Interferon Gamma Release Assay-Guided Latent Tuberculosis Prophylaxis in Israel

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ABSTRACT: **Background:** Determining the accuracy of interferon gamma-release assays (IGRAs) is difficult due to the lack of a gold standard test for diagnosing latent tuberculosis (LTB).

Objectives: To analyze the guidelines used for interpreting IGRAs in determining prophylactic treatment management for latent tuberculosis (LTB) in Israel.

Methods: We analyzed the retrospective data of 367 subjects who were referred to our laboratory during the period 2007–2011 for QuantiFERON Test-Gold In Tube (QFT-GIT) tests because of suspected LTB. Demographics and clinical data were retrieved from a questionnaire at enrollment, and 166/367 (45%) were further interviewed by phone in order to complete follow-up information on prophylactic TB treatment.

Results: The majority of subjects (116/166, 69.9%, $P < 0.0001$) were spared prophylactic treatment subsequent to QFT-GIT testing. Subjects with negative QFT-GIT and positive tuberculin skin test (TST) results who were BCG-vaccinated had the lowest treatment rates (6/68, 8.8%, $P < 0.0001$). Most BCG-vaccinated subjects with positive TST and negative QFT-GIT test results received treatment with anti-tumor necrosis factor-alpha (TNFα) (17/19, 89.5%, $P = 0.004$). We found more negative QFT-GIT test results in subjects who were receiving anti-TNFα or steroid and other immunosuppressive treatment prior to testing (11/11, 100%, $P = 0.029$; 22/26, 84.6%, $P = 0.06$; 15/17, 88%, $P = 0.06$, respectively).

Conclusions: Deciding on LTB prophylactic treatment in Israel is highly influenced by QFT-GIT test results. QFT-GIT findings contribute to clinical decisions, but their interpretation must also consider the patient's medical history and clinical characteristics.

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KEY WORDS: tuberculin skin test (TST), Bacillus Calmette-Guérin (BCG) vaccination, interferon gamma (IFNγ) release assays, latent tuberculosis (LTB), prophylactic treatment

Mycobacterium tuberculosis is an intracellular pathogen that initiates a delayed hypersensitivity T cell-mediated immune response. The tuberculin skin test (TST) has been used for over a century, despite high rates of false-positive results due to previous Bacillus Calmette-Guérin (BCG) vaccination [1], low patient compliance, operator dependence, and interpretation [1]. Although the specificity of the TST is estimated to be only 73% [2], it is still used worldwide for the diagnosis of latent tuberculosis (LTB) and as an aid for diagnosing active TB.

Between 5 and 10% of immunocompetent persons with LTB will develop the disease during their lifetime [3]. The efficacy of prophylactic 6–9 month isoniazid treatment for LTB was confirmed in many studies [4], but it is not free of side effects [5].

Interferon-gamma (IFNγ) release assays (IGRAs) are in vitro tests based on the fact that TB infection causes a secretion of IFNγ from TH1, CD4-type lymphocytes. The advantage of IGRAs is increased specificity [6-9] with the use of TB-specific antigens, which are absent in the BCG vaccine as well as in most *Mycobacteria* species.

Many studies have shown that IGRA is the preferential method for identifying LTB in patients vaccinated with BCG, but because of the lack of a gold standard test for diagnosing LTB it is difficult to determine the accuracy of IGRAs, although recent papers have shown that QuantiFERON Test-Gold In Tube (QFT-GIT) tests have 99% specificity and 83% sensitivity [9,10]. Despite considerable progress in the field of IGRAs, some issues have yet to be resolved, such as the influence of host and bacterial factors on IGRA results. The implementation of IGRA for LTBI screening of health care workers in non-endemic areas yielded a positive IGRA rate that was significantly lower than the positive TST results in non-endemic areas [11]. However, due to a lack of data on optimum cutoff values for repeated testing and imprecise interpretation of conversions and reversions [11], the use of IGRA continues to be problematic.

According to the latest guidelines from the U.S. Centers for Disease Control regarding persons with conflicting test results, medical management decisions must be individualized according to the quality and magnitude of each test result [9]. There are also no clear-cut guidelines for testing the target population in Israel, or with regard to the role of IGRA results in deciding on prophylactic treatment or medical management of individuals with discordant TST and IGRA results. The aims of this study were threefold: to compare QFT-GIT findings

with TST test results, determine the effect of selected clinical parameters on QFT-GIT test results, and evaluate the impact of QFT-GIT test results on the medical management protocol in the local community.

