Optimizing the Detection of Recent Tuberculosis Infection in Children in a High Tuberculosis–HIV Burden Setting

Anna M. Mandalakas^{1,2,3}, H. Lester Kirchner⁴, Gerhard Walzl⁵, Robert P. Gie³, H. Simon Schaaf³, Mark F. Cotton⁶, Harleen M. S. Grewal^{7,8}, and Anneke C. Hesselning³

¹Section on Retrovirology and Global Health, Department of Pediatrics, Baylor College of Medicine, Houston, Texas; ²The Global TB Program, Texas Children's Hospital, Houston, Texas; ³Desmond Tutu TB Centre, Department of Paediatrics and Child Health, and ⁴DST/NRF Centre of Excellence for Biomedical Tuberculosis Research and MRC Centre for TB Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa; ⁵Center for Health Research, Geisinger Clinic, Danville, Pennsylvania; ⁶Children's Infectious Diseases Clinical Research Unit, Department of Pediatrics and Child Health, Stellenbosch University, Tygerberg, Cape Town, South Africa; ⁷Department of Clinical Science, Infection, Faculty of Medicine, University of Bergen, Bergen, Norway; and ⁸Department of Microbiology, Haukeland Hospital, Bergen, Norway

Abstract

Rationale: Children who are young, malnourished, and infected with HIV have significant risk of tuberculosis (TB) morbidity and mortality following TB infection. Treatment of TB infection is hindered by poor detection and limited pediatric data.

Objectives: Identify improved testing to detect pediatric TB infection.

Methods: This was a prospective community-based study assessing use of the tuberculin skin test and IFN- γ release assays among children ($n = 1,343$; 6 mo to <15 yr) in TB-HIV high-burden settings; associations with child characteristics were measured.

Measurements and Main Results: Contact tracing detects TB in 8% of child contacts within 3 months of exposure. Among children with no documented contact, tuberculin skin test and QuantiFERON-TB Gold In-Tube positivity was greater than T-SPOT.TB. Nearly 8% of children had IFN- γ release assay positive and skin test negative discordance. In a model accounting for

confounders, all tests correlate with TB contact, but IFN- γ release assays correlate better than the tuberculin skin test ($P = 0.0011$). Indeterminate IFN- γ release assay results were not associated with age. Indeterminate QuantiFERON-TB Gold In-Tube results were more frequent in children infected with HIV (4.7%) than uninfected with HIV (1.9%), whereas T-SPOT.TB indeterminates were rare (0.2%) and not affected by HIV status. Conversion and reversion were not associated with HIV status. Among children infected with HIV, tests correlated less with contact as malnutrition worsened.

Conclusions: Where resources allow, use of IFN- γ release assays should be considered in children who are young, recently exposed, and infected with HIV because they may offer advantages compared with the tuberculin skin test for identifying TB infection, and improve targeted, cost-effective delivery of preventive therapy. Affordable tests of infection could dramatically impact global TB control.

Keywords: HIV; latent tuberculosis infection; pediatrics; IFN- γ release tests; tuberculin test

Following infection with *Mycobacterium tuberculosis*, the 5-year risk of tuberculosis (TB) is 33% in children younger than 5 years and 20% for 5–14 years of age (1).

TB risk is greatest in the year following infection and highest among young, malnourished, and immune-compromised children; up to 50% of children infected

during the first year of life develop disease in the absence of preventive therapy (PT) (2). PT decreases TB morbidity and mortality among adult contacts (3, 4) and

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Correspondence and requests for reprints should be addressed to Anna M. Mandalakas, M.D., Section on Retrovirology and Global Health, 1102 Bates Street, FC-630, Houston, TX 77030. E-mail: anna.mandalakas@bcm.edu

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At a Glance Commentary

Scientific Knowledge on the

Subject: Treating latent tuberculosis infection prevents tuberculosis disease. Improved detection of infection in children could increase the cost-effectiveness of postexposure preventive treatment.

What This Study Adds to the

Field: Contact tracing detects tuberculosis in nearly 8% of child contacts within 3 months of exposure. In a model accounting for confounders, all tests correlate with tuberculosis contact, but IFN- γ release assays correlate better than the tuberculin skin test ($P = 0.0011$). The frequency of IFN- γ release assay indeterminate results did not differ in younger compared with older children. Among children infected with HIV, the correlation between tuberculosis contact and all tests of infection declines as malnutrition worsens. Resources permitting, the use of IFN- γ release assays should be considered in children who are young, recently exposed, and infected with HIV.

markedly reduces TB risk in child contacts (5, 6). Nevertheless, in 2010, of 7.6 million children infected with *M. tuberculosis*, more than 650,000 developed TB (7), and 74,000 children not infected with HIV died of TB (8).

In low-burden countries, most TB occurs in foreign-born persons (9), making screening, diagnosing, and treating latent TB infection (LTBI) at immigration a cost-effective control strategy (10); diagnosis of *M. tuberculosis* infection guides LTBI treatment. In high-burden settings, guidelines recommend PT based on TB exposure history, with proof of infection not mandatory (11).

