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Qualitative and quantitative results of interferon-γ release assays for monitoring the response to anti-tuberculosis treatment

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Background/Aims: The usefulness of interferon-γ release assays (IGRAs) in monitoring to responses to anti-tuberculosis (TB) treatment is controversial. We compared the results of two IGRAs before and after anti-TB treatment in same patients with active TB.

Methods: From a retrospective review, we selected patients with active TB who underwent repeated QuantiFERON-TB Gold (QFN-Gold, Cellestis Limited) and T-SPOT.TB (Oxford Immunotec) assays before and after anti-TB treatment with first-line drugs. Both tests were performed prior to the start of anti-TB treatment or within 1 week after the start of anti-TB treatment and after completion of treatment.

Results: A total of 33 active TB patients were included in the study. On the QFN-Gold test, at baseline, 23 cases (70%) were early secreted antigenic target 6-kDa protein 6 (ESAT-6) or culture filtrate protein 10 (CFP-10) positive. On the T-SPOT.TB test, at baseline, 31 cases (94%) were ESAT-6 or CFP-10 positive. Most of patients remained both test-positive after anti-TB treatment. Although changes in interferon-γ release responses over time were highly variable in both tests, there was a mean decline of 27 and 24 spot-forming counts for ESAT-6 and CFP-10, respectively on the T-SPOT.TB test ($p < 0.05$ for all).

Conclusions: Although limited by the small number of patients and a short-term follow-up, there was significant decline in the quantitative result of the T-SPOT.TB test with treatment. However, both commercial IGRAs may not provide evidence regarding the cure of disease in Korea, a country where the prevalence of TB is within the intermediate range.

Keywords: Interferon-gamma release tests; Tuberculosis; Therapeutics

INTRODUCTION

One third of the world's population is infected with *Mycobacterium tuberculosis*, and tuberculosis (TB) ranks a leading cause of death among infectious diseases worldwide. In 2014, 9.6 million people fell ill with TB, an estimated 480,000 people developed multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) has been reported by 105 countries by 2015 [1]. An estimated 9.7% of people with MDR-TB have XDR-TB. Response to anti-TB treatment in patients with bacteriologically confirmed TB is monitored principally by serial bacteriologic examinations [2], whereas responses in patients with clinically diagnosed TB, including cases

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diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation, are usually monitored clinically and/or radiographically. Therefore, new monitoring tools for TB treatment are needed to improve TB treatment strategies in some clinical or experimental settings.

The search for improved tools for detection of latent TB infection has led to the development of in *vitro* assays based on interferon-γ production. Commercial *ex vivo* interferon-γ release assays (IGRAs) have been approved as indirect tests for *M. tuberculosis* infection (including infection resulting in active disease) when used in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations [3], QuantiFERON-TB Gold (QFN-Gold, Cellestis Limited, Carnegie, Australia), QuantiFERON-TB Gold In-tube (QFT-GIT, Cellestis Limited, Carnegie, Australia), and T-SPOT.TB (Oxford Immunotec, Oxford, UK) [4-7]. These assays use the *M. tuberculosis*-specific antigenic peptides, early secreted antigenic target 6-kDa protein 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are not present in Bacille Calmette-Guérin (BCG) strains and most nontuberculous mycobacteria, making them highly specific for detecting TB infection.

Although to date there have been several reports about the clinical usefulness of IGRA in the diagnosis of active TB and monitoring the response to TB treatment [8-14], there is no report on a direct comparison of two IGRAs for monitoring the response to TB treatment in Korea. Therefore, we evaluated the qualitative and quantitative IGRA results measured by both QFN-Gold and T-SPOT.TB assays before and after anti-TB treatment in same patients with active TB.

METHODS

Subjects and study design

From a retrospective review, we selected patients with active TB who underwent repeated QFN-Gold and T-SPOT.TB assays before and after anti-TB treatment with first-line drugs, from June 2004 through December 2005 at the Asan Medical Center (Seoul, Korea). Active TB was defined as bacteriologically confirmed or clinically diagnosed cases of TB by 2013 WHO revised definition. Pulmonary tuberculosis (PTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree. A patient with both PTB and extrapulmonary tuberculosis (EPTB) classified as a case of PTB. EPTB refers to any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs.