PATIENTS AND METHODS

The study population consisted of all individuals (n=367) referred to the Pulmonary and Allergic Diseases Laboratory at Tel Aviv Medical Center between 2007 and 2011. They all underwent QFT-GIT™ testing (Cellestis Limited, Australia) and signed an informed consent after the study was approved by the local Helsinki Committee. Included were health care workers referred for evaluation due to a positive TST result during medical screening, individuals after TB exposure, and patients prior to undergoing anti-tumor necrosis factor-alpha (TNFα) therapy or organ transplantation. They were asked to complete a questionnaire on demographics and clinical parameters at the time of enrolment into the study. During 2012 we were able to keep track of 166/367 (45%) who provided information on prophylactic TB treatment and adherence to physician recommendations.

The QFT-GIT test was performed according to the manufacturer's instructions. The result was considered positive when the amount of IFNγ was 0.35 IU/ml and 25% more than the nil control value, as recommended by the manufacturer and based on accepted values from previous adult studies.

STATISTICAL ANALYSIS

Comparisons of the categorical demographic and clinical variables were performed using the chi-square test. Pair-wise comparisons between groups were performed using Hochberg's False Discovery Rate correction for multiple comparisons when there was a significant difference between groups. The same analysis was applied to examine the association between QFT-GIT test results and categorical variables. Agreement between QFT-GIT and TST results was assessed using Cohen's Kappa. Kappa was calculated for the entire sample as well as for the subgroups of subjects according to the levels of the various clinical parameters. The subjects were also divided into two subgroups according to agreement between the TST and QFT-GIT test results: those with similar results for both were compared to subjects with no agreement for any of the clinical parameters by means of the chi-square test. All statistical analyses were performed using the SAS for Windows version 9.2.

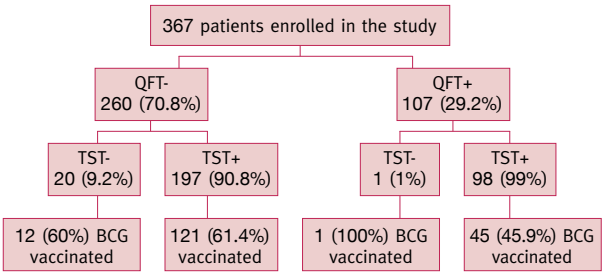
RESULTS

The study population's demographic parameters are shown in Table 1. Most of the subjects were referred from one of the nine dedicated centers for the diagnosis and treatment of tuberculosis established in 1997 by the Israeli Ministry of Health in cooperation with all four Israeli health insurance funds to "MALSACH," the National Center for the Diagnosis and Treatment of Tuberculosis. The majority of them had QFT-GIT and TST results as well (316/367, 84%). QFT-GIT test findings were undetermined in one subject, but he had a negative result on the T-SPOT TB test (Oxford Immunotec™, UK).

Table 1. Demographic parameters of the study population (n = 367)

Characteristic	n (%)
Gender	
Female	144 (39.3)
Male	223 (60.7)
Age (years)	
0–19	16 (4.4)
20–39	130 (35.4)
40–59	141 (38.4)
› 60	80 (21.8)
Country of birth	
Israel	249 (67.8)
Other	118 (32.2)
Occupation	
Health care worker	127 (34.4)
Other	240 (65.5)
Residence in Israel	
Center	201(57)
South	70 (19)
North	21 (6)
Jerusalem	66 (18)

Figure 1. Flow chart of study population according to QuantiFERON-Gold In Tube (QFT-GIT) test results



TST+ = positive tuberculin skin test result, TST- = negative tuberculin skin test result, BCG = Bacillus Calmette-Guérin

Most of the subjects (260/367, 70.8%) had a negative QFT-GIT test result [Figure 1]. Of the 197 responders, 121 (61.4%) with positive TST result and negative QFT result had been vaccinated with BCG; when compared to patients with positive TST and QFT results only 45 of 98 responders (45.9%) had been vaccinated with BCG. Table 2 presents the clinical parameters and their influence on the QFT-GIT findings. There were no significant differences between the percentage of positive and negative QFT-GIT test results regarding the source of referral, smoking habits, medical history, TB exposure, chest X-rays, and prior prophylactic treatment. Patients treated with immunosuppressive drugs had a higher percentage of nega-

tive QFT-GIT test results (84.6% for the 22/26 on steroids and 88.2% for the 15/17 on methotrexate, mercaptopurine or azathioprine), compared with the non-treated group (222 of 322 responders, 68.2%, $P = 0.06$) [Table 2]. Similar results were observed for the TST results (data not shown).

