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Comparison of a whole blood interferon-γ assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India

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Summary *Objective:* In vitro interferon-γ (IFN-γ) assays have emerged as novel alternatives to the tuberculin skin test (TST) for the diagnosis of latent tuberculosis (TB) infection. These assays have been evaluated in low incidence countries, mainly in adults, and have been shown to be more specific than TST. Because few studies have been done in high incidence countries, and because paediatric data are limited, we compared a whole-blood IFN-γ assay with TST among hospitalized Indian children.
Methods: Between July 2004 and June 2005, a total of 105 consecutively admitted children (median age 6 years; 82% had BCG scars) in whom TB was suspected or had history of contact with an index case were recruited at a rural hospital in India. All children underwent TST, and the QuantiFERON-TB-Gold *In Tube* (QFT) assay.
Results: The overall prevalence of TB infection was similar with both tests. With a TST cut-off point of ≥10 mm, 10 of 105 (9.5%; 95% CI 3.8, 15.2) children were TST positive. With a cut-off point of IFN-γ ≥ 0.35 IU/ml, 11 of 105 (10.5%; 95% CI 4.5, 16.4) were QFT positive. The concordance between TST and QFT was substantial (agreement 95.2%; kappa [κ] 0.73; 95% CI for κ 0.53, 0.92). Agreement between TST and QFT results was 100% (κ 1.0) in BCG scar-negative children as compared to 94% (κ 0.63) in scar-positive children. BCG was not associated with the results of either TST or QFT ($P > 0.05$ for both tests). The number of children with

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bacteriologically confirmed active TB was too small to permit the estimation of sensitivity of the tests.
Conclusions: In a rural, predominantly BCG-vaccinated paediatric population in India, the TST and QFT assay produced comparable results. BCG vaccination did not significantly affect either TST or QFT results. Larger studies are needed to compare the sensitivity of the IFN-γ assay with that of the TST in children with bacteriologically and/or clinically confirmed TB.

Introduction

Tuberculosis (TB) accounts for significant morbidity and mortality in children worldwide, with a majority of cases of latent TB infection (LTBI) and active disease occurring in developing countries.[1,2] Of the estimated 8.3 million new cases of TB in 2000, about 11% occurred in children younger than 15 years of age.[1,2] India accounts for one-third of the global burden of TB.[3] Although Indian data on TB in children are scarce, tuberculin surveys conducted in children suggest a high rate of infection in the community—the annual risk of TB infection is 1.5%.[4]

Paediatric TB poses diagnostic challenges.[5–8] Children often present with vague and non-specific signs and symptoms. TB is less often bacteriologically confirmed in children than adults. This is largely due to the paucibacillary nature of TB in children, greater likelihood of extrapulmonary and disseminated presentations, as well as the difficulty in obtaining clinical specimens.[2,5,6] Clinicians, therefore, frequently use indirect approaches to make a diagnosis.[5–7] This includes history of contact with a case of infectious TB, chest x-ray abnormalities, and a positive tuberculin skin test (TST) as evidence of infection. The TST, therefore, is widely used in paediatric practice.

Until recently, the TST, which uses purified protein derivative (PPD), was the only method available for the diagnosis of LTBI. The utility of this conventional test is hampered by technical and logistical problems: potential for false positive and false negative results; problems in administration and interpretation; and difficulty in separating true infection from the effects of prior BCG vaccination and infection due to non-tuberculous mycobacteria (NTM).[9–12]

Advances in genomics[13,14] and immunology have led to a promising alternative—in vitro interferon-γ (IFN-γ) assays, based on the principle that T-cells of individuals infected with *Mycobacterium tuberculosis* release IFN-γ when they re-encounter TB-specific antigens.[15,16] Latest versions of IFN-γ assays use antigens such as the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens, encoded within the region of difference 1 (RD1) of the *M. tuberculosis* genome, although not entirely specific to the *M. tuberculosis* complex, are significantly more specific to *M. tuberculosis* than PPD, as they are not shared with any BCG vaccine strains or selected NTM species including *M. avium*.[17–19]

Research evidence, extensively reviewed elsewhere,[9,17,20–24] suggests that RD1-based IFN-γ assays have higher specificity than TST, and are less influenced by previous BCG vaccination. IFN-γ assays that use more than one antigen (e.g. ESAT-6 and CFP-10) appear to be at least as sensitive as the TST in active TB (surrogate for LTBI). Other advantages include the need for fewer patient visits, avoidance of subjective readings, and the ability to perform serial testing without boosting. A major limitation of IFN-γ assays, particularly in developing countries, is their higher material costs and the need for laboratory support. The need for venous blood also poses problems for their use in young children and in community-based studies.

