RESEARCH ARTICLE

Prevalence and Diagnosis of Latent Tuberculosis Infection in Young Children in the Absence of a Gold Standard

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Abbreviations: BCG, Calmette-Guérin bacillus vaccine; CI, Confidence interval; IGRAs, Interferon-γ release assays; IQR, Interquartile range; HIV, Human immunodeficiency virus; LTBI, Latent

Abstract

Introduction

For adequate disease control the World Health Organization has proposed the diagnosis and treatment of latent tuberculous infection (LTBI) in groups of risk of developing the disease such as children. There is no gold standard (GS) test for the diagnosis of LTBI. The objective of this study was to estimate the prevalence of LTBI in young children in contact with a household case of tuberculosis (TB-HCC) and determine the accuracy and precision of the Tuberculin Skin Test (TST) and QuantiFERON-TB Gold in-tube (QFT) used in the absence of a GS.

Methods

We conducted a cross-sectional study in children up to 6 years of age in Manaus/Brazil during the years 2009–2010. All the children had been vaccinated with the BCG and were classified into two groups according to the presence of a TB-HCC or no known contact with tuberculosis (TB). The variables studied were: the TST and QFT results and the intensity and length of exposure to the index tuberculosis case. We used the latent class model to determine the prevalence of LTBI and the accuracy of the tests.

Results

Fifty percent of the children with TB-HCC had LTBI, with the prevalence depending on the intensity and length of exposure to the index case. The sensitivity and specificity of TST were 73% [95% confidence interval (CI): 53–91] and 97% (95%CI: 89–100), respectively,

tuberculous infection; Mtb, *Mycobacterium tuberculosis*; MTC-score, *Mycobacterium tuberculosis* contact score; PPD, Purified protein derivatives; QFT, QuantiFERON-TB Gold in-tube; TB, Tuberculosis; TB-HCC, Household case of tuberculosis; TST, Tuberculin Skin Test; TU, Tuberculin units; WHO, World Health Organization.

versus 53% (95%CI: 41–66) and 81% (95%CI:71–90) for QFT. The positive predictive value of TST in children with TB-HCC was 91% (95%CI: 61–99), being 74% for QFT (95% CI: 47–95).

Conclusions

This is one of the first studies to estimate the prevalence of LTBI in children and the parameters of the main diagnostic tests using a latent class model. Our results suggest that children in contact with an index case have a high risk of infection. The accuracy and the predictive value of the two tests did not significantly differ. Combined use of the two tests showed scarce improvement in the diagnosis of LTBI.

Introduction

Tuberculosis (TB) is one of the most prevalent contagious infections worldwide[1]. Nonetheless, although the incidence of TB is decreasing by approximately 1.5% annually, according to the elimination target set for 2050 proposed by the World Health Organization (WHO) this reduction is insufficient[2]. This may be explained by the underdiagnosis of the disease which has been estimated to be around one third of the incidence cases[1]. For adequate disease control the WHO has proposed the need for not only adequate diagnosis and treatment of TB cases but also the diagnosis and treatment of latent tuberculous infection (LTBI) in groups of risk of developing the disease such as children[2–4]. Most cases of pediatric age are due to household contact with adults with TB[5,6]. Therefore, pediatric control measures involve contact screening to detect and treat children with TB and LTBI in order to prevent the disease in those infected[7].

The tuberculin skin test (TST) and Interferon-γ release assays (IGRAs) are used for the diagnosis of LTBI and are based on challenging the immune system in order to detect whether it recognizes the antigens of *Mycobacterium tuberculosis* (Mtb)[8,9]. However, relatively few studies have been carried out in children, and the results described suggest that the performance of these assays differs from those obtained in adults, especially in countries with a high incidence of TB and TB in young children[10]. The absence of a reference test for these techniques makes their evaluation and comparison difficult[8]. With the latent class model defined by Dendukuri and Joseph (2001) the prevalence and the performance parameters of several diagnostic tests without gold standard can be estimated[11].

The objective of the present study was to estimate the prevalence of LTBI and determine the accuracy and safety of the TST and QuantiFERON-TB Gold In-Tube (QFT) tests in young children taking into account the intensity of exposure of the children to an index case. The population studied included children up to 6 years of age vaccinated with the BCG in Manaus (Brazil) during the years 2009–2010.

Material and Methods

Study design and population

We conducted a cross-sectional study of children to calculate the prevalence and accuracy of the TST and QFT. The study included a sample of children of up to 6 years of age without recent known contact with an index TB case and another group of children in contact with an index case within the previous 12 months. Case recruitment was performed in the *Policlínica*

Cardoso Fontes (regional reference center for TB) and in the *Fundação de Medicina Tropical Dr. Heitor Vieira Dourado* (FMT-HVD), Manaus, Amazonas, Brazil from March 2009 to February 2010. Adults (greater than 12 years of age) diagnosed with TB in both centers were questioned about contact with children from 0 to 6 years of age. Those responding affirmatively were asked to bring the children to the center for evaluation and were invited to participate in the study. All the adult index cases were sputum smear and/or culture positive. Subjects receiving treatment or prophylaxis for TB were excluded. The controls were selected among children attended in both centers for reasons other than TB. The study was undertaken at an outpatient level.

Ethics Statement

The study was approved by the Ethical Committee in Investigation of the FMT-HVD, October 26, 2007 (Protocol 2865–07). The legal guardians of all the participants provided written informed consent for inclusion in the study.

Data collection

Demographic data as well as the epidemiologic history of exposure to an index case of TB and the clinical history of the patient and physical examination were recorded. Chest X-ray, the TST, and blood analysis (prior to the TST) were performed. The human immunodeficiency virus (HIV) test was not obligatory but was recommended to all the participants.