Previous prophylactic treatment did not have any effect on QFT-GIT test results, but anti-TNF therapy seemed to have a negative effect: all subjects who had previously received anti-TNFα treatment had negative QFT-GIT test results compared to those without prior treatment ($P = 0.029$) [Table 2].

There were significant differences in the QFT-GIT test results associated with past vaccination with BCG (76.3% vaccinated subjects had negative results and 23.7% vaccinated subjects had positive results, $P = 0.0175$). The average IFNγ concentration for patients who were positive for the QFT-GIT assay was 0.97 IU/ml (range 0.4–10 IU/ml), while the average TST induration size was 13.5 mm (range 1–37 mm). The IFNγ levels ranged between 0.2 and 0.99 IU/ml in 61 of the entire study population (61/367, 16.6%). There were more positive QFT-GIT test results at all cutoff points of TST-positive results ($r = 0.27$, $P = 0.0192$) [Table 2], but they were more significant for TST results of 10–15 mm compared to results of 5–10 mm (94/271, 34.7% vs. 4/22, 18.2%, $P = 0.0192$) [Figure 2].

Most subjects (116/166, 69.9%, $P < 0.0001$) were spared prophylactic treatment after QFT-GIT testing: this was more significant for subjects vaccinated with BCG (79/106, 74.5%, $P < 0.0001$). Fewer BCG-vaccinated subjects with negative QFT-GIT and positive TST were prescribed treatment (6/68. 8.8%), which is significantly lower than the group of unvaccinated subjects (4/19, 21.05%, $P = 0.0001$) [Figure 2B]. There were no differences between groups when the BCG vaccination result was uncertain. More subjects with positive QFT-GIT and positive TST results were prescribed prophylactic treatment regardless of the BCG status of vaccination (17/22, 77.3% and 9/11, 81.8%) [Figure 2C]. Most candidates for anti-TNFα treatment (36/51, 70.6%) were qualified to receive it without prophylactic treatment following the QFT-GIT test results. Anti-TNFα treatment rates were high for patients who had been vaccinated with BCG and had negative QFT-GIT test results and positive TST results (17/19, 89.5%).

DISCUSSION

Many studies have confirmed that IGRAs have higher specificity than TST, especially in BCG-vaccinated populations [12]. The BCG vaccine was routinely given to neonates in Israel until 1982, and a second dose was given in the seventh grade according to the TST results between 1961 and 1987. TST is currently performed in the seventh grade only in high risk populations, and the BCG vaccine is given to infants of immigrants from endemic countries [13]. There is no gold standard test for diagnosing LTB, and guidelines for the use and interpretation of IGRAs are inconclusive. This retrospective study was conducted to regulate the informal policy on the use of prophylactic treatment and therapeutic regimens in Israel.

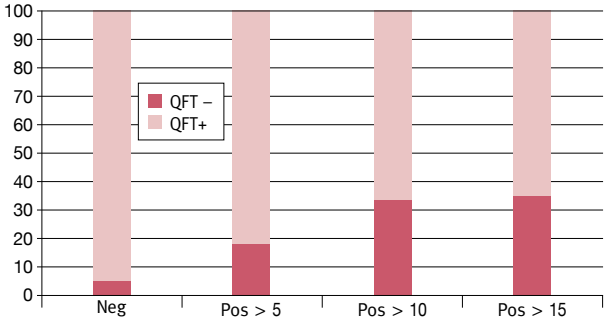
Table 2. QuantiFERON-Gold In Tube (QFT-GIT) test results according to clinical parameters

