Determining the Annual Risk of TB Infection among Health Care Workers in a Public Hospital in South Africa Using the Interferon Gamma Release Assay

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A Master's Paper submitted to the faculty of
the University of North Carolina at Chapel Hill
In partial fulfillment of the requirements for
the degree of Master of Public Health in
the Public Health Leadership Program.

Chapel Hill

2008

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April 10, 2008

Date

BACKGROUND AND RATIONALE

Epidemiology and historical context:

Tuberculosis (TB) has been a known cause of human suffering for at least 8,000 years[1]. It presents both as a chronic wasting condition and as a highly lethal epidemic. It thrives on poverty, overcrowding, and undernutrition. While TB has been endemic in many populations since the advent of agriculture, it rose to global pandemic levels with the industrialization of the 19th century[1, 2 3, 4].

Technical advances--such as the tuberculin skin test for diagnosis of latent infection, the advent of radiographic diagnosis, and the development of effective pharmacotherapy for treatment--raised the hope for disease eradication. Unfortunately, human social organization wasn't as adanced. In most of the world poverty and other social ills exacerbate poor individual adherence to treatment and governmental failure to support control programs. This allows Mycobacterium Tuberculosis (MTB) to develop resistance to treatment and spread uncontrolled. The appearance and rapid success of the Human Immunodeficiency Virus (HIV) in the last 30 years has magnified the TB epidemic accelerating global spread[2].

At present, TB is the leading cause of death from a curable infectious disease in adults[5]. In 2005 alone, TB infected 8.8 million people, and killed 1.6 million people. One third of the world's population is estimated to be infected with TB [6 7].

MTB has multiple forms of resistance to drug therapy. Multidrug resistant MTB (MDR-TB) is resistant to the two most important first-line drugs, Isoniazid and Rifampin. Extensively drug resistant MTB (XDR-TB) emerged in 2006. It is resistant to first- and second-line drugs, and was the only MTB infection growing in the United States in 2005[8]. These varieties threaten TB control efforts globally[9].

While TB remains a major public health threat, the epidemics in the U.S. and globally have turned in the last decade. In the U.S., rates of active TB disease and TB mortality are declining[10]. Rates of MDR-TB declined in 2006 among all cases, rising only among foreign-born cases of TB. Globally, the rates of new cases and mortality are stable or declining everywhere except sub-Saharan Africa[6, 11]. Even in sub-Saharan Africa more high-burden countries are reporting to the World Health Organization (WHO) and some national control programs are showing a reduced rate of increase in TB.

The TB pandemic, however, is not yet controlled. Drug resistant MTB varieties continue to develop and spread in Africa and Eastern Europe, and the disease frequently crosses international borders[8, 9, 12, 13]. TB and HIV stimulate one another, increasing pressure on the fragile health systems in the world's poorest and least-stable countries[11]. If TB is not controlled in the current hot-spots a new epidemic of incurable XDR-TB may spread unchecked[9, 14].

TB and HIV:

The HIV epidemic drives the TB epidemic in many parts of the world, especially in sub-Saharan Africa, where up to 70% of patients with TB are infected with HIV [15]. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), 33.2 million adults and children are living with HIV or AIDS as of 2007. HIV infection impairs immune function, which dramatically increases the risk of developing active TB. Tuberculosis, in turn, may stimulate and activate viral replication of HIV.[16, 17].

HIV weakens the immune system by causing the autolysis of macrophages and T-lymphocyte helper cells, the very cells responsible for the immune response to TB. In the absence of HIV, these cells "eat" the living mycobacterium and then isolate themselves by stimulating granuloma formation, which imprisons the mycobacteria so they do not cause disease. Once a person is infected with TB and has undergone this immune reaction he or she is said to have latent tuberculosis infection (LTBI). The lifetime risk for such a person to get

active TB disease is 10%. Active TB is most likely to occur when the body's immune quarantine system fails, usually due to old age or additional diseases. Because HIV attacks the very cells responsible for quarantining TB, HIV infection is a major risk factor for development of active TB. Among people with LTBI the risk of active TB disease in individuals who also have HIV rises from 10% per lifetime to 10% per year[18].

Conversely when MTB bacteria are consumed by an HIV infected macrophage it stimulates the viral production of HIV. HIV in the absence of TB can also sit dormant in a host for years. The host may go for 10 or more years without any symptoms and even without replicating virus. MTB exposure to an asymptomatic person with HIV activates both diseases. When MTB infects a previously HIV infected cell it activates the HIV genetic code in that cell to begin replicating viruses. Macrophages produce cytokines in response to contact with MTB and these cytokines increase the activity of HIV virus in nearby cells[18].

This complex immunological synergy results in progressive rapid immunosuppression and active TB disease. This can come either from activation of LTBI or rapid evolution to active TB from recent exposure. Persons with LTBI who are infected with HIV have been shown to develop active TB in as little as 2 years. Persons with HIV have been shown to get active TB in early as 60 days after exposure[18]. For these reasons, to ensure optimal treatment, it is important to know the HIV status of people infected with TB and the TB status of those infected with HIV.

Measuring TB status with the TST:

The most common method for evaluating TB status in asymptomatic people is the Tuberculin Skin Test (TST) [2, 19]. The TST measures delayed type hypersensitivity skin reaction to tuberculin proteins. It is a test of immune response to a purified protein derivative (PPD) of non-species specific mycobacterium. The main benefits of the TST are that it is inexpensive, well studied, and nurses can be trained to do it without the assistance of a lab.

The TST has a number of significant detriments. First, because the test is done in vivo, each time someone is tested, he or she is exposed to the tuberculin PPD. With repeated testing people can develop an immune response to the test, which causes a false positive result[20]. Second, the TST is not specific for MTB because the proteins used in the test are common to non-tuberculous mycobacteria and proteins in the Bacillus Calmette-Guerin (BCG) vaccine[2]. Thus it detects lifetime cumulative exposure to all forms of mycobacteria[21]. Third, immune response to MTB wanes over time if the initial infection is isolated effectively by the immune system. Thus in a healthy individual with LTBI there may be a false negative result on the TST. Also, people with any immunodeficiency, such as HIV and even those with active TB disease may receive false negative results because of reduced immune response. To partially make up for these variations the TST is read as positive at 5mm induration in people with HIV rather then the normal 10mm. Similarly for people in endemic areas with non-TB mycobacteria and high rates of BCG, the recommendation is 15mm for positive[6, 22 23]. The TST is both non-specific and non-sensitive in sub-Saharan Africa where community exposure is very high, non-TB mycobacteria and BCG vaccination are common, and many people are immunocompromised due to HIV, malnutrition, and other illnesses.

Health care workers and TB risk:

Health care workers (HCWs) are at risk of contracting MTB due to frequent or prolonged exposure to infectious patients[24]. In spite of the aforementioned problems the TST is used to estimate occupational risk of TB. One method is to use cross sectional data to evaluate the number of people with LTBI according to a positive TST in an occupational group vs. the general population. According to the Institute of Medicine report edited by Field some studies in the U.S. and other high-income and low-prevalence countries using this method reported the same or even lower rates of LTBI in HCWs vs. the general population[25]. HCWs however, are younger, healthier, and of a higher socioeconomic status than the general population, which are

known confounders not accounted for in these cross sectional evaluations[26]. In addition to the issue of confounding, the cross sectional approach is also problematic because the TST tends to measure lifetime cumulative exposure rather than recent exposure[2]. Cohort studies use TST tests over time to identify those who convert from negative to positive. This process takes a long time because the TST should not be repeated frequently. Other studies evaluated TB risk by measuring the number of cases of active disease among the populations, but applying the results from this method is complicated because active disease can occur many years after TB infection, and both the time delay and number of cases is very dependant on the HIV rates in the studied populations.

