



Interferon- γ release assays do not identify more children with active tuberculosis than the tuberculin skin test

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ABSTRACT: Data are lacking on the performance of interferon- γ release assays (IGRAs) in children. Although IGRAs are recommended for screening for latent tuberculosis infection (LTBI), many clinicians wish to employ them as a diagnostic test for active tuberculosis (TB). The objective of the present study was to compare the performance of the two commercially available IGRAs and the tuberculin skin test (TST) side-by-side in children with active TB and LTBI.

In a prospective study, 209 children were investigated for active ($n=91$) or latent TB ($n=118$). TST, QuantiFERON-TB Gold In-tube (QFG-IT; Cellestis, Carnegie, Australia) and T-SPOT.TB (Oxford Immunotec, Abingdon, UK) assays were simultaneously used.

For culture-confirmed active TB, the sensitivity of the TST was 83%, compared with 80% for QFG-IT and 58% for T-SPOT.TB. IGRAs did not perform significantly better than TST, although QFG-IT was significantly better than T-SPOT.TB. The agreement between QFG-IT and T-SPOT.TB in culture-confirmed TB was poor at 66.7%. In LTBI, the agreement between QFG-IT and T-SPOT.TB was very good (92%) with moderate agreement between TST and T-SPOT.TB (75%) and QFG-IT and TST (77%).

A negative interferon- γ release assay should not dissuade paediatricians from diagnosing and treating presumed active tuberculosis. If used for diagnosis of latent tuberculosis infection, interferon- γ release assays could significantly reduce the numbers of children receiving chemoprophylaxis. Very good concordance between both tests was found.

KEYWORDS: Children, interferon- γ release assays, skin test, tuberculosis

The identification of *Mycobacterium tuberculosis*-specific antigens has led to the successful development of commercially available immunoassays that are able to distinguish the production of interferon (IFN)- γ in response to *M. tuberculosis* infection from the bacillus Calmette-Guérin (BCG) vaccine effect, unlike the tuberculin skin test (TST) [1–3]. For the purposes of screening for latent tuberculosis infection (LTBI), and to avoid unnecessary chemoprophylaxis, these blood-based IFN- γ release assays (IGRAs) are now widely recommended for use in adults and children with a positive TST, and have found their way into national guidelines in several countries, including the UK [4]. In the UK, however, no recommendations have been made regarding which of the two commercially available assays

to use and the guidelines do not suggest using IGRAs for the diagnosis of active tuberculosis (TB).

However, clinicians (and paediatricians in particular) are searching for improved diagnostic tools [1] and have been exploring IGRAs for immunodiagnosis of active TB, since microbiological confirmation is the exception rather than the rule in children due to the paucibacillary nature of their disease [2].

Of the large number of publications assessing the performance of IGRAs *versus* the TST, most relate to adult cohorts, and the majority have only evaluated one of the two commercially available IGRAs in comparison with the TST. The few studies comparing both IGRAs with the TST have small paediatric cohorts including a minority of children with active TB [3, 5, 6]. Most investigators have chosen to analyse the combined results

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of performance of IGRAs *versus* TST in active TB as well as LTBI. The aim of these studies has been to assess concordance of IGRAs with TST, and the published data have generally shown superior sensitivity of IGRAs in LTBI [3, 7, 8]. Some studies have also demonstrated higher diagnostic sensitivity and specificity of IGRAs over TST in active TB [9–11]. However, several recent reviews have concluded that performance of IGRAs in young children and immunocompromised individuals deserve further investigation and that a gap of knowledge persists in this area [12, 13].

It was postulated that the performance of IGRAs in children might be discrepant between active TB and LTBI, since the underlying immunological phenomena are probably different.

The aim of the present study was to describe the performance of both commercially available IGRAs that are recommended by the National Institute of Health and Clinical Excellence (NICE) in the UK, side-by-side with the TST, in the setting of active and latent TB. Children were recruited from two National Health Service (NHS) hospital trusts in north-west London, UK, where the TB incidence of the local population varies 40–100 per 100,000 population [14].

METHODS

Study participants

Study approval was obtained from the ethics committees of Imperial College Healthcare NHS Trusts and the North West London Hospitals Trust. Written consent was provided by all study participants or their parents or legal guardians. Verbal consent was also sought from children aged >10 yrs.

Between February 2006 and February 2008, children with suspected active TB or LTBI attending the inpatient and outpatient services at Imperial College Healthcare NHS Trust St Mary's Hospital or North West London Hospitals NHS Trust were prospectively enrolled.

Inclusion criteria

All children aged between 2 months and 16 yrs who were investigated for active TB were included, as were referrals for contact tracing after exposure to a case of active TB.

