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APLICABILITAT CLÍNICA DE LES TÈCNIQUES DE DETECCIÓ *IN VITRO* DE L'INTERFERÓ-GAMMA EN LA INFECCIÓ I LA MALALTIA TUBERCULOSA

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

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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*.

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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 (⩽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 ⩾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 ⩽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 ⩾5 mm in contacts and in children with abnormal chest radiographs consistent with active TB, and ⩾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±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±SD age was 9.08±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 ⩾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 ⩾5 mm.

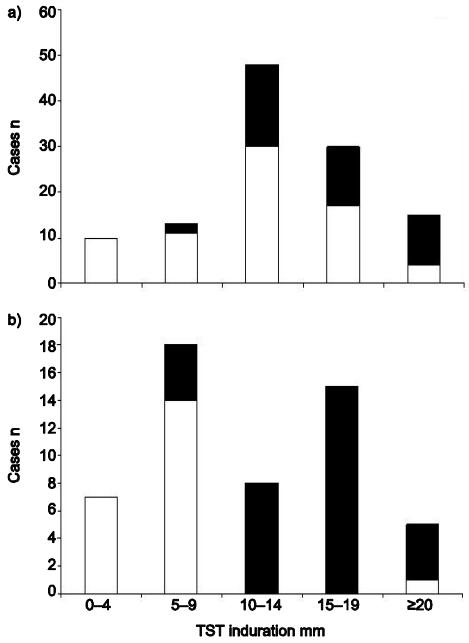

FIGURE 1. Interferon-γ tests result distribution (■: positive; □: negative) according to the tuberculin skin test (TST) induration in a) bacille Calmette–Guérin (BCG) and b) non-BCG-vaccinated children from the contact and screening groups.

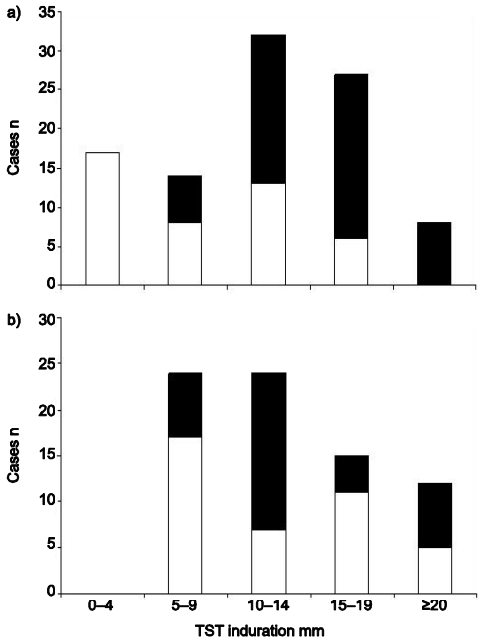

FIGURE 2. Interferon-γ test results distribution (■: positive; □: negative) according to the tuberculin skin test (TST) induration in the a) contact and b) screening groups, including both bacille Calmette–Guérin (BCG) and non-BCG-vaccinated children.

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

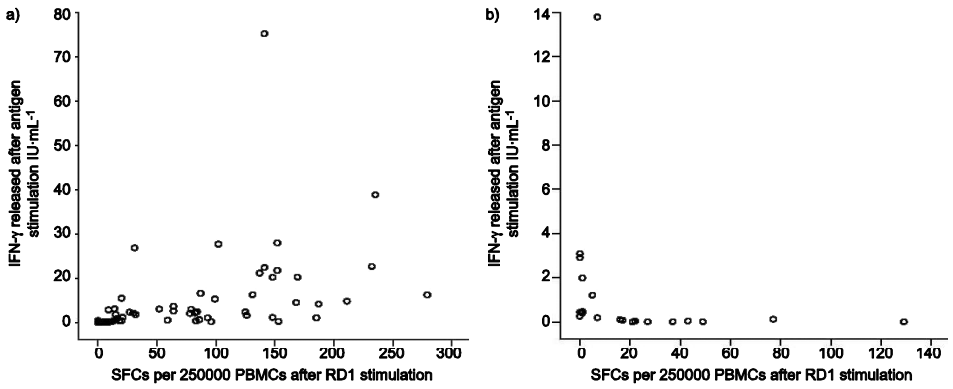

FIGURE 3. Correlation between the number of spot forming cells (SFCs) after stimulation with specific *Mycobacterium tuberculosis* antigens and the amount of interferon (IFN)-γ released in children with a) concordant and b) discordant results between the T-SPOT®.*TB* and QuantiFERON®-TB Gold In-Tube 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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4.3. Article 3
Correlation between tuberculin skin test and IGRAs with risk factors for the spread of infection in close contacts with sputum smear positive in pulmonary tuberculosis.

Maria Luiza de Souza-Galvão, Irene Latorre, Neus Altet-Gómez, María Ángeles Jiménez-Fuentes, Celia Milà, Jordi Solsona, Maria Asunción Seminario, Adela Cantos, Juan Ruiz-Manzano, and José Domínguez.

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La prevenció de la TB es basa en la identificació i el tractament preventiu de les persones amb un risc més alt de desenvolupar la TB, com són els contactes íntims i recents de pacients amb TB pulmonar bacil·lífera, les persones infectades pel VIH o els pacients sotmesos a tractaments immunosupressors.

La propagació de la TB en espais tancats està fortament influenciada pel nombre de nuclis de gotetes infectades presents en l'aire, la viabilitat dels bacils de *M. tuberculosis* i la durada de l'exposició. Viure en condicions d'amuntegament sembla augmentar el risc d'infecció tuberculosa. La presència de tos disminueix el retard en el diagnòstic de la TB augmentant la sospita, però també augmenta la propagació de la malaltia.

L'objectiu de l'estudi va ser avaluar en els contactes de pacientes amb TB pulmonar bacil·lífera, la correlació entre la PT i els tests d'IF-γ (QFN-G-IT i T-SPOT.TB) amb alguns dels factors de risc per a la propagació de la infecció tuberculosa: les condicions d'amuntegament, nombre de persones per metre quadrat i mida de l'habitació on va tenir lloc el contacte, la presència de tos en el cas índex i els dies de retràs en el diagnòstic de la TB.

La concordança entre els resultats de la PT i els IGRAs va ser baixa, excepte en el grup de nens no vacunats amb la BCG, mentres que entre ambdós tests de IF-γ va ser substancial. Els resultats positius de la PT no es van correlacionar amb el grau d'exposició, mentres ambdos IGRAs van presentar una major quantitat de resultats positius amb l'augment d'hores d'exposició. També va ser major la quantitat de cèl·lules T estimulades amb CFP-10 ($p = 0,029$), la combinació ESAT6 / CFP10 ($p = 0,042$), així com la quantitat de IF-γ alliberat ($p = 0,015$) en els contactes amb més de 6 h de l'exposició, en comparació amb els contactes amb menys de 6 h d'exposició al cas índex.

Tanmateix, ambdós IGRAs van mostrar una associació significativa amb la presència de tos i la mida de l'habitació. A més, T-SPOT.TB va mostrar una tendència a l'associació amb el retard diagnòstic sense arribar a la significació estadística.
En conclusió, QFN-G-IT i T-SPOT.TB s'associen més que la PT amb certs factors de risc implicats en la transmissió de la malaltia, com la presència de tos, i la mida de l'habitació on es produeix el contacte.

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RESEARCH ARTICLE Open Access

Correlation between tuberculin skin test and IGRAs with risk factors for the spread of infection in close contacts with sputum smear positive in pulmonary tuberculosis

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Abstract

Background: The aim of the study was to assess the correlation between the tuberculin skin test (TST) and *in vitro* interferon-gamma released assays (IGRAs) with risk factors for the spread of infection in smear positive pulmonary tuberculosis (TB) contacts.

