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Research Article

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Performance of Commercial Interferon-γ Release Assays for Screening Latent Tuberculosis Infection: A Meta-Analysis

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Abstract

Context: This meta-analysis was performed to systematically evaluate two interferon gamma release assays (IGRAs): ELISA and ELISPOT along with Tuberculin Skin Test (TST) in the detection of latent tuberculosis infection.

Methods: PUBMED, MEDLINE, and OVID data bases were searched between January 2000 and October 2012 for serial IGRA and TST results in screening LTBI among children. Meta-analysis was performed to estimate the sensitivity, specificity and predictive value of the two commercial IGRAs and TST.

Results: A gradient of exposure among contacts was used as the gold standard for evaluating the sensitivity of the IGRA and TST. For IGRA, the positive result rates gradually decreased with the reduced degree of exposure in ten studies, and for TST, the same trend was seen in seven studies. In children with an 88.6% BCG vaccination rate, IGRA had a high specificity (88% for QFT-IT and 90% for T-SPOT, respectively). The pooled specificity of TST was only 65%. Two articles conducted a longitudinal cohort study to determine the prognostic value of positive IGRA and TST results for development of active tuberculosis, and suggested IGRA has a very high negative predictive value; children with positive IGRA results were 3.4 times more likely than those with negative results to develop active disease.

Conclusion: Current evidence suggests that IGRA tests are likely to be specific tools to diagnose tuberculosis infection especially in children with BCG vaccination. Additional large cohort studies are needed to draw a clear conclusion of the prognostic value of the two tests.

Keywords: Meta-analysis; Interferon gamma; Diagnosis; Tuberculosis

Introduction

Tuberculosis (TB) in children is a significant, but neglected, worldwide health problem [1]. Of the estimated 8.3 million new cases of TB globally, about 11% occurred in children younger than 15 years of age [2,3]. It is well known that children in contact with contagious active tuberculosis are at high risk of infection and disease progression. Accordingly, contact investigations in children and adolescents are a major component of tuberculosis control activities. Multiple studies monitored large cohorts of children for screening latent tuberculosis infection (LTBI) in household Diagnosis of LTBI is mainly based on the tuberculin skin test (TST). This test suffers from a number of limitations, including false positive results because of the previous bacillus Calmette–Guérin (BCG) vaccination or nontuberculous mycobacteria (NTM) infection, poor responses in the condition of severe active tuberculosis or immune suppression, operator errors and a second visitation for reading the results. Thus, that could lead either to over diagnosis or to underestimation of TB.

In vitro interferon gamma release assays (IGRAs) have recently become available as alternative or complementary tests to TST. Two new tests are available as commercial kits: T-SPOT.TB test (TSPOT) (Oxford Immunotec, Oxford, United Kingdom) and QuantiFERON-TB Gold In-Tube (QFT-IT) (Cellestis, Carnegie, Australia). Although some authors highlight the ability of these tests to predict the risk of LTBI according to the exposure level [4,5], relatively fewer studies evaluate the assay in children.

The aim of this study is to better understand the value of IGRAs and TST in the diagnosis of tuberculosis infection in childhood contacts. Because there is no gold standard for testing LTBI, we estimated test sensitivity from studies of children who are categorized into different gradients of exposure (such as high or low exposure). We estimated specificity from studies of low-risk participants with no identified exposure.

Materials and Methods

Study strategy

We conducted a literature search of databases (PUBMED, MEDLINE, OVID) for articles published between January 2000 and October 2012. Search terms included latent tuberculosis infection and contact and interferon gamma release assay, or T-SPOT.TB test, or QuantiFERON-TB Gold In-Tube, or ESAT-6, or CFP-10, and childhood, or pediatrics. Only English-language articles were included. The references cited in the original studies concerning the relevant content were also retrieved.

Only studies that met the following selection criteria were included in the meta analysis: (a) articles that reported original data, reviews, case report and editorials were excluded, (b) studies that presented data on the commercial tests, QFT-IT and T-SPOT, (c) participants must not be co-infected with HIV or present with other immune compromises, (D) patients must not have received antituberculosis treatment.