Finally, patients who completed the treatment or who were cured were enrolled in the study. In all cases, both tests were performed prior to the start of treatment or within 1 week after the start of treatment (T_0) and after completion of treatment (T_1), respectively. This study was part of a larger study comparing the abilities of tuberculin skin tests (TST) and *ex vivo* IGRAs to detect TB infection and to monitor the response to anti-TB treatment [15]. All patients provided written informed consent, and the study protocol was approved by the Institutional Review Board of Asan Medical Center.

QuantiFERON-TB Gold assay

The QFN-Gold assay was performed according to the manufacturer's instructions [15]. Immediately before TST, 4 mL of peripheral venous blood was collected into a heparinized tube. Within 4 hours of collection, 1 mL aliquots of blood were mixed with 3 drops each of one of the following provided solutions: saline (negative control), phytohemagglutinin (mitogenic positive control), ESAT-6 peptides or CFP-10 peptides. The samples were incubated at 37°C for 20 hours and then frozen until analysis. T cell responses were determined by quantitative measurement of plasma interferon-γ levels using the enzyme-linked immunosorbent assay technique. For each subject, the negative control value was subtracted from the values obtained from the antigen-stimulated plasma samples. An interferon-γ concentration of 0.35 IU/mL was used as the cut-off for a positive response as recommended by a manufacturer, regardless of the result of the positive control (mitogen well). The result was considered "negative" if the response to the specific antigens was < 0.35 IU/mL and if the interferon-γ level of the positive control was ≥ 0.5 IU/mL. The result was considered "indeterminate" if both antigen-stimulated samples were negative and the value of the positive control was < 0.5 IU/mL.

T-SPOT.TB assay

T-SPOT.TB analysis was performed according to the

Table 1. Clinical characteristics of 33 patients with active tuberculosis

Characteristic	Total (n = 33)	Positive at T_0 on QFN-Gold (n = 23)	Positive at T_0 on T-SPOT.TB (n = 31)
Age, yr	46.1 ± 15.0	46.1 ± 16.4	46.3 ± 15.0
Sex, male:female	19:14	13:10	18:13
History of TB treatment	3/29 (10)	2/20 (10)	3/27 (11)
Presence of BCG scar	17/29 (59)	10/20 (50)	16/27 (59)
TST, mm	11.6 ± 8.9 (0–37)	14.0 ± 8.9 (0–37)	12.1± 8.9 (0–37)
TST ≥ 10 mm	22 (67)	18 (78)	21 (68)
No. of patients with risk factor for immunosuppression	11 (33)	6 (26)	10 (32)
Uncontrolled DM	5 (15)	3 (13)	5 (16)
Cancer on chemotherapy	2 (6)	2 (9)	2 (6)
Kidney transplantation	1 (3)	0	1 (3)
Liver transplantation	1 (3)	1 (3)	1 (3)
On prednisolone > 1 mon	2 (6)	0	1 (3)
PTB:EPTB	31:2	22:1	29:2
Bacteriological TB[a]:clinical TB[b]	25:8	17:6	23:8

Values are presented as mean ± SD, number (%), or mean ± SD (range).
TB, tuberculosis; BCG, Bacille Calmette-Guérin; TST, tuberculin skin test; DM, diabetes mellitus; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.
[a]A bacteriologically confirmed TB case is one from whom a biological specimen is positive by smear microscopy, culture or TB polymerase chain reaction.
[b]A clinically diagnosed TB case is one who does not fulfil the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation.

manufacturer's instructions [15-17]. Immediately before TST, peripheral venous blood samples were collected and processed within four hours. The samples were incubated for 20 hours, and the number of spots was counted using an automated AID ELISPOT Reader (AID GmbH, Strasbourg, Germany). ESAT-6- or CFP-10-stimulated wells with at least six spots more than the negative control well, which had ≤ 5 spots, were scored as positive. When the negative control well had 6 to 10 spots, the result was defined as positive when either the ESAT-6- or CFP-10-stimulated well contained at least twice as many spots. If the negative control well had more than 10 spots, the result was considered "indeterminate."