Definition of study groups

Active TB

Cases of active TB were defined according to the following three categories. 1) Definite TB: all children in this group had culture-confirmed TB. 2) Probable TB: all children in this group had (in the absence of culture confirmation) symptoms and signs of active TB; abnormal radiography consistent with TB and/or abnormal cerebro-spinal fluid (CSF) consistent with tuberculous meningitis; response to TB therapy; and either a history of TB contact or a history of travel to TB-endemic countries within the previous 24 months. 3) Possible TB: signs and symptoms consistent with active TB and existing risk factors for active TB, such as history of TB contact or history of travel to TB-endemic countries. Subsequently, however, the treating paediatrician made an alternative diagnosis or felt that TB was unlikely. Therefore, none of the children in this group ever received TB treatment and none subsequently developed active TB.

LTBI

Children with suspected LTBI were children with documented exposure to active TB. Results for these children were stratified according to their BCG vaccination record by a TST ≥ 15 mm (BCG vaccinated) or a TST of ≥ 6 mm (not BCG vaccinated), following the UK guidelines.

Consent for the study, and demographic and clinical data were obtained by the paediatricians in charge of the patients' clinical care. All blood samples were handled by laboratory staff blinded to the clinical status of the patients. Results of IGRA testing were not available to the treating physicians.

Investigations for active TB

Children admitted with suspected active pulmonary TB (PTB) had sputum samples and/or gastric washings collected as per routine protocol. Any child with suspected TB meningitis had CSF sent for microscopy and culture. For other sites of presumed disseminated disease, tissue biopsies were obtained where possible and appropriate. Any extrapulmonary manifestation of TB is referred to as EPTB.

Tuberculin skin testing

TSTs were sited by the medical or TB nursing staff, according to routine hospital procedure.

Two units of purified protein derivative RT23 (Staten Serum Institute, Copenhagen, Denmark) were applied to the volar surface of the left lower forearm according to the intradermal Mantoux method. The transverse diameter of skin induration was recorded in millimetres 48–72 h later. In line with the cut-offs selected by NICE for interpretations of TST results for LTBI, TST results were stratified into two groups of ≥ 6 mm, and ≥ 15 mm, depending on the absence or presence of previous BCG vaccination. For active TB, sensitivities of cut-offs of 15 mm and 10 mm were evaluated.

Laboratory assays

Peripheral blood samples were obtained simultaneously for both assays by paediatric phlebotomists who were trained in the use of the specific blood collection tubes, according to the manufacturer's recommendations. All blood samples were processed within 4 h of phlebotomy, as recommended.

The commercially available T-SPOT.TB (Oxford Immunotec, Abingdon, UK) and the QuantiFERON-TB Gold In-tube (QFT-GIT) system (Cellestis, Carnegie, Australia) were used in all assays.

T-SPOT.TB

Blood samples were collected in 4-mL CPT tubes (BD Bioscience, Oxford, UK) and peripheral blood mononuclear cells (PBMCs) were prepared within 2 h, following standard procedures. Commercially available T-SPOT.TB kits were used for all assays. All procedures, including interpretation of T-SPOT.TB results, were carried out according to the manufacturer's recommendations. Absolute numbers of spot-forming cells (SFCs) were counted using an Elispot counter (AID, Strassberg, Germany) and numbers of antigen-specific SFCs in positive T-SPOT.TB assays were plotted following subtraction of numbers in the negative control well.

QFG-IT

The commercially available blood bottles and ELISA kits were used for all assays, following manufacturer's recommendations. IFN- γ values for TB-specific antigens, mitogen and negative control were calculated by the Cellestis software program. The cut-off value for a positive result was set at >0.35 IU of IFN- γ , as recommended by the manufacturers. The test result was considered to be indeterminate for TB antigen responsiveness if the IFN- γ level was <0.35 IU·mL⁻¹ in the TB antigen well and the mitogen minus nil was <0.5 IU·mL⁻¹ or if the nil value was >8.0 IU·mL⁻¹. Absolute levels of antigen specific IFN- γ were calculated by subtracting the value of the negative control from the antigen-specific value.

Statistical analysis

Sensitivity of the assays was measured by the proportion of positive results identified in the whole cohort and the

following subgroups: active TB (which was further categorised as "definite", "probable", "definite or probable" and "possible") and LTBI, stratified by BCG vaccination status.

Failed or indeterminate assay results were excluded from statistical analysis for sensitivity or agreement between tests.

Agreement between assays was measured by total per cent agreement and the κ statistic. The strength of agreement was defined as poor with $\kappa \leq 0.2$, fair if κ was 0.21–0.4, moderate if κ was 0.41–0.6, good if κ was 0.61–0.8 and very good if κ was 0.8–1 [15].

RESULTS

Between February 2006 and February 2008, 209 children who attended the NHS paediatric services for active or latent TB were enrolled into the study.

Demography

The mean (range) age of children presenting with active TB was 9.2 yrs (6 months to 15 yrs), and that of children investigated for LTBI was 6.9 yrs (3 months to 16 yrs). Table 1 summarises the demographic details of the cohort.

The male/female ratio was 1:1 for the active cases and 1:0.9 for the LTBI cohort. In line with the mixed community living in north-west London and attending NHS facilities, most children were from ethnic minorities. The majority of children (129 out of 209; 62%) were born outside of the UK.

