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Diagnosing TB infection in children: analysis of discordances using *in vitro* tests and the tuberculin skin test

N. Altet-Gómez*,#, M. De Souza-Galvao*,#,¶, I. Latorre¶,+, C. Milà*,#, M.A. Jiménez*,#, J. Solsona*,#, A. Cantos*,#, J.J. Zamora#, J. Ruiz-Manzano¶,§, V. Ausina¶,+ and J. Domínguez¶,+

ABSTRACT: The aim of the present study was to compare the performance of the interferon (IFN)-γ tests (QuantiFERON®-TB Gold In-Tube (QFT-G-IT) and T-SPOT®.*TB*) with the tuberculin skin test (TST) in diagnosing tuberculosis (TB) infection in children, and to analyse discordant results.

This was a prospective study including 98 children from contact-tracing studies and 68 children with TST indurations ⩾5 mm recruited during public health screenings.

Positive IFN-γ tests results were associated with risk of exposure ($p<0.0001$). T-SPOT.*TB* was positive in 11 (78.6%) out of 14 cases with active TB and QFT-G-IT in nine (64.3%) out of 14 cases. Sensitised T-cells against *Mycobacterium avium* were detected in six out of 12 children not vaccinated with bacille Calmette-Guérin (BCG), a TST induration 5–9 mm in diameter and both IFN-γ tests negative. In concordant IFN-γ tests results, a positive correlation was found ($p=0.0001$) between the number of responding cells and the amount of IFN-γ released. However, in discordant IFN-γ tests results this correlation was negative ($p=0.371$): an increase in the number of spot-forming cells correlated with a decrease in the amount of IFN-γ released.

The use of IFN-γ tests is helpful for the diagnosis of TB infection, avoiding cross-reactions with BCG immunisation and nontuberculous mycobacterial infections. The analysis of highly discordant results requires further investigation to elucidate possible clinical implications.

KEYWORDS: Agreement, children, interferon-γ release assays, nontuberculous mycobacterial sensitins, tuberculin skin test, tuberculosis

In 2007, the estimated global incidence of tuberculosis (TB) cases was 9.27 million. Approximately 11% of these cases were children. In the developed world, the estimated proportion of children with TB is around 3–6%, but in developing countries this percentage can reach 15–20%, with an approximate mortality of 30% [1]. Latent TB infection (LTBI) treatment is an essential strategy to eliminate TB [2], although in order to achieve any epidemiological impact, this strategy must target groups with a high risk of infection and development of the disease if they become infected. Children merit special consideration, as they can develop the disease very quickly after primary infection, with the most severe forms prevailing in younger children [3].

The advantages of techniques based on the detection of interferon (IFN)-γ secreted by effector T-cells stimulated with specific *Mycobacterium tuberculosis* antigens to diagnose LTBI over the tuberculin skin test (TST) are the lack of cross-reactivity with vaccinal *Mycobacterium bovis* bacille Calmette–Guérin (BCG) strains and nontuberculous mycobacteria (NTM), and the absence of booster effect [4, 5]. These antigens are the early secretory antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10 encoded in region of difference (RD)1, and TB7.7 encoded in RD11, which are absent in all BCG strains and in the majority of NTM. Two commercial *in vitro* assays based on this technology are currently available: QuantiFERON®-TB GOLD In-Tube (QFT-G-IT; Cellestis, Carnegie, Australia) and T-SPOT®.*TB* (Oxford Immunotec, Oxford, UK). Studies in adults have shown these tests to have a high sensitivity and specificity for TB diagnosis [5–8]. In a recent systematic review [5], using active TB as a surrogate for LTBI, sensitivities values were 70% (95% CI 63–78%) for QFT-G-IT and 90% (95% CI 86–92%) for T-SPOT.*TB*, and specificity values were 96% (95% CI 94–98%) for QFT-G-IT and 93% (95% CI 86–100%) for T-SPOT.*TB*.

AFFILIATIONS
*Unitat de Prevenció i Control de la Tuberculosi de Barcelona, Institut Català de la Salut,
#Red de Investigación en Atención Primaria (REDIAP),
¶Universitat Autònoma de Barcelona,
+Servei de Microbiologia, and
§Servei de Pneumologia, Hospital Universitari Germans Trias i Pujol, Fundació Institut d'Investigació en Ciències de la Salut ''Germans Trias i Pujol'', CIBER Enfermedades Respiratorias, Badalona, Barcelona, Spain.

CORRESPONDENCE
N. Altet-Gómez
Unitat Clínica de Prevenció i Control de la Tuberculosi de Barcelona
CAP Drassanes
Av Drassanes 17–21
08001 Barcelona
Spain
E-mail: naltet.bcn.ics@gencat.cat

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The objectives of this study were: 1) to compare QFT-G-IT and T-SPOT.*TB* results with those obtained by TST for the diagnosis of TB infection in children in a referral clinical centre for TB control; and 2) to analyse their concordant or discordant results.