Methods: We recruited prospective contacts with smear positive pulmonary TB cases. We looked at human immunodeficiency virus (HIV) infection and other conditions of immunosuppression, presence of BCG vaccination and the degree of exposure to the index case. Patients underwent the TST, chest radiography, sputum analysis when necessary, and IGRA assays (QFN-G-IT and T-SPOT.TB). Presence of cough, diagnostic delay (days between first symptoms and TB diagnostic), contact conditions: room size (square meters) and index of overcrowding (square meters per person) were investigated in the index case.

Results: 156 contacts (119 adults, 37 children) of 66 TB patients were enrolled, 2.4 (1-14) contacts per TB case. The positivity of the TST did not correlate with the risk factors studied: presence of cough ($p = 0.929$); delayed diagnosis ($p = 0.244$); room size ($p = 0.462$); overcrowding ($p = 0.800$). Both QFN-G-IT and T-SPOT.TB, showed significant association with cough ($p = 0.001$, and $p = 0.007$) and room size ($p = 0.020$, and $p = 0.023$), respectively.

Conclusions: Both IGRA associated better than TST with certain host-related risk factors involved in the transmission of disease, such as the presence of cough.

Keywords: Tuberculosis infection, Tuberculin skin test, Interferon gamma release assays, IGRA, Overcrowding, Diagnostic delay, Cough

Background

The World Health Organization estimates approximately 8.6 million new cases of tuberculosis (TB) annually [1]. Studies published in the 80s showed that 5-10% of recently infected contacts develop active disease within the subsequent 2-5 years after exposure to an infectious source; while another 5-10% percent develop TB sometime during the rest of their lives [2]. In Barcelona, a low TB burden city (incidence: 24.9/100,000 in 2011), a study showed that 25.5% of new TB cases were due to recent transmission [3].

TB prevention relies on the targeted identification and preventive treatment of individuals who carry an increased risk of developing TB [4]. Identification of individuals from these groups, showing the highest risk of developing TB is performed by the evaluation of the presence of an adaptive immunity against *Mycobacterium tuberculosis*, in the absence of active disease. For many decades the tuberculin skin test (TST) has been the immunodiagnostic method of

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choice for this risk analysis. In the last years, interferon–γ (IFN-γ) release assays (IGRAs), the Quantiferon-TB Gold in Tube (QFN-G-IT) (QIAGEN, Düsseldorf, Germany), and the T-SPOT.TB (Oxford Immunotec Limited, Abingdon, UK), have been introduced into clinical practice as laboratory methods for the immune-diagnosis of latent TB infection (LTBI) [5].

The spread of TB in confined spaces is strongly influenced by the number of infected droplet nuclei present in the air, the viability of the *M. tuberculosis* bacilli and the duration of exposure [6]. Living in a crowded household increases the risk of TB infection [7]. The presence of cough decreases the delay in TB diagnostic, improving suspicion, also increasing the spread of the disease [8]. While several studies have compared the frequency of positive TST and IGRA results in relation to the exposure time to case index [9,10], no study comparing the performance of the TST and IGRAs was targeted directly in living conditions and on overcrowding, as risk factors for the development of LTBI in contacts.

The aim of this study was to assess the association between the positivity of TST and IGRAs with some risk factors for the spread of the disease as: presence of a cough and diagnostic delay in the index case; and the size of the room and the overcrowding in the environment where the contact took place. Furthermore, we compared the performance of the 3 tests, the agreement between them as well as their correlation with the time of exposure.

Methods

Study design

We prospectively recruited people who came to the TB Unit in Drassanes (Barcelona, Spain) in our routine contact investigation tasks. The Ethics Committee of the "IDIAP Jordi Gol Research Foundation" provided ethics approval for this study. Patients and parents of children were asked to sign a consent form. Detailed questionnaires were completed with data about prior contact or any prior TST result; data about HIV infection or other causes of immunosuppression were collected and we looked for the presence of BCG scars. We tested using TST and whenever possible, also with QFN-GIT and with T-SPOT.TB. We drew blood only from untreated patients or from patients receiving LTBI treatment for less than 30 days, in order to avoid the effects of the therapy skewing IGRA results [11]. Chest x-Ray and sputum analysis were also done if necessary.

Contacts were eligible if they were exposed to a pulmonary TB sputum smear positive patient index, and excluded if they had active TB, were diagnosed with HIV infection, were pregnant, or if the result of the culture of the patient index was not confirmed to be *M. tuberculosis*. Contacts of sputum smear positive patients were from two different origins: familial (relatives or friends referred to the Unit for TB screening) and community (colleagues or students referred to the Unit for screening with chest x-Ray due to a positive TST). They were also classified according to degree of exposure to the case index in 2 groups: 6 or more hours daily and less than 6 hours daily. Clinicians were blind to IGRA results and microbiologists did not know the clinical history of the patients.

Clinical and environmental risk factors for TB infection

In the index case we checked for the presence or absence of a cough and considered the days of diagnostic delay, defined as days between the first symptoms and TB diagnostic. As far as the environmental contact conditions, we looked for data on the room size (in square meters) and we used the Floor Area per Person Index. The room size was classified according to the Statistical Institute of Catalonia, slightly adapted to the characteristics of the neighbourhood of our study population. This indicator of Sustainable Development was used by the United Nations, to measure the adequacy of living space, not taking into account cultural differences, as an indicator of overcrowding. We considered overcrowding when the index was less than 15 square meters per person [12]. The data about the index case were obtained by direct interview when the patient was diagnosed in our institution or collected from the database of the TB Programs of Barcelona and Catalonia.

Tuberculin skin testing

TST was performed by the Mantoux method using two tuberculin units of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) and read within 48-72 h, using the ball-point pen and ruler method by trained nurses and doctors. All TST ≥ 5 mm were classified as positive result regardless of the BCG defined by the Spanish Pneumology and Thoracic Surgery Society (SEPAR) guidelines [13,14].

QFN-gold in tube

One ml of blood was drawn per each of the 3 tubes with antigens ready for incubation: (Nil, TB antigens and mitogen). Samples were incubated with stimulatory antigens for 16 h-24 h and the test was performed according to the manufacturer's instructions. The cut-off value for a positive test was IFN-γ of at least 0.35 IU/mL in the sample after stimulation with the specific antigens, regardless of the result of the mitogen control. The result of the test was considered indeterminate if the antigen-stimulated sample was negative and if the value of the positive control was less than 0.5 IU/mL after subtraction of the value of the nil control, and/or if the nil control was higher than 8.0 IU/ml.

T-SPOT.TB

The test was performed following manufacturer's recommendations. 8 ml of blood were drawn from each subject by venipuncture for the isolation of peripheral blood mononuclear cells (PBMCs) in CPT tube (Beckton Dickinson Diagnostics, Franklin Lakes, NJ). The isolated PBMCs were washed twice by centrifugation with RPMI medium (Invitrogen, Auckland, N.Z.), and later resuspended in AIM-V medium (Invitrogen, Auckland, N.Z.). Finally, viable cells were counted with an inverted microscope using the tripan blue method.

After that, the PBMCs were stimulated in each well by medium alone (as nil control), phytohaemagglutinin (as positive control), different peptide panels encompassing the antigens ESAT-6 and CFP-10. Spots were scored using an automated AID ELISPOT plate reader (AID Systems, Strasberg, Germany) and also by naked eye. Test wells were scored as positive if they contained at least six spot-forming cells more than the nil control well, and if this number was at least twice the number of the nil control well. The result of the test was considered undetermined if the antigen-stimulated sample was negative and if the value of the positive control was less than 20 spots, and/or if the number of SFC in the negative control was greater than 10. All blood samples for both tests were processed within 4 h of phlebotomy in the Microbiology Department of the Hospital Universitari Germans Trias i Pujol in Badalona (Spain).

