Interferon-Gamma-Release Assay Prevents Unnecessary Tuberculosis Therapy

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ABSTRACT: **Background:** The mass influx of immigrants from tuberculosis-endemic countries into Israel was followed by a considerable increase in the incidence of tuberculosis (TB). All contacts of active TB patients are obliged to be screened by tuberculin skin tests (TST) and, if found positive, prophylactic treatment is considered.

Objectives: To assess the utility of interferon-gamma (IFNγ)-release assay with a prolonged follow-up in preventing unnecessary anti-TB therapy in individuals with suspected false positive results.

Methods: Between 2008 and 2012 the QuantiFERON TB gold-in-tube test (QFT-G) was performed in 278 sequential individuals who were mostly TST-positive and/or were in contact with an active TB patient. In all, whole blood was examined by the IFNγ-release assay. We correlated the TST diameter with the QFT-G assay and followed those patients with a negative assay.

Results: The QFT-G test was positive in only 72 (42%) of all 171 TST-positive individuals. There was no correlation between the diameter of TST and QFT-G positivity. Follow-up over 5 years was available in 128 (62%) of all QFT-G-negative individuals. All remained well and none developed active TB.

Conclusions: A negative QFT-G test may obviate the need for anti-TB therapy in more than half of those with a positive TST.

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KEY WORDS: QuantiFERON TB gold-in-tube test (QFT-G), purified protein derivative (PPD), latent tuberculosis infection (LTBI), interferon-gamma (IFNγ), tuberculin skin tests (TST)

Tuberculosis (TB) is a highly communicable disease caused by *Mycobacterium tuberculosis* complex organisms such as *M. tuberculosis, M. bovis* and *M. Africanum*. Typically, full-blown tuberculosis develops in 10% of newly infected individuals, occurring in 5% during the first 2 years after infection and in 0.1% each year later [1]. The remaining 90% of newly infected individuals remain well and are defined as having latent tuberculosis infection (LTBI). LTBI is a non-communicable asymptomatic condition, which may remain so for life. Whereas most of these individuals maintain a normal life, immune-suppressed patients are frequent candidates to develop active TB even after many years of latency. This is particularly true for human immunodeficiency virus (HIV)-infected patients with low CD4 counts in whom T cell function fails to control infection [2]. This is also true for patients receiving chemotherapy, and those under prolonged high dose steroid and/or biological treatment [3].

Tuberculin skin testing (TST) has been used for many decades for the diagnosis of TB infection (both active and latent). The test is based on a delayed-type hypersensitivity response, evidenced by a dermal reaction appearing 48–72 hours after intradermal injection of purified protein derivative (PPD). False positive results of TST are frequently noticed due to prior exposure to non-tuberculous Mycobacteria or to previous administration of Bacillus Calmett-Guérin (BCG) vaccine. At present, it is common practice that each individual with a positive TST is referred to a pulmonologist for the decision whether or not "prophylactic" anti-TB therapy is required. However, in many cases, due to the possible false positivity of TST, this treatment is obviously unnecessary. Aiming to look for a more specific and easy-to-perform test, QuantiFERON TB gold-in-tube test (QFT-G) was approved by the FDA (Food and Drug Administration) in 2005 as a reliable test for the diagnosis of LTBI as well as for assessing active tuberculosis [4]. A whole-blood sample from a suspected individual is mixed with the specific TB antigens and a control (Nil). The QFT-G test is based on assessing the release of interferon-gamma (IFNγ) following the stimulation of CD4 T cells with peptides of three specific TB antigens (ESAT-6, CFP-10, TB 7.7). Interferon, both types I and II, are important in a wide range of immune responses. In this respect, IFNα was reported in association with systemic lupus erythematosus disease activity [5]. Compared to the TST, the QFT-G test has many advantages: it requires only a single patient visit, results are usually available within 24 hours, and the test is not subject to reader bias that can occur with TST, and finally it is not affected by a prior BCG vaccination [6,7]. Previous studies have noted that the QFT-G test has a sensitivity as high as 92% and a specificity of 99%, which are considerably higher than those of the TST whose specificity is only 49% [8-10].

During the last two decades, Israel has absorbed more than one million immigrants from the former Soviet Union, Eastern Europe, and Ethiopia – countries with a high prevalence of TB. This was followed by an increased incidence of both active cases of TB and LTBI [11,12]. The QFT-G test thus became a valuable tool for assessing people with a positive TST. We designed this study with the aim of evaluating the risk of those individuals with TST-positive/QFT-G test-negative to develop active TB over a follow-up of up to 5 years. In addition, we assessed the contribution of the QFT-GT in preventing unnecessary anti-TB therapies in individuals suspected to have LTBI.