MATERIALS AND METHODS

Study design

This was a prospective study in children ($\leqslant$15 yrs of age) who attended the Unitat Clínica de Prevenció i Control de la Tuberculosi, Barcelona, Spain between September 2005 and September 2007. This study was approved by the Ethics Committees of Fundació Jordi Gol i Gurina and of the Hospital Universitari Germans Trias i Pujol, Barcelona. Parents were asked to sign an informed consent form.

Children were divided in two groups: a contact group (CG) including children studied due to a close contact with a smear-positive active TB patient diagnosed within the previous 15 days and a screening group (SG) consisting of healthy children with a positive TST result detected during an epidemiological screening at school or by their paediatrician.

Data were collected by means of a structured interview. A clinical examination, TST, chest radiography and both IFN-γ tests were performed. The presence of a BCG scar was recorded. Children were excluded if they had a previous history of any TB treatment.

The risk of infection was classified into three groups as follows. 1) ''High'' risk was defined as living in the same household as the contagious index case or a different household but with a daily contact of $\geqslant$6 h with the index case. 2) ''Medium'' risk was defined as nondaily contact with the contagious index case, at least once weekly. 3) ''No risk known'' included children from the SG without any TB index case known.

A blood draw was performed $\leqslant$5 days after the TST. The study was double-blinded: the clinical diagnosis of TB was made without knowing the IFN-γ test results and the researchers in the laboratory did not see the clinical data prior to the performance of the tests.

TST

The TST was performed with 2 U purified protein derivative RT23 [1]. TST was considered positive when the induration was $\geqslant$5 mm in contacts and in children with abnormal chest radiographs consistent with active TB, and $\geqslant$10 mm for children in the SG, irrespective of BCG immunisation.

Active TB diagnosis

We followed national guidelines for the diagnosis of the active TB cases [9, 10]. A TB case was considered to be a child with *M. tuberculosis* isolated from clinical specimens, or with the presence of symptoms, signs and/or radiological images compatible with TB (when chest radiography was doubtful, thoracic computed tomography was performed), and/or a positive TST (as defined previously), and who responded clinically to antituberculous chemotherapy. Close contact with a bacillary TB case was used as a diagnostic support.

T-SPOT.TB

Specific peripheral blood mononuclear cells (PBMCs) were stimulated with ESAT-6 and CFP-10 separately, following the manufacturer's recommendations. Positive, negative and indeterminate results were strictly interpreted according to the manufacturer's instructions. Nonstimulated cells were washed with RPMI medium (Invitrogen, Auckland, New Zealand) and resuspended in freezing medium (80% RPMI and 20% fetal bovine serum; PAA Laboratories GmbH, Pashing, Austria) adding dimethylsulfoxide (Merck, Darmstadt, Germany) dropwise to a final concentration of 10%, and then frozen at -80°C. We considered the sum of spot-forming cells (SFCs) obtained after ESAT-6 and CFP-10 stimulation as an overall RD1 response [11].

Ex vivo *detection of T-cells sensitised against* M. avium *sensitin*

In order to investigate the influence of NTM infections on non-BCG-vaccinated children with a TST induration 5–9 mm in diameter and both IFN-γ tests negative, we performed an *ex vivo* ELISPOT, stimulating the cells with *M. avium* sensitin. Cells were thawed and resuspended in RPMI medium. Then, cells were washed, resuspended and stimulated with medium alone, phytohaemagglutinin (PHA) or *M. avium* sensitin (10 $\mu g \cdot mL^{-1}$) (Statens Serum Institut, Copenhagen, Denmark) as previously described [12]. Sensitised cells were detected by ELISPOT. The interpretation of the results followed the same criteria as that for detecting ESAT-6 and CFP-10 immunoresponse.

QFT-G-IT

The QFT-G-IT test detects IFN-γ released from T-cells stimulated with the specific antigens in whole blood. QFT-G-IT incorporates specific antigens (ESAT-6, CFP-10 and TB7.7) inside the same blood collection tube. The test was performed and the results were interpreted according to the manufacturer's instructions.

Statistical methods

Qualitative data are presented as n (%) and quantitative data are presented as mean$\pm$SD. The Chi-squared test and two-tailed Fisher's exact test were used to compare qualitative variables. Odds ratios and 95% confidence intervals were calculated. The associated variables with a p-value <0.05 were analysed at a multivariate level by means of logistic regression. Nonparametric tests (Mann–Whitney, Kolmogorov–Smirnov and Kruskal–Wallis) were used to compare quantitative variables according to the categories of the group variable. Graphical analysis and Pearson correlation techniques (CC) were used to study the association. Cohen's κ coefficient was used to analyse the concordance, and its p-value and standard error (according to Landis and Cock estimation). The area under the receiver operating characteristic curve was calculated to compare the diagnostic performance of the TST, T-SPOT.*TB* and QFT-G-IT in the diagnosis of active TB. The data were analysed using SPSS (version 14.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical performance

A total of 166 children were included in the study, of whom 84 (50.6%) were female. The mean$\pm$SD age was 9.08$\pm$4.85 yrs. 98 (59%) subjects were contacts and 68 (41%) belonged to the SG.