The *Mycobacterium tuberculosis* contact score (MTC-score) was used to evaluate the intensity of exposure. The MTC-score is from 0 to 15 and is based on the assumption that the gradient of Mtb exposure is a composite function of the infectivity of the index case (0–4), the duration of exposure in hours per day (0–4), the relationship to the index case (0–4) and the type of exposure (0–3)[12].

The time (months) from symptom onset to the time of initiation of treatment of the index case was calculated to measure the total exposure time (contagion) of the child to the index case.

Procedures

The TST was performed with an intradermic injection of 2 tuberculin units (TU) of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark) and read 72 hours thereafter. A strong TST reaction (TST+) was defined when induration was $\geq$10 mm [7].

The QFT test (Cellestis, Carnegie, Australia) was carried out and interpreted according to the manufacturer's instructions by experienced laboratory technicians who were unaware of the data of the study subjects.

Statistical analysis

Categorical variables were described using absolute frequencies and percentages and quantitative variables using the 25, 50 and 75 percentiles. The chi-square test was used to study the association between the results of the two tests and the variables of gender and age groups (less than or equal to 24 months and greater than 24) and the Mann Whitney U test was used for the exposure time and the MTC-score variables. Concordance between the QFT and the TST tests was measured by the kappa index.

The statistical analysis was divided into two phases. In the first phase the accuracy of the two tests was estimated, and the prevalence of LTBI was determined based on whether the children had been in contact with an index case or not. To estimate these parameters the proposal

of Dendukuri N. and Joseph L. (2001) was used[11], stratifying the model to estimate the prevalence of LTBI in children with and without contact with an index case. Then, it was assumed that the combinations of both test results were distributed by a multinomial distribution with probabilities equal to:

$$P(\text{QFT} = +, \text{ TST} = +) = P_i \cdot [(S_1 \cdot S_2) + \text{covS}_{12}] + (1 - P_i) \cdot [((1 - C_1) \cdot (1 - C_2)) + \text{covC}_{12}]$$

$$P(\text{QFT} = +, \text{ TST} = -) = P_i \cdot [(S_1 \cdot (1 - S_2)) - \text{covS}_{12}] + (1 - P_i) \cdot [((1 - C_1) \cdot C_2) - \text{covC}_{12}]$$

$$P(\text{QFT} = -, \text{ TST} = +) = P_i \cdot [((1 - S_1) \cdot S_2) - \text{covS}_{12}] + (1 - P_i) \cdot [(C_1 \cdot (1 - C_2)) - \text{covC}_{12}]$$

$$P(\text{QFT} = -, \text{ TST} = -) = P_i \cdot [((1 - S_1) \cdot (1 - S_2)) + \text{covS}_{12}] + (1 - P_i) \cdot [(C_1 \cdot C_2) + \text{covC}_{12}]$$

Where P_i is the prevalence of LTBI for the i*th* stratum (1: children with contact with an index case, and 2: without contact with an index case), S_1 and S_2 are the sensitivity of QFT and the TST test respectively, and C_1 and C_2 are the specificities. CovS_{12} and covC_{12} are the covariance between tests among subjects with latent infection and non-latent infection, respectively.

Both prevalences were assumed to have a Beta(1,1) prior distribution. Based on the literature of LTBI, we reasonably considered that the sensitivity and the specificity of the two tests lie within the range of 50%-100%. Consequently, the prior distribution for these parameters was a Beta(8.25, 2.75). The covariance parameters are taken to have Beta(1,1) prior distribution, and their feasible ranges were given as $0 \leq covS_{12} \leq \min(S_1,S_2) - S_1S_2$ and $0 \leq covC_{12} \leq \min(C_1,C_2) - C_1C_2$,

According to the proposal of Martinez E.Z. (2008),[13], in the second phase the previous model is widened and rather than study the prevalence based on whether there was or was not exposure to an index case it is studied based on the magnitude of intensity and the time of exposure to an index case. Denoting the true infection status (latent) of the k*th* subject as D_k (0: among non-infected subjects and 1 among infected subjects), the prevalence of LTBI is the probability of being truly infected $P(D_k = 1)$, and it is given as covariates:

$$P(D_k = 1) = \Phi(\beta_0 + \beta_1 \times ExpositionTime + \beta_2 \times MTC - Score)$$

Φ: represents the cumulative distribution function of the Normal(0,1) distribution

The conditional dependence between tests is modeled using a random effects model[14]. In this case, the sensitivities and specificities of the tests are modeled as a function of subject-specific random effect, i_k. Thus, the probability that the k*th* subject has a positive result in the j*th* test (1: QFT and 2: TST) is given as:

$$P(T_{jk} = +|D_k = 1, I_k = i_k) = \Phi(\beta_{3j} + b_1 i_k)$$

And has a negative result

$$P(T_{jk} = -|D_k = 0, I_k = i_k) = \Phi(\beta_{4j} + b_0 i_k)$$

where:

i_k: is the intensity of the *k*th subject, and it is a random variable following a Normal(0,1) distribution.

