Identification of False-Positive QuantiFERON-TB Gold In-Tube Assays by Repeat Testing in HIV-Infected Patients at Low Risk for Tuberculosis

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The QuantiFERON-TB Gold In-Tube assay (QFT) is increasingly being used for latent tuberculosis screening in patients infected with human immunodeficiency virus (HIV) in the United States. This is a retrospective analysis of repeating positive QFT assays as a strategy to identify false-positive results in HIV-infected patients at low risk for tuberculosis.

The incidence of tuberculosis and the proportion of individuals coinfected with human immunodeficiency virus (HIV) in the United States is falling [1]. HIV-infected patients remain an important group to target for latent tuberculosis infection (LTBI) testing and treatment due to their increased risk of progression to active tuberculosis [2]. Isoniazid preventative therapy in HIV-infected persons has proven beneficial if given to patients with positive tuberculin skin tests (TSTs) but has risk of toxicity without proven benefit in patients who are TST negative [3].

The commercially available interferon (IFN)–γ release assays (IGRAs), QuantiFERON-TB Gold In-Tube (QFT; Cellestis) and the T-Spot.TB (Oxford Immunotec), are alternatives to TST for screening HIV-infected patients [4]. IGRAs are increasingly used because of their higher specificity and requirement of 1 patient visit [5–10]. As with TSTs, IGRAs rely on functioning cell-mediated immunity and therefore have higher rates of failed tests in HIV-infected patients [11–13]. There are no data on the predictive value of IGRAs in HIV-infected patients in the United States, and poor concordance between the TST and IGRAs has been reported [14]. One study of 336 HIV-infected patients in Atlanta, Georgia, found that just 1 of 27 patients who tested positive by any test was positive by all 3 tests. This suggests a poor positive predictive value for all 3 tests in populations with low probability of LTBI [15].

The current Centers for Disease Control and Prevention (CDC) guidelines on IGRA testing recommend LTBI treatment in HIV-infected patients with a positive test result by any method (QFT, T-SPOT, or TST) [4]. Due to poor rates of LTBI screening using TST, the HIV clinics at Denver Health and University of Colorado switched to QFT in 2009. Rates of testing increased, but unexpected positive QFTs were observed in patients with low risk for tuberculosis exposure, such as US-born Coloradans among whom the tuberculosis incidence in 2010 was 0.6 per 100 000. To assess the validity of the QFT results, both clinics began repeating unexpected positive QFTs prior to recommending LTBI therapy.

METHODS

We performed a retrospective review of HIV-infected patients seen at Denver Health and University of Colorado infectious disease clinics. Both clinics began using QFT in 2009. Shortly after implementation, a recommendation was made to repeat the QFT in patients with a positive test and no identified risk for tuberculosis exposure. All HIV-infected patients with a positive QFT between 1 July 2009 and 30 June 2010 were reviewed. Patients with a history of treatment for LTBI or active tuberculosis were excluded.

The medical records were abstracted for all QFT results, HIV-related characteristics, and factors related to risk of tuberculosis exposure. The dates, qualitative results, and quantitative results of all QFTs were documented. Data on demographics and tuberculosis exposure risk, including age, sex, race, country of origin, history of healthcare work, history of known tuberculosis contact, history of previous incarceration, and history of homelessness, were collected. Current HIV status was assessed using the CD4 cell count, CD4 percentage, HIV RNA level, and use of antiretroviral therapy at the time nearest the QFT. Medical records were reviewed for LTBI treatment and the development of active tuberculosis from date of QFT testing through the most recent clinic visit.

QFTs were performed and interpreted per the manufacturer’s guidelines (Cellestis) at the Denver Health Medical Center.
laboratory and at National Jewish Health laboratory. The number and frequency of positive, indeterminate, and negative QFT results were recorded. Any repeat QFT result was also recorded for patients with an initial positive result. SAS version 9.2 software was used to generate logistic regression models to evaluate patient characteristics associated with QFT reversion and to analyze the association of QFT reversion with the IFN-γ response in the TBAg, Nil, and Mitogen tubes.

This study was approved by the Denver Health Hospital Authority and Colorado Multidisciplinary Institutional Review Board.

RESULTS
From 1 July 2009 to 30 June 2010, 1364 HIV-infected patients were tested with QFT: 94 (6.9%) had positive test results, 27 (1.98%) indeterminate, and 1243 (91.13%) negative. Of the 94 QFT-positive patients, 36 (38.3%) had risk factors of tuberculosis (34 born outside the United States). Repeat QFTs were performed on 49 of 94 (52.1%) patients with positive results. Of the 45 QFT-positive patients not retested, all were offered LTBI treatment, and none developed tuberculosis during follow-up. Twenty-eight of the 45 (62%) were not retested because they had risk factors for tuberculosis exposure, and 17 (38%) were not retested during the policy implementation period. All repeated QFT tests were performed on a separate blood draw with a median time of 40.5 days (interquartile range, 14–105) between testing. No patients were started on LTBI therapy prior to the repeat QFT. There were no significant differences in CD4 cell count, HIV RNA level, and use of antiretroviral therapy between testing. Of the 49 repeated QFT tests, 35 (71.4%) reverted to negative, 12 (24.5%) remained positive, and 2 (4.1%) were indeterminate.