Statistical analysis

The qualitative variables description is based on the calculation of the number and its percentage, and for quantitative variables, based on calculation of the mean and the standard deviation (SD). The chi-squared test and two-tailed Fisher's exact test were used to compare qualitative variables. Non-parametric tests (Mann–Whitney, Kolmogorov-Smirnov, Kruskal-Wallis) were used to compare quantitative variables according to the categories of the group of variable. Cohen's kappa coefficient (k) was used to analyse the concordance and its p value. All analyses were made with SPSS statistical software for Windows (SPSS version 15.0; SPSS, Chigago, IL).

Results

Demography

Table 1 summarizes the demographic and clinical details. 230 contact patients with smear positive TB were recruited for the study. 11 patients were excluded by diagnosis of active TB; 5 by absence of growth of *M. tuberculosis* in the culture sputum of the index case and 34 because the blood samples were collected more than 30 days after they had started treatment of TB infection. 24 contacts did not have all three testing techniques applied. In the end, a total of 156 contacts (119 adults and 37 children) from 66 cases of pulmonary TB sputum smear positive were enrolled (Figure 1). The average number of close contacts per source case was 2.4 and varied from 1 to 14 individuals.

Table 1 Characteristics of the study population (n = 156)

Characteristics	Adults n = 119	Children n = 37
Mean age (SD)	29.3 (10.5)	9.2 (2.4)
Gender		
Female	67 (56.3)	21 (56.8)
Male	52 (43.7)	16 (43.2)
BCG-vaccinated		
Yes	69 (58)	25 (67.6)
No	50 (42)	12 (32.4)
Place of birth		
Spain	60 (50.4)	12 (32.4)
Outside Spain	59 (49.6)	25 (67.6)
America	42 (71.2)	19 (76)
Eastern Europe Mediterranean	7 (11.9)	1 (4)
Europe	6 (5)	5 (13.5)
South Asia	1 (1.7)	0 (0)
Western Pacific	3 (5.1)	0 (0)
Type of contact		
Familial	45 (37.8)	25 (67.6)
Community	74 (62.1)	12 (32.4)
Degree of exposure		
≥ 6 hours daily	46 (38.6)	16 (43.2)
< 6 hours daily	73 (61.4)	21 (56.8)
Cough in index case		
Yes	37 (52.1)	23 (92)
No	34 (47.9)	2 (8)

Data are presented as n (%) or means ± SD. *BCG*: Bacille Calmette-Guérin.

Agreement between tests

Overall agreement of TST and both IGRAs was mostly slight (Table 2). Nevertheless, in BCG-unvaccinated children reached the best agreement. The overall agreement between both IGRAs was substantial; with no differences in the agreement in BCG-vaccinated and non-BCG-vaccinated taking into account children and adults together.

Positive test results for all techniques

The results of TST and IGRAs are summarised separately into adults and children according with the origin of the contact (familial or community contact) in Figure 2, and also according to the presence or absence of BCG vaccine and the degree of exposure (Tables 3 and 4).

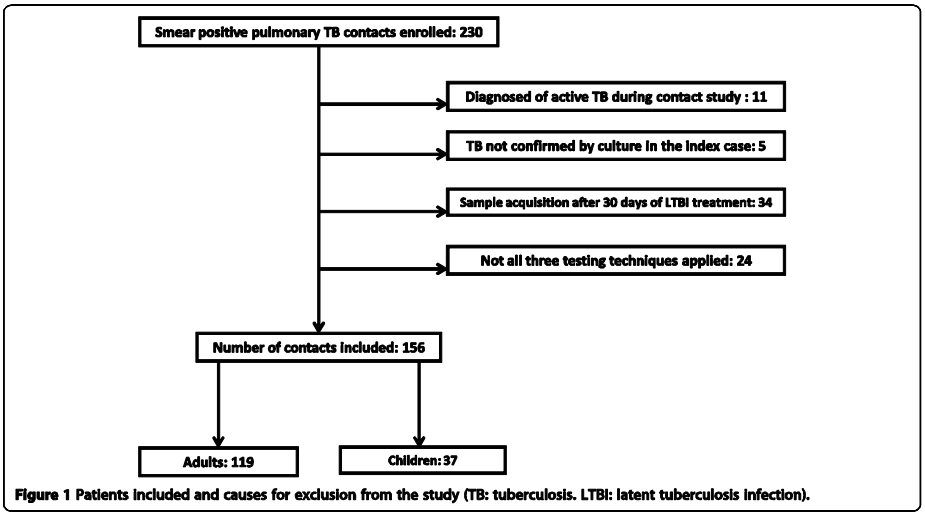

Figure 1 Patients included and causes for exclusion from the study (TB: tuberculosis. LTBI: latent tuberculosis infection).

BCG-unvaccinated

The results of the TST showed 46 (92%) positive adults and 11 (91%) positive children (Table 3). The QFN-G-IT was positive in 27 (54%) adults and 9 (75%) children, while the T-SPOT.TB was positive in 33 (66%) and indeterminate in 2 (4%) adults; and positive in 10 (83.3%) children with no indeterminate results.

BCG-vaccinated

Percentages of positive results in TST were similar to those of non-vaccinated individuals (92.8% and 92% in adults and children, respectively) (Table 4). In contrast, IGRAs detected less positive results compared to the unvaccinated groups, except for T-SPOT.TB in children.

Positive test results according to degree of exposure

BCG-unvaccinated contacts

TST positive results did not correlate with the degree of exposure in adults, showing identical percentage of positive results independently of the degree exposure (Table 3). In contrast, although IGRAs showed a higher amount of positive results with the increase of hours of

Table 2 Agreement between tests and K coefficients

Patient characteristics	TST *versus* T-SPOT-TB		TST *versus* QFN-IT		T.SPOT-TB *versus* QFN-IT	
	Agreement (%)	Kappa	Agreement (%)	Kappa	Agreement (%)	Kappa
Total						
All	62.6	0.172	50.91	0.121	82.82	0.659
BCG	51.58	0.119	42	0.078	83.84	0.664
No BCG	79.37	0.310	64.62	0.224	81.25	0.604
Adults						
All	58.97	0.148	45.6	0.100	81.45	0.629
BCG	47.83	0.101	34.72	0.056	80.82	0.580
No BCG	75	0.256	60.38	0.195	82.35	0.637
Children						
All	75	0.288	69.23	0.235	86.84	0.721
BCG	64	0.199	62.96	0.182	92	0.838
No BCG	93.33	0.634	83.33	0.429	76.92	0.418

BCG: Bacille Calmette-Guérin. *TST*: tuberculin skin test. *QFN-G-IT*: Quantiferon-TB Gold in tube.

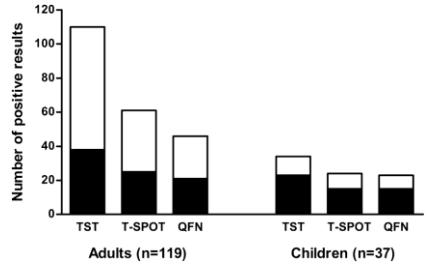

Figure 2 Number of positive results obtained by TST and both IGRA tests (T-SPOT.TB and QFN-G-IT) in adults and children, including information in each case of the origin of the contact: familial (■) or from the community (□).

exposure to the index case, the differences did not reached statistical significance.

Taking into account the same degree of exposure, significant differences were found between the positive results using the 3 techniques. TST compared with QFN-G-IT and T-SPOT.TB showed more significant positive results in both degrees of exposure: more than 6 hours ($p = 0.007$ and $p = 0.022$, respectively) and less than 6 hours ($p < 0.001$, respectively) in adults. The low number of BCG-unvaccinated children included, did not allow us to establish if there was a statistical association in this group.

BCG-vaccinated contacts

TST did not show a significant difference in the number of positive results according to the degree of exposure, neither in adults nor in children (Table 4). In opposite, although IGRAs showed a higher amount of positive results with the increase of hours of exposure to the index case, the differences did not reached statistical significance.

Taking into account the same degree of exposure, significant differences were found between the positive results using the 3 techniques. TST compared with QFN-G-IT and T-SPOT.TB showed more positive results in both degrees of exposure: more than 6 hours ($p < 0.001$, respectively) and less than 6 hours ($p < 0.001$, respectively) in adults and the same in children ($p = 0.018$ and 0.016, respectively).