Thus, the mean sensitivity of the *jth* test in all the subjects was:

$$S_j = P(T_j = +|D = 1) = \int_{-\infty}^{\infty} P(T_{jk} = +|D_k = 1, I_k = i_k)d\Phi(i_k) = \Phi\left(\frac{\beta_{3j}}{\sqrt{1 + b_1^2}}\right)$$

and the mean specificity of the *jth* test in all the subjects was:

$$C_j = P(T_j = -|D = 0) = \int_{-\infty}^{\infty} P(T_{jk} = -|D_k = 0, I_k = i_k) d\Phi(i_k) = \Phi\left(-\frac{\beta_{4j}}{\sqrt{1 + b_0^2}}\right)$$

Using the estimates obtained in the first phase of the prevalence, sensitivity, specificity and the covariance between test, the parameters of the prior distributions from the constants of the model were $\beta_0 \sim$ Normal(-1.59,1.8), $\beta_{31} \sim$ Normal(0.24,0.32), $\beta_{32} \sim$ Normal(0.83,0.49), $\beta_{41} \sim$ Normal(1.15,0.32) and $\beta_{42} \sim$ Normal (2.01,0.48) and for the b_1 and b_0 were Normal(0.77,0.5) and Normal(0.99,0.5), respectively. The prior distribution for the coefficient of the exposure time and MTC-score was a Normal (0,0.1), respectively[15].

Results

A total of 121 children were studied, 92 (76.0%) of whom had had contact with an index case and 29 (24.0%) had not. All the children studied had received the BCG vaccination in the first 2 months of life. Sixteen children (13.2%) were excluded from the analysis because they presented an indeterminate result with QFT. Of the 105 children analyzed, 41.9% were male and 23.8% were less than 24 months of age. Twenty-five children (23.8%) had had no contact with an index case but in 3 children result was positive for the QFT test but not for the TST test (Fig 1). There were no significant differences when comparing age and gender in exposed children and controls. Of the 80 (76.2%) children having contact with an index case, the median MTC-score was 12.0 points (IQR = 3.75), and the time of exposure was 2 months (IQR = 4.0). Thirty-one children (38.8%) showed a positive QFT result and 33 (41.2%) did so with the TST, but only 19 children (23.9%) were positive for both tests. None of the positive TST results were weak (between 5–9 mm). The two tests showed a low concordance of 0.364 (p< 0.001).

The results of the two tests were not associated with either the gender or age of the children. The time and the intensity of the exposure were associated with the QFT and TST results (Table 1). The time and the intensity of the exposure of the children with a positive QFT result (median time of exposure = 2; median MTC-score = 12.0) was greater than that of children with a negative QFT (median time of exposure = 1.0; p = 0.026; median MTC-score = 10.0, p = 0.021). The time and the intensity of the exposure of the children with a positive TST result (median time of exposure = 4; median MTC-score = 12.0) was greater than in those with a negative TST result (median time of exposure = 1.0, p<0.001, median MTC-score = 9.5, p<0.001).

Bayesian Analysis

The prevalence of infection in the group of children without contact with an index case was 0.04 (95% CrI [0.00, 0.20], being 0.50 in children with contact (95% CrI [0.28, 0.81]). The sensitivity of the QFT test was 0.58 (95% CrI [0.41, 0.78]) and 0.75 (95% CrI [0.49, 0.94]) for the TST test. The specificity of the QFT was 0.79 (95% CrI [0.67, 0.91]) and 0.92 (95% CrI [0.78, 0.98]) for the TST test (Table 2). The positive predictive values of both tests were very low in the group of children with no contact with an index case and thus, the prevalence of infection was very low. In the group of children with contact with an index case, the probability of a child diagnosed as positive really being infected was 0.74 (95% CrI [0.47, 0.95]) for QFT and 0.91 (95% CrI [0.61, 0.99] for TST. Moreover, the probability of a child diagnosed as negative really not being infected was 0.65 (95% CrI [0.27, 0.88]) for QFT and 0.79 (95% CrI [0.31, 0.96]) for TST. On evaluating the positive predictive value with the results of both tests together we found that the probability of really being infected when the two tests were positive was 0.93
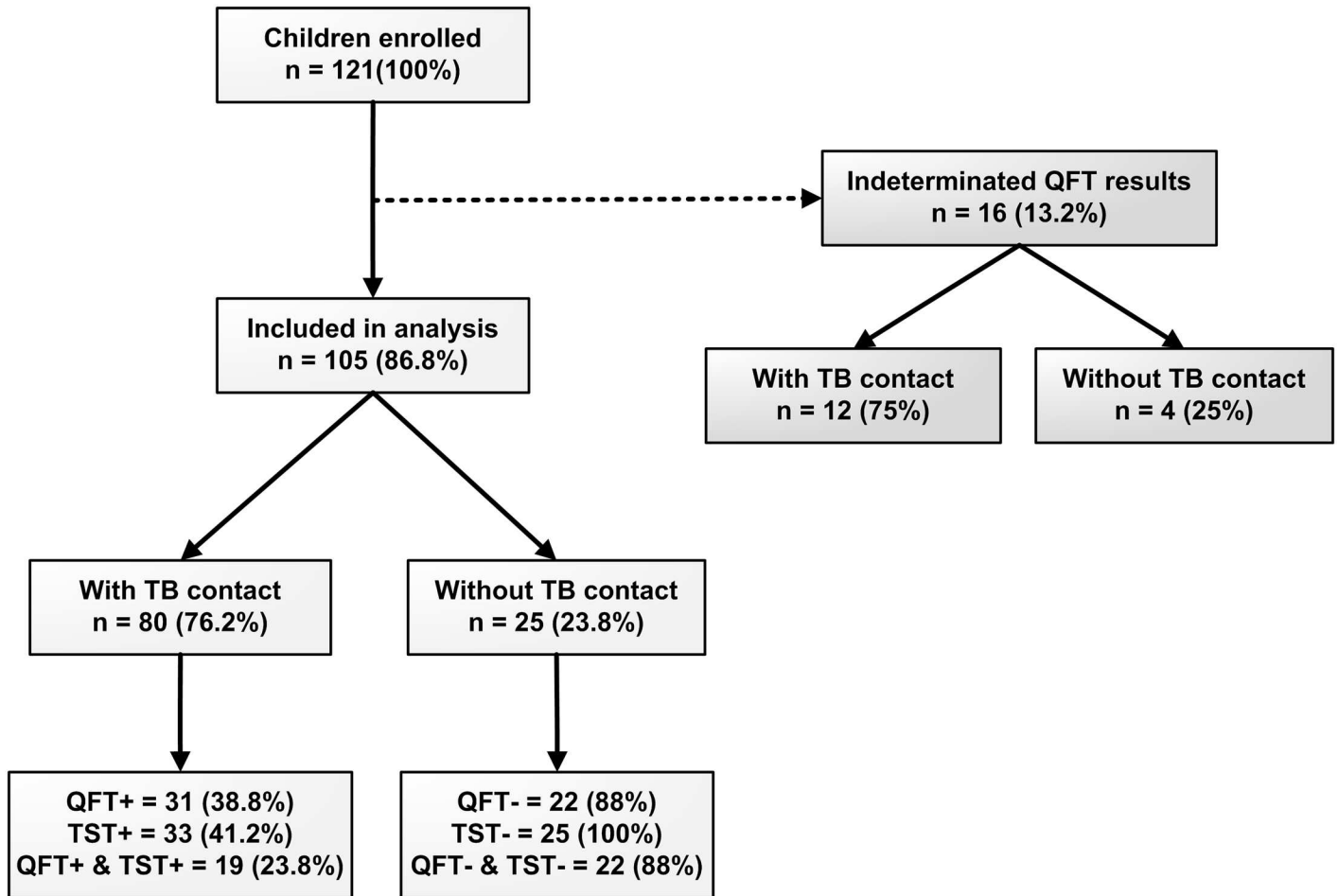
Fig 1. Flow diagram of enrollment. TB: tuberculosis, QFT: QuantiFERON-TB Gold In-Tube, TST: Tuberculin Skin Test.