Statistical methods

Statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD for continuous variables, and percentages for categorical variables. Paired numeric data were compared using Student *t* test. All tests of significance were two sided; *p* values < 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

A total of 33 active TB patients were included in the analysis; their baseline demographic and clinical data are summarized in Table 1. Active TB was diagnosed bacteriologically in 25 patients (76%) and clinically in eight patients (24%). Of bacteriologically confirmed TB, 21 had *M. tuberculosis* cultured from their specimens. In 20 of these 21 patients, the isolates were sensitive to all anti-TB medications; the isolate from one patient showed isoniazid (INH) monoresistance. Clinical TB patients had high adenosine deaminase level in pleural effusion

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Table 2. Change in the IFN-γ level and the number of SFCs of patients with active TB during anti-TB treatment

	IFN-γ level, IU/mL			No. of SFCs		
	T_o[a]	T_1[b]	*p* value	T_o[a]	T_1[b]	*p* value
ESAT-6	2.7 ± 6.3	2.9 ± 7.8	NS	58.9 ± 53.6	31.9 ± 7.43	0.011
CFP-10	1.0 ± 2.7	1.5± 4.8	NS	48.7± 59.8	25.0 ± 39.6	0.029

Values are presented as mean ± SD.

IFN-γ, interferon-γ; SFC, spot-forming cell; TB, tuberculosis; ESAT-6, early secreted antigenic target 6-kDa protein 6; CFP-10, culture filtrate protein 10.

[a]T_o: before or within 1 week after the start of anti-TB treatment.

[b]T_1: after completion of treatment.

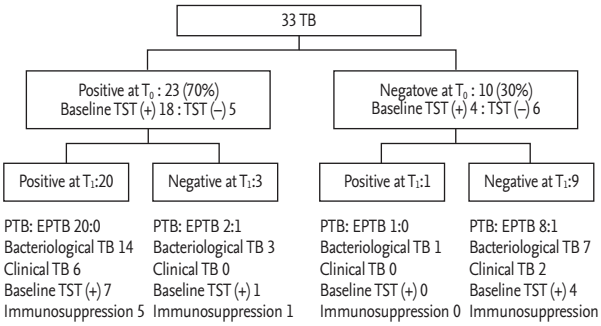

Figure 1. Change in the QuantiFERON-TB Gold (QFN-Gold, Cellestis Limited) test results for patients with active tuberculosis (TB) before and after anti-TB treatment. TST, tuberculin skin test; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.

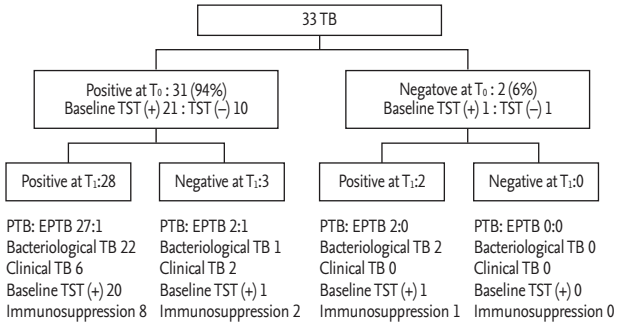

Figure 2. Change in the T-SPOT.TB (Oxford Immunotec) test results for patients with active tuberculosis (TB) before and after anti-TB treatment. TST, tuberculin skin test; PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis.

or typical CT findings, including tree-in-bud appearance accompanying lung nodules, suggesting active TB; these lesions improved after anti-TB treatment. All patients completed anti-TB treatment successfully with first-line anti-TB medication; 32 patients completed treatment in 6 months and one patient with INH monoresistance in 9 months. Both interferon-γ tests were performed at baseline (T_o) and treatment completion (T_1), with a mean interval of 6.9 ± 0.9 months between T_o and T_1.

