

# Rate of tuberculosis infection in children and adolescents with household contact with adults with active pulmonary tuberculosis as assessed by tuberculin skin test and interferon-gamma release assays

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## SUMMARY

Tuberculosis (TB) infection was evaluated in Brazilian immunocompetent children and adolescents exposed and unexposed (control group) to adults with active pulmonary TB. Both groups were analysed by clinical and radiological assessment, TST, QFT-IT and T-SPOT.TB. The three tests were repeated after 8 weeks in the TB-exposed group if results were initially negative. Individuals with latent tuberculosis infection (LTBI) were treated and tests were repeated after treatment. Fifty-nine TB-exposed and 42 controls were evaluated. Rate of infection was 69·5% and 9·5% for the exposed and control groups, respectively. The exposed group infection rate was 61% assessed by TST, 57·6% by T-SPOT.TB, and 59·3%, by QFT-IT. No active TB was diagnosed. Agreement between the three tests was 83·1% and 92·8% in the exposed and control groups, respectively. In the exposed group, T-SPOT.TB added four TB diagnoses [16%, 95% confidence interval (CI) 1·6–30·4] and QFT-IT added three TB diagnoses (12%, 95% CI 0–24·7) in 25 individuals with negative tuberculin skin test (TST). Risk factors associated to TB infection were contact with an adult with active TB [0–60 days: odds ratio (OR) 6·9; >60 days: OR 27·0] and sleeping in the same room as an adult with active TB (OR 5·2). In Brazilian immunocompetent children and adolescents, TST had a similar performance to interferon-gamma release assays and detected a high rate of LTBI.

**Key words:** Adolescents, BCG, children, IGRA, latent tuberculosis infection, TST.

## INTRODUCTION

Approximately 2 billion people worldwide have latent *Mycobacterium tuberculosis* infection (LTBI) and are at risk of progression to tuberculosis (TB) disease. Young children are at increased risk of developing serious disease due to decreased ability of their immune system to contain infection [1]. An essential strategy

to eliminate TB is treatment of LTBI in order to avoid illness in infected target groups [2, 3].

Tuberculin skin test (TST) has been used as the sole immunodiagnostic test for the detection of *Mycobacterium* infection. However, it has limitations such as low specificity due to cross-reaction with non-tuberculous mycobacteria (NTM) and *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine strains. Moreover, low sensitivity is described in young children and immunosuppressed individuals [2, 4].

At present two commercial interferon-gamma release assays (IGRAs): T-SPOT.TB (Oxford

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Immunotec, UK) and QuantiFERON-TB Gold In-Tube assay (QFT-IT; Cellestis, Australia) based on the detection interferon-gamma (IFN- $\gamma$ ) by T cells after *in vitro* exposure to antigens from *M. tuberculosis* are available.

Although IGRAs are expensive and require an equipped laboratory with trained staff, they have operational advantages over TST, and they appear to have higher specificity compared to TST. However, like TST, they cannot distinguish between latent and active TB disease [4].

Most early studies comparing TST and IGRA were performed in adults [5] or in mixed samples of adults and children [6, 7], but few studies were performed exclusively in children [8, 9]. More recently, other studies have been conducted exclusively in children [10–14].

To the best of our knowledge, no studies comparing TST and IGRA in children only who were household contacts of adults with active pulmonary TB have been performed in Brazil.

The goals of this study were:

- (1) To describe the total rate of *M. tuberculosis* infection in children and adolescents with active TB household contact across three tests: TST, T-SPOT.TB and QFT-IT and to compare this rate in children and adolescents without known TB exposure.
- (2) To describe the LTBI rate for each test in the same groups of children and adolescents.
- (3) To describe the diagnostic increment rate with TST repetition, as recommended by the Ministry of Health in Brazil when the TST is negative at the first evaluation and the diagnostic increment rate when each IGRA is performed in parallel to TST.

## METHODS

This is a prospective study performed at the Federal University of São Paulo, Brazil, from August 2010 to June 2013. Two groups of children and adolescents aged <15 years were investigated: one group with household contact with adults with active TB (known exposure group) and another group without known contact with TB (control group).

The control group was composed of children and adolescents without known contact with TB and who lived with healthy adults without respiratory symptoms and who had never been investigated or treated for TB. They were age- and gender-matched

to the children and adolescents from the known exposure group.

Adult cases of active TB had sputum and/or bronchoalveolar lavage (BAL) positive smear or a positive culture for *M. tuberculosis* complex. The exposure period was defined as the period of time in days from the onset of clinical symptoms of the index case until study entry.

Children and adolescents in both groups with previous TB treatment or with known primary or secondary immunodeficiency or a clinical condition suggestive of possible immunodeficiency were excluded.