In young children with highest risk of TB, bacillus Calmette-Guérin (BCG) vaccination limits tuberculin skin test (TST) specificity and consequent targeted delivery of PT. IFN- γ release assays (IGRAs) are more specific tests quantifying the *in vitro* production of IFN- γ by T cells after *ex vivo* stimulation with *M. tuberculosis*-specific antigens (12). In children, the estimated specificity for LTBI

is 94, 91, and 88% for the ELISPOT-based test, ELISA-based test, and TST, respectively (13). Systematic reviews in children failed to identify a superior test of infection (13, 14), but suggest that host factors affect test performance (13).

Programmatic TB screening guidelines vary considerably (15). U.S. guidelines preferentially recommend IGRAs in BCG-vaccinated individuals, whereas the TST is preferred in young children because data on IGRAs are limited (16). U.S. guidelines further recommend IGRA in conjunction with TST to improve sensitivity in high-risk populations including young and HIV-infected persons. The UK National Institute for Health and Clinical Excellence guidelines recommend stepwise IGRA use in TST-positive individuals (17). In contrast, the World Health Organization (WHO) does not recommend IGRAs for high-burden settings (18), in the absence of data on benefit. Additional data regarding IGRA sensitivity in children could inform current guidelines.

We assessed the use of the TST and commercial IGRAs for the detection of *M. tuberculosis* infection in children living in a TB-HIV high-burden setting while measuring associations between test outcome and host characteristics.

Methods

Study Setting and Design

We conducted a prospective, household-contact study in Cape Town, Western Cape Province, South Africa, of children recently exposed to a TB source case identified at community TB clinics, and children with documented recent TB exposure from routine outpatient care. Children with no documented TB exposure were recruited from neighboring households and outpatient care to measure background community exposure. BCG coverage (single vaccination at birth; Danish strain, intradermal) is greater than 98% (19).

Eligibility

Children infected and uninfected with HIV aged 3 months to 15 years with and without documented TB exposure were eligible. Children with documented TB exposure were recruited within 3 months of the source case starting treatment. Weight less than 5 kg, hemoglobin less than 9 g/dl, and

current TB treatment were exclusion criteria. Enrollment was deferred in the presence of TST (preceding 12 wk); live attenuated vaccination (preceding 6 wk); or acute severe respiratory, diarrheal, or neurologic illness.

Study Measures

Data on age, ethnicity, BCG vaccination (scar and written history), household death (preceding year), passing a worm (preceding 3 mo), and previous TB exposure or disease were collected from parents and caregivers and data verified from TB registers. Source case sputum specimens underwent smear and culture.

We used well-quantified TB exposure as a surrogate measure of *M. tuberculosis* infection (20). Pediatric-specific exposure data from a subset of children negative for HIV with documented TB exposure were used to derive a contact score (range, 0–10) assigning a higher value as exposure intensity increases (20). Additional detail on these methods is provided in the online supplement.

HIV testing and counseling was completed on children with unknown or negative status (Abbott Determine HIV-1/2 rapid test). Positive or indeterminate HIV rapid tests were confirmed with ELISA (children >18 mo) or DNA polymerase chain reaction (children $\leq$ 18 mo). Children infected with HIV had access to combination antiretroviral therapy (cART).

Children completed three tests to assess *M. tuberculosis* infection status, at enrolment and 3 months. TST (two Tuberculin Units RT-23; Statens Serum Institute, Copenhagen, Denmark) was placed and read by study nurses (who completed repeat standardization training) 48–72 hours after administration. Children with history of TST ulceration did not complete TST. IGRAs were completed following manufacturers' guidelines: QuantiFERON-TB Gold In-Tube (QFT; Cellestis Limited, Carnegie, Victoria, Australia) and T-SPOT.TB (TSpot; Oxford Immunotec, Oxford, UK). IGRA samples were obtained immediately before TST and transported to the research laboratory within 3 hours. Because of funding limitations, children older than 5 years of age recruited from two entry points did not complete TSpot. Laboratory and clinical teams were masked to clinical data and IGRA results, respectively.

Children were screened for TB through standardized symptom screening (21), chest radiography (anteroposterior and lateral), and mycobacterial culture (MGIT; Becton-Dickinson, Franklin Lakes, NJ) of gastric aspirates or sputum. Two masked independent experts read chest radiographs, using a standardized pediatric tool (22). If discordant, a third masked reviewer's classification was used. Standard weight and height measurements were completed. Additional detail regarding TB evaluation and the protocol-specific TB case definition are provided in the online supplement.

Children younger than 6 years and children infected with HIV with a positive TST or documented TB exposure were referred for 6-month PT (isoniazid, 10–15 mg/kg/d); children diagnosed with TB were referred for 6-month directly observed treatment.

The research ethics committees of Baylor College of Medicine, Stellenbosch University, Case Western Reserve University, and local health authorities approved the study. Written informed consent was obtained from all parents and

caregivers; children older than 7 years gave assent. Standards for studies of diagnostic accuracy were followed (23).

Statistical Analysis

The TST was coded positive if greater than or equal to 10 mm in children uninfected with HIV and greater than or equal to 5 mm in children infected with HIV. IGRAs were interpreted following manufacturers' guidelines. Indeterminate results were recorded and excluded from analysis. Operational challenges recorded included failed phlebotomy, laboratory error, and insufficient specimen. To capture children with test conversions, *M. tuberculosis* infection status was also defined by a composite measure defining a child as infected by a specific test if positive at either baseline or 3 months. The proportion of positive results was compared across tests using McNemar chi-square test.

