

REVIEW ARTICLES

ROLE OF QUANTIFERON GOLD TEST (INTERFERON GAMMA RELEASE ASSAY) FOR DIAGNOSIS OF TUBERCULOSIS IN CHILDREN

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Introduction

Infection with *Mycobacterium tuberculosis* (MTB) without causing disease results in so-called latent TB infection (LTBI). (1) Diagnosis of LTBI is difficult and there is no gold standard test available. Diagnosis of TB in children is often based on circumstantial evidence such as clinical symptoms, imaging findings, tuberculin skin test (TST) and contact with a patient having tuberculosis as the disease is paucibacillary and specimen collection is difficult in this population. However, TST has its own fallacies as a false positive TST would reflect a past BCG vaccination or atypical mycobacterial infection whereas a false negative TST could occur due to malnutrition, young age, severe TB disease, HIV-related impaired cellular immunity, and other forms of immune suppression. (1) Recognition that interferon gamma plays a critical role in regulating cell mediated immune responses to MTB infection led to development of in-vitro Interferon Gamma Release Assays (IGRAs) for detection of MTB infection. (2)

Interferon Gamma Release Assays (IGRAs)

There are two commercial IGRAs available. Both assays work on the principle that the T-cells of an individual who have acquired TB infection will respond to re-stimulation with *M. tuberculosis*-specific antigens by secreting interferon-gamma. The QuantiFERON-TB Gold (QFT-G, Cellestis, Australia) and the newer version QuantiFERON-TB Gold In-Tube (QFT-GIT, Cellestis, Australia) are whole-blood based enzyme-linked immunosorbent assays (ELISA) measuring the amount of IFN- γ produced in response to specific *M. tuberculosis* antigens [QFT-G: early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10), QFT-GIT: ESAT-6, CFP-10, TB7.7]. In contrast, the enzyme-linked immunospot (ELISPOT)-based T-SPOT.TB (Oxford Immunotec, UK) measures the number of peripheral mononuclear cells that produce INF- γ after stimulation with ESAT-6 and CFP-10. (1) The MTB antigens used in IGRAs are absent from BCG and most of non tuberculous mycobacteria (NTM) thus decreasing incidence of false positive results due to these infections or previous BCG exposure as compared to TST. However, ESAT-6 and CFP-10 are present in *M. kansasii*, *M. szulgai*, and *M. marinum*, and sensitization to these organisms might contribute to the release of IFN- γ in response to these antigens and cause false-positive IGRA results. (3)

TST versus IGRAs

Neither the TST nor IGRAs can distinguish TB infection from active TB disease. (1) Several studies show that QFT-G/QFT-GIT to be more specific than the TST for MTB detection in children. (4-8) Thus IGRAs seem to be very useful to rule out tuberculosis. A recent meta-analysis evaluated IGRAs (QFT-G, QFT-GIT and ELISPOT) and the tuberculin skin test (TST). The authors found that the sensitivities of all three tests in active tuberculosis were similar. The pooled sensitivity was 70% for QFT based tests, 62% for ELISPOT studies and 71% for TST. The pooled specificity was 100%

for QFT based tests and 90% for ELISPOT, but was much lower for TST [56% in all included studies and 49% in children with bacillus Calmette-Guerin (BCG) vaccination]. (9) The reason why the TST could be false positive may be due to previous BCG vaccination or due to environmental exposure to NTM. (10)

IGRAs have the following operational advantages over TST: the tests can be completed in 1 visit with the results available within 24 hours; are able to avoid boosting due to repeat testing; and have a standardized interpretation. (11) IGRAs have limitations in that they are expensive, require an equipped laboratory with trained personnel, and, like TST, they cannot distinguish between latent and active TB disease. (12)