Quantitative evaluation of T-cell response according to the degree of exposure

We studied T-cell quantitative enumeration after stimulation with ESAT-6, CFP-10 and also the sum of both antigens together (ESAT6/CFP10) with T-SPOT.TB; and the amount of IFN-γ release in QFT-G-IT, in the contacts included, according to the different degrees of exposure to the index case. In adults, we found a higher amount of responding T cells after stimulation with CFP-10 ($p = 0.029$), ESAT6/CFP10 ($p = 0.042$) and in the amount of IFN-γ released ($p = 0.015$) (Figure 3) in contacts with >6 h of exposure, in comparison to the contacts with <6 h of exposure. In children, this analysis was not possible, given the low number of children included.

Positive results according to clinical and environmental risk factors

We studied 4 risk factors likely associated with TB infection: "presence of cough", "days of diagnostic delay", "size of the household contact" and "overcrowding", and their relation with the positivity of the TST, QFN-GIT and T-SPOT.TB. In the paediatric population, the positivity of the techniques was not correlated with any of the risk factors studied. Data concerning the adult population is presented in Table 5. For the TST, the positivity of the test was not correlated with any of the risk factors studied. On the other hand, we found that the positivity of both IGRAs tests were associated with presence of

Table 3 Results of interferon-γ release assays and tuberculin skin test in adults and children BCG-unvaccinated

Technique		Adults				Children			
		Degree of exposure				Degree of exposure			
		> 6 h daily	< 6 h daily	Total	p	> 6 h daily	< 6 h daily	Total	p
TST	Positive	18 (94.7)	28 (90.3)	46 (92)	0.820	2 (100)	9 (90)	11 (91)	0.999
	Negative	1 (5.3)	3 (9.7)	4 (8)		0 (0)	1 (10)	1 (9)	
QFN-G-IT	Positive	13 (68.4)	14 (45.2)	27 (54)	0.173	2 (100)	7 (70)	9 (75)	0.999
	Negative	6 (31.6)	17 (54.8)	23 (46)		0 (0)	3 (30)	3 (25)	
	Indeterminate	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	
T.SPOT-TB	Positive	15 (78.9)	18 (58)	33 (66)	0.223	2 (100)	8 (80)	10 (83.3)	0.999
	Negative	4 (21.1)	11 (35.5)	15 (30)		0 (0)	2 (20)	2 (16.7)	
	Indeterminate	0 (0)	2 (6.5)	2 (4)		0 (0)	0 (0)	0 (0)	

Data are presented as n (%). *BCG*: Bacille Calmette-Guérin. *TST*: tuberculin skin test. *QFN-G-IT*: Quantiferon-TB Gold in tube.
Two-tailed statistical test was used to compare the qualitative results of TST, QFN-G-IT and T-SPOT.TB, respectively, between the two degree of exposure categories in adults and children.

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Table 4 Results of interferon-γ release assays and tuberculin skin test in adults and children BCG-vaccinated

Technique		Adults				Children			
		Degree of exposure				Degree of exposure			
		> 6 h daily	< 6 h daily	Total	p	> 6 h daily	< 6 h daily	Total	p
TST	Positive	27 (100)	37 (88.1)	64 (92.7)	0.998	13 (92.9)	10 (90.9)	23 (92)	0.859
	Negative	0 (0)	5 (11.9)	5 (7.3)		1 (7.1)	1 (9.1)	2 (8)	
QFN-G-IT	Positive	9 (33.3)	10 (23.8)	19 (27.5)	0.622	9 (64.3)	5 (45.5)	14 (56)	0.350
	Negative	17 (63)	32 (76.2)	49 (71)		5 (35.7)	6 (54.5)	11 (44)	
	Indeterminate	1 (3.7)	0 (0)	1 (1.5)		0 (0)	0 (0)	0 (0)	
T.SPOT-TB	Positive	14 (51.8)	14 (33.3)	28 (40.6)	0.175	9 (64.3)	5 (45.5)	14 (56)	0.350
	Negative	13 (48.2)	28 (66.7)	41 (59.4)		5 (35.7)	6 (54.5)	11 (44)	
	Indeterminate	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	

Data are presented as n (%). BCG: Bacille Calmette-Guérin. TST: tuberculin skin test. QFN-G-IT: Quantiferon-TB Gold in tube.
Two-tailed statistical test was used to compare the qualitative results of TST, QFN-G-IT and T-SPOT.TB, respectively, between the two degree of exposure categories in adults and children.

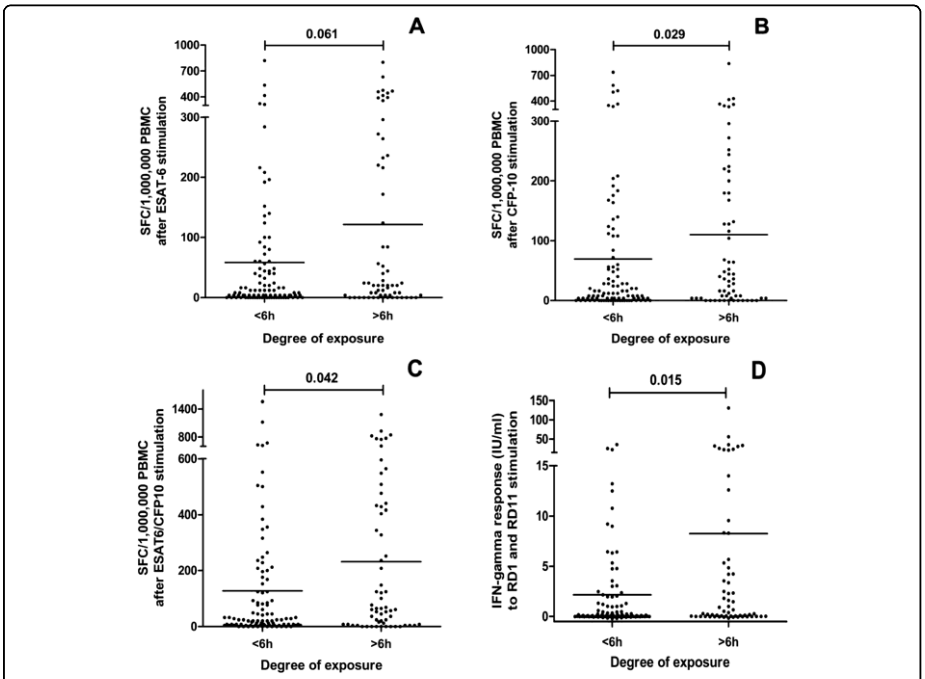

Figure 3 Number of responder T cells to ESAT-6 (A), CFP-10 (B), ESAT6/CFP10 (C), and IFN-γ released after specific antigens stimulation (D) in more and less than 6 hours of exposition to the index case.