PATIENTS AND METHODS

The QuantiFERON TB Gold In-Tube test was performed in 278 sequential individuals referred to the Division of Clinical Immunology at Bnai-Zion Medical Center in Haifa, during the period 2008–2012. Of these, 171 (62%) were TST-positive and were referred for a QFT-G test as a double check in order to limit possible false positivity. The other 107 individuals (38%) were referred for QFT-G as a primary test. The following tested individuals were referred:

- Individuals in whom the TST was found positive and therefore anti-TB therapy was advised. In this case, the possible finding of a negative QFT-G test could spare this unnecessary treatment
- Patients who are candidates for anti-tumor necrosis factor (TNF) therapy and in whom anti-TB treatment had to be given once their TST or QFT-G was positive
- New medical staff in whom a routine TST is required
- Individuals who reported to have been in recent contact with active TB patients.

POSITIVE TST/PPD

Intradermal injection of 0.1 ml of PPD, consisting of more than 200 antigens (many of which are Mycobacterium not specific to TB) including the homologous antigen BCG. Induration (palpable raised area) diameter across the forearm in millimeters was measured after 72 hours and assessed for test positivity. The decision whether to consider a diameter of 5 mm, 10 mm or 15 mm as a positive test depends on the medical condition of the tested individual; namely, if the individual is healthy, immune suppressed, or had been in contact with a patient with active TB. A TST ≥ 5 mm was considered positive in HIV-positive patients and in individuals with a history of recent exposure to a proven TB patient. TST ≥ 10 mm was considered positive in hospital medical staff (with increased chances of exposure to patients with active TB) and drug users, whereas TST ≥ 15 mm was considered positive in healthy individuals with no risk factors [Table 1].

Table 1. Interpretation of PPD positivity

PPD diameter	Medical condition
≥ 5 mm positive	• HIV-positive • Recent exposure to a TB patient • Immunosuppressed
≥ 10 mm positive	• Recent arrivals (≤ 5 years) from high prevalence countries • Intravenous drug users • Hospital staff • Patients with chronic disease
≥ 15 mm positive	• Persons with no known risk factors for TB

The patient's medical condition is important for determining what diameter of PPD (in mm) should be considered as positive and thus requiring further investigation and treatment (Centers of Disease Control and Prevention www.cdc.gov)

QFT-G ASSAY

QuantiFERON TB Gold In-Tube test (Cellestis/Qiagen, Victoria, Australia) is a blood test that detects ex vivo IFNγ production by peripheral blood mononuclear cells exposed to peptides designed to simulate *Mycobacterium tuberculosis* antigens. Heparinized whole blood was incubated with the three TB antigens (ESAT-6, CFP-10, and TB7.7) with Nil control tube and control mitogens (as a positive control) for 16–24 hours. Following this incubation the amount of IFNγ released from mononuclear cells was measured, using enzyme-linked immunosorbent assay (ELISA). A test is considered positive for an IFNγ response to the TB antigen tube that is significantly above the Nil IFNγ IU/ml value (TB antigen minus Nil IU/ml ≥ 0.35 and ≥ 25% of Nil value).

FOLLOW-UP

Patients with negative QFT-G test were followed by their general practitioner and/or a pulmonologist during 2–5 years. During this period, all individuals positive for PPD but negative for QFT-G were under close follow-up including checkups and periodic chest X-rays in search of the possible development of active TB.

RESULTS

A total of 278 QFT-G tests were performed during the period 2008–2012. The mean age of all individuals was 43.8 ± 15.5 years ± SD (51.4% females, 48.5% males). It is noteworthy that awareness regarding performance of the QFT-G increased significantly during the period 2010–2012, with a yield of 125 in 2012 only [Figure 1].

Of 278 tested individuals 171 were PPD-positive. The other 107 individuals were referred for QFT-G as a primary test. In those individuals with positive PPD (n=171), the QFT-G test was positive in only 72 (42%). This is in accord with previous studies where half of all PPD-positive individuals had a negative QFT-G test [8]. The characteristics of the QFT-G-positive individuals were as follows: a) individuals who were in contact

Figure 1. Number of individuals undergoing the QFT-G test each year

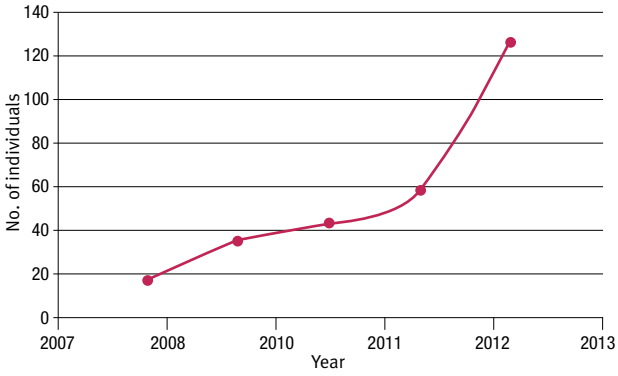
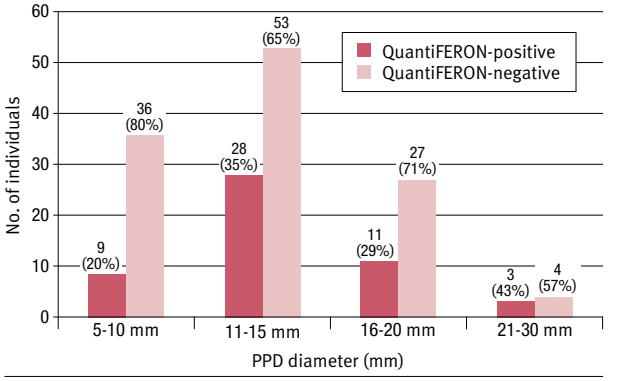


