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Risk Factors Associated with Positive QuantiFERON-TB Gold In-Tube and Tuberculin Skin Tests Results in Zambia and South Africa

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Abstract

Introduction: The utility of T-cell based interferon-gamma release assays for the diagnosis of latent tuberculosis infection remains unclear in settings with a high burden of tuberculosis.

Objectives: To determine risk factors associated with positive QuantiFERON-TB Gold In-Tube (QFT-GIT) and tuberculin skin test (TST) results and the level of agreement between the tests; to explore the hypotheses that positivity in QFT-GIT is more related to recent infection and less affected by HIV than the TST.

Methods: Adult household contacts of tuberculosis patients were invited to participate in a cross-sectional study across 24 communities in Zambia and South Africa. HIV, QFT-GIT and TST tests were done. A questionnaire was used to assess risk factors.

Results: A total of 2,220 contacts were seen. 1,803 individuals had interpretable results for both tests, 1,147 (63.6%) were QFT-GIT positive while 725 (40.2%) were TST positive. Agreement between the tests was low (kappa = 0.24). QFT-GIT and TST results were associated with increasing age (adjusted OR [aOR] for each 10 year increase for QFT-GIT 1.15; 95% CI: 1.06–1.25, and for TST aOR: 1.10; 95% CI 1.01–1.20). HIV positivity was less common among those with positive results on QFT-GIT (aOR: 0.51; 95% CI: 0.39–0.67) and TST (aOR: 0.61; 95% CI: 0.46–0.82). Smear positivity of the index case was associated with QFT-GIT (aOR: 1.25; 95% CI: 0.90–1.74) and TST (aOR: 1.39; 95% CI: 0.98–1.98) results. We found little evidence in our data to support our hypotheses.

Conclusion: QFT-GIT may not be more sensitive than the TST to detect risk factors associated with tuberculous infection. We found little evidence to support the hypotheses that positivity in QFT-GIT is more related to recent infection and less affected by HIV than the TST.

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Introduction

Tuberculosis continues to be a major public health problem in sub-Saharan Africa. The incidence of tuberculosis [1] is being accelerated by high rates of HIV co-infection [2,3]. Targeted testing and treatment of latent tuberculosis infection (LTBI) especially among HIV positive individuals is an important strategy to reduce the incidence of tuberculosis [4].

Currently, LTBI detection relies on the tuberculin skin test (TST) in most countries with high incidence of tuberculosis although this is not routinely performed and is perceived as a barrier to accessing TB preventive therapy [5]. However, TST has many reported limitations. These include low specificity in populations with high levels of BCG vaccination or significant exposure to non-tuberculosis mycobacteria (NTM), and reduced sensitivity in immunocompromised individuals such as those with HIV infection [6,7].

T-cell based interferon-gamma release assays (IGRAs) such as the QuantiFERON-TB Gold In-Tube (QFT-GIT) can now also be used to detect LTBI [8]. IGRAs are in-vitro blood tests based on interferon-γ release after T-cell stimulation by antigens more specific to Mycobacterium tuberculosis (Mtb) than the purified protein
derivative used in TST. IGRAs are therefore designed to have high specificity that is unaffected by BCG vaccination and cross-reactivity with most NTM [8]. There is also some evidence of greater sensitivity among HIV positive individuals [9,10] compared to the TST. Current literature suggests that IGRAs detect responses of effector T-cells that have recently encountered antigens in vivo, while TST reflects the mobilization of a wider spectrum of memory T-cells that are long-lived [11].

The use of IGRAs in developed countries is rapidly expanding but their performance in settings with a high prevalence of tuberculosis and HIV still requires further research [12,13]. There is growing evidence that IGRAs performance varies in different settings [14]. In high-TB burden settings, the results of IGRAs may be influenced by factors that affect the immune response [15] such as HIV co-infection, BCG vaccination, malnutrition, tropical infections and widespread exposure to NTM. Recent studies done in low and middle income settings [16] showed a large reduction in the proportion of positive test results for both QFT-GIT and TSPOT in HIV infected individuals. Another recent study in Bangladesh showed that malnutrition and helminth infections were associated with indeterminate QFT-GIT results in children [17].

