

Interferon Gamma Assays for Tuberculosis in Children

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Abstract

The data of interferon gamma assays for tuberculosis in children is still in its infancy. It is further complicated by the designs and methodology of available studies. These tests do have much advantage over Tuberculin Skin Test (TST) in malnourished or HIV positive children where TST is mostly negative. Both TST or interferon gamma assays are not diagnostic in 100% cases of childhood tuberculosis. Even combined TST and interferon gamma assays cannot diagnose tuberculosis in 100% cases. Similar and well designed follow up studies at multiple centers are needed to evaluate these tests and to compare with TST.

Introduction

Tuberculosis (TB) in children is an important, but neglected, global health problem¹. It was estimated in 1991 that, from a reservoir of 180 million children with primary or latent *Mycobacterium tuberculosis* infection, there were 1.3 million cases of childhood tuberculosis and 450,000 deaths². The overwhelming majority of these cases occur in developing countries.

Paediatric tuberculosis poses diagnostic challenges³⁻⁶. Children often present with vague and non-specific signs and symptoms. TB is less often bacteriologically confirmed in children than adults. This is largely due to the paucibacillary nature of tuberculosis in children, greater likelihood of extrapulmonary and disseminated presentations, as well as the difficulty in obtaining clinical specimens^{3,4,7}. Clinicians, therefore, frequently use indirect approaches to make a diagnosis³⁻⁵. This includes history of contact with a case of infectious tuberculosis, chest x-ray abnormalities, and a positive tuberculin skin test (TST) as evidence of infection. The TST, therefore, is widely used in paediatric practice.

Until recently, the TST, which uses purified protein derivative (PPD), was the only method available for the diagnosis of latent tuberculosis infection. The utility of this conventional test is hampered by technical and logistic problems: potential for false positive and false negative results; problems in administration and interpretation; and difficulty in separating true infection from the effects of prior BCG vaccination and infection due to non-tuberculous mycobacteria⁸⁻¹¹.

Advances in genomics^{12,13} and immunology have led to a promising alternative-in vitro interferon gamma assays, based on the principle that T-cells of individuals infected with *Mycobacterium tuberculosis* release interferon gamma when they re-encounter TB-specific antigens^{14,15}. Latest versions of interferon gamma assays use antigens such as the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These antigens, encoded within the region of difference 1 (RD1) of the *Mycobacterium tuberculosis* genome, although not entirely specific to the *Mycobacterium tuberculosis* complex, are significantly more specific to *Mycobacterium tuberculosis* than PPD, as they are not shared with any BCG vaccine strains or selected non-tuberculosis mycobacteria species including *mycobacterium avium*¹⁶⁻¹⁸.

The Quantiferon-TB Gold assay (Cellestis Ltd, Carnegie, Australia) is a commercial test, recently approved by the U.S. Food and Drug Administration. In December 2005, the U.S. Centers for Disease Control and Prevention recommended that the Quantiferon-TB Gold assay can be used instead of TST in all situations where the TST is currently used¹⁹. The Quantiferon-TB Gold in Tube assay, a simplified variant of the Quantiferon-TB Gold assay, uses tubes coated with ESAT-6, CFP-10, and TB7.7 for stimulating T-cell response; this version is not currently approved by U.S. Food and Drug Administration. Other interferon gamma assays, including those using ELISPOT (e.g. T-SPOT, Oxford Immunotec, UK) are also available now. These tests are promising and have been successfully used by independent investigators in many settings, including low-income countries.

The following is the discussion on interferon gamma assays in children.

Limitations of the Studies

A small number of studies have been performed in children, all of which did not use the similar type of interferon gamma assay test. Few studies²⁰⁻²³ used Quantiferon-TB Gold in Tube assay; others²⁴⁻²⁶ used Quantiferon-TB Gold; some used ELISPOT²⁷⁻²⁹, whereas only one study compared Quantiferon-TB Gold and ELISPOT³⁰.

These studies were heterogeneous because the

populations selected were suspected of having active tuberculosis^{24,27}, had contact with infected persons²³⁻²⁹, or were school children²⁵⁻²⁸. Some were healthy²³⁻²⁸, whereas others were hospitalized²⁷. Some studies^{20,24-26,30} were conducted in low prevalence/intermediate prevalence while others^{21-23,27-29} in high prevalence countries for tuberculosis. Most of the studies were cross sectional; only a few studies^{21,22,25,30} were prospective or follow up studies. The type of TST and the strength of tuberculin used were not uniform in all the studies. Moreover, the criteria for positivity of the TST were not the same. In some studies interferon gamma assays and TST were not performed on the same day. Interferon gamma assays were done after 2-3 months of TST in the study by Ewer K et al²⁰ and about 35 days after TST in the study by Higuchi K et al²⁵. BCG vaccination status was also not uniform in these studies; some studies^{21-24,27,29} analyzed the effects of age on these tests, while the effects of nutrition were examined in a few other studies^{21,22,27,28}. In addition, interferon gamma assays were the occurrence of indeterminate results^{21,23,24,26} and failed phlebotomy²⁸.

