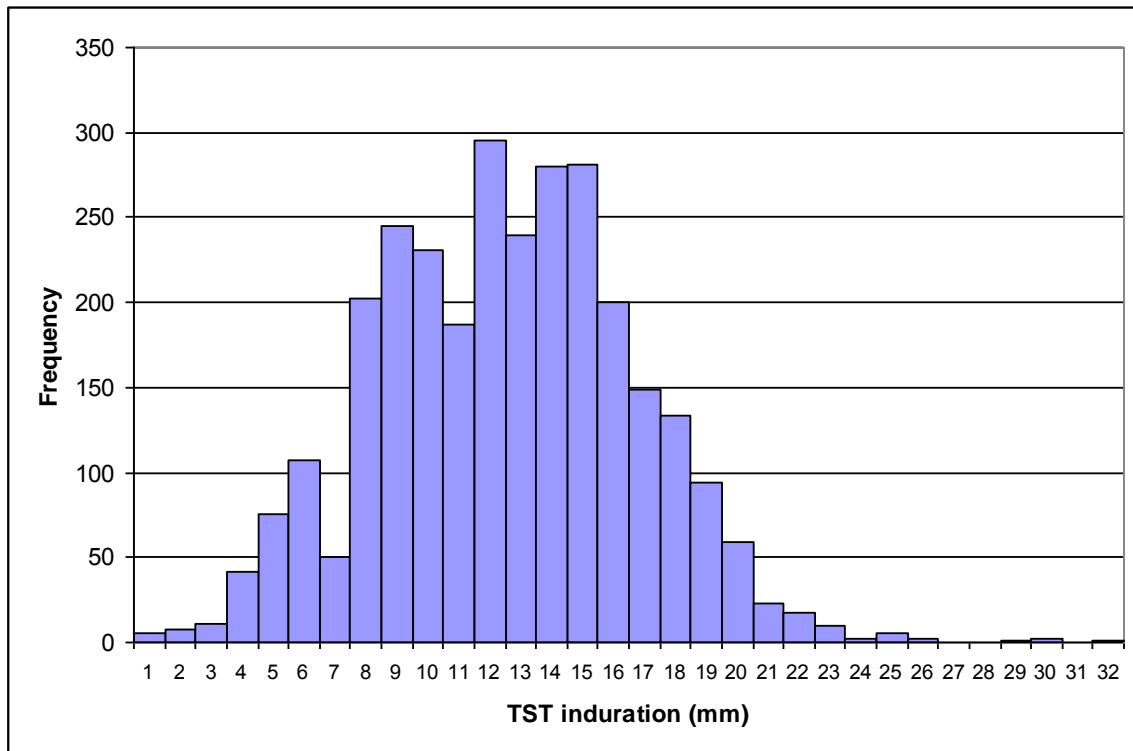
Figure 1 All TB incidence rate by age (years) in Cape Town, July 2002 to June 2003 (N=5039)



Source: City Health Directorate of the City of Cape Town (notified TB cases) and Census 2001 (population estimates)

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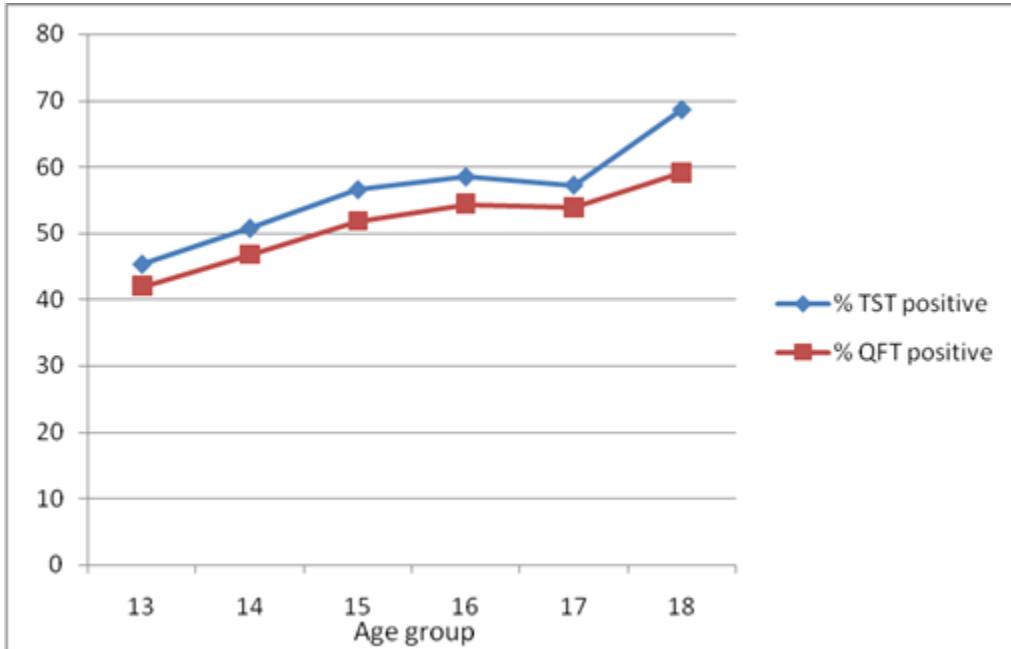
Figure 2 TST distribution amongst adolescents aged 12-18 years (N= 5244)*



*TST = 0 mm N= 2284 (43.6%)

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Figure 3: Latent TB infection by age, using 5 mm cut-off point for TST (data for 12 year olds excluded) (N=5193)



(Chi squared for linear trend, $p < 0.01$ for both).

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Chapter 4: Screening for TB in high school adolescents in a high burden setting in South Africa

Paper overview

This analysis reports on the prevalence of TB disease in the adolescent cohort that was studied and examines the utility of different screening methods – symptoms, household contact, the tuberculin skin test and the QuantiFERON TB Gold assay. The sensitivity, specificity, positive predictive value and negative predictive values of these methods alone and in combination are reported.

Contribution to the thesis and novelty

This paper addresses the second objective of the thesis. Little data on the population prevalence of TB disease are available for adolescents. No data on the use of these different screening tools for detecting TB disease in adolescents were found. These data will be of use to those planning clinical trials of TB in adolescents and for TB programs considering screening interventions amongst adolescents.

Contributions of candidate

I was the first author on the publication and was primarily responsible for overseeing the conduct of the study on which the publication is based. I was also primarily responsible for the data analysis and write-up of the study and this is clear from the roles of co-authors as described below.

H Mahomed: Involved in study conceptualisation, oversaw the conduct of the study, analysed the data and wrote the manuscript.

R Ehrlich: Reviewed the analysis and manuscript.

T Hawkrige: Involved in study conceptualisation and reviewed the manuscript.

M Hatherill: Reviewed the manuscript.

L Geiter: Involved in study conceptualisation and reviewed the manuscript.

F Kafaar: Was the study co-ordinator responsible for day to day management of the study and reviewed the manuscript.

D Abrahams: Responsible for processing of QuantiFERON specimens and reviewed the manuscript.

H Mulenga: Managed the study database and reviewed the manuscript.

M Tameris: Approved microbiology results and reviewed the manuscript.

H Geldenhuys: Approved microbiology results and reviewed the manuscript.

WA Hanekom: Involved in study conceptualisation and reviewed the manuscript.

S Verver: Involved in study conceptualisation, reviewed the analysis and reviewed the manuscript.

GD Hussey: Involved in study conceptualisation and reviewed the manuscript.

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7.

A few further amendments have been made to this paper subsequent to publication.

Screening for TB in high school adolescents in a high burden setting in South Africa

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Abstract

Screening for tuberculosis (TB) disease is important for TB control and TB vaccine efficacy trials but this has not been evaluated in adolescents. We conducted a study to determine the prevalence of active TB and performance of specific screening tests for TB in adolescents in a high burden setting. Adolescents aged 12-18 years were recruited from high schools in a rural town in South Africa. Participants were screened for active TB using symptoms, household TB contact, positive interferon gamma release assay (IGRA) and positive tuberculin skin test (TST). Of 6363 adolescents recruited, 21 were newly diagnosed with TB of whom 19 were culture positive. After exclusions, the derived prevalence of smear positive TB was $16/5682 = 3/1000$ (95% confidence interval (CI) 1-4/ 1000). The sensitivity of TST and IGRA for active TB were 85% (95% CI 62-100%) and 94% (95% CI 79-100%) respectively. None of the methods alone or in combination had positive predictive values greater than 2%. The screening tools evaluated in this study may not be practical for routine use owing to low positive predictive values but may be useful in TB vaccine clinical trials.

Word count: 187

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Key words: tuberculosis, adolescence, prevalence, screening

Introduction

Tuberculosis (TB) remains a significant public health problem globally.¹ While TB Control Programmes have previously focused on optimal cure rates of smear positive TB patients, active case finding is receiving increased attention as an important component of strategies aimed at TB elimination. Early and improved case detection are both components of the Global Plan to Stop TB.^{2,3} In a different context, efficacy trials of new TB vaccines need effective methods to exclude cases of TB prior to study enrolment and to detect TB cases during follow up.

Based on TB control programme data, age distributions of TB incidence show adolescence to be a period of increasing incidence.^{4,5} There are, however, no studies that have specifically investigated prevalence of active TB in adolescents. Knowledge of prevalent TB in adolescents is relevant to public health programmes since these cases represent a source of transmission. Resulting new infections in turn present a high risk of progressing to active TB.⁶

It has been shown that occurrence of TB in high burden settings is often due to exposure outside of the home environment.⁷ Thus, adolescents may become infected in the school environment owing to the presence of active TB cases and may rapidly progress to active TB while still at school, representing a further risk to others. Early diagnosis in adolescents, who have been described as having a high “force of infection”,⁸ is therefore important to reduce the risk of transmission in school settings where large numbers of adolescents congregate in closed spaces.

Adolescents are also an important target group for TB vaccine trials.⁹ Knowledge of prevalence would assist with planning for TB vaccine trials since it is important to know the burden of TB for calculating trial sample sizes.

The identification of prevalent cases requires effective screening tools. TB screening has been evaluated in communities in Africa,¹⁰⁻¹² amongst immigrants in a low burden setting¹³ and health workers.¹⁴ The World Health Organisation has also proposed strategies for TB screening

in population-based surveys.¹⁵ However, we have found no studies that have investigated TB screening specifically in adolescents. In the context of TB vaccine clinical trials, the value of good screening tools would be to exclude cases of TB at enrolment prior to vaccination for safety reasons and to detect TB cases during follow up as case ascertainment is crucial to the success of efficacy trials. Low cost, efficient screening tools are therefore needed to make trials as cost effective as possible.

We thus conducted a study in adolescents to determine the prevalence of TB and the performance of various TB screening tools in detecting TB disease. As the routine use of the chest radiograph was not feasible in our setting, this was not included as a screening tool. Other publications on this study population include: 1) An examination of risk factors for latent TB infection based on the tuberculin skin test (TST) and QuantiFERON TB Gold (in-tube) (QFT) assay results at enrolment¹⁶ 2) A comparison of the predictive value of baseline TST and QFT for TB disease during follow-up¹⁷ 3) The determination of the predictive value of a QFT conversion for TB disease in a subset of participants who underwent extended follow up¹⁸ and 4) TB incidence in an adolescent cohort in South Africa (manuscript under review).

Methods

Study setting: The study took place in the town of Worcester, approximately 100 kilometres from Cape Town, South Africa between July 2005 and April 2007.

Study participants: All adolescents aged 12-18 years attending 11 high schools in the study area were approached to participate.

Study procedures: At enrolment, demographic and clinical information (including history of current or prior household TB contact) was collected. Blood was taken for QuantiFERON[®] TB Gold In-tube (Cellestis, Victoria, Australia) (QFT) and a TST was administered. Those with previous or current TB or with a previous severe reaction to a TST did not have a TST performed in order to reduce the risk of severe reactions.

The first 481 participants enrolled were all screened with a single sputum culture. For logistical reasons (the microbiology laboratory was not able to cope with a single sputum for culture on all study participants), the protocol was then amended to only screen the following: those with (1) TB symptoms (unexplained cough, night sweats, loss of weight or loss of appetite for two weeks or more, or haemoptysis), (2) a household contact within three years of enrolment, (3) a TST ≥ 10 mm, or (4) a positive QFT. These participants were considered TB suspects and were investigated by the collection of two sputum samples for smears on two separate occasions. The sputum was concentrated and examined with a fluorescent stain (auramine). A positive smear was followed by a culture (liquid, Mycobacteria Growth Indicator Tube (MGIT, Bactec MGIT 960 system, Beckton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and solid, Loewenstein-Jensen) to confirm the presence of *Mycobacterium tuberculosis*, a chest radiograph and an HIV test.

Definitions: TB disease was defined by at least two positive sputum smears and/or one positive sputum culture. However, data on all individuals placed on TB treatment by a physician were recorded.

Data analysis: Data were captured in a Microsoft Access database, and analysed with STATA version 11.0 (Statacorp, Texas, USA). Data were verified and validated prior, during and after data entry according to a data entry standard operating procedure. Prevalence was calculated based only on the number of sputum smear positive cases diagnosed through study procedures excluding the first 481 who were screened with a single sputum culture. Sensitivities, specificities, positive predictive values and negative predictive values were calculated with 95% confidence intervals, using these prevalent cases as the reference standard. The screening tests were evaluated individually and in combination. Since recruitment was done in clusters (11 schools), confidence intervals of rates and proportions were adjusted to take clustering into account (cluster design effect).

Ethics: This study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town. Written informed consent was obtained from the parents of adolescents and assent was obtained from adolescents.

Results

Study participants

6,363 adolescents aged 12-18 years were enrolled. This represented 58% of 10,492 registered learners at the high schools where recruitment took place. Enrolment rates by school ranged from 22.2% to 74.4% (31.8% in schools in affluent areas and 66.6% in schools in poorer areas. Schools in affluent areas made up 13.3% of the study population and 24.2% of the source population). The study population had a predominance of females of 54.3% (3458 participants). There were a greater proportion of younger adolescents (56.6% [3603] were ≤ 15 years of age) since there were fewer students in the higher than in the lower school grades. Figure 1 shows the TB related profile of the study population. Prevalence of latent TB infection based on both the TST (induration ≥ 10 mm) and QFT, was present in 42.1% and 53.5% of the population respectively. There were 639 (10.0%) adolescents who reported having had TB before, while 1728 (27.2%) had a current or prior household TB contact. Among the latter, 1055 (16.6%) had had a current or prior household contact within three years of enrolment. Two hundred and one (3.1%) reported at least one TB related symptom at enrolment.

Prevalence of TB

The distribution and classification of cases is set out in Figure 2. In the cohort as a whole (N=6363), 21 adolescents were diagnosed with TB, of whom 17 were smear positive (all confirmed by culture), two were smear negative but culture positive (making up 19 culture positive cases in total), while two were diagnosed on clinical grounds. All 21 cases had pulmonary TB; none were classified as primary TB and no extra-pulmonary TB cases were diagnosed. Both cases diagnosed on clinical grounds had chest radiographs compatible with TB disease. Amongst the culture positive cases, 15 out of 19 had abnormal chest radiographs compatible with TB disease with the remaining 4 chest radiographs being reported as normal.

Sixteen of the nineteen persons with culture confirmed TB agreed to HIV testing. Results were negative in all those tested. Four of the 19 culture positive cases (21.1%) reported having had TB before. Of the 19 culture positive cases, 3 (one smear and culture positive and the two smear negative culture positive cases) were diagnosed amongst the first 481 participants on whom a single culture was automatically done (single culture group). Of the remainder (5882) who underwent step-wise screening (step-wise group), 3683 (63%) had at least one criterion for TB investigation (symptoms, recent household contact, positive TST or positive QFT). Of these, 3483 (95%) were investigated with at least one smear amongst whom 16 cases of smear positive TB were diagnosed (all were confirmed by a positive culture). Those not investigated (200) were excluded from the analyses below. Thus, excluding the first 481 participants for whom screening procedures were different, we obtained a smear positive TB prevalence of 16/5682 (3/1000 population, 95% CI 1-4/1000).

Sensitivity, specificity, positive predictive value and negative predictive value

The individual performance of each screening test is set out in Table 1. While sensitivities of the TST and QFT were high for TB disease, 84.6% (95% CI 61.9-100%) and 93.8% (95% CI 78.8-100%) respectively, sensitivities of “any symptoms” and recent household contact were very low, 12.5% (95% CI 0.0-30.2%) and 31.3% (95% CI 0.0-63.9%) respectively. The reverse was true for specificity. Unexplained cough was the most common TB related symptom reported and the only one associated with diagnosed TB cases. Positive predictive values of all tests were all very low (< 2%) and negative predictive values very high (>99%). The lower number with data available for the TST as seen in Table 1 was due to those with prior or current TB not having had a TST done. If one assumed that those who did not have a TST done were TST positive, then sensitivity of the TST increased to 87.5% (95% CI 68.4-100.0%), specificity decreased to 51.9% (95% CI 42.3-61.4%) while positive predictive value and negative predictive value remained unchanged at 0.5% (95% CI 0.4-0.6%) and 99.9 (95% CI 99.8-100.0%) respectively. QFT has slightly less data available than the other screening tests due to QFT kits not being available at certain times or due to misbleeds i.e. not for any reason that might have introduced a bias. The performance of combined screening tools is outlined in Table 2. Sensitivity increased when combining screening tools and was above 85% when either a TST or QFT was part of the combination. The combination of either a positive TST or a positive QFT was 100% sensitive. However, all combinations had low positive predictive values (< 1%).

Discussion

The first objective of the study was to measure the prevalence of TB disease amongst adolescents. We found a prevalence of smear positive TB of 3/1,000 amongst school-going adolescents in a high burden area in South Africa. In the British MRC trial of BCG which started in 1950¹⁹, of 58,900 adolescents aged 14.5 to 15.5 years screened at study entry, 156 (3/1000) were found to have “definite” TB based on chest radiograph and chest clinic physician review. This 1950s British prevalence was similar to that found in our study, although case definitions differed. As we did not find any recent studies of TB prevalence amongst adolescents, the finding in our study is a useful benchmark for future studies of adolescents in high burden settings such as South Africa, especially given that screening took place in a modern TB control programme environment. The World Health Organisation reported a global TB prevalence of 2/1000 in 2011 and in the Africa Region as 3/1000.¹ Since adolescence is a period of low but increasing TB disease, the prevalence figure reported for our study should be regarded as high for this age group.

A Kenyan community based prevalence survey in a population aged ≥ 15 years, found a TB prevalence of 6.0/1000 based on smear or culture positive TB.¹¹ A prevalence study in high TB burden suburbs of Cape Town, South Africa, also in those aged ≥ 15 years, found a TB prevalence of 10/ 1000 based on smear or culture positive TB.²⁰ In our analyses, we used cases who were both smear and culture positive since sputum smear testing was the first step in our TB investigation algorithm and cultures were only done on those with positive smears. Also, there were none that were smear positive but culture negative. The DETECTB study in Zimbabwe found TB prevalences of 6.5/1000 and 3.7/ 1000 in adults before and after an intervention to reduce TB incidence and prevalence.¹² The prevalences summarized above from studies conducted mainly in adults are not inconsistent with our findings since higher rates are expected in adults than in adolescents. However, comparisons have limited value given different TB burdens in different countries.

The prevalent cases detected reflect a substantial proportion of untreated TB disease cases. This should be of concern since they represent a source of TB infection and disease in a congregate

environment. A substantial proportion of cases (>50% of smear positive cases) in the Cape Town study had had TB before.²⁰ This proportion was 21% in our study, more than double those reporting having had TB before (10%). This confirms previous TB as a risk factor for further episodes of TB.

The high proportion of cases with normal chest x-rays (21%) was probably due to disease not being substantial enough to be visible on chest x-ray as a result of early detection. In children, positive cultures can be obtained with recent infection without disease on CXR – this certainly could also be a possibility in adolescents. Five of the 19 adolescents with a positive culture had a household TB contact within three years of enrolment. Only one of these had an x-ray reported as normal. The less likely possibility is that there was laboratory contamination and that these positive cultures were false positive cases. Chest x-rays were done prior to treatment start and most (15 out of 19) were abnormal making “transient infection” or contamination an unlikely explanation in most cases. Also, we had a system of quality control which involved the use of dummy sputa and this process did not show laboratory contamination to be a concern.

Case series describing the profile of adolescent TB have been published.²¹⁻²⁴ These series found primary TB and extra-pulmonary TB among their cases. However, they were mainly from hospital settings or across different age ranges in contrast with our own community based study.

The second objective of this study was to examine the performance of different screening methods for TB disease. The World Health Organisation promotes three approaches to TB screening in population-based surveys :¹⁵ 1) Symptom and chest radiograph screening on all. Those with symptoms or abnormal chest radiographs are referred for a sputum smear and culture, 2) Symptom and chest radiograph screening, and sputa taken for smears on all. Cultures are only done if any of the former are positive, 3) Sputa are taken for smears and cultures on all. These range from the more affordable option, 1, to the more expensive option, 3. There is, however, a loss in sensitivity with the less expensive methods of screening. Options 2 and 3 also

require substantial laboratory capacity. Option 3 would not miss any case of pulmonary TB while options 1 and 2 would miss those cases not detected through their screening approach i.e. option 1 would miss smear or culture positive pulmonary TB cases without symptoms or chest radiograph abnormalities while option 2 would miss smear negative, culture positive cases without symptoms or chest radiograph abnormalities. These approaches were published after the completion of our study.

We did not use chest radiographs in our study as the numbers recruited were too high to be radiographed at routine health care facilities in the area, and a specially purchased mobile radiograph facility was not financially feasible. We did, however, explore the use of measures of latent infection, the TST and QFT, and a history of a recent household contact in addition to symptom screening. While a positive QFT and TST were both sensitive indicators of prevalent TB alone, and 100% sensitive in combination, all screening methods, alone or in combination, had very low positive predictive values, suggesting that they would not be cost effective as case finding tools.

The prevalence surveys in Cape Town and Kenya^{10, 11} found chest radiographs to be sensitive screening tools for detecting TB, 94% and 97% respectively. Positive predictive values were not provided and we calculated them based on the data provided. The positive predictive value for any abnormal radiograph finding was 2% in the Kenyan study and 6% in the Cape Town study. We confirmed their findings that symptom screening has a low sensitivity for detecting TB disease. We speculate that adolescents may be reluctant to disclose TB symptoms, given that 14 of the 16 smear and culture positive cases in our study did not report any symptoms. This aspect should be investigated in more depth in similar future studies of adolescents.

The screening algorithm used in our study of collecting sputa for smears only on those with symptoms, a recent household contact, a positive TST or a positive QFT, reduced the proportion of the sample that needed sputum smears by about 40%. However, the fact that just over 60% of our participants had indications for TB investigation raises questions about the cost-

effectiveness of the screening strategy we used. The prevalence of latent infection was higher than expected, reducing the value of TST and QFT as a screening tool. Given the low positive predictive values of the screening tools used in this study, it is clear that none of these screening methods would be effective in a routine health care setting i.e. many who test positive would have to submit sputum samples to find relatively few confirmed TB cases.