Significant challenges exist in directly assessing whether IGRAs are superior to TST in diagnosing LTBI as there remains no gold

Figure 1. Flow diagram of study participants. QFT-GIT (Quantiferon-TB Gold In Tube) not done was due to refusal (18.4%), being absent (18.3%), insufficient blood samples (0.4%) or missing data (1.6%). TST (Tuberculin skin test) not done was due to refusal (5.6%), not returning for reading (3.6%) or missing data (0.8%). Individuals with QFT-GIT/TST not done and those with indeterminate QFT-GIT results were excluded from analysis.

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standard against which to compare either test. In the absence of a practical gold standard for 
*Mtb* infection, exposure to an infectious TB index case has been used as a surrogate measure of infection [18,19,20,21]. Studies from low-TB burden countries indicate that the IGRAs correlate better, along a gradient of exposure, than the TST [14]. Nevertheless, in high-TB burden settings, the TST performs reasonably well and correlates as well, or better, with proxy measures of exposure [14]. There are limited data on the comparison of QFT-GIT and TST in relation to *Mtb* exposure as a surrogate measure of infection and the influence of age [19].

In this study, we describe the prevalence of tuberculous infection among household contacts of recently diagnosed tuberculosis patients as measured by QFT-GIT and TST in 24 communities with a high prevalence of TB and HIV in Zambia and South Africa. We also determine risk factors associated with positive QFT-GIT and TST results and the level of agreement between the tests in each community. We use data from two recent TST surveys [22] to explore the correlation between community TB transmission and infection prevalence in contacts as measured by QFT-GIT and TST. A TST survey, if conducted correctly and technically interpretable, allows an estimation of the extent of *Mtb* transmission that has occurred in the community [23].

Finally, we formally assess the extent to which our results are compatible with expected findings on the basis of a number of prior hypotheses about the characteristics of each test. Previous studies have given rise to prevailing views about the expected performance of both TST and IGRAs [7,11,14,16]. We therefore explore whether our data support the hypotheses that positivity in QFT-GIT is more related to recent infection and less affected by HIV than the TST.

### Methods

#### Ethics statement

Ethics approval for the study was obtained from the research ethics committees of the University of Zambia, the London School of Hygiene and Tropical Medicine and Stellenbosch University. All individuals involved in the study gave written informed consent.

#### Study setting

This cross-sectional study was nested within a large community randomized trial of interventions to reduce tuberculosis transmission, the Zambia South Africa TB and AIDS Reduction Study (ZAMSTAR) in 24 selected communities in Zambia and South Africa [24]. We defined a “community” as the population (minimum size of 25,000) accessing one tuberculosis diagnostic centre and this was the unit of randomization for the ZAMSTAR trial. The communities selected were in five provinces of Zambia (16 communities) and in Western Cape Province of South Africa (8 communities) and included both urban and rural communities. The design of the ZAMSTAR study is described elsewhere [24,25].

Baseline measurement of tuberculous infection in all ZAMSTAR communities was estimated by means of TST surveys among primary school children [22]. These community-wide surveys served three objectives: to characterize ZAMSTAR communities, with regards to TB infection, in relative terms; to inform the randomization of the communities into intervention arms; and to provide data for one of ZAMSTAR’s secondary outcomes. The design of the ZAMSTAR study is described elsewhere [24,25].

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Zambia and South Africa have among the highest tuberculosis incidence [26] and HIV seroprevalence rates [27] in Africa and globally. The estimated HIV prevalence in new tuberculosis cases is 70% [26].

#### Participants

From April 2007 to July 2008 we recruited newly notified adult tuberculosis cases from the 24 ZAMSTAR communities, subse-

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**Table 1. Characteristics of the study population.**