The QuantiFERON-TB Gold (QFT-G) assay (Cellestis Ltd, Carnegie, Australia) is a commercial test, recently approved by the U.S. Food and Drug Administration (FDA). In December 2005, the U.S. Centers for Disease Control and Prevention (CDC) recommended that the QFT-G assay can be used instead of TST in all situations where the TST is currently used.[22] The QuantiFERON-TB Gold *In Tube*, a simplified variant of the QFT-G assay, uses tubes coated with ESAT-6, CFP-10, and TB7.7 for stimulating T-cell response; this version is not currently FDA approved. Although data are limited, the *In Tube* version of QFT-G appears to yield higher positivity than the FDA approved version.[25] Other IFN-γ assays, including those using ELISPOT (e.g. T-SPOT.*TB*, Oxford Immunotec, UK) are also available now and have shown promise in various settings.[9,20,21,26,27]

Although several studies have evaluated IFN-γ assays, most have been done in low incidence countries, mainly in adults.[21] Few studies have been conducted in countries such as India where high TB prevalence, universal BCG vaccination, and widespread exposure to NTM pose challenges for the evaluation of new diagnostics. Further, few studies and none in India have evaluated IFN-γ assays in children-a vulnerable group that is at high risk of progressing from latent infection to active disease. The need to evaluate IFN-γ assays in children has been emphasized by several investigators.[9,20,21,24,27–29] The CDC guidelines on QFT-G state that no published data exist on the performance of QFT-G in children.[22]

In a previous study in India, we estimated the prevalence of LTBI among health care workers (HCW) using the TST and the QFT-G *In Tube* assay (henceforth called ''QFT'').[30] Our results showed a high prevalence of LTBI among HCWs, substantial concordance (81%) between TST and QFT, and similar association between risk factors and positive test results.[30] Another interesting finding in this study was the lack of an effect of previous BCG vaccination on both TST and QFT results.[30] However, other studies in low incidence settings have showed that BCG vaccination has a greater impact on TST than IFN-γ assay.[31,32] To our knowledge, the QFT assay has not been evaluated in the Indian paediatric population. Therefore, we evaluated the QFT assay in hospitalized children. The objectives were to estimate the prevalence of LTBI in children using TST and

QFT, determine concordance between the tests, and estimate the sensitivity of both tests in children with active TB.

Materials and methods

Study setting and participants

We conducted a cross-sectional study at the Mahatma Gandhi Institute of Medical Sciences (MGIMS) hospital, Sevagram, a rural medical school in India. This hospital averages about 2700 in-patient paediatric admissions annually. TB is a major public health concern in this setting. In the MGIMS hospital, about 300–400 patients are diagnosed with active TB each year. The annual risk of TB infection in the community is 1.2–1.6%, indicating a high rate of TB transmission in the population.[33]

In our study, children aged 1–12 years admitted to the paediatric wards were screened for eligibility using a set of pre-specified inclusion and exclusion criteria (Table 1). Between July 2004 and June 2005, a total of 105 consecutively admitted children who met the inclusion criteria were enrolled. Written parental informed consent was obtained, and a verbal assent was obtained from older children (aged 10 years or more). The study was approved by the Institutional Review Boards at the MGIMS hospital in India, and the University of California, Berkeley.

Table 1 Inclusion and exclusion criteria for participant selection

Inclusion criteria

1. Child aged between 1 and 12 years
2. Parent willing to provide informed consent
3. Admitted in the paediatric ward for clinical suspicion of tuberculosis or history of contact with a patient with infectious TB
   - Clinical suspicion of tuberculosis was based on any of the following:
     - Fever more than 2–3 weeks
     - Cough more than 2–3 weeks
     - Progressive unexplained weight loss or malnutrition
     - Lymphadenopathy
     - Meningitis of presumed tubercular origin (CSF lymphocytosis/high protein levels/culture negative for pyogenic organisms, and mycobacterial culture pending)
     - Pleural effusion of presumed tubercular origin (pleural fluid lymphocytosis and exudate)
     - Radiological abnormalities consistent with TB (e.g. hilar shadows)
     - History of contact with an individual with pulmonary tuberculosis (e.g. household contact with an index case with TB)

Exclusion criteria

1. Refusal of consent or assent by parent/guardian/child
2. Unstable cardio-respiratory condition
3. Past history of allergy to tuberculin skin testing
4. Past history of active tuberculosis

TB, tuberculosis; CSF, cerebrospinal fluid.

