The Effect of Age on Whole Blood Interferon-Gamma Release Assay Response among Children Investigated for Latent Tuberculosis Infection

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Objective To evaluate the effect of age upon QuantiFERON-TB Gold-In-Tube (QFT-IT) assay outcome among children examined for latent tuberculosis infection (LTBI).

Study design A cross-sectional study was conducted among 761 children (mean age ± SD: 7.84 ± 4.68 years) evaluated for LTBI. Participants were examined with both tuberculin skin test and QFT-IT (Cellestis, Australia) and categorized into 4 age groups. Multivariate logistic and linear regressions were used to evaluate the association between selected demographic and patient characteristics upon the qualitative and quantitative QFT-IT outcomes. Agreement between the tuberculin skin test and QFT-IT within groups was evaluated with the κ statistic.

Results QFT-IT indeterminate results occurred more frequently among young children (8.1%; $P < .0001$) and children (2.7%; $P = .025$) than adolescents (0.7%). Among QFT-IT positive patients, infants had higher mean (±SD) interferon-gamma (IFNγ) concentration than adolescents. QFT-IT positive (vs negative) outcome was associated with origin from a high tuberculosis endemicity setting (AOR = 4.54; 95% CI, 3.22-6.25) and lack of previous Bacille Calmette Guerin immunization (AOR = 2.70; 95% CI, 1.89-3.85), but not patient age (AOR = 0.96; 95% CI, 0.92-0.99). However, among QFT-IT positive patients, the IFNγ concentration was inversely associated with patient age ($P = .009$) and positively with mitogen response ($P = .0002$). Agreement between tests was not significantly different between younger and older children in the different risk groups.

Conclusions Qualitative QFT-IT assay results are not affected by patient age. However, indeterminate results occur more frequently among younger children. Among patients with LTBI the quantitative QFT-IT result (ie, IFNγ) is inversely associated with patient age. *(J Pediatr 2012;161:632-8)*.

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Tuberculosis (TB) persists globally as an important public health problem both in adults and children.[1] It has been estimated that about one-third of the world's population has been infected with *Mycobacterium tuberculosis*.[2] The prevalence of latent tuberculosis infection (LTBI) is notable not only in resource poor settings, but also in areas with a low disease burden. In particular, it is estimated that approximately 1 million children and adolescents in the United States have asymptomatic TB infection.[3] Early diagnosis and treatment of LTBI is vitally important for disease control and eradication.[4]

For approximately 1 century, the tuberculin skin test (TST) has been used as the sole immunodiagnostic test for the detection of *M tuberculosis* infection. The recently developed interferon-gamma release assays (IGRAs) have been a breakthrough in the diagnosis of tuberculosis infection in adults and children.[5-7] Compared with the TST, IGRAs have enhanced specificity for *M tuberculosis* infection because the result is unaffected by previous Bacille Calmette Guerin (BCG) immunization or infection with most nontuberculous mycobacteria.[8-10] In addition, preliminary studies have indicated that IGRAs may have a higher positive predictive value for the development of TB compared with the TST among close contacts of patients with TB.[11,12] However, it has been suggested that young age may have a negative effect on the performance of IGRAs, mainly because of frequent indeterminate results due to lower interferon-gamma (IFNγ) production in response to the mitogen control.[13-17] Concerns about lower sensitivity of IGRAs to detect *M tuberculosis* infection among young children also have been expressed.[13,15]

Our primary aim was to evaluate the effect of multiple factors, including age, on the qualitative (positive/negative) and quantitative (IFNγ concentration) whole blood IGRA response in children investigated for LTBI. In addition, the agreement between IGRA and TST was compared between children and adolescents in the study population and according to risk of TB infection.

BCG	Bacille Calmette Guerin
IFNγ	Interferon-gamma
IGRA	Interferon-gamma release assay
LTBI	Latent tuberculosis infection
QFT-IT	QuantiFERON-TB Gold-In-Tube
TB	Tuberculosis
TST	Tuberculin skin test

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Methods

A cross-sectional study was conducted among children evaluated for TB infection who were referred to the outpatient Tuberculosis Clinic at the P. & A. Kyriakou Children's Hospital in Athens, Greece, during the period from January 2007 through July 2010. The study protocol was approved by the Human Research and Ethics Committee of the P. & A. Kyriakou Children's Hospital and informed consent for study participation was requested from the legal guardians of all eligible participants. Demographic and socioeconomic characteristics, as well as complete personal and family histories in relation to TB exposure, were recorded. All patients were evaluated clinically and tested with both the TST and QuantiFERON-TB Gold-In-Tube (QFT-IT) (Cellestis Limited, Carnegie, Victoria, Australia) assay.