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Table 1. Descriptive analysis and comparisons between the results of the QFT and the TST test in children up to 6 years of age in Manaus, Brazil.

	QFT			TST		
	+	-	p-value	+	-	p-value
Male	14 (31.8%)	30 (68.2%)	0.917	14 (31.8%)	30 (68.2%)	0.942
Female	20 (32.8%)	41 (67.2%)		19 (31.1%)	42 (68.9%)	
Age< = 24 months	7 (28.0%)	18 (72.0%)	0.592	8 (32.0%)	17 (68.0%)	0.944
Age >24 months	27 (33.8%)	53 (66.2%)		25 (31.2%)	55 (68.8%)	
Age (months)*	47.76 (22.5)	43.68 (21.5)	0.384	43.91 (22.7)	45.50 (22.4)	0.737
Exposure Time**	2 (5.25–1)	1 (0–3)	0.026	4 (1–10)	1 (0–3)	<0.001
MTC-score**	12 (14.0–8.75)	10 (0–12)	0.021	12 (10–14)	9.5 (0–12)	<0.001

* mean (standard deviation). Student t.

**median (quartile 25-quantile 75). Mann-Whitney U.

TST: Tuberculin Skin Test,QFT: QuantiFERON-TB Gold In-Tube, MTC-SCORE: *Mycobacterium tuberculosis* contact score.

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Table 2. Results of the prevalence of LTBI according to exposure to an index TB case and the sensitivity and the specificity of QFT and TST in children up to 6 years of age in Manaus, Brazil (first phase).

	Median	95% CrI
P(LTBI) Unknown exposure of children	0.04	0.00–0.20
P(LTBI) Exposed Children	0.50	0.28–0.81
Sensitivity of QFT	0.58	0.41–0.78
Specificity of QFT	0.79	0.67–0.91
Sensitivity of TST	0.75	0.49–0.94
Specificity of TST	0.92	0.78–0.98

CrI: Credibility interval, TST: Tuberculin Skin Test, QFT: QuantiFERON-TB Gold In-Tube, MTC-SCORE: *Mycobacterium tuberculosis* contact score, LTBI: latent tuberculosis infection.

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(95% CrI [0.69, 0.99]). When only the QFT was positive this value was 0.35 (95% CrI [0.04, 0.90]), being 0.90 (95% CrI [0.30, 0.99]) when only the TST was positive. In relation to the negative predictive values, the probability of not being infected when both tests were negative was 0.83 (95% CrI [0.37, 0.97]), and when only the QFT was negative this value was 0.1 (95% CrI [0.01, 0.7]), being 0.65 (95% CrI [0.10, 0.96]) (Table 3) when only the TST was negative.

Taking into account the magnitude of the intensity (MTC-score) and the time of exposure, according to the model used, the prevalence of LTBI increased with the intensity (beta = 0.14, 95% CrI [0.06, 0.23]) and the time of exposure (beta = 0.19, 95% CrI [0.05, 0.49]) (Tables 4 and 5). Fig 2 shows the values of prevalence for the times of exposure of 0, 1, 3, 6 and 12 months and MTC-score values of 4, 8, 13 and 15. Children with an exposure time of 12 months and MTC-score greater than 8 presented prevalences greater than 75% while children with low exposure times (less than or equal to 1 month) and a MTC-score less than 5 presented prevalences of less than 25%. On taking into account the intensity and time of exposure in this model, the sensitivity and specificity of the tests was slightly modified. Fig 3A and 3B show the positive predictive values based on the intensity and time of exposure. The results indicate that with high exposure times (greater than or equal to 6 months) and exposures of greater than or equal to 13, the predictive value of QFT was greater than 0.70, being greater than 0.9 for TST. The negative predictive values of both tests showed a similar behavior, being high with a low time and intensity of exposure and low when the time and intensity were high (Fig 3C and 3D).

Table 3. Probability of LTBI in children up to 6 years of age according to the results of both tests in the latent class model (first phase).