| Study participants n (column %) |  |
|---------------------------------|--|
| **Total**                       | 2220 |
| **Sex**                         |     |
| Male                            | 666 (30.1) |
| Female                          | 1545 (69.9) |
| Missing                         | 9   |
| **Age group (years)**           |     |
| 15–24                           | 835 (38.3) |
| 25–34                           | 556 (25.5) |
| 35–44                           | 302 (13.9) |
| 45–54                           | 246 (11.3) |
| 55–64                           | 134 (6.1) |
| ≥65                             | 106 (4.9) |
| Missing                         | 41  |
| **Age in years:** Median 28 (IQR:21–42); mean 33 | |
| **Highest level of education**  |     |
| Not attended school             | 142 (6.5) |
| Primary school                  | 689 (31.9) |
| Secondary school                | 1126 (52.1) |
| College or University           | 203 (9.4) |
| Missing                         | 60  |
| **Smoking habits**              |     |
| Daily smoker                    | 220 (10.0) |
| Occasional smoker               | 73 (3.3) |
| Ex-smoker                       | 104 (4.7) |
| Never smoked                    | 1801 (81.9) |
| Missing                         | 22  |
| **Alcohol consumption**         |     |
| No                              | 1648 (75.2) |
| Yes                             | 544 (24.8) |
| Missing                         | 28  |
| **Household size (adults)**     |     |
| 1–3                            | 331 (15.0) |
| 4–6                            | 848 (38.4) |
| 7–9                            | 554 (25.1) |
| ≥10                            | 477 (21.6) |
| Missing                         | 10  |
| **HIV status**                  |     |
| Negative                        | 1271 (62.4) |
| Positive                        | 765 (37.6) |
| Missing                         | 184 |
| **Smear status of index**       |     |
| Smear negative                  | 741 (49.2) |
| Smear positive                  | 766 (50.8) |
| Missing                         | 713 |

1Defined as alcohol consumption four weeks prior to the interview. 

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| Community code | Geography | Urban/rural | ¹HIV prevalence | Number with both TST & QFT results | QFT-GIT positive (%) | TST positive (10 mm) (%) | Kappa |
|----------------|-----------|-------------|-----------------|------------------------------------|---------------------|------------------------|-------|
| SA1            | Province  | Urban       | Moderate        | 109                                | 78                  | 42                     | 0.17  |
| SA 3           | Metropole | Urban       | High            | 98                                 | 77                  | 77                     | 0.49  |
| SA 6           | Province  | Rural       | Moderate        | 70                                 | 77                  | 27                     | 0.15  |
| Z 4            | Lusaka    | Urban       | High            | 69                                 | 72                  | 41                     | 0.25  |
| SA 5           | Metropole | Urban       | Moderate        | 52                                 | 71                  | 62                     | 0.28  |
| Z 5            | Copperbelt| Urban       | High            | 45                                 | 71                  | 13                     | -0.09 |
| SA 2           | Province  | Rural       | Moderate        | 87                                 | 70                  | 28                     | 0.16  |
| Z 1            | Lusaka    | Urban       | High            | 93                                 | 67                  | 49                     | 0.36  |
| Z 15           | Luapula   | Rural       | Moderate        | 24                                 | 67                  | 33                     | 0.25  |
| SA 7           | Metropole | Urban       | High            | 74                                 | 66                  | 76                     | 0.38  |
| Z 11           | Luapula   | Rural       | Moderate        | 40                                 | 65                  | 73                     | 0.02  |
| SA 8           | Metropole | Urban       | High            | 124                                | 65                  | 24                     | -0.01 |
| Z 7            | Lusaka    | Urban       | High            | 92                                 | 61                  | 38                     | 0.19  |
| Z 6            | Lusaka    | Urban       | High            | 84                                 | 61                  | 61                     | 0.48  |
| Z 8            | Southern  | Urban       | High            | 121                                | 60                  | 42                     | 0.41  |
| Z 3            | Copperbelt| Urban       | High            | 48                                 | 60                  | 17                     | 0.16  |
| Z 10           | Central   | Urban       | High            | 68                                 | 59                  | 34                     | 0.08  |
| Z 12           | Copperbelt| Urban       | High            | 97                                 | 59                  | 15                     | 0.19  |
| Z 13           | Central   | Urban       | High            | 103                                | 50                  | 23                     | 0.16  |
| Z 2            | Copperbelt| Urban       | High            | 90                                 | 48                  | 28                     | 0.32  |
| Z 16           | Southern  | Rural       | Moderate        | 18                                 | 44                  | 17                     | 0.16  |
| Z 9            | Southern  | Urban       | High            | 29                                 | 38                  | 7                      | 0.22  |
| Z 14           | Southern  | Rural       | Moderate        | 28                                 | 29                  | 11                     | 0.25  |

Communities arranged from highest to lowest TB infection prevalence estimates as defined by Quantiferon-TB Gold In-Tube (QFT-GIT) test. SA: South African community; Z: Zambian community; TST (Tuberculin skin test). Geography, urban/rural and HIV prevalence as described elsewhere [22,25].