Two individuals independently extracted data and reached consensus conclusions. One individual abstracted information, for example author's first name, year of publication, country of origin, number of cases and controls. The second individual reviewed the data for accuracy.

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Statistical analysis

For each study, positive rate, specificity and 95% CIs were calculated and summarized in forest plots. The between-study heterogeneity was investigated by Chi-square based *Q*-test. When heterogeneity was present, random effects model (DerSimonian and Laird; ($p<0.05$ and $I2>50\%$) was performed, and heterogeneity was not present the fixed effects model (Mantel- Haenszel) was performed. Statistical analysis was conducted using Meta-Disc, version 1.4 (Hospital Ramony Cajal, Madrid, Spain).

Results

Eligible studies

A total of 30 articles published between January 2000 and October 2012 were selected for full-text review. Eleven articles screened pediatric tuberculosis contacts were excluded because contacts were not grouped according to gradients of exposure resulting in 19 articles for analysis (Table 1). Twelve articles screened LTBI in 2691 children who are categorized into gradients of exposure, 10 articles were available for specificity evaluation and included healthy participants or respiratory tract infection (RTI) children without identified exposure, Figure 1). Studies ranged from 15 participants to more than 700 participants.

Sensitivity evaluated using a gradient of exposure

Among children recruited in the twelve articles available for screening LTBI who are categorized into gradients of exposure, some were household-exposed children contacts of TB cases diagnosed recently [6-10], some were referred to the outpatient clinic or health center to evaluate for TB infection [5,11-13], and some were school-contacts which have the identified contact history with a active TB teacher [14]. When participants were grouped according to their gradients of exposure, it is difficult to identify equivalence of the exposure categories because each study characterized exposure differently [15]. The risk of infection reference standard was TB exposure, defined dichotomously (exposed or not) or as a gradient [5], or based on index case bacteriological indicators (sputum smear) [9,10], or sleeping proximity of contact [7,14,16,17]. Therefore, we compared the positive result rates of the tests transversely in the same article.

For studies assessing sensitivity, a gradient of exposure was used as the gold standard. Table 2 shows the positive results of IGRA and TST in different risk groups. For IGRA, twelve studies could be included. When the positive result rates were compared transversely in each article, the prevalence of positive results was highest in the high exposure groups in eleven studies, and gradually decreased with the reduced degree of exposure in ten studies. For TST, eleven studies were included. The prevalence of positive results gradually decreased in the three risk groups in seven studies. Among seven studies that involved mostly BCG vaccinated populations [6,7,9,10,14,16,30], the prevalence of positive results on TST was higher than that of IGRA in four studies in all exposure groups. For example, one prospective study [16] included 98 children from contact-tracing studies, 69.4% of which were BCG vaccinated. The prevalence of positive results of IGRA and TST was 69.2% and 80.0% in high exposure group, 62.1% and 87.9% in moderate exposure group, and 35.3% and 75.0% in low exposure group, respectively.

Specificity evaluated in no risk factor participants

Because of the lack of gold standard for diagnosing LTBI, we

Table 1: Characteristics of studies included for Meta-analysis.

Study	Country	Participants,N.	BCG vaccination,%	Case resource	Index test
Rutherford et al. [30]	Indonesia	371	73.3	household contact	QFT- IT \TST
Adetifa et al. [7]	UK	215	59.1	household contact	QFT- IT \TSPOT\TST
Altet-Gómez et al. [16]	Spain	98	69.4	epidemiological screening & contact	QFT- IT \TSPOT\TST
Tsolia et al. [11]	Greece	99	45.3	hospital	QFT- IT \TST
Bergamini et al. [8]	Italy	91	38.9	household contact & immigration	QFT- IT \TSPOT\TST
Higuchi et al. [14]	Japan	308	98.7	school contact	QFT- IT\TST
Chun et al. [6]	South Korea	71	100	household contact	QFT- IT \TST
Okada et al. [9]	Japan	195	88	household contact	QFT- IT \TST
Nakaoka et al. [10]	UK	158	91.8	household contact	QFT- IT \TST
Critselis et al. [18]	Greece	761/341	45.0	hospital & healthy children	QFT- IT
Lighter et al. [5]	America	204/30	36.3	hospital & healthy children	QFT- IT\TST
Lighter et al. [12]	America	120/21	NR	hospital & healthy children	QFT- IT \TST
Alsleben et al. [31]	Germany	15	NR	RTI children	QFT- IT \TST
Yassin et al. [32]	Ethiopia	156	76.9	healthy children	QFT- IT \TST
Sun et al. [26]	China	51	90.2	RTI children	TSPOT\TST
Warier et al. [27]	India	47	92.5	healthy children	TSPOT
Hansted et al. [17]	Lithuania	52	100	healthy children	TSPOT\TST
Detjen et al. [28]	Germany	22	0	healthy children	QFT- IT \TSPOT\TST
Soysal et al. [29]	Turkey	209	100	healthy children	TSPOT\TST