Sensitivity and Specificity of Interferon Gamma Assays and TST in Latent Tuberculosis

The major limitation in estimation of sensitivity or specificity was the use of a cross-sectional design in most of the studies. With this design, there is no gold standard for latent tuberculosis infection. The only certain measure that latent tuberculosis infection exists is when the risk for active tuberculosis associated with a particular test result has been defined. This requires large-scale cohort studies with long-term follow-up of untreated populations with positive results at baseline. In addition to being expensive and complex, such studies are ethically impossible in most high-income countries, where the standard of care is to offer treatment to such persons.

Because there is no gold standard for testing latent

tuberculosis infection, the estimated sensitivity can be calculated from studies of patients with active tuberculosis, persons in contact with active tuberculosis patients who were categorized into gradients of exposure and concordance of interferon gamma assays and the tuberculin skin test. The situation is further complicated by the fact that confirming or excluding active tuberculosis is even more difficult in children. Hence, information is currently insufficient to estimate sensitivity, specificity, and reproducibility of interferon gamma assays in children. Menzies D et al³¹ calculated sensitivity of TST from pooled estimates from studies in children and found it to be 0.55 (95%CI 0.43-0.67) while sensitivity of Quantiferon was 0.66 (95%CI 0.5-0.83) and for ELISPOT was 0.62 (95%CI 0.43-0.81) by using active cases as surrogate for latent infection. It is interesting to note that even combined TST and interferon gamma assay failed to give 100% sensitivity^{21,27}. Furthermore all the three tests (TST, ELISPOT and Quantiferon-TB Gold) were not similar³⁰.

It is difficult to ensure equivalence of the exposure categories because each study characterized exposure differently, nevertheless, overall findings were similar. The prevalence of positive results on interferon gamma assays and the TST was highest in the most-exposed groups. In the less-exposed groups, the prevalence of positive results on the TST was higher²⁰⁻²⁹ than that of interferon gamma assays in studies that involved populations which were less BCG vaccinated²³. Lee et al showed that the specificity of ELISPOT was 84.7%, Quantiferon-TB Gold 91.6% and TST 78.6%³⁰.

Tables I and II show the concordance and discordance between interferon gamma assays (IGA) and TST.

Interferon Gamma Assays in the Monitoring of Therapy of Tuberculosis

Only one has been conducted in South African

TABLE-I: Concordance of Quantiferon and TST in Healthy Populations with Varying Risk for Latent Tuberculosis Infection*

Study, year (reference)	Country	Risk group	Total Participants n**	BCG %	Concordant Results		Discordant Results	
					TST+ve and IGA -ve n (%)	TST -ve and IGA +ve n (%)	TST -ve and IGA +ve n (%)	TST+ve and IGA -ve n (%)
Connel TG et al 2006 (24)	Australia	Pediatric contacts	75	49	10 (13)	38 (51)	4 (5)	23 (31)
Lee JY et al 2006 (30)	Korea	Healthy students	131	100	3 (2)	95 (73)	8 (6)	25 (19)
Dogra S et al 2006 (27)	India	Hospitalized children	97***	82	3 (3)	89 (92)	3 (3)	2 (2)
Tsiouris SJ et al 2006 (28)	South Africa	Pediatric contacts	184	73	51 (28)	94 (51)	10 (5)	29 (16)
Nakaoka H et al 2006 (29)	Nigeria	Pediatric contacts	179	37	40 (22)	106 (59)	19 (11)	14 (8)
Total			666	67.27	107 (16.07)	422 (63.36)	44 (6.61)	93 (13.96)

* modified from the TABLE-IV reference 31

n** number

***Eight participants with active tuberculosis were excluded from this analysis

TABLE-II: Concordance of ELISPOT with TST in Healthy Populations with Varying Risk for Latent Tuberculosis Infection*