Individuals from known exposure and control groups were submitted to a clinical evaluation performed by one of the authors (M.A.G.F.). They were then sent for chest radiography and tested for TST, T-SPOT.TB, QFT-IT and HIV infection.

Those from the control group were discharged after the first evaluation if TST and the two IGRAs proved negative, and the chest radiography did not present any image suggestive of pulmonary disease.

Children and adolescents from the known exposure group had another set of tests performed after 8 weeks if they were all negative at the first evaluation. When one of the three tests was positive either at the first evaluation or after 8 weeks and there was no evidence of disease, treatment for LTBI was initiated. They were then seen every 30 days by the same paediatrician (M.A.G.F.) for 6 months, after which laboratory re-evaluation was performed (Fig. 1).

The study protocol was approved by the Ethics Committee of the Federal University of São Paulo, Brazil (protocol no. 2121/08). All parents or guardians of eligible participants gave written informed consent for study participation before enrolment.

## Sample size assessment

Because no previous study of this kind had been performed in the Brazilian population, the expected percentage of discordance between the three tests (TST and the two IGRAs) was arbitrarily set at 10% for the control group and 15% for the known exposure group.

A sample size of 39 individuals without known contact with TB was necessary in order to obtain an estimate of percentage of discordance between the three tests that did not differ from the true value by more than 30% (margin of relative error), with a 95% confidence interval (CI). For the group of individuals with known contact with TB, a sample size of 53 was necessary in order to obtain an estimate of percentage of

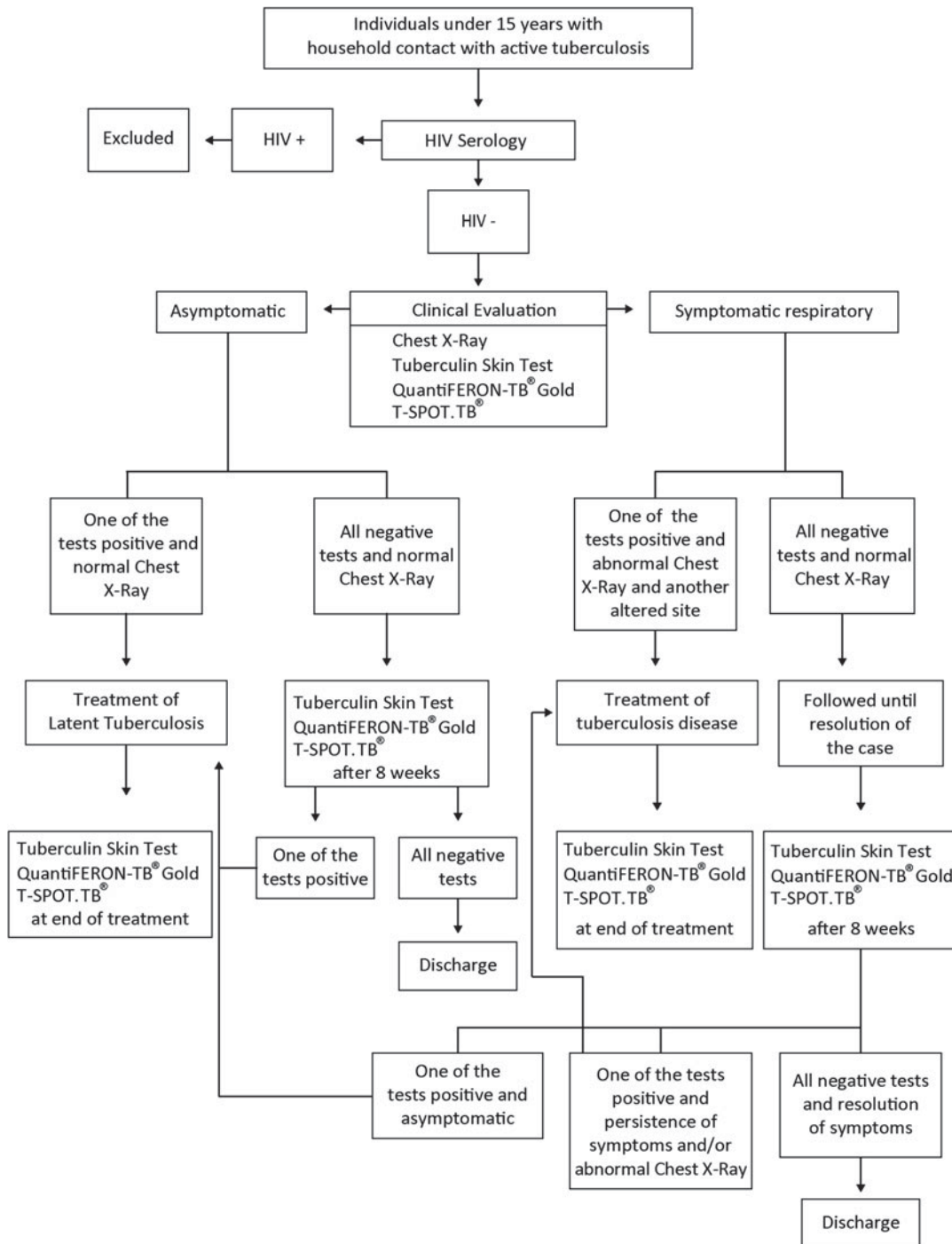


Fig. 1. Algorithm of the known exposure group of children and adolescents.

