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Significance of interferon-gamma response to mitogen in serial QuantiFERON-TB Gold In-Tube assay of routine laboratory practice

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ABSTRACT

Background: Clinical data of serial interferon-γ release assay (IGRA) testing in routine laboratory practice are limited. IFN-γ response to mitogen is used as a positive control in IGRA. We assessed the association between IFN-γ response to nil, mitogen, tuberculosis (TB) mycobacterial antigens, and the variations from the results of the serial testing.
Method: A total of 299 patients with serial QuantiFERON-TB Gold In-Tube (QFT-GIT) were enrolled. The medical records of patients were reviewed for demographic information, status of *Mycobacterium tuberculosis* infection, treatment of tuberculosis, and the quantitative response to nil, mitogen, and TB antigen.
Results: The initial QFT-GIT result was positive in 142 patients (47.5%), negative in 139 (46.5%), and indeterminate in 18 (6.0%). Of total, 79.6% showed concordant results in serial testing. The discordance in serial tests was significantly high in patients with a low mitogen response (≤3.93 IU/ml) ($p < 0.0001$). Quantitative TB responses around the cut-off point in serial QFT-GIT were associated with an increased conversion and reversion rates ($p = 0.01$, $p = 0.0005$), respectively.
Conclusion: Because IGRAs are dynamic assays, integrated interpretation of quantitative TB response with mitogen and nil response would be helpful in serial QFT-GIT. Recommendations for the interpretation of results of serial testing for active TB will be required.

1. Introduction

Interferon-gamma (IFN-γ) release assays (IGRAs) are in vitro tests to determine both latent *Mycobacterium tuberculosis* infection (LTBI) and active infections manifesting as tuberculosis diseases. The QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis, Carnegie, Australia) is one of the commercially available IGRAs that have been recommended as an alternative to the tuberculin skin test in screening for *M. tuberculosis* infection, especially in populations that have received bacillus Calmette-Guérin (BCG) as a vaccine [1]. Although IGRAs have some limitations in their performance in predicting active tuberculosis, several studies have been done to review the ability of IGRA to detect the presence of a latent infection and to diagnose active disease in different populations [2–4].

The performance of IGRA in serial testing is usually evaluated in several settings like among health-care workers, and patients treated with immunosuppressive agents, and while monitoring treatment efficacy in tuberculosis diseases [5–7]. In a systemic review, repeating IGRAs in health care workers has shown a revision rate (22–71%) that is higher than the conversion rate (1–14%) [8]. In these studies, the variation of QFT-GIT in a serial setting was evaluated during the examinations for LTBI screening. There are few reports on the variations in sequential QFT-GIT measurement in routine laboratory practice.

The IFN-γ response to polyclonal T cell stimulus, phytohemagglutinin (PHA) as a mitogen is used as a positive control in QFT-GIT, and such use is especially warranted where there is doubt about the individual's immune status. It also serves as a control for correct blood handling and incubation. A low response to the 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 with lymphopenia, reduced lymphocyte activity due to improper specimen handling, incorrect filling/mixing of the mitogen tube, or inability of the patient's lymphocytes to generate IFN-γ [9,10].

The Centers for Disease Control and Prevention Guidelines on IGRAs recommend that clinical laboratories report both standard qualitative interpretation and quantitative assay measurement [1]. The quantitative measurement includes the IFN-γ response to TB antigens. Although there has been no need to report the nil and mitogen response, these

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two measurements have been reported since the IGRA was adopted in our laboratory. There is no report that analyzes the association between variations in serial QFT-GIT tests and nil and mitogen responses.

2. Materials and methods

2.1. Study populations

From March 1, 2009 to January 31, 2013, 2821 patients who were consecutively referred by clinicians to the immunology laboratory in Dong-A University Hospital for a QFT-GIT were initially enrolled in this study. Of these, 300 patients (10.6%) had serial QFT-GIT results. The medical records of patients were reviewed for demographic information, status of *M. tuberculosis* infection, therapy for LTBI or active infection, and the level of IFN-γ to nil, mitogen, and TB antigen. This study was approved by the Institutional Review Board at Dong-A University Hospital.

2.2. QFT-GIT assay

The QFT-GIT was performed according to the manufacturer's instructions [11]. Whole blood was collected directly into the manufacturer-provided nil, TB antigen and mitogen tubes and transported to the laboratory as soon as possible. All tubes were incubated for 16–24 h at 37 °C according to the manufacturer's instruction. ELISA optical density (OD) was read using a CODA microplate processor (Biorad). Four IFN-γ standard calibrators (4, 1, 0.25, and 0 IU/ml) in duplicate were used to create standard curves for each assay. The OD values were then inputted into the QFT Gold analysis software v 2.50.4. The software reported test results automatically according to the manufacturer's specifications.

