Evaluation of an Interferon Gamma Release Assay to Detect Tuberculosis Infection in Children in San Diego, California

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QuantiFERON-TB Gold In-Tube (QFT-GIT) and tuberculin skin test (TST) results are reported in 23 children with active tuberculosis due to Mycobacterium tuberculosis and Mycobacterium bovis. Overall QFT-GIT (96%) was more sensitive than TST (74%) for detecting tuberculosis infection in these patients.

Interferon gamma release assays (IGRAs) are blood tests that detect tuberculosis infection by quantifying the patient’s interferon gamma (IFN-γ) response to specific peptides associated with pathogens causing tuberculosis. QuantiFERON-TB Gold In-Tube (QFT-GIT) test is an IGRA test licensed in the United States. The peptides used in this IGRA simulate the proteins ESAT 6, CFP 10, and TB 7.7. These proteins are present in Mycobacterium tuberculosis complex organisms including Mycobacterium tuberculosis and Mycobacterium bovis but are absent in Bacille-Calmette-Guérin (BCG) vaccine strains and most non-tuberculous mycobacteria [1, 2]. In adult studies, IGRAs show similar sensitivity but greater specificity compared with tuberculin skin tests (TSTs) in the diagnosis of latent tuberculosis infection [3]. Fewer data are available on use of these tests in children. There are also no studies validating IGRAs in children or adults known to have M. bovis infection. Children with disease defined as tuberculosis may have infection caused by either M. tuberculosis or M. bovis.

In 2009, San Diego County had a tuberculosis disease rate of 7.0 per 100 000, which is nearly double the national average of 3.8 per 100 000 population, with 6% of disease occurring in children aged <15 years. Between 2004 and 2008, 129 (10%) of the culture-confirmed tuberculosis cases in San Diego were due to M. bovis, and 21% of cases were seen in children aged <15 years [4]. This paper reports the results of IGRA testing with the QFT-GIT test compared with the TST in patients with culture-confirmed tuberculosis disease caused by M. tuberculosis and M. bovis.

MATERIALS AND METHODS

The protocol for this study was approved by the joint Institutional Review Board for the University of California San Diego and Rady Children’s Hospital. Enrollment was carried out from September 2007 to February 2010. Patients were recruited from the inpatient hospital service and outpatient clinics at Rady Children’s Hospital and the San Diego County Health and Human Services Agency tuberculosis clinic. Patients were eligible for enrollment if they were aged 0–16 years and were suspected of having active pulmonary or extrapulmonary tuberculosis disease. Only patients with culture-confirmed disease are reported. Once informed consent was obtained, demographic and clinical data were collected. The QFT-GIT testing and TST placement were the only study interventions. All other diagnostic testing and treatment was carried out per hospital or clinic routine for patients with suspected active tuberculosis.
The TST results (using a standard test dose of 5 tuberculin units) were collected. Tests were not repeated if results were documented by a licensed medical provider. Patients with prior positive TST results did not have a repeat TST performed if documentation was obtained from a licensed medical provider. For those with no prior documented test, a TST was placed and read by study personnel, the child’s medical provider, or Health and Human Services Agency staff. A positive TST is defined as ≥10 mm induration for healthy patients and ≥5 mm for patients that are immunocompromised or suspected of having active tuberculosis disease after exposure or close contact to an active case. Demographic and historical information, including ethnicity, travel, BCG vaccination, medication, and exposure history, was obtained by patient questionnaire at the time of enrollment. Patient charts were reviewed for clinical information, including imaging studies, microbiologic data, and histologic data from tissue samples. Mycobacterial cultures were performed at the San Diego County Public Health laboratory. Cultures were grown on solid and liquid media. Mycobacterium bovis was distinguished from M. tuberculosis by nitrate reduction assay and niacin accumulation tests that are negative with M. bovis and positive with M. tuberculosis.

Blood for QFT-GIT tests was collected in specified tubes and incubated per package insert instructions (Cellestis, Inc) [5]. Samples were processed for enzyme-linked immunosorbent assay measurement of IFN-γ levels per manufacturer instructions at the San Diego County Public Health laboratory. Positive tests were defined using a standard cutoff of 0.35 IU/mL. Indeterminate results were possible with either lack of control mitogen response or high background IFN-γ levels. Data analysis was performed using SPSS software version 16.0.

RESULTS

Twenty-three patients were diagnosed with culture-confirmed tuberculosis disease with cultures obtained from the site of infection that grew M. tuberculosis or M. bovis. The average age of the patients was 8.52 years, with 9 (39%) aged <5 years and 5 (22%) aged <2 years. Twenty-one (91%) were of Hispanic ethnicity, and 2 (9%) were foreign born.

Table 1 shows the location of disease and culture results. Twelve patients (52%) yielded M. tuberculosis on culture, and 11 (48%) yielded M. bovis. Eleven (92%) of the patients with M. tuberculosis disease and 11 (100%) of those with M. bovis disease had a positive QFT-GIT test. One indeterminate result, due to a low mitogen response, was obtained in a 12-year-old with pulmonary disease (M. tuberculosis) and a history of a TST positive with 15 mm of induration 1 year prior to enrollment. The TST was not repeated at the time of the current evaluation. In comparison, TST results were positive in 10 (83%) of the patients with M. tuberculosis disease and 7 (64%) of the patients with M. bovis disease. Six (18%) patients (2 M. tuberculosis, 4 M. bovis) had a negative TST with positive QFT-GIT test results, including 2 patients with disseminated disease and a 12-month-old with tuberculosis meningitis. Three of these 6 patients received at least 1 dose of steroids.