Comparisons between children infected and uninfected with HIV were performed using Pearson chi-square and the Wilcoxon rank-sum tests. Agreement between tests was assessed using the κ statistic (24). The association between

covariates and each test was assessed using logistic regression while controlling for *M. tuberculosis* exposure (20). Models with common variables and interaction terms were developed to support simultaneous comparison of the index tests. Interactions with HIV status were considered and retained if *P* less than 0.05. Final models were also run in subgroups stratified by age and subgroups excluding children diagnosed with TB within 3 months of enrollment. To estimate and compare contact score association across index tests a generalized estimating equation model (25, 26) was fitted, treating tests as the multivariate dependent variable. A covariate was included to distinguish between tests and was interacted with the contact score. An assessment was performed to understand if any differences were found between children with complete or missing TST or IGRA results at baseline and 3-month visit. Because no significant differences were found, no adjustment for missing data was considered. Analyses used Stata v10 (StataCorp LP, College Station, TX) and SAS 9.2 (SAS Institute, Inc., Cary, NC).

Table 1. Baseline Characteristics of Children Infected and Uninfected with HIV (n = 1,343)

	All Children (n = 1,343) [Median (IQR) or n/N (%)]	Children Infected with HIV (n = 299) [Median (IQR) or n/N (%)]	Children Uninfected with HIV (n = 1,044) [Median (IQR) or n/N (%)]	P Value*
Age, mo	58.6 (27.3 to 102.7)	46.4 (25.0 to 72.8)	61.8 (29.0 to 108.5)	<0.0001
Male	645/1,343 (48)	152/299 (51)	493/1,044 (47)	0.2701
Race				
African/black	451/1,343 (34)	220/299 (74)	231/1,044 (22)	
Mixed-race	887/1,343 (66)	78/299 (26)	809/1,044 (78)	<0.0001
Indian/Asian/white	5/1,343 (0.4)	1/299 (0.3)	4/1,044 (0.4)	
Height–age z score	−1.57 (−2.31 to −0.81)	−1.94 (−2.77 to −1.18)	−1.46 (−2.19 to −0.70)	<0.0001
Weight–height z score	0.51 (−0.22 to 1.27)	0.68 (−0.02 to 1.47)	0.45 (−0.31 to 1.14)	0.0002
Reported recent worm infection	84/1,340 (6)	23/298 (8)	61/1,042 (6)	0.2775
Prior TB treatment	162/1,340 (12)	113/297 (38)	49/1,043 (5)	<0.0001
BCG vaccination†	1,165/1,343 (87)	261/299 (87)	904/1,044 (87)	0.7526
cART at enrollment‡		251/289 (88)		
CD4 count		1,317 (867 to 1,804)		
CD4 percentage		30 (23 to 36)		
Viral load§		1,882 (140 to 20,176)		
Current TB contact	836/1,340 (62)	54/298 (18)	782/1,042 (75)	<0.0001
TB contact score	3 (0 to 5)	0 (0 to 0)	4 (0 to 6)	<0.0001
TB contact score	5 (4 to 7)	4 (2 to 6)	5 (4 to 7)	0.0051
Recent household death	118/1,338 (9)	26/298 (9)	92/1,040 (9)	1.0000

Definition of abbreviations: BCG = bacillus Calmette-Guérin; cART = combination antiretroviral treatment; IQR = interquartile range; TB = tuberculosis.

*Represents comparison of proportions (chi-square test) or medians (Wilcoxon rank-sum test) between children infected and not infected with HIV.

†Children were considered BCG vaccinated if a BCG scar was noted or a record of vaccination was available.

‡Among the 38 children infected with HIV not receiving cART at baseline, five had started cART by the 3-month follow-up.

§Viral load was lower than detectable limits in 126 children and missing in 31 children. Hence, the median and IQR were calculated in 142 children with detectable viral load values.

||Calculated among all children.

||Calculated only in children with a current TB contact.

Results

Characteristics of Study Population

Between January 2008 and July 2012, a total of 1,343 children (median age, 59 mo; interquartile range [IQR], 27–103 mo) were enrolled including 836 with documented TB exposure and 507 without documented

TB exposure. A total of 912 children were recruited from the community and 431 from the hospital outpatient department. A total of 22% (299 of 1,343) were infected with HIV; 169 from community and 130 from hospital-based antiretroviral clinics. Children infected with HIV were more likely of black race, stunted, to have acute

malnutrition, and report past TB treatment, but less likely to have documented TB exposure in the past 3 months. Children infected with HIV had a median CD4 count of 1,317 per microliter (IQR, 867–1,804), CD4% of 30 (IQR, 23–36), and viral load of 1,882 copies per milliliter (IQR, 140–20,176);

Table 2. Measures of *Mycobacterium tuberculosis* Infection in Children Infected and Uninfected with HIV