1A panel of eight experts critically examined data from ante-natal clinic surveillance, prevention of mother-to-child transmission programmes, voluntary counseling and testing clinics and provincial demographic and health survey data and made an informed decision whether to categorize HIV prevalence as ‘high’ or ‘moderate’ for each community [25].

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Figure 2. Scatter plot of positive QFT-GIT results in contacts and infection prevalence results from previous TST surveys. Previous TST surveys among children were conducted within the same communities as those of contacts. Infection prevalence in children was defined as TST ≥10 mm.

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quently referred to as index cases, to the study. All tuberculosis cases (pulmonary smear positive, smear negative or extrapulmonary) were eligible if recruited within a month after being notified in the tuberculosis register and started on treatment at a government clinic. We obtained written informed consent from those accepting to take part in the study. In addition, we sought permission from these index cases to visit their households where we invited household members to participate. We made at least three attempts to visit household members who were absent during the first visit to the household.

We defined household contacts as individuals at least 15 years old, who generally slept in the home, ate with the index case and who identified a common household head. We asked all household contacts for individual signed consent before participating in the study. This study focuses on this population of household contacts of newly diagnosed tuberculosis cases.

**Measures**

Consenting household contacts had blood drawn for HIV antibodies and QFT-GIT testing. Tuberculin skin tests were also performed. A standardized questionnaire was administered to all contacts by trained interviewers, who collected information on risk factors associated with tuberculous infection. Sputum microscopy for index cases was performed as part of the clinic routine services and the results were recorded in the TB registers.

HIV testing was done using the Abbot Murex HIV Ag/Ab combination ELISA (Murex Biotech, Dartford, United Kingdom). All individuals were encouraged to attend counseling and HIV testing at the local health centre. In South Africa, HIV positive individuals were advised to go for TB preventive therapy in accordance with National Tuberculosis Control Program guidelines [28] while in Zambia this is not yet government policy. However, in Zambia, preventive therapy was offered to eligible contacts through collaboration with another study operating in the ZAMSTAR sites.

**QFT-GIT procedure.** QFT-GIT test was performed according to the manufacturer's instructions [29]. For four Zambian and all the South African communities, QFT-GIT processing was done centrally at our research laboratories in Lusaka and Stellenbosch University Medical School respectively. However, for twelve Zambian remote communities, QFT-GIT processing was decentralized. In these communities, blood samples were collected, incubated, separated and stored locally. Tubes were incubated for 16–24 hours at 37°C and plasma was harvested and frozen at –20°C. Frozen samples from these sites were transported monthly to the central laboratory in Lusaka where the ELISA was performed manually in batches.

**TST procedure.** The skin testing was conducted using 2 TU (Tuberculin Units) of PPD RT23 with Tween, supplied by the Statens Serum Institut (Copenhagen, Denmark). All tests were administered and read by nurses who were trained according to the standard IUATLD protocol [23]. A dose of 0.1 ml was injected intradermally on the left forearm. Skin reactions were read using calipers 72 hours later. A positive TST was defined as an induration of ≥ 10 mm. Blood for QFT-GIT was drawn before TST was administered usually on the same day.

**Statistical analysis**

Data were double entered into a “Microsoft SQL Server” database and checked for errors. Analysis was performed using STATA (version 11.0).

The characteristics of the study population were described using frequencies and percentages for categorical variables and the median and interquartile range for quantitative variables. Prevalence of infection was defined as the number of QFT-GIT or TST positive results divided by the total number of individuals with interpretable (positive and negative) results. Individuals having missing TST or indeterminate QFT-GIT results were excluded from the analysis. These did not differ significantly from those that had interpretable results (results not shown). Furthermore, household contacts on TB treatment were excluded from analysis.