QFT in younger children

Since it is known that cell-mediated immunity matures from infancy through to early childhood, an important question that is whether age affects the IFN- γ production measured by QFT. Kampmann et al (2006) showed a significantly lower production of IFN- γ in response to the positive control mitogen phytohaemagglutinin in children younger than 4 years old compared with children 4 to 15 years old ($p < 0.0001$). (13). In a meta-analysis by Machingaidze et al, indeterminate results with QFT were seen more common in younger children. (12) These findings highlight the need for pediatric studies of larger groups of children, stratified by age. Kampmann et al have thus stated that before advocating the routine use of new assays, appropriate validity studies are needed in pediatric populations. Laboratory cut-off values used for adults are not necessarily correct when dealing with pediatric samples and might lead to false conclusions. (13) Pavic et al have suggested that in a high-risk population of children up to 5 years of age, both tests (QFT and TST) should be performed and the child should be considered infected if both tests are positive. (14)

QFT in children already on treatment for TB

Children with LTBI and TB have increased IFN- γ levels during follow up period, in some children even one year after the beginning of treatment. The reason for persistently increased IFN- γ levels even after anti-TB therapy has not been established. (15) Recently, Pai stated that serial determination of IFN- γ can reveal 4 different underlying phenotypes, i.e., 1) persistently positive (subjects who are repeatedly IFN- γ positive for a prolonged period), 2) stable conversions (individuals who convert IFN- γ results and stay converted at least over a short period), 3) unstable conversion (individuals who convert IFN- γ results from negative to positive and then revert again to negative results) and 4) persistently negative (subjects who are repeatedly IFN- γ negative for a prolonged time period). (16) Thus, IGRAs can also remain positive over a prolonged period of time in children with TB or LTBI. (15) Also exact time of testing with QFT-GIT cannot be established and whether a negative QFT-GIT needs serial testing needs to be established.

QFT in immunocompromised children

Limited data are available regarding the use of QFT-GIT for testing immunocompromised persons. In two studies with a total of 34 HIV-infected subjects with culture-confirmed active tuberculosis, the sensitivities of QFT-GIT were 81% and 88% (17,18). In one study, the sensitivities of QFT-GIT and TST were similar (81% and 85% respectively, $p>0.99$) (17). QFT-GIT sensitivity was not significantly different among persons with HIV infection than among those without infection (81% and 73%, respectively; $p=0.59$). In another study in Zambia involving 112 persons (59 were infected with HIV, 37 were not infected with HIV, and 16 were not tested) in whom active tuberculosis was diagnosed on the basis of sputum smear (19), QFT-GIT and TST were significantly less sensitive in persons infected with HIV than in persons not infected with HIV (76% compared with 97% for QFT-GIT; $p=0.02$ and 55% compared with 81% for TST, $p=0.04$). Among children, a study in Great Ormond Street Hospital for Children, UK found that impaired immunity ($P < 0.001$) was independently associated with a higher probability of an indeterminate QFT-IT. Thus, currently role of QFT-GIT is not established in immunocompromised patients to diagnose or rule out LTBI and active TB.

QFT in drug resistant Tuberculosis

The problem of drug resistant tuberculosis (DR-TB) especially multidrug-resistant tuberculosis (MDR-TB) and extensively resistant tuberculosis (XDR-TB) is on the rise in children. (20) To identify LTBI in children having contact with a patient with DR-TB is necessary to prevent progression to active disease. A study in Korea screened close contacts of patients with bacteriologically confirmed MDR-TB with TST and the QFT-GIT. Of the 48 individuals who were TST positive, 34 (70.8%) were positive for the QFT-GIT assay. Of the 53 subjects who were TST negative, 33 (62.5%) were negative for the QFT-GIT assay. Thus, TST and QFT-GIT assays showed poor correlation in close contacts of patients with MDR-TB. (21) Thus role of QFT in children exposed to drug resistant TB is still not established.

Conclusion

Quantiferon gold test has a higher specificity as compared to tuberculin skin test to rule out tuberculosis. However, it cannot differentiate between active TB and LTBI. Role of QFT in younger children, in immunocompromised children, in children with drug resistant TB needs further evaluation.