Given the low background prevalence of TB, it may thus be difficult to find a cost-effective method of screening. In clinical trials, however, where there is an imperative both to exclude TB disease at baseline and to detect TB disease during follow up, there would be greater incentive to provide the costly resources needed for TB active case finding. A TST or QFT at least should be included as part of a screening strategy for adequate sensitivity. History of symptoms and/or household contact may be easily added to improve sensitivity. **There is however a need to carefully discuss future disease endpoint definitions if symptomatic screening has poor sensitivity and measures of infection are too non-specific to be of value.**

Effective screening tools should meet certain criteria: ^{25, 26} the disease being screened for should be important and treatable; there should be a recognizable latent or early symptomatic phase; and a suitable acceptable test should be available which is cost-effective. While high sensitivity is an important characteristic of a good screening tool, positive predictive value is relevant to determine operational feasibility and cost effectiveness. TB meets the criteria for a disease amenable to screening ^{25, 26} since it is a common and serious disease and is treatable. On the other hand, the prevalence is relatively low and the effective screening tools available have low positive predictive values and are therefore unlikely to be cost-effective. Besides the costs of diagnostic tests themselves, one should take into account transport of specimens (sputum screening), repeated visits (TST), laboratory infrastructure (sputa, QFT) and personnel required. Cost effectiveness analyses in other contexts have shown various outcomes. A single post travel TST was most cost effective for TB screening for Canadians returning from travel abroad ²⁷ but chest radiographs were more cost effective when screening immigrants from high burden countries. ²⁸ A chest radiograph in Kenya was more cost-effective than a sputum smear in screening for TB when treatment was taken into account. ²⁹ In Cape Town, a strategy of

screening all HIV negative adults with TB symptoms and all HIV positive persons in a high TB and HIV burden area for TB disease was found to be feasible and cost-effective.³⁰ Given these results from other studies, a formal cost-effectiveness analysis of TB screening in adolescents may be necessary before any final conclusions are drawn about the cost effectiveness of screening for TB in adolescents.

Limitations

This study was focused on school going children as a feasible target group for vaccination rather than the whole community. Adolescents who had dropped out of school would probably be at higher risk of TB and this study is likely to have underestimated the true prevalence of TB in the adolescent population as a whole. Refusals were dictated mainly by fear of blood draws, so we do not believe that the included population is substantially different from those who declined to participate. We were dependent on adolescents reporting symptoms and household contacts and these may not have been accurately reported. As cultures were done mainly to confirm smear positive TB, our TB prevalence is an underestimate of the actual prevalence of culture positive TB. Another source of potential under- or overestimation was that 7% of participants who met the criteria for investigation did not undergo sputum testing. Variations in recruitment rate by school might have biased these results as well. Since schools in affluent areas were less well represented, these results probably overestimate the true prevalence of TB. However, we think that this was to a limited extent given the proportions of school-going adolescents attending schools in affluent areas. The impact of those not having TSTs due to having prior or current TB was tested by assuming that these would be TST positive. We found that sensitivity increased by 3% which we felt was relatively small.

Conclusion

This study in a high burden setting found a TB prevalence of 3/ 1000 but the screening methods used all had low positive predictive values. They may thus not be feasible for use in a routine health care setting but may be suitable for use in a TB vaccine clinical trial context given sufficient resourcing.

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Conflicts of interest statement

Hassan Mahomed – holds other grants for research funded by the Aeras Foundation.

Rodney Ehrlich – no conflicts of interest

Tony Hawkrige – has previously worked for and is a medical monitor on trials funded by the Aeras Foundation

Mark Hatherill - holds other grants for research funded by the Aeras Foundation.

Lawrence Geiter – has previously worked for the Aeras Foundation.

Fazlin Kafaar – no conflicts of interest.

Deborah Ann Abrahams – no conflicts of interest.

Humphrey Mulenga - no conflicts of interest.

Michele Tameris - holds other grants for research funded by the Aeras Foundation.

Hennie Geldenhuys - no conflicts of interest.

Willem Albert Hanekom- holds other grants for research funded by the Aeras Foundation.

Suzanne Verver – no conflicts of interest.

Gregory Dudley Hussey – previously held other grants for research funded by the Aeras Foundation

References

1. World Health Organisation. *Global Tuberculosis Report 2012*. World Health Organisation 2012. Available directly at http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf or by visiting http://www.who.int/tb/publications/global_report/en/. Accessed 24 May 2013..
2. Raviglione MC. The Global Plan to Stop TB, 2006-2015. *Int J Tuberc Lung Dis* 2006; **10**(3): 238-9.
3. Stop TB Partnership. *The global plan to stop TB 2011-2015: transforming the fight towards elimination of tuberculosis*: World Health Organisation; 2010. Available at: http://www.stoptb.org/assets/documents/global/plan/TB_GlobalPlanToStopTB2011-2015.pdf. Accessed 24 May 2013.
4. Donald PR. Childhood tuberculosis: the hidden epidemic. *Int J Tuberc Lung Dis* 2004; **8**(5): 627-9.
5. Wood R, Lawn SD, Caldwell J, Kaplan R, Middelkoop K, Bekker LG. Burden of new and recurrent tuberculosis in a major South African city stratified by age and HIV-status. *PLoS One* 2011; **6**(10): e25098.
6. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* 1970; **26**: 28-106.
7. Verver S, Warren RM, Munch Z, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet* 2004; **363**(9404): 212-4.

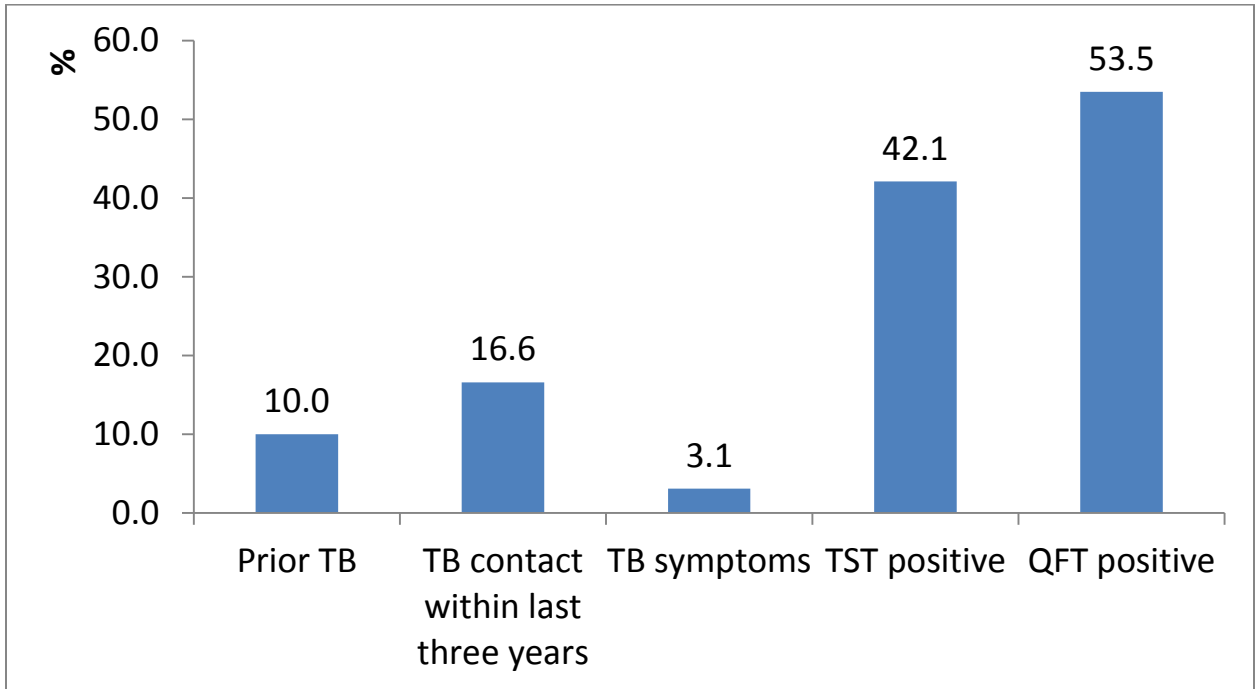
8. Middelkoop K, Bekker LG, Liang H, et al. Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study. *BMC Infect Dis*; **11**: 156.
9. Brennan MJ, Thole J. Tuberculosis vaccines: a strategic blueprint for the next decade. *Tuberculosis* 2012; **92 Suppl 1**: S6-13.
10. den Boon S, White NW, van Lill SW, et al. An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *Int J Tuberc Lung Dis* 2006; **10**(8): 876-82.
11. Van't Hoog AH, Meme HK, Laserson KF, et al. Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. *PloS One* 2012; **7**(7): e38691.
12. Corbett EL, Bandason T, Duong T, et al. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster-randomised trial. *Lancet* 2010; **376**(9748): 1244-53.
13. Verver S, van Soolingen D, Borgdorff MW. Effect of screening of immigrants on tuberculosis transmission. *Int J Tuberc Lung Dis* 2002; **6**(2): 121-9.
14. Joshi R, Patil S, Kalantri S, Schwartzman K, Menzies D, Pai M. Prevalence of abnormal radiological findings in health care workers with latent tuberculosis infection and correlations with T cell immune response. *PloS One* 2007; **2**(8): e805.
15. World Health Organisation. *Tuberculosis prevalence surveys: A handbook*: World Health Organisation; 2011. Available at

http://whqlibdoc.who.int/publications/2011/9789241548168_eng.pdf. Accessed on 24 May 2013..

16. Mahomed H, Hawkridge T, Verver S, et al. Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. *Int J Tuberc Lung Dis* 2011; **15**(3): 331-6.
17. Mahomed H, Hawkridge T, Verver S, et al. The tuberculin skin test versus QuantiFERON TB Gold(R) in predicting tuberculosis disease in an adolescent cohort study in South Africa. *PLoS One* 2011; **6**(3): e17984.
18. Machingaidze S, Verver S, Mulenga H, et al. Predictive Value of Recent QuantiFERON Conversion for Tuberculosis Disease in Adolescents. *Am J Respir Crit Care Med* 2012; **186**(10): 1051-6.
19. B.C.G. AND vole bacillus vaccines in the prevention of tuberculosis in adolescents; first (progress) report to the Medical Research Council by their Tuberculosis Vaccines Clinical Trials Committee. *BMJ* 1956; **1**(4964): 413-27.
20. den Boon S, van Lill SW, Borgdorff MW, et al. High prevalence of tuberculosis in previously treated patients, Cape Town, South Africa. *Emerging Infect Dis* 2007; **13**(8): 1189-94.
21. de Pontual L, Balu L, Ovetchkine P, et al. Tuberculosis in adolescents: A French retrospective study of 52 cases. *Pediatr Infect Dis J* 2006; **25**(10): 930-2.
22. Nemir RL. Perspectives in adolescent tuberculosis: three decades of experience. *Pediatrics* 1986; **78**(3): 399-405.

23. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR. The clinical and radiological features of tuberculosis in adolescents. *Ann Trop Paediatrics* 2000; **20**(1): 5-10.
24. Kam A, Ford-Jones L, Malloy P, Khan K, Kitai I. Active tuberculosis among adolescents in Toronto, Canada: clinical features and delays in diagnosis. *Pediatr Infect Dis J* 2007; **26**(4): 355-6.
25. Wilson JM, Jungner YG. [Principles and practice of mass screening for disease]. *Boletin de la Oficina Sanitaria Panamericana Pan American Sanitary Bureau* 1968; **65**(4): 281-393.
26. Hennekens C, Buring JE. Chapter 13 Screening in Epidemiology in Medicine. First ed. Mayrent SL, editor. Little, Brown and Company, New York, USA; 1987.
27. Tan M, Menzies D, Schwartzman K. Tuberculosis screening of travelers to higher-incidence countries: a cost-effectiveness analysis. *BMC public health* 2008; **8**: 201.
28. Schwartzman K, Menzies D. Tuberculosis screening of immigrants to low-prevalence countries. A cost-effectiveness analysis. *Am J Respir Crit Care Med* 2000; **161**(3 Pt 1): 780-9.
29. van Cleeff MR, Kivihya-Ndugga LE, Meme H, Odhiambo JA, Klatser PR. The role and performance of chest X-ray for the diagnosis of tuberculosis: a cost-effectiveness analysis in Nairobi, Kenya. *BMC Infect Dis* 2005; **5**: 111.
30. Kranzer K, Lawn SD, Meyer-Rath G, et al. Feasibility, yield, and cost of active tuberculosis case finding linked to a mobile HIV service in Cape Town, South Africa: a cross-sectional study. *PLoS Med* 2012; **9**(8): e1001281.

Figure 1



TB profile of the adolescent population (%) (n=6363).

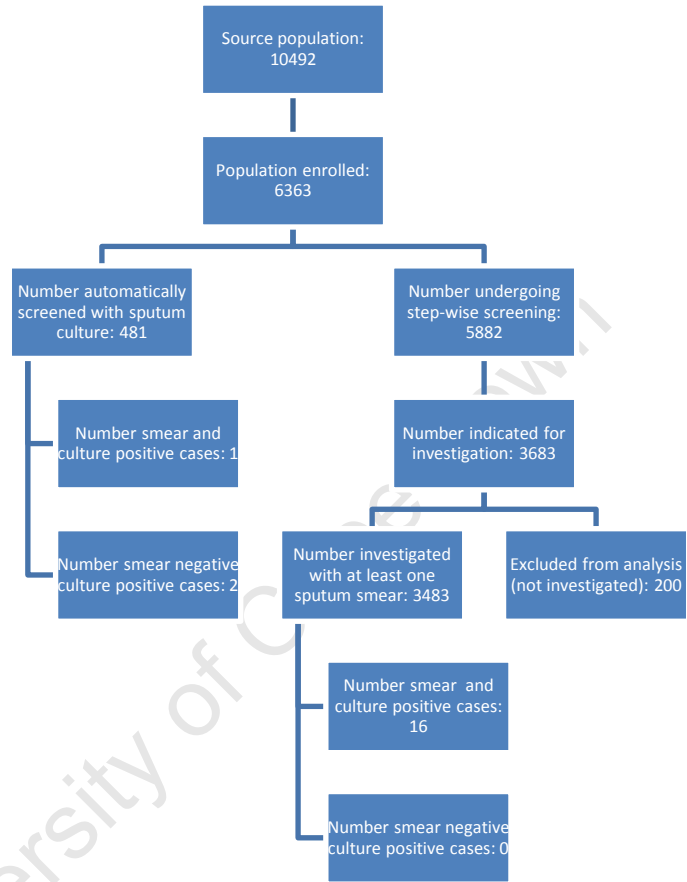
TB – tuberculosis.

TST – tuberculin skin test.

TST positive – induration of 10 mm or more.

QFT – QuantiFERON TB Gold (in-tube) assay

Figure 2



Flow chart of enrolment and TB case identification.

Table 1: Sensitivity, specificity, positive predictive value and negative predictive value of screening tests for smear and culture positive TB disease^a

Screening test	Number with data available (N)	Feature present/ absent	TB cases detected	TB cases not detected	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Unexplained cough ≥ 2 weeks	5682	Present	2	105	12.5	98.1	1.9	99.7
		Absent	14	5561	(0.0-30.2)	(97.4-98.9)	(0.0-4.6)	(99.6-99.8)
Unexplained loss of weight ≥ 2 weeks	5682	Present	0	56	0	99.0	0	99.7
		Absent	16	5610		(98.7-99.3)		(99.6-99.8)
Unexplained fever ≥ 2 weeks	5682	Present	0	10	0	99.8	0	99.7
		Absent	16	5656		(99.6-100.0)		(99.6-99.8)
Haemoptysis	5682	Present	0	17	0	99.7	0	99.7
		Absent	16	5649		(99.4-100.0)		(99.6-99.8)
Unexplained night sweats ≥ 2 weeks	5682	Present	0	39	0	99.3	0	99.7
		Absent	16	5627		(98.9-99.7)		(99.6-99.8)
Any of above symptoms	5682	Present	2	172	12.5	97.0	1.1	99.7
		Absent	14	5494	(0.0-30.2)	(95.6-98.3)	(0.0-2.8)	(99.6-99.8)
Household contact within 3 years	5682	Present	5	963	31.3	83.0	0.5	99.8
		Absent	11	4703	(0.0-63.9)	(78.8-87.3)	(0.01-1.1)	(99.6-99.9)
TST positive (≥ 10 mm cut-off)	5071	Present	11	2119	84.6	58.1	0.5	99.9
		Absent	2	2939	(61.9-100.0)	(49.5-66.7)	(0.3-0.8)	(99.8-100.0)

QFT positive	5524	Present	15	2931	93.8	46.8	0.5	100.0
		Absent	1	2577	(78.8 -100.0)	(36.3-57.2)	(0.3-0.7)	(99.9-100)

CI – confidence interval, PPV – positive predictive value, NPV – negative predictive value.

^a95% confidence intervals corrected for the cluster design effect.

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Table 2: Sensitivity, specificity, positive predictive value and negative predictive value of combinations of screening tests for smear and culture positive TB disease^a

Screening test	Number with data available (N)	Feature present/ absent	TB cases detected	TB cases not detected	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Any TB symptom or recent household TB contact only								
Any TB symptom or household contact within 3 years	5682	Present	6	1077	37.5	81.0	0.6	99.8
		Absent	10	4589	(5.8-69.2)	(76.3-85.7)	(0.1-1.0)	(99.6-99.9)
Any TB symptom + either TST or QFT								
Any TB symptom or a positive TST ≥ 10 mm	5110	Present	12	2227	85.7	56.3	0.5	99.9
		Absent	2	2869	(64.7-100.0)	(47.6-65.0)	(0.3-0.7)	(99.8-100.0)
Any TB symptom or a positive QFT	5526	Present	16	3006	100.0	45.4	0.5	100.0
		Absent	0	2504		(35.4-55.5)	(0.4-0.7)	
Recent household TB contact + either TST or QFT								
Household contact within 3 years or a positive TST ≥ 10 mm	5199	Present	12	2575	85.7	50.3	0.5	99.9
		Absent	2	2610	(63.9-100.0)	(40.5-60.1)	(0.2-0.7)	(99.8-100.0)
Household contact within 3 years or a positive QFT	5548	Present	15	3225	93.8	41.7	0.5	100.0
		Absent	1	2307	(78.8-100.0)	(31.1-52.3)	(0.3-0.6)	(99.9-100.0)
Any TB symptom or recent household TB contact + either TST or QFT								

Any TB symptom, a household contact within 3 years or a positive TST \geq 10mm	5225	Present	13	2647	86.7	49.2	0.5	99.9
		Absent	2	2563	(66.4-100.0)	(39.3-59.1)	(0.3-0.7)	(99.8-100.0)
Any TB symptom, a household contact within 3 years or a positive QFT	5548	Present	16	3277	100.0	40.8	0.5	100.0
		Absent	0	2255		(30.3-51.2)	(0.4-0.6)	

CI – confidence interval, PPV – positive predictive value, NPV – negative predictive value.

^a95% confidence intervals corrected for the cluster design effect.

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Chapter 5: TB incidence in an adolescent cohort in South Africa

Paper overview

This analysis reports on the incidence of TB disease in the adolescent cohort that was studied and the risk factors at baseline that were predictive of TB disease onset within 2- 4 years. A multivariate analysis using a Cox regression was used to determine these factors. The profile of cases and incidence rates using different case definitions are also reported.

Contribution to the thesis and novelty

This paper addresses the third objective of the thesis. Little data on the population incidence of TB disease are available for adolescents. No previous data on the factors predicting TB disease in adolescents were found. These data will be of use to those planning clinical trials of TB in adolescents especially with respect to estimating sample sizes and designing case definitions.

Contributions of candidate

I was the first author on the publication and was primarily responsible for overseeing the conduct of the study on which the publication is based. I was also primarily responsible for the data analysis and write-up of the study and this is clear from the roles of co-authors as described below.

H Mahomed: Involved in study conceptualisation, oversaw the conduct of the study, analysed the data and wrote the manuscript.

R Ehrlich: Reviewed the analysis and manuscript.

T Hawkrige: Involved in study conceptualisation and reviewed the manuscript.

M Hatherill: Reviewed the manuscript.

L Geiter: Involved in study conceptualisation and reviewed the manuscript.

F Kafaar: Was the study co-ordinator responsible for day to day management of the study and reviewed the manuscript.

D Abrahams: Responsible for processing of QuantiFERON specimens and reviewed the manuscript.

H Mulenga: Managed the study database and reviewed the manuscript.

M Tameris: Approved microbiology results and reviewed the manuscript.

H Geldenhuys: Approved microbiology results and reviewed the manuscript.

WA Hanekom: Involved in study conceptualisation and reviewed the manuscript.

S Verver: Involved in study conceptualisation, reviewed the analysis and reviewed the manuscript.

GD Hussey: Involved in study conceptualisation and reviewed the manuscript.

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TB incidence in an adolescent cohort in South Africa

Mahomed H, Ehrlich R, Hawkrige T, Hatherill M, Geiter L, Kafaar F, Abrahams DA, Mulenga H, Tameris M, Geldenhuys H, Hanekom WA, Verver S, Hussey GD.

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A few further amendments have been made to this paper subsequent to publication.

TB incidence in an adolescent cohort in South Africa

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At the time of this study, Lawrence Geiter was employed by the Aeras Foundation.

Short title: TB incidence in adolescents

Key words: tuberculosis, adolescence, incidence, risk factors

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Abstract

Background

Tuberculosis (TB) is a major public health problem globally. Little is known about TB incidence in adolescents who are a proposed target group for new TB vaccines. We conducted a study to determine the TB incidence rates and risk factors for TB disease in a cohort of school-going adolescents in a high TB burden area in South Africa.

Methods:

We recruited adolescents aged 12 to 18 years from high schools in Worcester, South Africa. Demographic and clinical information was collected, a tuberculin skin test (TST) performed and blood drawn for a QuantiFERON TB Gold assay at baseline. Screening for TB cases occurred at follow up visits and by surveillance of registers at public sector TB clinics over a period of up to 3.8 years after enrolment.

Results

A total of 6,363 adolescents were enrolled (58% of the school population targeted). During follow up, 67 cases of bacteriologically confirmed TB were detected giving an overall incidence rate of 0.45 per 100 person years (95% confidence interval 0.29-0.72). Black or mixed race, maternal education of primary school or less or unknown, a positive baseline QuantiFERON assay and a positive baseline TST were significant predictors of TB disease on adjusted analysis.

Conclusion

The adolescent TB incidence found in a high burden setting will help TB vaccine developers plan clinical trials in this population. Latent TB infection and low socio-economic status were predictors of TB disease.

Word count: 232

Introduction

Tuberculosis (TB) is a major public health problem globally with 8.8 million cases being diagnosed in 2010 of whom 1.1 million died who were HIV negative and 0.35 million who were HIV positive [1]. Incidence rates start to rise in adolescents and precede a peak in adulthood in developing countries [2]. New TB vaccines are being developed as part of the strategy to combat TB and adolescents are a proposed target for such vaccines [3]. However, relatively little has been published on the incidence of TB in adolescents. Two clinical trials of BCG in adolescents provide limited incidence data [4,5] and studies on TB in adolescents have focused on the clinical features of TB in this group [6-9]. A latent TB infection prevalence of about 50% has been reported for the population described in this report and for another adolescent group from South Africa [10,11].