discordance between the three tests that did not differ from the true value by more than 25% (margin of relative error), with a 95% CI.

**Nutritional evaluation**

Participants from both groups were evaluated for Z score body mass index using the World Health

Organization reference values for children aged <5 years [15] and for those aged 5–19 years [16].

**Socioeconomic classification**

The socioeconomic classification of each adult case was assessed using a questionnaire from the Brazilian Association of Research Companies [17], which

estimates the purchasing power of individuals and families according to the possession of items and the level of education of the head of the family. The final score ranks economic class from A (the highest) to E (the lowest).

### TST

Two units of purified protein derivative (PPD) RT23 (Statens Serum Institute, Denmark) were applied to the volar surface of the left lower forearm according to the intradermal Mantoux method. A trained nurse assessed the transverse diameter of skin induration in millimetres after 72 h of PPD administration. TST results were considered positive depending on previous BCG vaccination:  $\geq 5$  mm, when the child had received BCG more than 2 years previously and  $\geq 10$  mm, when BCG was administered in the last 2 years [18].

### Laboratory assays for TB immunodiagnostic

Peripheral blood samples were obtained simultaneously for both assays and all blood samples were processed within 2 h of phlebotomy, as recommended. The commercially available T-SPOT.TB (Oxford Immunotec, UK) and the QuantiFERON-TB Gold In-tube (QFT-IT) system (Cellestis, Australia) were used in all assays and performed according to the manufacturers' instructions.

### HIV testing

All children and adolescents were submitted to a HIV serology test. The Determine™ HIV-1/2 kit (Abbott, Japan) was used following the manufacturer's recommendations.

### Chest radiography

All children were submitted to chest radiography at the first evaluation. All chest radiographies were analysed by a single experienced staff radiologist (H.M.L.), who was blind to the clinical data.

### Definitions and treatment

The criteria established by III Brazilian Thoracic Association on Tuberculosis (2009) [18] were employed.

### LTBI

LTBI was considered in children and adolescents aged <15 years who presented a positive TST with no signs of active disease. Alternatively, for this study, children or adolescents with positive results for QFT-IT or T-SPOT.TB were also considered infected.

LTBI treatment consisted of isoniazid at a dose of 5–10 mg/kg per day (maximum 300 mg/day) for 6 months [18].

### Active pulmonary TB

Diagnosis of active pulmonary TB was considered according to a scoring system recommended by the Brazilian National Ministry of Health which was based on clinical and radiological findings, known contact with an adult with TB in the last 2 years, TST result and nutritional status [19].

### Statistical analysis

Categorical variables were compared using  $\chi^2$  test or Fisher's exact test. Continuous variables with non-normal distributions were compared with the non-parametric Mann–Whitney test. Agreement between tests was evaluated using the Kappa test. McNemar's test was employed to compare qualitative response change of QFT-IT and T-SPOT.TB after treatment. The paired *t* test or Wilcoxon's test were used to assess quantitative alterations of TST and T-SPOT.TB after treatment.

Univariate and multivariate logistic regression analyses were used to evaluate the association between selected demographic and patients' characteristics and LTBI diagnosis. The following risk factors for TB infection were analysed: gender, age, period of exposure to index case and sleeping in the same room as index case.

A *P* value <0.05 was considered statistically significant. All analyses were performed using Minitab® 16-1 (Minitab, USA) and SPSS statistics v. 20.0 (IBM, USA).

### RESULTS

Of the initial sample of 111 children and adolescents, 10 were excluded (six from the known exposure group and four from the control group), so that 101 children and adolescents (59 from known exposure group and 42 from control group) were included in the study.