In total, 68% (142 out of 209) of children had a known household contact, with 118 (56.45%) investigated for LTBI and 91 (43.55%) for active TB.

The majority of children were BCG vaccinated (142 (68%) out of 209). Children were not routinely tested for HIV, but all children with active TB and additional epidemiological risk factors for the presence of HIV (n=35) were screened for HIV and all found to be negative.

Test results for all children

Of the 209 children enrolled, 204 had available results for TST, 206 had a T-SPOT.TB sample analysed and 209 had available data for QFG-IT. The results per test conducted are summarised in table 2, stratified for children above and below 5 yrs of age.

Failures of QFG-IT and T-SPOT.TB

There were no technical failures with the QFG-IT, but 14 (6.7%) indeterminate results were obtained; eight in active TB and six in LTBI. The mean (range) age of children with indeterminate results was 6.4 (0.5–16) yrs. There were 18 failures (8.7%) of the T-SPOT.TB assay, consisting of sample errors prior to generating the PBMCs, such as clot in the blood sample (n=4), insufficient blood taken (n=3), and uninterpretable results (n=11) after processing of PBMCs because of high backgrounds (n=9) or low phytohaemagglutinin response (n=2). The mean age of children whose sample failed the T-Spot.TB was 4.74 (0.7–16) yrs. The mean age of the children with failed tests was not significantly different from the mean age of the

TABLE 1 Demographic details of the study cohort

	Active TB	LTBI
Subjects	91	118
Age yrs		
<1	2 (2.2)	18 (16.3)
1–2	7 (7.7)	8 (6.7)
2.1–5	18 (19.8)	31 (26.0)
5.1–10	18 (19.8)	22 (18.0)
10.1–16	46 (50.5)	39 (33.0)
Sex		
Female	47 (51.6)	57 (48.3)
Male	44 (48.4)	61 (51.7)
Ethnicity		
Black African	49 (54.4)	50 (42.0)
South Asian	21 (23.3)	39 (33.0)
South-east Asian	6 (6.6)	9 (8.0)
Afro-Caribbean	0 (0)	4 (3.0)
Arab	7 (7.7)	3 (2.5)
Mixed	1 (0.01)	7 (6.0)
Caucasian	7 (7.7)	6 (5.0)
Country of birth		
UK	30 (33.0)	50 (42.3)
Other	61 (67.0)	68 (57.7)
Known TB contact		
Yes	40 (44.0)	102 (86.0)
No	47 (51.6)	13 (11.0)
Unknown	4 (4.4)	3 (3.0)
BCG vaccinated		
Yes	55 (60.4)	87 (74.0)
No	23 (25.3)	17 (14.0)
Unknown	13 (14.3)	14 (12.0)
Travel to TB endemic countries		
Yes	58 (63.7)	55 (47.0)
No	25 (27.5)	53 (44.5)
Unknown	8 (8.8)	10 (8.5)

Data are presented as n or n (%). TB: tuberculosis; LTBI: latent TB infection. BCG: bacillus Calmette–Guérin.

TABLE 2 Performance of interferon-γ release assays and tuberculin skin test (TST) in all children according to age

	Subjects		<5 yrs							≥5 yrs							
			Subjects		QFG-IT			T-SPOT.TB		Subjects		QFG-IT			T-SPOT.TB		
	>15 mm		Pos.	Neg.	Ind.	Pos.	Neg.	TF	>15 mm		Pos.	Neg.	Ind.	Pos.	Neg.	TF	
	TST	TST							TST	TST							
All	209	82	19 (24)	13 (16)	62 (76)	7 (8)	11 (14)	59 (74)	10 (12)	127	72 (57)	67 (53)	53 (42)	7 (5)	59 (47)	59 (47)	8 (6)
Active	91	26	6 (23)	6 (23)	19 (73)	1 (4)	3 (12)	19 (73)	4 (15)	65	33 (51)	36 (55)	22 (34)	7 (11)	31 (48)	29 (45)	4 (6)
Definite	25	2	1 (50)	1 (50)	1 (50)	0	0	1 (50)	1 (50)	23	19 (82)	19 (82)	2 (9)	2 (9)	14 (64)	8 (36)	0
Probable	38	11	4 (36)	4 (36)	7 (64)	0	2 (18)	7 (64)	2 (18)	27	14 (52)	16 (59)	9 (33)	2 (7)	15 (56)	10 (37)	2 (7)
Possible	28	13	3 (23)	1 (8)	11 (84)	1 (8)	1 (8)	11 (84)	1 (8)	15	0	1 (7)	11 (73)	3 (20)	2 (13)	11 (73)	2 (13)
LTBI	118	56	13 (23)	7 (13)	43 (76)	6 (11)	8 (15)	40 (74)	6 (11)	62	39 (63)	31 (50)	31 (50)	0	28 (45)	30 (48)	4 (7)

Data are presented as n or n (%). QFG-IT: QuantiFERON-TB Gold In-tube system (Cellestis, Carnegie, Australia); pos.: positive; neg.: negative; ind.: indeterminate result; TF: test failure; LTBI: latent tuberculosis infection. T-SPOT.TB is manufactured by Oxford Immunotec, Abingdon, UK.

children with successful tests. Failed or indeterminate test results were not included in the further analysis.

