Interferon-gamma Release Assays for the Diagnosis of Latent Tuberculosis Infection: an Updated Review

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Abstract. Interferon-gamma (IFN-γ) release assays (IGRAs) are in vitro immune tests that have recently been introduced as an alternative to the tuberculin skin test (TST). An increasing body of evidence supports the use of IGRAs for the diagnosis of latent tuberculosis infection (LTBI) in various clinical situations. Currently, two commercial IGRAs are available: the QuantiFERON-TB Gold In-Tube test (QFT-GIT, Cellestis Ltd, Carnegie, Australia) and the T-SPOT TB assay (T-SPOT, Oxford Immunotec Ltd., Abingdon, UK). Though the clinical performances of these two IGRAs varied widely according to the characteristics of each study, they were reported to be better than those of TST in most cases. In this concise review, the performance characteristics, the use in special clinical situations, and the guidelines of IGRAs are presented.

Introduction

Tuberculosis (TB) infection is an enormous burden worldwide [1-2]. In 2010, there were an estimated 8.8 million cases of TB infection globally [3], and one-third of the world’s population is thought to be latently infected with Mycobacterium tuberculosis (M. tuberculosis) [4]. People with latent TB infection (LTBI) do not manifest overt symptoms of active TB; however, they are at increased risk for developing active diseases (5% – 10% lifetime progression) and constitute a significant number of future epidemics [5]. For people with known risk factors, a targeted approach is recommended for the screening of LTBI, especially in regions of low prevalence, such as the US [6]. Risk factors for the progression of LTBI to active TB include human immunodeficiency virus (HIV) infection, an age of < 5 years, certain cancers and chronic diseases, inadequate treatment of TB, and immunosuppressive therapy – e.g., tumor necrosis factor–alpha (TNF-α) antagonists, systemic corticosteroids, or following organ transplantation [4]. Treatment with isoniazid reduces the risk of LTBI progression by 75% – 90%; thus, identifying individuals with LTBI is an important step towards TB eradication [5]. The development of an accurate method for the identification of LTBI is thus essential to this end.

There is no gold standard for the diagnosis of LTBI. Therefore, prophylactic treatment is performed on the basis of epidemiologic, clinical, and laboratory criteria with patient acceptance [7]. Until recently, the tuberculin skin test (TST) has been the only test for LTBI detection. The TST uses the principles of delayed hypersensitivity to recruit memory T-cells to the site of an intradermal injection of purified protein derivative (PPD) of Mycobacterium bovis. Because the PPD used in the TST is a mixture of antigens, a response to PPD can occur due to the presence of Mycobacterium bovis, bacille Calmette-Guérin (BCG), or some non-tuberculous mycobacteria (NTM) [2, 8]. Moreover, the interpretation of TST results can be subjective, and false negative results can be produced in the cases of immunocompromised individuals, young persons, and the elderly [7].

Interferon-gamma (IFN-γ) release assays (IGRAs) are in vitro immune tests that have been recently introduced as an alternative to the TST for the diagnosis of LTBI. IGRAs are based on the detection of a T-cell immune response towards M. tuberculosis complex-specific antigens. To date, there are two commercially available IGRAs: the QuantiFERON-TB Gold In-Tube test (QFT-GIT) and its predecessor the QuantiFERON-TB Gold (QFT-G) test (Cellestis Ltd, Carnegie, Australia); and the T-SPOT TB assay (T-SPOT) (Oxford Immunotec Ltd., Abingdon, UK). In this review, we give an overview of current IGRAs and their clinical applications.
Interferon-gamma (IFN-γ) release assays (IGRAs)

In 2001, the QuantiFERON-TB test (QFT; Cellestis Ltd.) became the first IGRA to be approved by the Food and Drug Administration (FDA) as an aid for diagnosing *M. tuberculosis* infection [9]. Initially, this test used an enzyme-linked immunosorbent assay (ELISA) to measure the amount of IFN-γ released in response to PPD as compared to controls. Due to its low specificity, the QFT was replaced by a new version, the QFT-G, which assesses the response to synthetic overlapping peptides that represent specific *M. tuberculosis* proteins, such as early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10) [10]. These proteins are present in all *M. tuberculosis* and stimulate a measurable release of IFN-γ in most infected persons; however, they are absent from BCG vaccine strains and from most NTM [4]. Although these proteins allow the test to be more specific than does PPD, they are also present in *M. kansasii*, *M. szulgai*, and *M. marinum*, and sensitization to these organisms may cause false-positive IGRA results.