References

1. World Health Organization (WHO) - Use of Tuberculosis Interferon-Gamma Release Assays (IGRAs) In Low- And Middle-Income Countries. Available at URL: http://www.who.int/tb/features_archive/policy_statement_igra_oct2011.pdf. Accessed on 5th August 2012.
2. Bellete B, Coberly J, Barnes GL, Ko C, Chaisson RE, Comstock GW, Bishai WR. Evaluation of a whole-blood interferon-gamma release assay for the detection

of Mycobacterium tuberculosis infection in 2 study populations. *Clin Infect Dis*. 2002; 34: 1449-1456

3. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K, et al. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. *MMWR Recomm Rep*. 2010; 59(RR-5):1-25.
4. Lighter J, Rigaud M, Eduardo R, Peng CH, Pollack H. Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold In-Tube test. *Pediatrics*. 2009; 123: 30-7.
5. Tsiouris S, Austin J, Toro P, et al. Results of a tuberculosis-specific IFN-[gamma] assay in children at high risk for tuberculosis infection. *Int J Tuberc Lung Dis*. 2006;10:939-941.
6. Hesselting A, Mandalakas A, Kirchner H, et al. Highly discordant T cell responses in individuals with recent exposure to household tuberculosis. *Thorax*. 2009;64:840-846
7. Bianchi L, Galli L, Moriondo M, et al. Interferon-[gamma] release assay improves the diagnosis of tuberculosis in children. *Pediatr Infect Dis J*. 2009;28:510-514.
8. Chun J, Kim C, Kim H, et al. The role of a whole blood interferon-[gamma] assay for the detection of latent tuberculosis infection in Bacille Calmette-Guerin vaccinated children. *Diagn Microbiol Infect Dis*. 2008;62:389-394.
9. Sun L, Xiao J, Miao Q, Feng WX, Wu XR, Yin QQ, et al. Interferon gamma release assay in diagnosis of pediatric tuberculosis: a meta-analysis. *FEMS Immunol Med Microbiol*. 2011; 63: 165-173.
10. Debord C, De Lauzanne A, Gourgouillon N, Guerin-El Khourouj V, Pedron B, Gaudelus J, Faye A, Sterkers G. Interferon-gamma release assay performance for diagnosing tuberculosis disease in 0- to 5-year-old children. *Pediatr Infect Dis J*. 2011; 30: 995-997
11. Chang K, Leung E, Leung C. Interferon-gamma release assays in childhood tuberculosis: a systematic review. *Hong Kong J Paediatr*. 2009;14:86-95.
12. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, Hatherill M, Moyo S, Hanekom W, Mahomed H. 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.
13. Kampmann B, Tena-Coki G, Anderson S. Blood tests for diagnosis of tuberculosis [letter]. *Lancet*. 2006;368:282
14. Pavic I, Topic RZ, Raos M, Aberle N, Dodig S. Interferon- γ release assay for the diagnosis of latent tuberculosis in children younger than 5 years of age. *Pediatr Infect Dis J*. 2011; 30: 866-70.
15. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. *PLoS One*. 2009; 4: e8517
16. Santin M, Muoz L, Rigau D. Interferon- γ release assays for the diagnosis of tuberculosis and tuberculosis infection in HIV-infected adults: a systematic review and meta-analysis. *PLoS One*. 2012;7:e32482. Epub 2012 Mar 5.
17. Tsiouris SJ, Coetzee D, Toro PL, Austin J, Stein Z, El-Sadr W. Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis. *J*

- Clin Microbiol 2006;44:2844-50.
18. Aichelburg MC, Rieger A, Breiteneker F, et al. Detection and prediction of active tuberculosis disease by a whole-blood interferon-gamma release assay in HIV-1-infected individuals. Clin Infect Dis 2009;48:954-62.
 19. Raby E, Moyo M, Devendra A, et al. The effects of HIV on the sensitivity of a whole blood IFN-gamma release assay in Zambian adults with active tuberculosis. PLoS ONE 2008;3:e2489.
 20. Shah I, Chilkar S. Clinical Profile of Drug Resistant Tuberculosis in Children. Indian Pediatr. 2012 Jun 10. pii: S097475591101060-2.
 21. Jo KW, Jeon K, Kang YA, Koh WJ, Kim KC, Kim YH, et al. Poor correlation between tuberculin skin tests and interferon- γ assays in close contacts of patients with multidrug-resistant tuberculosis. Respirology. 2012 Jul 4.

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E-published: 1st August 2012. **Art#**54

DOI No. 10.7199/ped.oncall.2012.54

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