For each child enrolled in the study, data on clinical history, physical examination, nutritional status, data on prior treatment for TB, BCG scar per visual inspection, and contact history with active TB patients was recorded on a pilot-tested questionnaire. Data such as history of prior TB and contact with active TB cases were ascertained by interviewing parents. To diagnose active TB, sputum microscopy was done on three serial sputum samples (two spot and one overnight, as per the guidelines of India's Revised National Tuberculosis Control Programme [RNTCP]).[34] However, sputum samples were not obtained in most cases. In children with signs and symptoms of extra-pulmonary TB, specimens such as cerebrospinal fluid, pleural fluid, peritoneal fluid, lymph node biopsy/aspirate, and gastric fluids were collected and subjected to microscopy and/or culture. These samples were sent for cytology and histopathological examination in addition to microbiological investigations. HIV testing was only done in selected cases, when requested by the attending paediatricians.

Because microbiological diagnosis is often difficult to establish in the paediatric population, we also collected data on the number of children who were initiated on anti-TB therapy by the paediatricians in charge of case management. This group included a subset of children with laboratory-confirmed TB.

Tuberculin skin testing

All the children were investigated for LTBI using both QFT-G *In Tube* assay and the TST. The TST was administered using the Mantoux technique. 1 TU PPD RT23 (Staten Serum Institut, Denmark) was administered intra-dermally, and induration was read 48–72 h later by an experienced, certified tuberculin reader. Although not the internationally recommended dosage,[35] the 1 TU dose is the standard in India,[36] as recommended by India's Revised National Tuberculosis Control Programme (RNTCP).[34] An induration of at least 10 mm was considered positive, in tune with the standard practice in India, and consistent with the high-risk population we enrolled.[34,35] For comparison, 5 mm and 15 mm cut-off points were also evaluated. The TST reader was blinded to the clinical diagnosis and QFT results. However, paediatricians in charge of case management were not blinded to the TST results.

IFN-γ assay

The QFT assay was performed as per the manufacturer's instructions. The assay involved two stages: the first stage involved incubation of whole blood with antigens, and the second stage involved measurement of IFN-γ production in harvested plasma by ELISA. Venous blood was directly collected, prior to TST administration, into three 1 ml heparin-containing tubes. One tube contained only heparin as negative control, another also contained mitogen as positive control, and the third tube had overlapping peptides representing the entire sequences of ESAT-6 and CFP-10 and another peptide from a portion of the TB antigen TB7.7 (Rv2654). Within 2–6 h of blood draw, the tubes were incubated at 37 °C. After exactly 24 h of incubation, the tubes were centrifuged and plasma was harvested and

frozen at −70 °C until the ELISA was performed (on average, ELISA was performed within 4–6 weeks of blood collection). The IFN-γ response was quantified using ELISA. IFN-γ values (IU/ml) for TB-specific antigens and mitogen were corrected for background by subtracting the value obtained for the respective negative control. As recommended by the manufacturer, and based on previous studies,[25,30,37,38] the cut-off value for a positive test was IFN-γ ≥ 0.35 IU/ml. The QFT ELISA is not designed to estimate the absolute IFN-γ values when they exceed 10 IU/ml.[30] Therefore, IFN-γ values >10 IU/ml were reported as 10 IU/ml. Paediatricians in charge of case management were blinded to QFT results.

Statistical analyses

Analyses involved estimation of the prevalence of LTBI using both tests, along with the 95% confidence intervals (CI). Because there is no gold standard for LTBI, concordance was evaluated between TST and QFT assay using two indices: proportion agreement, and kappa (κ) coefficients. Bivariate and multivariate analyses (logistic regression) were used to identify risk factors associated with positive test results. Unadjusted and adjusted odds ratios (OR) were used to determine the magnitude of the associations. Risk factors evaluated included age, sex, nutritional status, BCG scar status, and history of contact with infectious TB. Because HIV testing was not done in most children, we were unable to evaluate HIV as a risk factor.