The QFT-GIT was developed in 2007. In the QFT-GIT, controls and TB-specific antigens are contained in the blood collection tubes for the test, allowing more direct testing of fresh blood. The QFT-GIT uses a peptide cocktail of ESAT-6, CFP-10, and TB7.7 proteins to stimulate cells. A test is considered positive if an IFN-γ response to the TB antigen tube is significantly above the Nil IFN-γ value (≥ 0.35 IU/mL), which adjusts for background, heterophile antibody effects, or non-specific IFN-γ samples. The IFN-γ level of the Nil tube is subtracted from the IFN-γ level for the TB antigen tube and Mitogen tube (positive control). A low response to Mitogen (< 0.5 IU/mL) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens. This pattern may occur because of insufficient lymphocytes, reduced lymphocyte activity due
to prolonged specimen transport or improper specimen handling, including filling/mixing of blood tubes, or the inability of the patient’s lymphocytes to generate IFN-γ [11]. According to the 2005 Centers for Disease Control and Prevention (CDC) guidelines, the QFT-G may replace the TST in situations such as contact investigations, the evaluation of recent immigrants, and serial-testing surveillance programs for infection control [10].

Another IGRA, the T-SPOT, was approved by the US FDA in 2008. The T-SPOT is a simplified variant of the enzyme-linked immunospot (ELISPOT) assay technique. In ELISPOT assays, the target cytokine is captured directly around the secreting cell before it is diluted in the supernatant, captured by the receptors of adjacent cells, or degraded. The T-SPOT is designed to detect effector T-cells that respond to the stimulation with antigens specific for M. tuberculosis, ESAT-6, and CFP-10, and counts the individual spot of activated TB-specific T-cells. The T-SPOT uses peripheral blood mononuclear cells (PBMCs), which are incubated with antigens to allow stimulation of any sensitized T-cells present. Secreted cytokine is captured by specific antibodies on the membrane and a second antibody, conjugated to alkaline phosphatase, binds to the cytokine captured on the membrane surface. A soluble substrate is added to each well, which is then cleaved by bound enzymes to form a spot of insoluble precipitate at the site of the reaction. Each spot represents the footprint of

an individual cytokine-secreting T-cell, and the number of resulting spots provides a measure of the abundance of M. tuberculosis-sensitive effector T-cells in the peripheral blood. T-SPOT results are interpreted by subtracting the spot count in the Nil Control well from the spot count in each panel of the antigens. The test result is positive if the spot count of at least one TB antigen panel minus the spot count of the Nil Control is ≥ 6 [12]. The main steps of two IGRAs are presented in Figure 1 and Figure 2.
Performance characteristics

The performance of IGRAs is difficult to assess, because there is no gold standard to confirm LTBI or culture-negative active TB. Most studies have analyzed the results of IGRAs by testing populations with known characteristics, such as culture-confirmed active TB patients and patients at high or low risk for LTBI.

**Sensitivity.** Comparing sensitivities from previous studies is challenging and should be done with caution given that these studies varied in population, interpretation criteria, and the types of tests used [4, 13]. For culture-confirmed cases with active TB, the sensitivity of the QFT-GIT ranged from 65% to 93%, and the pooled sensitivity was 81% based on 14 published studies [4].