The nil response (Nil) was the concentration of IFN-γ in plasma from unstimulated blood. The mitogen response was the concentration of IFN-γ in plasma from PHA stimulated blood minus Nil. The TB response was the concentration of IFN-γ in plasma from blood stimulated by a single cocktail of peptides representing ESAT-6, CFP-100, and part of TB7.7 minus Nil. We interpreted the test results according to both the results of the software and quality control. The decision to initiate treatment of the TB infection was at the discretion of the referring clinician. The conversion of the result was defined as negative QFT-GIT at the baseline and positive QFT-GIT at the follow-up, and reversion of QFT-GIT was defined as positive QFT-GIT at the baseline and negative QFT-GIT at the follow-up.

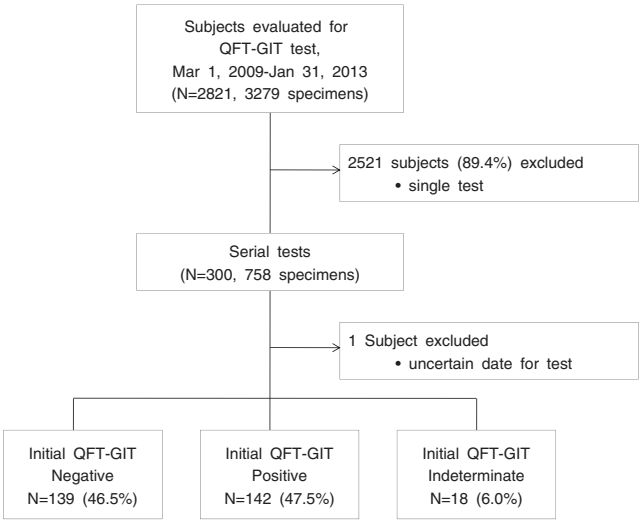

Fig. 1. Study flow diagram.

2.3. Statistical analysis

Data are expressed as medians and ranges or numbers (percentages). Pearson's χ^2 test or Fisher's exact test was used to analyze categorical variables. The highest outlier of the mitogen response and the lowest outlier of the nil response in the first specimens were used for the categorization for the analysis of subsequent discordant results. For results classified according to the manufacturer's specifications, the agreement among three subsequent serial tests was assessed with the kappa statistic. The fourth and subsequent results of the tests were excluded because of small sample sizes. The likelihood ratio for conversion and reversion according to the quantitative TB response was obtained. For quantitative results, we used Bland–Altman plots of the first–second response differences for nil, mitogen, and TB responses against their means. All p values <0.05 were considered to be statistically significant. Data analysis was carried out using MedCalc Software (ver. 12.6.1, MedCalc Software, Mariakerke, Belgium).

3. Results

3.1. Characteristics of patients

Three hundred patients with serial QFT-GIT tests were recruited in this study. One of them had an uncertain date of test and was excluded. Therefore, 299 patients were analyzed (Fig. 1). The characteristics of the population with serial QFT-GIT are summarized in Table 1. The indications for the test were varied and patients with suspected symptoms for TB disease were the most common (37.1%). One third of the patients had anti-TB treatment and isoniazid preventive therapy was taken by 22.1% of patients. The median length of the interval between the first and second tests was 140 days (range 0–1370), and most (90.3%) of the subjects were tested two or three times for QFT-GIT. Patients tested for QFT-GIT four or more times tended to use TNF-blocker (23/29, 97.3%).

3.2. Agreement of serial tests

A total of 756 QFT-GIT were performed. The first QFT-GIT result was positive in 142 patients (47.5%), negative in 139 (46.5%), and indeterminate in 18 (6.0%). When the first and second results of the tests were

Table 1
Characteristics of the population with serial QFT-GIT tests.

Characteristic	Total (n = 299)
Age, y	42 (2 months–87 y)
Male sex	161 (53.8)
Indication of test	
Pul TB	24 (8.0)
Extrapul TB	70 (23.4)
Combined pul TB and extrapul TB	10 (3.3)
Contact investigation	32 (10.7)
Before TNF-blocker use	52 (17.4)
Suspected symptoms for TB disease	111(37.1)
Medication for TB	
None	122 (40.8)
Isoniazid preventive therapy	66 (22.1)
Anti-TB treatment	103 (34.4)
Therapeutic anti-TB trial	8 (2.7)
Interval between first and second tests, days	140 (0–1370)
Under 7 days	31 (10.4)
8–30 days	41 (13.7)
1–6 months	92 (30.8)
6 months–1 y	88 (29.4)
Over 1 y	47 (15.7)
Number of tests per subject	
2	178 (59.5)
3	92 (30.8)
4	22 (7.4)
≥5	7 (2.3)

Data are presented as no. (%) or median (range).