Interferon-γ release assay

Fig. 1 shows qualitative change in QFN-Gold test results for patients with active TB before and after anti-TB treatment. At baseline, 23 cases (70%) were ESAT-6 or CFP-10 positive. Of these, three cases (13%) were ESAT-6 and CFP-10 negative after completion of anti-TB treatment. Fig. 2 shows qualitative change in T-SPOT.TB results for patients with active TB before and after anti-TB treatment. At baseline, 31 cases (94%) were ESAT-6 or CFP-10 positive. Of these, three cases (10%) were ESAT-6 and CFP-10 negative after completion of anti-TB treatment. Fig. 3 shows the quantitative changes in the interferon-γ level in QFN-Gold test between before treatment and after treatment. Eighteen cases (55%) had decreased in their interferon-γ level using ESAT-6. Also, 18 cases (55%) had decreased in their interferon-γ level using CFP-10. Fig. 4 shows the quantitative changes in the number of spot-forming cells (SFCs) in T-SPOT.TB test between before treatment and after treatment. Twenty-three cases (70%) had decreased in their number of SFCs using ESAT-6. Twenty-two cases (67%) had decreased in their number of SFCs using CFP-10. The mean value of interferon-γ level (QFN-Gold test) including all cases remained unchanged on a comparison test results between before and after treatment. However, the mean value of number of SFCs (T-SPOT.TB test) significantly decreased on a comparison test results between before and after treatment (Table 2).

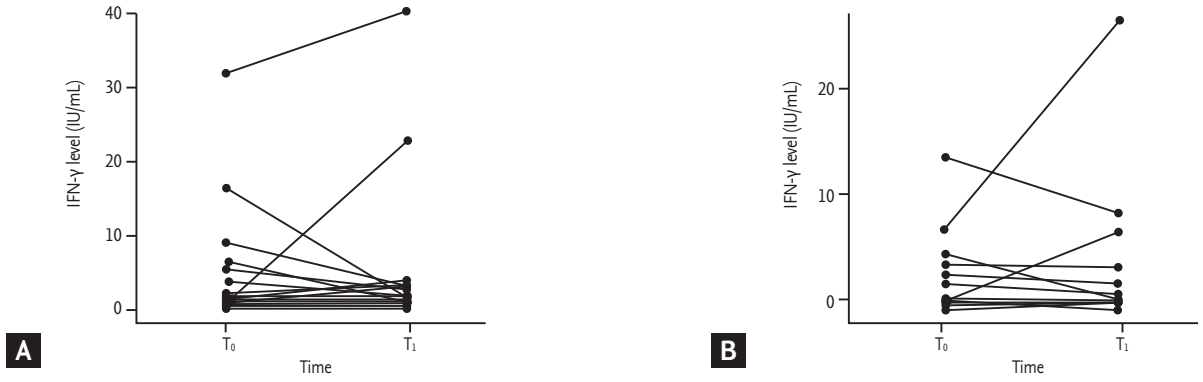

Figure 3. Paired before and after treatment quantitative interferon-γ (IFN-γ) level on the QuantiFERON-TB Gold (QFN-Gold, Cellestis Limited) test using (A) early secreted antigenic target 6-kDa protein 6 and (B) culture filtrate protein 10.

Figure 4. Paired before and after treatment quantitative interferon-γ level in on the T-SPOT.TB (Oxford Immunotec) test using (A) early secreted antigenic target 6-kDa protein 6 and (B) culture filtrate protein 10.

DISCUSSION

Although this report presents short-term follow-up results of two commercial blood tests based on small number of patients, this study is the first report on a comparison of two IGRAs for monitoring the response to TB treatment in Korea, where the incidence of TB is within the intermediate range (90 per 100,000) and BCG vaccination is mandatory [18].