The level of agreement between test results was assessed for each community using Cohen’s kappa coefficient. By convention, kappa values of less than 0.4 generally indicate poor agreement. Correlation between continuous interferon-γ values (IU/ml) and TST induration (mm) was assessed using Spearman’s correlation coefficient for each community.
Table 3. Univariable and multivariable odds ratios showing risk factors associated with positive QuantIFERON-TB Gold In Tube assay results.

| QFT positive | Crude OR (95% CI) | Adjusted OR (95% CI) |
|--------------|-------------------|----------------------|
| n (row %)    |                   |                      |
| Total        | 1147/1803 (63.6%) |                      |
| **Sex**      |                   |                      |
| Male         | 333 (63.5)        | 1                    |
| Female       | 809 (63.7)        | 1.08 (0.76–1.25)     |
| Missing      | 5                 | 0.93 (0.72–1.20)     |
| **Age group (years)** |   |                      |
| 15–24        | 417 (60.8)        | 1                    |
| 25–34        | 276 (61.3)        | 1.00 (0.74–1.34)     |
| 35–44        | 159 (66.5)        | 1.35 (0.93–1.96)     |
| 45–54        | 139 (68.5)        | 1.49 (1.00–2.22)     |
| 55–64        | 87 (75.6)         | 2.46 (1.43–4.23)     |
| ≥65          | 53 (64.6)         | 1.42 (0.79–2.53)     |
| Missing      | 16                |                      |
| **Highest level of education** |   |                      |
| Not attended school | 75 (64.7) | 1 | 1 |
| Primary school | 378 (67.1) | 1.11 (0.68–1.81) | 1.40 (0.82–2.38) |
| Secondary school | 551 (60.9) | 0.79 (0.49–1.28) | 0.99 (0.57–1.71) |
| College or University | 111 (67.3) | 1.12 (0.62–2.02) | 1.40 (0.73–2.69) |
| Missing | 32 | | |
| **Smoking habits** |   |                      |
| Never smoked | 913 (62.7)        | 1                    |
| Ex-smoker    | 42 (53.9)         | 0.73 (0.43–1.24)     |
| Occasional smoker | 43 (70.5) | 1.40 (0.73–2.66) | 1.13 (0.57–2.25) |
| Daily smoker | 137 (72.9)        | 1.69 (1.14–2.50)     |
| Missing      | 12                |                      |
| **Alcohol consumption** |   |                      |
| No           | 844 (62.9)        | 1                    |
| Yes          | 285 (65.4)        | 1.11 (0.85–1.45)     |
| Missing      | 18                | 1.04 (0.78–1.38)     |
| **Household size (adults)** |   |                      |
| 1–3          | 170 (61.8)        | 1                    |
| 4–6          | 415 (62.7)        | 1.03 (0.73–1.46)     |
| 7–9          | 302 (64.4)        | 1.14 (0.78–1.67)     |
| ≥10          | 258 (65.3)        | 1.16 (0.77–1.75)     |
| Missing      | 2                 | 1.68 (1.09–2.61)     |
| **HIV status** |   |                      |
| Negative     | 728 (69.0)        | 1                    |
| Positive     | 335 (54.6)        | 0.48 (0.37–0.61)     |
| Missing      | 84                | 0.51 (0.39–0.67)     |
| **Smear status of index** |   |                      |
| Smear negative | 373 (60.8) | 1 | 1 |
| Smear positive | 426 (67.6) | 1.48 (1.09–2.01) | 1.25 (0.90–1.74) |
| Missing      | 348               |                      |
| **Sleeping proximity to index** |   |                      |
| Different house | 72 (57.6) | 1 | 1 |
| Same house   | 355 (63.6)        | 1.31 (0.78–2.18)     |
| Same room    | 36 (56.3)         | 0.92 (0.42–2.02)     |

Risk Factors Associated with Tuberculous Infection
The distribution of positive reactions to each test in relation to established individual and household level risk factors for LTBI was described. The strength of relationship between risk factors and QFT-GIT/TST positivity was assessed using random effects logistic regression. The random effects approach specified the clustering variable. All models were adjusted for age, sex and community of residence. We present adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for each risk factor.

Finally, we formally assessed four hypotheses related to the expected performance of the tests. We explored whether our data were compatible with expected findings on the basis of a number of prior hypotheses about the characteristics of each test. Ideally, we would have had a gold standard measure of LTBI against which to compare both tests. However, no such test currently exists. Furthermore, the natural history of LTBI remains a source of debate. Compare both tests. However, no such test currently exists. Additionally, the natural history of LTBI remains a source of debate.

We then explored: (ii) whether age was more strongly associated with a positive TST result than with a positive QFT-GIT result, since we expect HIV infection to cause more false negative results with TST than QFT-GIT. We restricted the analysis to individuals with a positive QFT-GIT result and used random effects logistic regression models as described previously.