Performance of tests in children with active TB

The cohort of children that was investigated for active TB (n=91) was divided into three subgroups, as specified in the Methods section. All children classified as definite (culture proven) TB (n=25) and probable TB (n=38) received treatment for active TB. No children classified as possible TB (n=28) at study entry subsequently received anti-TB therapy. Additionally, results are shown for the combination of definite and probable cohorts in order to illustrate the potential value of IGRAs and TST in the reality of paediatric practice, where TB treatment might be administered on clinical grounds in the absence of culture confirmation (table 3).

Sensitivities for the TST were calculated using two different cut-offs of 15 mm and 10 mm to examine the relative effect on sensitivity. In active, culture-confirmed TB the sensitivity was 83% for a TST ≥15 mm, 87.5% for a TST ≥10mm, 80% for

QFG-IT and 58% for T-SPOT.TB. Since the different cut-offs for TST did not result in a significant difference, further analysis was pursued with the cut-off of 15 mm, which is likely to be more specific for *M. tuberculosis* infection than a cut-off of 10 mm.

IGRAs did not perform significantly better than the TST in this group, but QFG-IT performed significantly better than T-SPOT.TB (p=0.015) in identifying culture-confirmed, active TB. There was no significant difference between any test in predicting probable (p=0.62) or possible TB (p>0.99). When results for definite and probable active TB were combined, TST ≥15 mm had a sensitivity of 60%, QFG-IT of 64% and T-SPOT.TB of 50%, as summarised in figure 1a.

In order to test for improved sensitivity by combining tests, the sensitivity for TST ≥15 mm combined with QFG-IT or T-SPOT.TB was calculated. In definite TB, a combination of TST and QFG-IT (n=22) achieved 91% sensitivity, compared with 100% for TST plus T-SPOT.TB (n=22). For probable TB,

TABLE 3 Summary of interferon-γ release assay and tuberculin skin test (TST) results in children with active tuberculosis (TB) and latent tuberculosis infection (LTBI)

	TST mm			QFG-IT			T-SPOT.TB		
	≥15	≥10	<6	Pos.	Neg.	Ind.	Pos.	Neg.	TF
Active TB									
Definite	20/24 (83.3)	21/24 (87.5)	2/24 (8.3)	20/25 (80.0)	3/25 (12.0)	2/25 (8.0)	14/24 (58.3)	9/24 (37.5)	1/24 (4.2)
Probable	17/38 (44.7)	24/38 (63.1)	9/38 (23.6)	20/38 (52.6)	16/38 (42.1)	2/38 (5.2)	17/38 (44.7)	17/38 (44.7)	4/38 (10.5)
Definite and probable	38/63 (60.3)	45/63 (71.4)	12/63 (19.0)	40/63 (63.5)	18/63 (28.5)	4/63 (6.3)	31/62 (50.0)	26/62 (41.9)	5/62 (8.0)
Possible	2/28 (7.1)	5/28 (17.8)	22/28 (78.5)	2/28 (7.1)	22/28 (78.5)	4/28 (14.2)	3/28 (10.7)	22/28 (78.5)	3/28 (10.7)
LTBI									
BCG vaccinated	38/84 (45.2)	51/84 (60.7)	59/84 (70.2)	28/87 (32.1)	53/87 (60.9)	6/87 (6.9)	25/85 (29.4)	51/85 (60.0)	9/85 (10.6)
Not BCG vaccinated	14/30 (46.7)	17/30 (56.7)	19/30 (63.3)	10/31 (32.2)	21/31 (67.8)	0/31 (0.0)	11/31 (35.5)	19/31 (61.3)	1/31 (3.2)

Data are presented as n (%). QFG-IT: QuantiFERON-TB Gold In-tube system (Cellestis, Carnegie, Australia); pos.: positive; neg.: negative; ind.: indeterminate result; TF: test failure; BCG: bacillus Calmette-Guérin. T-SPOT.TB is manufactured by Oxford Immunotec, Abingdon, UK.