In addition, we calculated the sensitivity of the IFN-γ assay and compared that against the sensitivity of TST. Sensitivity was defined as the proportion of individuals that had a positive result in the cohort deemed as having laboratory confirmed active TB (laboratory confirmation involved either bacteriological or pathological verification). All analyses were performed using Stata® (Version 9, Stata Corp., College Station, TX).

Results

Description of study participants

Of a total of 1840 paediatric admissions between July 2004 and June 2005, 133 (7%) children met eligibility criteria. Of these 133 children, 105 (79%) children with clinical suspicion of TB or history of contact with an adult with active TB were enrolled after informed consent. Of the 105 children, 55% were included because they were malnourished and therefore TB workup was considered necessary, 30% were included because of symptoms suggestive of TB, and 15% had a history of contact with a patient with pulmonary TB. The median age of the children enrolled was 6 years (range 1–12 years). As shown in Table 2, a majority of the children (88%) were from a lower socio-economic background. Malnutrition was common as 60 of 105 (57%) children had weight-for-age Z-score of < −2. Nine of 105 (9%) children were tested for HIV status and only one was found to be positive for HIV. Apart from malnutrition and HIV, none of the children had any known immunocompromising condition (e.g. due to use of steroids). A total of 97 of 105 (92%) children had a history of BCG vaccination in past but BCG scars could be visually ascertained in only 86 (82%). None of the children had ever been tuberculin tested in past, and none of the parents had received treatment for LTBI.

Table 2 Characteristics of the study participants ($N = 105$)

Characteristics	Male (%)	Female (%)	Total (%)
Age (completed years)			
1–4 years	25 (60)	17 (40)	42 (40)
5–8 years	17 (52)	16 (48)	33 (31)
9–12 years	12 (40)	18 (60)	30 (29)
Educational level			
Kindergarten	6 (40)	9 (60)	15 (14)
Primary school	19 (54)	16 (46)	35 (33)
High school	5 (31)	11 (69)	16 (15)
No schooling[a]	24 (62)	15 (38)	39 (37)
Socio-economic status (SES)[b]			
Low	46 (50)	46 (50)	92 (88)
Middle	8 (62)	5 (38)	13 (12)
High	0	0	0
HIV status			
Total tested	5 (56)	4 (44)	9
Positive	1 (100)	0 (0)	1 (11)
Negative	4 (50)	4 (50)	8 (89)
Weight-for-age Z-score			
> −2 (well nourished)	20 (44)	25 (56)	45 (43)
< −2 (malnourished)	34 (57)	26 (43)	60 (57)
BCG vaccination scar present			
Yes	44 (51)	42 (49)	86 (82)
No	10 (53)	9 (47)	19 (18)
History of direct contact with a TB index case[c]			
Yes	10 (63)	6 (37)	16 (15)
No	44 (49)	45 (51)	89 (85)

HIV, human immunodeficiency virus; BCG, bacille Calmette–Guerin.

[a] Includes children below ≤3 years of age ($N = 33$) and those who never received any kind of school education ($N = 6$).

[b] Based on SES scores calculated from highest parental occupation, ownership of materials (e.g. car, house), and monthly household income of the parents as proxy markers of SES.

[c] History of direct contact was defined as any child who lived in a household with an adult taking anti-TB therapy.

TST results

Valid TST results were available in all 105 children. With the standard cut- point of ≥10 mm, 10 of 105 (9.5%; 95% CI 3.8, 15.2) children were TST-positive. In comparison, with cut-off points of ≥5 mm and ≥15 mm, a total of 16 (15.2%; 95% CI 8.2, 22.2) and 4 (3.8%; 95% CI 0.08, 7.5) were positive, respectively.