In 2007 Menzies published a review of TB risk among Health Care workers that included published works from 1960 to 2005 in low and middle income countries (LMIC), and from 1990 to 2005 in high-income countries (HIC)[27]. It is common to separate HICs from LMICs in any discussion of TB because the risks, treatments, and recommendations vary for each group. In HICs Menzies Joshi and Pai found that the average prevalence of LTBI for HCWs was 24%. Positive status for LTBI in HICs was associated with non-occupational factors related to lifetime risk such as low socioeconomic status or foreign birth. The risk of TB attributable to heath care work in these settings was only 1.1% [0.2 – 12%].

In LMICs there was a much higher burden of disease as expected. On average 63% of HCWs in LMICs were LTBI positive. The risk of TB attributable to heath care work in these settings was 5.8% [0 – 11%]. In both groups the nosocomial risk was related to both the burden of TB in the health setting and the implementation of engineering and administrative TB control measures [27].

Earlier in the year the same authors published the review of only LMICs. In this paper Joshi discussed the subgroups of HCWs in greater detail and presented the little data available on the effect of TB control measures on the occupational risk of TB[26]. In LMICs, health care settings are crowded and even low-cost strategies to reduce transmission of MTB are seldom

implemented. High risk of occupational exposure among these HCWs contributes to a prevalence of LTBI ranging from 33 to 79%, and an annual risk of infection ranges from 0.5 to 14.3%. Because the numbers are so high, the attributable-risk of active TB disease due to nosocomial exposure ranges from 25 to 5,361 per 100,000 HCWs per year[26].

These high rates of active TB among HCWs in low- and middle-income countries are particularly worrisome and often considered to be HIV–associated[6] . The increase of disease burden from HIV and TB directly reduces the working life expectancy of health care workers in an already severely strained environment [11]. There is great concern about nosocomial risk for TB because HCWs have frequent contact with people suffering from other chronic diseases who are likely susceptible to TB. HCW who are not protected have the potential to spread the disease even more than other groups if they become infectious.

Prevention and TB Infection Control:

TB Control strategy varies from country to country depending on national resources and the burden of disease. In HICs such as the U.S. and Western Europe there is a low burden of TB. People at risk for TB in HICs are identifiable as groups such as HCWs, the homeless, immigrants, and others. The goal of TB control for these countries is to prevent and eliminate the disease[28]. They generally use screening tests for at-risk groups and treat anyone found positive for LTBI with preventive therapy to avoid active disease. Since Isoniazid for preventive therapy (IPT) is inexpensive and safe, and the number of people with LTBI is small, HICs screen with the TST to maximize sensitivity. Engineering and other measures are taken to reduce occupational risk.

In low-income high-burden countries TB control prioritizes reduction of active transmission of TB, not prevention[29, 30]. LMICs rely on identification and treatment of active cases to stop transmission. In these settings it is unclear how effective it is to treat someone with IPT because that person might be reinfected once therapy is stopped. Also the prevalence of LTBI is so high

that treating everyone is not feasible. Tests need to be highly specific to be useful and even more so, they need to identify those most likely to be spreading the disease. For this reason, LMICs rely on direct investigation of sputum smears for mycobacteria. People with MTB in their sputum are most infectious and therefore receive priority treatment.

Very little is published on the effects of infection-control measures, especially in LMICs. It is difficult for policy makers to allocate resources away from the more obvious need to identify and treat the most infectious cases and toward preventive interventions. In order to make the argument for prevention one must be able to estimate the nosocomial risk of TB to the workers and the populations they serve and to demonstrate the reduction of that risk through implementation of some or all of the available control measures.

Prevention and Occupational TB infection Control for Health Care Workers:

In 1994 the Centers for Disease Control (CDC) established comprehensive infection control practices to protect HCWs and prevent TB transmission to the general population in U.S. health care settings [31] . This action was taken as a response to increasing rates of nosocomial transmission and multiple TB outbreaks in the 1990s[1, 25]. The CDC guidelines were not based on evidence of effectiveness; instead they were implemented to theoretically address known mechanisms of transmission. The guidelines include triage methods, behavioral systems of isolation, and regular screening and treatment for LTBI, engineering procedures, such as negative pressure rooms and antimicrobial radiation through UV lights, as well as the use of personal protective equipment such as respirators and masks. Implementation of these recommendations has led to a dramatic decline in the burden of TB among HCWs in the U.S.[10].

In order to find any information on the effects of these or other occupational infection control programs we searched medical literature available from Medline and Google Scholar in November 2007 for Tuberculosis or TB with combinations of secondary phrases such as health worker, infection control, prevention or control. We further used the "cited by" in Google Scholar

and PLOS in order to find more recent articles. Results were limited to papers published since 1995, in which there was an evaluation of the effectiveness of TB control measures on HCWs in a health care setting. We found five studies evaluating the impact of infection-control strategies on the risk of TB disease among HCWs [32-36]. In three of these studies the investigators (Blumberg et al, Yanai et al and Baussano et al.) evaluated reduction in TST conversion at large referral hospitals after the full CDC recommendations for TB control (including isolation procedures, engineering, protocol measures, and personal protective equipment for HCWs) were implemented. Blumburg did a cohort study of house staff in the U.S. before and after implementing TB control measures and found that the TST conversion rate dropped to less than 20% of the baseline value (5.8 down to 1.1 conversions per 100 person years (py)). Yanai et al demonstrated a similar benefit in Thai HCWs after implementing full TB control measures (9.3 down to 2.2 conversions per 100 py) as did Baussano in Italy (2.19% per 100py down to 0.84% per 100py). Roth et al. evaluated TST conversion in four Brazilian hospitals; two implemented full CDC recommended TB control measures while the other two had no control measures. The rate of conversion was more than twice as high in the hospitals with no control measures. None of these attempted to evaluate the effect of specific parts of the overall nosocomial prevention guidelines.

Harries et al. evaluated a less comprehensive intervention. They measured TB indirectly by evaluating TB registries before and after limited local guidelines were imposed on hospitals in Malawi. The guidelines were only for faster recognition and treatment of smear positive TB cases so they were not the same as implementing the TB control guidelines. They found that while the goal of the intervention was rapid recognition, the time to recognition and treatment was unchanged and there was no difference in the number of HCWs being registered with TB.

One need not address nosocomial risk as an all-or-nothing investment. It may be possible to compare interventions to estimate the benefits and the costs of practical control measures

that could be implemented at different levels of investment. These would range from the simplest, such as outdoor waiting rooms and open windows, to more expensive interventions, like masks for respiratory isolation or UV lights, to the most intensive interventions, such as positive pressure rooms and full CDC guidelines. This would allow Ministries of Health to identify which prevention measures are effective enough to complement identification and treatment of active cases. This might be done through a cohort study that measures baseline and follow up LTBI rates in a cohort of at-risk people (such as HCWs) over time. To improve upon TST, an LTBI test for this purpose should be repeatable at shorter intervals and compared with the exposure level of each individual. It should be free of interaction with non-TB mycobacteria so that it can be accurate in a high-burden setting. It should also be independent of BCG vaccination. Ideally, it would be more specific for recent infection than lifetime infection.

Interferon Gama Release Assays

A new group of tests for LTBI has recently been developed. These tests measure interferon-γ production by T-cells in response to in-vitro stimulation of whole blood with MTB specific proteins not present in BCG and non-TB mycobacteria. As a group they are known as interferon-γ release assays (IGRAs)[19, 37, 38]. Two commercially available FDA-approved IGRAs have been studied in recent years and are being compared with TSTs in a variety of settings[38-50]. These are the Quantiferon (QFT) and Elispot tests. The IGRAs avoid some of the problems of the TST. They use proteins that do not cross react with response to BCG vaccination or non-TB mycobacteria. They are ex-vivo tests and so do not stimulate immunity as the TST can.

There is no gold standard for evaluating LTBI[2, 19]. In order to evaluate the IGRAs, studies have compared to TST for agreement and made correlation with expected levels of exposure, or used active TB disease as a surrogate for LTBI. In a meta-analysis comparing the three tests to active TB, the pooled sensitivity of the TST was 70% compared to 76% for the QFT test and 88% for the Elispot. Pooled specificity of the three tests in populations at low risk for LTBI was 66% for the TST, 97% for the QFT and 92% for the Elispot[43]. The results of studies completed

to date leave more questions as to the benefits and usefulness of IGRAs for the diagnosis of LTBI. For instance the IGRA is less likely to be positive after treatment for TB[51, 52]. This could represent an accurate evaluation of infection or a change in immune response. IGRA has also shown marked variability without treatment and this should be evaluated in longer cohort studies of untreated people that will demonstrate risk of disease for those who test IGRA positive.