Since there is currently no immune correlate of protection against TB, end points based on evidence of TB disease will be needed for new TB vaccine efficacy trials and these would need to be specific to optimize efficacy assessments [12,13]. Knowledge of TB incidence rates based on specific clinical disease features will be crucial for planning the size of efficacy trials.

We conducted a study to determine the TB incidence rate and risk factors for TB disease in a cohort of school-going adolescents in a high TB burden area in South Africa. Other publications on this study population include: 1) An examination of risk factors for latent TB infection based on the tuberculin skin test (TST) and QuantiFERON TB Gold (in-tube) (QFT) assay results at enrolment [11] 2) A comparison of the predictive value of baseline TST and QFT for TB disease during follow-up [14] 3) The determination of the predictive value of a QFT conversion for TB

disease in a subset of participants who underwent extended follow up [15] and 4) The measurement of the prevalence of TB disease at enrolment and the value of certain screening tests for detecting TB (manuscript under review).

Materials and Methods:

Study setting: The study took place in the town of Worcester and surrounding villages, approximately 100 kilometres from Cape Town, South Africa. The total population of the municipal area from which the adolescents were drawn was estimated as 146,101 by the Department of Health in 2005, the year when recruitment started. According to the national Census of 2001, there were 21,056 adolescents aged 12-18 (14% of the total population) in the municipal area targeted and 83% of these adolescents were attending schools. The study area was a subset of the municipal area and consisted of a major town and two villages within this area. Based on the Census 2001 population data for the municipal area and high school attendance data of 10,492 in the study area, we estimated that there were 12,641 adolescents aged 12-18 years in the study area in total.

Study participants: All adolescents aged 12-18 years attending all 11 publicly funded high schools in the study area were approached to participate. The very few small private schools in the study area were not approached.

Study procedures: At enrolment, demographic, socio-economic and clinical information were collected through interview of parents and the participating adolescent [11]. Blood was taken for QuantiFERON[®] TB Gold In-tube (Cellestis, Victoria, Australia) (QFT) and a TST was administered at baseline. Those with previous or current TB or with a previous severe reaction to TST did not have a TST performed, in order to prevent severe allergic reactions. The reading of the TST took place between 48 and 96 hours after administration, slightly longer than the

more commonly used limit of 72 hours but there are data and recommendations which suggest that this is acceptable [16,17]. A trained nurse examined each adolescent for a BCG scar. Participants were screened for TB at baseline (the overall number of cases diagnosed will be reported here but details of these cases are the subject of another publication (4 above)).

All participants enrolled were scheduled for follow up visits after two years which included a blood draw for QFT and the administration of a TST. About half underwent three monthly visits prior to this which included six monthly QFTs and annual TSTs to compare follow up strategies while the other half were seen only at baseline and two year visit (details of the comparison of the two follow up strategies will be reported separately). At follow up visits, those with new symptoms or a new household contact compared to baseline, a converted TST (≥ 10 mm increase from baseline) or converted QFT (change from negative to positive) were investigated for TB disease. In addition, passive surveillance was conducted of TB clinic and hospital admission registers in the area for any TB cases diagnosed between visits. Investigation for TB involved the collection of two sputum samples for smear examination on two separate occasions. For persons with at least one positive smear, a culture was performed, a chest x-ray done and an HIV test offered. The radiologist's report on the chest x-ray was used to classify chest x-ray findings.

Study duration: Enrolment started in July 2005 and was completed in April 2007. Follow up was completed at the end of February 2009. Owing to financial constraints, about 10% of the two year visits were performed one to two months short of two years towards the end of the study. Follow up was thus continued for a minimum of 22 months. Those completing their two year

visits were followed up passively until all other subjects had completed their two year visits. This gave a maximum follow up time of 3.8 years.

Definitions:

The protocol definition of a TB case was a diagnosis of intrathoracic TB with either two positive sputum smears and/ or one single positive sputum culture (“bacteriologically confirmed TB”). However, data on all individuals placed on TB treatment by a physician were recorded (“all TB”). A chest x-ray consistent with active TB was defined as “compatible with TB” - this included pleural effusions. An “abnormal chest x-ray” was defined as any abnormality judged to be evidence of active disease including TB and evidence of old/ previous disease.

Sample size determination

Those agreeing to participate determined the sample size. Based on routine TB programme data, we expected to find an incidence rate of bacteriologically confirmed TB of 0.5 per 100 person years. With an anticipated sample size of 6,500 and an expected incidence rate of bacteriologically confirmed TB of 0.5 per 100 person years over two years of follow up, we expected to yield a 95% confidence interval (precision) of approximately 0.4 to 0.6 /100 person years.

Data analysis and statistical considerations:

Data were captured in a Microsoft Access database, and analysed with STATA version 11.0 (Statacorp, Texas, USA). Data were verified and validated prior, during and after data entry according to a data entry standard operating procedure.

Total person-time for TB incidence analysis was calculated from date of enrolment to date of the last visit of the last participant, TB diagnosis or death, whichever occurred first. Those lost to follow-up were assigned the duration of time to when last seen plus half the duration between that visit and the next missed visit. Univariate analysis was performed on demographic, socio-economic and clinical characteristics examining their association with “bacteriologically confirmed TB”. Hazard rates with 95% confidence intervals were calculated using Poisson regression. The design effect was accounted for during statistical analysis, the clusters being the 11 schools from which the participants were enrolled. Kappa statistics were used to evaluate collinearity amongst the potential risk factor variables to avoid over-matching in building models for multivariate analysis. The risk factors for TB disease were analysed in a multivariate Cox regression model using the statistically significant variables ($p < 0.05$) on univariate analysis to determine adjusted hazard ratios.

Ethics: This study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town. Written informed consent was obtained from the parents of adolescents and assent obtained from adolescents.

Results

6,363 adolescents were enrolled (58% of 10,492 registered at local schools and 50% of the estimated total adolescent population of 12,641 in the study area). Enrolment by school ranged from 22.2% to 74.4%. 5203 (81.8%) participants completed their two year visits, 660 (10.4%) withdrew, 11 (0.2%) died and 489 (7.7%) were lost to follow up prior to their two year visit. 84% (1,939/ 2,310) of participants who met the criteria for investigation underwent the required tests.

Participant baseline profile

The demographic and clinical profile of participants at enrolment is shown in Table 1. There was a slight predominance of females (54%) in the study population compared to the source population data supplied by the Department of Education (50% were female). There was a greater proportion of younger adolescents (≤ 15 years) than those >15 years since there were fewer adolescents in the higher school grades. BCG scars were faded in some adolescents so it was difficult to be sure about their presence in certain instances. Twenty one cases of TB were diagnosed at baseline of whom 19 were culture positive.

Profile of incident cases

There were 87 TB incident cases detected during follow up, 67 (77.0%) of whom met the protocol definition (bacteriologically confirmed TB). A profile of the cases is set out in Table 2.

Please note that 7 non-

tuberculous mycobacteria (NTMs) were cultured at baseline and 2 during follow up. Most incident cases (61 or 70%) were culture positive and most (63 or 72%) had chest x-ray changes suggestive of TB. All those diagnosed with TB (87) were offered HIV testing, 61 (70.1%) of whom accepted and of whom only one (1.6%) was found to be positive.

Incidence rates

Incidence rates using varying definitions of what constitutes a TB case are set out in Table 3. This resulted in a range of incidence rates from 0.30 to 0.59/ 100 person years. The highest annual number of cases occurred during the second year of follow up i.e. 33 (49%) of bacteriologically confirmed cases (Table 4). Ten additional cases (15%) were detected through surveillance after the two year visit. The rates of disease in the successive years of follow up were not statistically significantly different from each other. Incidence rates varied widely amongst the different

schools from 0 to 2% cumulative incidence and 0 to 0.9/100 person years incidence rates suggesting the possibility of micro-epidemics in certain schools but confidence intervals were wide making these data difficult to interpret. Eight bacteriologically confirmed cases were diagnosed within 6 months of enrolment. If we consider the possibility that these may have been cases missed at baseline and were in fact prevalent cases, then the incidence rate based on the bacteriologically confirmed cases drops to 0.40 per 100 person years (pyrs) (95% CI 0.27-0.58). Incidence rates by TST induration size were as follows: <5 mm - 0.20/100 pyrs (95% CI 0.11-0.38), 5-9 mm - 0.17/100 pyrs (95%CI 0.04-0.71), 10-14 mm - 0.40/100 pyrs (95% CI 0.30-0.53) and ≥ 15 mm - 1.18/100 pyrs (95% CI 0.77-1.81). There was thus a trend of increasing incidence with increasing induration with an induration of ≥ 15 mm being significantly higher than the other categories.

Risk factors for TB

An unadjusted univariate analysis of the relationship between demographic, socio-economic and clinical variables, and TB incidence using hazard ratios is set out in Table 1. A comparison between those having three monthly follow up versus those with just a baseline and two year visit showed no statistically significant difference in incidence rates and is not shown in this analysis. In unadjusted analysis, statistically significant risk factors for bacteriologically confirmed TB were being of black or mixed race origin compared to being of white or Indian origin, maternal education primary school or less and maternal education unknown, current or prior household contact, a positive TST and a positive QFT. Absence of a BCG scar was of borderline statistical significance. These risk factors were considered for fitting in a Cox regression model. We could not include ethnic group since there were no cases in the reference group. Maternal education primary school or less and maternal education unknown were combined into a single variable. BCG coverage in South Africa is high at more than 95%. We

therefore assumed that those with an “unknown/ unsure” scar were BCG vaccinated and these were grouped with those with a definite scar present for the Cox regression analysis. The following comparisons were tested for collinearity using the kappa statistic: TST versus QFT, prior or current household TB contact versus TST and prior or current household TB contact versus QFT since all three were potential indicators of TB exposure. Only the TST and QFT outcomes were found to be collinear (percentage agreement 85.0%, kappa 0.7). They could thus not be fitted into a model simultaneously. In adjusted analysis (Table 5), absence of a BCG scar, maternal education primary school or less, or unknown and a positive QFT were statistically significant predictors of TB disease while prior household contact was not. When QFT was replaced with TST in the model (not shown), the TST was also a significant predictor with a hazard ratio of 2.4 (95% CI 1.2-4.8). In this model, maternal education primary school or less or unknown remained a predictor of TB disease (hazard ratio 1.9, 95% CI 1.1-3.1) but not absence of a BCG scar. When we defined the presence of a BCG scar strictly and excluded those with scar “unknown/ not sure” and repeated the Cox regression (N = 4061), BCG scar and QFT were both only of borderline significance, hazard ratios 1.7 (95% CI 0.9 - 3.2, p=0.06) and 1.7 (95% CI 1.0-2.9, p=0.07) respectively while maternal education of primary school or less or unknown remained significant at hazard ratio 1.4 (95% CI 1.0-1.9, p=0.04). With this reduced dataset, when TST replaced QFT in the model, only TST remained significant 2.4 (95%CI 1.3-4.3, p=0.007).

Discussion

We found a bacteriologically confirmed TB incidence rate of 0.45/100 person years of TB in school-going adolescents in a high burden area. Taking this incidence of 0.45/100 person years and the prevalence of 3/1000 or 0.3/100 reported in chapter 4 and using the formula of incidence = prevalence X duration, this amounts to an average duration of prevalent TB of 1.5 years. The clinical profile of TB cases was mainly of the adult type: most were confirmed

bacteriologically and most had evidence of chest x-ray abnormalities. Significant predictors of TB disease during follow up included demographic, socio-economic and latent TB infection variables. While it was notable that most incident cases occurred in the second year of follow up, differences across the years were not statistically significant.

The British MRC trial in adolescents aged 14.5-15.5 years conducted from 1950-1970 showed an annual incidence rate of 0.25 per 100 in the first five years of the study in the BCG unvaccinated group who were TST negative at enrolment with a similar rate in those with a TST of >15mm at enrolment [5]. This rate was similar to the 0.2 per 100 found in the TST negative group in our study but not to those with an induration of ≥ 15 mm who had a significantly higher incidence rate than those with a negative TST. One important difference was that the adolescents in our study had mostly been vaccinated with BCG at birth. Those who were BCG vaccinated in the MRC trial had a lower rate of 0.04 per 100 but had been vaccinated in adolescence as part of the trial so cannot be compared to the adolescents in our study. Children who were aged 7-14 years of age in the Brazilian BCG revaccination trial had rates of 0.03 per 100 person years [4], much lower than in our study. This is probably due to the difference in burden of TB in each country given that South Africa (981/100,000) has a much higher overall incidence rate of TB than Brazil (85/100,000) (2010) [1]. The TB incidence rate found in this study is similar to that based on routine TB control programme data in our study area. It was also similar but not identical to the rate reported in a prior publication on this cohort (0.43/100 person years) due to that analysis focusing on a subset of the cohort (5244) where participants with both baseline TST and QFT results were required for analysis [14].

The profile of cases in our study was typical of adult type disease, a finding similar to that shown in a different adolescent population in South Africa by Weber et al [6]. They reviewed the TB diagnosis records of 324 adolescents aged 10-18 years and found 78% to have had bacteriological evidence of disease and 94%, intra-thoracic lesions on chest x-ray. They found 10% with evidence of primary TB whereas we found none, but their lower age limit was younger (10 years) than in our study (12 years). Very few extra-thoracic cases of TB were detected in our study similar to the 6% of cases reported as extra-thoracic by Weber et al [6]. At least 87% of cases in our study had abnormal chest x-rays confirming the microbiological findings of evidence of TB disease.

The choice of TB case definition resulted in some variation in the TB incidence calculation. Given the need for a specific definition in a clinical trial setting to avoid undermining efficacy estimates [12,13], careful consideration needs to be given to the endpoint case definition for clinical trials. Unlike the pediatric setting where the disease is pauci-bacillary and where reaching consensus on endpoints for infant trials has been difficult [18], most cases in adolescents were microbiologically confirmed suggesting that the use of a highly specific definition is feasible.

We have shown previously that latent TB infection as demonstrated by a positive TST or QFT is associated with a higher risk of progressing to TB disease [14]. The analysis in this paper shows specifically that this relationship persists even when confounders are controlled for. The treatment of latent TB infection with isoniazid as prophylaxis (IPT) is not standard of care in South Africa for HIV negative persons over the age of 5. Fifty percent of adolescents as shown in our study and a higher proportion of adults are latently infected [19]. Implementation of IPT in

South Africa would thus be very resource intensive. The benefits of IPT in high TB burden settings for HIV negative adolescents and adults are not clear. A recent large study of IPT in miners in South Africa showed benefit in reducing the risk of TB only while isoniazid was being taken but this benefit was lost when treatment was completed [20]. The university ethics committee which approved this study did not require the provision of INH prophylaxis in adolescents who were latently infected.

The fact that the absence of a BCG scar was shown to be linked to a higher risk of developing TB is surprising given the high population burden of TB in a country with a high coverage of BCG (>95%) [21]. The benefit was only found when QFT was part of the multivariate model but not when it was replaced with TST nor when the presence of the BCG scar was strictly classified and those whose scars were classified as “unsure” were excluded from analysis. There should therefore be caution in interpreting this finding and the absence of a BCG scar may not be indicative of an absent immune response to BCG or indicative of not receiving BCG. A study in India showed no difference in leukocyte migration levels in young BCG vaccinated children between those who developed a BCG scar and those who did not [22]. A recent editorial referencing this and other studies also commented on the lack of correlation between adaptive cellular immune responses and BCG scar formation, and the authors recommend further studies to clarify the differences between those with and those without a BCG scar [23].

The fact that a low or unknown level of maternal education is linked to progression to TB disease is interesting and requires further investigation but surprisingly, parental low income was not, given the known relationship between poverty and TB. Also unexpected was the fact

that household TB contact was not shown to be a statistically significant risk factor for TB on adjusted analysis. This may be because the study was not sufficiently powered to show this relationship or due to household contact being very common in this high burden setting. Black or mixed race was a perfect predictor of the onset of TB. It is likely that this association is linked to underlying socio-economic factors given South Africa's history [24] but this could not be properly tested in multivariate analysis.

The TB incidence rates measured in this study show that large sample sizes will be needed to power clinical trials evaluating the efficacy of new TB vaccines in adolescents. For a vaccine trial expected to show a vaccine efficacy of 60% at a 95% significance level and with 80% power, a study duration of 2 years with completion rate of 82% and cumulative incidence of bacteriologically confirmed TB of 1.05% in the unvaccinated group (as found in this study [Table 3]), a sample size of 6400 participants would be needed in a study with a 1:1 vaccine to placebo ratio. Since pre and post infection TB vaccines are being developed, sample sizes for trials would have to be calculated separately for those latently uninfected and those latently infected. This is further addressed in more detail in chapter 8.

This study has a number of limitations: HIV testing was not done routinely because it was believed that this population would not have a high prevalence of HIV. This decision was vindicated by the finding that among participants diagnosed with TB, only one was confirmed to be HIV positive with most agreeing to be tested. Besides the data on gender distribution, no other data was available on those not enrolled in the study. A proportion was older than 18 but there was no exact data on this. In addition, many refused to participate due to fear of blood

draws. Our results may thus be biased because of differences between the enrolled and unenrolled populations but we do not believe that this was major given the main reason given for refusal to participate and given that others were not eligible due to their age.

The selections of schools as the source of participants introduced a cluster effect and this produced incidence rates with wider confidence intervals than would have been produced by a purely random sample. While the majority of participants who met the criteria for investigation for TB underwent the required tests, some (371) did not which may have resulted in the actual number of cases being underestimated. However, we believe that the surveillance system would in most instances have eventually picked up cases missed in this way. Hypothetically, given a cumulative incidence of 1.05%, 4 TB cases could have been missed in this group. Smear negative, culture positive TB cases diagnosed early in year one might possibly be cases missed at baseline given that smears were the main method of investigation. In a clinical trial setting using more rigorous screening methods, such cases would have been picked up at baseline and the rates in this study may therefore be an overestimate of incidence relative to what might be expected in a clinical trial. An endpoint committee was not used for case classifications. The case classifications were purely data driven with chest x-ray findings being based only on the radiologists reports. Given that most data items were clear-cut, we do not believe that an endpoint committee would have classified cases significantly differently.

Finally, this study represents the burden of disease at schools only. Adolescents not at school are likely to be located in poorer environments and the TB incidence rate in the total population of adolescents is likely to be higher.

In conclusion, the incidence rates in school-going adolescents in a high burden TB setting ranged from 0.30 to 0.59 per 100 person years using varying case definitions. These data will be essential for the planning of clinical trials of new TB vaccines in adolescents. The risk factors shown to be associated with TB will be useful in estimating TB rates in various risk groups in these clinical trials.

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References

1. World Health Organisation. (2011) Global Tuberculosis Control 2011. World Health Organisation.
2. Wood R, Lawn SD, Caldwell J, Kaplan R, Middelkoop K, et al (2011). Burden of new and recurrent tuberculosis in a major South African city stratified by age and HIV-status. PLoS One 6: e25098.
3. Brennan MJ, Thole J (2012) Tuberculosis vaccines: a strategic blueprint for the next decade. Tuberculosis (Edinb) 92 Suppl 1: S6-13.
4. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, et al. (2005) Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 366: 1290-1295.
5. Hart PD, Sutherland I (1977) BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life. Br Med J 2: 293-295.
6. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR (2000) The Clinical and radiological features of tuberculosis in adolescents. Ann Trop Paediatr 20: 5-10.
7. de Pontual L, Balu L, Ovetchkine P, Maury-Tisseron B, Lachassinne E, et al. (2006) Tuberculosis in adolescents: A French retrospective study of 52 cases. Pediatr Infect Dis J 25: 930-932.
8. Kam A, Ford-Jones L, Malloy P, Khan K, Kitai I (2007) Active tuberculosis among adolescents in Toronto, Canada: clinical features and delays in diagnosis. Pediatr Infect Dis J 26: 355-356.

9. Nemir RL (1986) Perspectives in adolescent tuberculosis: three decades of experience. *Pediatrics* 78: 399-405.
10. Middelkoop K, Bekker LG, Liang H, Aquino LD, Sebastian E, et al (2011). Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study. *BMC Infect Dis* 11: 156.
11. Mahomed H, Hawkridge T, Verver S, Geiter L, Hatherill M, et al. (2011) Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. *Int J Tuberc Lung Dis* 15: 331-336.
12. Mahomed H, Fourie PB Clinical trials of TB vaccines: harmonization and cooperation. *Tuberculosis (Edinb)* 92 Suppl 1: S21-24.
13. Rustomjee R, McLeod R, Hanekom W, Steel G, Mahomed H, et al (2012). Key issues in the clinical development and implementation of TB vaccines in South Africa. *Tuberculosis (Edinb)* 92: 359-64.
14. Mahomed H, Hawkridge T, Verver S, Abrahams D, Geiter L, et al (2011). The tuberculin skin test versus QuantiFERON TB Gold(R) in predicting tuberculosis disease in an adolescent cohort study in South Africa. *PLoS One* 6: e17984.
15. Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, et al. (2012) Predictive Value of Recent QuantiFERON Conversion for Tuberculosis Disease in Adolescents. *Am J Respir Crit Care Med* 186: 1051-1056.
16. Arnadottir T, Rieder HL, Trebucq A, Waaler HT (1996) Guidelines for conducting tuberculin skin test surveys in high prevalence countries. *Tuber Lung Dis* 77 Suppl 1: 1-19.

17. Cobelens F, Van Deutekom H, Draayer-Jansen I, Schepp-Beelen A, Van Gerven P, et al. (2003) Tuberculin skin test reactions by time of reading among Dutch travellers. *Int J Tuberc Lung Dis* 7: 758-763.
18. Hatherill M, Verver S, Mahomed H (2011) Consensus Statement on Diagnostic End Points for Infant Tuberculosis Vaccine Trials. *Clin Infect Dis* 15: 493-501.
19. Mahomed H, Hughes EJ, Hawkrigde T, Minnies D, Simon E, et al. (2006) Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. *Int J Tuberc Lung Dis* 10: 310-316.
20. AIDSMAP (2012) Thibela TB: Community-wide IPT did not improve TB control in South Africa's mines at the population level. <http://www.aidsmap.com/Thibela-TB-Community-wide-IPT-did-not-improve-TB-control-in-South-Africas-mines-at-the-population-level/page/2285760/>. Accessed 14 December 2012.
21. Corrigan J (2006) Vaccination coverage of the Western Cape Province. Cape Town: Provincial Government of the Western Cape.
22. Rani SH, Vijayalakshmi V, Sunil K, Lakshmi KA, Suman LG, et al. (1998) Cell mediated immunity in children with scar-failure following BCG vaccination. *Indian Pediatr* 35: 123-127.
23. Lalvani A, Sridhar S (2010) BCG vaccination: 90 years on and still so much to learn. *Thorax* 65: 1036-1038.
24. Harling G, Ehrlich R, Myer L (2008) The social epidemiology of tuberculosis in South Africa: a multilevel analysis. *Soc Sci Med* 66: 492-505.