Table 1. Characteristics of children and adolescents from the known exposure group and control group

Parameter	Known exposure group ( <i>n</i> = 59)	Control group ( <i>n</i> = 42)	<i>P</i> value
Median age (years) (Q1-Q3)	5.3 (3.2–10.5)	7.3 (5.4–10.5)	0.167†
Female gender (%)	31/59 (52.5%)	24/42 (57.1%)	0.799†
Relationship	Daughter/son: 22/59 (37.3%) Grandchild: 12/59 (20.3%) Niece/nephew: 15/59 (25.4%) Stepson: 2/59 (3.4%) Brother: 2/59 (3.4%) Cousin: 3/59 (5.1%) Foster care contact: 3/59 (5.1%)	Daughter/son: 38/42 (90.5%) Grandchild: 1/42 (2.4%) Niece/nephew: 3/42 (7.0%)	n.a.
Exposure period	≤30 days: 15/59 (25.4%) 31–60 days: 15/59 (25.4%) 61–90 days: 14/59 (23.7%) ≥91 days: 15/59 (25.4%)	0/42 (0%)	n.a.
Nutritional assessment	Eutrophic: 44/59 (74.6%) Overweight risk: 4/59 (6.8%) Overweight: 5/59 (8.5%) Obesity: 2/59 (3.4%) Slimness: 4/59 (6.8%)	Eutrophic: 29/42 (69.0%) Overweight risk: 2/42 (4.8%) Overweight: 6/42 (14.3%) Obesity: 5/42 (11.9%)	0.933‡
Relevant clinical findings	Pneumonia responsive to antibiotics: 2/59 (3.4%)	0/42 (0%)	n.a.
Altered chest radiography on admission	1/59 (1.7%)*	0/42 (0%)	n.a.

n.a., Not applicable.

\* Interpreted as primary complex, with resolution in the following radiography.

† Mann–Whitney test.

‡  $\chi^2$  test.

Individuals from both groups were comparable with regard to age, gender and nutritional status (Table 1). Median BCG vaccination age was comparable between groups (exposure group 19 days vs. control group 15 days; Mann–Whitney,  $P = 0.361$ ). Only one child from the exposure group did not have a BCG scar or records to prove their BCG vaccination. The largest number of index cases in the contact group were parents (22/59, 37.3%) and they belonged to socioeconomic group C (60.6%) and group B (30.3%) (Tables 1 and 2).

Of the 59 children and adolescents from the known exposure group, 38 (64.4%) were submitted to treatment for LTBI due to positivity of one or more tests in the first assessment. Twenty-one (21/59, 35.6%) had negative results and were invited to repeat the three tests, but two refused, therefore 19 children repeated the tests after 8 weeks. Of these, three were diagnosed with LTBI and commenced treatment.

Of the 42 children and adolescents from the control group, 38 presented negative results in all the tests at the first evaluation and were then discharged. Four (4/42, 9.5%) presented one or more positive tests and

commenced treatment for LTBI and were retested after finishing. Of those four controls, only one child had positive results in the three tests. Two children were positive only for TST and one child was positive for QFT-IT and negative for TST and T-SPOT.TB.

No cases of children or adolescents with active TB were diagnosed.

Of 45 LTBI children who started treatment, three abandoned the follow-up, one in the second month and two in the fifth month (Table 3).

The agreement between the three tests was 83.1% (49/59) for the known exposure group at the first evaluation and 92.8% (39/42) for the control group (Kappa TST vs. QFT-IT = 0.807, TST vs. T-SPOT.TB = 0.798 and QFT-IT vs. T-SPOT.TB = 0.888) (Fig. 2).

In the known exposure group, TST was positive in 36/59 children (61%), 34 at the first evaluation and two in the second series of tests, with an increment of 10.5% (95% CI 0.0–24.3).

With respect to IGRAs, two indeterminate results for the T-SPOT.TB at the first evaluation were excluded from the analyses.

Table 2. Demographic data of adult index cases with active tuberculosis

Parameter	Description
Median age (years) (Q1–Q3)	34 (26–40)
Female gender (%)	21/33 (64.0%)
Socioeconomic classification (%)	A: 2/33 (6.1%) B: 10/33 (30.3%) C: 20/33 (60.6%) D: 1/33 (3.0%)
Number of contacts (%)	One: 13/33 (39.4%) Two: 15/33 (45.5%) Three to five: 5/33 (15.1%)
AFB in sputum (%)	Negative: 3/33 (9.1%)* +: 8/33 (24.2%) ++: 4/33 (12.1%) +++: 6/33 (18.2%)
Sputum culture for <i>Mycobacterium tuberculosis</i> complex (%)	Positive without quantification: 12/33 (36.4%) Done: 16/33 (48.5%) Positive: 13/16 (81.3%)† Negative: 3/16 (18.7%)‡ Not performed: 17/33 (51.5%)§

\* The three smears with negative acid-fast bacilli (AFB) had a positive culture result.

† Thirteen positive cultures for *M. tuberculosis* complex.

‡ Despite negative culture, the three were positive for AFB.

§ Two patients did not present sufficient secretion and 14 did not collect it.