- TSPOT: T-SPOT.TB test; QFT- IT : QuantiFERON-TB Gold In-Tube; TST: Tuberculosis skin test; NR: not reported; RTI: respiratory tract infection.

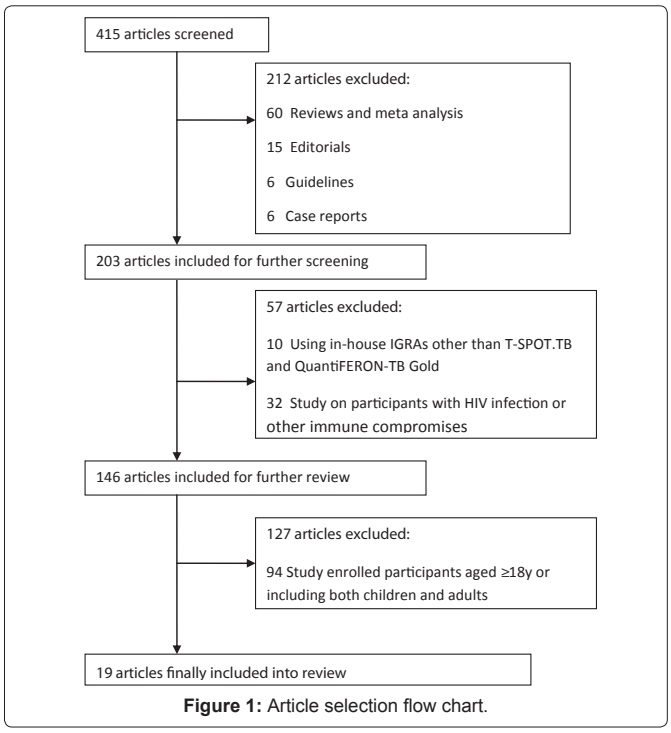

Figure 1: Article selection flow chart.

estimated specificity in participants with no known risk factors. In studies children considered at very low risk for latent TB infection (healthy or RTI), IGRA had a high specificity (Figure 2). The rate of BCG vaccination of the participants varied, and the pooled BCG vaccinated rate for all participants enrolled in this analysis was 88.6%. Pooled specificity was 88% in QFT-IT (95% CI, 85%-90%), and 90% in T-SPOT (95% CI, 86%-93%), respectively. On the other hand, the pooled specificity of TST was only 65% (95% CI, 61%-69%), which was statistically lower than that of IGRA.

Follow-up study of incidence of tuberculosis

In actuality, the only proof for LTBI is the later development of active TB. Two studies conducted a longitudinal cohort study to determine the prognostic value of positive IGRA and TST results for development of active tuberculosis in a key high-risk population: children with recent exposure to tuberculosis [14,19]. Higuchi et al. [14] evaluated usefulness of IGRA in primary school students exposed to a tuberculosis patient and observed that none of the students who were QFT-G negative developed active TB in the 3 year follow-up suggesting QFT-G has very high negative predictive value. Bakir et al. [19] studied children contacted for clinical followed-up every 6 months for 2 years, and were asked to return for further clinical assessment. Of the 381 contacts who had positive IGRA results, 11 (2.9%) progressed to active TB. Of the 550 contacts with positive TST results, 12 (2.2%) progressed to TB. Moreover, a significantly higher proportion of contacts had positive TST results than positive IGRA results (550 of 908 vs. 381 of 908, respectively $p<0.001$); data consistent with the higher specificity of IGRA.