Table 1: Unadjusted analysis of risk factors for TB disease for bacteriologically confirmed TB cases.

N=6,363	N Participants	No of	Hazard	Hazard	95% CI**#
	(%)	TB	rate	ratio	
		cases	/100 pyrs*		
Gender					
Female	3,458 (54.3%)	45	0.55	1.7	0.5-5.3
Male (ref***)	2,905 (45.7%)	22	0.33	1.0	-
Age (years)					
>15	2,760 (43.4%)	31	0.49	1.2	0.7-2.0
≤15 (ref)	3,603 (56.6%)	36	0.42	1.0	-
Racial group					
Black and mixed race	5,921 (93.1%)	67	0.48	Perfect predictor	
Indian/ white (ref)	442 (6.9%)	0	0.00		
Parent income: classified on at least one parent's income					
<R4000 /month	5,313 (83.5%)	62	0.50	2.3	0.6-8.7
>R4000 /month (ref)	1,050 (16.5%)	5	0.22	1.0	-
Maternal highest education level					
Primary school or less	1,893 (29.8%)	28	0.63	1.7	1.1-2.7

High school or more (ref)	3,482 (54.7%)	24	0.29	1.0	-
Unknown	988 (15.5%)	15	0.68	1.6	1.1-2.4

Paternal highest education level

Primary school or less	849 (13.3%)	12	0.59	1.4	0.6-3.0
High school or more(ref)	2,042 (32.1%)	15	0.31	1.0	-
Unknown:	3,472 (54.6%)	40	0.50	1.3	0.8-2.0

BCG scar

Absent	1,813 (28.5%)	24	0.59	1.5	1.0-2.2
Present (ref)	2,565 (40.3%)	22	0.36	1.0	-
Unknown (Not sure)	1,985 (31.2%)	21	0.46	1.0	0.6-1.6

Current or prior TB household contact

Yes	1,728 (27.2%)	31	0.77	2.3	1.1-4.8
No (ref)	4,635 (72.8%)	36	0.45	1.0	-

Previous TB

Yes	639 (10.0%)	7	0.481	1.5	0.7-3.2
No (ref)	5,709 (89.7%)	60	0.45	1.0	-
Unknown	15 (0.3%)	0	0.00	0	

TST status at baseline

TST positive (≥ 5 mm)	3,115 (49.0%)	44	0.61	3.0	1.4-6.5
TST negative (ref)	2,456 (38.6%)	12	0.20	1.0	-
TST unknown****	702 (12.4%)	11	0.68	1.6	0.7-3.7

QFT status at baseline

QFT positive	3,233 (50.8%)	48	0.65	2.4	1.4-4.2
QFT Negative (ref)	2,804 (44.1%)	17	0.27	1.0	-
QFT indeterminate or unknown	326 (5.1%)	2	0.21	0.5	0.05-4.7

*pyrs – person years

**95% CI = 95% confidence interval

#adjusted for the design effect

***ref – reference

**** Those with previous or current TB or with a previous severe reaction to TST did not have a TST performed, in order to prevent severe allergic reactions.

Normal	3 (3%)	1 (1%)
Not done/ missing/ no report	8 (9%)	5 (7%)

Table 3: TB incidence rates using different case definitions.

	Cases	Cumulative incidence (%) (Mean 2.3 years of follow up) N= 6,363	Incidence rate (per 100 pyrs*) (95% CI**)***. pyrs = 14,786
All	87	1.36	0.59 (0.36-0.96)
Bacteriologically confirmed	67	1.05	0.45 (0.29-0.72)
Culture confirmed	61	0.96	0.41 (0.23-0.71)
Chest x-ray compatible with TB	63	0.99	0.43 (0.27-0.68)
Culture positive and chest x-ray compatible with TB	45	0.71	0.30 (0.17-0.54)
Culture positive and abnormal chest x-ray (includes those compatible with TB)	54	0.85	0.37 (0.21-0.62)
Bacteriologically confirmed TB and chest x-ray compatible with TB	51	0.80	0.34 (0.21-0.56)
Bacteriologically confirmed TB and abnormal chest x-ray	61	0.96	0.41 (0.27-0.64)

*pyrs = person years

**95% CI = 95% confidence interval

***adjusted for the design effect

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Table 4: Annual incidence rate by year of study up to February 2009 for bacteriologically confirmed TB* (n=6,363)

Year	Persons	Cases	Pyrs	Incidence rate (per 100 pyrs**) (95% CI***)****
Yr 1	6363	23	6,245	0.37 (0.19-0.72)
Yr 2	5784	33	5,557	0.59 (0.40-0.88)
Yr 3	4884	11	2,762	0.40 (0.21-0.75)
Total		67	14,786	0.45(0.29-0.72)

*No cases were reported in year 4 of follow up so this rate has not been included in the table.

**pyrs = person years

***95% CI = 95% confidence interval

****adjusted for the design effect

Table 5: Adjusted analyses of risk factors for bacteriologically confirmed cases of TB (N=6,036)

Variables	Hazard Ratio	P > z	95% CI*#
BCG scar absent	1.5	0.04	(1.0-2.1)
Positive baseline QuantiFERON	2.0	0.02	(1.2-3.3)
Mother's education primary school or less, or unknown	1.8	0.01	(1.2-2.7)
Prior or current household TB contact	1.7	0.15	(0.8-3.9)

*95% CI = 95% confidence interval

#adjusted for the design effect

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Chapter 6: The tuberculin skin test versus QuantiFERON TB Gold(R) in predicting tuberculosis disease in an adolescent cohort study in South Africa.

Paper overview

This analysis reports on the predictive value of a tuberculin skin test compared to a commercial interferon gamma release assay as measured at baseline for TB disease in the adolescent cohort. The data from this study are compared to other studies assessing predictive value

Contribution to the thesis and novelty

This paper addresses the fourth objective of the thesis. Little data on the predictive value of interferon gamma release assays compared to the tuberculin skin test for TB disease (and even less for high TB burden settings) were available at the time of this study. These data has been utilised in two systematic reviews and for guideline development on interferon gamma release assays by the Centers for Disease Control (CDC), Atlanta, USA and the World Health Organisation.

Contributions of candidate

I was the first author on the publication and was primarily responsible for overseeing the conduct of the study on which the publication is based. I was also primarily responsible for the data analysis and write-up of the study and this is clear from the roles of co-authors as described below.

H Mahomed: Involved in study conceptualisation, oversaw the conduct of the study, analysed the data and wrote the manuscript.

T Hawkrige: Involved in study conceptualisation and reviewed the manuscript.

S Verver: Involved in study conceptualisation, reviewed the analysis and reviewed the manuscript.

D Abrahams: Responsible for processing of QuantiFERON specimens and reviewed the manuscript.

L Geiter: Involved in study conceptualisation and reviewed the manuscript.

M Hatherill: Reviewed the manuscript.

R Ehrlich: Reviewed the analysis and manuscript.

WA Hanekom: Involved in study conceptualisation and reviewed the manuscript.

GD Hussey: Involved in study conceptualisation and reviewed the manuscript.

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A few further amendments have been made to this paper subsequent to publication.

The Tuberculin Skin Test Versus QuantiFERON TB Gold® in Predicting Tuberculosis Disease in an Adolescent Cohort Study in South Africa

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Abstract

Setting: This study was conducted in a high tuberculosis (TB) burden area in Worcester, South Africa, with a notified all TB incidence rate of 1,400 /100,000.

Main objective: To compare the predictive value of a baseline tuberculin skin test (TST) with that of the QuantiFERON TB Gold (In-tube) assay (QFT) for subsequent microbiologically confirmed TB disease among adolescents.

Methods: Adolescents aged 12-18 years were recruited from high schools in the study area. At baseline, blood was drawn for QFT and a TST administered. Participants were followed up for up to 3.8 years for incident TB disease (median 2.4 years).

Results: After exclusions, 5244 (82.4%) of 6,363 adolescents enrolled, were analysed. The TB incidence rate was 0.60 cases per 100 person years (pyrs) (95% CI 0.43-0.82) for baseline TST positive (≥ 5 mm) participants and 0.64 cases per 100 pyrs (95% CI 0.45-0.87) for baseline QFT positive participants. TB incidence rates were 0.22 per 100 pyrs (0.11-0.39) and 0.22 per 100 pyrs (0.12-0.38) among those with a negative baseline TST and QFT respectively. Sensitivity for incident TB disease was 76.9% for TST and 75.0% for QFT ($p=0.81$). Positive predictive value was 1.4% for TST and 1.5% for QFT.

Conclusion: Positive TST and QFT tests were moderately sensitive predictors of progression to microbiologically confirmed TB disease. There was no significant difference in the predictive ability of these tests for TB disease amongst adolescents in this high burden setting. Therefore, these findings do not support use of QFT in preference to TST to predict the risk of TB disease in this study population.

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Introduction

Latent tuberculosis (TB) infection has historically been diagnosed with a tuberculin skin test (TST). However, with this method, cross reactivity with BCG and non tuberculous mycobacteria (NTMs) undermines the specificity of the test[1]. As an alternative to the TST, interferon γ release assays (IGRAs) have been developed which utilise antigens not present in BCG and most NTMs[2]. The advantages of these assays over TSTs are: a second visit for reading of the test is no longer necessary; boosting due to repeated testing is avoided; they have greater specificity for latent TB infection; and they are less prone to the biases encountered when measuring the size of skin reactions, such as digit preference[3]. However, IGRAs are more expensive, need a blood draw and require a sophisticated laboratory.

The QuantiFERON TB Gold (In-tube method) (Cellestis Limited, Carnegie, Victoria, Australia) (QFT) is one such commercially available assay which has been adopted by many countries as an alternative to TST or as part of a two step approach which uses both tests – a TST is done first and those with a positive test result, have a QFT done. QFT uses ESAT-6, CFP10 and antigen 7.7 of *Mycobacterium tuberculosis* as stimuli to determine if T cells in whole blood are sensitized to such antigens thus indicating prior exposure and/ or evidence of latent TB infection.

The predictive value of a positive TST for TB disease has been shown in isoniazid prevention trials and contact investigations[4,5,6]. When our study was initiated, there was a call to conduct longitudinal studies with the new assays so as to determine their validity in predicting TB disease[7,8]. Some longitudinal studies have been done to date but the results have been contradictory in that some show a better predictive ability for the IGRAs than for the TST while

others show no difference between them[9,10,11,12,13,14,15]. References 9 and 14 concluded that IGRAs had superior prognostic ability for TB over the TST whereas references 10-13 and 15 did not. Most of these studies were designed as follow up of contacts of cases diagnosed with TB so it is not clear what the relative value of these tests would be in a general population setting.

We conducted a longitudinal study to determine the predictive value for subsequent TB disease of QFT compared to TST in a large cohort of adolescents in a high burden setting. The baseline characteristics of this adolescent cohort have been published with 55.2% of participants being TST positive (using a 5mm cut-off) and 50.9% being QFT positive at baseline[16]. Adolescents are currently under investigation as a target group for TB vaccines and the predictive value of IGRAs for the subsequent onset of TB disease will be helpful in planning clinical trials of novel TB vaccines. Such data are also necessary for policy makers, researchers, and clinicians developing guidelines for the use of such tests in TB Control Programmes and in clinical practice.

Methods

Ethics Statement: Written informed consent was obtained from the parents of participating adolescents and written informed assent obtained from each adolescent. This study was approved by the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town. Isoniazid prevention therapy (IPT) is not standard of care in South Africa for individuals with latent TB infection except for infants under the age of 5 and HIV positive persons (National TB Management Guidelines, Department of Health, South Africa 2009).

Participants with a positive TST or QFT were investigated for TB disease and referred for treatment at public sector facilities if needed.

Study setting: The study took place at an established TB vaccine trial site in the town of Worcester (and surrounding villages) approximately 100 km from Cape Town. This area has a high burden of TB with a total notified TB incidence rate among all ages of approximately 1,400 per 100,000 population, based on official TB programme data for 2006 [17].

Study subjects: Adolescents aged 12 to 18 years were recruited from high schools in the study area. They were not participating in a TB vaccine trial at the time of this study.

Study procedures: Demographic data were collected at baseline, as well as data on current and prior household TB contact. At baseline, blood was drawn for QuantiFERON[®] TB Gold In-tube (Cellestis). A TST was then immediately administered using the Mantoux method on either forearm, using 2 tuberculin units of RT23 (Statens Serum Institut, Denmark). Induration at the TST site was read 48 - 96 hours later with a ruler or a caliper by trained study personnel. Those with previous or current TB did not have a TST performed, because of the increased risk of severe allergic reactions. The QuantiFERON test (QFT) was performed as recommended by the manufacturers. Any participant who had TB related symptoms, a recent household contact, a positive TST ≥ 10 mm induration or a positive QFT were referred for two sputum smears. If either or both were sputum samples were smear microscopy positive for acid fast bacilli, the sputums were cultured, a chest x-ray performed and an HIV test done. Those diagnosed with TB were

referred to the public health services for evaluation and treatment. About half of participants were allocated to active follow up three monthly and half to passive follow up being seen at baseline and at their two year visit only. At follow up visits, those with new symptoms, a new household contact, a converted TST (>10 mm increase from baseline) or a converted QFT test (change from negative to positive) were investigated for active TB. Investigation for TB included two sputum samples for smear microscopy on two separate occasions. If any single sputum was smear positive, a mycobacterial culture, chest x-ray, and HIV test were performed. In addition, surveillance was conducted at TB clinics and of hospital registers in the area to find any TB cases diagnosed in between visits in all participants. All subjects were scheduled to be seen for a two-year close-out visit unless they were lost to follow up or had died. Due to financial constraints, a small proportion of two-year visits were brought forward at the end of the study. Follow up was therefore continued for a minimum of 22 months. Those completing “two-year” visits were still observed for the occurrence of cases through surveillance of health facility records until the last subject had their final visit, giving a maximum follow up time of 3.8 years. The study took place from 2005 to 2009.

Analysis: Data were captured in a Microsoft Access database and analysed with STATA version 11.0 (Statacorp, Texas, USA). A TST cut-off of 5 mm was used to define a positive or negative test based on the distribution of TST indurations[16]. Analyses based on the 10 mm and 15 mm cut-offs are also given. A QuantiFERON value of 0.35 international units or more was deemed positive as per manufacturer’s specifications. Any participant diagnosed with pulmonary TB based on at least two positive sputum smears or a single positive sputum culture was defined as a case of TB. Incidence rates were calculated by dividing the number of incident cases by the

total person time of observation. Observation time was calculated from date of enrolment to study end date except where there was loss to follow up, diagnosis of TB, consent withdrawal or death. When there was loss to follow up, person time of observation was calculated from baseline to a halfway point between the date when last seen and the date of the next scheduled visit when loss to follow up was established. 95% confidence intervals for incidence rates were calculated using Poisson regression. Incidence rate ratios (IRRs) were then calculated and the 95% confidence interval for each IRR was calculated. Exposure to TB was based solely on reported household TB contact at baseline. Reported time between household TB contact and enrolment was calculated by subtracting the year of reported contact from the year of enrolment. The sample size for this study was based on estimation of an incidence rate that would be useful for planning clinical trials of new TB vaccines in adolescents. The sample size analysed would have been sufficient to detect a significant difference in predictive value between the TST positive and QFT positive groups at an incidence rate ratio of 1.54 given an incidence rate of 0.60 per 100 person years in the TST positive group.

Results

Study participants

6,363 participants were enrolled at baseline but after exclusion of those with prior or current TB, indeterminate QFT results, or missing QFT or TST results, as described elsewhere[16], 5,244 participants were included in this analysis. 82% of participants completed follow up at two years. Of the 18% of participants who did not complete their two year visits, 8 (0.2%) had died during follow up. More detail on attendance at follow up visits and reasons for visits not being completed is provided separately as Supporting Information (Table S1). Mean follow up time

was 2.3 years, median 2.4 years and range 1.5 to 46 months (3.8 years). The most common reason for non-participation in this study when a reason was given was fear of blood draws. Important baseline characteristics of the recruited population were as follows: 54.2% female, 56.9% under the age of 16, 93.8% reported having received BCG at birth and 25.4% reported a prior or current household contact. More details on baseline characteristics are laid out in Table 1.

Baseline TST and QFT results

There was good agreement at baseline between QFT and TST at the 5 mm (84.8%, kappa 0.70) and 10 mm cut-off (81.4%, kappa 0.63) but not the 15 mm cut-off (64.3%, kappa 0.30) (details described elsewhere[16]).

Incident cases and rates by TST/QFT status

67 participants were diagnosed with TB, of whom 52 met the *a priori* case definition and 42 were culture positive. Percentage agreement between TST and QFT among the 52 cases was 86.5% (kappa 0.63). The incidence rates of TB by baseline QFT and TST status are shown in Table 2 and Figure 1. These show that the TB incidence rate was 0.60 cases per 100 person years (pyrs) (95% confidence interval [CI] 0.43-0.82) for baseline TST positive (≥ 5 mm) participants and 0.64 cases per 100 pyrs (95% CI 0.45-0.87) for baseline QFT positive participants. There were 7 cases diagnosed within 6 months of enrolment and excluding these changed the rates to 0.53 (95% CI 0.37-0.73) and 0.55 (95% CI 0.38 – 0.77) cases per 100 pyrs for positive TSTs and QFTs respectively. An additional analysis (not shown in the table) gives an incidence rate of 0.74

per 100 pyrs (95% CI 0.53-1.02) in participants with a baseline TST \geq 10 mm (42.2% of participants). There was a significantly higher rate of TB in those with a positive QFT or TST than in those with a corresponding negative test result. The incidence rate ratios (IRRs) were 2.7 (95% confidence interval [CI] 1.4-5.0) for TST (Incidence Rate (IR) TST+/ IR TST-) and 2.9 (95% CI 1.6-5.2) for QFT (IR QFT+/ IR QFT-). The number of persons that needed to be tested for TST and/ or QFT and followed up to predict one TB case over a period of one year ranged from 157 to 520 depending on the test result. The proportion of cases who were test positive and diagnosed with TB within the first year compared to subsequent years was 84.2% versus 72.7% ($p=0.34$) for TST and 73.7% versus 75.8% ($p=0.86$) for QFT respectively.

Sensitivity, specificity, positive predictive value and negative predictive value (Table 3).

Approximately three quarters of cases, 76.9% for TST and 75.0% for QFT ($p=0.81$), were test positive at baseline. Specificity was less than 50% for both tests: 45.0% for TST and 49.3% for QFT ($p < 0.01$). While this constituted a statistically significant difference, it was not viewed as clinically relevant. The positive predictive value was similarly very low for both (TST 1.4% and QFT 1.5%) and the negative predictive value was equally high for both tests (99.5%).

Relationship between time of exposure and risk of disease

In one subset of participants ($n=1328$) for whom a history of a household TB contact was reported at baseline, the prevalence of a positive baseline TST and QFT was significantly and negatively associated with time since TB contact exposure (Table 4); i.e. the longer the time since last recalled contact, the lower the proportion with a positive result on either test. Of 20

cases for whom a TB contact prior to enrolment was reported, 15 (75.0%) had had a contact less than 5 years before enrolment and 5 (26.3%), 5 years or more. The proportion positive TST and QFT results among cases who reported recent compared to remote contacts were similar. Rates of disease were higher amongst those more recently exposed than among those exposed ≥ 5 years previously. These differences were not significant since all incidence rate ratios had very wide confidence intervals which included one (Table 5).

Discussion

This is the largest study investigating the predictive value of TST versus an IGRA and it is one of few from a high burden setting (Table 6). A positive TST and QFT were both indicative of a higher risk of developing subsequent TB disease, and were equivalent in predicting incident TB disease. While the sensitivity was moderate, specificity and positive predictive values were relatively low, and negative predictive values were high. There was a trend for more recent reported exposure (<5 years) to be associated with a higher risk of disease than more remote exposure but the confidence intervals were very wide.

This study is significant in that most of the other longitudinal studies examining this question were based largely on household contacts of TB cases[9,10,12,13,15,18,19,20] while those using a cohort methodology were much smaller by comparison[11,14]. The finding in this study of a similar predictive value for QFT and TST accords with that of studies in Turkey, the Gambia, Senegal, Norway and the Netherlands [10,11,12,13,15], but contradicts the findings of the studies from Germany and Hong Kong[9,14]. It is thus still unclear whether IGRAs offer better predictive value for subsequent TB disease than the TST. There is increasing evidence that IGRAs

perform differently in high burden compared to low burden countries and this may partly explain the differences seen amongst the different studies[21]. The study in The Netherlands was done amongst immigrants and the one in Norway amongst asylum seekers, populations that represent high incidence home countries rather than low incidence host countries[11,13]. In countries with a high burden of TB disease and therefore exposure to active TB cases, the greater specificity of IGRAs is apparently not useful. Also, where BCG is given at birth as is common in high burden countries, it has been shown that the effect of BCG on the TST is limited after the age of 10 and the TST thus retains its specificity[22]. These tests may therefore perform better in low incidence settings. Therefore, many low incidence countries have shifted to using IGRAs. The setting in which this study was done possibly has a low burden of NTMs. It may be that the IGRAs will perform better in settings where there is higher exposure to NTMs due to IGRAs not being affected by most NTMs while the TST is.

Table 6 summarises the studies comparing the predictive value of TST and IGRAs for TB disease and includes the data from this study. There are important differences amongst the studies – different products are compared, different cut-offs are used for TST and different populations have been studied. These design and measurement differences may also explain some of the different results obtained. This limits the degree to which these studies are comparable.

The TB incidence rates in our cohort study are lower than in the contact investigation studies. Contacts are likely to have been more recently exposed whereas in a cohort study, time since exposure will vary. Recent contacts are at higher risk of progressing to disease within the first two to five years of follow up as demonstrated in early studies in the USA which give a 10 year

rate of disease of 36.9 per 1000 amongst household contacts compared to a 10 year rate of 6.6 in a cohort of mental patients who were followed up[4]. In the household contact group in this American paper, most cases occurred in the first 5 years of follow up. The kind of cohort study reported in this manuscript helps to quantify risk in a clinical context where time since exposure is often not known.

The lower proportion of TST and QFT positive results in those with a longer reported time between exposure and latent TB infection measurement by TST and QFT indicates waning of responses to TB antigens over time although this may also be affected by recall bias. When we examined risk of progression to disease in those who reported a prior household contact, there was a trend towards an increased risk in those with recent exposure but this was not significant. This study may have been inadequately powered to detect this difference. Also, one would need to recognize that in this community, the high burden of TB means that exposure is likely to be a common occurrence whether reported or not. A study amongst immigrants to the Netherlands showed that remote exposure is common particularly among those from high burden settings[23].

There was no difference in proportion TST or QFT positive at baseline between cases diagnosed in the first year after enrolment versus those diagnosed afterwards. This may be due to the fact that follow up was not continued for long enough to detect a drop off in risk. The studies reviewed by Ferebee show a sustained high risk in those with a recent contact in the first five years of follow up before the risk starts declining[4].