The T-SPOT.TB was positive in 4/25 children (16%) who had negative TST at the first evaluation; therefore, T-SPOT.TB added LTBI diagnosis to 16% (95% CI 1.6–30.4) of cases. The QFT-IT was positive in 3/25 children with negative TST, therefore it added LTBI diagnosis to 12% (95% CI 0.0–24.7) of cases.

When both tests were associated with TST, they added four diagnoses of LTBI for the 25 children with negative TST or 16% (95% CI 1.6–30.4).

Considering 24 children with negative T-SPOT.TB, there were two positive QFT-IT (8.3%, 95% CI 0.0–19.4); in the 24 negative QFT-IT, two were positive T-SPOT.TB (8.3%, 95% CI 0.0–19.4).

A multivariate logistic regression analysis was performed to assess the simultaneous influence of period of exposure and sleeping in the same room as index case upon the diagnosis of LTBI. Age ( $P=0.688$ ) and gender ( $P=0.561$ ) were not included in the model because they were not associated with LTBI in the univariate analysis.

The interaction between period of exposure and sleeping in the same room as index case was initially investigated and proved not to be significant ( $P=0.839$ ). It was therefore excluded from the model.

In the adjusted model, both the period of exposure ( $P<0.001$ ) and sleeping in the same room as index case ( $P=0.024$ ) were significant. Hence, the chance

of LTBI in children and adolescents with a period of exposure of  $\leq 60$  days was 6.9 times (95% CI 1.9–26.0) higher than for those without known exposure to TB. For those with period of exposure  $>60$  days, the chances of LTBI were 27 times higher (95% CI 5.5–131.1). Moreover, the chances of LTBI in those who slept in the same room as an index case were 5.2 times (95% CI 1.2–22.1) higher than for those who did not sleep in the same room (Table 4, Fig. 3).

Regarding the evolution of the test after treatment, TST had a significant incremental value of 1.8 mm ( $P=0.0021$ , 95% CI 0.289–3.310) in the 40 children who were assessed after treatment. Only one adolescent was negative and became positive after the treatment. However, he had already been diagnosed by the other two tests.

The IGRAs were evaluated in 42 children after treatment, and no significant qualitative changes ( $P>0.999$ ) were observed for QFT-IT or T-SPOT.TB ( $P=0.724$ ) results. For T-SPOT.TB, there was a significant median reduction of 3.5 spots ( $P=0.010$ ) in panel A (ESAT-6 antigen) with a variation of  $-123$  to  $+74$ , and a median reduction of 3 spots ( $P=0.035$ ) in panel B (CFP-10 antigen), with a variation of  $-143$  to  $+16$ .

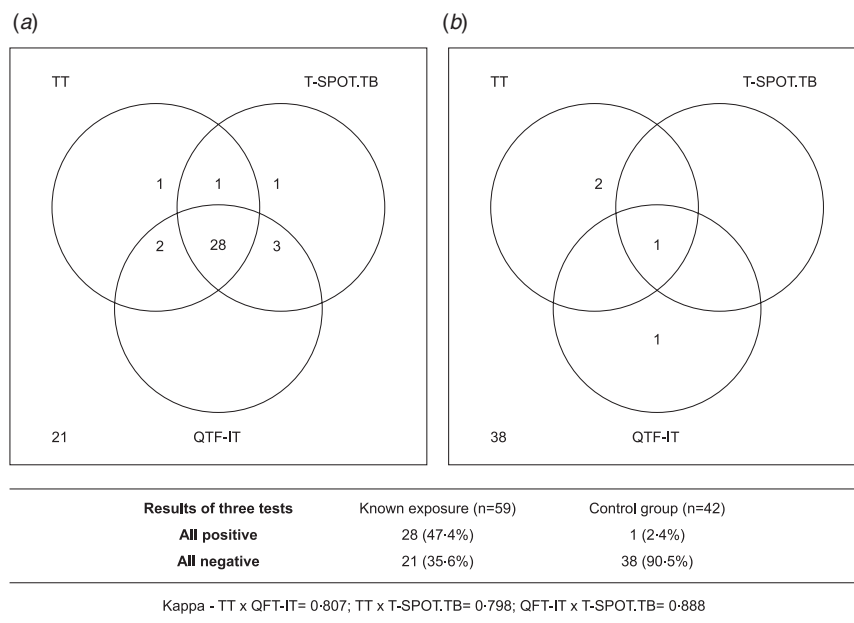
The concomitant use of three tests allowed the LTBI diagnosis in 41/59 (69.5%) children exposed to

Table 3. Results of three tests of the the known-exposure and control groups at study entry and 24 weeks after treatment. Three patients (nos. 6, 12, 18) were retested after 8 weeks because they were negative at study entry and became positive on this occasion