To date, only a few published studies have focused on the IGRA in HCWs [52, 42, 48, 53]. The studies by Neinhaus and Harada are done in high income countries (Germany and Japan) and examine the benefits of using an IGRA in place of TST due to its possibly superior specificity for people with BCG history.

Pai studied IGRA in a high risk environment. Working in India, Pai et al documented an annual risk of MTB infection of 5% among HCWs, which is more than three-fold the risk in the general population. They also observed that some HCWs had reversion of the IGRA on serial testing. The authors suggest that transient MTB infections are occurring and can be cleared by the immune system, a process which goes undetected with the traditional TST. Alternatively, this could represent a high level of variability in the IGRA thus implying reduced accuracy or reproducibility. In a study of 10 HCWs from the same cohort who were positive for LTBI and treated with Isoniazid, Pai documented changes in the IGRA values that might demonstrate reversion with treatment and re-conversions with continued exposure in the health care setting[51].

Corbett studied the IGRA in nursing students in a high risk setting in Harare, Zimbabwe, in 2006 [54] They found an annual conversion rate with TST of 19.3 per 100 person-years in nursing students as compared to 6.0 per 100 person-years in other polytechnic students (AR 13.3, RR 3.2). In recent conference proceedings the same authors revealed preliminary results of a comparison of TST to IGRA for evaluating conversion [55]. They reported in the same group of nursing students a conversion rate by IGRA of 27.6 per 100 person-years vs the 19.3 per 100 person-years by TST (AR 8.3, RR 1.4). Corbett et al. concluded that TST markedly under-

estimates the TB conversion rate and that the IGRA may be a more accurate method of assessing infection.

Summary

While TB disease rates are leveling off or declining in most of the world, with one third of humanity infected and 1.8 million deaths a year, it remains a major public health problem. The main threat to global public health from TB is the continually increasing number of infections and increasing resistance to treatment in sub-Saharan Africa. Health care workers are at high risk for TB and their loss to the infections of TB and HIV is adding further strain to an already fragile health system. Research done thus far on the rate HCWs become infected with TB is limited, and the difference in risk among various occupational groups is unclear. Methods of TB control and prevention have been effective in wealthy countries and in the few middle income countries that have implemented them in limited settings. It remains unclear, however, if these methods can protect health care workers in high risk areas. Also unclear is which infection control interventions, if any, carry practical benefits that outweigh their costs in high risk areas. It is too difficult to evaluate these questions with the common test for LTBI (TST) because the TST is a poorly effective test, both non-sensitive and non-specific in the high-risk TB settings of sub-Saharan Africa. A new test for LTBI is now available, the IGRA. The IGRA has some qualities that may make it a better test for evaluating TB exposure in HCWs.

This pilot study proposes to use an IGRA to estimate the annual risk of newly established and transient MTB infections in health care workers employed in a setting with high rates of TB and HIV. We will focus on two different groups of HCWs. First we will follow the risks of conversion as well as reversion and variability of the IGRA as compared to TST in nurses and technicians working in TB clinics. This is an expansion of the study of 10 HCWs done by Joshi et al in India. We will use the data to gain a better understanding of the use of the IGRA in high risk settings. Second we will evaluate baseline risk of conversion in students whose risk profile should parallel the general population at baseline then increase to that of HCWs as they begin

working in clinical medicine and have increased patient contact. We aim to use this methodology and baseline information to plan future studies to assess the effectiveness of TB infection control measures in prospective controlled trials within health care settings in sub-Saharan Africa.

SPECIFIC AIMS

Specific aim 1: Describe the fluctuations in IGRA results over a 1 year period among HCWs involved in direct TB care.

Hypothesis: We will observe distinct patterns of IGRA responses among these HCWs: (1) sustained high IGRA responses among HCWs with LTBI at baseline who do not receive Isonizid Preventive Therapy (IPT); (2) conversions among HCW with negative IGRA at baseline; and (3) reversion of IGRA response among HCW with baseline LTBI who receive IPT. A fourth possible pattern is fluctuations of IGRA above and below the recommended threshold for a positive test. Each of these patterns is in contrast to null pattern of no change or persistent negative test.

Rationale: Insight into IGRA responses over time among highly exposed HCW will increase our understanding and interpretation of IGRA in this population of continuously highly exposed individuals.

Specific aim 2: Assess the rate of IGRA and TST conversion among medical students and nursing staff in a high prevalence area for TB and HIV. Determine the rate of agreement between IGRA and TST conversion.

Hypothesis: The annual IGRA conversion rate will be ≥10% among those negative at baseline, and higher than the TST conversion rate. Agreement between IGRA and TST by kappa score will be approximately 80%.

Rationale: Establishing an accurate annual *M. tuberculosis* infection rate will allow future evaluations of effectiveness of different infection control measures.

Specific aim 3: Evaluate knowledge and attitudes among HCWs and medical and nursing students towards TB, LTBI, and infection control measures.

Hypothesis: Students and clinic HCWs will have good knowledge on active TB but insufficient knowledge on LTBI and TB infection control measures.

Rationale: Insight into the current knowledge and attitudes of HCW towards the occupational risk of *M. tuberculosis* infection and TB infection control measures will help guide implementation plans for TB infection control in high risk settings.

Specific Aim 4: Estimate the prevalence of LTBI in both HCWs and medical students.

Hypothesis: HCWs will have high levels of LTBI (75%). Medical students will have low levels (10%).

Rationale: While prevalence of LTBI has been documented in aggregated groups of HCWs it has not been shown for physicians or medical students. Insight into the level of risk of TB infection for different segments of the healthcare workforce may influence decisions regarding worker protection and disease prevention.

MATERIALS AND METHODS

Setting:

This study takes place in South Africa, which according to the WHO ranks 7th on the list of 22 high TB burden countries. South Africa reported more than 270,000 new and relapsed TB cases in 2006. This is an incidence rate of 600/100,000 population. Successful treatment completion rates, recorded in 2004, were 70% with interruption rates of 13% and 7.4% mortality with treatment [6].

The burden of HIV disease affects both the incidence of TB and the mortality. South Africa is the country with the largest number of HIV infections in the world. HIV prevalence data estimate that 29% of 15 to 24 year old pregnant women are HIV positive in South Africa[56]. South Africa has 0.7% of the world's population but 19% of the world population of people co-infected with

TB and HIV. South Africa is beset by TB as well as severe HIV epidemics and is working to improve cure rates of active disease to 85% [30].

Prevention of TB transmission through infection control measures is becoming increasingly important to the South African TB strategy. As stated by acting Minister of Health, Mr. Jeff Radebe, on World Tuberculosis Day[57], "TB is one of the major health challenges currently facing South Africa....Realizing the challenges, the Department of Health developed the National TB Crisis Management plan .... The Plan seeks to improve systems that are necessary to support the TB control programme....We have paid particular attention on strengthening infection control precautions at our health facilities in order to reduce possible transmission of TB in these facilities. Infection control measures are aimed at reducing direct or indirect contact transmission by isolating patients, creating adequate bed floor space and improving ventilation in wards."

Johannesburg is South Africa's largest city; it consists of a diverse population and a complex public health system under Gauteng province. There are forty-eight community health centers for the treatment of TB as well as an inpatient TB hospital for the treatment of drug resistant varieties. Johannesburg boasts a prominent medical school and research center in the University of Witwatersrand. In Johannesburg there is both a formidable burden of disease and a large number of professionals and resources able to complete this study and use the results to develop a more comprehensive study in the future.