Despite the higher risk of subsequent TB represented by a positive TST or QFT, the number of people that must be followed up and screened for TB in order to find a single case is substantial, even in this high burden setting where infections and re-infections are common[24]. The cost effectiveness of such population screening would need to be evaluated, including the value of treating test positive persons with isoniazid preventive therapy to prevent the onset of future TB disease. Given that across most studies, more than 95% of persons who are IGRA positive do not progress to TB disease, emphasises the need for biomarkers other than interferon gamma for risk prediction, or a combination of interferon gamma with risk factors (e.g. age, contact history, conversion) to enhance predictive value.

Positive and negative predictive values are dependent on prevalence. Since a low positive predictive value was achieved in a high burden setting, an even lower PPV can be expected in a low burden setting. The findings on sensitivity, specificity, positive and negative predictive value are similar to those in The Netherlands study[13] while the other studies did not describe these. While a positive test is not very helpful in predicting disease, a negative test suggests that risk of progression to disease is low although it does not rule out the possibility entirely.

These results are not representative of adolescents with prior TB since they were excluded from having a TST. Since those with past TB are at higher risk of getting TB again [25], our incidence rates were possibly all underestimated. Incidence rates of those with prior TB could not be calculated based on those with a positive baseline TST as they did not undergo this test.

However, incidence rates could be calculated for those with a history of prior TB with a positive baseline QFT and the rate for them was 0.66/ 100 pyrs (95% confidence interval 0.27-1.37). This is similar to those with a positive baseline TST or QFT without a history of prior TB. One can then reasonably assume that the rate would have been similar in those who had prior TB who would have had a positive baseline TST. A positive QFT (and presumably TST) did not therefore predict a higher risk of TB in those with prior diagnosis of TB in this study. Since those with past TB are also more likely to be TST and QFT positive, it is unclear how sensitivity, specificity, PPV and NPV would change, had we included those. Also, any of those negative at baseline could have converted during study follow up due to new exposures prior to the onset of TB disease and would be misclassified as test negative. There is no reason to think that this would have occurred differentially between the tests. Those with a positive TST or QFT at baseline were investigated for TB. If diagnosed with TB, they were not included in the 5244 participants analysed in this predictive analysis. Those with a QFT or TST conversion were investigated at follow up and if diagnosed were included in the analysis. However, most cases (>80%) were diagnosed by local health services who would not normally have enquired about TST and QFT results since these are not routinely used in the diagnosis of TB in this age group. Therefore neither incorporation bias nor lack of blinding were felt to be major factors influencing the analysis. While the screening methods for TB at baseline were insensitive for smear negative culture positive TB, a sensitivity analysis which excluded cases diagnosed within six months of enrolment did not appreciably change the main findings showing no difference in the predictive value of TST versus QFT for TB disease. Not all participants completed follow up; if any of these had developed TB, this may have influenced the findings in that rates would have been higher. Finally, since recruitment took place at schools and a substantial proportion (41.8%) did not

participate, these results are not representative of all adolescents in this area. None of these limitations are thought to affect the comparison between TST and QFT in this study.

These findings suggest TST and QFT are equally predictive of progression to TB disease in a cohort of adolescents in a high TB burden population and may be used interchangeably. Our results do not support the hypothesis that QFT is superior to TST in its predictive value. These findings should assist policy makers attempting to develop guidelines for IGRA use in high and low TB burden countries. More studies in high burden settings and in adolescents are needed to indicate whether either the TST or QFT may be used as a screening tool in planning TB vaccine trials in adolescents.

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References

1. Pai M, Riley LW, Colford JM, Jr. (2004) Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 4: 761-776.
2. Richeldi L (2006) An update on the diagnosis of tuberculosis infection. *Am J Respir Crit Care Med* 174: 736-742.
3. Pai M, Zwerling A, Menzies D (2008) Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 149: 177-184.
4. Ferebee SH (1970) Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc* 26: 28-106.
5. Comstock GW, Livesay VT, Woolpert SF (1974) The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 99: 131-138.
6. Moran-Mendoza O, Marion SA, Elwood K, Patrick DM, FitzGerald JM (2007) Tuberculin skin test size and risk of tuberculosis development: a large population-based study in contacts. *Int J Tuberc Lung Dis* 11: 1014-1020.
7. Jason E Stout DM (2008) Predicting tuberculosis does the IGRA tell the tale? *Am J Respir Crit Care Med* 177: 1055-1057.
8. Adetifa IM, Brookes R, Lugos MD, de Jong BC, Antonio M, et al. (2007) Rising ELISPOT count prior to the onset of symptoms of full-blown tuberculosis disease. *Int J Tuberc Lung Dis* 11: 350-352.

9. Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus. (2010) A negative and positive predictive value of a whole-blood IGRA for developing active TB - an update. *Am J Respir Crit Care Med*. doi:10.1164/rccm.201006-0974OC.
10. Bakir M, Millington KA, Soysal A, Deeks JJ, Efee S, et al. (2008) Prognostic value of a T-cell-based, interferon-gamma biomarker in children with tuberculosis contact. *Ann Intern Med* 149: 777-787.
11. Harstad I, Winje BA, Heldal E, Oftung F, Jacobsen GW. (2010) Predictive values of QuantiFERON-TB Gold testing in screening for tuberculosis disease in asylum seekers. *Int J Tuberc Lung Dis* 14: 1209-1211.
12. Hill PC, Jackson-Sillah DJ, Fox A, et al. Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts. *PLoS One* 2008;3:e1379.*
13. Kik SV, Franken WP, Mensen M, Cobelens FG, Kamphorst M, et al. (2009) Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts. *Eur Respir J* 35(6):1346-53.
14. Leung CC, Yam WC, Yew WW, Ho PL, Tam CM, et al. (2010) T-Spot.TB outperforms tuberculin skin test in predicting tuberculosis disease. *Am J Respir Crit Care Med* 182: 834-840.
15. Lienhardt C, Fielding K, Hane AA, Niang A, Ndao CT, et al. (2010) Evaluation of the prognostic value of IFN-gamma release assay and tuberculin skin test in household contacts of infectious tuberculosis cases in Senegal. *PLoS One* 5: e10508.

16. Mahomed H, Hawkrigde T, Verver S, Geiter L, Hatherill M, et al. (2011) Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. *Int J Tuberc Lung Dis* 15: 331-336.
17. Groenewald P (2007) Annual Health Status Report 2006. Worcester: Department of Health, Boland-Overberg Region.
18. Nsutebu E, Moffitt SJ, Mullarkey C, Schweiger MS, Collens T, et al. (2008) Use of QuantiFERON-TB Gold test in the investigation of unexplained positive tuberculin skin tests. *Public Health* 122: 1284-1287.
19. Ohno H, Ikegami Y, Kishida K, Yamamoto Y, Ikeda N, et al. (2008) A contact investigation of the transmission of *Mycobacterium tuberculosis* from a nurse working in a newborn nursery and maternity ward. *J Infect Chemother* 14: 66-71.
20. del Corral H, Paris SC, Marin ND, Marin DM, Lopez L, et al. (2009) IFN γ response to *Mycobacterium tuberculosis*, risk of infection and disease in household contacts of tuberculosis patients in Colombia. *PLoS One* 4: e8257.
21. Dheda K, Smit RZ, Badri M, Pai M (2009) T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. *Curr Opin Pulm Med* 15: 188-200.
22. Farhat M, Greenaway C, Pai M, Menzies D (2006) False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 10: 1192-1204.

23. Kik SV, Franken WP, Arend SM, Mensen M, Cobelens FG, et al. (2009) Interferon-gamma release assays in immigrant contacts and effect of remote exposure to Mycobacterium tuberculosis. *Int J Tuberc Lung Dis* 13: 820-828.
24. van Helden PD (2002) Molecular epidemiology of TB: challenging dogmas and asking new questions. *IUBMB Life* 53: 219-223.
25. Rieder H. (1999) *The Epidemiologic Basis for Tuberculosis Control*, First Edition. International Union Against Tuberculosis and Lung Disease. Available at: <http://www.theunion.org/index.php/en/resources/technical-publications/item/165-epidemiologic-basis-of-tuberculosis-control>-. Accessed 24 May 2013.

*please note that the incorrect reference 12 was reflected in the published manuscript – a correction is now reflected when the article is accessed on the internet. The correct reference has been inserted here.

Figure 1: Incidence rates by baseline TST and QFT status.

Figure 1 is a chart of incidence rates of tuberculosis per 100 person years of observation by baseline TST and QFT status. Firstly, rates are shown individually for either positive or negative TST or QFT result. Then rates are shown with combined results, either concordant negative or positive TST and QFT results, or discordant negative/ positive combinations.

Figure 1: Incidence rates by baseline TST and QFT status

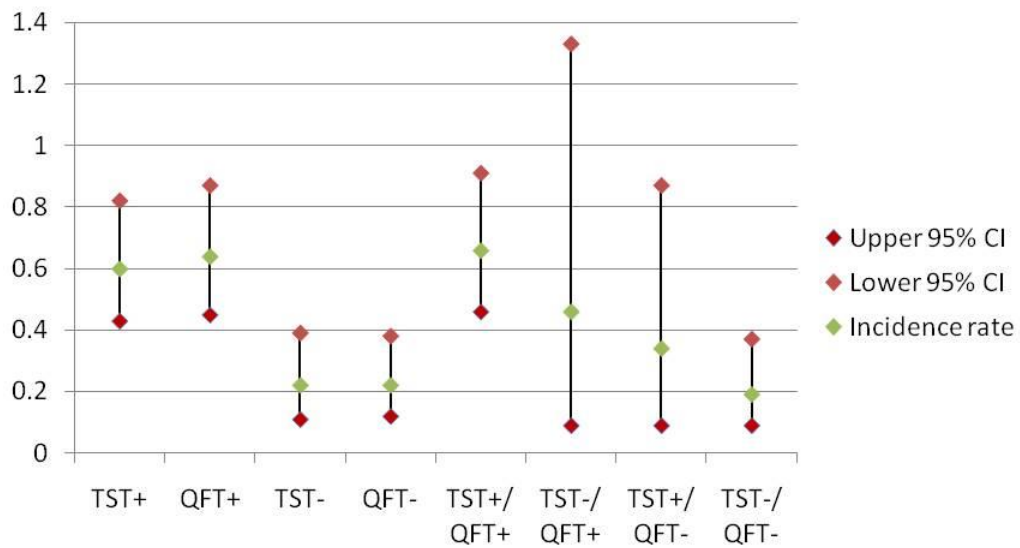


Table 1: Demographic profile of study participants analysed (n=5244)

Category	Numbers (column %)
Gender	
Male	2,402 (45.8%)
Female	2,842 (54.2%)
Age (years)	
>15	2,261 (43.1%)
≤15	2,983 (56.9%)
Racial group	
Black	995 (19.0%)
Mixed race	3,839 (73.2%)
Indian/ white	410 (7.8%)
Parent income: classified on at least one parent's income	
≤R4000 /month	4,243 (80.9%)
>R4000 /month	921 (17.6%)
Unknown	80 (1.5%)
Maternal highest education level	

≤ Primary school 1,510 (28.8%)

≥ High school 2,890 (55.1%)

Unknown: 844 (16.1%)

Paternal highest education level

≤ Primary school 686 (13.1%)

≥ High school 1,720 (32.8%)

Unknown: 2,838 (54.1%)

BCG reported as being given

No 46 (0.9%)

Yes 4,917 (93.8%)

Unknown 281 (5.4%)

BCG scar

Absent 1,490 (28.4%)

Present 2,064 (39.4%)

Unknown (Not sure) 1,690 (32.2%)

Current or prior TB household contact

Yes 1,332 (25.4%)

No 3,911 (74.6%)

Unknown 1 (0.02%)

Chronic allergy-related conditions e.g. asthma, hay fever,
eczema

Yes 53 (1.0%)

No 5,191 (99.0%)

History of hospitalization within the 6 months prior to
enrolment

Yes 46 (0.9%)

No 5,198 (99.1%)

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Table 2: Incidence rates by TST ($\geq 5\text{mm}$) and QFT status at baseline.

	N	Incident TB Cases	Person years of follow up	Incidence Rate (per 100 person years, 95%CI)	No of persons followed up per year to detect 1 case
TST+	2,894	40	6651	0.60 (0.43-0.82)	166
QFT+	2,669	39	6137	0.64 (0.45-0.87)	157
TST-	2,350	12	5337	0.22 (0.11-0.39)	444
QFT-	2,575	13	5851	0.22 (0.12-0.38)	450
TST+/QFT+	2,383	36	5480	0.66 (0.46-0.91)	152
TST-/QFT+	286	3	657	0.46 (0.09-1.33)	219
TST+/QFT-	511	4	1171	0.34 (0.09-0.87)	293
TST-/QFT-	2,064	9	4680	0.19 (0.09-0.37)	520
Total*	5,244	52	11988	0.43 (0.32-0.57)	231

*all participants analysed

Table 3: Sensitivity, specificity, positive predictive value and negative predictive value of TST and QFT for predicting TB disease.

	TST % (95% CI)	QFT %(95% CI)
Sensitivity	76.9 (63.6 – 87.5)	75.0 (61.1 – 86.0)
Specificity	45.0 (43.7 – 46.4)	49.3 (48.0 – 50.7)
Positive Predictive value	1.4 (1.0 – 1.9)	1.5 (1.0 – 2.0)
Negative predictive value	99.5 (99.1 – 99.7)	99.5 (99.1 – 99.7)

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Table 4: Proportion TST and QFT positive by time since recalled exposure to household TB contact (N=1,328)

	QFT positive (%)	TST positive (%)
0-4 years (n=890)	69.4	72.3
5-9 years (n=235)	65.1	72.8
10-14 years (n=168)	57.1	67.3
≥15 years (n=35)	57.1	54.3
Chi squared for trend:	p<0.001	P=0.036

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Table 5: Incidence rates by time since recalled exposure to household TB contact*

Time since contact	Numbers reporting contact	Incident cases	Incidence rate per 100 person years	QFT + incident cases	QFT + incidence rate per 100 person years	TST + incident cases	TST + incidence rate per 100 person years
Recent <5 years	890	15	0.74	15	1.07	14	0.95
Remote ≥5 years	438	5	0.49	3	0.48	4	0.56
Incidence rate ratio (Recent/ remote) (95% confidence intervals)			1.5 (0.5-5.3)		2.2 (0.6– 11.9)		1.7 (0.5-7.0)

*As reference, those with no exposure had an overall incidence rate of 0.36/ 100 pyrs (95% CI 0.25-0.51), those with a positive TST 0.49/100 pyrs (95% CI 0.31-0.75) and positive QFT 0.51 (95% CI 0.32-0.78) with the latter 2 results being similar to those with remote exposure.

Table 6: A summary of longitudinal studies comparing the predictive value of TSTs and IGRAs.

Authors, Country and Year of publication	Study Type	Number	Population description	Cases	Length of Follow up	Products and TST cut-offs	Baseline prevalence	Cumulative incidence (%) or Incidence rate per 100 person years (pyrs)*
R Diel et al. Germany (2010)	Contact investigation	903	All ages - range 1-62	19	Up to 4 yrs	TST \geq 5 mm	63%	3.2%
						TST \geq 10 mm	25%	4.8%
						QFT	21%	12.9%
P Hill et al. Gambia (2008)	Contact investigation	2348	All ages	26	2 yrs	TST \geq 10 mm	36%	0.9/ 100 pyrs
						Elispot	28%	0.9/ 100 pyrs
S Kik et al. The Netherlands (2009)	Contact investigation	339	Immigrants \geq 16 years	9	2 yrs	TST \geq 10 mm	54%	3.1%
						QFT	55%	2.8%
						T-Spot	63%	3.3%
M Bakir et al. Turkey (2008)	Contact investigation	908	Age \leq 16 years	15	2 yrs	TST \geq 5 mm	61%	1.7/ 100 pyrs
						Elispot	42%	2.1/ 100 pyrs

CC Leung et al. Hong Kong (2010)	Cohort study	308	Adult males with silicosis	17	1-5 yrs	TST \geq 5 mm	74%	2.3/ 100 pyrs
						TST \geq 10 mm	66%	2.6/ 100 pyrs
						T - Spot	66%	3.2/ 100 pyrs
C Lienhardt et al. Senegal (2010)	Contact investigation	2679	All ages	52	2 yrs	TST \geq 5 mm	78%	1.5/ 100 pyrs
						TST \geq 10 mm	65%	1.2/ 100 pyrs
						TST \geq 15 mm	37%	1.6/ 100 pyrs
						Elispot	65%	1.4/ 100 pyrs
H del Corral et al. Colombia (2009)	Contact investigation	2052	All ages	37	2-3 yrs	Elisa (IFN- γ responses to CFP-10)	66.3%	0.8/100 pyrs
H Mahomed et al. South Africa	Cohort study	5244	Adolescents aged 12-18	52	2-4 yrs	TST \geq 5 mm	55%	0.6/ 100 pyrs
						TST \geq 10 mm	42%	0.7/ 100 pyrs
						QFT	51%	0.6/ 100 pyrs

*Incidence rates adjusted to per 100 person years from original articles to enable comparison

Table S1: Flow of visits and reasons for visits not taking place. (Supplementary table)

This table describes frequency of visits, the number of visits at each time point and the reasons for visits not being completed. It also provides the number for each reason with percentages.

Visit	Died	Other	Loss to follow up	Withdrew	Missed	Seen	Not Seen ^a	Total
90	1	0	18	12	5	2,765	2,443	5,244
%	0.02	0.00	0.34	0.23	0.10	52.73	46.59	100.00
180	1	0	62	73	29	2,636	2,443	5,244
%	0.02	0.00	1.18	1.39	0.55	50.27	46.59	100.00
270	1	0	69	77	19	2,635	2,443	5,244
%	0.02	0.00	1.32	1.47	0.36	50.25	46.59	100.00
360	1	1	87	159	33	2,520	2,443	5,244
%	0.02	0.00	1.66	3.03	0.63	48.05	46.59	100.00
450	2	1	90	162	23	2,523	2,443	5,244
%	0.04	0.02	1.72	3.09	0.44	48.11	46.59	100.00
540	2	1	133	261	52	2,352	2,443	5,244
%	0.04	0.02	2.54	4.98	0.99	44.85	46.59	100.00
630	2	1	146	282	150	2,220	2,443	5,244

%	0.04	0.02	2.78	5.38	2.86	42.33	46.59	100.00
720	8	0	409	537	0	4,290	0	5,244
%	0.15	0.00	7.80	10.24	0.00	81.81	0.00	100.00

^a Roughly half of participants were followed up three monthly and half were seen at baseline and two year visit. These figures reflect the group only seen at baseline and day 720 visits. This component of this study will be dealt with in a separate manuscript and does not have any bearing on the analysis shown in this manuscript.

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Chapter 7: *Are adolescents ready for tuberculosis vaccine trials?*

Paper overview

This analysis reports on a quantitative and qualitative assessment of adolescents and their parents' attitudes towards participating in tuberculosis (TB) vaccine trials.

Contribution to the thesis and novelty

This paper addresses the fifth objective of the thesis. TB vaccine trials in adolescents are planned for the future. While studies in the HIV and human papilloma virus vaccine fields have been done to assess knowledge and attitudes towards vaccine trials, no studies had been done in any populations examining knowledge of TB and attitudes towards TB vaccine trials. This study was thus unique and provided useful insights that would be helpful in planning TB vaccine trials in adolescents.

Contributions of candidate

I was the first author on the publication and was primarily responsible for overseeing the conduct of the study on which the publication is based. I was also primarily responsible for the data analysis and write-up of the study and this is clear from the roles of co-authors as described below.

Role of authors:

H Mahomed: Conceived the study, wrote the protocol, responsible for study design, oversaw the conduct of the study, analysed the data and wrote the manuscript.

J Shea: Was responsible for qualitative component of the study design, conducted the qualitative component, arranged the qualitative data in themes and reviewed the manuscript.

F Kafaar: Was the study co-ordinator responsible for day to day management of the study and reviewed the manuscript

T Hawkrige: Reviewed and commented on the protocol and reviewed the manuscript

WA Hanekom: Reviewed the manuscript

GD Hussey: Reviewed and commented on the protocol and reviewed the manuscript

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A few further amendments have been made to this paper subsequent to publication.

Are adolescents ready for tuberculosis vaccine trials?

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Abstract:

Tuberculosis vaccine trials are planned in adolescents in a high tuberculosis burden rural area near Cape Town, South Africa. To determine the knowledge and attitudes of adolescents about tuberculosis, vaccines and vaccine trials, a representative sample of adolescent learners was chosen from high schools in the trial area. A questionnaire was administered and focus group discussions held with the group and a sample of their parents. The questionnaire response rate was 65%. Knowledge of tuberculosis was fair (63.7%) but knowledge of vaccines poor (41.9%) based on a TB and vaccine knowledge score respectively. Willingness to participate in vaccine trials will be influenced by the type of procedures involved (60% willing to answer questions, 43% willing to be examined, 32% willing to undergo skin tests and 39% willing to undergo blood draw). In general, better knowledge was statistically associated with greater willingness to participate in study procedures except for the blood draw. The focus group discussions showed that adolescents and their parents were positive about participating in vaccine trials but concerns about safety and the provision of adequate information should be considered when planning TB vaccine trials. This study suggests that TB vaccine trials would be acceptable amongst adolescents in this community with certain provisos.

Key words. : Tuberculosis, Vaccine, Survey, Adolescents, Parents.

Running Head: Are adolescents ready for TB vaccine trials?

1. Introduction

Tuberculosis (TB) is a major part of the disease burden in South Africa and worldwide with one third of the world's population estimated to be infected with *Mycobacterium tuberculosis* [1].

The existing vaccine against tuberculosis, bacillus Calmette Guérin (BCG) has had a limited impact on the tuberculosis epidemic and new tuberculosis vaccines are being developed. [2,3].

The South African Tuberculosis Vaccine Initiative (SATVI) and the Aeras Global Tuberculosis Vaccine Foundation are planning clinical trials of new TB vaccines in adolescents in a rural area just outside of Cape Town hereafter referred to as "the study area". In the study area, the reported new smear positive rate of pulmonary TB is high (564/100 000 in 2004) [4].

Adolescents are considered a target group for TB vaccines due to the fact that the rate of TB in high burden countries starts to increase in adolescence (5).

The success of a TB vaccine trial in adolescents would be reliant on adolescent willingness to participate and the consent of their parents. The authors did not come across any studies reporting specifically on the feasibility and acceptability of conducting TB vaccine trials in adolescents. It was thus considered to be important to investigate the knowledge and attitudes to TB, vaccines and research amongst adolescents and their parents.

While some studies have investigated knowledge levels with regard to HIV in adolescents [6], none were found that looked at knowledge levels in adolescents with regard to TB or vaccines.

To our knowledge, no study involving procedures such as drawing of blood and skin tests has been conducted before in the study area's adolescent population.