Table 5 Risk factors associated with the positivity of the TST, QFN-G-IT and T-SPOT.TB in adults

Risk factors	TST			QFN-G-IT			T-SPOT.TB		
	n (%)			n (%)			n (%)		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p
Cough									
Yes	34 (94.4)	2 (5.6)	0.929	22 (61.1)	14 (38.9)	0.001	25 (69.5)	11 (30.5)	0.007
Not	31 (93.9)	2 (6.1)		7 (21.2)	26 (78.8)		12 (36.4)	21 (63.6)	
Diagnostic delay									
<30	35 (97.2)	1 (2.8)	0.244	11 (30.5)	25 (69.5)	0.565	14 (38.9)	22 (61.1)	0.077
30-59	13 (76.5)	4 (23.5)		7 (41.2)	10 (58.8)		7 (41.2)	10 (58.8)	
60-89	17 (85)	3 (15)		7 (35)	13 (65)		9 (45)	11 (55)	
>89	25 (100)	0 (0)		12 (48)	13 (52)		18 (72)	7 (28)	
Room's size (m^2)									
≤50	2 (66.7)	1 (33.3)	0.462	1 (33.3)	2 (66.7)	0.020	2 (66.7)	1 (33.3)	0.023
51-79	15 (78.9)	4 (21.1)		4 (21.1)	15 (78.9)		6 (31.6)	13 (68.4)	
≥80	19 (90.5)	2 (9.5)		14 (66.7)	7 (33.3)		16 (76.2)	5 (23.8)	
Overcrowding									
≤15	7 (87.5)	1 (12.5)	0.800	2 (28.6)	5 (71.4)	0.480	5 (62.5)	3 (37.5)	0.820
>15	26 (83.9)	5 (16.1)		16 (51.6)	15 (48.4)		18 (58.1)	13 (41.9)	

TST: tuberculin skin test. QFN-G-IT: Quantiferon-TB Gold in tube.
Diagnostic delay: days between first symptoms and TB diagnostic. Overcrowding: floor area index per person (square meters per person).
Two-tailed statistical test was used to compare the qualitative results of TST, QFN-G-IT and T-SPOT.TB, respectively, between the different categories of the risk factors studied.

cough and with the household size, but in this case, inversely to what was expected: we found more positive results, the more square meters the room contained. In T-SPOT.TB results, there was a trend towards an association with diagnostic delay. Overcrowding was not associated with the positivity of any of the tests. The unexpected result of the household size was attributed to the association found between household size and the degree of exposure: contacts living in larger homes had been exposed longer to the index case. Logistic regression analysis was not performed since the number of cases having all the variables to be analyzed, was insufficient.

Discussion

To our knowledge, this is the first study that has investigated the role of the environment and of host-related risk factors for TB infection using TST and IGRAs. We compared the risk of positivity of TST, QFN-GIT and T-SPOT.TB and related them to risk factors of the index case, such as presence of a cough and delayed diagnosis, and also included environmental characteristics, such as household size and overcrowding.

The use of IGRAs has increased specificity in the diagnosis of LTBI, and has proven greater cost efficiency, especially in the use of preventive therapy [15,16]. Several studies have compared the performance of the IGRAs against the performance of TST, taking into account the time of exposure from a contagious source and the infectiousness of the source [17-19].

In our experience, the agreement between IGRA tests and TST was slight (especially in BCG-vaccinated), while the agreement between both IGRA tests was substantial and similar in BCG-unvaccinated and BCG-vaccinated contacts. The results suggest, as in previous studies, that IGRA tests are less affected by BCG vaccination than TST [9,19].

In general there is a recognized low capacity of IGRAs to detect LTBI in children, especially in young children. In our study, it is important to point out that the percentage of TST-positive results (in children and adults) was high because most were referred to the Unit for study by positive TST after a contact. This may explain the high percentage of positive results obtained in children included in the study.

A possible explanation for the lack of relation observed between the positivity of the tests and the degree of exposure, could be the fact that we enrolled only contacts of patients showing smear-positive TB. This may have biased the sample of patients towards being more advanced in the disease, thus underestimating the effects of other variables. This inclusion criteria, was used in order to increase the likelihood of positive TST, attempting to get the majority of cases due to recent contact with the case study and not simply due to a previous infection.

The number of responder T cells and the amount of IFN-γ released could be a better indicator of infectiousness, than the qualitative positivity of an IGRA test, as seen in other studies [20]. We even found correlation between the degree of exposure and the number of responder T cells to CFP-10 and ESAT6/CFP10 by TSPOT.TB and the amount of IFN-γ released in adults.

Most transmissions occur between the onset of coughing and the starting of the treatment [21]. It is known that the delay in diagnosis determines disease progression, increasing affected lung areas with the occurrence of cavitated lesions, extending the probability of infecting a new contact. In this study, neither TST nor IGRAs demonstrated to be related to diagnostic delay.

Sputum smear positivity should be considered a risk factor for infectiousness but there are both epidemiologic [22] and experimental studies [23] that have shown considerable variability of infectiousness among sputum smear-positive patients. Some studies suggested that the presence of a cough could generate potentially infectious aerosols that may increase the risk of disease transmission, regardless of the sputum smear status in the index case [24,25]. Our results showed that the positivity of both IGRA tests was associated with the presence of a cough. The potential of infectiousness is related to the patient's ability to aerosolise bacilli and to the number of bacilli that are aerosolised. In a study in Uganda, contacts of TB patients who produced high aerosols were more likely to have a new infection, as opposed to those who produced low aerosols, and the aerosol negative cases [26].

Since we enrolled only contacts with smear positive TB patients, it was not possible to examine the effect of smear negative and the positivity of tests when the index case had a cough. Guwatudde et al. [27], using TST, studied 1,206 household contacts with smear positive pulmonary. All the TB index cases had a cough for more than 3 weeks and 86% had an advanced disease. Among the host-related risk factors (including overcrowding), they found that only cavitary disease and a prolonged contact with an index case were independently associated with TB in the contact, and the "muzigo" type of housing; a building with multiple rooms that often share air space.

These findings are similar to those of our study, where we did not find a correlation between the overcrowding with the positivity of the tests. The number of patients living in crowded conditions was low in our sample, probably due to the characteristics of the individuals: almost 50% natives and 50% immigrants from Latin American countries, a kind of population with enough resources to live in family groups, in relatively good living conditions. The other type of population was from the community group, usually people sharing the same air for a full working day. In our study, we found a reverse correlation between the size of the room and the positivity of the IGRAs, but associated this correlation with a higher degree of exposure.

Although data about ventilation was not collected in this study, it suggests that in relation to environmental risk factors, more than the number of people in contact with the TB case in a limited environment, it is the ventilation of the room that is the main determinant in the transmission of infection in addition to the time of exposure. A study carried out in Peru found that opening windows and doors provided more than double the amount of ventilation of the mechanical ones and the risk of TB infection was lower [28].

The main limitation of our study was the small number of patients included, given the difficulty of enrolling patients with smear-positive pulmonary TB in our environment. However, the results are sufficiently consistent to show the strong association between the IGRAs and the risk factors involved in the LTBI.

Conclusions

In conclusion, comparing the performance of the TST and IGRA tests in contacts with smear positive pulmonary TB, we found that both IGRAs were associated with certain host-related risk factors involved in the transmission of disease, such as the presence of cough. On the other hand, the study suggests that some environmental risk factors, such as the lack of ventilation associated with the time of exposure, may be crucial for the transmission, aside from the status smear of the index case and the diagnostic delay. A larger study would be needed, in order to confirm all the findings and better characterize the true role of the different variables involved in the transmission of the disease.

Abbreviations

TST: Tuberculin skin test; IGRA: Interferon-gamma released assays HIV, Human immunodeficiency virus; TNF: Tumor necrosis factor; QFN-G-IT: Quantiferon-TB Gold in tube; LTBI: Latent tuberculosis infection.

Competing interests

None of the investigators have relevant financial interest in or a competing interest with the subject matter or materials discussed in this manuscript. None of the Scientific Societies, neither QIAGEN nor Oxford Immunotec (Abingdon, UK) had a hole in the study, conduct, collection, management, analysis, or interpretation of the data, or preparation, review, or approval of the manuscript.

Authors' contributions

MLSG, IL and NAG conceived and designed the study. MLSG, NAG, CM, MAJF, JS, MAS, AC and JRM contributed to acquisition and analysis of data from hospital wards, and IL and JD to acquisition and analysis of data in the laboratory. MLSG, IL and JD wrote the manuscript with significant contributions from NAG, MAJF, CM, JS, MAS, AC and JRM. All authors read and approved the final manuscript.

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5. DISCUSSIÓ

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5.1 Habilitat del QFN-G-IT i el T-SPOT.TB en diagnosticar la TB activa en adults en comparació amb la PT en diferents etapes de la malaltia.