Too few patients (n = 2) had a CD4 count <200 cells/mm³ to appropriately analyze an association with low CD4 cell count. There was a significant association between undetectable HIV RNA and QFT reversion (OR, 5 [95% CI, 1.2–23.5]; P = .031), but this did not remain significant after excluding patients born in countries with high incidence of tuberculosis. QFT reversions were not significantly associated with the IFN-γ responses in the TBAg, Nil, and Mitogen tubes.

Patients with a positive repeat QFT result were offered LTBI treatment, none of whom developed tuberculosis. None of the 35 patients with QFT reversion were offered LTBI treatment, and none developed tuberculosis after an average and cumulative follow-up of 500 days and 41.1 patient-years, respectively.

DISCUSSION
In our population of HIV-infected patients living in Denver, Colorado, positive QFT results reverted to negative in 80.5% of US-born patients at low risk for tuberculosis exposure. Only 2 of 8 (25%) repeat QFTs reverted in patients from high-tuberculosis-incidence countries, suggesting that patients with true LTBI remain positive on retesting. Without a “gold standard” for LTBI diagnosis, the clinical significance of variable test results is unknown. We believe the correlation between country of birth and likelihood of reversion supports the conclusion that transiently positive QFTs represent false-negative results.
positive initial tests, a conclusion supported by the lack of observed tuberculosis cases in patients with a QFT test reversion not treated for LTBI. Our findings also suggest that the positive predictive value of QFTs is quite low when testing a large number of HIV-infected patients at low risk for tuberculosis, providing a likely explanation for the high rate of discordance between QFT and TST results in studies of HIV-infected patients in the United States [15, 16].

The potential causes of variability in serial IGRA testing could be intrinsic to the assay or result from variable immune response. Potential causes of intra-assay variability include improper collection, storage, incubation, and processing of blood tubes, and variation of the enzyme-linked immunosorbent assay IFN-γ measurements that are done in 96-well plates [17]. Potential sources for variable immune responses include changing CD4 cell count, medications, stress, and infection. The high reversion rates we observed cannot be explained entirely by variable immune responses because the repeat QFTs were performed within a median of 40 days, only 2 patients had <200 CD4 cells/mm³, and reversions were not associated with the use of HIV treatment. Unexplained positive to negative QFT test reversion has been described in healthcare workers where rates of QFT reversion have ranged from 24% to 41% [18–20]. Strategies that have been proposed to reduce QFT variability include raising the cutoff for a positive QFT or creating an “uncertainty zone” between a positive and negative test result [20, 21], would have eliminated few of the QFT reversions in our low-risk population (Table 1).

The CDC guidelines recommend IGRA testing for BCG-vaccinated individuals and those unlikely to return for TST readings [4]. False-positive IGRA results are expected when testing low-risk populations, and the guidelines recommend repeat testing in people at low risk for progression. In contrast, these guidelines recommend treating HIV-infected patients for LTBI with any positive test irrespective of discordance due to the high rate of tuberculosis reactivation in HIV-infected patients. Our data suggest that identifying false-positives by repeating the QFT would avoid unnecessary LTBI treatment. Although more prospective data are needed on the predictive value of positive and transiently positive QFTs, we support a strategy of retesting positive QFTs in HIV-infected patients with no tuberculosis exposure risks.

Table 2. Results of Repeated Positive QuantiFERON-TB Gold In-Tube Assays by Tuberculosis Exposure Risk and HIV Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total No.</th>
<th>Negative, No (%</th>
<th>Positive, No (%)</th>
<th>Indeterminate, No (%)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>49</td>
<td>35 (71.4%)</td>
<td>12 (24.5%)</td>
<td>2 (4.1%)</td>
<td>1.73 (.2–12)</td>
<td>.577</td>
</tr>
<tr>
<td>No tuberculosis risk</td>
<td>41</td>
<td>33 (80.5%)</td>
<td>6 (14.6%)</td>
<td>2 (4.9%)</td>
<td>7 (1.2–57.7)</td>
<td>.034</td>
</tr>
<tr>
<td>From high-tuberculosis-incidence country</td>
<td>8</td>
<td>2 (25%)</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>0.14 (0.2–9)</td>
<td>.034</td>
</tr>
<tr>
<td>CD4 cell count &lt;200 cells/mm³</td>
<td>2</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>0 (%)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>CD4 count &gt;200 cells/mm³</td>
<td>47</td>
<td>35 (74.5%)</td>
<td>10 (21.3%)</td>
<td>2 (4.2%)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>On ART</td>
<td>43</td>
<td>31 (72.1%)</td>
<td>11 (25.6%)</td>
<td>2 (4.9%)</td>
<td>1.73 (1.2–12)</td>
<td>.577</td>
</tr>
<tr>
<td>HIV RNA &lt;48 copies/mL</td>
<td>35</td>
<td>27 (77.1%)</td>
<td>7 (20%)</td>
<td>1 (2.9%)</td>
<td>5 (1.2–23.5)</td>
<td>.031</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; QFT, QuantiFERON-TB Gold In-Tube assay.

* Defined as a country with >20/100 000 tuberculosis cases per year.

Notes

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

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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