IFN-γ assay results

Valid QFT assay results were available for all 105 children; none of the results were indeterminate due to low mitogen response. With a cut-off point of ≥0.35 IU/ml, 11 (10.5%)

Table 3 Agreement between TST and QFT assay results ($N = 105$)

TST results	QFT results[a]			Agreement (%)	Kappa (95% confidence interval)
	QFT+	QFT−	Total		
TST cut-off point of ≥5 mm					
TST+	8	8	16	89.5	0.53 (0.34–0.71)
TST−	3	86	89		
Total	11	94	105		
TST cut-off point of ≥10 mm					
TST+	8	2	10	95.2	0.73 (0.53–0.92)
TST−	3	92	95		
Total	11	94	105		
TST cut-off point of ≥15 mm					
TST+	4	0	4	93.3	0.50 (0.33–0.66)
TST−	7	94	101		
Total	11	94	105		

TST, tuberculin skin test; QFT, QuantiFERON-TB Gold *In Tube* assay.
[a] QFT cut-off point of IFN-γ ≥ 0.35 IU/ml.

were QFT positive (95% CI, 4.5, 16.4). The IFN-γ levels ranged from 0.58 IU/ml to 10 IU/ml in the QFT-positive children, with a mean of 5.9 IU/ml. Of the 11 children with positive QFT results, 5 had IFN-γ levels of 10 IU/ml.

Agreement between TST and IFN-γ assay results

Data on agreement between TST and QFT assay were available for all children (Table 3). With a cut-off point of 10 mm, both the TST and QFT assay were positive in 8, and negative in 92 subjects respectively, resulting in an agreement of 95% ($\kappa = 0.73$; CI 0.53, 0.92). In comparison, agreement was slightly lower when 5 mm and 15 mm cut-off points were used (differences not statistically significant). There were 5 discordant results when a 10 mm cut-off point was used (2 children with TST-positive/QFT-negative results and 3 with TST-negative/QFT-positive results). Table 4 summarizes the clinical and laboratory characteristics of subjects with discordant results.

Analysis of the distribution of IFN-γ levels in TST positive and negative subjects showed a significant correlation between TST positivity and IFN-γ levels: the median IFN-γ level in TST-positive subjects was 8 IU/ml, whereas the median level in TST-negative subjects was 0 IU/ml ($P < 0.05$).

Effect of BCG vaccination on agreement between TST and QFT

As seen in Table 5, in BCG scar-negative children, the agreement between the tests was 100% ($\kappa = 1.0$) as compared to 94% ($\kappa = 0.63$; CI 0.42, 0.84) in scar-positive children. BCG scar-positivity was not associated with any definite pattern of discordance. Among the scar-positives, two children had TST+/QFT− pattern of discordance, whereas three children had the reverse pattern (TST−/QFT+).

Test results in children with confirmed active TB

Table 6 shows the data on TST and QFT in 8 children ultimately diagnosed to have laboratory confirmed TB. Of these 8 children, only 5 had a microbiological confirmation, with either smear ($N = 4$) or culture ($N = 1$) positivity. In the remaining 3 cases, TB was confirmed using cytology and/or histopathology. It is seen that there is a complete agreement between the two tests irrespective of the final diagnosis. Of the 4 subjects with extra-pulmonary TB, 3 were negative by both the tests indicating that the sensitivity of both TST and QFT assay was possibly low in extra-pulmonary TB. The sensitivity of both TST and QFT assay

Table 4 Discordance between tuberculin skin test and QuantiFERON-TB Gold results ($N = 5$)

Sample no.	Age/sex	BCG scar	QFT result	TST result (mm)	Final clinical diagnosis	Anti-TB therapy started	6 months follow-up
1	5/M	+	+	2	Viral encephalitis	No	No evidence of TB
2	11/M	+	+	0	Fever of unknown origin	No	No evidence of TB
3	5/F	+	+	0	Measles	No	No evidence of TB
4	5/M	+	−	12	Pulmonary tuberculosis	Yes	Lost to follow-up
5	11/F	+	−	12	Pulmonary tuberculosis	Yes	No evidence of TB

BCG, bacille Calmette–Guerin; TST, tuberculin skin test, QFT, QuantiFERON-TB Gold *In Tube* assay.

Table 5 Effect of previous BCG vaccination on agreement between tuberculin skin test and QuantiFERON-TB Gold results

TST results[a]	QFT assay results[b]			Agreement (%)	Kappa (95% confidence interval)
	QFT+	QFT−	Total		
BCG scar-negative					
TST+	3	0	3	100	1.0 (0.55−1.44)
TST−	0	16	16		
Total	3	16	19		
BCG scar-positive					
TST+	5	2	7	94	0.63 (0.42−0.84)
TST−	3	76	79		
Total	8	78	86		

BCG, bacille Calmette−Guerin; TST, tuberculin skin test, QFT, QuantiFERON-TB Gold *In Tube* assay.
[a] TST cut-off point of >10 mm induration.
[b] QFT cut-off point of IFN-$\gamma \geq 0.35$ IU/ml.

in microbiologically confirmed TB cases (5 out of 8) was found to be identical (62.5%).