This study was designed to determine knowledge levels about TB and vaccines amongst adolescents, their attitudes and that of their parents towards research and invasive procedures (including HIV testing), the factors which influenced their attitudes and what factors an advocacy or recruitment campaign should take into account when trying to promote adolescent participation in clinical research. Potential barriers to the success of the TB vaccine trials were explored in focus group discussions with adolescents and their parents. The adolescents studied in this research were learners attending high schools in the study area. Because TB rates are higher in poorer communities, differences in responses between high and low socio-economic groups were explored. Prior to this study, the Director of the local Department of Education, school managers (known as circuit managers), school heads (principals) and teachers were engaged through meetings with respect to conducting TB vaccine trial research amongst school learners. All these stakeholders responded positively to the planned research activities and it was felt not necessary to specifically survey their views as part of this study.

In South Africa, anonymous HIV testing is conducted annually amongst pregnant women to determine epidemiological trends. The women tested are not informed of their results. In preparation for TB vaccine trials in adolescents, HIV trends amongst adolescents would be important to know because of the increased risk of TB amongst those who are HIV positive. The authors wished to explore how adolescents would feel about such epidemiological prevalence surveys particularly with respect to the disclosure of the results. HIV testing is also likely to be

part of the screening process for entry into new TB vaccine clinical trials and adolescents not wishing to know their HIV status may be reluctant to participate in such trials as a result.

2. Materials and Methods

Study population: High school learners in all grades at the 11 schools in the study area and their parents.

Study design: A cross-sectional study involving questionnaires and focus group discussions.

Sample: For the questionnaires, one class from each of the eleven schools was selected with an even distribution by grade as follows: 2 classes from grades 8, 9, 11 and 12, and 3 classes from grade 10.

Grades were randomly chosen by drawing the name of a school from one box and then the name of a grade from another box.

For the focus group discussions, one class per grades 8-11 was chosen from amongst the above classes (the highest grade, grade 12 was excluded from the focus group discussions to minimise disruption of their final year of school). Ten learners were then randomly selected from the class lists. The parents of these learners were interviewed separately for the parent focus group discussions.

The focus groups were asked to attend specially arranged sessions of one and a half to two hours led by a trained facilitator (J Shea).

Study tools: A self administered, multiple choice, paper questionnaire made up of 19 closed questions explored knowledge levels of infectious diseases and vaccines, and attitudes to research amongst learners. The questionnaires were administered in the two languages used in the study area, Afrikaans and Xhosa and took learners about half an hour to complete them. The content of the questionnaires was pre-tested amongst a group of adolescents from Cape Town and this resulted in a few minor changes to the questionnaire. The first focus group discussion with learners was used to check the suitability of the questions in the questionnaire and this also resulted in a few minor changes. Thereafter, the questionnaire survey and focus groups were conducted over the same period. Those answering the questionnaire were told that more than one answer could be marked per question. No demographic information such as age and gender or any other additional information was collected as part of the survey in order to reduce the risk of deductive disclosure of the survey participants and to encourage honest responses. (Sometimes, young adults still attend high school in South Africa. However, all participants required parental consent for participation and therefore must have been under the age of 21.)

Focus groups discussions were held separately with learners and parents to determine attitudes towards medical research, invasive procedures and infectious diseases using a set of guide questions. Guide questions were thus used to solicit responses initially but further discussions were based on the responses given to these questions. When an Afrikaans speaking group was interviewed, the facilitator conducted the discussion in Afrikaans. When a Xhosa speaking group was interviewed, the assistance of a translator was obtained.

Ethical issues: Permission was obtained from the Department of Education to do the study. Individual consent and assent were obtained from parents and learners for the study. The study

was conducted in the language of preference of the participants. The study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town.

Data capture and analysis: The questionnaires were captured in Microsoft Excel and analysed with EPI info 3.3.2. When “unsure” was indicated as an answer, these were counted as incorrect for the purpose of knowledge measurement. The schools were divided into two groups according to financial subsidies received from the Department of Education based on socio-economic status. Group A, the schools from a higher socio-economic status comprised one grade 8, two grade 9, one grade 10 and one grade 11 class and group B, the lower socio-economic status schools comprised one grade 8, two grade 10, one grade 11 and two grade 12 classes. Responses to the questionnaire were then compared between the 2 groups. The responses to knowledge questions were compared with willingness to undergo procedures using summary measures of the TB and vaccine answers. Binary variables were created for vaccine knowledge and TB knowledge. Three correct answers were considered knowledgeable for TB knowledge, and two for vaccine knowledge. Proportions were compared using relative risks and 95% confidence intervals. Knowledge of TB was compared to knowledge of vaccines using a kappa statistic.

The focus group discussions were recorded using an electronic dictaphone. A recorder manually kept notes of the discussion. The tape recordings were transcribed and translated verbatim. Field notes were translated and transcribed and all transcripts were reviewed for completeness by two independent reviewers. The transcripts were used for content analysis to identify the main themes and were then coded for retrieval and analysis. Where appropriate, quotations were used to illustrate the themes.

3. Results

270 (65%) of 414 learners targeted completed the questionnaire. The response rate ranged from 31-98% amongst the schools. It was 75% in more affluent schools (group A) and 59% in the less well-off schools (group B) (Relative Risk 1.28; 95% confidence interval 1.12 – 1.47).

The learner focus groups were well attended with 10 participants attending sessions at each of the 4 schools selected. The parent groups were less well attended with attendance ranging from 4-8 per focus group.

Questionnaire results

Knowledge results for all learners are set out in the first column of Table 1. Approximately three quarters correctly answered 3 of the 5 TB knowledge questions. Only half knew that the direct cause of TB was a germ while half incorrectly felt that smoking was the direct cause.

Only 41% knew that vaccines were given to prevent infections, 25% knew that vaccines were made of “bits of dangerous germs that have been made safe” and 41% knew that smallpox no longer occurred due to a worldwide vaccination programme. The percentages reporting “unsure” as an answer for these 3 questions were 29%, 33% and 31% respectively. 74% knew that research is “when scientists want to find out something new”. The learners from the better resourced schools serving a higher socio-economic group (Group A) tended to have correct answers more often than the less resourced schools’ learners (Group B) particularly with respect to the questions on vaccines (table 1). This is confirmed by the summary data given below.

In summarising the knowledge data, 63.7% (Group A: 67.5% and Group B: 60.4%, Relative Risk 1.12; 95% confidence interval 0.93 – 1.34) of participants were knowledgeable about TB (as defined above) and only 41.9% (Group A 55.6%: and Group B: 29.9%, Relative Risk 1.86; 95% confidence interval 1.39 – 2.50) for vaccines. Percentage agreement between those knowledgeable about TB and those knowledgeable about vaccines was 61.9% ($\kappa = 0.27$).

Fifty percent said they would like to know if they have HIV, 24% felt that they were not at risk of HIV and only 3% admitted to having already had an HIV test.

Participants indicated that their willingness to participate (Figure 1) would be positively influenced by the provision of more information (66%), the research benefiting the community (33%), benefiting the school (33%), personal benefiting the participants (31%) or if someone known to the participants monitored the research (21%).

In response to the following question “Who would assist you in deciding on whether to participate in a research project?”, parents were by far the most commonly cited (70%), followed by friends (27%), teachers (24%), siblings (13%) and other (11%). As far as source of health information was concerned, parents (54%), teachers (47%) and health facilities (41%) were rated the highest.

When comparing attitudes to research procedures by socio-economic group, little difference was observed (table 2) with the greater willingness of learners from more affluent schools to answer questions being the only variable with a statistically significant difference. However, learners from less well resourced settings tended to be more interested in knowing their HIV status than those from the better off schools. Benefiting personally from the research was more important to learners from the better resourced schools.

Summary knowledge of TB, summary knowledge of vaccines and a correct response to the question on research was compared with willingness to participate in different procedures (Table 3). In general, greater knowledge was likely to be associated with greater willingness to participate in procedures. However, knowledge of TB and vaccines was not statistically significantly associated with willingness to undergo a blood draw.

Analysis by grade was conducted but no clear trends emerged with socio-economic group tending to have a greater influence on responses than grade.

Focus group discussion (FGD) results for the parent group

Reasons for Participating:

The general feeling amongst parents was that the research would have educational, societal and health benefits. However, some parents expressed fears regarding certain aspects of research,

as reflected in the verbatim quotes provided as follows: “How do we know that you are not going to inject our children with TB so that you can study them?”, “But is the vaccine safe to use in people?” and “What if we get side-effects?”

Research Exposure:

Parents reported minimal prior exposure to research apart from market surveys conducted in shopping centres.

Perceptions about TB research and TB itself

Generally, parent participants felt very strongly that TB vaccine research is needed and that a new vaccine would be of enormous benefit to their communities. Those from a more affluent community had fewer family members with TB than those with a lower socio-economic status.

TB Stigma:

Participants had faith in TB treatment and felt that as long as people remained faithful to taking their medication, they had no reason to ostracise anyone who had TB.

TB Screening Site:

Parents had no particular preference in terms of the TB screening location. They agreed that a clean and safe environment is a significant prerequisite for conducting the research. A designated area at a school or the local health facility was acceptable.

Study/Screening Staff:

Parents, like their adolescent children identified professional competence, neatness and cleanliness, confidentiality, trustworthiness and friendliness as very important characteristics for research staff. Gender and age were not considered important. They expressed a hope that the research team present at the FGDs would be conducting the research as they felt confident in the team.

Vaccination:

Parents were particularly concerned about the safety and side-effects of the proposed new vaccine. They referred to the scarring on the right upper arm, the site of BCG vaccination, and they wanted reassurance that the new vaccine had been approved as being safe for use in human subjects.

Focus group results for the adolescent group

Reasons for Participating:

The reasons given for their participation in the research included curiosity, an interest in understanding more about TB and most importantly a desire to make a contribution to a body of knowledge that can be of benefit to the larger, and often less privileged, population.

Research Exposure:

As with their parents, the exposure of adolescents in this community to research was limited to market type surveys or no exposure at all.

Perceptions about TB research and TB itself

Most participants regarded the TB research as being very important and acceptable. Given the high prevalence of TB in the region, it was not surprising that there was a heightened awareness of the need for TB –related research. Those adolescents attending the predominantly Xhosa-speaking school expressed a fear of TB and associated it with death. Participants also wanted the assurance that needles would be opened in their presence and discarded immediately once used.

TB Stigma:

The degree of stigmatization varied from: “If I am exposed to a person with TB then my immune system must take care of it, I can’t go about avoiding people” to “Yes I would feel a bit uncomfortable [being with a person with TB]” and finally an admission that “It would be better if a person with TB kept to themselves until they got better.”

TB Screening Site:

Absolute assurance of privacy and confidentiality was considered essential if a dedicated space on the school premises were to be used for the study.

Adolescent participants drawn from the predominantly “Xhosa” school were more likely to associate TB with shame and stigma. Consequently their preference for TB screening was a venue away from the school.

One respondent said: “If you come to school and then everybody will see me going to that TB room and then they will talk and I will feel bad.”

Study/Screening Staff:

The highly valued characteristics for research staff were as mentioned above for the parent group. Gender and age were not considered to be significant characteristics.

HIV testing

Most were opposed to the idea of anonymous HIV testing and if tested would want to know the result. Most indicated a willingness to undergo an HIV test.

Vaccination:

Immunization was not known to be a TB prevention strategy and a large proportion of participants did not know whether they had been immunized. A common concern expressed in every focus group session related to the safety and side-effects of a new TB vaccine. This concern was expressed by a 15 year-old boy: "I heard that vaccines can be dangerous because they have traces of mercury. Is that true?"

Once an explanation of the procedures of testing a vaccine in the laboratory and the standards to which vaccines have to conform prior to testing on human subjects was given, the majority of these parents and their children found TB vaccination to be highly acceptable, suggesting that there will be great interest in these vaccines once they become available.

4. Discussion

These results showed a fair knowledge of TB amongst the high school adolescents in this community but a poor level of knowledge about vaccines. Willingness to participate in research varied depending on the type of procedures involved. While there were differences in knowledge levels between lower and higher socio-economic groups, attitudes to research were similar. However, there was a distinct difference in attitudes towards HIV testing with children from poorer families being more interested in knowing their HIV status than children from more well off families. The provision of information on the research and the involvement of parents would assist in the decision-making process of whether to participate in a study or not.

Willingness to participate in research procedures was in general associated with knowledge of TB and vaccines. However, this was different with phlebotomy as knowledge of TB and vaccines were not significantly associated with willingness to undergo a blood draw.

The fair levels of knowledge about TB may reflect the high degree of prior contact with community or family members with TB which these adolescents have experienced as indicated by the focus group discussions. The poor level of knowledge about vaccines is of concern. The normal school curriculum does not include any health education on vaccines. This policy should be reviewed since vaccines for human papilloma virus (HPV), HIV and TB as well as boosters for other vaccines are being considered in adolescence. Lack of knowledge may inhibit participation in clinical trials and underscores the need by organisations considering vaccine trials to have an education campaign regarding vaccines amongst adolescents. This is supported by the finding that knowledge of vaccines and research was significantly associated with willingness to participate in research procedures in most instances. A review of HIV/AIDS knowledge amongst South African youth showed that while youth knew about AIDS, they were less familiar with HIV [7]. Specifically, youth were less familiar with how HIV is transmitted and about the asymptomatic HIV carrier status. The implication of this for this study is that while the adolescents surveyed in this study showed a fair level of knowledge about TB, there may be a lack of depth in their understanding. In a study on HPV in Finland, 79% of parents and only 33% of adolescents had heard of HPV but 86% of adolescents were willing to accept vaccination against HPV should it become available [8]. The authors of this study also recommended improving knowledge and awareness of HPV as a way of dealing with potential resistance to vaccination. In Kentucky, USA, 44.4% of a sample of adult women in two counties indicated that

they had heard of HPV [9]. Of course, knowledge itself would not be sufficient to change attitudes. The national risk survey in South Africa [6] indicated high levels of exposure to HIV education but adolescents still participated at high rates in high risk sexual practices. This suggests that any education strategy with respect to vaccines should be carefully thought through if the intention is to change attitudes and behaviour.

The differences in levels of knowledge between high and low socioeconomic groups are probably a reflection of differences in resources between the schools as well as differences in home circumstances. The usefulness of this information is that it could help educational campaigns in their design and targeting. Poorer communities would have the higher rates of TB and would benefit most from a TB vaccine. For these reasons, their participation in TB vaccine trial would be crucial but informed consent principles would require that their participation must be based on a sound understanding of what such participation would mean.

Anonymous HIV testing in this group was not perceived positively and this should be taken into account when anonymous HIV testing is considered in other studies. Anonymous HIV testing is utilised as a means of determining HIV trends in epidemiological studies. However, since ARVs and TB preventive therapy are benefits that are available to someone who knows his or her HIV status, it would not be easy to justify the anonymous testing.

Differences seen in willingness to test for HIV between the socioeconomic groups probably reflect differences in perceived risk. Perceived risk may be based on community misconceptions

about what type of person is at risk or current sexual practices. Certainly, the proportion of school learners participating in high risk behaviour as indicated by a national survey [6] emphasises the importance of HIV testing in this group. The one HPV study quoted above from the USA found that women from lower income families were more willing to accept HPV vaccination for girls than higher income families [9].

The differences in willingness to participate in different procedures are understandable given that some procedures such as phlebotomy and tuberculin skin testing involve some degree of pain or discomfort. This is likely to be a key determinant in recruitment for TB vaccine trials as both of these latter procedures are likely to be part of study screening procedures. A South African study amongst adolescents looking at willingness to participate in HIV vaccine trials found that increasing age and length of residence were significantly associated with willingness to participate [10]. Provision of study information is likely to play a key role in getting adolescent agreement to participate and also that parents will play a key role in influencing adolescent participation. This allows for clear strategies to be formulated for achieving high levels of adolescent participation in TB vaccine trials. Roughly, one third of participants (31%) would want some personal benefit from participating. This option was the least highly rated but still represents a substantial proportion. A study looking at the recruitment and retention of Latino adolescents in clinical trials in the USA mentions incentives as being important to enrolling and maintaining adolescent participation. [11]. Parental and peer support also came out as important factors in this study.

C Mathews et al found in a study in South Africa on parental consent for school-based HIV/AIDS prevention research that while a large majority of parents provided postal consent for their children to participate, subsequent follow up showed that a lower proportion seemed to have actively participated in the consent process [12]. There would thus need to be clear processes to ensure both parental and adolescent informed consent in any planned TB vaccine trial. Trial organisers will have to prepare carefully regarding how procedures are explained during the consent process and then conducted in practice so as to minimise impact on recruitment.

The response rate of 65% and the variation amongst the schools probably indicates that those less interested in research are under-represented in the results and that this would need to be considered in interpreting the findings. The statistically significantly higher response rate for affluent schools means that their views are over represented in these findings. Because one class per school was selected, and because better resourced schools tend to have fewer classes per grade, this is another reasons why the views of better resourced schools are probably over represented in this study. Literacy levels may have affected the results particularly with respect to the comparison between the socioeconomic groups although every attempt was made to phrase the questions as simply as possible. Since the questions in this study were not particularly sensitive except for the one on HIV testing, it is felt that the answers given were reasonably reliable. The review on HIV/AIDS knowledge amongst South African youth referred to above [23] indicated that surveys using open ended questions showed lower levels of knowledge than those using closed questions. Because the questionnaire part of this study used closed questions in a multiple choice format, knowledge amongst adolescents might have been overestimated in this study. The lack of data on age and gender is also a limitation as this would

have added useful information on the age and gender distribution of the parameters assessed such as “fear of blood draws”.

5. Conclusion

TB vaccine trials amongst adolescents in this community are clearly acceptable provided that a number of preparatory steps are undertaken. Knowledge about vaccines in general and about TB vaccines specifically needs to be improved. A clear explanation of the types of procedures involved in TB vaccine trials and the risks involved should be outlined. Mechanisms should be put in place to minimise the negative aspects of invasive procedures that are undertaken in clinical TB vaccine trials. Parents should be fully involved at an early stage in the research process as their attitudes would have a strong influence on adolescent participation. Different strategies may be needed for different socio-economic groups.

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References

1. Global tuberculosis control: surveillance, planning, financing. WHO report 2006. Geneva, World Health Organisation (WHO/HTM/TB/2006.362).
2. Wiker HG, Mustafa T, Malen H, Riise AMD. Vaccine approaches to prevent tuberculosis. *Scand J Imm* 2006;64:243-50.
3. Skeiky YAW, Sadoff JC. Advances in tuberculosis vaccine strategies. *Nat Rev Microbiol* 2006;4:469-476.
4. Groenewald P, Dept of Information Management, Boland-Overberg Regional Office Annual Health Status Report 2004, Worcester, South Africa: Department of Health, Boland-Overberg Region, 2005.
5. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR. The clinical and radiological features of tuberculosis in adolescents. *Ann Trop Paediatr* 2000;20:5-10.
6. Reddy SP, Panday S, Swart D, Jinabhai CC, Amosun SL, James S, Monyeki KD, Stevens G, Morejele N, Kmabaran NS, Omardien RG and Van Den Borne HW. Umthenthe Uhlaba Usamila - The South African Youth Risk Behaviour Survey 2002. Cape Town: South African Medical Research Council, 2003.
7. Eaton L, Flisher AJ. HIV/AIDS knowledge amongst South African Youth. *SA J Child Adolesc Ment Hlth* 2000;12:97-124.

8. Woodhall SC, Lehtinen M, Verho T, Huhtala H, Hokkanen M, Kosunen E. Anticipated acceptance of HPV vaccination at the baseline of implementation: a survey of parental and adolescent attitudes in Finland. *J Adolesc* 2007; 40:466-469.
9. Hopenhayn C, Christian A, Jay Christian W, Schoenberg NE. Human papillomavirus vaccine: knowledge and attitudes in two Appalachian Kentucky counties. *Cancer Causes Control* 2007;DOI 10.1007/s10552-007-9007-7.
10. Jaspan HB, Berwick JR, Myer L, Mathews C, Flisher AJ, Wood R, Bekker LG. Adolescent HIV prevalence, Sexual Risk, and Willingness to participate in HIV vaccine trials. *J Adolesc* 2006;39:642-48.
11. Villarruel AM, Jemmott LS, Jemmott JB, Eakin BL. Recruitment and retention of Latino Adolescents to a research study: lessons learned from a randomised clinical trial. *J Spec Pediatr Nurs* 2006;11(4):244-50.
12. Mathews C, Guttmacher SJ, Flisher AJ, Mtshizana Y, Hani A, Zwarenstein M. Written parental consent in school-based HIV/AIDS prevention research. *Am J Public Health* 2005;95(7):1266-9.

Table 1: A comparison of responses to knowledge based questions with respect to TB vaccine trials and procedures by socio-economic group

Correct answer selected	All (N=270) n (%)	Group A* (N=126) n (%)	Group B* (N=144) n (%)	RR	95% CI
TB					
TB is a kind of infection affecting mainly the lungs	190 (70%)	102 (81%)	88 (61%)	1.32	1.13-1.55
TB is spread by germs that have been coughed into the air	211 (78%)	90 (71%)	121 (84%)	0.85	0.75-0.97
With treatment TB can be cured	207 (77%)	95 (75%)	112 (78%)	0.97	0.85-1.11
The direct cause of TB is a germ	144 (53%)	85 (68%)	59 (41%)	1.65	1.31-2.07
Vaccines					
Vaccines are given to prevent infections	110 (41%)	72 (57%)	38 (26%)	2.14	1.56-2.92
Vaccines are made of bits of dangerous germs that have been made safe	68 (25%)	40 (32%)	28 (19%)	1.63	1.07-2.48
Smallpox no longer occurs in the world due to a worldwide vaccination programme	110 (41%)	65 (52%)	45 (31%)	1.65	1.23-2.22

Research

Research is when scientists try to find out something new	199 (74%)	105 (83%)	94 (65%)	1.28	1.11-1.47
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*Group A, the schools from a higher socio-economic status comprised one grade 8, two grade 9, one grade 10 and one grade 11 class and group B, the lower socio-economic status schools comprised one grade 8, two grade 10, one grade 11 and two grade 12 classes.