Parameter	Total n (%)	QFT (-) n (%)	QFT (+) n (%)	*P* value
Source of referral (n=313)				0.7955
MALSHACH[a]	105 (33.6)	75 (73.4)	30 (28.6)	
Pulmonologist	47 (15.0)	33 (70.2)	14 (29.8)	
Specialist physician[b]	72 (23.0)	55 (76.4)	17 (23.6)	
Self	36 (11.5)	26 (72.2)	10 (27.8)	
Place of work	8 (2.6)	7 (87.5)	1 (12.5)	
Primary care physician[c]	45 (14.4)	30 (66.7)	15 (33.3)	
Smoking (n=356)				0.4134
Yes (current/ former)	115 (32.3)	85 (73.9)	30 (26.7)	
No	241 (67.7)	168 (69.7)	73 (30.3)	
Medical history (n=367)				0.1107
No inflammatory diseases	257 (70.0)	175 (68.1)	82(31.9)	
Inflammatory diseases	92 (25.0)	73 (79.3)	19 (20.7)	
Candidates for transplantation / chemotherapy/steroid treatment	11 (3.0)	6 (54.6)	5 (45.4)	
Immunocompromised due to immunodeficiency disease[d]	6 (1.6)	5 (83.3)	1 (16.7)	
Immunosuppressive drugs (n=365)				0.066
Steroids[e]	26 (7.1)	22 (84.6)	4 (15.4)	
Other immunosuppressive drugs[f]	17 (4.7)	15 (88.2)	2 (11.8)	
None	322 (88.2)	222 (68.2)	100 (31.1)	
Tuberculosis exposure (n = 247)				0.5059
Yes	147 (42.4)	101 (68.7)	46 (31.3)	
No	200 (57.6)	144 (72.0)	56 (28)	
Chest X-ray n (%) (n=177)				0.6419
Normal	154 (87.0)	111 (72.1)	43 (27.9)	
Pathologic findings	23 (13)	18 (78.3)	5 (21.7)	
Bacillus Calmette-Guérin vaccinated (n=352)				0.0175
Yes	194 (55.1)	148 (76.3)	46 (23.7)	
No	77 (21.9)	54 (70.1)	23 (29.9)	
Unknown	81 (23)	48 (59.3)	33 (40.7)	
Tuberculin skin test (n=316)				0.0192
Positive › 5	22 (7.0)	18 (81.8)	4 (18.2)	
Positive › 10	139 (44.0)	92 (66.2)	47 (33.8)	
Positive ›1 5	132 (42.4)	97 (64.9)	47 (35.1)	
Negative	21 (6.6)	20 (95.2)	1 (4.8)	
Previous tuberculosis treatment (n=360)				0.2421
Yes	22 (6.2)	18 (82.8)	4 (18.2)	
No	338 (93.9)	237 (70.1)	101 (29.9)	
Previous anti-TNFα treatment (n=361)				0.0299
Yes	11 (3.0)	11 (100)	0 (0)	
No	350 (97.0)	244 (69.7)	106 (30.3)	

[a]National Center for the Diagnosis and Treatment of Tuberculosis
[b]Gastroenterologist/rheumatologist/dermatologist/nephrologist /ophthalmologist/oncologist
[c]Internal medicine specialist/military physician/family physician
[d]HIV/autoimmune hepatitis/congenital immunodeficiency
[e]Prednisone
[f]One or more of the following: methotrexate, mercaptopurine, azathioprine

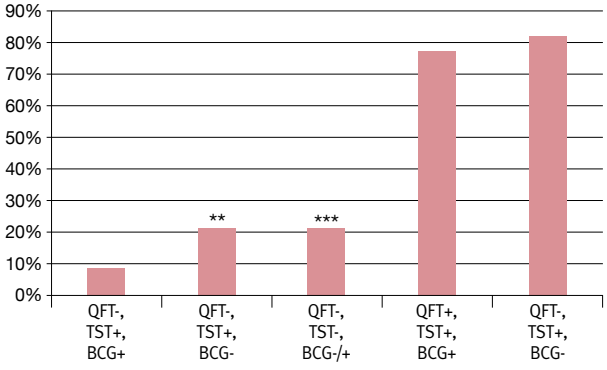
Notably, only a few subjects were referred by primary care physicians for definitive testing due to suspected LTB. This points to the lack of awareness among general practitioners regarding this additional and more specific diagnostic test for LTB.