Les persones amb TB activa han de ser ràpidament identificades i tractades per a interrompre la transmissió de la malaltia a la comunitat. Aquest diagnòstic moltes vegades és difícil, en especial en els casos amb bacil·loscopia negativa i en nens. Noves eines diagnòstiques són necessàries per a la detecció precoç de la malaltia, ja que el "gold standard" segueix sent la detecció de *M. tuberculosis* en el cultiu de mostres biològiques. Ni la determinació de IGRAs en sang, ni la PT semblen ser capaços de distingir entre la infecció latent i la malaltia activa o passada.

Els estudis que han avaluat els IGRAs en sang en el diagnòstic de la TB activa han descrit un ampli rang de sensibilitats (78). Pocs estudis han fet una comparació directa entre T-SPOT.TB i QFN-G-IT en la mateixa població d'estudi (142) (149) (150) (151) (152). Encara que hi ha algunes diferències entre els estudis publicats, en tots ells T-SPOT.TB té una lleugera major sensibilitat (de 81,8% a 100%) que QFN-G-IT (de 70,1% a 89%) en pacients amb TB activa, inclosos els pacients amb algun tipus de immunosupressió. D'acord amb l'experiència acumulada en aquesta població, el T-SPOT.TB presenta un major nombre de resultats positius i un menor nombre de resultats indeterminats que el QFN-G-IT, degut al fet de que en aquesta tècnica, el nombre de cèl·lules estimulades està estandarditzada en 250.000, cosa que fa minimtizar els resultats negatius i indeterminats secundaris a un recompte limfocitari baix (Annex II)

Els resultats del nostre estudi son consistentes amb la literatura (Article 1). La sensibilitat dels tests en pacients amb TB pulmonar va ser més alta per T-SPOT.TB (83,3%) que per QFN-G-IT (69,4%) en pacients estudiats a l'inici del tractament i es va reduir a 69,8% i 48,8%, respectivament durant el tractament. Encara que les diferències en la sensibilitat de les proves avaluada al principi i durant el tractament no va ser significativa per a T-SPOT.TB (p= 0,209) o per QFN-G-IT (p=0,078), el nombre de les cèl·lules T responedores després de l'estimulació amb ESAT-6 i la quantitat de IF-γ alliberat en resposta

a l'estimulació antigènica específica, sí van presentar diferències significatives (p=0,004 i p=0,030, respectivament). No vam observar una relació entre resultats negatius dels IGRAs i la duració del tractament. No obstant això, els resultats obtinguts en els 10 pacients monitoritzats des del principi i durant el tractament va mostrar que hi ha una considerable variació interindividual en la disminució de la resposta antigènica.

Altres autors també han demostrat que la resposta d' IF-γ disminueix amb el tractament (153) (154) . Les cèl·lules T efectores que han estat recentment en contacte amb l'antigen *in vivo* alliberen IF-γ al ser reexposades al antigen *in vitro*. La quantitat de cèl·lules T activades i la quantitat d'IF-γ alliberat reflecteixen la dinàmica del procés de càrrega antigènica, fet que va suggerir que aquesta resposta podria ser usada per monitoritzar l'eficàcia de la teràpia.

Aquest aspecte el vam estudiar comparant T-SPOT.TB amb un ELISPOT experimental basat en pèptids seleccionats de ESAT-6 i CFP-10 (155) en pacients amb TB activa, en diferents fases del tractament (Annex V). La sensibilitat global utilitzant T-SPOT.TB, i ELISPOT experimental amb pèptids seleccionats de ESAT-6 i CFP-10 en els pacients inclosos en l'inici del tractament van ser 89,7% i 79,3%. En contrast, durant el tractament, la sensibilitat es va reduir a 87,5% i 25%, respectivament. Les diferències en la sensibilitat entre els pacients avaluats en el inici i durant el tractament van ser significatives per ELISPOT experimental (P <0,0001). Aquestes troballes suggereixen que l'assaig immunològic basat en pèptids RD1 seleccionats s'associa amb la replicació activa de *M. tuberculosis* i es correlaciona millor amb la càrrega bacteriana que el T-SPOT.TB i per tant podría ser un marcador de la resposta al tractament.

Encara hi ha pocs estudis que hagin avaluat els IGRAs en l'estudi de la TB residual. Joshi et al (156) van classificar les troballes radiològiques del personal sanitari en una regió rural d'alta incidència de TB a l'Índia en normal, suggestiva de TB activa i suggestiva de TB residual, i no va trobar diferències

entre els nivells d'IF-γ utilitzant el QFN-G-IT. En canvi, Jeong et al (157) a Corea, van trobar que la positivitat tant de la PT com del QFT-G-IT era més alta en els individus amb lesions radiològiques sugestives de TB residual que en els que tenien normalitat radiològica.

En la nostra experiència, no hi vam trobar diferències significatives entre pacients amb malaltia activa i lesions residuals probablement pel fet que, en la nostra experiència, la resposta als antígens específics, encara que va disminuir amb l'inici del tractament, va retornar a valors similars al final de la teràpia curativa.

Més recentment, els IGRAs han estat avaluats per al diagnòstic de la TB activa directament en mostres biològiques com el rentat broncoalveolar (158), vessament pleural (159) i el líquid cefaloraquidi (160), amb resultats prometedors. Les dades semblen indicar que l'utilització del T-SPOT.TB a fluids extrasanguinis és actualment el millor mètode immunodiagnòstic disponible per al diagnòstic de la tuberculosi activa, tot i que es necessiten més estudis per confirmar-ho.

El que sembla evident, segons l'últim metaanàlisi publicat, és que la sensibilitat d'ambdós IGRAs és major que la de la PT per al diagnòstic de la tuberculosi, però encara no prou alta com per utilitzar-los per excloure la TB activa (161). No obstant, la revisió suggereix que una combinació d'un dels dos IGRAs amb la PT o un altra prova diagnòstica com la bacil·loscòpia o la radiografia de tòrax, pot augmentar la probabilitat post-test de descartar tuberculosi activa (162) (163), però es requereixen estudis addicionals per a avaluar aquest abordatge.

La baixa especificitat dels IGRAs en el diagnòstic de la malaltia tampoc permet distingir entre la TB activa i la infecció latent. El nostre grup va dissenyar un estudi per tal d' intentar diferenciar entre infecció activa i latent mitjançant un punt de tall de la resposta quantitativa específica de cèl·lules T enfront a ESAT-6, CFP-10 (i TB7.7), en adults i nens mitjançant T-SPOT.TB i QFT-G-IT.

(Annex III). Vam avaluar la millor sensibilitat de la corba ROC tenint en compte una especificitat òptima major o igual al 80%.

Encara que hi va haver superposició, es va poder definir un llindar per distingir entre la TB activa i la infecció tuberculosa. El llindar òptim va ser de 69 cèl·lules T per l'estimulació amb CFP-10, amb una sensibilitat del 45,9% i una especificitat del 81,2%, mentres que estimulant amb RD1 (la combinació ESAT-6/CFP-10) va ser de 116 cèl·lules T amb una sensibilitat del 43,2% i una especificitat del 81,2%.

En la nostra experiència, en pacients adults, el nombre de cèl·lules T responedores després de l'estimulació amb antígens RD1 és significativament més gran en la TB activa que en els pacients amb infecció tuberculosa. No obstant això, hi ha una gran quantitat de superposició de valors que fa que de moment, sigui difícil distingir la tuberculosi activa de la latent amb aquests tests.

En els nens, el nombre de cèl·lules T de resposta davant els antígens específics i la producció d' IF-γ amb el QFT-G-IT també han estat més grans en la TB activa que en la infecció latent, però les diferències no van ser significatives.

L'absència de diferències significatives en la resposta entre la tuberculosi activa i la infecció tuberculosa latent podria explicar-se pel fet que la infecció pediàtrica en general és recent. Per tant, la resposta segueix sent intensa, similar a l'obtinguda durant la TB activa.