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For these final three hypotheses we conducted matched-pairs analysis using conditional logistic regression in an approach similar to that used by Ewer and others (21), where the outcome is the positivity of the index and QFT-GIT (aOR: 1.25; 95% CI: 0.90–1.20; p = 0.025 for linear trend). HIV positivity was less common among those with positive results on QFT-GIT (aOR: 0.51; 95% CI: 0.32–0.82; p = 0.001). Overall, infection prevalence as measured by QFT-GIT was higher for South African communities (arithmetic mean 50%, range 24–77%) than for the Zambian communities (arithmetic mean 31%, range 7–73%). Results were similar for QFT-GIT (results not shown).

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For these final three hypotheses we conducted matched-pairs analysis using conditional logistic regression in an approach similar to that used by Ewer and others (21), where the outcome is the positivity of the index and QFT-GIT (aOR: 1.25; 95% CI: 0.90–1.20; p = 0.025 for linear trend). HIV positivity was less common among those with positive results on QFT-GIT (aOR: 0.51; 95% CI: 0.32–0.82; p = 0.001). Overall, infection prevalence as measured by QFT-GIT was higher for South African communities (arithmetic mean 50%, range 24–77%) than for the Zambian communities (arithmetic mean 31%, range 7–73%). Results were similar for QFT-GIT (results not shown).
Table 4. Univariable and multivariable odds ratios showing risk factors associated with positive tuberculin skin test results.

| TST ≥10 mm | n (row %) | Crude OR (95% CI) | Adjusted OR (95% CI) |
|------------|-----------|-------------------|----------------------|
| Total      | 725/1803 (40.2%) | | |
| **Sex**    |           |                   |                      |
| Male       | 203 (38.7) | 1                 | 1                    |
| Female     | 520 (40.9) | 1.22 (0.92–1.62)  | 1.18 (0.90 – 1.56)   |
| Missing    | 2         |                   |                      |
| **Age group (years)** |   |                   |                      |
| 15–24      | 263 (38.4) | 1                 | 1                    |
| 25–34      | 177 (39.3) | 1.10 (0.79–1.54)  | 1.05 (0.77–1.45)     |
| 35–44      | 102 (42.7) | 1.41 (0.93–2.13)  | 1.49 (1.00–2.21)     |
| 45–54      | 88 (43.3)  | 1.34(0.87–2.07)   | 1.34 (0.89–2.04)     |
| 55–64      | 58 (50.4)  | 2.01(1.17–3.47)   | 2.03 (1.19–3.45)     |
| >65        | 28 (34.1)  | 1.05(0.55–2.00)   | 1.12 (0.58–2.17)     |
| Missing    | 9         |                   |                      |
| **Highest level of education** |   |                   |                      |
| Not attended school | 39 (33.6) | 1 | 1 |
| Primary school | 236 (41.9) | 1.47 (0.85–2.55) | 1.53 (0.88–2.66) |
| Secondary school | 365 (40.3) | 1.22 (0.72–2.10) | 1.29 (0.72–2.31) |
| College or University | 74 (44.8) | 1.49 (0.77–2.90) | 1.13 (0.57–2.25) |
| Missing | 11 | | |
| **Smoking habits** | | | |
| Never smoked | 573 (39.3) | 1 | 1 |
| Ex-smoker | 32 (41.0) | 1.19 (0.64–2.20) | 1.09 (0.59–2.02) |
| Occasional smoker | 31 (50.8) | 1.51 (0.75–3.02) | 1.26 (0.63–2.54) |
| Daily smoker | 85 (45.2) | 1.33 (0.87–2.02) | 1.10 (0.70–1.73) |
| Missing | 4 | | |
| **Alcohol consumption** | | | |
| No | 544 (40.6) | 1 | 1 |
| Yes | 174 (39.9) | 0.93 (0.68–1.27) | 0.94 (0.69–1.28) |
| Missing | 7 | | |
| **Household size (adults)** | | | |
| 1–3 | 127 (46.2) | 1 | 1 |
| 4–6 | 261 (39.4) | 0.68 (0.44–1.03) | 0.77 (0.52–1.13) |
| 7–9 | 192 (40.9) | 0.72 (0.46–1.14) | 0.91 (0.59–1.40) |
| ≥10 | 144 (36.5) | 0.57 (0.35–0.94) | 0.71 (0.44–1.13) |
| Missing | 1 | | |
| **HIV status** | | | |
| Negative | 465 (44.1) | 1 | 1 |
| Positive | 207 (33.8) | 0.57 (0.43–0.76) | 0.61 (0.46 – 0.82) |
| Missing | 53 | | |
| **Smear status of index** | | | |
| Smear negative | 230 (37.5) | 1 | 1 |
| Smear positive | 290 (46.0) | 1.65 (1.15–2.36) | 1.39 (0.98 – 1.98) |
| Missing | 205 | | |
| **Sleeping proximity to index** | | | |
| Different house | 43 (34.4) | 1 | 1 |
| Same house | 202 (36.2) | 1.08 (0.62–1.89) | 0.76 (0.44-1.30) |
| Same room | 20 (31.2) | 0.80 (0.34–1.92) | 0.94 (0.41–2.15) |
| Same bed | 91 (39.9) | 1.37 (0.74–2.55) | 0.80 (0.44–1.46) |
Discussion