	Unknown exposure of children		Exposed Children	
	Median	95% CrI	Median	95% CrI
P(LTBI|QFT+)	0.01	0.00–0.51	0.74	0.47–0.95
P(LTBI|TST+)	0.27	0.01–0.79	0.91	0.61–0.99
P(LTBI|QFT+ TST+)	0.31	0.01–0.83	0.93	0.69–0.99
P(LTBI|QFT+ TST-)	0.02	0.00–0.34	0.35	0.04–0.90
P(LTBI|QFT- TST+)	0.29	0.01–0.82	0.90	0.31–0.99

CrI: Credibility interval, TST: Tuberculin Skin Test, QFT: QuantiFERON-TB Gold In-Tube, MTC-SCORE: *Mycobacterium tuberculosis* contact score, LTBI: latent tuberculosis infection.

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Table 4. Probability of non LTBI (LTBI^c) in children up to 6 years of age according to the results of both tests in the latent class model (first phase).

	Unknown exposure of children		Exposed Children	
	Median	95% CrI	Median	95% CrI
P(LTBI^c|QFT-)	0.98	0.88–1.00	0.65	0.27–0.88
P(LTBI^c|TST-)	0.99	0.90–1.00	0.79	0.31–0.96
P(LTBI^C|QFT+ TST-)	0.98	0.66–1.00	0.64	0.10–0.96
P(LTBI^c|QFT- TST+)	0.76	0.18–0.99	0.10	0.01–0.69
P(LTBI^c|QFT- TST-)	0.99	0.93–1.00	0.83	0.37–0.97

CrI: credibility interval, TST: Tuberculin Skin Test, QFT: QuantiFERON-TB Gold In-Tube, MTC-SCORE: *Mycobacterium tuberculosis* contact score, LTBI: latent tuberculosis infection.

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Discussion

Estimation of the prevalence of a disease and the parameters of sensitivity, specificity and predictive values of the tests used to study this disease in the absence of a gold standard are an important advance for the evaluation of diagnostic tests. This is of special relevance in the current epidemiological and diagnostic context of the study of LTBI in which it is impossible to determine if an individual is really infected or whether this person presents immune response to the previous infection.

The prevalence of LTBI in the group exposed to an index case was 50.4% (27.1%-83.3%), and this prevalence or probability of becoming infected increases with both the intensity (MTC-score) and the time of exposure to the index case[16]. The prevalence in the control group was 3.9% (0.10%-20.6%).

The prevalence obtained suggests that young children are very susceptible to exposure to an index case, with this prevalence possibly being greater than in other population groups[17]. On the other hand, most of the cases of LTBI and TB disease in young children are the result of exposure to a known close index case of TB[5]. These two reasons reinforce the crucial importance of diagnostic strategies of infection and disease in household pediatric contacts in order to control TB[18].

Knowledge of the prevalence of LTBI based on the time and intensity of exposure allows estimation of the prevalence and thus, the risk of LTBI in a young child before obtaining the result of the diagnostic tests. Indeed, we found that the high intensity and time of exposure result in very high prevalences (greater than 70%).

Table 5. Estimation of the sensitivity and specificity of QFT and TST, and the coefficients of regression of the variables of time of exposure and MTC-Score associated with the prevalence (second phase).

Variable	Median	95% CrI
Exposure Time	0.19	0.05–0.49
MTC-Score	0.14	0.06–0.23
Sensitivity of QFT	0.53	0.41–0.66
Specificity of QFT	0.81	0.71–0.90
Sensitivity of TST	0.73	0.53–0.91
Specificity of TST	0.97	0.89–1.00

CrI: credibility interval, TST: Tuberculin Skin Test, QFT: QuantiFERON-TB Gold In-Tube, MTC-SCORE: *Mycobacterium tuberculosis* contact score, LTBI: latent tuberculosis infection.
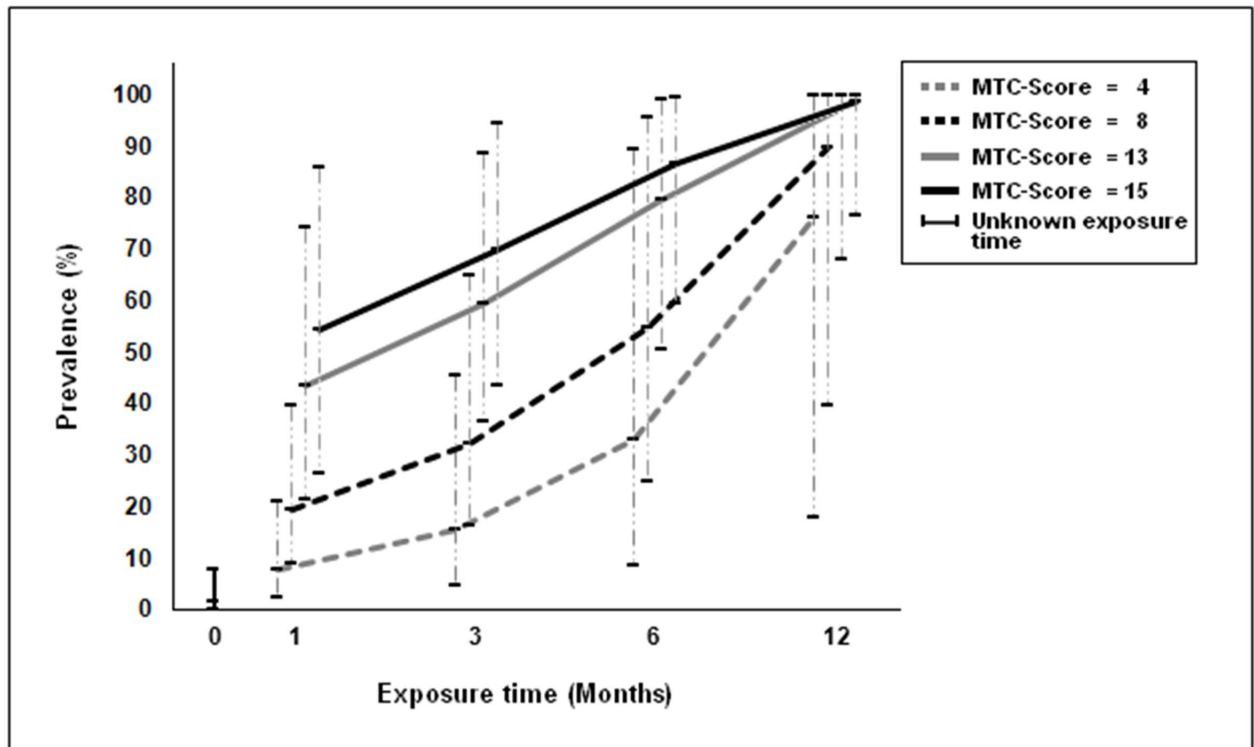
doi:10.1371/journal.pone.0164181.t005