	All Children (n = 1,343) [Median (IQR) or n/N (%)]	Children Infected with HIV (n = 299) [Median (IQR) or n/N (%)]	Children Uninfected with HIV (n = 1,044) [Median (IQR) or n/N (%)]	P Value*
TST, mm [†]	14.5 (11.0–17.3)	13.8 (10.0–16.3)	14.6 (11.5–17.5)	0.0390
Baseline TST				
Positive [‡]	529/1,325 (39.9)	93/295 (31.5)	436/1,030 (42.3)	0.0008 [§]
Missing	18	4	14	
Follow-up TST [¶]				
Conversions	71/645 (11.0)	13/157 (8.3)	58/488 (11.9)	0.2094
Reversions	58/391 (14.8)	11/62 (17.7)	47/329 (14.3)	0.4825
TST composite**	603/1,332 (45.3)	107/296 (36.2)	496/1,036 (47.8)	0.0004
Baseline QFT				
Positive	520/1,261 (41.2)	59/271 (21.8)	461/990 (46.6)	<0.0001 [§]
Indeterminate	34/1,343 (2.5)	14/299 (4.7)	20/1,044 (1.9)	0.0114
Insufficient volume	4/1,343 (0.3)	0/299 (0)	4/1,044 (0.4)	0.5810
Failed runs/error	8/1,343 (0.6)	3/299 (1.0)	5/1,044 (0.5)	0.3870
Missing ^{††}	36/1,343 (2.7)	11/299 (3.7)	25/1,044 (2.4)	0.2259
Follow-up QFT ^{‡‡}				
Conversions	55/602 (9.1)	11/180 (6.1)	44/422 (10.4)	0.0925
Reversions	32/387 (8.3)	6/47 (12.8)	26/340 (7.6)	0.2323
QFT composite**	591/1,318 (44.8)	73/293 (24.9)	518/1,025 (50.5)	<0.0001
TSpot sample size	n = 1,093 ^{§§}	n = 297	n = 796	
Baseline TSpot				
Positive	302/991 (30.5)	39/263 (14.8)	263/728 (36.1)	<0.0001 [§]
Indeterminate	2/1,093 (0.2)	1/297 (0.3)	1/796 (0.1)	0.4698
Insufficient volume	13/1,093 (1.2)	4/297 (1.4)	9/796 (1.1)	0.7580
Failed runs/error	27/1,093 (2.5)	9/297 (3.0)	18/796 (2.3)	0.5114
Missing ^{††}	60/1,093 (5.5)	20/297 (6.7)	40/796 (5.0)	0.2960
Follow-up TSpot				
Conversions	61/576 (10.6)	18/178 (10.1)	43/398 (10.8)	0.5173
Reversions	27/255 (10.6)	3/30 (10.0)	24/225 (10.7)	0.0730
TSpot composite**	377/1,057 (35.7)	59/290 (20.3)	318/767 (41.5)	<0.0001

Definition of abbreviations: IQR = interquartile range; QFT = QuantiFERON-TB Gold In-Tube; TSpot = T-SPOT.TB; TST = tuberculin skin test.

*Represents comparison of proportions (chi-square) or medians (Wilcoxon rank-sum) between participants infected and uninfected with HIV.

[†]Data only used if TST is greater than 0 mm.

[‡]TST greater than or equal to 10 mm were considered as positive for participants uninfected with HIV and TST greater than or equal to 5 mm for participants infected with HIV.

[§]Statistical test is comparing positive and negative index test results.

[¶]Reasons for missing baseline TST include two TSTs read but result not recorded; three TSTs deferred because of history of extensive blistering following prior TST; one family refused because child had been previously treated for TB; one TST placed but did not return for reading; and 11 subjects with missing data.

^{¶¶}Follow-up TST result not available in 158 children: 96 did not complete testing because of current tuberculosis treatment (47), refusal of parent or child (20), recent receipt of live vaccination (two), prior TST ulceration (12), and withdrawal from study because of relocation (24). Data are missing for 53 children.

^{**}*M. tuberculosis* infection status defined considering both baseline and 3-month test results. A child was defined as infected by a specific test if positive at either baseline or 3 months.

^{††}Blood was taken according to source documents, but samples were not logged or received at laboratory.

^{‡‡}Follow-up QFT results were not available in 162 children because of the following reasons: indeterminate (eight), insufficient blood volumes (two), failed runs/laboratory error (21), refusal to provide a sample at the 3-month visit (36), withdrawal from study because of relocation (24), and missing data (71).

^{§§}Because of funding limitations, children older than 5 years of age recruited from two entry points did not complete TSpot testing. Hence, the TSpot sample size is reduced compared with that of TST and QFT.

^{||||}Follow-up TSpot results were not available in 150 children because of the following reasons: indeterminate (11), insufficient blood volumes (five), failed runs/laboratory error (53), refusal to provide a blood sample at the 3-month visit (23), withdrawal from the study because of relocation (20), and missing data (38).

88% were receiving cART (median, 22 mo; IQR, 9–43 mo) (Table 1).

Six percent (85 of 1,343) of children were diagnosed with TB within 3 months following enrollment (1). Of these, 76% (65 of 85) were diagnosed within 6 weeks; the sensitivity of the TST (75%), TSPot (71%), and QFT (79%) were similar. Test sensitivity increased if two tests were considered: TST/TSPot (83%), TST/QFT (84%), and TSPot/QFT (88%). Initial test sensitivity was lower among children diagnosed with TB greater than 6 weeks following enrollment: TST (48%), TSPot (37%), and QFT (47%).

Summary of Test Results, Agreement, and Concordance in Univariate Analyses

All baseline tests and composite measure of infection were more likely positive in children not infected with HIV (Table 2). TST distribution varied by HIV and TB exposure status (Figure 1). Positivity of all tests varied along the contact score continuum and would therefore result in differences in targeted treatment (Figure 2).