We conducted a large scale evaluation of the prevalence of LTBI as detected by TST and QFT-GIT among household contacts of tuberculosis patients in 24 high HIV and TB prevalence communities in Zambia and South Africa. Our findings suggest a high prevalence of LTBI among this population. QFT-GIT estimates were higher than those of TST in all but two communities. LTBI prevalence was higher in South African communities compared to the Zambian ones, as in previous findings [22].

LTBI was more common among older individuals and those who were HIV negative, similar to previous studies in this setting [9,10,30]. HIV positivity was less common among those with positive results on QFT-GIT and TST. We found little evidence to support the hypothesis that HIV infection was associated with TST negativity among QFT-GIT positive individuals as would have been expected if HIV causes more false negatives with TST than QFT-GIT. Both TST and QFT-GIT are prone to false negatives results among different population groups [9,30]. In a study done in Zambia, low CD4+ counts in HIV positive TB patients were associated with increases in both indeterminate and false-negative QFT-GIT results [9]. Current evidence suggests that IGRAs perform similarly to the TST at identifying HIV-infected individuals with LTBI [16].

For both QFT-GIT and TST, prevalence of infection was higher in contacts exposed to smear positive index cases compared to smear negative ones, consistent with findings of other studies [31]. Sleeping proximity of the contact to the index case was not associated with either QFT-GIT or TST results. In contrast, a study done in Cape town found an association between Mtb contact scores and increasing exposure [19], similar to findings in the Gambia [21]. Both of these studies had smaller sample sizes compared to our study and were done among HIV negative [19] or few HIV positive contacts [21]. There is growing evidence suggesting a stronger and better defined association between surrogate markers for TB exposure and QFT-GIT results in low TB incidence settings compared to high-TB incidence settings [14,32,33,34] although this is still inconclusive.

Our results suggest that tuberculous infection in adults may often be unrelated to household transmission. It is well recognized that transmission of tuberculosis in high incidence settings occurs not only within households but in the community as well [35,36] among various social locations [37]. A study in Zimbabwe found that the proportion of ELISpot positive contacts was not different from community controls [31]. In another study done in two communities in Zambia, almost 50% of community controls were QFT-GIT positive [38]. In our study, positive QFT-GIT results in contacts correlated well with infection prevalence results from previous TST surveys, providing further evidence that community transmission seems to play a bigger role in positivity than household exposure. However, in a large study in Colombia, IFN-γ responses to CFP-10 were consistently higher in household contacts of all ages compared to subjects in the source population [34]. Nevertheless, a seven day whole blood culture in-house assay was used, which primarily detects central memory responses. It has been argued that, in settings of high endemicity where a mixture of recent and old infections are commonly found, long term assays are more sensitive than those with shorter culture times [34].

We found little evidence in our matched pair analysis to support the idea that age was more strongly associated with a positive TST result than with a positive QFT-GIT result since we anticipated that TST was more likely to detect evidence of lifetime infection with Mtb while QFT-GIT was more likely to detect recent infections. Our results using conditional logistic regression showed that age was associated with positive QFT-GIT and TST results and there was a trend to increased responses with increasing age for both tests suggesting cumulative exposure to Mtb. In the study done in Colombia [34], exploration of IFN-γ variations by age revealed a trend to increased responses up to adulthood with CFP, but not with CFP-10, similar to observations in Uganda [39]. However, children were included in both of these studies.