149 (89.8%) children were TST-positive. This high percentage of TST-positive results is due to the fact that all children included in the SG group were TST-positive. The IFN-γ tests (either one or both) were positive in 72 (43.4%, 95% CI 35.7–51.3%) children: 54 (55.1%, 95% CI 44.7–65.2%) contacts and 18 (26.5%, 95% CI 16.5–38.6%) from the SG. T-SPOT.*TB* was positive in 64 (38.6%, 95% CI 31.1–46.4%) children and QFT-G-IT in 61 (36.7%, 95% CI 29.4–44.6) children (table 1). Treatment of LTBI was considered according to the TST result; consequently, children who had positive TST and negative IFN-γ tests were treated, and conventional follow-up and control was performed.

All children considered non-TB-infected according to the TST result obtained a negative IFN-γ-based test result. Of the 20 non-BCG-vaccinated children from the SG, both IFN-γ tests were negative in 14 children with a TST induration 5–9 mm in diameter. There were 48 BCG-vaccinated children in the SG: T-SPOT.*TB* was positive in 11 (22.9%) out of 48 and QFT-G-IT was positive in nine (18.75%) out of 48. In the three BCG-vaccinated children from the SG with TST induration of 5–9 mm, both IFN-γ tests were negative. Therefore, in the 45 children who had a positive TST (induration $\geqslant$10 mm), T-SPOT.*TB* was positive in 11 (24.4%) out of 45 subjects and QFT-G-IT was positive in nine (20%) out of 45 subjects. Distribution of IFN-γ tests and TST results according to BCG- and non-BCG-vaccinated status, and CG and SG are shown in figures 1 and 2. No indeterminate results were detected by QFT-G-IT, but by T-SPOT.*TB*, in three (1.8%) cases, the test failed because the blood volume drawn was insufficient.

IFN-γ tests were in agreement in 146 out of 166 children (table 2). None of the variables that might have influenced the level of concordance between both tests was significantly associated with the outcome. There were no significant differences in age, sex or study group between the children with concordant and discordant IFN-γ results (data not shown). IFN-γ tests were discordant in 20 (12.04%) children. The three (3.06%) failed cases in the T-SPOT.*TB* belonged to the CG and, among them, there was a 3-yr-old patient with active TB. The overall agreement was 89.6% (κ=0.778) after excluding the failed cases.

Variables related to the positivity of IFN-γ tests

Variables significantly associated with IFN-γ test positivity are shown in table 1. In the multivariate analysis, a positive T-SPOT.*TB* was associated with being a contact ($p<0.001$) and having an abnormal chest radiogram, and a positive QFT-G-IT was associated with being a contact ($p<0.001$) and not being BCG-vaccinated ($p=0.01$) (table 1).

In table 3, the positivity of the IFN-γ tests according to the risk of exposure to an infectious source is shown. The probability of a positive IFN-γ test (OR 3.60, 95% CI 1.85–7.04) was significantly associated with an increasing risk of exposure independent of age and sex in the multivariate analysis ($p<0.001$). In addition, in the multivariate analysis, the main factors associated with a positive T-SPOT.*TB* in the CG were a daily contact >6 h (OR 3.5, 95% CI 1.1–12.1; $p=0.03$) and an exposure time >30 days (OR 1.9, 95% CI 1.1–6.9; $p=0.04$). However, no significant associations were found for the QFT-G-IT.

Clinical performance of the IFN-γ tests in active primary TB

14 cases were finally classified as active primary TB. In four cases, a microbiological confirmation was possible (positive culture for *M. tuberculosis*: three gastric aspirates samples and one sputum sample). In eight cases, the children were from the CG and in six cases, they were from the SG. T-SPOT.*TB* was positive in 11 (78.6%) out of 14 cases and the QFT-G-IT in nine (64.3%) out of 14 cases. Both IFN-γ tests were positive in eight (57.1%) children and negative in two (21.4%) cases. For one patient, the T-SPOT.*TB* failed and the QFT-G-IT was positive, and three patients had a negative QFT-G-IT and a positive T-SPOT.*TB*. However, the differences in the number of

TABLE 1 Variables associated with a positive interferon-γ test result: bivariate and multivariate analysis in the 166 children included in the study

Variable	Total	T-SPOT.*TB*#					QFT-G-IT#				
		Positive	Unadjusted		Adjusted		Positive	Unadjusted		Adjusted	
			OR¶ (95% CI)	p-value	OR (95% CI)	p-value		OR (95% CI)	p-value	OR (95% CI)	p-value
Initial inclusion group											
SG	68 (41.0)	16 (23.5)	1		1		14 (20.6)	1		1	
CG	98 (59.0)	48 (49.0)	3.3 (1.7–6.6)	<0.001	7.2 (3.1–16.5)	<0.001	47 (48.0)	3.6 (1.7–7.6)	<0.001	6.2 (2.8–13.5)	<0.001
BCG immunisation											
Yes	116 (69.9)	39 (33.6)	1		1		36 (31.0)	1		1	
No	50 (30.1)	25 (50.0)	2.0 (1.01–3.9)	0.049	1.3 (0.3–5.3)	0.662	25 (50.0)	2.2 (1.1–4.4)	0.021	3.03 (1.3–7.03)	0.01
Chest radiography											
Normal	152 (91.6)	53 (34.9)	1		1		52 (34.2)	1		1	
Compatible with TB	14 (8.4)	11 (78.6)	10.1 (2.15–47.1)	0.003	12.3 (2.1–70.6)	0.005	9 (64.3)	3.5 (1.1–10.9)	0.033	2.4 (0.6–9.5)	0.217

