Reversion and Conversion of Interferon-γ Release Assay Results in HIV-1–Infected Individuals

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In this prospective study, human immunodeficiency virus type 1 (HIV-1)–infected subjects underwent QuantiFERON-TB Gold In-Tube interferon-γ release assay (IGRA) testing at baseline and after 24 months in a low tuberculosis incidence country.

Concordant baseline and follow-up results were observed in 86% (n = 686 of 794) of subjects. IGRA conversions occurred in 9% (n = 63 of 718), whereas IGRA reversions were seen in 33% (n = 25 of 76) of individuals. Of the 10 active tuberculosis cases during follow-up, 5 had concordant positive IGRA results and 2 were IGRA converters.

Although the clinical significance of IGRA conversions and reversions remains to be established, repeated IGRA testing seems to be of value in HIV-1–infected individuals.

Keywords. human immunodeficiency virus type 1; interferon-gamma release assay; serial testing; conversion; reversion.

Individuals with human immunodeficiency virus type 1 (HIV-1) infection are at increased risk of latent tuberculosis infection (LTBI) and subsequent active tuberculosis reactivation and develop more severe clinical disease manifestations [1] and thus represent an important target group for LTBI testing and treatment.

For more than a century, the tuberculin skin test (TST) has been the most extensively used immune-based test for detecting LTBI [2]. More recently, laboratory T-cell–based interferon-γ (IFN-γ) release assays (IGRAs) have been developed as an alternative in vitro immunodiagnostic approach to the TST, possessing logistical conveniences [3]. Despite the fact that their performance is dependent on the actual CD4+ T-cell count, these tests have been demonstrated to detect LTBI with higher reliability than the TST in individuals infected with HIV-1 [4].

The interpretation of serial TST results may be complicated by nonspecific variations in test results, including boosting, conversions (negative to positive test), and reversions (positive to negative test) upon repetitive testing [5]. Serial testing with IGRA is attractive because it avoids subjective measurements, can be repeated without sensitization and boosting in subsequent tests, and requires only a single patient visit. However, there is only limited data on the reproducibility of IGRAs, particularly with regard to within-person variability of T-cell responses during serial testing. Thus, the updated US Center for Disease Control and Prevention guidelines 2010 ask for more research on the serial testing performance of IGRAs, especially in individuals with HIV-1 infection [6].

This study was performed in a cohort of HIV-1–infected individuals in a country with a low risk of tuberculosis reinfection after inclusion. The objectives of this prospective, single-center study were to determine (1) the incidence of IGRA reversions and conversions in a cohort of HIV-1–infected individuals and (2) to identify socio-epidemiological and clinical risk factors associated with reverted or converted IGRA results.

METHODS

Study Setting and Recruitment

This study is an extension of a project initiated in 2006 to evaluate the QuantiFERON-TB Gold In-Tube IGRA for routine use in HIV-1–infected individuals in a low-incidence country [7]. Briefly, HIV-1–infected patients aged ≥18 years attending the HIV outpatient clinic of the Vienna General Hospital who voluntarily underwent IGRA testing were prospectively enrolled. Written informed consent for inclusion was provided by all participants. Patients with current active tuberculosis disease were excluded. The study protocol was approved by the Ethics Committee of the Medical University of Vienna, Austria.

Laboratory Assays

Venous blood samples were drawn in 3 QuantiFERON-TB Gold In-Tube evacuated tubes, precoated with either Mycobacterium...
tuberculosis–specific antigens (ESAT-6, CFP-10, TB7.7), phytohemagglutinin for the positive control, or no antigen for the negative control. Within 4 hours of drawing, specimens were processed according to the manufacturer’s instructions, as described elsewhere [8].

The IGRA was done at baseline and repeated after approximately 2 years using identical protocols. In accordance with US Center for Disease Control and Prevention guidelines, IGRA conversion was defined as baseline IFN-γ < 0.35 IU/mL and follow-up IFN-γ ≥ 0.35 IU/mL, whereas IGRA reversion was defined as baseline IFN-γ ≥ 0.35 IU/mL and follow-up IFN-γ < 0.35 IU/mL [6].

All subjects underwent chest x-ray screening for tuberculosis at least once a year and were clinically monitored for tuberculosis-related clinical symptoms, as described elsewhere [7]. In case of IGRA positivity, subjects were recommended to start both antiretroviral therapy (ART) and tuberculosis chemoprevention.

Statistical Analyses
Statistical analyses were conducted employing Statistica version 6.0 for Windows. Median values were compared using the non-parametric Wilcoxon–Mann–Whitney U rank-sum test. Fisher’s exact test was used to evaluate differences in proportions. The intertest agreement of serial dichotomized IGRA testing was assessed by Cohen’s kappa (κ) statistics. All P values were two-tailed, and P < .05 was considered to denote statistical significance.