The sensitivity of T-SPOT was superior to that of QFT-GIT in most studies [4, 13]. For culture-confirmed cases with active TB, the sensitivity of T-SPOT ranged from 50% to 100%, and the pooled sensitivity was 91% based on 12 published studies [4]. Most studies demonstrated similar sensitivities for the QFT-GIT, T-SPOT, and TST [14-17]. However, some studies showed higher sensitivities for the T-SPOT compared to the TST or QFT-GIT [16-19].

**Specificity.** Specificity can be assessed in low-risk populations. Comparing the specificities of published data is difficult, since the results can be influenced by background risk for infection and unrecognized LTBI in the study population [4]. The QFT-GIT and T-SPOT generally showed higher specificity, as expected, especially in BCG-vaccinated populations because of the use of MTB-specific antigens [13, 15, 20-21]. In a BCG-vaccinated population, the pooled specificity of the TST decreased to 59%, compared to 97% in a non-vaccinated population [13]. The specificities of the QFT-GIT ranged from 99% to 100%, and the pooled specificity was 99% [15, 22-23]. Although the sample size of data for the T-SPOT was small, the specificities of the T-SPOT ranged from 85% to 100%, and the pooled specificity was 88% [15, 18, 24].

**Predictive value.** The predicted risk for subsequent active TB is an important issue. In individuals with positive TST results, the lifetime risk for active TB is known to be 5% – 10% [25]. However, there is little data on the predictive value of IGRAs. Diel et al. recently conducted a systematic review and meta-analysis to compare the accuracy of QFT-GIT and T-SPOT with that of TST for the diagnosis of LTBI. In that study, the negative predictive value (NPV) for the progression of LTBI to active TB within 2 years was 97.8% for the T-SPOT and 99.8% for the QFT-GIT in a low-incidence population. The rate of progression to active TB among subjects having tested positive for LTBI was 2.3% – 3.3% for the TST, 2.8% – 14.3% for the QFT-GIT, and 3.3% – 10% for the T-SPOT [14, 26-29] (Table 1). These studies included HIV-infected patients, recent immigrants, adults in contact with infected persons, and children. Another meta-analysis by Rangaka et al. [30] demonstrated the predictive values of IGRAs and the TST. This report showed a moderate association between positive results and subsequent TB (poled unadjusted incidence rate ratio [IRR], 2.10; 95% CI, 1.42 – 3.08). Although there was a statistical difference in the IRR between the IGRA and the TST, the use of the IGRA may reduce the number of subjects considered for treatment for LTBI since the proportion of positive results were lower in the IGRA than in the TST (Table 1).

**Reproducibility of IGRA.** Published studies of serial testing have revealed considerable fluctuations in IFN-γ responses in individual patients [31-33]. Conversion from negative to positive can occur with new infection or boosting following the TST [34], and reversion from positive to negative in antimycobacterial treatment. However, these fluctuations have been observed without any known factors, with frequencies of 12% – 50% [31-32, 35]. This may be due to actual fluctuations in IFN-γ responses in the patient; however, laboratory factors including limitations in assay precision or variation of procedures are also possible [5]. These fluctuations occur more readily in individuals with results near the cut-off; thus, results in this grey area should be interpreted with caution. To solve these problems, well-controlled studies are needed to further
define the causes of individual variations in IFN-γ response, and assays with better precision should be developed.

Special clinical situations

Use of QFT-GIT and T-Spot to test children. The rates of progression from LTBI to severe active diseases such as meningitis, disseminated disease, or even death are higher in infants. However, the use of IGRA as a predictive marker for progression is especially risky in children aged < 5 years [36]. Use of IGRAs in children has several limitations: 1) unknown performance of IGRAs in children due to the limited number of studies; 2) higher portion of indeterminate results for children due to low mitogen response from immunologic immaturity; and 3) reported sensitivities similar to or lower than that of TST [37-38]. In recent studies, sensitivities were 78% – 93% for the QFT-GIT, 58% – 93% for the T-Spot, and 82% – 100% for the TST [15-16, 37], while the specificities of IGRAs were reported to be high (98% – 100%) [15]. The use of IGRAs in children should continue to be evaluated in further studies.