Investigators

This project was organized and developed at UNC-CH under a grant for students and faculty funded by the North Carolina Institute of Occupational Health and Safety to Patrick Keller MD in the department of Social Medicine and Annelies Van Rie MD PhD in the department of Epidemiology. Co-investigators in South Africa were recruited by Dr Van Rie through prior contacts. These are: Charles Feldman MD PhD, Professor and Head of the Division of Pulmonology and Deputy Chair of the Department of Medicine for the University of

Witwatersrand; Kerrigan McCarthy MBBCh DTM&H FCPath (Micro), consultant microbiologist responsible for TB/HIV integration activities with the Reproductive Health and HIV research Unit, Department of Obstetrics and Gynecology, University of the Witwatersrand; And Francois Venter MD FCP, Clinical Director of the HIV Management Cluster, RHRU, and lecturer at the Department of Medicine, University of the Witwatersrand and President of the Southern African HIV Clinicians Society. Program design was a collaborative process between all of the co-investigators. Management of the project in South Africa is being done by Kerrigan McCarthy.

Study Design:

This study is a prospective 1-year cohort study of two distinct populations: (1) a cohort of health care workers involved in inpatient or outpatient TB care and (2) a cohort of medical students beginning their clinical training.

Study Population:

Cohort 1: Approximately 50 HCWs involved with inpatient or outpatient TB care in Gauteng province. Study sites will be selected among the 48 non-mobile clinics that provide TB care and the hospitals that provide inpatient TB care. The study sites are selected based on consultation with the South African Ministry of Health and Department of Health for Gauteng province in South Africa, and as a convenience sample of sites located near the University of Witswatersrand. The actual number of volunteers recruited will depend on financial feasibility of the study budget as well as on negotiations for permission with the National and provincial health departments as well as individual site managers. 50 is used as a reasonable number that can be reached. Below is a list of potential sites:

Inpatient TB care: SANTA tuberculosis hospital in Soweto, JHB; TB referral clinic at Baragwanath Hospital

Outpatient health centers: Esselen, Jeppe, Yeoville, Urban Health, Joubert Park, and Rosettenville, Sandown, Randburg, Parkhurst, Crosby, Claremont, Riverlea Major,

Roodepoort, Weltevreden Park, Helderkruin, Davidsonville, Princess Clinic, Zandspruit Clinic, Siphumlille, Nokuphila, Bophelong, Itireling, Zola, Klipspruit West, Klispruit West, Senaoane, Tshiawelo, Protea South, Tladi, Mofolo South, Jabavu, Eastbank, Fourth Avenue Alexandra, St Mary's, Marshalltown, Malvern, Eldorado Park, Elia Mostoaledi, Michael Maponia, Orlando East, Noordgesis Clinic, Meadowlands, Diepkloof, Ennerdale Ext, Lawley Ext , Lenasia Ext, Lenasia South, Mid Ennerdale.

Inclusion criteria:

HCW employed in the selected clinics

Informed consent

Exclusion criteria

Currently receiving treatment for active TB

Plans to move outside of the Gauteng area in the next 12 months

Refusal to have blood tested for HIV

Cohort 2: All students enrolled in the 3rd year in medical school at the University of Witwatersrand will be invited to participate. These students are selected because they will be making the transition from predominantly classroom teaching to bedside teaching and clinical training. Students will conduct part of their training in hospital wards with high risk of transmission of *M. tuberculosis*, including the Emergency and ID wards. We expect a participation rate of 70% or 154 students enrolled at baseline. Among these, an estimated 111 students will be eligible to participate in the follow-up study.

Inclusion criteria:

Student enrolled in the 3rd year in medical school or 1st clinical year in nursing school

Informed consent

Exclusion criteria for enrollment at baseline:

Refusal to have blood tested for HIV

Currently receiving treatment for active TB

Exclusion criteria for participation in longitudinal study:

LTBI at baseline assessment as documented by concordant TST and IGRA test.

Recruitment and consent procedures:

Cohort 1: Following approval by the Department of Health, selected clinics will be visited by study investigators to inform HCWs about the study and obtain informed consent of those interested in participating. Participation is voluntary and the DOH will not be informed which HCWs participated and which declined participation.

Cohort 2: Medical students will be recruited through an announcement at the end of a core class by a University of the Witwatersrand faculty member not otherwise involved in the study. A study flyer and consent form will be distributed at the end of class to interested students. Interested students will be asked to return the completed consent form at the time of a specified date. At that time, any questions regarding the study will be answered. The University will not be informed which students participated and which students declined participation.

Study procedures

TST: The TST will be a single step Mantoux technique using 5 TU PPD RT23. The Mantoux procedure is performed by injecting 5 TU of PPD subcutaneously (usually on the ventral forearm). The procedure can be done in a single step fashion where the test is placed once and the skin is read for reaction 48 – 72 hours later or in a two step fashion where a test is placed a second time on those with a negative result. The two step is helpful in populations at low risk for TB where sensitivity is to be maximized. In this case the placement of the first test stimulates immune activity that has been dormant for the second test. The single step will be used

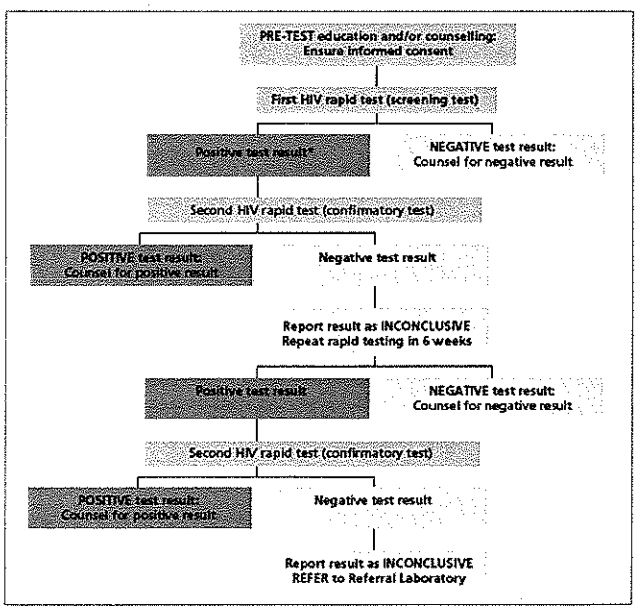
because the two-step procedure loses significant specificity without much gain in sensitivity due to the high rates of non-tuberculous mycobacteria and BCG vaccination [58].

Quantiferon Gold In-tube Interferon-γ-release-assay: The QuantiFERON ® -TB Gold assay detects cell mediated immunity responses in-vitro to tuberculosis infection by measuring interferon-gamma (IFN-γ) harvested in plasma from whole blood incubated with the M. tuberculosis-specific antigens, ESAT-6 & CFP-10. Five mL of venous blood will be collected and heparinized by inverting the tube several times. Aliquots of heparinized whole blood will be incubated with ESAT-6, CFP-10, T cell mitogen (PHA) and negative control antigens. Following 16 to 24 hours incubation, the plasma will be harvested. The amount of IFN-γ in the plasma samples will be quantified by enzyme-linked immunosorbent assay (ELISA). Results are reported in International Units relative to recombinant human IFN-γ standard preparation.

In order to minimize test-related variability, identical protocols will be used for baseline and follow-up testing, with previous results masked. All assays will be performed in the National Health Laboratory Services, led by Wendy Stevens, who has been involved in over 600 clinical research projects and provides laboratory support for several NIH networks (HPTN, ACTG, PACTG, and CIPRA).

HIV test: HIV will be tested by the use of rapid tests according to the algorithm recommended by DOH and WHO. An HIV diagnosis requires two positive HIV tests. The blood sample taken at visit 1 is enough to perform both tests.

FLOW CHART 1. ALGORITHM FOR USE OF RAPID HIV TESTS IN TESTING AND COUNSELLING SERVICES



Knowledge and attitudes questionnaire (appendix A and B): A self administered questionnaire will be used to assess knowledge and attitudes about TB, LTBI and infection control. The questionnaire will evaluate knowledge attitudes and beliefs among both cohorts on TB transmission, diagnosis and treatment of LTBI, and methods for HCWs to prevent nosocomial infection. The questionnaire will also assess TB associated stigma, and feasibility of interventions for TB infection control. By understanding the perception of HCWs with respect to the most feasible methods of TB infection control, we can prepare future studies of the effectiveness of those interventions most acceptable to the local workers.