<table>
<thead>
<tr>
<th>Location</th>
<th>M. tuberculosis (n = 12)</th>
<th>M. bovis (m = 11)</th>
<th>Total (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>6a</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Pleural</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cervical lymph node</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Meningitis</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pericardial</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disseminated (3 with central nervous system disease)</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>QFT-GIT positive</td>
<td>11/12 (92%) (95% CI, 0.6–0.99)</td>
<td>11/11 (100%) (95% CI, 0.68–1.00)</td>
<td>22/23 cx confirmed (96%) (95% CI, 0.76–0.99)</td>
</tr>
<tr>
<td>TST positive</td>
<td>10/12 (83%) (95% CI, 0.51–0.97)</td>
<td>7/11 (64%) (95% CI, 0.32–0.88)</td>
<td>17/23 cx confirmed (74%) (95% CI, 0.51–0.89)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; M. bovis, Mycobacterium bovis; M. tuberculosis, Mycobacterium tuberculosis; QFT-GIT, QuantiFERON-TB Gold In-Tube; TST, tuberculin skin test.

*One patient with culture-confirmed pulmonary M. tuberculosis disease had an indeterminate QFT-GIT test and history of a positive TST.
DISCUSSION

In our study, the QFT-GIT test was 96% sensitive in detecting tuberculosis infection in patients diagnosed with culture-confirmed tuberculosis disease caused by either \( M. \) \( \text{tuberculosis} \) or \( M. \) \( \text{bovis} \). In comparison, the sensitivity of the TST was calculated as 74%. There was a trend toward greater sensitivity of the QFT-GIT test over TST that with a greater sample size would likely reach significance. The QFT-GIT test performed similarly with patients with \( M. \) \( \text{tuberculosis} \) and \( M. \) \( \text{bovis} \) disease. The TST was less sensitive with \( M. \) \( \text{bovis} \) cases, but this difference was not significant. Compared with our findings, studies in adults report lower sensitivity of the QFT-GIT test (64%–93%) [3, 6–9] with comparable sensitivity of the TST. Some studies in children report similar sensitivity of the QFT-GIT test to what we have found (93%) [10, 11]; however, others reported lower sensitivities of 43%–80% [12–15]. Age and ethnicity have been associated with differential responses to IGRA responses [8]. These studies were done in different populations, and severity of disease, genetic variables, and underlying patient conditions may account for differences in test performance. An inability of young children to produce high levels of IFN-\( \gamma \) in response to tuberculosis proteins has been postulated to explain decreased sensitivity of IGRA assays in this population [12, 13]. False-negative TSTs are frequently observed in children with disseminated tuberculosis and tuberculosis meningitis [16, 17]. However, our study included 5 children aged <2 years, all of whom had positive QFT-GIT test results (2 negative TSTs). Specificity of the QFT-GIT test and TST were not calculated because our population did not include patients with a low likelihood of tuberculosis infection.

\( \text{Mycobacterium bovis} \) is a significant cause of tuberculosis disease in the San Diego population [18] and was confirmed in our study. Our study is the first to report the sensitivity of the QFT-GIT test compared with the TST in children with \( M. \) \( \text{bovis} \) disease. The QFT-GIT test was sensitive in detecting disease caused by both \( M. \) \( \text{bovis} \) and \( M. \) \( \text{tuberculosis} \). This is expected because the tuberculosis proteins used in the QFT-GIT test are present in both \( M. \) \( \text{tuberculosis} \) and \( M. \) \( \text{bovis} \).

Earlier studies have reported a higher rate of indeterminate QFT-GIT test responses in young and immunocompromised children. Haustein et al [14] reported a 35% indeterminate rate in 269 children in the United Kingdom. Factors associated with an indeterminate result were immune compromise and age \( \leq \) 5 years. Bergammini et al [19] reported higher rates of indeterminate results in children aged <4 years. All 9 of our patients aged <5 years had positive QFT-GIT test results.

Potential weaknesses in our study included not repeating the TST in patients with a history of a positive TST. It is possible that the TST would have been negative because anergy has been reported in patients with active tuberculosis disease [20]. Also a larger sample size may have revealed a higher number of indeterminate QFT-GIT test results. An additional potential problem in our study, inherent in many studies using the TST, is the variability in the accuracy of placement of the tests and in their interpretation when these factors are not strictly controlled.

Advantages of the QFT-GIT test over the TST are that it requires a single visit and results are not dependent on test placement and interpretation of tissue induration. There should be fewer false positive reactions due to BCG vaccination or nontuberculous mycobacteria infection. The largest practical hurdle related to the use of IGRAs in children is the need for phlebotomy, which can be challenging in young children. The issue of indeterminate results also adds to the complexity of test interpretation.

CONCLUSIONS

The IGRA QFT-GIT test is a sensitive test to detect tuberculosis infection in children with disease caused by both \( M. \) \( \text{tuberculosis} \) and \( M. \) \( \text{bovis} \).

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**Potential conflicts of interest.** All authors: No reported conflicts.

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