Data are presented as n (%), unless otherwise stated. QFT-G-IT: QuantiFERON®-TB Gold In-Tube test; SG: screening group; CG: contact group; BCG: bacille Calmette–Guérin; TB: tuberculosis. #: n=166. ¶: for the positivity threshold of tuberculin skin test $\geqslant$5 mm.

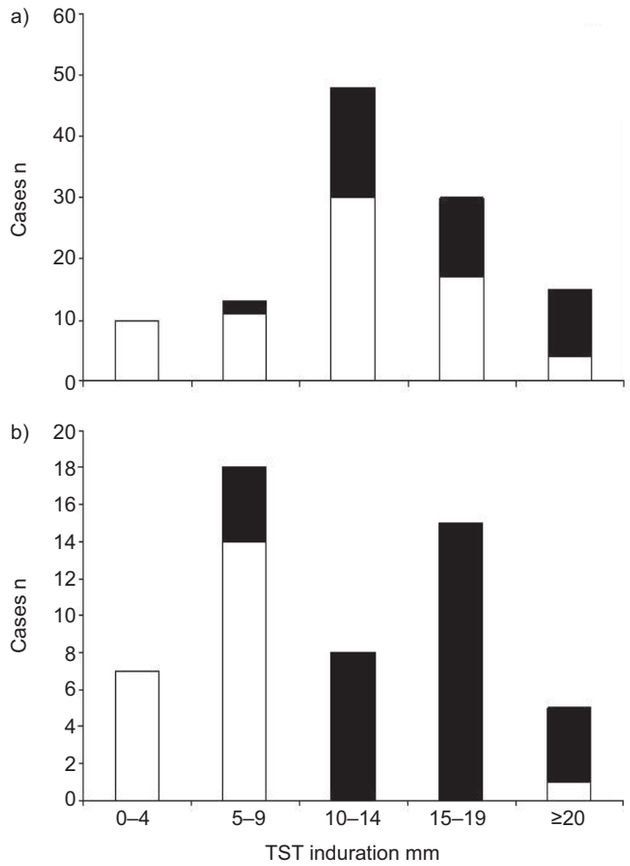

FIGURE 1. Interferon-γ tests result distribution (■: positive; □: negative) according to the tuberculin skin test (TST) induration in a) bacille Calmette–Guérin (BCG) and b) non-BCG-vaccinated children from the contact and screening groups.

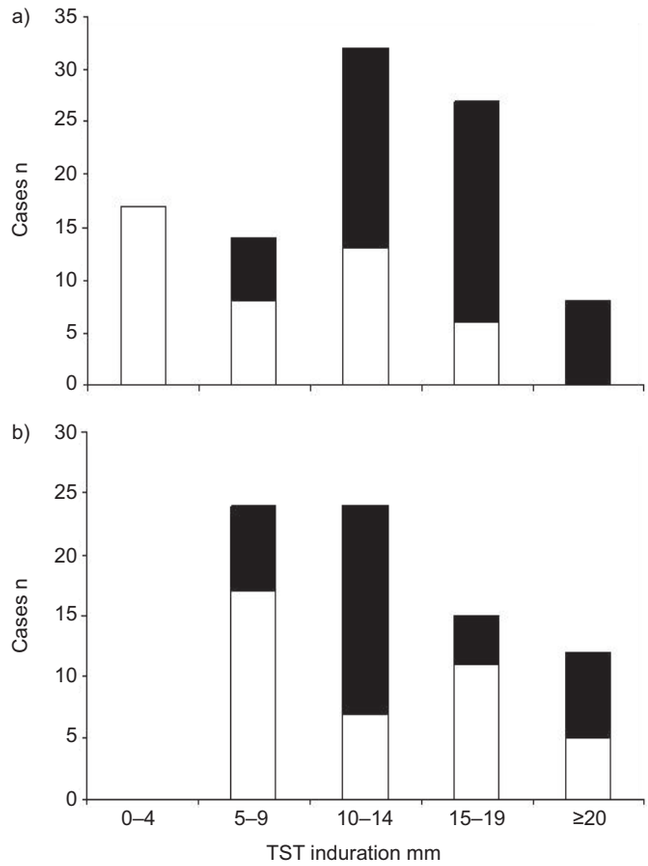

FIGURE 2. Interferon-γ test results distribution (■: positive; □: negative) according to the tuberculin skin test (TST) induration in the a) contact and b) screening groups, including both bacille Calmette–Guérin (BCG) and non-BCG-vaccinated children.

responding T-cells after stimulation with the specific antigens in the comparison between children diagnosed with active TB and all children without disease was significant ($p=0.01$ for ESAT-6 and CFP-10, respectively, and $p=0.009$ for RD1), but differences in the IFN-γ released did not reach statistical significance ($p=0.09$). However, if we exclude from the analysis children who were not diagnosed with LTBI by IFN-γ tests (both T-SPOT.*TB*- and QFT-G-IT-negative), then there are no statistical significant differences in the number of responding T-cells and the amount of IFN-γ released after antigen stimulation between active and LTBI children (table 4).