RESULTS

Baseline Characteristics of the Study Participants
A total of 1525 HIV-1–infected individuals were asked to participate in this study. Seven patients declined participation, 13 were diagnosed with active tuberculosis at baseline, and in 659 individuals no repeat IGRA was performed. A total of 846 subjects (representative of the entire study population) with both baseline and repeat IGRA results were included in this study. The mean age was 39.6 ± 11.5 years; 71% (n = 597 of 846) of the subjects were male and 29% (n = 249 of 846) were female. The majority of individuals were white (86%; n = 730 of 846), 8% (n = 68 of 846) were black, 3% (n = 26 of 846) were Hispanic, and 2% (n = 18 of 846) were Asian. The majority of subjects originated from the United States, 63.5% (n = 40 of 63) originated from a high tuberculosis incidence country according to the World Health Organization, compared with 21.6% (n = 169 of 783) of nonconverters (odds ratio [OR], 2.3; P = .02). Patients with a converted IGRA were more likely to have a history of concurrent tuberculosis disease compared to nonconverters (OR, 2.3; 95% confidence interval [CI], 1.7–3.2).

Concordance of Baseline and Follow-up IGRA Results
At baseline, the IGRA yielded positive results in 9% (n = 76 of 846), negative results in 85% (n = 718 of 846) and indeterminate results in 6% (n = 52 of 846) of subjects. After a mean of 614 ± 195 days, the repeat IGRA was performed. Of the 76 patients who initially tested positive, 58% (n = 44 of 76) were concordantly positive, and 9% (n = 7 of 76) were indeterminate on serial testing. In 33% (n = 25 of 76) of patients, the serial IGRA result was negative, and thus a reversion of IGRA result was observed. At follow-up testing of the 718 patients with a negative IGRA at baseline, 89% (n = 642 of 718) individuals remained IGRA negative, whereas 9% (n = 63 of 718) converted to a positive IGRA. Among the 52 subjects with indeterminate baseline IGRA results, the second IGRA was positive in 3, negative in 43, and indeterminate in 6 individuals. The intertest agreement of serial QFT testing was moderate as indicated by a Cohen κ coefficient of 0.448 (95% confidence interval [CI], .439–.452). The observed intertest agreement was 0.887 (95% CI, .847–.899), whereas the probability of random agreement of serial QFT testing was calculated as 0.796 (95% CI, .786–.803) (Table 1).

When applying a more stringent criteria for IGRA conversion (follow-up IFN-γ response >0.70 IU/mL), 30% (n = 19 of 63) of converters would have tested negative at follow-up. Changes in quantitative IFN-γ responses for converters and reverters are shown in Supplementary Table 1. Although not part of the initial study protocol, a third IGRA was performed in 60% (n = 38 of 63) of converters, with 55% (n = 21 of 38) showing negative IGRA results. These 21 patients had a lower IFN-γ response than those who remained IGRA positive (median IFN-γ value, 0.75, interquartile range [IQR], 0.49–1.8) IU/mL vs 1.37, IQR: 0.93–2.175 IU/mL). patients who initially tested positive, 58% (n = 44 of 76) were

Table 1. Agreement of Baseline and Follow-Up Interferon-γ Release Assay Results

<table>
<thead>
<tr>
<th>IGRA 1</th>
<th>IGRA 2</th>
<th>No. (% or 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>642 (82.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>63 (8.1)</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>44 (5.8)</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>25 (3.2)</td>
</tr>
<tr>
<td>Intertest agreement</td>
<td>0.887 (.847–.899)</td>
<td></td>
</tr>
<tr>
<td>Cohen κ coefficient</td>
<td>0.448 (.439–.452)</td>
<td></td>
</tr>
<tr>
<td>Probability of random agreement</td>
<td>0.796 (.786–.803)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IGRA, interferon-γ release assay.

Independent Predictors for IGRA Conversion and Reversion
In multivariable regression analysis, there were no statistically significant differences between converters and nonconverters in terms of sex, age, and HIV-1–related parameters, including actual and nadir CD4+ T cell counts, HIV-1 RNA level, ART status, and prior AIDS manifestation. Of patients with a converted IGRA, 63.5% (n = 40 of 63) originated from a high tuberculosis incidence country according to the World Health Organization, compared with 21.6% (n = 169 of 783) of nonconverters (odds ratio [OR], 2.3; P = .02). Patients with a converted IGRA were more likely to have a history of concurrent tuberculosis disease compared to nonconverters (OR, 2.3; 95% confidence interval [CI], 1.7–3.2).
have acquired HIV-1 infection by intravenous drug abuse (28.6% vs 17.9%; OR, 2.5; P = .01) (Table 2).