Fig 2. Prevalence of LTBI according to the time of exposure of 0, 1, 3, 6 and 12 months and the MTC-score values of 4, 8, 13 and 15 in children up to 6 years of age in Manaus, Brazil. MTC-SCORE: *Mycobacterium tuberculosis* contact score.

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Since it is difficult to precisely know at what time an individual is contagious, we estimated the period of contagion from the time at which the index case shows symptomatology compatible with TB until the time of treatment. Likewise, to evaluate the intensity of exposure with the index case we used the MTC-score which assesses the capacity of contagion of the index case and the grade of relationship with the same. Our results suggest that the use of the time of exposure and MTC-score are feasible for study models of LTBI in children.

Concordance between the TST and QFT was lower than expected. This has been reported previously in young children, especially in regions with a high incidence of TB[19] and may be related to coinfection with helminths, malnutrition, short exposure times, immunosuppression and reversion phenomena in the IGRAs[16,20,21].

Despite the low concordance, the tests presented similar sensitivities and specificities, without significant differences. It is of note that the specificity of the TST was not lower than QFT. This result is surprising in view of the widespread opinion that the BCG vaccine produces false positives in the TST[22]. Some authors have suggested that this effect is lower in tropical regions[23], and the type of BCG strain vaccinated may also have different effects on the TST [24].

The predictive values which depend on the prevalence and accuracy of the tests did not show significant differences according to the test applied, despite the TST being superior in all the values. Likewise, the results obtained suggest that both tests have a very high predictive capacity when the intensity and time of exposure are high and are therefore useful for diagnosis in the study of contacts. In the case of controls we observed very low positive predictive values thereby indicating the scarce utility of these tests in population screenings [2].
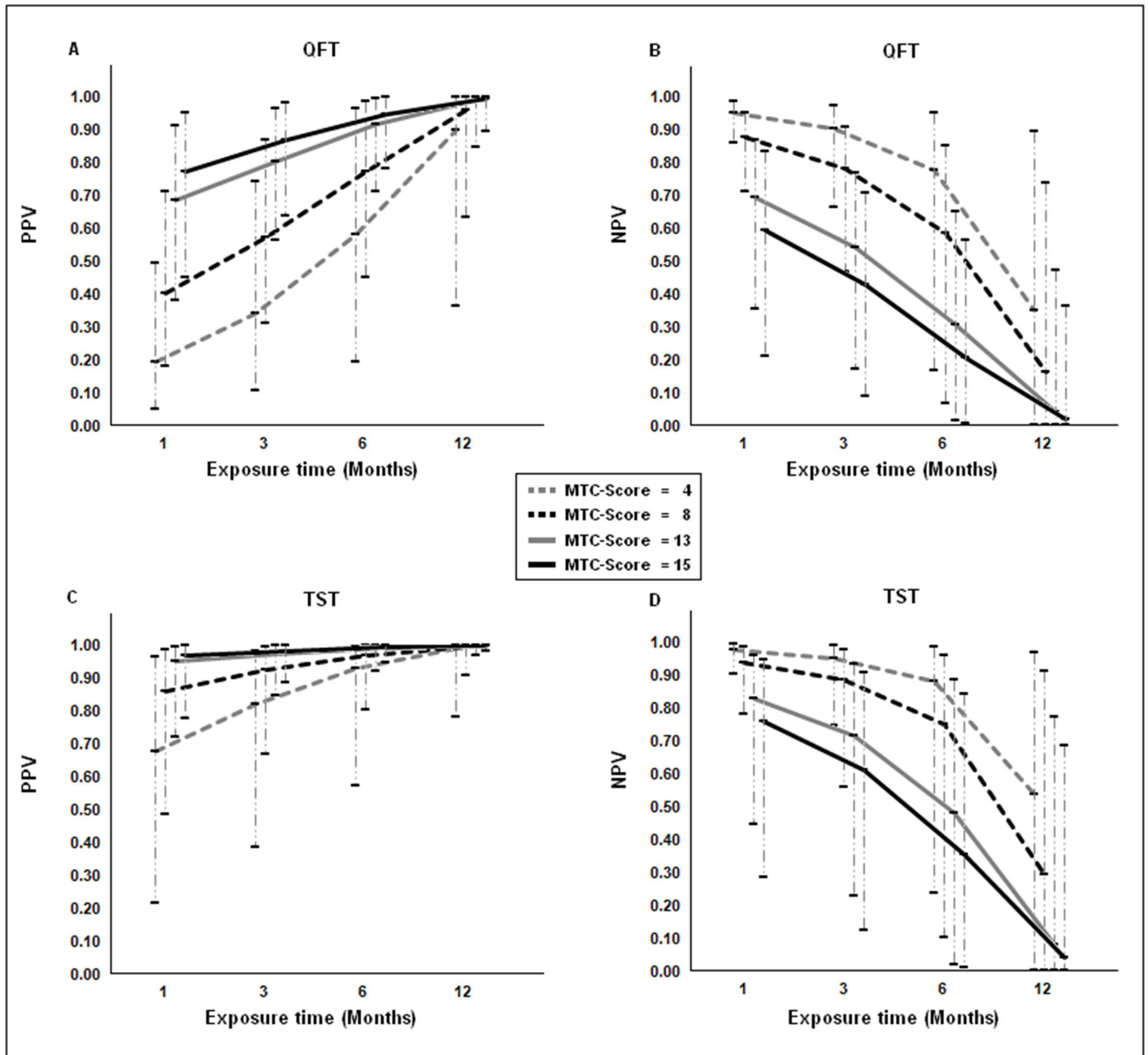
Fig 3. Positive and negative predictive values for QFT for times of exposure of 1, 3, 6, and 12 months and intensity of exposure of 4, 8, 13 and 15. TST: Tuberculin Skin Test, QFT: QuantiFERON-TB Gold In-Tube, PPV: positive predictive values, NPV: negative predictive values.