	Study entry			8 weeks after study entry			24 weeks after treatment			Abandon
	TST	T-SPOT.TB	QFT- IT	TST	T-SPOT.TB	QFT- IT	TST	T-SPOT.TB	QFT- IT	
<b>LTBI</b>										
1	P	I	N				N	N	P	
2	P	P	P				P	P	P	
3	P	I	P				P	N	N	
4	P	N	P				P	P	P	
5	P	P	P				P	P	P	
6	N	N	N	P	N	N	P	N	N	
7	P	P	P				P	P	P	
8	P	P	P				P	N	N	
9	P	N	P				P	P	P	
10	N	P	P				N	N	P	
11	P	P	P				P	P	P	
12	N	N	N	P	P	P	P	P	P	
13	P	P	P				P	P	P	
14	P	P	P				P	N	P	
15	P	P	P				P	P	P	
16	P	P	P				P	P	P	
17	P	P	P							2nd month
18	N	N	N	N	P	N	N	P	N	
19	N	P	N				N	P	N	
20	N	P	P				P	P	P	
21	P	P	P				P	P	P	
22	P	P	P				n.d.	P	P	
23	P	P	P				n.d.	P	P	
24	P	N	N				P	N	N	
25	P	P	P				P	P	P	
26	P	P	N				P	P	P	
27	P	P	P				P	P	P	
28	P	P	P				P	P	P	
29	P	P	P				P	P	P	
30	P	P	P				P	P	P	
31	P	P	P				P	P	P	
32	P	P	P				P	P	P	
33	P	P	P							5th month*
34	P	P	P							5th month*
35	P	P	P				P	P	P	
36	N	P	P				N	P	P	
37	P	P	P				P	P	P	
38	P	P	P				P	P	P	
39	P	P	P				P	P	P	
40	P	P	P				P	N	N	
41	P	P	P				P	P	P	
<b>Control group</b>										
42	P	P	P				P	P	P	
43	P	N	N				N	N	N	
44	N	N	P				N	N	N	
45	P	N	N				P	N	N	

P, Positive; N, negative; I, indeterminate; n.d., not done.

\* Abandoned follow-up in the 5th month, but came back for clinical evaluation 2 months after and were both well.



**Fig. 2.** Agreement and disagreement between the three tests (TST, QFT-IT, T-SPOT.TB) in (a) the known exposure group and (b) the control group at the first evaluation.

active TB and in 4/42 (9.5%) children without known contact with a TB patient.

In the known exposure group, if only TST was employed, LTBI would be diagnosed in 61% (95% CI 48.6–73.5) of cases; if only QFT-IT was employed, 59.3% (95% CI 46.8–71.9) of LTBI cases would be diagnosed. If only T-SPOT.TB was used, LTBI would be diagnosed in 57.6% (95% CI 45.0–70.2) of cases.

## DISCUSSION

This is a prospective study conducted exclusively on children and adolescents who presented a high rate of LTBI in those exposed to an adult with active pulmonary TB. No child progressed to active disease during the follow-up. There was a high agreement in results obtained with TST and IGRAs. However, an increment in LTBI diagnosis was observed either with TST repetition or with the association of IGRAs to TST. After treatment, no significant change was observed in the results of the three tests.

The infection rate of 69.5% was higher than what has been reported by other Brazilian studies that evaluated children by TST, with rates between 30% and 58% [20–22]. Today, Brazil has a moderate prevalence of TB with an incidence rate of 36.1/100 000 concentrated in some risk groups such as prisoners, Native Indians,

homeless people and HIV-infected individuals [23]. However, this study was performed in the city of São Paulo, which has a high TB coefficient incidence (46.9/100 000) [24]. That is probably due to the presence of urban areas that concentrate a high proportion of TB patients who live in conditions of extreme poverty and substandard housing that facilitate the transmission of the disease. Therefore it is very important that LTBI in children and adolescents is diagnosed and treated to avoid progression to TB disease [3].

Studies that evaluated the risk of TB infection in countries with high incidence of the disease using other tests combined with TST detected rates of infection in contacts from 24% to 74% [8, 9, 25].

The risk of an exposed individual becoming infected is related to the infectiousness of the index case [20], duration and proximity of contact [13, 20] and the susceptibility of the exposed person [26].

The assessment of the infection rate is determined by the way the diagnostic investigation is undertaken, i.e. which test is selected [20, 22].

The diagnostic value of TST depends on the cut-off value used to distinguish positive from negative results [18], on the use of BCG vaccine in the population analysed, on the cross-reactivity with non-tuberculous environmental mycobacteria (NTM), on nutritional status and on immunosuppressive conditions present at contact [27].