Figure 2. [A] QuantiFERON-Gold In Tube (QFT-GIT) test results in four tuberculin skin test (TST) induration categories

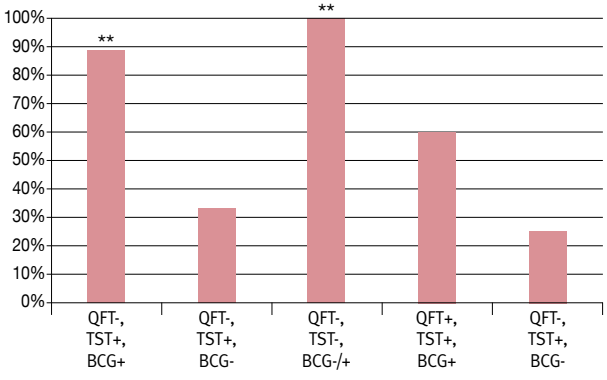


*P < 0.0001: QFT-, TST+, BCG+ vs. QFT+, TST+, BCG+/-
**P < 0.01: QFT-, TST+, BCG- vs. QFT+, TST+, BCG+/-
***P < 0.01: QFT-, TST-, BCG+/- vs. QFT+, TST+, BCG+/-

[B] Prophylactic treatment for tuberculosis following QFT-GIT testing



[C] Anti-TNFα treatment following QFT-GIT testing



*P = 0.06: QFT-, TST+, BCG+ vs. QFT+/-, TST +, BCG-
**P = 0.06: QFT-, TST-, BCG+/- vs. QFT+/-, TST+, BCG-
TST = tuberculin skin test, BCG = Bacillus Calmette-Guérin, (+)/Pos = positive, (-)/Neg = negative

It is well known that immunization with BCG significantly increases the likelihood of a positive TST [3]. That feature is consistent with our finding of a higher percentage of negative QFT-GIT test results among subjects vaccinated with BCG. We could not include the individual's age at the time of vaccination in our analysis because most of the subjects could not remember when they were vaccinated. This may pose a problem in interpreting our findings since BCG vaccinations given more than 15 years previously should not be considered the cause of a current positive TST result, especially if the induration is > 15 mm [12].

A small subgroup of patients with human immunodeficiency virus (HIV), autoimmune hepatitis and congenital immunodeficiency had a high rate of negative QFT-GIT test results, as did patients treated with immunosuppressive drugs. Our results are in agreement with a study conducted on an immunocompromised population that showed a positive correlation between lymphocyte count and QFT-GIT results [14]. It should be noted that current data do not suggest that IGRAs have a clear-cut advantage over TST in immunocompromised patients [10].

A positive correlation was found between the intensity of TST induration and the level of IFNγ production in the QFT-GIT results. Subjects with a TST result > 10 mm had higher rates of positive QFT-GIT results than those with TST results of 5–10 mm. These findings are consistent with an Ethiopian study [15] that showed a positive correlation between the IFNγ levels in IGRAs and the size of skin test indurations in the TST of patients with LTB. Another study found that subjects with high levels of IFNγ are at higher risk of developing active TB than QFT-GIT-positive subjects with lower levels of IFNγ [16]. Notably, 16.6% of our subjects had IFNγ levels around the positive cutoff value (0.2–0.99 IU/ml). A recent study on health care workers showed no active TB development among 2.8% of their subjects with IGRA results conversion, when 71% of them had a positive result ≤ 1 IU/ml [17]. This emphasizes the caveat that using a single cutoff point for IGRA may lead to over-diagnosis of new TB infections.

With regard to cost-effectiveness of IGRAs compared to TST, QFT-GIT testing is more expensive (US$ 167, 2014 update). This additional cost of IGRAs is offset by the reduction of the number of people who would be receiving unnecessary prophylactic treatment for TB. This mainly holds true for people who have been vaccinated with BCG [18]. In our study, the majority of BCG-vaccinated subjects were spared prophylactic antibiotic treatment subsequent to QFT-GIT testing. Subjects with a negative QFT-GIT test result and a positive TST result who were vaccinated with BCG had the lowest rate of treatment.

Finally, several studies reported that the use of IGRAs reduces the number of patients who need prophylactic antibiot-

ics prior to anti-TNFα therapy [19,20]. Similarly, in our study, most of the subjects with negative QFT-GIT test results received anti-TNF treatment without prophylactic treatment.

The study has several limitations. First, the study population was followed for a relatively short period, which can be significant in terms of antibiotic treatment and the development of active TB. Second, the self-reported questionnaire-based data may contain inaccuracies and gaps in information. Finally, most subjects were referred because of positive TST findings, which can introduce bias into the results.

CONCLUSIONS

The results of the present study demonstrate the role of QFT-GIT testing in the diagnosis of LTB in Israel, especially for BCG-vaccinated individuals, and emphasize the fact that most prophylactic treatments are guided by the results of IGRA tests in patients who were tested.

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