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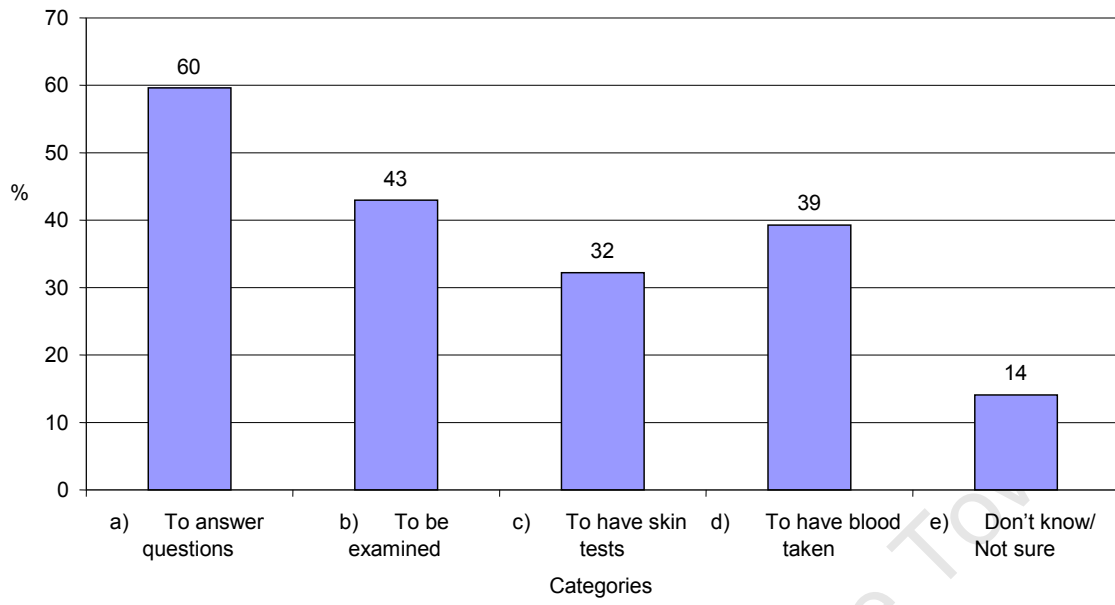
Table 2 A comparison of responses to attitude based questions with respect to TB vaccine trials and procedures by socio-economic group

Answer indicated	Group A (n=126) n (%)	Group B (n=144) n (%)	RR	95% CI
I would be willing to participate in research to answer questions	85 (68%)	76 (53%)	1.28	1.05-1.56
I would be willing to participate in research to be examined	51 (41%)	65 (45%)	0.90	0.68-1.18
I would be willing to participate in research to have skin tests	45 (36%)	42 (29%)	1.22	0.87-1.73
I would be willing to participate in research to have blood taken	49 (39%)	57 (40%)	0.98	0.73-1.73
Yes, I do want to know if I have HIV or not	50 (40%)	85 (59%)	0.67	0.52-0.87
I would be more willing to take part in research if I personally got something in return	50 (40%)	34 (24%)	1.68	1.17-2.42

Table 3 A comparison of knowledge of TB, vaccines and research versus willingness to undergo different procedures

	Willingness to							
	Answer questions		Be examined		Have a skin test		Have blood drawn	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Knowledgeable about TB	1.42	1.13-1.8	1.27	0.93-1.72	2.04	1.31-3.18	1.21	0.87-1.67
Knowledgeable about vaccines	1.41	1.16-1.71	1.39	1.06-1.82	1.56	1.1-2.2	1.29	0.96-1.73
Correct answer to research question	1.77	1.3-2.42	1.61	1.1-2.38	2.03	1.2-3.43	1.66	1.08-2.48

Figure 1:



Willingness to take part in research amongst adolescents (n=270)

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Chapter 8: Discussion and Conclusion

This thesis set out to measure tuberculosis (TB) infection and disease prevalence and incidence, willingness to participate in TB vaccine trials and factors influencing these outcomes amongst school-going adolescents aged 12-18 years in a high TB burden rural setting. The following was found:

The prevalence of latent TB infection was 55% on the basis of the tuberculin skin test (TST) and 51% using the QuantiFERON TB Gold (in-tube) (QFT) assay. The main risk factors for latent infection on multivariate analysis were being of black/mixed race racial group compared to being white or Indian, male sex, older age, having had a household TB contact, low income and low education level.

The prevalence of TB disease was 3/1000 (95% CI 1-4/1000). A positive TST (sensitivity 85% [95% CI 62-100%]) and QFT (sensitivity 94% [95% CI 79-100%]) were sensitive indicators of TB disease. In contrast, TB related symptoms (12.5% [95% CI 0.0-30.2%]) and household TB contact (31.3% [95% CI 0.0-63.9%]) were not. However, all these screening tests alone or in combination had positive predictive values of less than 2%.

The incidence of bacteriologically confirmed TB was 0.45/100 person-years (95% CI 0.29-0.72) using a protocol defined case definition. When the case definition was varied, rates ranging from 0.30 to 0.59/100 person-years were obtained. The clinical profile of TB cases was mainly of the adult type and most had radiographic abnormalities on chest x-ray. Black or mixed race versus white/ Indian, maternal education of primary school or less or unknown, a positive baseline QFT and a positive baseline TST were significant predictors of TB disease in adjusted analysis.

Both a positive TST and a positive QFT were equally predictive of the onset of TB on follow up at 0.60 cases per 100 person years (pyrs) (95% CI 0.43–0.82) and 0.64 cases per 100 pyrs (95% CI 0.45–0.87) respectively; incidence rate ratio [IRR] of TST to QFT was 0.95 (95% CI 0.59-1.51).

Adolescents had fair knowledge about TB and were willing to participate in clinical trials of new TB vaccines but the degree of likely participation varied depending on the procedures involved.

These results and their limitations were discussed in detail in the individual chapters and will not be repeated to any great extent here. Instead, specific discussion areas will be addressed.

Feasibility of doing trials in adolescents

The discussion below draws on different chapters of this thesis so as to address one of the underlying themes, viz feasibility of TB vaccine trials in adolescents.

Adolescents as a target for TB vaccines

There has been an important shift in approach in the TB vaccine world as outlined in the recently published Blueprint for TB vaccines (1, 2). Previously, the focus of TB vaccine development has been on infants, HIV positive persons and adolescents. There has been a shift towards focusing efficacy trials of new TB vaccines on HIV negative adults and adolescents, at least initially, for the following reasons: 1) clinical endpoints are difficult to define in infants (3) and an infant vaccine will have limited immediate impact on the TB epidemic (4), 2) immune impairment in HIV positive persons may limit TB vaccine efficacy, and 3) HIV negative adults and adolescents are the main source of disease transmission (5). Once efficacy has been established in adults and adolescents, efficacy trials or bridging trials will be done in infants and HIV positive persons. Although TB incidence rates are lower in adolescents than adults (6), it is still proposed to include them in these initial trials. A recent paper discussing a model Phase III TB vaccine efficacy trial proposes HIV negative adults *and* adolescents as a target group but provides an alternative option of focusing initially on adults with bridging studies to adolescents to follow (7). This latter option was based on concerns raised by the Medicines Control Council of South Africa about trials being conducted in adolescents (the Council views adolescents as a vulnerable group) and also on the lower rates of TB in adolescents than in adults. Nevertheless, adolescents were still seen as an attractive target due to their being accessible through schools for mass TB vaccine campaigns should these be proposed. Given the above, the data produced in this thesis remain topical and relevant to TB vaccine development.

Implications of the prevalence of latent TB infection in adolescents for TB vaccines

The fact that there was a prevalence of latent TB infection in this adolescent population of just over 50% has implications for the type of vaccine to be tested in this group. Certain vaccines target those who are TB uninfected while others focus on those who are infected and some target both (8, 9). The data in this thesis show that substantial numbers of both groups are available for recruitment. However, TB incidence rates differ between the two groups and this would mean that much larger numbers would be needed to evaluate efficacy in latent TB negative adolescents. The identified risk factors may assist with targeting recruitment should one or other group be preferentially required to be enrolled. Poorer communities, those that have had household contacts, age above 15 years or males may be preferentially enrolled should latently infected persons be required for recruitment and the opposite (those without household TB contacts, age 15 years or less or females) should latent infection negative persons be required. However, the ethics of such preferential selections would need to be carefully considered as well as the implications for eventual licensure should the vaccine prove efficacious. Also, screening for latent TB infection prior to vaccination may not be practical with an approved vaccine suggesting that using latent TB infection as an inclusion or exclusion criterion in a licensure trial may bring challenges when the vaccine is available for public use.

Screening for TB amongst adolescents in TB vaccine trials

Exclusion of participants with TB disease would be necessary in all TB vaccine trials at baseline. The thesis data show a relatively low prevalence of TB disease in adolescents of 3/1000. This is good news for recruitment potential. However, the detection methods used underestimated the prevalence because smear negative culture positive TB was not screened for. Nevertheless, prevalence would still be relatively low even if this group were included. While tests for latent infection were sensitive indicators of TB disease, the low positive predictive values mean that screening for TB, which is necessary in TB vaccine trials, will be costly and have a low yield. Other studies have shown chest radiography to be more sensitive at detecting TB (10, 11) and this may be an area for further investigation taking due cognizance of the practicality of using this method of screening in the efficacy trial context. A licensed TB vaccine, should this become a reality, would also require the exclusion of TB prior to administration but in depth screening may not be practicable. Only simple symptom screening with its limitations may be feasible. Thus, vaccine safety testing in persons with active TB will be required prior to licensure.

Implications of the incidence rate of TB in adolescents for planning TB vaccine trials

The incidence rate of TB in adolescents of 0.45/100 person years and the implications for the sample size of a phase IIb efficacy trial was discussed in chapter 5 (confidence interval for efficacy above 0). There is no agreement as yet on the target efficacy for a new TB vaccine (2). However, there is a view that efficacy targets at this stage should be modest. A sixty percent reduction in disease between the vaccine and placebo groups appears to be a commonly used efficacy target based on current efficacy trials with MVA 85A in infants (12) and HIV positive adults (ClinicalTrials.gov identifier: NCT01151189) and from that which was used in a modelling exercise (4). Using this efficacy target and accepting a lower confidence interval bound for an efficacy of 30%, the sample size for a Phase III trial in adolescents would be approximately 20,000 to 25,000 if one applies the data from this thesis and depending on assumptions made about loss to follow up. Even higher sample sizes would be needed in other countries which are likely to have a lower TB incidence rate in adolescents. This will be too large a sample for enrolment at one site but phase III trials of such a magnitude would usually be multi-centred and thus feasible from that perspective. Given the geographical diversity of efficacy measures for BCG (13), a multi-centred approach to trials of new TB vaccines would in fact be desirable. As indicated above, the risk factors for TB disease show that measures of latent TB infection will have a strong influence on sample sizes. The adult type profile of TB seen in adolescents will make endpoint definition easier than in infants and make efficacy results of trials in adolescents easier to interpret given that a specific case definition could be applied.

Willingness to participate by adolescents in TB vaccine trials

Adolescents and their parents in a high burden setting expressed a desire to participate in TB vaccine trials. While only 40% said they would be willing to undergo phlebotomy and even fewer a skin test (32%), we managed to recruit 58% in the cohort study. This shows that with correct marketing, adolescents may be persuaded to participate even when there are fears about procedures such as phlebotomy. Nevertheless, fear of blood draws appeared to be a common reason for refusal to participate and for withdrawal. Consideration should be given to this when planning trials in adolescents.

In summary, based on the prevalence and incidence of TB in adolescents and the risk factors for these that were found in the studies reported in this thesis, and on the fact that adolescents remain an important target for TB vaccine trials, adolescent trials or trials including adolescents are feasible.

Implications for TB control

Another broad aim of this thesis was to examine the implications of TB epidemiology amongst adolescents for TB Control Programmes. This thesis has presented the prevalence and incidence of TB amongst adolescents in schools.

The role of age and gender

There is an increasing incidence of TB in adolescence after the phase of low incidence in the pre-adolescent childhood years as shown by other studies (14, 15) and by this thesis. There has been speculation that this is due to hormonal changes and/or viruses but this has not been established (16, 17). The initial predominance of TB in females in adolescence followed by a male predominance in adulthood (14, 15) also point towards a hormonal explanation. The influence of psychosocial factors has been examined, but little evidence has been found to support these as an explanation of the increasing incidence (18). Recent work in Cape Town is enlightening in that it confirms adolescence to be a period of high social interactions (19). In the context of communities with a high TB burden, this would mean an increased risk of exposure to active TB cases. The research group conducting the work on social interactions amongst adolescents also showed adolescence to be a period of high force of infection thereby supporting this hypothesis (14). Adolescents at schools represent a high proportion of all adolescents (83% in the thesis based study area according to Census 2001). Of note is that the distribution of TB amongst different schools was not uniform, with schools in poorer communities having a higher burden than those in more affluent communities. The role of HIV in the increasing TB incidence during adolescence is not clear since HIV prevalence tends to be lower amongst adolescents (20). It may not be the key driver in the age related rise in TB incidence but may accelerate the increase in the older age groups.

Adolescents as an accessible group for intervention

Given the high volumes of TB suspects being processed in current TB Control programme conditions, the number of cases being diagnosed and treated, and the rising prevalence of drug resistance, it may be difficult to address prevention

(contact tracing, active case finding, TB education) in adolescence and to make adolescent TB a focus of interest. Nevertheless, a case can be made for giving this group attention. Adolescents represent an accessible group for screening and health promotion programmes through the schools. The TB exposures in adolescence may in fact be responsible for the peak seen in adulthood. Thus, interventions in schools may be important for TB Control Programmes. A booster vaccination for diphtheria and tetanus is currently scheduled for adolescents aged 12 years in the routine EPI programme. HPV vaccine is a vaccine being considered for administration in early adolescence. New HIV and TB vaccines (should these be discovered in the future) could be added to the above in the adolescent period. Thus, there may be scope for greater involvement by the public health system in schools in the future which may then provide a framework and infrastructure within which TB control could be exercised. Clearly, primary prevention with an efficacious vaccine would be the best intervention but this does not currently exist. In the absence of such a vaccine, secondary prevention through contact screening may be a suitable alternative intervention particularly when adolescents are diagnosed while at school. Contact tracing usually focuses on household contacts and including screening of school class contacts for adolescents may be needed.

The challenges of screening for TB

The fact that symptoms were not reported in most cases diagnosed at baseline is cause for concern. This may be due to early detection of disease, a reluctance by adolescents to report symptoms or due to adolescents not recognising cough as a symptom due to smoking. Active untreated cases in the school environment where adolescents congregate in classrooms may be the cause of the high force of infection found in adolescents (21). The lack of symptoms complicates screening efforts since carrying out TSTs or drawing blood for QFT may not be feasible or cost-effective. Thus, routine screening is not likely to be feasible in the current environment. However, since TB incidence has an uneven distribution, with higher rates in poorer communities, some targeting of interventions may be needed.

Education interventions

The data in this thesis (chapter 7) showed that knowledge of TB was fair amongst adolescents. Improving adolescent knowledge of TB may be of value in improving early detection of TB as a means of limiting the spread of TB. Knowledge of vaccines was poor. This should be improved so that acceptability of a new TB vaccine is high should one become available in the future.

GeneXpert screening

Sputum GeneXpert screening of TB suspects may be worthwhile exploring given its higher sensitivity than normal sputum smears and its rapid turnaround of results, with rifampicin resistance screening as an additional benefit (22). This diagnostic test was not evaluated in the work contributing to this thesis but is currently being rolled out in the public health system of South Africa (23). It may more correctly be described as a diagnostic tool rather than a screening tool since a positive result is enough to initiate treatment without the need for further investigations. There may be implementation obstacles in trying to use it as a screening tool since it requires the provision of an adequate sputum sample. It could be used as part of a screening algorithm where only symptomatic individuals who have a productive cough are tested.

In summary, a case can be made for targeting adolescents at school for interventions to support TB control efforts. Re-establishing contact tracing using GeneXpert as a screening tool with an emphasis on poorer communities, in combination with health education may be the most feasible option currently given the absence of an efficacious booster TB vaccine.

Isoniazid prophylaxis

A repeated concern raised by international reviewers of the published work from this thesis was the lack of isoniazid preventive therapy (IPT) for adolescents confirmed to be latently infected with *M. tuberculosis*. Firstly, they argued that a higher standard of care might be expected in a research environment since more resources may be available through international funding. Secondly, prior trials have shown that IPT is beneficial in reducing the risk of progression to TB (24) and it is thus standard of care in many countries. Thirdly, the data from this thesis showed a three times higher risk of progression to TB when latently infected (chapters 5 and 6). The current standard of care in South Africa is that IPT is only provided to children under the age of 5 years exposed to an infectious adult with TB disease and to HIV positive persons shown not to have evidence of active TB disease (25, 26). The Human Research Ethics Committee of the University of Cape Town did not require IPT to be provided in this trial.

Prior trials of IPT done in the 1950s and 60s were done in both developed and developing countries (24). These studies were done in the in pre HIV era and thus represent data for efficacy in the HIV negative population. These studies showed a protective efficacy of isoniazid therapy of 90% when the analysis was done only on compliant participants. Importantly, the benefit of IPT in preventing TB was shown to last up to 19 years after administration (27). These findings with respect to INH prophylaxis in HIV negative populations were confirmed by a more recent Cochrane review published in 2005 which included 11 studies from developed and developing countries (28). In this review, only studies with a minimum 2 year follow up were included and they found a relative risk of 0.4 (95% CI 0.31-0.52) of contracting TB if on isoniazid prophylaxis for 6 or 12 months. They also found the risk of death from TB was significantly reduced when on prophylaxis.

Contrasting results have been found in other trials. A recent trial amongst South African gold miners (HIV positive and negative) investigated the value of IPT as an intervention strategy (29, 30). Miners on IPT had a reduced risk of developing TB disease but once they had stopped the course of treatment, their risk of TB was not different from those not on IPT. The IPT trial in Botswana evaluating 36 months of isoniazid treatment in HIV positive adults similarly showed benefit while trial participants were on IPT (particularly for those that were TST positive at baseline) but this benefit was also lost once treatment had been stopped (31). The high risk of reinfection may be responsible for this lack of long term benefit in these studies (32). Thus, the long term benefit of IPT when given for a limited duration of time is not as clear cut in a high burden setting as in a low risk setting and studies on lifelong IPT have not yet been done. Recent World Health Organisation and South African guidelines on IPT for HIV positive persons both clearly recommend IPT for HIV positive persons (26, 33). The CDC (Atlanta, USA) currently recommend IPT for latent TB infection more broadly than HIV positive persons and children under 5 years but their most recent guidelines only focus on testing high risk groups for latent TB infection (34). These high risk groups include HIV positive persons, persons on immunosuppressive therapy, recent immigrants who have been in the country for less than 5 years, children under 5 years, workers in high risk occupations and persons with illnesses which predispose to contracting TB. In other words, current practice in the USA is that IPT is not provided universally to those with latent TB infection.

The prevalence of latent TB infection is high (>50%) in South African adults and adolescents in the high burden area where the studies for this thesis were conducted. There would be enormous resource considerations should IPT be implemented for HIV negative persons. Adverse events due to the medication would be a concern since hepatitis (28) and peripheral neuropathy can occur with isoniazid administration. While these side effects are uncommon, there should be a clear benefit to justify the exposure to these risks. Given the inconsistent benefits and potential harm, it is difficult to justify its use as routine for all persons who are infected. Nevertheless, the high burden of TB in South Africa requires us to explore all avenues to bring TB under control. It may be worthwhile exploring IPT in an adolescent setting given the limited number of studies of IPT in high burden settings amongst HIV negative populations. Responses may be different from gold miners who have been exposed to silica dust, a known risk factor for TB.

The nature of latent TB infection has come under scrutiny (35, 36). There is now a common view that latent TB infection represents a continuum of states rather than a single entity and that despite the absence of symptoms, host-organism interaction is occurring at a local level. The presence of *Mycobacterium tuberculosis* (*Mtb*) in tissues of individuals not diagnosed with TB is still being demonstrated in the modern era (37). Further investigation and insight may be needed to understand the nature of latent infection before implementing interventions such as IPT.

In summary, there is currently not enough evidence to support IPT in adolescents in high burden settings but further discussion and research on this issue are needed.

The IGRA debate: IGRA versus TST

Data from this thesis were used to support US policy development on IGRAs (38). A review and meta-analysis done in children has shown that IGRAs appear to behave differently in high burden compared to low burden settings (39). A key issue has been the predictive value of TST versus IGRA for TB disease. One recently published systematic review and meta-analysis found that both IGRAs and TST were both poor predictors of TB disease (based on positive predictive values) and that other factors such as costs, relative specificity in different populations, etc., should guide which to use rather than predictive ability(40). A second more recent review found IGRAs to have a superior predictive ability to that

of the TST and recommended their use over the TST, particularly when targeted at high risk groups (41). Data from this thesis were quoted in both reviews. The first review included 15 studies in its meta-analysis but then focused its main analysis on 5 studies because of concerns that the data from certain studies were subject to incorporation bias or differential work-up bias and the data in this thesis was classified as such. This was a valid concern in that a positive IGRA was an indication for investigation for TB in the study reported in this thesis. However, this author's view is that the impact of this potential bias was limited since there were other criteria for investigation such as symptoms, household contact and a positive TST and in addition, surveillance of clinics was conducted for any cases diagnosed by the health services. The authors of the first review (40) found a predictive value based on the restricted group of 5 studies of 2.11 (95% CI 1.29-3.46) for IGRAs versus 1.60 (0.94 – 2.72) for the TST using the 10 mm cut-off.

The second meta-analysis included 28 studies since it was published later and more data had become available. They did a subset analysis on a group of studies which recruited high risk populations. They reported a predictive value for TB disease of 2.7% (95% CI 2.3-3.2%) for the IGRA compared to 1.5% (95% CI 1.2-1.7%) for the TST when all 28 studies were included. When only the high risk group was analysed, the PPV was 6.8% (95% CI 5.6-8.3%) for IGRA and 2.4% (95% CI 1.9-2.9%) for TST. They excluded the study published from this thesis from their "high risk" group since they argued that only 25% of participants had reported a household contact, a high proportion were positive for TST and the IGRA amongst those with a recent contact, and because there was no difference in TB incidence rates between those recently exposed compared to those more remotely exposed in time. Clearly, these authors misunderstood that absence of evidence of contact in high burden settings does not define these settings as low risk. A higher proportion positive for latent TB infection in those recently exposed could be due to waning of responses over time or could confirm that exposures are more recent. The data comparing incidence rates amongst those recently exposed to those remotely exposed shows a clear trend of higher incidence amongst those more recently exposed – the lack of significance was more likely to be due to a lack of power rather than a lack of a difference. Their reasons for excluding this thesis's study from the high risk group do not appear to be valid. The authors of this second review did not address the question of heterogeneity which was reported as high but not discussed as a limitation. They used cumulative incidence rather than incidence rates which were used in the first review.

Currently, guidelines on IGRAs in various countries around the world have adopted varying approaches (42). The WHO which also used the data reported in this thesis has produced a policy document on the use of IGRAs in low and middle income countries and has concluded that both IGRAs and the TST cannot accurately predict the onset of TB and neither should be used for the diagnosis of TB disease (43). Most individuals (>95%) with a positive IGRA test do not develop TB. In addition, because of cost and complexity, IGRAs are not recommended for use in low and middle income countries. South Africa does not have a policy on the use of IGRAs although IGRAs are available in the private sector. While the WHO policy document provides a useful guide for the use of IGRAs, the data in this thesis and those from other studies done in South Africa and elsewhere on the African continent should be able to provide the basis to support policy formulation on IGRAs in the region.

In summary, the use of IGRAs remains controversial and its value in high burden settings remains moot. South Africa and other high burden countries should develop guidelines on the use of IGRAs given the inconsistent findings in the literature. In line with the recommendations of the WHO, this author would recommend a cautious attitude towards introducing IGRAs in high burden settings for routine use. However, given the lack of data on their performance in high burden settings, their use and evaluation in research projects should be allowed and encouraged so that there is a comprehensive basis for policies on their use in high burden settings in the future.