Study, year (Reference)	Country	Risk group	Participants n**	BCG %	Concordant Results		Discordant Results	
					TST+ve and IGA -ve n (%)	TST -ve and IGA +ve n (%)	TST -ve and IGA +ve n (%)	TST+ve and IGA -ve n (%)
Ewer K et al 2003 (20)	United Kingdom	Contacts in high school outbreak	535	87	118 (22)	353 (66)	32 (6)	27 (5)
Hill PC et al 2006 (23)	Gambia	contacts	693	46	165 (24)	413 (60)	60 (9)	55 (8)
Total			1228	70.12	283 (23.05)	766 (62.38)	92 (7.49)	82 (6.68)

* modified from the table V reference 31
n** number

children²² that monitored interferon gamma assay during therapy of tuberculosis. The mean levels of serial ELISPOT assay done on patients treated for active tuberculosis increased by 45% after one month of therapy and then decreased with continued therapy.

Effect of BCG on TST and Interferon Gamma Assays

Studies^{20,23,24,28} failed to reveal any effect of BCG on TST or interferon gamma assays. Dogra S et al²⁷ reported that in BCG scar-negative children, the agreement between the tests was 100% (K=1.0) as compared to 94% (K=0.63) in scar-positive children. BCG scar-positivity was not associated with any definite pattern of discordance. Taylor REB et al²⁶ showed that Quantiferon gamma interferon and TST results were the same in 71% with prior BCG, and 51% cases without BCG vaccinated children.

Size of TST Positivity and Interferon Gamma Assay

The size of TST positivity does affect the concordance of the results between interferon gamma assay and TST although this may be insignificant^{21,27,28}.

Interferon Gamma Assays and TST in Relation With HIV and Nutrition Status

One study²¹ has compared TST with ELISPOT in HIV positive cases and found that ELISPOT was more sensitive. Interferon gamma assay tests were found to be more sensitive than TST in children suffering from moderate to severe malnutrition^{21,27}. Nicol MP et al²² noted that the response to ESAT 6 and CFP 10 was same irrespective of the weight-for-age Z scores.

Interferon Gamma Assays, TST and Perinatal Tuberculosis

Three cases of perinatal tuberculosis have been

reported in literature utilizing interferon gamma assays. TST was negative while interferon gamma assays were found to be positive in all the three cases.^{32,33}

Effect of Age and Sex

There is general trend in increasing likelihood of positivity of interferon gamma assay and TST with increasing age.^{24,27-29} Liebeschuetz S et al²¹ showed that ELISPOT was more sensitive in children below 36 months of age. A couple of studies^{27,28} did not show any relation of these tests with reference to sex of the child.

Interferon Gamma Assays in Reference to Nontuberculous Mycobacteria

The effect of nontuberculous mycobacteria on interferon gamma assays has been poorly studied in children. None of the reviewed studies assessed the effect of nontuberculous mycobacteria on interferon gamma assays response. Adults with disease caused by *M. marinum* and *M. kansasii* could have positive results on RD1-IGRA³⁵ which might be of interest because the genomes of these nontuberculous mycobacteria organisms contain the RD1 region³⁶.

Ewer K et al²⁰ showed that both TST and ELISPOT were more likely to be positive in students who had a history of household tuberculosis contact (a marker of *M. tuberculosis* exposure outside school) than in students without such a history. By contrast, for the students born in high-prevalence countries, mainly Africa and Asia (a risk factor for environmental mycobacterial exposure and *M. tuberculosis* exposure) only the TST was significantly more likely to be positive.

Cost

These tests are quite expensive. The cost of Quantiferon-TB Gold test kit including laboratory and other expenses for one patient in USA is \$ 29.22 - 33.7 and almost half of the total cost was due to the commercial kit itself³⁶. The cost of Quantiferon-TB Gold in Tube test kit including laboratory and other expenses for one patient in USA is

\$25.09-26.74 and almost 75% of the total cost was due to the commercial kit itself³⁶ while the cost of T-SPOT (ELISPOT) test kit including laboratory and other expenses for one patient in USA is \$57.79 and almost 70 % of the total cost was due to the commercial kit itself³⁶.

Future Implications

Interferon gamma assays are widely commercially available. Independent field studies are needed to evaluate the feasibility, utility, and costs of these tests in different populations and under different conditions. These studies will be able to report on the actual completion of tests and the subsequent evaluation and treatment of patients with positive test results. Much of the value of a test is lost if persons who are tested do not return to learn the significance of their test result or if those with positive results are not evaluated further.

Finally, large-scale cohort studies are needed that estimate risk for progression to active disease in persons who have had positive results on TST and interferon gamma assays. Of particular interest is the risk for disease in persons with discordant reactions.

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