En resum, en pacients amb sospita clínica de TB, encara que hi ha superposició en el nombre de cèl·lules T de resposta entre tots dos grups, un recompte de cèl·lules T per sobre del llindar descrit podria suggerir la TB activa, especialment en pacients amb una alta probabilitat de tenir tuberculosi activa (per sospita clínica i radiològica) i baixa probabilitat de tenir infecció latent (poblacions de baix risc en països de baixa incidència). A més, els resultats són consistents amb l'evidència actual de que la resposta de cèl·lules T pot indicar la càrrega micobacteriana i l'activitat de la malaltia.

5.2 El diagnòstic de la infecció tuberculosa en nens: anàlisi de les discordances utilitzant assaigs *in vitro* i la prova de la tuberculina.

La població pediàtrica, en especial els nadons i nens de menys de 6 anys, presenten un alt risc de progressió de la infecció tuberculosa a formes greus pulmonars o disseminades de malaltia tuberculosa degut a la immaduresa del seu sistema immune en contenir les infeccions per patògens intracel·lulars.
El diagnòstic ràpid i específic de la malaltia activa i la infecció tuberculosa latent són estratègies claus en el control de la TB en la infància. No obstant això, aquest diagnòstic segueix sent un desafiament ja que la fase inicial de la malaltia pot ser asimptomàtica o amb símptomes i signes inespecífics; l'examen microscòpic de les mostres d'esput sovint és inútil perquè els nens petits no són capaços de expectorar i generalment tenen una malaltia paucibacilar.

El diagnòstic de la infecció tuberculosa sovint es basa en la PT que té certes limitacions, com una sensibilitat reduïda en pacients amb una resposta immune cel·lular alterada i una baixa especificitat en vacunats amb la BCG i infectats per MNT.
El rendiment dels IGRAs en la població pediàtrica és encara objecte de debat i es recomana precaució en el seu ús i interpretació (73). Alguns autors van descriure una bona sensibilitat dels IGRAs en els nens (164) (165) però aquesta troballa no ha estat confirmada per d'altres (166). L'última metaanàlisi publicada ha avaluat la sensibilitat i especificitat dels IGRAs en nens, agrupant els estudis segons la procedència, l'edat i l'estatus immunològic i també una sub-anàlisi amb els estudis que comparen simultàniament els 2 IGRAs i la PT per a intentar reduir el potencial biaix a causa de les diferències individuals.
A primera vista, els resultats de la metaanàlisi van mostrar una més gran sensibilitat del QFT-G-IT envers la PT en els països de renda alta (79% vs 75%), mentre el T-SPOT.TB semblava tenir menor sensibilitat que les altres dues proves (67%).
No obstant això, aquest resultat no es va confirmar quan es van incloure només

els estudis amb TB amb confirmació microbiològica on el T-SPOT.TB va tenir una sensibilitat del 80% (IC del 95%: 59-90), mentre que la sensibilitat del QFT-G-IT va disminuir, però no significativament, a 66% (IC 95% :55-76).
A la sub-anàlisi, que inclou només els estudis que realitzen simultàniament les 3 proves, totes realitzades en països d'alts ingressos, no es va observar diferències en quant a la sensibilitat d'ambdós IGRAs i la PT, mentre que sí va confirmar una major especificitat dels IGRAs en vers la PT (97% vs 84%) (167).
En la nostra experiència, en el primer estudi que van comparar les 3 tècniques, la concordança entre ambdos tests d' IF-γ amb la PT va ser moderada-baixa. En els nens no vacunats amb BCG, el QFN-G-IT va ser negatiu en el 53,3% dels nens que presentàven una PT positiva, i el T-SPOT.TB va ser negatiu en el 50% dels casos. (Annex I). Aquest estudi ens va fer sospitar un possible l'efecte de la infecció per MNT com a causa de PT positiva i IGRAs negatius en aquesta població.

En l'estudi realitzat posteriorment amb població pediàtrica provenient d'estudis de contactes i de cribratge, la concordança entre la PT i els IGRAs va ser alta en aquells nens que no estaven vacunats amb la BCG (Article 2. Taula 5). Les variables associades amb resultats discordants entre la PT i els IGRAs en l'anàlisi multivariada van ser: pertànyer al grup de cribratge; estar vacunat amb BCG i tenir una induració de la PT entre 5-9 mm.

En aquests nens que presentàven induracions de PT entre 5 i 9 mm i IGRAs negatius vam estimular les cèl·lules limfocitàries amb sensitines de *M. avium* utilitzant l'ELISPOT per a investigar la possible influència de les infeccions per MNT. Dels 98 nens amb PT positivas, enrolats per estudi de contactes i els 68 per cribratge, hi van haver-hi 12 que van acomplir aquestes condicions. D'aquests, en 6 d'ells la detecció de cèl·lules T sensibilitzades contra *M. avium* va ser positiva.

Aquest estudi el vam ampliar a 21 pacients pediàtrics no vacunats amb BCG amb PT positiva i T-SPOT.TB negatiu. (Annex IV) Vam estimular les cèl·lules limfocitàries amb sensitines de *M. avium* amb un ELISPOT ex vivo. Dels 21 pacients, en 10 casos (47,6%), es va obtenir un resultat positiu, en 6 (28,6%) el resultat va ser negatiu i en els restants 5 casos (23,8%), el resultat va ser donat com invàlid.

El nombre de cèl·lules T responedores després de l'estimulació amb sensitines de *M. avium* va ser significativament superior al nombre de cèl·lules T responedores tras estimulació específica amb ESAT-6 i CFP-10 ($p=0,001$ i $p< 0,001$, respectivament).

Per tal de reforçar aquesta hipòtesi, vam començar un nou estudi utilitzant glicopeptidolípidos específics (GPLs) del complex *M. avium*. (GPLs). Vam aïllar cèl·lules monocitàries de cada individu i les vam estimular amb *M. avium* serovar 4 GPLs. La presència de cèl·lules T sensibilitzades contra els antígens es va analitzar mitjançant un ELISPOT "*in house".* Els resultats preliminars en els primers 38 individus estudiats van trobar que en el grup d'estudi (6 nens no vacunats amb BCG amb PT positiva i T-SPOT.TB negatiu), el 100% va respondre a l'estimulació amb GPLs mentre que cap dels controls sans negatius (8 nens amb PT i T-SPOT.TB negatius), va presentar ELISPOT positiu després de l'estimulació. La resposta a GPLs en nens amb infecció tuberculosa latent (16 pacients pediàtrics amb PT i T-SPOT.TB positius) va ser negativa en tots els casos. Es van obtenir resultats positius en resposta a GPLs en tots els casos vàlids de pacients amb infecció confirmada per *M. avium complex* (5/5). Finalment, un dels dos pacients amb tuberculosi van respondre a l'estimulació amb GPLs (168).

Aquests resultats preliminars suggereixen que els nens no vacunats amb BCG que responen a la PT i tenen IGRAs negatius podríen estar sensibilitzats a MNT i podrien no requerir tractament per a la infecció tuberculosa latent.

En resum, els nostres resultats mostren suficient evidència de que la sensibilització prèvia per MNT en nens provoca resultats falsament positius de la PT en el diagnòstic de la infecció latent i que els IGRAs podrien evitar l'ús

innecessàri de quimioprofilaxi entre les poblacions infantils i el consum innecesari de recursos en la recerca del cas índex infectant inicial.

5.3 Correlació entre la prova de la tuberculina i els IGRA amb els factors de risc per a la propagació de la infecció en contactes amb bacil·loscòpia positiva en la tuberculosi pulmonar.

L'estudi de contactes és una de les actuacions preventives més rendibles en termes de cost-efectivitat, amb una rendibilitat diagnòstica en quant a nous casos de TB detectats, entre el 3-5 % dels contactes estudiats (66) (67) (68) amb el voltant del 50% de casos d'infecció en contactes de bacil·lífers. La capacitat de transmissió de la malaltia depèn de la durada de l'exposició, la viabilitat dels bacils de *M. tuberculosis* i del nombre de nuclis de gotetes infectades presents en l'aire.