If we consider children diagnosed with active TB as truly infected, and children from the contact group with a tuberculin skin test (TST) induration <5 mm in diameters and children from the screening group with a TST <10 mm as truly not infected, then we could assume that the sensitivity and specificity of the IFN-γ tests is 78.57% (11 out of 14), and 100% (35 out of 35), respectively.

Agreement between IFN-γ tests and TST

The agreement between the TST and IFN-γ tests is high in non-BCG-vaccinated children (table 5). Variables associated with discordant results between TST and IFN-γ tests in multivariate analysis were: belonging to the SG (adjusted OR 6.9, 95% CI 3.4–14.4; $p<0.001$), being vaccinated with BCG (adjusted OR 10.1, 95% CI 3.3–30.9; $p<0.001$) and a TST with induration 5–9 mm in diameter (adjusted OR 10.4, 95% CI 3.5–31.1; $p<0.001$).

Among the 17 autochthonous children from Spain who were non-BCG-vaccinated with a TST result of 5–9 mm induration and negative IFN-γ tests, the detection of sensitised T-cells against *M. avium* sensitin was performed in 12 cases. In three cases, the test failed due not having a sufficient number of cells recovered. It was negative in three cases and in the remaining six, it was positive.

Relationship between number of sensitised T-cells and the amount of IFN-γ released

When both IFN-γ tests agreed, high SFC counts by T-SPOT.*TB* also showed high amounts of released IFN-γ (measured by QFT-G-IT). However, this correlation is negative in those children with a discordant result, where an increase in SFCs correlates with a decrease in IFN-γ released. In this case, the amount of IFN-γ tends to plateau. At this point, few cells produce high quantities of IFN-γ (negative T-SPOT.*TB* and

TABLE 2 Concordance and agreement (Cohen's κ coefficient) between the interferon (IFN)-γ tests results for the different groups of children according to their bacille Calmette–Guérin (BCG) immunisation status

IFN-γ results and agreements	Initial inclusion group				Total
	Contact group		Screening group		
	BCG	No BCG	BCG	No BCG	
Subjects n	68	30	48	20	166
Negative T-SPOT.*TB* and negative QFT-G-IT	36 (52.9)	7 (23.3)	35 (72.9)	15 (75.0)	93 (56.0)
Positive T-SPOT.*TB* and positive QFT-G-IT	24 (35.3)	17 (56.7)	7 (14.6)	5 (25.0)	53 (31.9)
Negative T-SPOT.*TB* and positive QFT-G-IT	2 (2.9)	2 (6.7)	2 (4.2)	0	6 (3.6)
Positive T-SPOT.*TB* and negative QFT-G-IT	4 (5.9)	3 (10.0)	4 (8.3)	0	11 (6.6)
Failed T-SPOT.*TB* and positive QFT-G-IT	1 (1.5)	1 (3.3)	0	0	2 (1.2)
Failed T-SPOT.*TB* and negative QFT-G-IT	1 (1.5)	0	0	0	1 (0.6)
Patients with concordant results n/N (%)	60/68 (88.2)	24/30 (80.0)	42/48 (87.5)	20/20 (100)	146/166 (88.6)
Cohen's κ coefficient	0.765	0.561	0.622	1	0.750
Excluding failed results					
Patients with concordant results n/N (%)	60/66 (90.9)	24/29 (82.8)	42/48 (87.5)	20/20 (100)	146/163 (89.6)
Cohen's κ coefficient	0.810	0.609	0.622	1	0.778

Data are presented as n (%), unless otherwise stated. QFT-G-IT: QuantiFERON®-TB Gold In-Tube assay.

positive QFT-G-IT), whereas the total amount of IFN-γ decreases or remains constant despite an increase in the SFCs (positive T-SPOT.*TB* and negative QFT-G-IT) (fig. 3).

However, as the diameter of the TST induration increases there is an increase in the SFCs (CC 0.09; $p<0.0001$) and in the total amount of IFN-γ released (CC 0.03; $p<0.01$); similarly, as the number of SFCs increases there is also an increase in the IFN-γ released (CC 0.27; $p<0.0001$). However, the correlation between TST induration, and the SFCs and the IFN-γ released varies depending on whether the IFN-γ tests agree or not. When both IFN-γ tests agree, as the diameter of the TST induration increases, there is an increase of responding SFCs (CC 0.315; $p<0.0001$), the regression line slope being 2.986 ($p<0.0001$); and there is also an increase of the IFN-γ released (CC 0.167; $p=0.045$), with a regression line slope of 0.343 ($p=0.046$). When there is no agreement between the IFN-γ tests, there is no correlation between the TST and the SFCs produced (CC 0.065; $p=0.786$), the slope of the line being almost null (0.189; $p=0.910$); nor is there a correlation with the amount of IFN-γ produced (CC 0.362; $p=0.117$), the slope of the regression line being 0.298 ($p=0.069$).