Discussion and Conclusion

Children merit special consideration because they have sub-optional cellar immune responses and can develop severe forms of active disease following primary MTB infection. Childhood LTBI provides a large reservoir for future transmission and poses the risk of future reactivation disease. Accordingly, timely detection of LTBI and effective anti-tuberculosis therapy is critical to reduce the risk of disease progression. However, LTBI detection is hampered by the unavailability of an ideal gold standard. As a result, researchers and clinicians often use the measurement of the exposure gradient as surrogate standards.

Herein, IGRA and TST were compared in children with varying levels of exposure to TB patients with the aim of evaluating the sensitivity of the two tests in detecting LTBI. When a gradient of exposure among contacts was used as the gold standard, IGRA

Table 2: Studies comparing interferon gamma release assays (IGRAs) with tuberculin skin test (TST) according to the clinical gold standard of exposure gradients.

Study	Participants/BCG vaccinated rate, N/%.	Results by exposure gradient					
		High exposure		Moderate exposure		Low exposure	
		IGRA-positive,%	TST-positive,%	IGRA-positive,%	TST-positive,%	IGRA-positive,%	TST-positive,%
Critselis et al. [18] [a]	761(45.0)	37.4	-	43.6	-	14.1	-
Rutherford et al. [30] [a]	371(73.3)	52.2	48.2	20.8	9.7	-	-
Altet-Gómez et al. [16] [a]	98(69.4)	69.2	80.0	62.1	87.9	35.3	75.0
Tsolia et al. [11] [a]	99(45.3)	36.6	46.6	32.2	64.3	9.1	63.3
Lighter et al. [5] [a]	204/30 (36.3)	100.0	61.5	14.0	61.5	0	26.7
Lighter et al. [12] [a]	120/21(NR)	75.0	83.3	23.0	96.6	0	14.3
Higuchi et al. [14] [a]	308(98.7)	9.8	34.2	1.8	28.7	-	-
Chun et al. [6] [a]	71(100.0)	19.0	33.3	6.9	24.1	-	-
Okada et al. [9] [a]	195(88.0)	32.0	43.0	18.0	27.0	19.0	22.0
Nakaoka et al. [10] [a]	158(91.8)	74.2	54.5	8.8	15.9	-	-
Adetifa et al. [7] [b]	215(59.1)	56.0	60.0	33.1	27.1	25.7	14.0
Bergamini et al. [8] [b]	91(38.9)	33.2	39.2	5.5	10.1	-	-

- a: The study uses the kit of QuantiFERON-TB Gold In-Tube; b:The study uses both QuantiFERON-TB Gold In-Tube and T-SPOT.TB test. This study does not include the low exposure group.