We show a low level of agreement between the tests in all communities, consistent with findings of studies done in high-TB burden settings [19,31,40]. As IGRAs are designed to be more specific than TST, perfect agreement is not expected [41]. However, better agreement has been shown when the comparison is done within specific risk groups like HIV positives [42]. Although kappa statistics have been widely used as a measure of agreement between IGRAs and TST, alternative statistical approaches have recently been proposed such as latent class analysis [43] but have yet to gain wider acceptance. Similar to other studies reported from poor-resource settings [44], there were particularly high number of QFT-GIT+/TST- discordant results, in contrast to studies done in settings with low TB incidence [45,46] where TST+/QFT-GIT- discordance is more common.

Our study had both strengths and limitations. Ours is among the first studies to conduct both TST and QFT-GIT tests using a large sample size which illustrates the realistic implementation of QFT-GIT in a setting with a high burden of TB and HIV. However, as for all studies of this nature we had no gold standard measure of LTBI against which to compare our tests. As such, we were unable to comment directly on the accuracy of either test, but rather to compare the findings of each test in relation to prior beliefs about their properties. It is plausible that our failure to prove our hypotheses may have been due to test limitations typical in such high-TB burden settings. In addition, individuals in these settings may have mixed infections due to multiple Mtb exposure.

Our results may have been severely compromised by missing data on some risk factors. Despite efforts to standardize TST training and reading across the two countries, use of different teams may have contributed to inter-reader variability. Although most contacts reported that they had never smoked or taken alcohol, we believe this was due to reporting bias. We had no data
on likely exposure to NTM which may provide an alternative reason for false positive results, especially with TST. Probably the most important characteristic of tests of LTBI is the extent to which they predict subsequent clinical tuberculosis. The data we present here are cross-sectional in nature; however, they come from a larger longitudinal study whose participants have been followed up for later development of active TB.

Conclusion

QFT-GIT may not be more sensitive than the TST to detect risk factors associated with tuberculosis infection. Given the lack of strong associations with either TST or QFT-GIT with risk factors generally accepted to be related to household infectivity, these results suggest that tuberculous infection in adults in these communities may often be unrelated to household transmission. We found little evidence to support the hypotheses that positivity in QFT-GIT is more related to recent infection and less affected by HIV than the TST.

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Author Contributions

Conceived and designed the experiments: HA NB CS PG-F. Performed the experiments: KS AM AS K-AL. Analyzed the data: KS JH KF PG-F. Contributed reagents/materials/analysis tools: AS K-AL CS. Wrote the paper: KS JH KF HA PG-F NB. The authors acknowledge the invaluable contributions of the ZAMSTAR study team which made this study possible. We would also like to thank the Ministry of Health, District Health Management teams and the communities where the study was undertaken for their help and advice.

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Table 5. Hypotheses of expected performance of QFT-GIT and TST in our setting and the results obtained.

| Prevaling Understanding | Hypothesis | Result |
|-------------------------|------------|--------|
| TST is more likely to give false negative results in HIV positives than QFT-GIT. | 1. HIV is a risk factor for TST negativity conditional on a QFT-GIT positive result. | Adjusted odds ratio for HIV on TST positivity among QFT-GIT positives = 0.94 (95% CI 0.62-1.40) Wald-test p = 0.75 |
| QFT-GIT positivity is related to recent acquisition of MTB infection whilst TST detects old infections. | 2. Age trend is stronger for TST than QFT-GIT because age is as proxy for likelihood of lifetime exposure to MTB. | Wald-test for age**diagnostic test** interaction parameter in conditional logistic regression; p = 0.94 |
|  | 3. Stronger association between residence with a smear positive TB case and QFT-GIT positivity than for TST positivity, because smear status is a marker of infectivity and thus of likelihood of recent exposure to MTB. | Wald-test for ‘smear status of index case’**diagnostic test’ interaction parameter in conditional logistic regression; p = 0.45 |
|  | 4. Stronger association between sleeping in same room as index case and QFT-GIT positivity than for TST positivity, because sleeping in the same room is a marker of likelihood of recent exposure to MTB. | Wald-test for ‘sleeping in same room as index’**diagnostic test’ interaction parameter in conditional logistic regression; p = 0.76 |

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