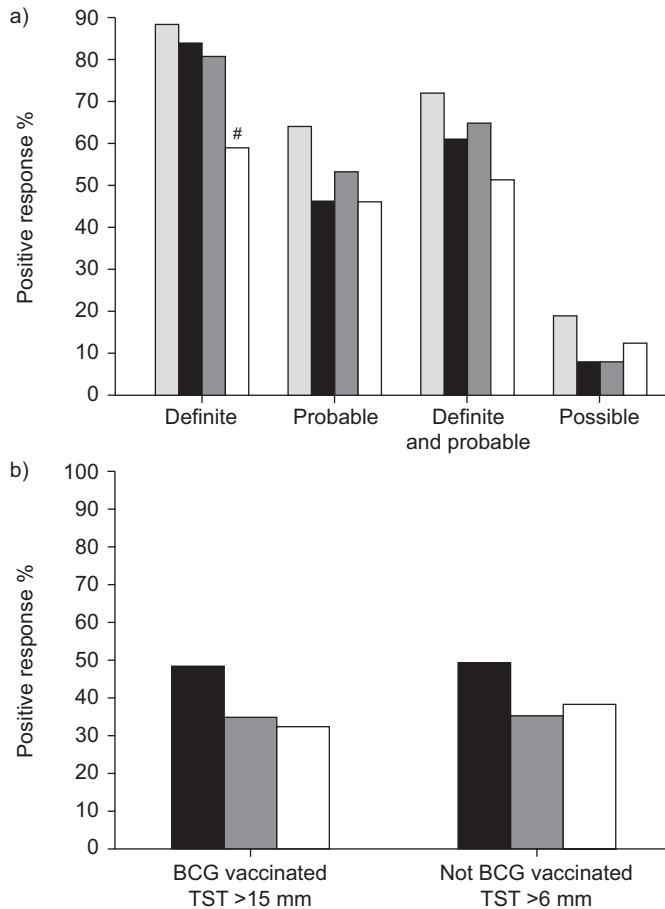


FIGURE 1. a) Percentage of positive responses in tuberculin skin test (TST) and interferon- γ release assays (IGRAs) according to TST cut-off and categories of active tuberculosis (TB). ■: TST ≥ 10 mm; ■: TST ≥ 15 mm; ■: QFG-IT; □: T-SPOT.TB. #: $p=0.012$ sensitivity of QuantiFERON-TB Gold In-tube (QFG-IT) system (Cellestis, Carnegie, Australia) and T-SPOT.TB (Oxford Immunotec, Abingdon, UK; 80% versus 58%) in culture-confirmed TB. b) Percentage of positive responses in TST and IGRAs in latent TB infection (LTBI) according to bacillus Calmette-Guérin (BCG) status. BCG vaccinated: $n=87$, TST cut-off ≥ 15 mm; not BCG vaccinated: $n=31$, TST cut-off >6 mm, in accordance with National Institute of Health and Clinical Excellence guidelines [4]. ■: TST; ■: QFG-IT; □: T-SPOT.TB.

sensitivities were 64% ($n=36$) versus 59% ($n=34$) and for combined definite and probable TB, sensitivities were 74% ($n=58$) and 75% ($n=56$) respectively.

Of the 25 children with culture-confirmed TB, 15 presented with PTB and 10 with EPTB. A TST ≥ 15 mm was present in 93% of children with PTB, but in only 60% in children with EPTB. The sensitivity of QFG-IT was unaffected by the manifestation of TB (80% in both PTB and EPTB), but sensitivity of the T-SPOT.TB in EPTB was lower than for PTB (40% versus 67%).

At the time of diagnosis, a TST of <6 mm was found in two (8%) out of 24, a negative QFG-IT in three (12%) out of 25 and a negative T-SPOT.TB in nine (39%) out of 23 of children with culture-confirmed TB.

Of those children with possible TB, 79% had a negative result in all three tests.

Results for children with LTBI

Of 118 children investigated for LTBI, the majority were BCG vaccinated (87 (74%) out of 118), as summarised in table 3. More children had a positive TST than IGRAs, but a record of BCG vaccination was not associated with a positive result in either the TST or IGRAs (TST ≥ 15 mm in 45.2% of BCG-vaccinated children versus TST ≥ 6 mm in 46.7% in BCG-unvaccinated children; positive QFG-IT of 32% in BCG-vaccinated children versus 32.2% in BCG-unvaccinated children; positive T-SPOT.TB in 29.4% of BCG-vaccinated children versus 35.5% in BCG-unvaccinated children; nonsignificant).

Concordance between all three tests

To measure the agreement between all tests in both active and latent TB, κ statistics were calculated for TST ≥ 15 mm versus both IGRAs as well as agreement between the two IGRAs.

The concordance between the two IGRAs was higher (88%, κ 0.75) than between IGRAs and TST (78.5% for QFG-IT versus TST, κ 0.57; and 73.5% for T-SPOT.TB versus TST, κ 0.46), as shown in table 4.

In active TB, overall agreement between IGRAs was good (82.7%, κ 0.66). However, the agreement between QFG-IT and T-SPOT.TB in children with culture-confirmed TB was poor (66.7%, κ 0.15). QFG-IT correctly identified a significantly larger number of children with definite TB ($p=0.012$) compared with T-SPOT.TB.

In LTBI, very good agreement was found between both IGRAs (92%, κ 0.82), but the agreement between TST and IGRAs was only moderate (TST versus T-SPOT.TB, κ 0.49; TST versus QFG-IT, κ 0.53).