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On evaluating the results of the predictive values in the two tests combined it was found that these values in the two tests alone improved, albeit not significantly. According to the latent variable model, when the results between the tests were discordant, the TST presented a greater probability of correctly classifying the individual. Thus, in young children QFT results discordant with those of TST should be interpreted with caution since the predictive values fall markedly. This may explain, in part, the discordance observed between the two tests in short times of exposure similar to what has previously been described in this group[16].

The indeterminate results for QFT were high in our study. The proportion of indeterminate results varies greatly in studies in children [25–27] and may be associated with technical problems and probably also factors which alter Th1 immune response[28] such as age, the presence of helminths, and immunosuppression[29].

This study has several limitations. The relatively small sample size may have limited the power of the study to find significant differences. Nonetheless, there are few studies in small children and the results obtained are biologically logical. In addition, the sensitivity and specificity may vary widely among studies and this may affect the bayesian analysis. However, we have attempted to control this with the use of wide priors for the sensitivity and specificity of both tests. The third limitation was that we used the WHO definition of TST positivity ($\geq$10 mm), and other cut off points might have shown different results.

On the other hand, the strengths of this study are: 1. There are few studies in small children who are the most vulnerable to *M. tuberculosis*. 2. The methodology used is novel in TB but has been well contrasted in other infectious diseases [30]. This study allows calculation of the prevalence of the disease which, to date, has not been done since LTBI cannot currently be microbiologically demonstrated due to its low bacillary load. Up to now both the prevalence and the accuracy of the tests has, in many cases, been calculated using an imperfect gold standard (one of the two tests) or has been extrapolated from ill children, who are clinically different from those who are infected. 3. The results obtained are biologically logical since the test results are correlated with the time and intensity of exposure.

Conclusions

This is one of the first studies to estimate the prevalence of LTBI in children and the parameters of its main diagnostic tests using a latent class model. The study of LTBI in young children is of great importance due to the high possibility of developing the disease and increasing the reservoir of cases which these cases may represent in the future if not treated[5,6]. The results of the present study suggest that children in contact with an index case have a high risk of infection according to the intensity and length of exposure to the index case, and this should be taken into account in the design of TB control strategies.

The TST and QFT showed similar characteristics and behavior but concordance was lower than expected. Combined use of the two tests in our study showed scarce improvement in the diagnosis of LTBI. We know that diagnostic tests of LTBI are not perfect. However, the latent variable model allows better evaluation of these tests and estimation of the prevalence of LTBI, thereby facilitating the development of more adequate approaches to this crucial problem of TB control. The diagnosis of LTBI and disease is the key to the control of pediatric TB and requires more and greater efforts to this end.

Author Contributions

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References

1. WHO | Global tuberculosis report 2014. In: WHO [Internet]. [cited 21 Jan 2016]. Available: http://www.who.int/tb/publications/global_report/en/

2. WHO | Systematic screening for active tuberculosis: principles and recommendations. In: WHO [Internet]. [cited 21 Jan 2016]. Available: http://www.who.int/tb/tbscreening/en/

3. Marais BJ, Gie RP, Schaaf HS, Hesseling AC, Obihara CC, Starke JJ, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis. 2004; 8: 392–402. PMID: 15141729

4. Lewinsohn DA, Lewinsohn DM. Immunologic susceptibility of young children to *Mycobacterium tuberculosis*. Pediatr Res. 2008; 63: 115. doi: 10.1203/PDR.0b013e3181652085 PMID: 18209663

5. Dodd PJ, Gardiner E, Coghlan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Glob Health. 2014; 2: e453–459. doi: 10.1016/S2214-109X(14)70245-1 PMID: 25103518

6. Starke JR. Pediatric tuberculosis: time for a new approach. Tuberc Edinb Scotl. 2003; 83: 208–212. doi: 10.1016/S1472-9792(02)00088-4 PMID: 12758213

7. WHO | Guidance for national tuberculosis programmes on the management of tuberculosis in children. In: WHO [Internet]. [cited 21 Jan 2015]. Available: http://www.who.int/tb/publications/childtb_guidelines/en/

8. Pai M, Riley LW, Colford JM. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis. 2004; 4: 761–776. doi: 10.1016/S1473-3099(04)01206-X PMID: 15567126