Further areas of research

Not all the data produced from the studies that formed the basis for this thesis are included in this thesis and further publications are planned. These include a study of the role of QFT quantitative values in predicting TB disease, a comparison of surveillance methods for detecting TB disease and an analysis of conversions and reversions of both the TST and QFT.

However, arising out of this work, other future areas of research are proposed:

- *Chest radiography as a screening tool for TB in adolescents.* This could be studied in two ways: Purchasing a mobile x-ray unit for research purposes and screening all participants. Alternatively, using an investigation algorithm where selected participants are screened with a chest x-ray which is then performed by locally available services.
- *Cost-effectiveness of different screening tests for TB in adolescence.* Costs of the different screening methods would be collected and then evaluated against screening indicators such as sensitivity, specificity, positive and negative predictive values.
- *The yield of GeneXpert as a screening tool to screen adolescents at school for TB disease.* A cross-sectional study of adolescents in a school setting could be performed in which either universal or selective GeneXpert screening is done.
- *The potential benefit of IPT in adolescents in a high burden setting.* This would involve a randomised controlled trial in which adolescents who are randomly assigned to either IPT (at least 6 months) or placebo and followed up. It would be important to include at least two years of post-treatment follow up since it would be crucial to demonstrate persistence of benefit.

Conclusion

The data in this thesis have made a contribution to the literature on adolescent TB. The incidence and prevalence of TB infection and disease data, and the willingness to participate in clinical trials data should be of great value in planning clinical trials of new TB vaccines. This thesis has provided information for TB Control Programmes to support possible TB control interventions in adolescents. The IGRA data should contribute to the debate on the use of this technique in high burden settings, especially since the studies reported in this thesis are amongst the largest comparing the TST with an IGRA. The use of IPT in HIV negative persons remains a vexing question and there are still gaps in the literature with respect to adolescent tuberculosis. Thus, a number of further areas of research are proposed to build on this thesis.

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References

1. Brennan MJ, Thole J. Tuberculosis vaccines: a strategic blueprint for the next decade. *Tuberculosis*. 2012;92 Suppl 1:S6-13. Epub 2012/03/28.
2. Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, Hanekom W, et al. Predictive value of recent QuantiFERON conversion for tuberculosis disease in adolescents. *Am J Respir Crit Care Med*. 2012;186(10):1051-6. Epub 2012/09/08.
3. Hatherill M, Verver S, Mahomed H, Taskforce on Clinical Research Issues, Stop TB Working Group on TB Vaccines. Consensus statement on diagnostic end points for infant tuberculosis vaccine trials. *Clin Infect Dis*. 2012;54(4):493-501. Epub 2011/12/07.
4. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proc Natl Acad Sci*. 2009;106(33):13980-5. Epub 2009/08/12.
5. Corbett EL, Charalambous S, Moloi VM, Fielding K, Grant AD, Dye C, et al. Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. *Am J Respir Crit Care Med*. 2004;170(6):673-9. Epub 2004/06/12.
6. Wood R, Liang H, Wu H, Middelkoop K, Oni T, Rangaka MX, et al. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *Int J Tuberc Lung Dis*. 2010;14(4):406-12. Epub 2010/03/06.
7. Rustomjee R MR, McClain B, Brennan MJ, McCleod R, Chetty-Makan CM, McShane H, et al. Designing an adaptive phase II/III trial to evaluate efficacy, safety and immune correlates of new TB vaccines in young adults and adolescents. *Tuberculosis*. 2013;93(2):136-42. Epub 2012/12/23.
8. Hawkridge T, Mahomed H. Prospects for a new, safer and more effective TB vaccine. *Paediatr Respir Rev*. 2011;12(1):46-51. Epub 2010/12/22.
9. Stop TB Working Group on TB Vaccines. Tuberculosis vaccine candidates - 2011. 2012; Available from: http://www.stoptb.org/wg/new_vaccines/assets/documents/TB%20Vaccine%20Pipeline_rAug%202012.pdf. Accessed 25 May 2013.

10. Van't Hoog AH, Meme HK, Laserson KF, Agaya JA, Muchiri BG, Githui WA, et al. Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. *PloS One*. 2012;7(7):e38691. Epub 2012/07/14.
11. den Boon S, White NW, van Lill SW, Borgdorff MW, Verver S, Lombard CJ, et al. An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *Int J Tuberc Lung Dis*. 2006;10(8):876-82. Epub 2006/08/11.
12. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet*. 2013;S0140-6736(13):60177-4. Epub 2013/02/04.
13. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA*. 1994;271(9):698-702. Epub 1994/03/02.
14. Wood R, Lawn SD, Caldwell J, Kaplan R, Middelkoop K, Bekker LG. Burden of new and recurrent tuberculosis in a major South African city stratified by age and HIV-status. *PloS one*. 2011;6(10):e25098. Epub 2011/10/22.
15. Donald PR. Childhood tuberculosis: the hidden epidemic. *Int J Tuberc Lung Dis*. 2004;8(5):627-9. Epub 2004/05/13.
16. Weber HC, Beyers N, Gie RP, Schaaf HS, Fish T, Donald PR. The clinical and radiological features of tuberculosis in adolescents. *Ann Trop Paediatr*. 2000;20(1):5-10. Epub 2000/05/29.
17. Nagelkerke NJ, de Vlas SJ, MacDonald KS, Rieder HL. Tuberculosis and sexually transmitted infections. *Emerg Infect Dis*. 2004;10(11):2055-6. Epub 2005/07/14.
18. Geldenhuys H, Sorsdahl K, Kafaar F, Hatherill M, Hanekom WA, Stein DJ, et al. Risky behaviour and psychosocial correlates in adolescents - is there a link with tuberculosis? *Afr J Psychiatry*. 2011;14(5):383-7. Epub 2011/12/21.
19. Wood R, Racow K, Bekker LG, Morrow C, Middelkoop K, Mark D, et al. Indoor social networks in a South African township: potential contribution of location to tuberculosis transmission. *PloS One*. 2012;7(6):e39246. Epub 2012/07/07.

20. Directorate Epidemiology and Surveillance DoH, South Africa. The National Antenatal Sentinel HIV and Syphilis Prevalence Survey, South Africa, 2010, National Department of Health. South Africa: 2011. Available at: http://www.doh.gov.za/docs/reports/2011/hiv_aids_survey.pdf. Accessed on 25 May 2013.
21. Middelkoop K, Bekker LG, Liang H, Aquino LD, Sebastian E, Myer L, et al. Force of tuberculosis infection among adolescents in a high HIV and TB prevalence community: a cross-sectional observation study. *BMC Infect Dis.* 2011;11:156. Epub 2011/06/03.
22. World Health Organisation. Policy statement: Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System: World Health Organisation; 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf. Accessed 25 May 2013.
23. Mayosi BM, Lawn JE, van Niekerk A, Bradshaw D, Abdool Karim SS, Coovadia HM, et al. Health in South Africa: changes and challenges since 2009. *Lancet.* 2012;380(9858):2029-43. Epub 2012/12/04.
24. Ferebee SH. Controlled chemoprophylaxis trials in tuberculosis. A general review. *Bibl Tuberc.* 1970;26:28-106.
25. Department of Health Republic of South Africa. National Tuberculosis Management Guidelines: Department of Health, Republic of South Africa; 2009. Available at: http://familymedicine.ukzn.ac.za/Libraries/Guidelines_Protocols/TB_Guidelines_2009.sflb.ashx. Accessed on 25 May 2013.
26. Department of Health Republic of South Africa. Guidelines for Tuberculosis Preventive Therapy among HIV infected individuals. South Africa: Department of Health, Republic of South Africa; 2010. Available at: http://www.who.int/hiv/pub/guidelines/south_africa_hiv_tb.pdf. Accessed on 25 May 2013.
27. Comstock GW, Baum C, Snider DE, Jr. Isoniazid prophylaxis among Alaskan Eskimos: a final report of the bethel isoniazid studies. *Amer Rev Respir Dis.* 1979;119(5):827-30. Epub 1979/05/01.
28. Smieja MJ, Marchetti CA, Cook DJ, Smaill FM. Isoniazid for preventing tuberculosis in non-HIV infected persons (Review). *The Cochrane Collaboration.* 2005(2):1-24.

29. AIDSMAP. Thibela TB: Community-wide IPT did not improve TB control in South Africa's mines at the population level. 2012 [cited 2012 14 December]; Available from: <http://www.aidsmap.com/Thibela-TB-Community-wide-IPT-did-not-improve-TB-control-in-South-Africas-mines-at-the-population-level/page/2285760/>.
30. Fielding KL, Grant AD, Hayes RJ, Chaisson RE, Corbett EL, Churchyard GJ. Thibela TB: design and methods of a cluster randomised trial of the effect of community-wide isoniazid preventive therapy on tuberculosis amongst gold miners in South Africa. *Contemp Clin Trials*. 2011;32(3):382-92. Epub 2011/01/05.
31. Samandari T, Agizew TB, Nyirenda S, Tedla Z, Sibanda T, Shang N, et al. 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011;377(9777):1588-98. Epub 2011/04/16.
32. Verver S, Warren RM, Munch Z, Richardson M, van der Spuy GD, Borgdorff MW, et al. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet*. 2004;363(9404):212-4.
33. Department of HIV/AIDS STD, World Health Organisation. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource constrained settings: World Health Organisation; 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf. Accessed on 25 May 2013.
34. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. *MMWR Recomm Rep*. 2000;49(RR-6):1-51. Epub 2000/07/06.
35. Cardona PJ. Revisiting the natural history of tuberculosis. The inclusion of constant reinfection, host tolerance, and damage-response frameworks leads to a better understanding of latent infection and its evolution towards active disease. *Arch Immunol Ther Exp*. 2010;58(1):7-14. Epub 2010/01/06.
36. Zumla A, Atun R, Maeurer M, Mwaba P, Ma Z, O'Grady J, et al. Viewpoint: Scientific dogmas, paradoxes and mysteries of latent *Mycobacterium tuberculosis* infection. *Trop Med Int Health : TM & IH*. 2011;16(1):79-83. Epub 2011/02/24.
37. Barrios-Payan J, Saqui-Salces M, Jeyanathan M, Alcantara-Vazquez A, Castanon-Arreola M, Rook G, et al. Extrapulmonary locations of mycobacterium tuberculosis DNA during latent infection. *J Infect Dis*. 2012;206(8):1194-205. Epub 2012/06/27.

38. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K, et al. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. MMWR Recomm Rep. 2010;59(RR-5):1-25. Epub 2010/06/26.
39. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, Hatherill M, Moyo S, Hanekom W, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. Ped Infect Dis J. 2011;30(8):694-700. Epub 2011/03/24.
40. Rangaka MX, Wilkinson KA, Glynn JR, Ling D, Menzies D, Mwansa-Kambafwile J, et al. Predictive value of interferon-gamma release assays for incident active tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(1):45-55. Epub 2011/08/19.
41. Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon-gamma release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. Chest. 2012;142(1):63-75. Epub 2012/04/12.
42. Denkinger CM, Dheda K, Pai M. Guidelines on interferon-gamma release assays for tuberculosis infection: concordance, discordance or confusion? Clin Microbiol Infect. 2011;17(6):806-14. Epub 2011/06/21.
43. World Health Organisation. Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries. World Health Organisation. 2011. Available at: http://www.who.int/tb/features_archive/policy_statement_igra_oct2011.pdf. Accessed on 25 May 2013.

Appendices

Appendix A

Overall methodology of adolescent cohort study (to be read in conjunction with Appendix B which provides a graphic illustration of study design)

Study setting: The study took place in the town of Worcester (and surrounding villages), approximately 100 kilometres from Cape Town, South Africa.

Study participants: All adolescents aged 12-18 years attending 11 high schools in the study area were approached to participate.

Study procedures: At enrolment, a questionnaire was administered to study participants and their parents to collect demographic and socio-economic data. These included parental income and parental education. The racial classification of participants “White”, “Mixed race”, “Indian” and “Black” was based on a system used in the apartheid era, but still used today as a proxy for socio-economic status and to measure social change. History of BCG vaccination (reported), history of current and past TB disease, current and past TB household contacts, hospitalization in the past 6 months and information on chronic diseases were obtained. Participants were examined for the presence of a BCG scar.

Blood was taken for QuantiFERON® TB Gold In-tube (Cellestis). A tuberculin skin test was then immediately administered using the Mantoux method on either forearm, using 2 tuberculin units of RT23 (Statens Serum Institut, Denmark).

Induration at the TST site was read 48 - 96 hours later with a ruler or a caliper, by trained study personnel. Those with previous or current TB did not have a TST performed, in order to prevent severe allergic reactions. The QuantiFERON test (QFT) was performed as recommended by the manufacturers.

The first 481 participants enrolled were all screened with a single sputum culture. For logistical reasons (the microbiology laboratory was not able to cope with a single sputum for culture on all study participants), the protocol

was then amended to only screen the following: those with (1) TB symptoms (unexplained cough, night sweats, loss of weight or loss of appetite for two weeks or more, or haemoptysis), (2) a household contact within three years of enrolment, (3) a TST ≥ 10 mm, or (4) a positive QFT. These participants were considered TB suspects and were investigated by the collection of two sputum samples for smears on two separate occasions. The sputum was concentrated and examined with a fluorescent stain (auramine). A positive smear was followed by a culture (liquid, Mycobacteria Growth Indicator Tube (MGIT) and solid, Loewenstein-Jensen) to confirm the presence of *Mycobacterium tuberculosis*, a chest radiograph and an HIV test.

Study follow up: All participants enrolled were scheduled for follow up visits after two years which included a blood draw for QFT and the administration of a TST. About half underwent three monthly visits prior to this which included six monthly QFTs and annual TSTs to compare follow up strategies while the other half were seen only at baseline and two year. At follow up visits, those with new symptoms or a new household contact compared to baseline, a converted TST (≥ 10 mm increase from baseline) or converted QFT (change from negative to positive) were investigated for active TB. In addition, passive surveillance was conducted of TB clinic and hospital admission registers in the area for any TB cases diagnosed between visits. Investigation for TB involved the collection of two sputum samples for smear examination on two separate occasions. For persons with at least one positive smear, a culture was performed, a chest x-ray done and an HIV test offered. The radiologist's report on the chest x-ray was used to classify chest-x-ray findings.

Study duration: Enrolment started in July 2005 and was completed in April 2007. Follow up was completed at the end of February 2009. Owing to financial constraints, about 10% of the two year visits were performed one to two months short of two years towards the end of the study. Follow up was thus continued for a minimum of 22 months. Those completing their two year visits were followed up passively until all other subjects had completed their two year visits. This gave a maximum follow up time of 3.8 years.

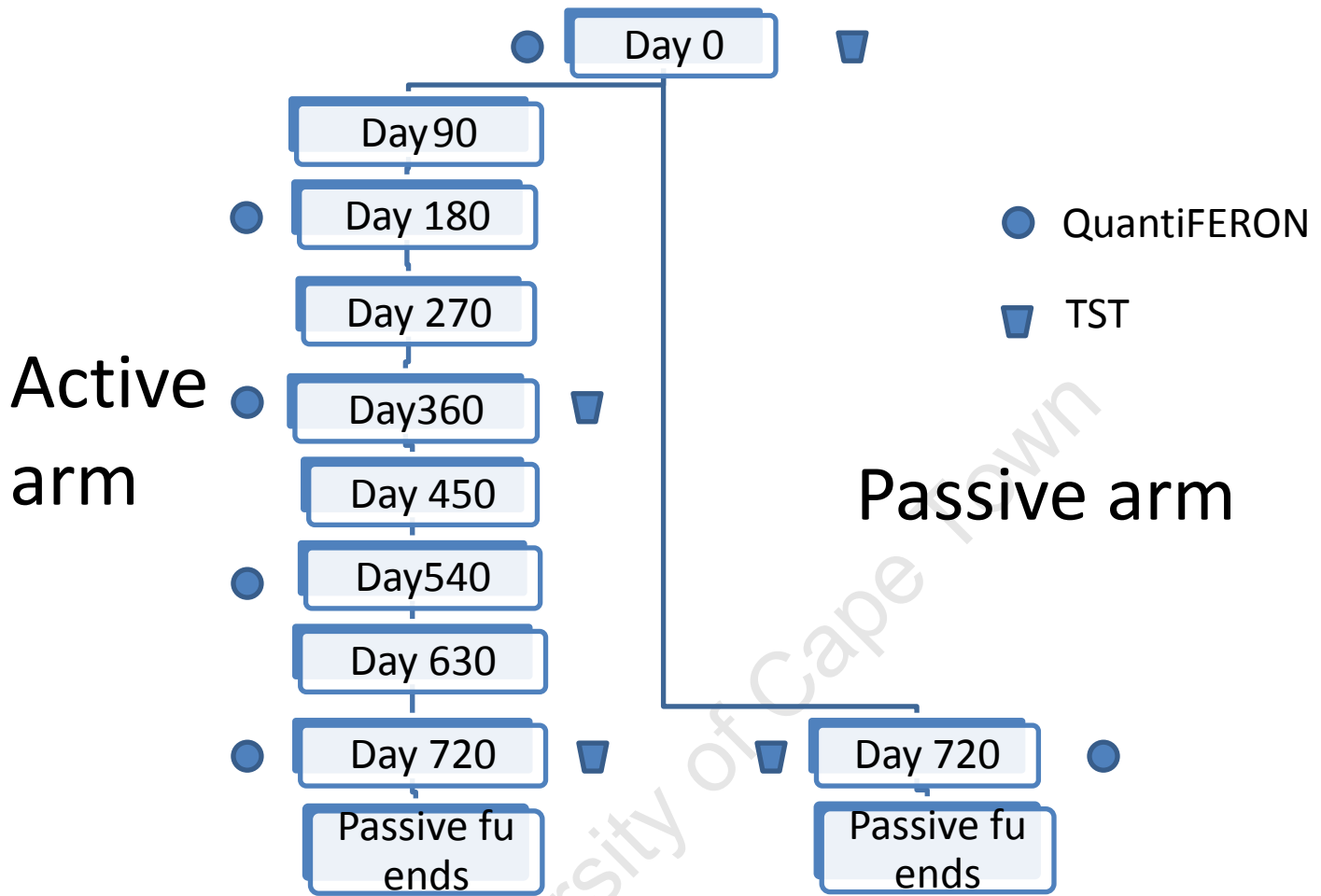
Definitions:

The protocol definition of a TB case was a diagnosis of intrathoracic tuberculosis with either two positive sputum smears and/ or one single positive sputum culture ("bacteriologically confirmed TB"). However, data on all individuals placed on

TB treatment by a physician were recorded (“all TB”). A chest x-ray consistent with active tuberculosis was defined as “compatible with TB” - this included pleural effusions. An “abnormal chest x-ray” was defined as any abnormality judged to be evidence of active disease including TB and evidence of old/ previous disease.

Appendix B

Study schema for overall adolescent cohort study



Questionnaire used in Knowledge and Attitudes study (Chapter 7)

South African TB Vaccine Initiative (SATVI)

University of Cape Town

Knowledge and attitudes questionnaire for school-going pupils in the Worcester, De Doorns and Rawsonville areas.

This questionnaire will not be used for exam purposes.

Instructions:

1. Do not put your name on this paper. It is an anonymous survey.
 2. Circle the answers which you think are correct or which reflects your point of view.
 3. Circle "Don't know/ Not sure" if do not know what answers are correct or if you are not sure.
 4. For each question, you may circle as many answers as you wish.
 5. Read through all the options before you make your choice.
-

- 1 TB is a:
- a) Kind of cancer affecting the lungs
 - b) Kind of infection affecting mainly the lungs.
 - c) Heart problem
 - d) Kind of asthma
 - e) Don't know/ Not sure

- 2 The direct cause of TB is :
- a) Smoking
 - b) Stormy weather
 - c) A germ
 - d) Exercise
 - e) Don't know/ Not sure

- 3 TB is made worse by:
- a) Poverty
 - b) Over-crowding
 - c) Smoking
 - d) All of the above
 - e) None of the above
 - f) Don't know/ Not sure

- 4 TB is mainly spread by:
- a) Blood
 - b) Touching
 - c) Germs which have been coughed into the air
 - d) Sexual intercourse
 - e) Don't know/ Not sure
- 5 With treatment, TB can be fully cured:
- a) True
 - b) False
 - c) Don't know/ Not sure
- 6 HIV is a:
- a) Kind of cancer
 - b) Kind of Infection
 - c) Heart problem
 - d) Kind of asthma
 - e) Don't know/ Not sure
- 7 The direct cause of HIV/AIDS is :
- a) Smoking
 - b) Poverty
 - c) A virus
 - d) Sexual intercourse
 - e) Don't know/ Not sure
- 8 The chances of getting HIV is higher when:
- a) Someone has many sexual partners
 - b) There is over-crowding
 - c) Someone smokes
 - d) All of the above
 - e) None of the above
 - f) Don't know/ Not sure
- 9 HIV is mainly spread by:
- a) Sexual intercourse
 - b) Touching
 - c) Kissing
 - d) Hugging
 - e) Blood transfusions
 - f) Don't know/ Not sure
- 10 Do you want to know if you have HIV or not?
- a) Yes

- b) No
 - c) I am not at risk of HIV
 - d) Don't know/ Not sure
 - e) Maybe
 - f) I don't want to answer this question
 - g) I know already (I have had an HIV test)
- 11 A vaccine or immunisation is given :
- a) To give energy
 - b) To help growth in a child
 - c) When a child is sick
 - d) To prevent the development of infections
 - e) Don't know/ Not sure
- 12 A vaccine or immunisation is made of:
- a) Chemicals
 - b) Vitamins
 - c) Bits of dangerous germs which have been made safe
 - d) Animal bits.
 - e) Don't know/ Not sure
- 13 The disease small pox no longer exists in the world because:
- a) The germ died out naturally
 - b) People around the world were vaccinated against small pox
 - c) Chemicals were used to kill off the small pox germ around the world.
 - d) The small pox virus changed into something harmless.
 - e) Don't know/ Not sure
- 14 Vaccines are given
- a) Mainly to children
 - b) By doctors and nurses
 - c) Free of charge
 - d) All of the above
 - e) None of the above
 - f) Don't know/ Not sure
- 15 Research is
- a) When scientists try to find out something new.
 - b) When an experiment is conducted on people without their permission.
 - c) Only used to develop new weapons
 - d) Only about reading books.
 - e) Don't know/ Not sure
- 16 Would you be willing to participate in research (you may circle more than one option):
- a) To answer questions
 - b) To be examined
 - c) To have skin tests

- d) To have blood taken
- e) Don't know/ Not sure

17 Would you be more willing to take part in research (you may circle more than one option):

- a) If you got more information
- b) If the community benefited
- c) If the school got something in return
- d) If you personally got something in return
- e) If someone you know monitored the research

18 Who would assist you in deciding on whether to participate in a research project?

- a) Parents
- b) Friends
- c) Teachers
- d) Brothers/ Sisters
- e) Other such as _____

19 Where do you get your information on health matters from?

- a) Parents
- b) Teachers
- c) Newspapers
- d) Radio/ TV
- e) Friends
- f) Magazines
- g) Your private doctor
- h) Clinics or hospitals
- i) Other, please specify _____

Thank you for your assistance