Use of QFT-GIT and T-Spot to test immunocompromised subjects. Most published studies have demonstrated the performance of IGRAs in HIV-infected patients. Because there is no ‘gold standard’ for LTBI, it is difficult to compare the sensitivities of IGRAs. Published comparisons have not demonstrated significant differences in the proportions of positive QFT-GIT or T-Spot results compared with TST results among HIV-infected patients screened for M. tuberculosis infection [4, 38]. The QFT-GIT and TST were both less sensitive in HIV-infected patients than in subjects without HIV infection [39].

Other important groups for LTBI screening are patients with immune-mediated inflammatory diseases (IMID), such as rheumatoid arthritis and ankylosing spondylitis [40]. Anti-tumor necrosis factor alpha (TNFα) therapy has emerged as an effective treatment for these diseases. However, TNFα plays a role in protecting the host against M. tuberculosis with TNF-dependent chemokines; thus, inhibition of TNFα and TNF-regulated chemokine networks can increase the risk of TB [41]. In fact, in patients with LTBI, a 4- to 5-fold higher incidence of TB is observed after initiation of anti-TNF therapy with infliximab [42-43]. Although several studies compared the performance of IGRAs with that of TST in patients with IMID, direct comparison data is limited [44-47]. In general, IGRAs performed better than TST in recent studies, which may underscore the value of IGRAs for

Table 1. Predictive value of interferon-gamma release assays for the progression to active tuberculosis based on two recent meta-analysis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Assay</th>
<th>TST</th>
<th>Incidence rate ratio*</th>
<th>Total (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rangaka et al.</td>
<td>ELISPOT†</td>
<td>Whole blood ELISA‡</td>
<td>TST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.9 - 4.5</td>
<td>1.09 - 2.60</td>
<td>1.05 - 2.10</td>
</tr>
<tr>
<td></td>
<td>Total (95% CI)</td>
<td>2.64 (1.41 - 4.93)</td>
<td>1.82 (1.11 - 2.97)</td>
<td>1.60 (0.94 - 2.72)</td>
</tr>
<tr>
<td>Diel et al.</td>
<td>T-SPOT</td>
<td>QFT-GIT</td>
<td>TST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>88.0% – 100%</td>
<td>98.0% – 100%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Pooled NPV (95% CI)</td>
<td>97.8% (94.5% - 99.4%)</td>
<td>99.8% (99.4% - 100%)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>3.3% - 10%</td>
<td>2.8% - 14.3%</td>
<td>2.3% - 3.3%</td>
</tr>
</tbody>
</table>

*The rate of disease progression in test-positive vs. test-negative individuals.
†ELISPOT includes T-SPOT and in-house method.
‡Whole blood ELISA includes QFT-GIT and in-house ELISA.

Abbreviations: ELISPOT, enzyme-linked immunosorbent spot; TST, tuberculin skin test; NPV, negative predictive value for progression; PPV, positive predictive value for progression; CI, confidence interval; ND, no data.
LTBI screening in patients receiving anti-TNF agents [44, 46, 48-50]. However, data on the predictive value of progression to TB in immunocompromised patients are still limited. A combination of results from TST and IGRA for LTBI diagnosis might be more appropriate in this high-risk patient population [48]. More data should be accumulated to validate the performances of IGRAs in this setting.

Screening of healthcare workers. Health care workers (HCWs) are at high risk for TB due to exposure to patients with TB or to specimens with *M. tuberculosis* [51-52]. According to the guidelines on preventing TB transmission, all HCWs should receive regular TB screening using the TST or the IGRA [51, 53]. The prevalence of LTBI in HCWs is influenced by age, duration or degree of exposure to the study populations, the incidence of TB in each region, and the method of LTBI detection. Using QFT-G, the prevalence of LTBI in HCWs was 9.9% –25.9% in low-incidence settings and up to 40% in high-incidence settings, higher than in general populations [2, 52, 54-55]. In BCG-vaccinated populations, TST is not appropriate for the screening of HCWs due to its high positivity. Although IGRA would be reliable and easy to perform in these BCG-vaccinated populations, its higher cost is regarded as a barrier to routine screening use.