Indeterminate QFT results were more frequent in children infected (4.7%) than children not infected with HIV (1.9%), whereas TSPot indeterminate results were rare (0.2%) and did not vary by HIV status. Test conversion, reversion, and other operational measures were not associated with HIV status, age, or nutritional status. Conversions were common among children diagnosed with TB following enrollment: TST (14.2%), TSPot (27.2%), and QFT (14.2%).

Children who were older, uninfected with HIV, or TB exposed were more likely to have positive results for each test (see Tables E1–E3 in the online supplement). Children with negative TST or QFT results were more likely to be BCG-vaccinated than children with positive results. Acute malnutrition was associated with negative QFT and TSPot ($P = 0.010$ and 0.010 , respectively), but not TST ($P = 0.052$). Children with a recent household death or who were receiving PT at the 3-month follow-up were more likely to have a positive TST (see Tables E1–E3); the latter reflecting TST positivity being an indication for referral.

Test agreement ranged from 0.71 to 0.78 in children not infected with HIV and 0.45 to 0.68 in children infected with HIV (Table 3). IGRA-positivity in children negative for TST occurred more often in children not infected with HIV and for the

Figure 1. Tuberculin skin test (TST) distribution stratified by HIV status and tuberculosis (TB) exposure. (A) Data from TB-exposed (red bars) and TB-unexposed (black bars) children infected with HIV and two normalized lines for recent contacts (red line) and nonexposed control subjects (black line). (B) Data from TB-exposed (red bars) and TB-unexposed (black bars) children not infected with HIV and two normalized lines for recent contacts (red line) and nonexposed control subjects (black line). The normalized lines are derived using polynomial (Poly.) expressions because this fits our bell-shaped curve data best. Nonresponsive children were excluded from the histogram but varied by

Figure 2. Test positivity and treatment implications in relationship to contact score. Bars represent the proportion of children with respective positive test results along the continuum of exposure ranging from no known exposure (contact score = 0) to the highest level of exposure (contact score = 10). Lines demonstrate the cumulative proportion of children that would be offered IPT based on test positivity if contact score were considered a cut-point for IPT eligibility. If testing were used to guide IPT among children with a contact score greater than or equal to 2, TST, Tspot, and QFT would result in 26, 21, and 29% of children being offered treatment, respectively. In contrast, if testing were used to guide IPT among children with a contact score of greater than or equal to 5, TST, Tspot, and QFT would result in 20, 16, and 22% of children being offered treatment. Finally, if testing were used to guide IPT among children with a contact score greater than or equal to 10, no child would be treated because the highest contact score possible is 10. IPT = isoniazid preventive therapy; QFT = QuantiFERON; RX QFT = treatment guided by QFT response; RX Tspot = treatment guided by Tspot response; RX TST = treatment guided by TST response; Tspot = T-Spot.TB; TST = tuberculin skin test.

QFT (6.8%) than the TSpot (4.3%). The mean QFT response was 18.5 IU/ml (SD, 9.8) in TST concordant and 16.7 IU/ml (SD, 7.8) in TST discordant samples. TST-QFT positive concordance was more common among children with a QFT response greater than 5 IU/ml and negative concordance with a QFT response less than 5 IU/ml. There was no association between discordance patterns and the magnitude of QFT response (*see* Table E5). Overall, IGRA-negativity in children positive for TST occurred in 11%, and in 19% of children infected with HIV. IGRA-negative/TST-positive discordance was more common in children with limited or no documented contact compared with those with more substantial contact (*see* Table E4). Test agreement did not vary

using the composite measure of TB infection rather than the baseline measure only.

Child Characteristics Associated with Test Performance in Multivariate Analysis

While controlling for the effect of all variables (adjusted models), the odds of TST, QFT, and TSpot positivity increased 17, 18, and 21% for each 1-point increase in the contact score, respectively. For all tests, the odds of test positivity increased 1% for each increasing month of age (Table 4).

The TSpot and QFT were 2 and 2.7 times more likely to be positive in children with previous TB treatment than children without (Table 4).

There was an interaction between HIV status and chronic malnutrition (stunting;

height-for-age *z* score) in all models. Among children infected with HIV, tests were less likely to be positive as stunting worsened (Table 4). The association between test positivity and acute malnutrition (wasting; weight-for-height *z* score) was not retained in multivariate regression models.

In the TST and QFT models, test positivity was not associated with BCG vaccination. In the TSpot model, test positivity was almost twofold higher in BCG-vaccinated than -unvaccinated children (Table 4); among children less than or equal to 5 years, BCG-vaccinated children were 5.5 times more likely to be TSpot positive. Results of TST and QFT regression analysis did not change in age-stratified subgroups.

For all tests, results of regression analysis did not change when children diagnosed with TB within 3 months of enrollment were excluded or when the composite measure of infection was considered.

Comparison of Test Performance for the Detection of *M. tuberculosis* Infection

Regression of the log of the odds ratios illustrated that positivity of all tests increases as TB exposure increases with TSpot having the highest rate of increase (Figure 3).

Generalized estimating equation modeling illustrated that the effect of the contact score on each test was significantly different ($P = 0.0004$). The association between an increasing contact score and test positivity was greater for both IGRAs than TST, and did not differ between TSpot and QFT.

In models using combinations of two baseline tests, test results and contact score were significantly associated ($P < 0.0001$); more so for the TSpot-QFT combination than the TST-TSpot and TST-QFT combination ($P = 0.0011$).