Adjusted analysis revealed the following risk factors of reverted IGRA results: male sex (OR, 15.9; P = .004) and origin from a high tuberculosis incidence country (OR, 1.9; P = .02). Age, mode of infection with HIV-1, actual and nadir CD4+ T cell count, and ART were not associated with a reverted IGRA result (Table 2).

There was no difference in terms of intraperson variability of CD4+ T cell count at baseline and follow-up testing among reverters and converters.

**Cases of Active Tuberculosis During Follow-up**

During the observational period, 10 individuals (1.2%; n = 10 of 846) were diagnosed with active tuberculosis, with a median delay of 435 days (IQR, 281–508) from inclusion. None of these subjects reported having had contact with an active tuberculosis case or having traveled to a country with high tuberculosis prevalence between study inclusion and tuberculosis diagnosis. Five patients suffered from pulmonary tuberculosis, 3 from extrapulmonary tuberculosis, and 2 from miliary tuberculosis. Seven of the 10 active tuberculosis cases had culture-confirmed disease. The median baseline IFN-γ release was significantly higher in the 7 IGRA positive patients with subsequent active tuberculosis as compared with the remaining 69 IGRA positive individuals who had not developed active tuberculosis (7 IU/mL vs 2 IU/mL; P = .04). Active tuberculosis was observed in 11% (n = 5 of 44) of subjects with concordant positive IGRA results. Only 1 patient progressed to active tuberculosis despite showing negative IGRA results both at baseline and at follow-up. Two subjects with tuberculosis were IGRA converters, whereas 1 had a reverted IGRA.

**DISCUSSION**

HIV-1–infected subjects represent a high-risk group for active tuberculosis development for which an accurate detection of LTBI is of paramount importance. Although broadly recommended and increasingly used, data on the interpretation of IGRA results in serial testing is scarce. In this prospective, longitudinal study on HIV-1–infected individuals in a low tuberculosis incidence country followed for 3 years, repeated IGRA testing showed promising results. Although interest agreement of serial IGRA testing was moderate, >86% of patients had...
concordant IGRA results at baseline and at follow-up. These results are consistent with previously published data in HIV-uninfected populations [9]. In terms of risk factors for converted IGRA results, we found that origin from a high tuberculosis incidence country according to the World Health Organization and injection drug abuse were associated with IGRA conversion. Interestingly, origin from a tuberculosis incidence country was also associated with IGRA reversion. However, a rationale with respect to biological plausibility is lacking.

Only a limited number of longitudinal studies assessing the value of IGRA in serial testing are available. The studies already published were predominantly performed in either contact [10] or healthcare worker investigations [11, 12]. Among contacts of active tuberculosis cases, IGRA conversions and reversions occurred with conversion rates depending on the test and definitions used [10]. Conversions, reversions, and nonspecific variations leading to discordance were observed with serial IGRA testing just as they were with TST in a study on healthcare workers in India [11]. A recent study by Fong et al showed that the clinical significance of IGRA conversions in serial testing remains a challenging task for clinicians and that the use of single cutoff point criteria for IGRA may lead to a significant number of false-positive results and overdiagnosis of LTBI [12].

So far, no study prospectively evaluated the performance of an IGRA in serial testing of HIV-1–infected patients in a low tuberculosis incidence country. To the best of our knowledge, this extension of our single-center study is the first to assess the serial testing performance of IGRA in a large cohort of individuals with HIV-1 infection. Gray et al recently identified false-positive IGRA results by repeat testing (within 40 days) in HIV-1–infected subjects in a low tuberculosis incidence setting (Denver, CO). However, the retrospective study design and the fact that only individuals with a positive IGRA result were presented in this study may have introduced a bias [13].

Although the reversion rate observed in our study was not as high as the 72% observed in the United States [13], still more than one-third of initially IGRA-positive patients tested negative at follow-up. Consistently, IGRA reversion of unknown origin has been described in healthcare workers, with rates of IGRA reversion ranging 25%–41% [14]. Thus, it might be conceivable to retest HIV-1–infected patients with positive IGRA results with no tuberculosis exposure risks to prevent unnecessary tuberculosis chemoprevention and possible drug–drug interactions. This strategy is in accordance with US Centers for Disease Control and Prevention guidelines [6].

We have to acknowledge the potential limitations of this study, including its single-center nature. Furthermore, because this study was a no-contact investigation, anamnesis regarding previous contact to an active tuberculosis index case was not part of the initial study protocol and is therefore missing.

In conclusion, we provide further evidence that IGRA are of clinical value both in immunocompetent individuals and in patients with moderate immunodeficiency. Because of objective read-out and independence from patient compliance, IGRA have the potential to replace the TST for the identification of HIV-1–infected individuals at risk for the development of active tuberculosis. However, IFN-γ variability should be kept in mind when interpreting repeated IGRA results.

**Supplementary Data**

**Supplementary materials** are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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

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