Exposure to TB among students (Appendix C): A data collection tool will be used to document exposure to TB during work/training.

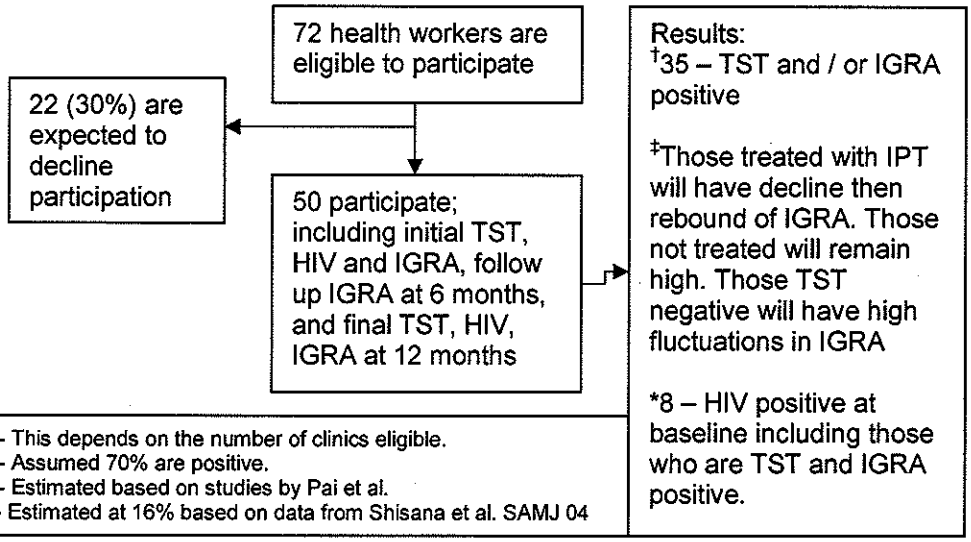
Study visits

Cohort 1: At the first study visit informed consent will be elicited in writing. Participants will be administered the TST, receive HIV pretest counseling, and blood will be collected for the IGRA and HIV tests. Participants will be informed that knowledge of HIV status is necessary to correctly interpret the TST test, and that HIV results will be masked from the investigators by separating out identifiable information. Participants will receive a questionnaire to complete (self administered) and will be given an appointment for the reading of the TST result 48 to 72 hours later. Participants will be encouraged to receive HIV results and posttest counseling at the time of their choosing with a qualified councilor. Study staff will return to the clinic 48 to 72 hours later (second study visit) to read the TST. At the second study visit staff will collect the completed questionnaire and give information about the posttest counseling. Participants will receive an appointment for the third study visit, 6 months later. One week prior to the third study visit, the participant will be contacted by phone to confirm (or reschedule) the appointment. At the third study visit, blood will be collected for IGRA and an appointment for the fourth study visit will be given. One week prior to the fourth study visit, the participant will be contacted by phone to

confirm (or reschedule) the appointment. At the time of the fourth study visit, blood will be collected for IGRA and HIV, a TST will be placed and an appointment 48 to 72 hours later for TST reading and posttest counseling will be given. At the time of the fifth study visit, the induration of the TST test will be read and posttest counseling will be offered.

A small incentive of R70 (approximately $10) will be given to participants in Cohort 1 at each time a blood sample for IGRA is collected (see Figure 1).

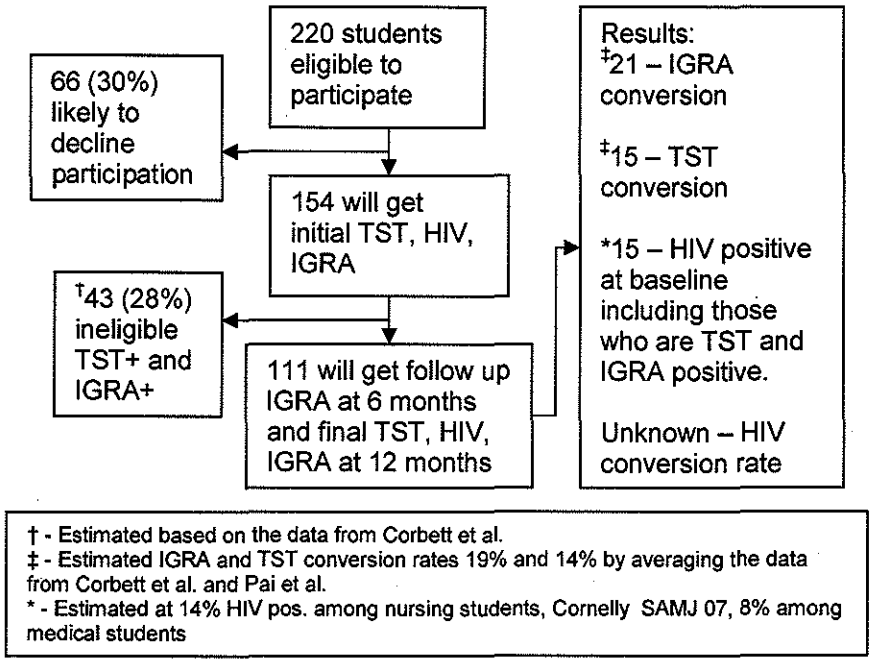
FIGURE 1: Expected participation and results for health workers in TB clinics.



Cohort 2: At the first study visit informed consent will be elicited in writing. Participants will receive group pretest HIV counseling. A TST will be administered and blood will be collected for IGRA and HIV tests. Participants will be informed that knowledge of HIV status is necessary to correctly interpret the TST. Participants will receive a questionnaire to complete (self administered) and will be given an appointment for the reading of the TST result 48 to 72 hours later. Participants will be encouraged to receive posttest counseling at the time of the second study visit. At the time of the second study visit, 48 to 72 hours later study staff will read the TST, collect the completed questionnaire and offer posttest counseling. Participants with concordant positive TST and IGRA test will be informed that they are not eligible for participation in the follow-up study. Eligible participants will receive an appointment for the third

study visit, 6 months later. One week prior to the third study visit, participants will be contacted by phone to confirm the appointment. At the time of the third study visit, blood will be collected for IGRA and an appointment for the fourth study visit will be given. One week prior to the fourth study visit, participants will be contacted by phone to confirm the appointment. At the time of the fourth study visit, blood will be collected for IGRA and HIV, a TST will be placed and an appointment 48 to 72 hours later for TST reading and posttest counseling will be given. At the time of the fifth study visit, the induration of the TST will be read and posttest counseling will be offered (see Figure 2).

FIGURE 2: Expected participation and results for medical students.



All study visits will be organized in groups. At each study visit, a small lunch will be provided to participants.

Care and treatment for participants with identified LTBI or HIV infection

LTBI and Preventive Therapy:

Currently, the South African department of Health only recommends TST-based screening for LTBI for children under 5 years of age in contact with an infectious adult case of TB and

among people living with HIV because of the high risk of active TB in these populations[30]. This is in line with WHO recommendations[22, 29]. There is no policy regarding screening and treatment for LTBI among HCWs. IGRA tests are available in South Africa, but test results are only used for research purposes. Results of the IGRA will not be given out nor will they be used for clinical decisions.

HIV seronegative individuals with conversion of TST will be informed of this result, referred for exclusion of active TB, and advised to seek care in case symptoms of TB develop.

HIV care and treatment:

All participants will receive pretest counseling (in group for students and individual for health care workers) prior to being tested for HIV. All study participants will also be offered convenient post test counseling. All HIV testing and counseling will be provided free of charge and will be performed by an NGO called New Start, which has expertise and an excellent reputation in the field of HIV counseling and testing. Those who are found to be HIV positive will be referred per local guidelines for HIV care and treatment.