Diversos estudis han comparat els IGRAs amb la PT, però pocs han fet servir simultàniament les 3 tècniques en els estudis de contacte (143) (169) (170).
Un major grau d'exposició recent mesurat per la durada del contacte o per la contagiositat del cas índex, va estar més associat amb resultats positius dels tests d'IF-γ més que de la PT, fet que va suggerir que els IGRAs podrien detectar millor l'exposició recent que la PT. En aquests estudis, les persones amb menys exposició recent van ser més propenses a ser positives per la PT que amb el IGRA, el que va suggerir que la PT podria haver estat millor que els IGRAs en detectar la infecció remota present prèviament a la infecció recent. En altres estudis, ni PT ni tampoc IGRA es van asociar amb exposició recent (165), (171), (172). En un altre estudi realitzat amb contactes amb exposició recent, el grau de proximitat (és a dir, la mateixa habitació, una altra habitació, o en cases diferents) estava més fortament relacionat amb els resultats de la PT que els resultats del QFT-G-IT (169).

En el nostre estudi vam comparar la positivitat de les 3 tècniques (PT, QFT-GIT i T-SPOT.TB) segons el grau d'exposició, però també vam estudiar certs factors de risc inherents al cas índex, com la presència de tos i els dies de retard

diagnòstic. També vam incloure les característiques ambientals, com la grandària del lloc on es va produïr el contacte i l'amuntegament expressat en número de persones per metre quadrat (Article 3).

La concordància entre els IGRAs i la PT va ser lleu, especialment en vacunats amb la BCG, mentres entre els tests immunològis la concordància va ser substancial i similar en els contactes vacunats i no vacunats amb BCG. Els resultats suggereixen , com en els estudis anteriors, que els tests d'IF-γ es veuen menys afectats per la vacuna BCG que la PT (Annex I; article 2).

La positivitat de la PT no es va correlacionar amb el grau d'exposició en els adults que van mostrar un percentatge idèntic de resultats positius independentment del grau d'exposició. En canvi, els IGRAs van detectar una major quantitat de resultats positius amb l'augment d'hores de exposició al cas índex, malgrat que les diferències no van assolir significació estadística. El fet d'haver inclós només els casos de TB amb bacil·loscòpia positiva pot haver esbiaixat la mostra cap a casos index amb malaltia més avançada i més contagiosa, cosa que fa augmentar la probabilitat de que aquests resultats positius siguin per infecció recent igual que els anteriors estudis citats.

En aquest estudi, el nombre de cèl·lules T activades com a resposta a l'estimulació amb CFP-10 i ESAT6/CFP10 per la tècnica del TSPOT.TB i la quantitat d'IF-γ alliberat mesurat per QFT-GIT van presentar una bona correlació amb el risc d'infecció, com vist en altres estudis (Annex III).
En relació als factors de risc d'infecció tuberculosa, la PT no es va correlacionar amb cap dels factors de risc estudiats mentres ambdos IGRAs es van relacionar amb la presència de tos i amb la mida de la habitació, mentres cap dels 3 tests no es va relacionar amb l'index d'amuntegament. En el cas del T-SPOT.TB, hi va haver una tendència cap a una associació amb el retard diagnòstic sense assolir significació estadística.

L'associació inversa entre la mida de l'habitació i el nombre d'infectats pot haver estat influenciada perquè els contactes que van conviure en ambients més amplis van ser els que van estar exposats durant més temps. Encara que no vam obtenir dades relatives a la ventilació de l'habitació, la majoria dels contactes adults estudiats van ser de l'ambit laboral on predominen els grans espais amb aire acondicionat i finestres tancades. Escombe et al (173) van demostrar que els vells hospitals assolellats i amb grans finestrals al Perú propiciaven el doble de ventilació i el risc d'infecció era més baix que les modernes sales amb pressió negativa. Aquests resultats suggereixen que més important que el nombre de persones en contacte amb el cas índex en un espai de petites dimensions, són la manca de ventilació afegit al temps d'exposició els factors determinants més importants en la transmissió de la malaltia.

Estudis recents han mostrat una considerable variabilitat en la contagiositat de pacients amb TB amb bacil·loscopia positiva, possiblement relacionat amb la capacitat del pacient d' aerosolitzar bacils i del nombre de bacils presents en el l'aerosol. La presencia de tos, produeix aerosols potencialment infecciosos, que augmenten el risc de transmissió independentment de la positivitat de bacil·loscopia d'esput (43) (174). En el nostre estudi, la positivitat d'ambdos IGRAs va estar relacionada amb la presència de tos en el cas índex.

En l'estudi de Jónes-Lopes et al (44) on van mesurar directament els aerosols generats per la tos, els pacients capaços de generar més de 10 unitats formadores de colònies van ser més contagiosos que els que generaven menors o nul·les quantitats.

En el nostre estudi, la PT no es va relacionar amb el grau d'exposició, la presència de tos, el retràs diagnòstic ni l'amuntegament.

En resumen, els resultats de tots els estudis presentats en aquesta Tesi Doctoral mostren que els nous mètodes de laboratori basats en la detecció del interferó-gamma, poden ajudar en la pràctica clínica diària en el diagnòstic de la tuberculosi, tant en la malaltia, com en la infecció. Encara que no són capaços de distingir entre la tuberculosi latent i activa, tenen utilitat com a mètode complementari per a el diagnòstic de la malaltia activa, i com la principal eina en el maneig dels estudis de contactes, tant en nens com en adults.

La gran contribució dels IGRA ha estat el fet de despertar l' interès dels investigadors per l'estudi de noves proves de laboratori basades en la immunologia de la tuberculosi i obrir l'esperança en el desenvolupament de tests de nova generació, capaços de diferenciar entre la infecció i la malaltia i de monitoritzar l'evolució del tractament. Aquesta línia d'investigació contribuirà en gran mesura a millorar el diagnòstic i el control del tractament de la tuberculosi.

6. CONCLUSIONS

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1. Utilitat dels IGRAs en tuberculosis activa. (Article 1)

T-SPOT.TB és més sensible en el diagnòstic de la tuberculosi activa que el QFN-G-IT.

La resposta d' IF-γ disminueix durant el tractament de la malaltia tuberculosa.

La quantitat de cèl·lules T activades i la quantitat d' IF-γ alliberat reflecteixen la dinàmica del procés de càrrega antigènica; encara que disminueix a l'inici, s'incrementa al final de la teràpia curativa a nivells similars als del començament del tractament.

Les proves de IF-γ malgrat no diferencien entre malaltia e infecció poden ser d'utilitat com a mètode complementari d'ajuda al diagnòstic de la tuberculosi activa en països de baixa incidència de tuberculosi.

2. Utilitat dels IGRAs en població pediàtrica. (Article 2)

La concordança entre la PT i els IGRAs és alta en aquells nens que no estan vacunats amb la BCG.

La discordança entre la PT i els IGRAs és més alta en els nens amb diàmetres de induració de la PT entre 5-9 mm.

La sensibilització prèvia per micobacteris no tuberculosos pot ser causa de resultats falsament positius de la PT en nens.

L'ús dels IGRAs pot optimitzar la indicació de tractament de la infecció tuberculosa latent en nens.

3. Utilitat dels IGRAs en l'estudi de contactes. (Article 3)

Els resultats dels IGRAs no s'afecten per la vacunació amb la BCG.

El nombre de cèl·lules T de resposta a CFP-10 i ESAT6/CFP10 per TSPOT.TB i la quantitat d'IF-γ alliberat mesurat per QFN-G-IT presenten bona correlació amb el risc de infecció.

Els resultats de T-SPOT.TB i QFN-G-IT es relacionen amb la presència de tos en el cas índex tuberculós.

Els resultats de T-SPOT.TB i QFN-G-IT es relacionen amb la mida de la habitació en què té lloc el contacte amb el cas de tuberculosi.

7. BIBLIOGRAFIA

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