In models including age dichotomized at 24 months, there was no association between age and TST. In contrast, IGRAs were more strongly associated with contact score in younger children ($P < 0.0001$) (Figure 4).

Discussion

The WHO aims to eliminate TB by the year 2050. Global TB elimination depends on

Figure 1. (Continued). HIV and exposure status: HIV-infected and TB-exposed (53%); HIV-infected and no documented TB exposure (70%); HIV-uninfected and TB-exposed (47%); HIV-uninfected and no documented TB exposure (62%).

Table 3. Agreement for Tests of *Mycobacterium tuberculosis* Infection and Discordance between Tests Stratified by HIV Status in Children

	TST versus TSPot Discordant Results			TST versus QFT Discordant Results			TSPot versus QFT Discordant Results		
	TSPot +ve TST –ve [n/N (%)]	TSPot –ve TST +ve* [n/N (%)]	κ (95% CI)	QFT +ve TST –ve [n/N (%)]	QFT –ve TST +ve* [n/N (%)]	κ (95% CI)	TSPot +ve QFT –ve [n/N (%)]	TSPot –ve QFT +ve [n/N (%)]	κ (95% CI)
Baseline measures									
All children	42/983 (4.3)	108/983 (11.0)	0.66 (0.61–0.71)	85/1,246 (6.8)	79/1,246 (6.3)	0.73 (0.69–0.77)	23/940 (2.4)	72/940 (7.7)	0.78 (0.73–0.82)
Children infected with HIV	4/259 (1.5)	49/259 (18.9)	0.45 (0.33–0.56)	12/267 (4.5)	41/267 (15.4)	0.50 (0.38–0.61)	5/245 (2.0)	19/245 (7.8)	0.68 (0.56–0.80)
Children not infected with HIV	38/724 (5.2)	59/724 (8.2)	0.71 (0.66–0.77)	73/979 (7.5)	38/979 (3.9)	0.77 (0.73–0.81)	18/695 (2.6)	53/695 (7.6)	0.78 (0.74–0.83)
Composite measures†									
All children	38/1,052 (3.6)	111/1,052 (10.6)	0.70 (0.66–0.75)	83/1,310 (6.3)	97/1,310 (7.4)	0.72 (0.68–0.76)	29/1,046 (2.8)	73/1,046 (7.0)	0.79 (0.76–0.83)
Children infected with HIV	8/287 (2.8)	56/287 (19.5)	0.46 (0.36–0.57)	14/290 (4.8)	50/290 (17.2)	0.49 (0.38–0.59)	11/287 (3.8)	23/287 (8.0)	0.66 (0.56–0.77)
Children not infected with HIV	30/765 (3.9)	55/765 (7.2)	0.77 (0.73–0.82)	66/1,020 (6.8)	47/1,020 (4.6)	0.77 (0.73–0.81)	18/759 (2.4)	50/759 (6.6)	0.82 (0.78–0.86)

Definition of abbreviations: +ve = positive; –ve = negative; CI = confidence interval; QFT = QuantiFERON-TB Gold In-Tube; TSPot = T-SPOT.TB; TST = tuberculin skin test.

*TST greater than or equal to 10 mm was considered as positive for participants uninfected with HIV and TST greater than or equal to 5 mm for participants infected with HIV.

†*M. tuberculosis* infection status defined considering both baseline and 3-month test results. A child was defined as infected by a specific test if positive at either baseline or 3 months.

many activities including LTBI treatment to reduce potential disease burden. Targeted LTBI treatment has been a TB control strategy for more than a decade (27), but remains hampered by poor LTBI detection and PT implementation. Our study provides new data and demonstrates some advantages of IGRAs for LTBI detection in children who are young, malnourished, and

infected with HIV with recent TB contact, those children at highest risk of TB progression (2).

The high proportion of children with undocumented or minimal TB exposure who had LTBI demonstrates substantial community exposure and past infection in this high-burden setting. Among children with no documented contact and increasing

TB exposure, TST and QFT positivity remained greater than TSPot positivity and would result in treating a higher proportion of children with little to no documented TB exposure. Positivity of all tests steadily increased among children with contact scores greater than or equal to 4, likely reflecting recent infection and supporting targeted

Table 4. Association between Tests of *Mycobacterium tuberculosis* Infection and Covariates of Interest in Children

Covariates	TST* [Odds Ratio (95% CI)]		TSPot [Odds Ratio (95% CI)]		QFT [Odds Ratio (95% CI)]	
	Unadjusted (n = 1,324)	Adjusted† (n = 1,315)	Unadjusted (n = 991)	Adjusted† (n = 986)	Unadjusted (n = 1,261)	Adjusted† (n = 1,253)
TB contact score	1.16 (1.11–1.22)‡	1.17 (1.11–1.23)‡	1.23 (1.16–1.29)‡	1.21 (1.14–1.29)‡	1.21 (1.16–1.26)‡	1.18 (1.12–1.24)‡
BCG vaccination (referent: unvaccinated)		1.30 (0.85–1.99)		1.91 (1.07–3.42)§		1.44 (0.92–2.24)
Prior TB treatment (referent: no prior treatment)		1.47 (0.93–2.31)		1.96 (1.13–3.41)§		2.74 (1.64–4.45)‡
Age (years) effect		1.01 (1.01–1.02)‡		1.01 (1.01–1.02)‡		1.02 (1.01–1.02)
HAZ score effect						
HIV-uninfected		0.99 (0.94–1.04)		0.99 (0.96–1.02)		0.99 (0.97–1.02)
HIV-infected		1.29 (1.07–1.57)§		1.19 (1.00–1.40)§		1.30 (1.05–1.61)§
HIV effect (referent: HIV-infected)						
HAZ score, 0		0.61 (0.35–1.04)		1.85 (1.04–3.28)§		1.55 (0.85–2.82)
HAZ score, –1		0.79 (0.51–1.23)		2.21 (1.32–3.70)§		2.02 (1.23–3.33)§
HAZ score, –2		1.04 (0.69–1.56)		2.64 (1.58–4.40)§		2.66 (1.65–4.28)‡