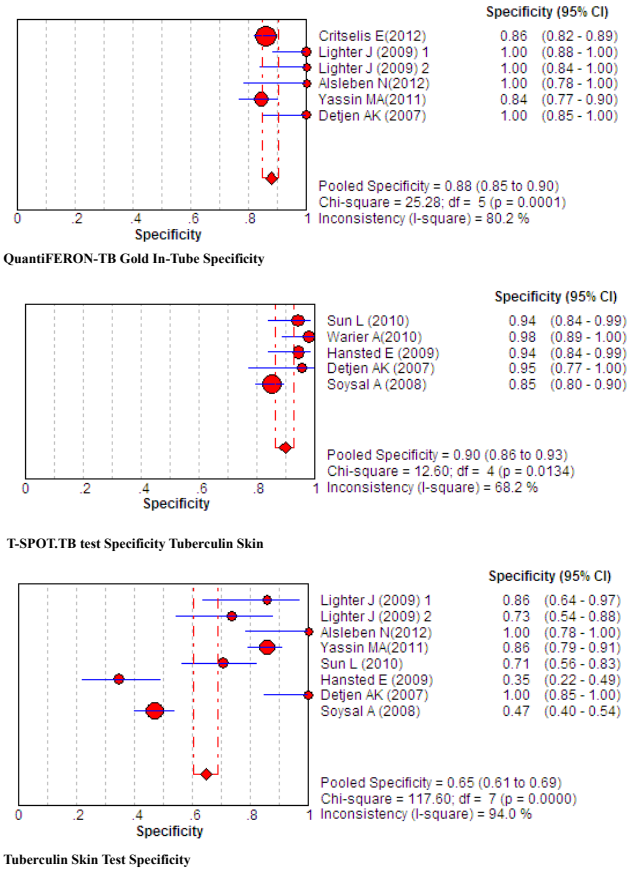

Figure 2: Specificity of Interferon Gamma Release Assays and Tuberculin Skin Test in No Risk Factor Participants.

correlated highly with TB exposure. In most of the studies enrolled (10 out of 12 articles), the gradient of exposure increased, so did the proportion of positive QFT-IT. In addition, TST showed the same trend in seven of eleven studies. However, as a whole, the difference of the sensitivities of the two tests is not remarkable. Although data from adults also showed that IGRAs correlate more closely with degree of exposure than the TST [20], one study enrolled 215 childhood contacts of newly diagnosed TB patients highlighted that TST correlated better with TB exposure compared with the IGRA [7]. Because of the mixed results, insufficient information, and heterogeneous samples conclusion were difficult to obtain, and our findings merit additional large cohort studies to draw a clearer picture of the sensitivity of the two tests.

BCG vaccination and non-tuberculosis mycobacterial (NTM) infections are important factors associated with high false positive results [15]; however, the effect of NTM on IGRA response is poorly studied in the pediatric population. Interestingly, our results identified the excellent specificity of IGRA in populations with high rate of BCG vaccination, and emphasized that BCG vaccination had little effect on IGRA. These data agrees with findings in other studies that the average specificity of IGRA is more specific than TST in cases of BCG vaccination [21]. TST is an established method to detect immunological memory to a poorly defined mix of antigens from M. tuberculosis, while IGRA reflects the recent or latest infection of M. tuberculosis because of the use of RD1 antigen which is absent in NTM and BCG. One study enrolled 99 childhood contacts grouped according to BCG vaccination emphasized that IGRA is particularly useful for the evaluation of recently exposed BCG immunized children [11]. Data from adults also showed that fewer BCG vaccinated individuals were identified as positive by IGRA than by TST [22]. As a result, IGRA will be increasingly helpful to exclude the cross-reaction with BCG immunization.

The timely detection of LTBI and the provision of chemoprophylaxis are critical to reduce the risk of disease progression. Currently, TST is one of the most important indexes of LTBI diagnosis. Since the cut-off value for a TST positive result varies greatly in different countries, from 5 to 15 mm of indurations, follow-up studies of incidence of tuberculosis are becoming increasingly vital. For IGRA, the study enrolled in this analysis suggests that QFT-G has a very high negative predictive value [14]. Another study observed that a significantly higher proportion of untreated household contacts with positive ELISA results progressed to active tuberculosis than contacts with positive TST results when using the 5 mm threshold [23]. One study evaluated the prognostic value of IGRA highlighted that a positive ELISpot result is prognostic of progression to tuberculosis, but the magnitude of this response does not further refine the risk for progression [19]. Although the TST has an inferior prognostic value compared with IGRA, some studies have documented that the size of the tuberculin reaction correlates with the risk for subsequent progression to tuberculosis [13,24,25].

The major limitation of our meta-analysis is the lack of gold standard for LTBI. Reactivation of LTBI is best determined through long-term follow-up of untreated individuals with positive IGRA or TST result; such studies are hampered by ethical issues and complexity of study design. Consequently, the number of studies in this meta-analysis is insufficient and most studies use small populations. An additional important limitation is the heterogeneity of the studies reviewed, which were conducted in samples with different degree and categorization of exposure.

The most interesting finding in current study is the high specificity of IGRA, which was unaffected by BCG vaccination. Because of the limited data of our analysis, our analysis results should be interpreted with some caution, although they could provide useful information to practicing clinicians. We expect that future research will determine the incidence of active TB in persons with positive and negative results on IGRA and TST.

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