Table 4. Logistic regression analyses for the assessment of demographic and clinical characteristics associated with latent tuberculosis infection (LTBI) diagnosis

Parameters	LTBI+ (n = 45)	LTBI- (n = 56)	P value
<b>Univariate analysis</b>			
Gender			0.561
Male	23	25	
Female	22	31	
Age			0.688
≤ 5 years	17	19	
>5 years	28	37	
Length of exposure			<0.001
No exposure	4	38	
1–60 days	16	14	
>60 days	25	4	
Sleeping			<0.001
In the same room	24	3	
In the same house	41	18	
No exposure	4	38	
Parameters	OR	(95% CI)	
<b>Multivariate analysis</b>			
No TB contact (n = 42)	–	–	
1–60 days of TB contact (n = 30)	6.9	(1.85–26.03)	
>60 days of TB contact (n = 29)	27.0	(5.55–131.08)	
Sleeping in the same room (n = 27)	5.2	(1.24–22.07)	

OR, Odds ratio; CI, confidence interval.

The recent reduction of the TST cut-off value in Brazil increased test sensitivity [28] and allowed the diagnosis of six children in the present study, who would not be considered infected if the previous cut-off was used. All these six children also had positive IGRAs (QFT-IT and T-SPOT.TB), suggesting that they were indeed infected.

However, the exclusive use of the TST would prevent the diagnosis of TB in a 3-month-old infant who had a TST result of 5 mm but who was treated because the two IGRAs turned positive.

In Brazil, the repetition of TST in individuals exposed to active TB is recommended after 6–12 weeks when the first result is negative [18]. In the present study, this procedure detected two cases that would otherwise have been considered negative, showing the importance of this recommendation.

Despite the known difficulties inherent to the TST – low sensitivity, low specificity, the possibility of errors in the application and reading and the need to return in 72 h to read the result – the infection rate detected exclusively by this test in this study was also higher (61% for the treatment group and 7.1% for

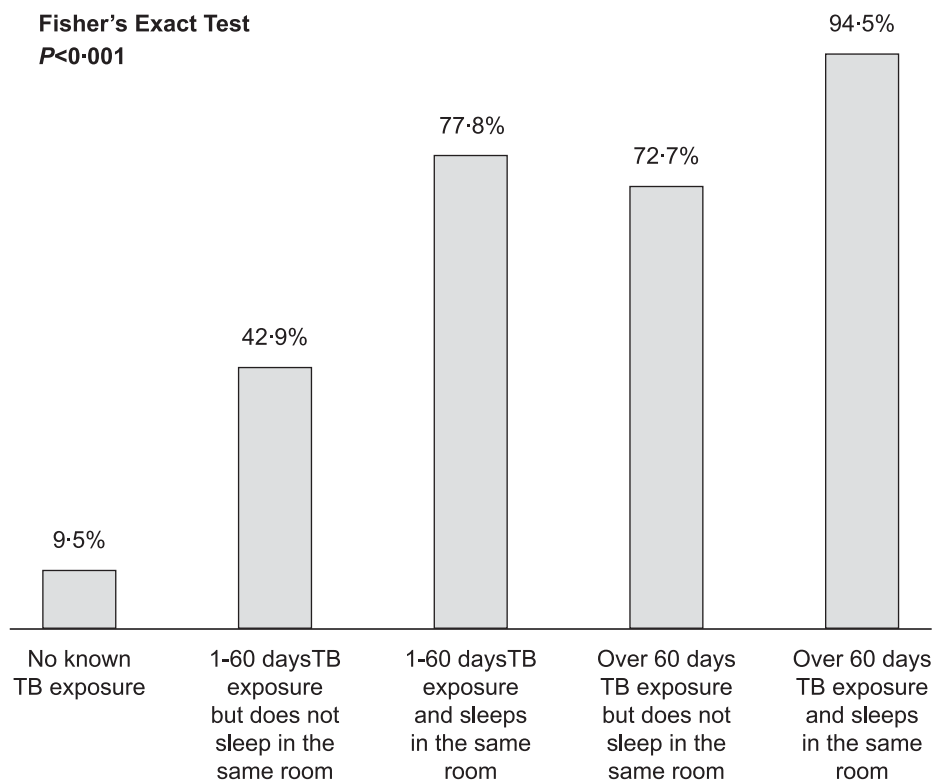
the control group) than what has been described in the literature [20–22].

BCG vaccine administered in the neonatal period has been shown to have minimal impact on the specificity of TST. In Spain, researchers also noted that BCG given at birth does not influence the outcome of TST 3 years after its administration [29].

Regarding the presence of NTM interfering with the specificity of TST, the State of São Paulo has a low number of cases reported compared to other regions in Brazil [30]. Therefore, it is unlikely that these infections have affected the response of TST in the present study.