The aim of the present study was to evaluate the results of two IGRAs before and after anti-TB treatment in same patients with active TB. We hypothesized that interferon-γ release would decrease as a response to anti-TB treatment in active TB patients. We have shown here that positive rates for QFN-Gold test and T-SPOT.TB test result remain unchanged after anti-TB treatment, although there was a significant reduction in mean ESAT-6 and CFP-10 T-SPOT.TB counts.

Several authors have made a direct comparison between the T-SPOT.TB and the QFN-Gold in the diagnosis of active TB in the same study population [15,19-21]. Although there are few differences between the sensitivities of the tests in these studies, the sensitivities are in the same range, with the T-SPOT.TB being more sensitive than QFN-Gold. The impact of the anti-TB treatment on the interferon-γ tests in reducing the response to the specific antigens has been described elsewhere. During TB treatment, the number of specific T cells decreases progressively, suggesting that the frequency of ESAT-6-specific T cells is related to antigen load [22,23]. It has been proposed that the interferon-γ could be used to monitor the patients' response to the treatment [8]. Although there have been several reports regarding the clinical usefulness of IGRA in the monitoring the response to TB treatment, there has not been any report on a direct comparison of two IGRAs for monitoring the response to TB treatment in Korea. In one Japan study [24], they compared of QFN-Gold test and T-SPOT.TB test in the same patients with active TB disease: positive results (for ESAT-6 and/or CFP-10) of both QFN-Gold

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test results and T-SPOT.TB test results decreased from 81% and 88% (before treatment), respectively, to 41% and 49% (12 months later). However, in our study, we showed that positive responses on both IGRAs were persistent after treatment. The reason might be long duration of exposure in TB endemic country and shorter follow-up period. However, in Norway study from a TB low-endemic country, most patients with latent TB infection were still QFT-TB positive after preventive therapy [25]. Therefore, further research is needed to better understand why T cell responses stay persistently elevated after treatment.

Although there was failure of reversion on the T-SPOT.TB test in our study, 23 cases (70%) had decreased number of SFCs using ESAT-6 and 22 cases (67%) had decreased number of SFCs using CFP-10. Also there was a mean decline of 27 and 24 SFCs for ESAT-6 and CFP-10, respectively ($p < 0.05$ for all). In a previous report on the T-SPOT.TB assay in Gambia [9], 82 cases (96%) successfully completed treatment: 44 (55%) were ESAT-6 and/or CFP-10 negative at 12 months, 17 (21%) were TST negative. Also, 64 (78%) had an ESAT-6 count decrease, 60 (73%) cured cases had a CFP-10 count decrease. There was a mean decline of 44 and 25 SFCs for ESAT-6 and CFP-10, respectively ($p < 0.001$ for all). They insist that a repeat T-SPOT.TB test at 12 months reflects the efficacy of a course of anti-TB treatment even in a TB endemic tropical setting. These findings were consistent with those of our study except for reversion rate.

In our study, changes in interferon-γ over time were highly inconsistent (Fig. 3) and median and the mean value of interferon-γ level (QFN-Gold test) remained unchanged on comparing the test results between before and after treatment. Pai et al. [10] reported that at baseline, 44 of 60 patients (73%) were positive by the QFN-Gold test and at the treatment completion, 31 of 39 patients (79%) were positive. Also, the average of interferon-γ level declined slightly during treatment, but it was not significant as in our results.

In conclusion, although limited by the small number of patients and a short-term follow-up, there was significant decline in the quantitative result of the T-SPOT.TB test with treatment. However, both commercial IGRAs may not provide evidence regarding the cure of disease, in Korea, a country where the prevalence of TB is within the intermediate range.

KEY MESSAGE

1. Although there was significant decline in the quantitative result of the T-SPOT.TB test with treatment, most of patients remained both test-positive after anti-tuberculosis (TB) treatment.
2. Interferon-γ release assays may not provide evidence regarding the cure of disease in Korea, a country where the prevalence of TB is within the intermediate-range.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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