Test results in children initiated on anti-tuberculosis therapy

Because microbiological confirmation of TB is difficult in children, we compared TST and QFT results in all children initiated on anti-TB therapy (based on either clinical or laboratory diagnoses). Eleven of 105 children (10.5%) were initiated on therapy (after recruitment), of which 8 had some form of laboratory confirmation. Of these 11 children, the TST was positive (10 mm cut-off point) in 9 (82% sensitivity). With QFT, 7 of 11 children (64%) were positive. The agreement between TST and QFT among these 11 children was 82% ($\kappa = 0.56$). When both tests were used in combination (positive by either TST of QFT), then the overall sensitivity was 9/11 (82%). Two of 11 children (18%) were negative by both tests.

Risk factors for latent TB infection

In order to evaluate the various risk factors associated with positive TST and QFT, the data were analysed using multivariate methods (Table 7). A logistic regression model was used to assess risk factors such as age, sex, socioeconomic status, nutritional status, BCG scar, and history of contact with index case. In the final model only age, sex, BCG scar, weight-for-age *Z*-scores and history of contact were retained as they appeared to be important risk factors. Increasing age was the most important risk factor for TST and QFT-positivity. However, only QFT was significantly associated with age >8 years (OR 5.92; [95% CI 1.02, 34.39]). None of the other covariates reached statistical significance. BCG scar was not associated with the results of both TST and QFT. This finding did not change when history of vaccination was used instead of scar as an indicator of BCG vaccination (data not shown).

Discussion

Paediatric TB is a diagnostic challenge and there is an urgent need to develop newer diagnostic tools to improve case detection.[5−8,24,39] Although IFN-γ assays have shown promise in several studies,[9,20,21,24] few published studies exist on their performance in children. Available studies suggest that these assays may be feasible, and in some cases, clinically useful in the evaluation of TB in children.[27,40−42] Recently, Liebeschuetz and colleagues showed that an ELISPOT based assay was more sensitive than TST

Table 6 Tuberculin skin test and QuantiFERON-TB Gold results in cases with confirmed active tuberculosis ($N = 8$)

Sample no.	Age/sex	BCG scar	QFT result	TST result (mm)	Positive diagnostic investigations	Final diagnosis
1	4/F	+	+	15	Peritoneal fluid cytology	Abdominal tuberculosis
2	3/M	+	−	0	Gastric aspirate smear, peritoneal fluid cytology	Abdominal tuberculosis
3	5/F	−	−	0	CSF smear and CSF cytology	Tuberculous meningitis
4	11/F	+	−	0	CSF cytology	Tuberculoma
5	12/F	−	+	13	Pleural fluid smear	Pulmonary tuberculosis
6	5/M	+	+	13	Peritoneal fluid cytology	Pulmonary tuberculosis
7	4/M	+	+	13	Gastric aspirate smear	Pulmonary tuberculosis
8	10/M	−	+	10	Sputum culture	Pulmonary tuberculosis

BCG, bacille Calmette−Guerin; TST, tuberculin skin test, QFT, QuantiFERON-TB Gold *In Tube* assay.

Table 7 Multivariate analysis showing risk factors associated with positive tuberculin skin test and QuantiFERON-TB Gold assay results