DISCUSSION

This study shows the results of IFN-γ test measurements in children seen in a reference centre for the diagnosis of TB infection, and compares the techniques currently available. Although the specificity for active TB for both tests was 100%, T-SPOT.*TB* obtained more positive results than QFT-G-IT in all groups analysed.

Our results highlight the usefulness of the IFN-γ tests compared with the TST in the diagnosis of LTBI in contacts, as an association was found with the increase in the risk of infection and the exposure. These data agree with findings in other studies that have investigated TB outbreaks and study contacts [4, 11, 13–18]. These results also show the usefulness of IFN-γ tests to diagnose LTBI in BCG-vaccinated children when they are screened as part of paediatric or epidemiological control.

We have found that both IFN-γ tests show sensitivity >75% and specificity of 100% for the diagnosis of active TB. LIEBESCHUETZ *et al.* [19] reported a sensitivity of 83% for T-SPOT.*TB* in African children. NICOL *et al.* [20] described T-SPOT.*TB* positive results in 70% of children with clinical TB.

TABLE 3 Interferon (IFN)-γ test results according to the risk of exposure to *Mycobacterium tuberculosis*

	Children n	Positive TST[#]	Positive IFN-γ tests[¶]	Unadjusted OR (95% CI)	p-value	Adjusted[+] OR (95% CI)	p-value
No risk known	68	51 (75)	18 (35.3)	1		1	
Medium risk	33	29 (87.9)	18 (62.1)	3.00 (1.06–8.64)	0.037	2.88 (1.22–6.80)	0.016
High risk	65	52 (80)	36 (69.2)	4.13 (1.68–10.27)	0.001	4.29 (2.01–9.18)	<0.001

Data are presented as n (%), unless otherwise stated. TST: tuberculin skin test. [#]: TST ⩾5 mm in the contact group and ⩾10 mm in the screening group was considered positive; [¶]: positive result of one or both IFN-γ tests; [+]: for age and sex.

TABLE 4 Number of spot forming cells (SFCs) after stimulation with early secretory antigenic target (ESAT)-6, culture filtrate protein (CFP)-10 and region of difference (RD)1 antigens, and the amount of interferon (IFN)-γ released measured by T-SPOT®.*TB* and QuantiFERON®-TB Gold In-Tube (QFT-G-IT) assays in children diagnosed with active tuberculosis (TB) and latent TB infection (LTBI) (in both cases, either or both of the *in vitro* tests were positive)

IFN-γ tests	Active TB		LTBI		p-value
	Cases	SFCs or IFN-γ	Cases	SFCs or IFN-γ	
QFT-G-IT	9	2.09 (0.23–13.23)	52	3.26 (0.65–9.57)	0.52
T-SPOT.*TB*					
ESAT-6	11	26.00 (5.00–69.00)	53	15.00 (6.00–40.00)	0.50
CFP-10	11	32.00 (18.00–75.00)	53	19.00 (7.00–70.00)	0.60
RD1	11	79.00 (37.00–137.00)	53	49.00 (15.00–125.00)	0.31

Data are presented as n or median (25–75th percentiles), unless otherwise stated.

DETJEN *et al.* [21] found a sensitivity of 93% for both IFN-γ tests when evaluating children with active TB. In addition, CONNELL *et al.* [22] also reported positive IFN-γ tests in the nine children diagnosed with active TB. In contrast with our results, KAMPMANN *et al.* [23] found better results for QFT-G-IT than for T-SPOT.*TB* in children with culture-confirmed TB. Even if IFN-γ tests have been developed to diagnose LTBI, an alternative approach to the evaluation of the sensitivity of the *in vitro* tests has been to test patients with active TB. Although patients with active TB are, by definition, infected with *M. tuberculosis*, they do not have a LTBI. In fact, active TB occurs when the host immune responses are unable to contain the latent infection. Therefore, it should be considered that the value of the IFN-γ assays in active TB diagnosis is limited. False negative results of both tests in active TB have been described previously [6, 24, 25]. In addition, it has been reported that young children with severe active TB can have a reduced number of lymphocytes or a reduced lymphocyte function that could affect the sensitivity of the IFN-γ tests. In fact, in our study, in six children aged <3 yrs, the T-SPOT.*TB* was negative in three cases, failed in two and was positive in only one case, and the QFT-G-IT was negative in three cases and positive in the remaining three cases. However, no very severe TB presentation was diagnosed in children with both IFN-γ tests negative. Other factors also involved could be the release of anti-inflammatory cytokines by PBMCs and the temporary depression of T-cell responsiveness [26, 27].