Definition of abbreviations: BCG = bacillus Calmette-Guérin; CI = confidence interval; HAZ = height for age z score; QFT = QuantiFERON-TB Gold In-Tube; TB = tuberculosis; TSPot = T-SPOT.TB; TST = tuberculin skin test.

*TST greater than or equal to 10 mm was considered as positive for participants uninfected with HIV and TST greater than or equal to 5 mm for participants infected with HIV.

†Logistic regression adjusted for age, prior BCG vaccination, prior TB treatment, chronic malnutrition status, and HIV status. All models included interaction for HIV status and HAZ score.

‡Significant difference at $P < 0.001$.

§Significant difference at $P < 0.05$.

^{||}Fixed HAZ score status (0, –1, and –2) represents no, mild, and moderate chronic malnutrition.

Figure 3. Regression slopes for exposure continuum expressed as contact score. To visually represent the association between the contact score and test outcome, the odds ratio for each positive level of the score versus a zero score was estimated from each adjusted logistic regression model. The natural logarithm of the odds ratio was then plotted against the contact score to allow for comparison between the tests. The slopes of the lines are estimated from the regression of the logs of odds ratios of each successive higher exposure compared with the least exposed group. Hence, a steeper slope represents a greater change in the log odds ratio as exposure increases. A greater change in the log odds ratio in response to increasing exposure (i.e., steeper slope) indicates that test results are better correlated with the likelihood of infection. This finding suggests that tests with a steeper slope are better able to detect infection. QFT = QuantiFERON; Tspot = T-Spot.TB; TST = tuberculin skin test.

screening among children with higher risk of LTBI (higher pretest probability) in conjunction with tests of infection to guide delivery of PT.

The lack of a gold standard for LTBI complicates studies of diagnostic accuracy (23). The association between IGRA positivity and TB exposure is well described in low-burden countries (28–30). Our regression analysis included a standardized measure of recent *M. tuberculosis* exposure (20) previously associated with IGRA and TST positivity in child contacts in high-burden settings (31, 32). These studies provide conflicting evidence and suggest both advantages of the TSpot (31) and no difference in TSpot performance compared with TST and QFT (32). Previously published pooled analysis retrospectively standardizing measures of *M. tuberculosis* exposure was unable to identify a superior

test of infection (13). Strengthened by our prospective approach, comprehensive quantification of TB exposure, and robust sample size, we developed a model that accounts for covariates and demonstrates that IGRAs correlate better with TB exposure than the TST ($P = 0.0011$). Further longitudinal research is needed to fully understand the practical significance of these findings.

Similar to data from other pediatric studies (32, 33), nearly 8% of children in our study were IGRA-positive/TST-negative, thus potentially identifying an additional 81 children with LTBI. Lack of association between this discordance pattern and the magnitude of the QFT response argues against QFT false-positivity. This pattern of discordance occurred more frequently with QFT than TSpot and more commonly in children not infected with HIV, who were also more likely to have had recent TB exposure because of our recruitment strategy. Among Indian children identified with presumed TB detected through active case finding, the IGRA-positive/TST-negative pattern occurred in only 3% (34). However, in that study, a positive TST was an inclusion criterion and introduced a selection bias decreasing the frequency of the IGRA-positive/TST-negative pattern. Using IGRA testing only after positive TST is recommended by UK guidelines (17), but as we have shown, may miss recent *M. tuberculosis* infection.

Similar to studies in low-burden settings (35, 36), IGRA-negativity in children positive for TST occurred more often in children with little to no contact, suggesting that a portion of these TST results were false positives. We noted this discordance pattern in 19% of children infected with HIV who were more likely to have been evaluated and treated for TB. We hypothesize that frequent TST may have had a boosting effect. This finding may suggest that TST lacks specificity for detecting recent *M. tuberculosis* infection in HIV coinfecting children. Because IGRAs were associated with past TB treatment in our study unlike TST, the IGRA-negative/TST-positive pattern is unlikely to reflect a subclinical, quiescent mycobacterial phase following treatment. Nevertheless, the absence of a gold standard of infection precludes confirmation of TST false positivity.

Among children infected with HIV, tests were less likely to be positive as chronic malnutrition (stunting) worsened. Delayed initiation of cART is associated with long-term impairment of *M. tuberculosis*-specific immune response (37). Adults infected with HIV can have an impaired effector memory pool of CD4 T cells despite normal or restored CD4 counts (38, 39), which may impair IFN- γ secreting ability. We suspect that children infected with HIV with evidence of stunting had advanced immune suppression at cART initiation with impairment of mycobacterial immune response and depressed IFN- γ production. Consistent with a smaller pediatric study (40), our findings in a larger, more diverse, community-based setting further confirm the compromised performance of TST and IGRAs in children who are malnourished and children infected with HIV highlighting the need for additional data to guide population-specific test interpretation.