Presence or absence of BCG vaccination did not significantly influence the proportion of positive tests (fig. 1b) or the agreement between the IGRAs (90% in BCG-vaccinated children versus 96% in BCG-unvaccinated children, nonsignificant), as shown in table 4.

Absolute values of TST and IGRAs

In order to assess whether absolute values of TST induration and/or production of IFN- γ in either IGRA could be used to distinguish between active and latent TB, all results were analysed according to their actual numeric values and not just according to positive or negative results. A significant difference in millimetres of induration was seen between both definite (median (range) 20 (6–35) mm) and probable TB (15 (6–14) mm; $p=0.018$) as well as between definite and latent TB (12.5 (0–41) mm; $p=0.0003$), as shown in figure 2a.

As summarised in figure 2b and c, there was no statistically significant difference between active and latent TB in absolute levels of secretion of IFN- γ or in numbers of SFCs, independent of the antigen investigated.

DISCUSSION

The present report is the first paediatric study to evaluate, side-by-side, the two commercially available IGRAs with TST, analysing for both active and latent TB in a large paediatric cohort, since the introduction of the new TB guidelines in the UK. Very good agreement was found between the IGRAs in

TABLE 4 Per cent concordance between tests and κ coefficients

Patient group	TST versus T-SPOT.TB		TST versus QFG-IT		QFG-IT versus T-SPOT.TB	
	Concordance %	κ (SEM)	Concordance %	κ (SEM)	Concordance %	κ (SEM)
All patients	73.5	0.46 (0.07)	78.5	0.57 (0.07)	88.0	0.75 (0.075)
Active TB[#]						
All	71.6	0.42 (0.11)	80.5	0.61 (0.11)	82.7	0.66 (0.11)
Definite	50.0	-0.17 (0.14)	86.4	0.49 (0.21)	66.7	0.15 (0.11)
Probable	79.4	0.59 (0.17)	75.0	0.5 (0.16)	87.5	0.74 (0.17)
Definite and probable	67.9	0.34 (0.13)	79.3	0.13 (0.13)	79.3	0.56 (0.13)
Possible	80.0	-0.11 (0.20)	83.3	-0.09 (0.20)	90.0	0.45 (0.21)
LTBI[†]						
All	75.0	0.49 (0.09)	77.0	0.53 (0.09)	92.0	0.82 (0.09)
BCG	74.0	0.48 (0.12)	76.0	0.51 (0.11)	90.0	0.78 (0.12)
No BCG	77.0	0.56 (0.16)	73.0	0.5 (0.16)	96.7	0.93 (0.18)

TST: tuberculin skin test; QFG-IT: QuantiFERON-TB Gold In-tube system (Cellestis, Carnegie, Australia); TB: tuberculosis; LTBI: latent TB infection; BCG: bacillus Calmette–Guérin. T-SPOT.TB is manufactured by Oxford Immunotec, Abingdon, UK. [#]: n=91; [†]: n=118.

the setting of LTBI, but low sensitivity of the T-SPOT.TB and, in particular, poor agreement between tests for the diagnosis of active TB were also found.

The diagnosis of active TB remains a challenge in paediatrics, as bacteriological confirmation is the exception rather than the rule, because of the paucibacillary nature of the disease and the relative difficulties in collecting adequate samples for microbiology [2]. Paediatricians around the globe will acknowledge that the majority of their patients are treated for TB in the absence of fulfilling the gold standard of bacteriological confirmation, and will instead receive a 6-month course of

TB treatment based on a combination of clinical signs and symptoms, suggestive radiology, history of household exposure or travel to TB-endemic countries, as well as the TST results [16]. Treatment is often justified, in retrospect, through the dramatic improvement of clinical symptoms.

In the present study, 91 children were enrolled and investigated for active TB, a third of which was culture confirmed. The calculated sensitivities for IGRAs and TST differ depending on whether only confirmed cases or children with probable TB were included, using a strict set of inclusion criteria. The current authors reasoned that it is important to assess a novel