9. Starke JR, Committee On Infectious Diseases. Interferon-γ release assays for diagnosis of tuberculosis infection and disease in children. Pediatrics. 2014; 134: e1763–1773. doi: 10.1542/peds.2014-2983 PMID: 25422024

10. Sollai S, Galli L, de Martino M, Chiappini E. Systematic review and meta-analysis on the utility of Interferon-gamma release assays for the diagnosis of *Mycobacterium tuberculosis* infection in children: a 2013 update. BMC Infect Dis. 2014; 14 Suppl 1: S6. doi: 10.1186/1471-2334-14-S1-S6 PMID: 24564486

11. Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. Am J Epidemiol. 1995; 141: 263–272. PMID: 7840100

12. Hesseling AC, Mandalakas AM, Kirchner HL, Chegou NN, Marais BJ, Stanley K, et al. Highly discordant T cell responses in individuals with recent exposure to household tuberculosis. Thorax. 2009; 64: 840–846. doi: 10.1136/thx.2007.085340 PMID: 18682523

13. Martinez EZ, Louzada-Neto F, Derchain SFM, Achcar JA, Gontijo RC, Sarian LOZ, et al. Bayesian estimation of performance measures of cervical cancer screening tests in the presence of covariates and absence of a gold standard. Cancer Inform. 2008; 6: 33–46. PMID: 19259401

14. Dendukuri N, Joseph L. Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. Biometrics. 2001; 57: 158–167. doi: 10.1111/j.0006-341X.2001.00158.x PMID: 11252592

15. Gelman A, Rubin DB. Markov chain Monte Carlo methods in biostatistics. Stat Methods Med Res. 1996; 5: 339–355. doi: 10.1177/096228029600500402 PMID: 9004377

16. Pérez-Porcuna TM, Ascaso C, Malheiro A, Abellana R, Martins M, Sardinha JFJ, et al. *Mycobacterium tuberculosis* infection in young children: analyzing the performance of the diagnostic tests. PloS One. 2014; 9: e97992. doi: 10.1371/journal.pone.0097992 PMID: 24879374

17. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2013; 41: 140–156. doi: 10.1183/09031936.00070812 PMID: 22936710

18. Cruz AT, Starke JR, Lobato MN. Old and new approaches to diagnosing and treating latent tuberculosis in children in low-incidence countries. Curr Opin Pediatr. 2014; 26: 106–113. doi: 10.1097/MOP.0000000000000049 PMID: 24299911

19. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, Hatherill M, Moyo S, Hanekom W, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. Pediatr Infect Dis J. 2011; 30: 694–700. doi: 10.1097/INF.0b013e318214b915 PMID: 21427627

20. Thomas TA, Mondal D, Noor Z, Liu L, Alam M, Haque R, et al. Malnutrition and helminth infection affect performance of an interferon gamma-release assay. Pediatrics. 2010; 126: e1522–1529. doi: 10.1542/peds.2010-0885 PMID: 21059723

21. Pai M, Joshi R, Dogra S, Zwerling AA, Gajalakshmi D, Goswami K, et al. T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis. 2009; 13: 84–92. PMID: 19105884

22. Seddon JA, Paton J, Nademi Z, Keane D, Williams B, Williams A, et al. The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: evidence to be considered when screening children for tuberculosis infection. Thorax. 2016; 71: 932–939. doi: 10.1136/thoraxjnl-2015-207687 PMID: 27335104

23. Hill PC, Ota MOC. Tuberculosis case-contact research in endemic tropical settings: design, conduct, and relevance to other infectious diseases. Lancet Infect Dis. 2010; 10: 723–732. doi: 10.1016/S1473-3099(10)70164-X PMID: 20883968

24. Ritz N, Dutta B, Donath S, Casalaz D, Connell TG, Tebruegge M, et al. The influence of bacille Calmette-Guerin vaccine strain on the immune response against tuberculosis: a randomized trial. Am J Respir Crit Care Med. 2012; 185: 213–222. doi: 10.1164/rccm.201104-0714OC PMID: 22071384

25. Bergamini BM, Losi M, Vaienti F, D'Amico R, Meccugni B, Meacci M, et al. Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. Pediatrics. 2009; 123: e419–424. doi: 10.1542/peds.2008-1722 PMID: 19254978

26. Connell TG, Ritz N, Paxton GA, Buttery JP, Curtis N, Ranganathan SC. A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. PLoSONE. 2008; 3: e2624. doi: 10.1371/journal.pone.0002624 PMID: 18612425

27. Haustein T, Ridout DA, Hartley JC, Thaker U, Shingadia D, Klein NJ, et al. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. Pediatr Infect Dis J. 2009; 28: 669–673. doi: 10.1097/INF.0b013e3181a16394 PMID: 19633512

28. Grare M, Derelle J, Dailloux M, Laurain C. QuantiFERON-TB Gold In-Tube as help for the diagnosis of tuberculosis in a French pediatric hospital. Diagn Microbiol Infect Dis. 2010; 66: 366–372. doi: 10.1016/j.diagmicrobio.2009.11.002 PMID: 20071130

29. Thomas TA, Mondal D, Noor Z, Liu L, Alam M, Haque R, et al. Malnutrition and helminth infection affect performance of an interferon gamma-release assay. Pediatrics. 2010; 126: e1522–1529. doi: 10.1542/peds.2010-0885 PMID: 21059723

30. Pai M, Dendukuri N, Wang L, Joshi R, Kalantri S, Rieder HL. Improving the estimation of tuberculosis infection prevalence using T-cell-based assay and mixture models. Int J Tuberc Lung Dis Off J Int Union Tuberc Lung Dis. 2008; 12: 895–902. PMID: 18647448