Some studies published over the last decade using IGRAs showed that BCG given in childhood might protect against *M. tuberculosis* infection [31, 32]. In the present study, 98.3% of children had been vaccinated with BCG at birth; however, despite that, a high rate of LTBI was observed.

No child developed active disease in our study. It is known that malnutrition is one of the factors that contributes to the development of TB disease [33]. Perhaps the good nutritional status of children and adolescents in



**Fig. 3.** Latent tuberculosis infection rate in children and adolescents according to the exposure period and sleeping in the same room.

our sample has contributed to the absence of TB disease: only four children were malnourished and still had a borderline body mass index  $Z$  score of  $-2.02$  to  $-2.21$ .

In the absence of a gold standard for the diagnosis of LTBI, interpreting discordant results is a challenge. Factors associated with discordance between tests are the age of the exposed child [14] the presence of BCG (age of administration and number of doses) [29] as well as the necessary period of time required by different tests to turn positive [34].

In general, in areas of low and moderate prevalence of TB, the agreement between the tests depends on the vaccination status of the participants, being higher in the unvaccinated population and lower in those BCG vaccinated [35].

The high agreement between tests observed in this study in a population so widely vaccinated suggests that a single dose of BCG at birth does not reduce the specificity of TST [29].

Another factor that may have contributed to the high agreement found could be the prolonged exposure to the index case – >30 days in 74.5% of the sample – probably enough for both TST and IGRAs to turn positive [34].

Despite this high agreement, the use of IGRAs contributed to the diagnosis in some cases. The literature suggests that the consecutive use of tests is possible when one desires to increase their sensitivity [13, 25].

On the other hand, only the repetition of TST in 6–12 weeks led to a diagnosis increment. There are no data on the use of this strategy in other countries.

Importantly, the repetition of TST should not be confused with the ‘PPD booster’ strategy [18].

It is worth mentioning the possible effect of TST on subsequent IGRA results. In previous studies, different IGRAs have been tested and varied periods of time between the two tests have been evaluated, providing some evidence that TST can boost a subsequent IGRA result; however, this is more apparent when the IGRA is positive to begin with and when the second IGRA is performed 7–28 days after the TST [36].

The fact that our results have shown that the three tests (TST, QFT-IT, T-SPOT.TB) have a similar performance in Brazilian children exposed to adults with active pulmonary TB is encouraging. Since 2013 Brazil has been facing a shortage of PPD. However, both QFT-IT and T-SPOT.TB are still quite expensive to use as a substitute for TST in the routine of TB

diagnosis for immunocompetent individuals and are not usually available in Brazil [18]. The introduction of IGRAs in Brazil might take place in the future, at least initially for immunosuppressed patients. Fortunately, PPD has recently been purchased by the Ministry of Health again and it is gradually being distributed to healthcare units in Brazil.

There is limited data on the use of IGRAs in some individuals, such as healthcare workers who are repeatedly tested and children aged <5 years [37]. In our study, 26/59 (44%) of TB-exposed children were aged <5 years (range 0.3–4.6 years). Interestingly, in this subgroup, the agreement between the three tests was also high.

In line with the literature [9, 25, 35, 38], logistic regression analysis showed that children with known exposure to an adult with active pulmonary TB were 6.9–27 times more likely to be infected than those with no known contact with TB. Sleeping in the same room as an adult with active pulmonary TB would increase this risk 5.2 times further.

Test results were not modified by treatment, suggesting that there is no advantage in their repetition. However, a *quantitative* variation after treatment was observed both after TST – with a significant increase in response – and after T.SPOT.TB, where there was a significant decrease in the number of IFN- $\gamma$ -producing cells when stimulated with specific antigens for *M. tuberculosis*.

A previous study using Quantiferon did not show differences pre- and post-treatment in children treated for LTBI or in the group treated for TB disease [39].

A decrease in the number IFN- $\gamma$ -producing cells in T.SPOT.TB does not necessarily indicate a change in immune response because other cytokines involved were not measured.

This study is limited by its small sample size, as reflected in the wide confidence intervals around effect estimates despite statistically significant results for some outcomes. Another limitation was the inability to collect sputum for mycobacteria culture from all index cases.

On the other hand, the high adherence to the protocol – a product of the follow-up of children performed by the same professional, of the attention given to the family in the health unit and of the reimbursement of the cost of transportation to all appointments – allowed the vast majority to pursue all proposed steps of the study. These factors have already been reported previously in Brazil [40] and also in Tanzania [41].

## CONCLUSION

In our setting, in immunocompetent children and adolescents exposed to an adult with active pulmonary TB, TST had a similar performance to IGRAs and detected a high rate of LTBI.

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## DECLARATION OF INTEREST

None.

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