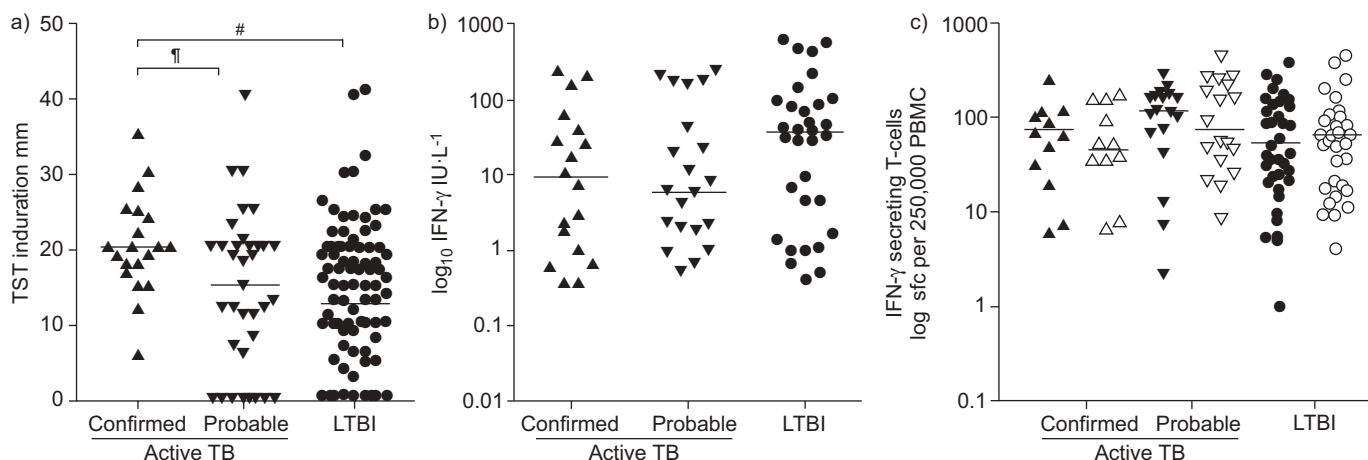


FIGURE 2. Absolute values of tuberculin skin test (TST) and interferon- γ release assays (IGRAs) in active tuberculosis (TB) and latent TB infection (LTBI). a) TST induration in children with either active TB (culture confirmed (n=20) or probable (n=35)) or LTBI (n=118). Horizontal lines indicate median values. #: p=0.0003; †: p=0.0188. b) Net concentration of interferon (IFN)- γ (antigen-stimulated level minus control level of IFN- γ) from positive QuantiFERON-TB Gold In-tube (Cellestis, Carnegie, Australia) assays performed on patients with either active TB (culture confirmed (n=18) or probable (n=22)) or LTBI (n=36). Horizontal lines indicate median values. There were no statistically significant differences between the groups. c) Spot-forming cells (sfc) per 250,000 IFN- γ producing T-cells in response to CFP-10 (closed symbols) and ESAT-6 (open symbols) antigens in T-SPOT.TB tests (Oxford Immunotec, Abingdon, UK) performed on patients with either active TB (culture confirmed (n=13) or probable disease (n=18)) or LTBI (n=34). Results were converted into a log₁₀ scale for ease of interpretation. There were no statistically significant differences between the groups. PBMC: peripheral blood mononuclear cell.

diagnostic test by not only using the gold standard of culture-confirmed TB, but also to assess its performance in the “real-life” situation of probable TB, since this evaluates the diagnostic potential of IGRAs more accurately in paediatric practice. As a result, a lower sensitivity of IGRAs in active TB may have been found compared with that reported from adult studies.

Young children are most vulnerable to developing active TB after a household contact, reflecting the immaturity of their immune responses. Primary TB elicits different immune responses from those seen in adults, in whom TB is usually caused by reactivation or reinfection [17]. As a consequence, TB-specific IFN- γ -producing T-cells might be present in lower numbers in children compared with adults [18–20], resulting in lower levels of IFN- γ , the target cytokine measured in IGRAs. As a consequence, assays relying on effector T-cell responses are more likely to be false-negative in young children compared with adults. In primary TB, antigen-specific effector cells need to be newly generated, while they simply need to be reactivated and undergo clonal expansion from the memory pool in reactivation or reinfection TB seen in adults. The limited findings of lower sensitivity of T-SPOT.TB in EPTB *versus* PTB support the hypothesis that initial immune responses might be compartmentalised at the site of disease [21] and sufficient numbers of antigen-specific effector T-cells still have to evolve in the peripheral circulation to be measurable in T-cell assays. The current authors have some evidence that an initially negative T-SPOT.TB can become positive during treatment, and longitudinal follow-up of paediatric patients is a part of ongoing studies (data not shown).

However, the relatively poor performance of IGRAs in accurately predicting active, culture-confirmed TB at the time of clinical presentation remains a particular concern. In the present study, the QFG-IT performed significantly better in culture-proven TB than the T-SPOT.TB (QFG-IT 80% *versus* T-SPOT.TB 58 %; $p=0.012$). Sensitivity for active TB in children was previously reported to be as high as 83% using the ELISPOT method and both recombinant protein as well as a range of overlapping peptides of ESAT-6 and CFP-10 [10]. A subsequent study by NICOL *et al.* [11] found the sensitivity to be 70% for clinical TB. Neither study used the commercially available T-SPOT.TB assay and both were carried out in the hospital setting in South Africa, where children are likely to present at a later stage of their disease compared with the European setting. The length of time to disease progression might influence the magnitude of measurable immune responses. Both studies compared ELISPOT with TST; QFG-IT was not included.

The presence of a third antigen (TB7.7) in addition to ESAT-6 and CFP-10 in the QFG-IT whole blood assay might have enhanced the sensitivity of this assay in comparison to T-SPOT.TB. Furthermore, additional factors, such as modulating cytokines, could play a role in whole blood assays. Such factors are no longer present when removing the plasma component for preparation of PBMC. Other groups have reported significant differences in cytokine production between whole blood assays and PBMC preparations [22, 23].