With the high annual and age-related risk of *M. tuberculosis* infection in high-burden TB settings, older children were more likely to have positive tests of infection. Unlike previous studies (33, 41, 42), we detected no difference in IGRA performance or the frequency of indeterminate results in younger children, even in children 6 months of age. These contradictory findings likely result from differences in covariates considered and study populations (i.e., contacts vs. children with broadly defined presumed TB and administrative screening). Consistent with our findings, the performance of IGRAs did not diminish among Thai children aged 2 months to 16 years (32) and children younger than 5 years of age have sufficient immunologic capacity for IFN- γ production quantified by the QFT (43). Our findings suggest that even in young children test performance improves in a population with increased risk of infection, such as household contacts.

Studies of children not infected with HIV have found an association between BCG vaccination and IGRA-negativity suggesting a BCG protective effect against LTBI (30, 33). However, we found no association between BCG vaccination and QFT, and a positive association between BCG vaccination and TSpot. Our current analysis does not support a definitive interpretation of this finding.

Figure 4. Odds ratio of test positivity in young children compared with older children. The figure compares the aOR of test positivity for each test in children younger than 24 months compared with children greater than or equal to 24 months. The aOR are indicated by the circles and the 95% confidence intervals are illustrated with the lines; Tspot in red, TST in black, and QFT in gray. For the QFT model and the Tspot, there were interactions between age and contact score. Hence, the association between the IFN- γ release assay results and contact score is most accurately measured within each of the respective age strata. The aOR of QFT positivity was higher for younger children ($P < 0.001$): children younger than 24 months, aOR of 1.44 (95% CI, 1.27–1.64) and children greater than or equal to 24 months, aOR of 1.11 (95% CI, 1.06–1.17). Similarly, the aOR of Tspot positivity was higher for younger children ($P < 0.001$): children younger than 24 months, aOR of 1.50 (95% CI, 1.30–1.74) and children greater than or equal to 24 months, aOR of 1.12 (95% CI, 1.06–1.20). For the TST model, there was no significant association between age and TST result. Therefore, the aOR of TST was 1.16 (95% CI, 1.10, 1.22) for children younger than and greater than or equal to 24 months. aOR = adjusted odds ratio; CI = confidence interval; QFT = QuantiFERON; Tspot = T-Spot.TB; TST = tuberculin skin test.

The sensitivity of detecting *M. tuberculosis* infection in children with TB was comparable among the three tests. Similar to the TST, IGRAs cannot differentiate between TB infection and disease and a negative result does not exclude TB. Clinically significant TB exposure, symptoms, and chest radiography remain the cornerstone of TB diagnosis in children.

Baseline test sensitivity was low among children diagnosed with TB more than 6 weeks following enrollment, suggesting that none of the tests have ability to forecast TB disease. Replication of these findings in a cohort with extended follow-up may provide additional insight.

Despite our large, representative cohort, our study has limitations. Our measure of TB exposure may most accurately measure household TB exposure in children not infected with HIV. To accommodate this limitation, we controlled for HIV status and age, and included a control group with no documented TB exposure to measure background community exposure. Analyses in this

control group supported interpretation of regression models and assessment of the influence of background community TB exposure. Our analysis did not consider the HIV status of the source case because of collinearity between HIV status of source case and child. We preferentially included the child's HIV status in models because it was biologically more relevant to our questions. Our recruitment strategy captured children infected with HIV routinely attending antiretroviral clinic who were less likely to have documented recent TB exposure. We adjusted for this difference by including a measure of TB exposure in all regression models. Assessment of prognostic utility and confirmation of the erroneous result in the case of test discordance requires analysis of longitudinal disease outcomes in the absence of PT beyond the scope of this manuscript. We did not measure micronutrient levels or helminth status, which may affect test performance (34, 42). Our results may underestimate the frequency of indeterminate and false-

negative results encountered in routine care settings.

Our study provides new evidence to inform TB screening and contact investigation guidelines. Contact tracing detects TB in nearly 8% of child contacts within 3 months of recent exposure. In a robust model accounting for confounders, IGRAs correlated better with TB contact than the TST in children with recent TB exposure. The IGRA-positive/TST-negative discordance pattern occurred at clinically significant rates, suggesting that screening algorithms using stepwise testing with IGRA only after positive TST (17) may under detect LTBI in children.

Where resources allow, IGRAs can potentially improve LTBI detection, guide targeted PT, and improve TB control in children. Paradoxically, increased investment in IGRAs may increase the value of PT in a public health setting. Because of financial constraints, IGRAs are currently not recommended by the WHO in low- and middle-income countries where TB incidence is greatest. Models using 2011 estimates of IGRA performance suggest that

provision of PT to contacts without testing for infection is the most cost-effective strategy in children younger than 3 years of age in high-burden settings (44). However, this strategy overburdens health systems struggling to provide PT to large numbers of TB-exposed children and children infected with HIV with consequent overtreatment, poor adherence, and incongruous allocation of limited resources.

An affordable and even more accurate test of infection is still urgently needed and could dramatically impact global TB control. ■

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