Figure 2. Comparison between PPD diameter and QFT-G results



with active TB cases (46%), b) routine TST for new medical staff in order to exclude latent TB (31%), c) the remaining QFT-G-positive individuals were candidates for biological therapy (19%), and d) others (4%) such as tourists and individuals before traveling to endemic countries.

We then looked into a possible correlation between the diameter of PPD positivity and the finding of positive and negative test results of the QFT-G test [Figure 2]. Of importance was the finding that positive QFT-G was almost equally distributed for all PPD diameters; therefore, we could not predict at what PPD diameter QFT-G is positive.

Active follow-up, including repeated physical examination and chest X-ray, was available in 128 (62%) of all QFT-G-negative individuals of whom 70 (55%) were PPD-positive. All remained well and did not convert to active TB and, therefore, were spared of receiving any anti-TB therapy.

DISCUSSION

The mass influx of immigrants from countries with a high prevalence rate of TB into Israel in the early 1990s was followed by a considerable increase of cases with active TB, mainly among the older immigrants. One of the consequences of this mass influx was the increased incidence of individuals with positive PPD tests. Most of these positive PPD tests were false positive, and the minority was true LTBI. False positivity is even more frequent among young, BCG-vaccinated individuals, leading in these cases to unnecessary anti-TB therapy, at least in some of them. Aiming to limit the number of unnecessary treatments and related medical expenses, we initiated the current project.

Many previous studies discussed the high sensitivity and specificity of the QFT-G test. In one of these, a specificity of 98.1% was reported in 216 BCG-vaccinated Japanese nursing students who were at low risk for *M. tuberculosis* infection, and a sensitivity of 89% was reported in 118 patients with culture-confirmed TB [8]. In another study, QFT-G was compared with TST in a group of 99 healthy, BCG-vaccinated medical students. Here also, the specificity of the QFT-G test was 96%, compared to 49% in the TST [9]. In a population of health care staff with low prevalence of TB and a significant rate of BCG vaccination, a positive QFT-G test result was associated with the presence of known risk for TB exposure, whereas a positive TST result was more strongly associated with a prior history of BCG vaccination [13,14]. In a TB-endemic population, the QFT-G test seemed to be more accurate than the TST in detecting LTBI in rheumatoid arthritis patients, and may potentially improve the targeting of prophylactic therapy before they start treatment with anti-TNF agents [15,16].

The aim of our study was to establish the results shown in previous studies that 55% of PPD-positive individuals are, in fact, QFT-G-negative and therefore can avoid unnecessary anti-TB therapies. In this respect our study showed that only 42% of TST-positive individuals are also positive for QFT-G, indicating that 58% of the individuals suspected of having latent TB (PPD-positive but QFT-G negative, and therefore assumed to be PPD false positive) may receive unnecessary prophylactic anti-TB treatment. In support of this assumption, 128 individuals who were QFT-G-negative were followed for 2–5 years and remained healthy and free of any evidence for active TB, indicating that the QFT-G test is indeed highly specific and should be performed routinely.

In addition, the current study is unique in demonstrating that the size (diameter) of the PPD test was not predictive of QFT-G positivity. We therefore propose that all PPD-positive individuals undergo QFT-G testing in order to limit false positivity. The last proposal holds until a firm recommendation calls to cancel PPD testing.

This study has a few limitations, the first of which is the possible false positivity of QFT-G which may occur due to the possible contact of some individuals with the following Mycobacteria*: M. szulgai*, *M. kansasii* and *M. marinum* (which have ESAT-6 and CFP-10 antigens). However, these types – even if they are responsible for some false positivity – are uncommon

in Israel. Secondly, our study lacked a subgroup of children since most of the patients in our study were adults. Thirdly, in more than half of all QFT-G-negative individuals, the follow-up lasted only 2 years. Although exacerbation of LTBI usually occurs in the first 2 years, a longer period of follow-up in future studies is required to better establish the usefulness of the QFT-G-negative test in predicting the lack of active TB.

In conclusion, this study was designed to assess the superiority of the QFT-G test over the classical PPD testing in preventing unnecessary anti-TB treatment in a large cohort of individuals who were referred in order to establish or exclude latent TB. We now recommend testing all PPD-positive individuals (especially those with BCG vaccination) with the QFT-G test in order to avoid bias, unnecessary treatments and their side effects, as well as shorten the process when recruiting health care workers.

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