Covariates	Tuberculin skin test (≥10 mm)			QuantiFERON-TB Gold assay (≥0.35 IU/ml)		
	No. positive/ No. tested (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	No. positive/ No. tested (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Age (completed years)						
1–4 years	2/42 (5)	1.0	1.0	2/42 (5)	1.0	1.0
5–8 years	2/33 (6)	1.29 (0.17, 9.68)	1.16 (0.14, 9.49)	3/33 (9)	2.0 (0.31, 12.7)	2.02 (0.30, 13.5)
9–12 years	6/30 (20)	5.0 (0.93, 26.7)	5.69 (0.95, 33.8)	6/30 (20)	5.0 (0.93, 26.7)	5.92 (1.02, 34.39)**
Sex						
Female	4/51 (8)	1.0	1.0	4/51 (8)	1.0	1.0
Male	6/54 (11)	1.46 (0.38, 5.5)	1.92 (0.43, 8.59)	7/54 (13)	1.75 (0.48, 6.37)	2.04 (0.50, 8.28)
Weight-for-age Z-score						
> −2 (well nourished)	5/45 (11)	1.0	1.0	4/45 (9)	1.0	1.0
< −2 (malnourished)	5/60 (8)	0.73 (0.19, 2.68)	0.63 (0.15, 2.59)	7/60 (12)	1.35 (0.37, 4.93)	1.26 (0.32, 4.90)
BCG scar						
Absent	3/19 (16)	1.0	1.0	3/19 (16)	1.0	1.0
Present	7/86 (8)	0.47 (0.11, 2.02)	0.69 (0.13, 3.44)	8/86 (9)	0.57 (0.13, 2.28)	0.82 (0.17, 3.91)
History of contact with TB index case						
No	7/89 (8)	1.0	1.0	8/89 (9)	1.0	1.0
Yes	3/16 (19)	2.70 (0.61, 11.8)	2.48 (0.51, 11.9)	3/16 (19)	2.33 (0.54, 9.96)	2.00 (0.42, 9.35)

**Statistically significant.
[a] Adjusted for age, sex, nutritional status, BCG scar status and history of contact.

among children with active TB, and was less affected by malnutrition.[27] To our knowledge, our study is the first evaluation of the QFT-Gold *In Tube* assay among children in India. Although limited by the small sample size, we believe our data will be helpful in understanding the performance of IFN-γ assays in children in high-burden settings for which data are scarce. Our data will also be helpful for designing larger prospective studies to assess the accuracy of these tests in children, especially as a rule-out test for active disease.

Our cross-sectional data suggest that TST and QFT are comparable in our study population of predominantly BCG-vaccinated, hospitalized children—the agreement between the tests was substantial, and risk factors for test positivity were similar for both tests. Increasing age was the most important risk factor for LTBI, with QFT showing a slightly better association with age than TST; whether this indicates a better sensitivity for QFT or not remains to be studied. These findings, overall, are consistent with the results of our previous evaluation of the QFT-Gold *In Tube* assay in health care workers.[30] However, comparability does not necessarily indicate that these tests are completely equivalent and interchangeable. The two tests, although measuring related aspects of the immune response, probably do not measure the exact same components of the cellular immune response.[43] It is worth noting that both TST and IFN-γ assay cannot distinguish between latent infection and active disease. This has important implications for high endemic countries.

Our data suggest that BCG vaccination, even in children with fairly recent history of vaccination, does not have significant effect on either TST or QFT results in our study population. This finding, although in tune with our earlier results on the QFT assay among health care workers,[30] may not be widely generalizable. The effect of BCG on subsequent TST responses is a complicated phenomenon affected by several factors including BCG strain used, dosage, timing of vaccination (infancy versus later in childhood), time elapsed since vaccination, frequency and method of administration, and age of the child at vaccination.[11,12,44,45] In India, the Danish 1331 BCG vaccine is administered intradermally at birth, and not repeated subsequently. In this context, the lack of a significant effect of BCG on TST results has been shown in several studies from India.[46–50] Similar findings have also been reported from other high burden countries such as The Gambia,[51,52] South Africa,[42] Uganda,[53] and Botswana.[54] In these countries BCG is usually administered at birth or in infancy. However, there are other countries, mostly low endemic, such as Korea, Japan, USA and UK, where BCG clearly has an adverse impact on the specificity of TST.[31,38,55–57]

In high-burden tropical countries, it is likely that BCG vaccination in infancy produces a transient increase in TST responses, but this rapidly wanes with age.[11,44,45,58] For example, the landmark south Indian BCG vaccine trial involving more than 200,000 subjects showed rapid waning of BCG-induced tuberculin sensitivity.[59] Waning was noticed as early as 2.5 months after vaccination. In contrast, BCG vaccination after infancy, or repeat vaccinations probably have a more discernible and persistent effect on TST responses.[11,44,45,58] There is some evidence that BCG induced tuberculin responses declines more rapidly in tropical than

in temperate environments.[58] Investigators have hypothesized that this may be due to high prevalence of exposure to other infections in tropical countries, leading to a bias away from a Th1 type of immune response.[58] These issues suggest that the accuracy and performance characteristics of IFN-γ assays, and the TST for that matter, may be greatly influenced by the setting (high burden versus low burden).[24]