TST

TST was performed with an intradermal injection of 0.1 mL or 2 tuberculin units PPD RT 23 (Statens SerumInstitut, Copenhagen, Denmark) in the forearm. A single trained TB clinic staff member, blinded to the study objectives, evaluated the transverse diameter of the induration 48-72 hours later with the palpation method. TST was considered positive if ≥5 mm among nonimmunized and ≥10 mm among immunized children with a positive contact history. The TST cut-offs for children without contact exposure, but who had high risk for infection was ≥10 mm and for those at low risk was ≥15 mm, regardless of BCG immunization history.[18]

All children with a positive contact history were tested with both the TST and QFT-IT concomitantly in our clinic. Universal (not targeted) TST screening of children is currently conducted in our settings by pediatricians in either the private or public sector. Screening of immigrants also is performed similarly. As a result, a considerable number of children belonging to both high risk or low risk groups for TB infection, had the TST performed during screening and were referred to our clinic for evaluation. In such cases, the QFT-IT test was performed within 7 days following the TST. Children who had a TST performed >7 days prior to enrollment were excluded from study participation to avoid possible boosting of QFT-IT outcome by prior TST.[19]

QFT-IT Assay

The whole blood was processed for the QFT-IT assay within 2 hours of sampling, according to manufacturer's instructions. One milliliter of whole blood was drawn into 3 separate tubes. The first tube contained heparin (negative control), the second phytohemagglutinin as a mitogen (positive control), and the third the *M tuberculosis* specific antigens ESAT-6, CFP-10, and TB7.7. Each tube was shaken vigorously and was incubated at 37°C for 24 hours within 2 hours from collection. Following centrifugation, the supernatants were stored at −70°C. The enzyme-linked immunosorbent assay method was used to measure the amount of IFNγ produced, and the amount released after stimulation with TB antigens was calculated by subtracting the amount of IFNγ released in the control tube. A positive QFT-IT result (≥0.35 IU/mL) was calculated according to the manufacturer's software. Indeterminate results were defined as those with IFNγ levels <0.35 IU/mL and either mitogen minus nil values <0.5 IU/mL or nil values >8.0 IU/mL. Because the QFT-IT assay does not accurately assess IFNγ values >10 IU/mL, higher values were treated as 10 IU/mL in all analyses. Response to mitogen (phytohemagglutinin) was considered positive if IFNγ concentration (minus nil) was >0.5 IU/mL. In addition, negative values after mitogen stimulation were evaluated as nil values and values >15 IU/mL were treated as 15 IU/mL in all analyses.[17]

Case Group Definitions

LTBI was identified in asymptomatic children with positive TST and/or QFT-IT, as well as chest radiographic findings, which were either normal or indicative of healed fibrotic changes and/or calcifications.[18] Participants were assigned into 3 groups based on the presence of risk factors for infection: (1) known exposure to an adult with active TB disease; (2) other children at high risk for TB infection[20] (negative contact history) because either they or their parents were born in a high prevalence country[21] (>24 cases/100 000 population) or if they had recently travelled to such a destination. Children with TST positive first degree family members were also characterized as being at high risk for TB infection; and (3) children at low risk for TB infection were defined as those with no known risk factors for TB infection.[21]

Statistical Analyses

The Kolmogorov–Smirnov test was used to test for the normality of the distributions of continuous variables. Continuous variables with non-normal distributions were compared between age groups with the nonparametric Mann–Whitney U test. The basis of comparison was adolescents aged ≥10 years. Categorical variables were compared with the χ^2 test or Fisher exact test in cases where sample sizes did not exceed 5 patients. Ordinal TST values were compared with the Wilcoxon rank sum test. The percent concordance between the TST and IGRA was computed and comparisons were made between age groups. The kappa (κ) statistic was computed to evaluate the agreement between the 2 tests examined. The criterion of significance was $P < .05$. The Mantel–Haenszel method was used to calculate OR and 95% CI for categorical variables between age groups. Univariate logistic regression was used to evaluate the association between selected demographic and patient characteristics upon a positive (vs negative) IGRA outcome. Independent variables with a Wald P value of <.05 were entered stepwise into the multivariate regression model for the evaluation of the factors associated with IGRA outcome following adjustment for sex. Linear regression was used to evaluate the association between the aforementioned variables and the quantitative IGRA response, mitogen response, and TST size among patients with positive IGRA outcome. Statistical