The TST was found to have better sensitivity than IGRAs for predicting definite TB and the data obtained by the current

authors provide no reason to abandon the TST as a diagnostic tool for active PTB in nonimmunocompromised children. Sensitivity is clearly increased when lower cut-off values are used for the TST, but this is at the expense of specificity, which is why it was decided to apply the most restrictive cut-off point of 15 mm to compare sensitivities and concordance.

It is well known that a TST can be negative in EPTB, and both TST and T-SPOT.TB lost sensitivity in the context of EPTB, unlike the QFG-IT assay, which was consistent in either manifestation of TB disease.

According to the present results a negative IGRA does not exclude active TB, and use of either IGRA cannot be recommended as a single tool to confirm or rule out active TB in children. However, as an additional test it can contribute value to the overall diagnostic process and the combination of TST and IGRA reached 100% in the definite case group. Furthermore, the current authors suggest that an initially negative IGRA should be repeated in cases of presumed active TB in which the clinicians felt certain enough to start the child on TB treatment. Several recent studies in adults have examined the predictive value of IGRAs in combination with the TST to exclude TB rather than to confirm it [24–26]. This analysis is difficult to conduct in paediatric populations, since there is significant overlap of symptoms with other childhood illnesses and the investigations of children do not routinely include bronchoscopy. Even in the best circumstances, bacteriological confirmation is the exception rather than the rule, and it is therefore extremely challenging to exclude TB, especially in a population with several risk factors, such as that described in the present report. Therefore, the current authors do not believe that it is possible to estimate the value of IGRAs and TST in excluding active TB in such a population.

The present data show that 79% of all children who were not commenced on TB therapy (possible TB cohort) by their paediatrician had a negative IGRA or TST, somewhat justifying the therapeutic decision. Conversely, 7% and 11% of children tested with QFG-IT and T-SPOT.TB, respectively, had a positive IGRA and were not treated. Given the high suspicion of TB in this group, the positive test may have indicated LTBI. Longitudinal follow-up studies of large cohorts are required to determine the positive predictive value of IGRAs in this circumstance. A recent publication by BAKIR *et al.* [27] showed that children with a positive IGRA had a 3- to 4-fold increased risk of progression to active TB, but the study could not detect a significant difference in the positive predictive value between TST and IGRAs, due to the limited numbers of children developing active TB.

In the present cohort, as well as in other published studies [5, 28, 29], significantly fewer children were found positive on either IGRA compared with the TST. Applying the results of IGRAs rather than the TST to guide prophylactic treatment, as recommended by the NICE guidelines, would have led to a reduction of chemoprophylactic therapy by ~30% in the present study cohort. However, there are no data assessing the incidence of active TB in children following a contact with smear-positive TB, who have a negative IGRA and do not receive chemoprophylaxis. To establish the negative predictive value of IGRAs in LTBI in children will require national and

international collaborative prospective studies in settings of low and high endemicity.

Interestingly, a higher proportion of positive TST was not seen in those children who had received BCG vaccination, even accounting for different cut-offs according to national guidelines. Although somewhat contradictory to reports from adult studies [6, 30], the same has been reported from other paediatric cohorts in TB endemic countries [31, 32], in which it was concluded that BCG vaccination was not an important cause of false-positive TST, except in communities with low TB prevalence. The findings of the present study are consistent with these observations.

In LTBI, the agreement between both IGRAs was very good (κ 0.82) and one IGRA cannot be recommended over the other in this context. The findings mirror the recently published work by CONNELL *et al.* [3], which showed a concordance of 93% between QFG-IT and T-SPOT.TB in LTBI in children and a κ of 0.83, compared with a concordance of 92% and a κ of 0.82 in the present study.

According to the present data, absolute values of either IGRA do not distinguish active from latent TB, in line with the published literature [11]. Although significant differences in induration between children with active and latent TB using TST were described, the considerable overlap and the lack of a defined cut-off between the groups makes it impossible for clinicians to distinguish the conditions *via* TST, which has been a long standing observation.

Although a sizeable population of children was enrolled, it could not be determined if IGRAs perform reliably in all age groups. The performance of IGRAs in very young children (aged <2 yrs) remains a concern and multicentre data sets are required to answer this question conclusively. The present study did not detect a significant difference in the age of children who failed or had an indeterminate IGRA result with either method; the percentage of indeterminate or failed IGRAs in the study is comparable to other published work [28, 33, 34].

In conclusion, a negative IGRA should not dissuade paediatricians from diagnosing and treating active TB. In the context of screening for LTBI, the use of IGRAs in place of TST would significantly reduce the number of children receiving chemoprophylaxis with good concordance between both assays.

However, to date there have been no prospective studies determining rates of active tuberculosis in children presenting with a negative interferon- γ release assay at the time of contact screening, who as a consequence do not receive chemoprophylaxis. Many paediatricians will be reluctant to leave these children untreated, especially children aged <2 yrs.

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