Several studies compared the costs for TB screening [56-58]. One study, using a computerized cost-comparison model for TB screening in HCWs, showed that the QFT-G-only model was cheaper than the TST-only model and required fewer clinical visits [59]. Although some studies raised concerns about the effectiveness of IGRAs as a sole screening test in HCWs, especially in low-incidence settings [60-61], a recent systemic review of the cost-effectiveness of different TB-screening strategies concluded that there is strong evidence in support of the use of IGRAs for screening risk groups, such as HCWs, immigrants from high-incidence countries, and close contacts [62]. The issue of cost may be different in each country with variable TB incidences and medical situations.

Current guideline for using IGRAs

The following is a summary of recommendations for using IGRAs that was recently published by the US Centers for Disease Control and Prevention (CDC) [4].

- The CDC suggests that an IGRA may be used in place of the TST in all situations in which the CDC recommends the TST as an aid in diagnosing *M. tuberculosis* infection. Each test has its advantages in certain situations, but the use of an alternative test (FDA-approved IGRA or TST) is an acceptable medical and public health practice.
  - **Situations in which an IGRA is preferred but a TST is acceptable**
    - Groups having low rates of returning to have TSTs read, such as homeless individuals
    - BCG-vaccinated populations
  - **Situations in which a TST is preferred but an IGRA is acceptable**
    - Children aged < 5 years, due to the low sensitivity of IGRAs
  - **Situations in which either a TST or an IGRA may be used without preference**
    - Recent contacts with persons known or suspected to have an active TB
    - Periodic screening of persons having occupational exposure to *M. tuberculosis*
  - **Situations in which testing with both an IGRA and a TST may be considered**
    - When the initial test (TST or IGRA) is negative in a situation where 1) the risks for infection, progression, and a poor outcome are increased, or 2) clinical suspicion exists for active TB.
    - When the initial test is positive in situations where additional evidence of infection is required to encourage compliance, or for healthy persons who have a low risk for both infection and progression.
    - When the initial IGRA result is indeterminate, borderline, or invalid.
- **Medical management after testing**
  - Diagnoses of TB should include consideration of epidemiologic and medical history and other clinical information along with TST or IGRA results.
A diagnosis of LTBI requires that active TB be excluded by medical evaluation. In persons with clinical evidence of active TB or who are at increased risk for progression to active TB, a positive result with either an IGRA or a TST should be taken as an evidence of *M. tuberculosis* infection. A single positive IGRA or TST result should not be taken as a reliable evidence of *M. tuberculosis* infection in healthy persons with low risk.

In persons with discordant test results, decisions require individualized judgments.

The strategy for LTBI screening differs across countries. Some countries, including Germany and Switzerland, recommend that the TST should be replaced by the IGRA; the USA and France recommend interchangeable use of the IGRA and the TST; others, including Canada and the UK, suggest a two-step approach that combines the TST and IGRA [4, 48, 63]. The strategy should be adjusted based on the burden of TB, medical environment, and vaccination policy of each country.

**Conclusions**

Screening LTBI using appropriate methods is fundamental to identify and treat patients who have an increased risk of progression to active TB. Although there is no gold standard for the diagnosis of LTBI, IGRA are now getting more attention as an alternative to the traditional TST for that purpose. The clinical performances of IGRA were variable across studies, but mostly superior to those of TST. Current guidelines recommend the use of IGRA in place of or in combination with the use of TST, and the cost-effectiveness of using IGRA was proven in some studies. From the perspective of medical and public health practices, routine screening of LTBI using IGRA could play a more crucial role in controlling TB burden worldwide. Individualized approaches would be necessary in each medical situation and country.

**Acknowledgements**

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