Our study had limitations that deserve discussion. The foremost limitation was the sample size, which comprised only 105 subjects of which only 8 (7.6%) could be diagnosed as confirmed TB cases. Because of the small number microbiologically confirmed TB, we were unable to estimate the sensitivity of the QFT assay. To partly overcome this limitation, we also analysed sensitivity of the tests in all children initiated in anti-TB therapy. Because of the cross-sectional design and the relatively few cases of discordant results, we were unable to adequately resolve the discordance between the two tests. Further, we used the 1 TU dose of PPD RT23. Although this is the standard dosage used in India,[36] it differs from the internationally used 2 TU RT23 dosage, considered equivalent to the 5 TU PPD-S formulation.[11] The use of 1 TU dosage may have lead to some loss of sensitivity. Also, we were unable to measure important covariates such as NTM infection and HIV infection, which would have enabled us to better evaluate the performance of both the tests in the Indian context. Lastly, because of study included only hospitalized children with a high prevalence of malnutrition, the results may not be generalizable to healthy, well-nourished paediatric populations in high income countries. Because of the small sample size, we were unable to determine the association between nutrition status and test results. The low yield of positive results by both TST and IFN-γ assay in our study suggests that malnutrition, to some extent, might have impacted both tests. However, a previous study in South Africa showed that an IFN-γ assay was less affected by malnutrition than TST.[27]

Our results and the external evidence suggest that both TST and IFN-γ assays have advantages and limitations, and both may have a useful role, depending on factors unique to each setting. The emergence of IFN-γ assays is an exciting new development. For the first time, it has expanded the range of tests available for LTBI. The decision to select one or the other test will depend on several factors unique to each setting, including the goal of testing, the population or clinical context, and the resources available. For example, because of its high specificity, RD1-based IFN-γ assays will be helpful in populations where cross-reactivity due to BCG hampers TST interpretation, and/or where background TB infection rate is relatively low. Also in urban and developed country settings, the IFN-γ assay may be more applicable because of availability of adequate laboratory infrastructure, and skilled human resources.

On the other hand, in high-burden, resource-limited settings such as rural India, the TST will probably continue to serve a useful purpose. In India, the TST is widely used in diagnosing childhood TB, and in epidemiological studies on annual risk of infection where blood testing is logistically difficult. In India, a 15-year follow-up of 280,000 subjects in the BCG vaccine trial showed that TST response is significantly associated with long-term development of active TB.[60] There is also strong evidence from several studies (including more than 100,000 children) that BCG does not significantly influence TST results in the Indian population.[30,46–50] Therefore, our findings, and evidence from previous Indian studies,[9,30] suggest that the TST remains a useful test, particularly because of the low cost, relatively easy accessibility, and because BCG does not significantly affect TST specificity in India. Although no formal costing studies have been done, IFN-γ assays may cost several times higher than the TST in India.[9] Cost, therefore, will be a critical factor in using IFN-γ assays in developing countries. Another factor that may affect applicability in paediatric practice is blood volume required for IFN-γ assays. Of the available commercial assays, the QuantiFERON-TB Gold *In Tube* requires the least amount of blood (3 ml). Further simplification of this assay (e.g. lateral flow or strip format that can use finger-prick blood) will greatly facilitate applicability in children.

In summary, in a rural, predominantly BCG-vaccinated paediatric population, the TST and the QFT assay produced comparable results. The QFT assay was feasible in children with no indeterminate results, even in malnourished children. BCG vaccination did not significantly affect either TST or QFT results. These results confirm those of a previous study of adults in our setting. Given the consistent results of these two studies, it appears that both TST and QFT may be useful in India. However, cost and technical considerations might favour the selection of the TST in rural settings with limited resources and poor laboratory infrastructure. Larger prospective studies are needed among children in high incidence countries to determine the sensitivity and specificity of IFN-γ assays, their ability to identify children who are at risk of progressing from latent infection to active disease, and their ability to serve as useful rule-out test for active disease. There is also a need to study the impact of HIV infection and malnutrition on TST and IFN-γ assay performance in high burden settings, particularly among children with active TB.

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