Sample size, variables and analysis plan

Sample size:

Cohort 1: Among the HCW employed in the selected TB clinics, an estimated 50 HCWs will participate. These HCWs are expected to have a very high rate of LTBI (75%). An estimated 15% of HCWs are expected to be HIV positive [59]. Analysis of baseline results will allow estimation of prevalence of latent *M. tuberculosis* infection and assessment of agreement between TST and IGRA results (Specific Aim 4). Analysis of follow-up results will provide a determination of the distribution of patterns of IGRA responses over time and assessment of conversion and reversion rates among heavily exposed HCWs (Specific Aim 1).

Cohort 2: Out of 220 eligible students an estimated 154 students will be recruited (70%). Analysis of baseline results will allow estimation of prevalence of latent *M. tuberculosis* infection

and assessment of agreement between TST and IGRA results (Specific Aim 2 and 4). We estimate that 28% of the students will have a concordantly positive TST and IGRA and will be excluded from the follow-up assessments [34]. Analysis of follow up IGRA and TST results in the remaining 111 students will allow an estimation of the annual risk of MTB infection with adequate precision (Specific Aim 2). Assuming a 15% annual risk, the sample size will result in a 95% CI of 0.08 to 0.22 for the annual risk of M. tuberculosis infection among medical students. An estimated 15% of students are expected to be HIV seropositive [59].

Some have suggested that this study should be done on a larger scale to include more students. An increase in sample size would result in a minor increase in precision of the point estimate of the annual risk of infection, the outcome of interest. In the table below an increase in the sample from 111 to 225 is shown as an example. We intend to limit the sample size to the Witswatersrand medical students.

Number of Participants	5% Loss to FU	Infection rate Per year	UL CI	LL CI	Number of Participants	5% Loss to FU	Infection rate Per year	UL CI	LL CI
111	106	10%	16	4	**225**	214	10%	14	06
		15%	**22**	**8**			**15%**	**20**	**10**
		20%	28	12			20%	25	15

Outcome measures:

Results of TST will be treated as a categorical variable and as a continuous measure. The categorical response will be defined as positive if the size of induration is ≥ 5mm in HIV infected individuals and ≥ 10 mm in HIV uninfected individuals. The continuous measure will be expressed as the size of induration in mm.

Results of IGRA will also be treated as a categorical and continuous variable. The categorical response will be defined as positive if the response is ≥ 35 IU interferon gamma, as per manufacturer instructions, and as negative if the response if < 35 IU interferon gamma.

Covariates:

Gender: Male or Female, per self report, will be treated as a categorical variable.

Age: Given per self report, will be treated as a continuous variable.

Race: Will be treated as a categorical variable the local standard classifications of race are: White, Black, Coloured, Indian or Other. This will be defined by self report on the survey.

Baseline HIV infection status: Will be treated as a categorical variable positive or negative per results of the two tests.

End HIV status: Will be treated as a categorical variable positive or negative.

Level of TB exposure: According to the tool developed for this study (see appendix) will be treated as a categorical variable.

Survey responses: each of the questions in the survey will act as a separate covariate. Some will be collated according to the categories detailed in the survey. These variables will be treated as categorical.

Analysis plan:

Specific aim 1: Define the fluctuations in IGRA results among HCWs involved in direct TB care.

We will present the IGRA results from HCWs in graphic form baseline result, by INH prophylaxis status and by HIV status. We will evaluate any inconsistencies with the expected 3 patterns by comparing other variables among those individuals where IGRA pattern does not follow one of the 3 preconceived patterns.

Specific aim 2: Assess the rate of IGRA and TST conversion among medical students in a high prevalence area for TB and HIV, and agreement between IGRA and TST conversion.

We will calculate the incidence of latent M. tuberculosis infection during the 1-year follow up. Incidence will be expressed as the number conversions per person-year observation.

Concordance between TST and IGRA conversions will be evaluated using agreement and kappa statistics. We will stratify the analysis by level of exposure during the one year observation. Univariate and multivariate analysis will be used to identify variables independently associated with annual risk of infection.

Specific aim 3: Evaluate knowledge and attitudes among HCWs and medical students towards TB, LTBI, and infection control measures.

We will evaluate the knowledge and attitudes in both groups and examine if there is a difference within the two cohorts.

Specific Aim 4: Estimate the prevalence of LTBI in both HCWs and medical students.

We will calculate the prevalence of latent *M. tuberculosis* infection at baseline in both cohorts and provide a 95% confidence interval around the point estimate. Concordance between infection determined by TST and IGRA will be evaluated using agreement and kappa statistics. Univariate and multivariate analysis will be used to assess which variables are associated with LTBI.

Results:

At the time of this writing the study visits for Cohort 1 (clinic HCWs) have been delayed pending logistics planning and preparation in concert with our South African partners and the Gauteng Provincial Department of Health. Cohort 2 (medical students) have completed study visits 1 and 2. According to information available prior to the start of the study 220 students were expected to be eligible in the 3rd year medical school class. Instead there were 190 eligible students. Of these, only 74 (39%) participated in the study. Mean age was 23.9 years. Forty-one (55%) of the students were female. Twenty-four (32%) students were black, 31 (41%) white, 19 (26%) Indian, 3 (4%) coloured, and 2 (3%) were Asian. Most (75%) grew up in an urban environment. Only one student had a personal history of TB. Ten students reported family with TB, and 22 a friend or distant relative with TB. All students' HIV tests were negative.

Eight students (11%) had a TST reaction greater than 15mm, 20 (27%) greater than 10mm, and 3 did not return for study visit 2 to have the test read. Twelve (17%) of the 70 students tested by IGRA were positive. Agreement between the IGRA and the TST with a cut off of 10mm was 70% (kappa 0.1508 [95% CI: -0.08 – 0.39]). Agreement between the IGRA and the TST with a cut off of 15mm was 82% (kappa 0.2997 [95% CI: 0.06-0.54].

The association between age, gender, race, childhood environment, and parent's occupation were evaluated with respect to TST interpretation or IGRA interpretation. Unadjusted odds ratios for each characteristic are noted in the table (Results3). None of the characteristics were associated with having a positive. People of black race were more likely to have a positive IGRA than whites (p = 0.003), and people with a rural childhood were more likely to have a positive IGRA than those with an urban upbringing (p = 0.006). Logistic regression was done with each of the variables mentioned above. Race was the only variable with a statistically significant (p=<0.05) coefficient. The results were tested with the likelihood ratio test. All variables other than race failed to add any explanatory power.

Results 1: Demographics	Medical Students (n = 75)
Age:	23.9 (21–32)
Gender:	
Female	41 (54.7%)
Male	34 (45.3%)
Race:	
Black	23 (30.7%)
White	29 (38.7%)
Coloured	2 (2.7%)
Indian	19 (25.3%)
Other	2 (2.7%)
Childhood:	
Urban	54 (75.0%)
Rural	9 (12.5%)
Mixed	9 (12.5%)
Were Parents Health Workers?	
Neither	56 (74.7%)
Mother only	10 (13.3%)
Father only	8 (10.7%)
Both	1 (1.3%)
TB exposure history	
None	45 (60.0%)
A friend had TB	19 (25.3%)
A close relative had TB	10 (13.3%)
I have had TB	1 (1.3%)

Results 2: Visit 1 Testing	Medical Students (n = 75*)
TST (in mm)	Mean 5.4 SD 7 (0-30)
> 10 mm	20 (27.4%)
> 15 mm	8 (11.1%)
IGRA Adjusted	Mean 0.21 SD 1.8 (-10.53 – 6.26)
IGRA positive	12 (17.4%)
HIV positive	None

* For testing n = 70 to 73 indeterminate results not included.