TABLE 5 Concordance and agreement (Cohen's κ coefficient) between the tuberculin skin test (TST), and T-SPOT®.*TB* and QuantiFERON®-TB Gold In-Tube (QFT-G-IT) assay results according to bacille Calmette–Guérin (BCG) immunisation status

BCG status	Tests compared	κ ± SD	p-value
BCG-immunised	TST and QFT-G-IT	0.087 ± 0.155	0.0048
	TST and T-SPOT.*TB*	0.095 ± 0.151	0.0032
Not BCG-immunised	TST and QFT-G-IT	0.844 ± 0.105	<0.0001
	TST and T-SPOT.*TB*	0.887 ± 0.062	<0.0001
Total	TST and QFT-G-IT	0.208 ± 0.111	<0.0001
	TST and T-SPOT.*TB*	0.272 ± 0.092	<0.0001

However, we have observed in our study that the IFN-γ assays are not able to distinguish between LTBI and active TB. No significant differences were detected between infected and diseased children in the number of responding T-cells and the amount of IFN-γ released after antigen stimulation. The absence of significant differences in the response between active TB and LTBI could be explained by the fact that paediatric infection is usually recent. Therefore, the response is still strong, being similar to the one obtained during active TB [28].

Indeterminate results can be due to different causes, though they are generally due to a failure of the positive control. These results have been associated with immunosuppression, young age (<5 yrs) and a negative TST [14, 19, 20, 29]. Interestingly, new information from different studies suggests that the increased frequency of indeterminate results in young children reflects a performance characteristic of the *in vitro* tests rather than a responding impairment to specific antigens and PHA [17, 30, 31]. An important source of failed results in young children has been related to an inadequate PBMC separation as a consequence of insufficient blood taken (especially in very young children) [13, 17, 23], which was the case in the three children who had a failed T-SPOT.*TB* result in our study. From our point of view, this kind of result should be considered, as in children (where blood drawn is not always easy), these problems are inherent to the *in vitro* tests. However, given that in the QFT-G-IT assays, no T-cell count is required, we can not assess the impact of this kind of failure in the performance of the test.

It is difficult to compare the agreement between IFN-γ tests and TST with the results obtained by other authors because in each case, the positivity cut-off needs to be taken into account. In published studies, this threshold can vary greatly, from 5, 10 and up to 15 mm of induration as indicative of TB infection [15, 17, 19, 20, 29], and generally depends on population groups (contacts and level of risk of development of active TB), and specific guidelines of the country.

The variables associated with discordance between the TST and IFN-γ test measurements were BCG immunisation, belonging

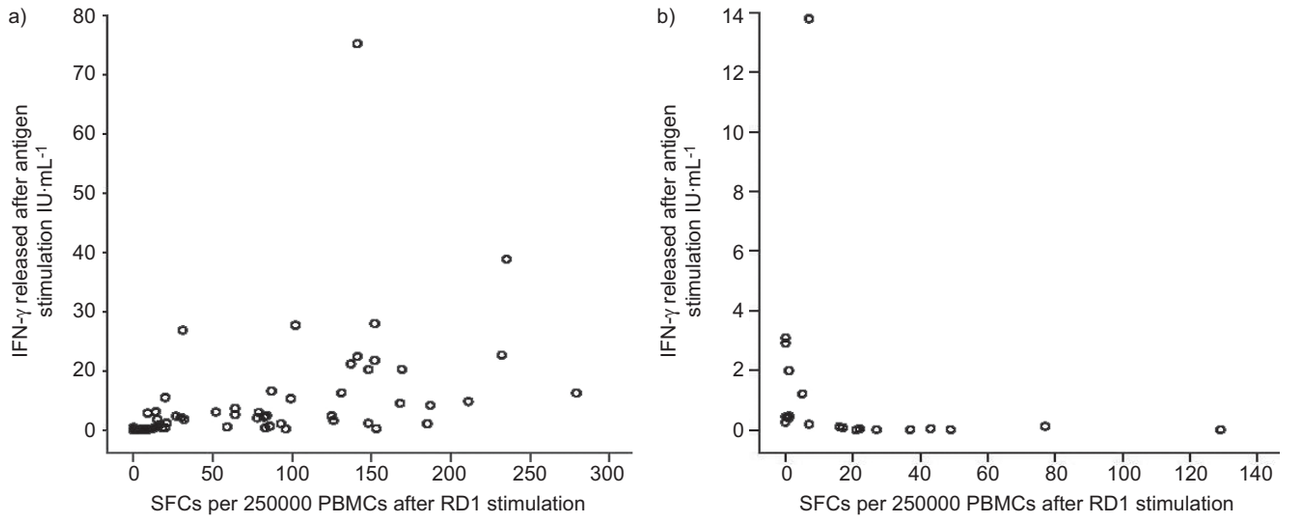

FIGURE 3. Correlation between the number of spot forming cells (SFCs) after stimulation with specific *Mycobacterium tuberculosis* antigens and the amount of interferon (IFN)-γ released in children with a) concordant and b) discordant results between the T-SPOT®.*TB* and QuantiFERON®-TB Gold In-Tube assays. The region of difference (RD)1 stimulation is the sum of SFCs obtained after early secretory antigenic target-6 and culture filtrate protein-10 stimulation. In children with a) concordant results, the Pearson's correlation coefficient was 0.530 (p=0.0001). In children with b) discordant results, the Pearson's correlation coefficient was -0.212 (p=0.371). PBMC: peripheral blood mononuclear cell.