Results 3: Unadjusted Odds Ratios for IGRA results and TST results						
	IGRA			**TST**		
	OR	P-value	95% CI	OR	P-value	95% CI
Age	1.20	0.137	0.944-1.52	0.98	0.901	0.66-1.43
Female Sex	0.686	0.526	0.180-2.63	2.83	0.198	0.485-29.60
Race						
White	1	Ref		1	ref	
Black	28.00	0.003	3.17-247.4	5.25	0.058	0.946-29.20
Coloured	14.00	0.097	0.62-317.4	Too few		
Indian	3.29	0.345	0.28-39.14	0.78	0.842	0.066-9.217
Other	Too few	Too few		Too few		
Childhood environment						
Urban	1	Ref		1	ref	
Rural	14.0	0.006	2.15-91.11	0.907	0.932	0.097-8.46
Mixed	2.33	0.352	0.39-13.91	1.16	0.894	0.122-11.2
Parent occupation as health care worker						
Neither	1	Ref		1	ref	
Mother	1.19	0.838	0.216-6.59	2.55	0.308	0.421-15.45
Father	2.04	0.459	0.443-9.47	2.91	0.249	0.472-17.99
Both	Too few			Too few		
TB exposure history						
None	1	Ref		1	ref	
Friend	2.57	0.128	0.762-8.68	1.37	0.690	0.295-6.343
Family	Too few			1.03	0.983	0.105-9.989
Self	Too few			Too few		

Data from the questionnaire are not yet available for analysis. Specific demographic information from the entire medical class to compare participants with non-participants is also not yet available.

The main findings of the preliminary results of this study are: 1) Far fewer medical students chose to participate than expected (39% as compared to 70%); 2) Among those who participated the prevalence of HIV was zero – far less than the 15% that was expected; 3) The prevalence of LTBI by TST was less than expected; 4) Race and Childhood environment were associated with IGRA.

Discussion:

In this study of medical students in Johannesburg, South Africa, a city with one of the highest TB incidence and HIV prevalence globally, we found that only 11% to 17.4% of medical students had evidence of latent tuberculosis infection when measured using TST and IGRA, respectively, and none of the participating medical students had a positive HIV test result.

Prior studies on TB in health workers from low and middle income countries show very high average rates usually above 60% [13, 26, 27, 62]. None of these studies specifically evaluated medical students but the Review by Joshi et al in 2006 presented combined numbers for medical and nursing students in multiple low and middle income countries. These rates were highly variable 7% - 40%. The only African country represented was Uganda and that was a 40% LTBI prevalence.

Two articles have been published since 2003 that measure HIV rates in South African health care workers [59, 61]. Neither of these studies included or identified medical students. Shisana et al. evaluated health care workers across South Africa in 2004. They used cluster methods to sample a representative 5% of the total health facilities in South Africa and surveyed and tested 595 out of 721 eligible health workers identified by their sample. They presented a total HIV rate of 15.7% for all HCWs. They specified their findings based on professionals (Physicians and Nurses, n = 349) and non-professionals (n= 246). They did not specify how many of the professionals were physicians, and they did not test students. The HIV rate for professionals was 11.7%. Shisana et al stratified HIV results by age for the full sample. HIV prevalence was higher at younger ages. Among 18 – 35 year olds HIV prevalence was 20% (n = 203), among 36-45 year olds it was 16.6% (n=221). Based on this study we expected a 15% HIV prevalence among medical and nursing students combined. This estimate is based on the 11.7% prevalence for professionals and the 20% prevalence for younger health workers.

The 0% HIV prevalence found in our study may be biased by the fact that only 39% of eligible students participated. In the original study design nursing students were to be included

along with medical students. This was changed because of practical limitations at the study site. It may be that there is a difference in the HIV risk of physicians as opposed to nurses or of students as opposed to professionals.

A study by Connelly et al was published since we developed our protocol. They tested 1493 out of 1813 HCWs at two hospitals in the Gauteng province of South Africa. They also stratified HIV prevalence by age but chose smaller intervals, 18 – 24 years, 25 – 34 years, etc. The overall HIV positive rate was 11.5%. For those 18 – 24 years old the prevalence was only 6.7% (n = 105). For 25 – 35 year olds HIV prevalence was 15.9% (n = 326) The prevalence in these two age groups combined is 13.6%, this is lower than the 20% calculated by Shisana and may be more relevant for our population because it is in Gauteng Province. It also shows that the younger group, aged 18-24, is much less likely to be positive than the group of 18-35 year olds.

The Connelly study also presented HIV rates for doctors, nurses, and student nurses separately. Physicians had a surprisingly low HIV prevalence at 2% (1 out of 49). Student Nurses were the professional group with the highest HIV prevalence at 13. 8% (n=65). It is interesting to note that while they had a response rate of 82.3% among all HCWs, among physicians it was less than 25%. This is consistent with our result of 39% participation and 0% HIV positivity. It may be that physicians and physician students are at much lower risk than other health workers in South Africa. It may also be that Connelly's study and ours are suffering from the same biased selection of only the lowest risk individuals.

Race was correlated with positive IGRA results, with Black race being associated with having a positive result. Childhood background was also associated with the IGRA with people from rural environments more likely to have a positive IGRA. These results are all limited by the small numbers in this study. Other studies have shown similar results for race but usually children from urban environments are more likely to test positive for TB [2, 4, 60].

In conclusion the level of participation, the prevalences of HIV and LTBI are all lower than expected in this sample. In order to investigate the participation – a group of medical students

involved in this study have started a student project to elicit reasons for participation or lack of participation from their peers. They hypothesize that the requirement of HIV testing is frightening and unacceptable for many because of the severe consequences that a positive result can entail in South Africa. If they are right and at-risk physicians and physician students systematically avoid any study that could identify HIV then it may be difficult to evaluate HIV or TB in this population. Others speculate that physicians in South Africa do not carry the risk of TB and HIV infection that other health workers do because of social separation and a distant approach to the practice of medicine. Regardless of the answer, for the purpose of this study follow up studies of HIV and TB risk for the goal of testing TB control interventions may have better results with nursing students and nurses as they are more likely to participate and more likely to suffer the highest burden of disease.

As we move forward with the analysis and further data are collected it will be important to evaluate any differences between participants and non-participants to judge how well our sample represents the medical students. Based on our data and information from Connelly, data from nursing students and other health workers will need to be analyzed separately from that of the medical students.

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Unique patient study Identifier:

TB INFECTION RISK AMONG HEALTH CARE WORKERS

Questionnaire

We ask you to complete this questionnaire. Please answer what you think is correct; do not ask others for their opinion. The questionnaire will be linked to your blood results and your tuberculin skin test. Your answers will only be accessible to investigators of the study and will not be shown to anyone else. Your name will never appear in any communication about this study.

First, we would like to ask you some *general information about yourself.*

YOU MAY CHOOSE NOT TO ANSWER ANY QUESTION

SECTION A: SOCIO-DEMOGRAPHIC INFORMATION

A1. Age |___|___| years

A2. Gender ☐ male ☐ female

A3. What is your ethnic or racial identity?

☐ Black ☐ White ☐ Coloured

☐ Indian ☐ Other_____

A4. How would you describe the environment where you spent most time as child?

☐ Urban (in city) ☐ Rural ☐ Mixed

A5. Were your parents healthcare workers?

☐ Neither ☐ Mother only ☐ Father only ☐ Both

A6. Are you a

☐ Medical student ☐ Nursing student

☐ Nurse ☐ Laboratory worker

A7. Do any of the scenarios below apply to you:

1. I have been diagnosed with TB and treated for TB before. ☐ applicable ☐ not applicable

2. An immediate family member of mine has been diagnosed with and treated for TB before ☐ applicable ☐ not applicable

3. Someone I know (colleague, friend or extended family) has been diagnosed with and treated for TB before ☐ applicable ☐ not applicable

SECTION B: TB KNOWLEDGE

Now we would like to ask you questions about tuberculosis. You can either choose one answer or tick all answers that apply if more than one answer is correct.

YOU MAY CHOOSE NOT TO ANSWER ANY QUESTION

B1. Which part of the body does TB most commonly affect?

☐ Glands. ☐ Lungs. ☐ Bones. ☐ Brain.

B2. How is pulmonary TB spread?

☐ Air. ☐ Water. ☐ Food.

B3. How should health workers identify TB suspects among those people attending health services in South Africa?

☐ Take a blood sample in all people attending health services
☐ Ask everyone attending if they have had a cough for more than two weeks,
☐ Request a chest X-ray in all people attending health services who have been coughing for more than 5 days

B4. List two safety precautions when collecting a sputum sample.

_____

_____

B5. Why is a different TB treatment regimen give to previously treated patients?

☐ They are more likely to have HIV
☐ They are more likely to have resistant TB
☐ They are more likely to have side effects of the TB drugs

B6. Compared to those not infected with HIV, people living with HIV are

☐ Less likely to have active TB disease

☐ More likely to have active TB disease

☐ Equally likely to have active TB disease

B7. Which of the following actions are effective in keeping TB from spreading to a patient's family and other people?

☐ Close windows and doors in patient's home.
☐ Open windows and doors in patient's home.
☐ Tell the patient to cover his or her mouth and nose when coughing.
☐ Tell the patient to eat special foods.
☐ Make sure that the patient takes regular TB treatment.
☐ Sterilize all dishes and other items touched by the patient.

B8. Which explanations below most accurately describe the presentation of latent tuberculosis infection?

- ☐ People with latent tuberculosis infection present with the same symptoms as people with active TB disease.
- ☐ People with latent tuberculosis infection present with different symptoms as people with active TB disease.
- ☐ People with latent tuberculosis infection have no symptoms.

B9. What may happen to people who have latent tuberculosis infection?

- ☐ All people with latent tuberculosis infection will develop TB disease.
- ☐ Some people with latent tuberculosis infection will develop TB disease.
- ☐ Some people with latent tuberculosis infection will develop TB disease, especially those who are also infected with HIV

B10. How can health care workers in South Africa diagnose latent tuberculosis infection?

- ☐ Ask person for symptoms of latent tuberculosis infection.
- ☐ Perform chest X-ray.
- ☐ Perform skin test.

B11. Can people with latent infection transmit *Mycobacterium tuberculosis* to others?

- ☐ People with latent TB infection are not infectious.
- ☐ People with latent TB infection are as infectious as people with active TB disease.
- ☐ People with latent TB infection are less infectious as people with active TB disease.

B12. Which of the following is true regarding the mechanism of isoniazid preventive therapy (IPT)?

- ☐ IPT prevents a person from being infected with *Mycobacterium tuberculosis*.
- ☐ IPT prevents a person from developing reactivation TB disease.
- ☐ IPT sterilizes latent TB infection

B13. According to South African guidelines, who should receive Isoniazid preventive therapy?

- ☐ Health care workers in contact with adults with TB disease.
- ☐ Everyone in contact with adults with TB disease.
- ☐ Young children without symptoms who were in contact with an adult with TB.
- ☐ People living with HIV who also have active TB disease
- ☐ People living with HIV who do not have active TB

B14. What do you know about the effect of BCG on the tuberculin skin test?

- ☐ No effect
- ☐ People vaccinated with BCG are likely to have a positive skin test when they are not infected with tuberculosis (false positive test)
- ☐ People vaccinated with BCG are likely to have a negative test when they are infected with tuberculosis (false negative test).

B15. What do you know about the effect of HIV on the tuberculin skin test?

- ☐ No effect
- ☐ People living with HIV are likely to have a positive skin test when they are not infected with tuberculosis (false positive test)
- ☐ People living with HIV are likely to have a negative test when they are infected with tuberculosis (false negative test).

SECTION C: OCCUPATIONAL TB

We would like to ask you questions about the risk of tuberculosis among health care workers. You can either choose one answer or tick all answers that apply if more than one answer is correct.

YOU MAY CHOOSE NOT TO ANSWER ANY QUESTION

C1. Do you think you have been exposed to TB (at work/place of study or elsewhere) in the past 2 months? ☐ yes ☐ no

C2. How would you rate the possibility that you are infected with tuberculosis?
☐ very likely ☐ likely ☐ unlikely ☐ highly unlikely

C3. How would you rate the possibility that you become infected with tuberculosis in the next 5 years?
☐ very likely ☐ likely ☐ unlikely ☐ highly unlikely

C4. How would you rate the possibility that you become ill with tuberculosis disease in the next 5 years?
☐ very likely ☐ likely ☐ unlikely ☐ highly unlikely

C5. How would you rate the possibility that you will die from tuberculosis if you were to fall ill with tuberculosis disease?
☐ very likely ☐ likely ☐ unlikely ☐ highly unlikely

C6. Have you had training or received instructions on how to prevent becoming infected with tuberculosis while working in a clinic or hospital?
☐ yes ☐ no

C7. Do you try to protect yourself from exposure to tuberculosis?
☐ yes ☐ no

If yes, how?

C8. If the government would offer free testing for latent tuberculosis infection using the skin test for all health care workers, would you agree to be tested when the skin test is used?

☐ yes If yes, why?

☐ no If no, why not? (check all that apply)

- ☐ I know it will be positive
- ☐ I know it will be negative
- ☐ I don't want to know the result
- ☐ Others might think badly of me if I am infected
- ☐ It might hurt my opportunity to work if I am infected
- ☐ I do not believe the skin test result
- ☐ Even if it was positive, I do not want to take 6 months of isoniazid
- ☐ I would only do the test for tuberculosis it if I am infected with HIV
- ☐ Other reason – please explain:

C9. The government offers free testing for HIV infection for all health care workers, have you been tested for HIV?

☐ yes ☐ no

If yes, why?

If no, why not? (check all that apply)

- ☐ I know it will be positive
- ☐ I know it will be negative
- ☐ I don't want to know the result
- ☐ Others might think badly of me if I am infected
- ☐ It might hurt my opportunity to work if I am infected
- ☐ Other reason: please explain

C10. As we have explained to you, there is a new blood test for tuberculosis infection. If it is demonstrated that this test works better than the skin test (but is still not perfect), and the government would offer this blood test for free, would you want to be tested?

☐ yes ☐ no

If yes, why?

If no, why not? (check all that apply)

- ☐ I know it will be positive
- ☐ I know it will be negative
- ☐ I don't want to know the result
- ☐ Others might think badly of me if I am infected
- ☐ It might hurt my opportunity to work if I am infected
- ☐ I do not believe the blood test result
- ☐ Even if it was positive, I do not want to take 6 months of isoniazid
- ☐ Other reason – please explain:

C11. There are several measures that can help reduce the risk of TB infection. We want your opinion on these measures. For each of the following possible infection control measures, please answer which one you think it should be done in South African clinics and hospitals? Which ones you think could work in South African clinics and hospitals? Which ones you think should be a priority?

a. Have people who cough jump the queue by placing all those who cough at the front of the line, so one can quickly provide care and reduce the amount of time that others are exposed to them.

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

b. Hang up posters that show patients respiratory hygiene (cover mouth and nose with hand or tissue when coughing or sneezing)

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

c. Ask all patients to wear a mask while in the clinic

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

d. Ask all patients suspect of having TB to wear mask while in the clinic

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

e. Ask all patients known to have TB to wear a mask while in the clinic.

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

f. Ask those who cough to wait in a different area.

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

g. Have all patient wait outside or in rooms with open windows, even in winter.

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

h. Always ask patients to collect their sputum sample outside

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

i. Train and educate health care workers on prevention, transmission, and symptoms of TB

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

j. Start TB screening program: screening and evaluating health care workers who are at risk for TB disease or who might be exposed to tuberculosis.

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

k. Have all health care workers wear a mask whenever in contact with any patient

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

l. Have all health care workers wear a mask whenever in contact with TB suspects

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

m. Have all health care workers wear a mask whenever in contact with known TB patients

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

n. Install UV (ultra-violet) lights in waiting rooms

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

o. Install ventilation systems in clinics and hospitals (expensive)

☐ do ☐ not do

☐ could work ☐ would not work

☐ priority ☐ not priority

If you have any comments or suggestions, feel free to write them here.

THANKYOU VERY MUCH FOR YOUR PARTICIPATION IN THIS STUDY