to the SG and a TST induration of 5–9 mm. In the SG, an induration ⩽10 mm is most likely caused by a NTM (nonspecific sensitisation). In fact, in our study, we detected T-cell sensitisation against *M. avium* sensitins in six (66.7%) out of nine of these children. The existence of NTM in Spain was shown by BLEIKER [32] and, recently, our group has described its presence in Catalonia [33]. Also, DETJEN *et al.* [21] showed the specificity of the IFN-γ tests in infections caused by NTM and other authors have also described low agreement between IFN-γ tests and positive TST [7, 34]. Our group, in a previous publication, reported that 10 (47.6%) out of 21 children with TST-positive and -negative T-SPOT.*TB* had sensitised T-cells against *M. avium* sensitins [12]. Given that *M. avium* sensitin is not totally specific, we cannot totally exclude the possibility that we are detecting, in some cases, a response of specific T-cells against some *M. tuberculosis* antigens different from ESAT-6, CFP-10 and TB7.7. In order to reduce this possibility we have focused our study on unexposed children with 5 to 9 mm of TST induration. Based in the classical studies performed by NYBOE *et al.* [35], the main guidelines in screening children population consider as a cut-off for *M. tuberculosis* infection a TST induration ⩾10 mm, in order to avoid false positive TST results induced by NTM immunisation [36]. Therefore, our results, in part, reinforce the guidelines [10], in that unnecessary chemoprophylaxis treatment in unexposed population could be avoided, and that IFN-γ based assays could help to confirm a positive TST result. Nevertheless, indurations >15 mm [21] and >20 mm [37] have been reported in children with NTM infections.

The main limitation of our study is that 89.75% of patients included had a positive TST (*i.e.* all children from the SG). This fact could introduce a bias in the comparison between the TST and IFN-γ tests due to the low number of negative TST results. Nevertheless, despite this limitation, the results obtained are sufficiently consistent to draw some conclusions about their utility in the diagnosis of LTBI in a referral centre.

Both IFN-γ tests have high agreement in our study. Although previous reports have described similar levels of agreement, very few of these studies have been carried out in children. DETJEN *et al.* [21] found an agreement of 95.6% (κ=0.91). FERRARA *et al.* [14] reached a high agreement (κ=0.699), independently of the BCG vaccination status, but T-SPOT.*TB* detected a higher number of positive cases (38%) than QuantiFERON®-TB Gold (26%). Furthermore, KAMPMANN *et al.* [23] found a poorer agreement of IFN-γ tests (66.7%) in culture-confirmed TB cases, but the agreement was high (92%) in LTBI.

The analysis of discordant results needs to be researched further. This study has shown that when there is disagreement between both IFN-γ tests, a negative correlation exists between the number of SFCs and the amount of IFN-γ produced. In our study, in 11 cases, the T-SPOT.*TB* was positive and the QFT-G-IT negative and in six cases, the T-SPOT.*TB* was negative but the QFT-G-IT positive. There may have been false positive or false negative IFN-γ tests. But it is also possible that there was an immunological dysfunction in these children. In fact, three of the children with a discordant result had discordant IFN-γ tests results again 3 months later. Recently, RICHELDI *et al.* [38] performed a comparative study on three different groups of immunocompromised individuals. They described highly discordant results, *i.e.* those clearly negative with one IFN-γ test and clearly positive with another, representing 12.1% of the study population. These results suggest an immunological dysfunction related with a decreased production of IFN-γ or a decrease in the number of IFN-γ-producing cells. Both situations have been associated with increased risk of developing active TB.

TB infection control in animals and humans is associated with the production of IFN-γ by the CD4+ T-helper (Th)-cells [39]. It has been shown, in animal models, that a decreased production of IFN-γ and a decrease in the number of IFN-γ-producing cells are predictive of an increased risk of developing TB [40]. In contact patients, it has been observed that the progression to disease was associated with a decrease in IFN-γ, and an increase in interleukin (IL)-10 and IL-4 levels [41, 42]. Some data suggest that in individuals with a recent exposure to TB, the protective response shifts from Th1 to Th2, even before the clinical symptoms appear [43]. Perhaps children with discordant IFN-γ tests could be a high-risk group for developing TB and, therefore, this could constitute a group that would benefit most from TB infection treatment.

In conclusion, in the daily practice of a referral centre for TB control, the use of IFN-γ tests is helpful for the diagnosis of TB infection. Its use eliminates the cross-reactions with BCG immunisation and may help to exclude NTM infections. The analysis of highly discordant results requires further investigation to elucidate any possible clinical implications. The use of both techniques simultaneously can contribute to improving the knowledge of TB immunity.

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STATEMENT OF INTEREST

None declared.

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