Hirama et al., J Mycobac Dis 2016, 6:4
DOI: 10.4172/2161-1068.1000225

Mycobacterial Diseases
ISSN: 2161-1068

Short Communication Open Access

The Quantiferon®-TB Gold In-Tube Assay Detects Interferon-γ Release Responses to Mycobacterium Tuberculosis Antigens for Extended Periods of Time

Takashi Hirama[1,2,3*], Shohei Minezaki[1], Minoru Kanazawa[1], Hidetoshi Nakamura[1], Makoto Nagata[1]

[1]Department of Respiratory Medicine, Saitama Medical University, 38 Morohongo Saitama, 3500495, Japan
[2]Program in Cell Biology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, ON, M5G 1X8, Canada
[3]Division of Respirology, Toronto General Hospital, 585 University Ave, Toronto, Ontario, M5G 2N2, Canada

Abstract

The development and wide usage of interferon (IFN)-γ release assays (IGRAs) brought remarkable advances in the diagnosis of tuberculosis (TB). QuantiFERON®-TB Gold In-Tube (QFT-GIT), one of the IGRAs, employs three TB antigens, ESAT-6, CFP-10, and TB7.7, to which cell-mediated immune responses are measured in a single tube. In this regard, the QFT-GIT in patients with active TB was hypothesized to detect TB positivity for a longer period after the initiation of treatment. The change of IFN-γ values in patients with pulmonary TB serially registered to the study was examined with QFT-GIT before initiating anti-TB drugs, after completion of treatment and 12 months after the cessation of treatment. The data demonstrates that the IFN-γ levels remained consistently positive for a period of one year after treatment.

Introduction

One of the remarkable advances in the diagnosis of tuberculosis (TB) in the last few decades has been the development and wide usage of interferon (IFN)-γ release assays (IGRAs). These assays quantify the in vitro release of IFN-γ from T-cells stimulated with *Mycobacterium tuberculosis* (*M. tuberculosis*) antigens. The innovation, together with their prevailing employment, facilitates the early identification of at-risk individuals allowing for early intervention treatment for latent TB infection, thereby preventing the spread of TB in health care settings and communities where the TB patients reside[1,2].

Two major IGRAs are widely utilized;one is QuantiFERON®-TB Gold In-Tube (QFT-GIT) (Cellestis, Victoria, Australia) and the other is T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). IGRAs, although not recommended for diagnosis of active TB in low-income countries [3], have been used to rule out latent TB infection especially for individuals who have received BCG or screening of health care workers [2,4].QFT-GIT simultaneously employs three *M. tuberculosis* antigens, ESAT-6, CFP-10, and TB7.7, to which cell-mediated immune responses are measured in a single tube [5].QFT-GIT replaces QuantiFERON®TB-Gold (QFT-G), which was the former generation of QuantiFERON applying two *M. tuberculosis* antigens with a separate reaction. QFT-GIT, therefore, is more sensitive and specific relative to QFT-G due to the additive effect [6]. With those features in mind, we hypothesized that, in patients with TB, the QFT-GIT should detect TB positivity for a longer period after the initiation of treatment. To this end, the change of IFN-γ values in patients with active TB serially registered to the study, and receiving anti-TB drugs, was examined for a period of one year after treatment.

Material and Methods

Patients

Adult patients aged 18 and over and diagnosed with active pulmonary tuberculosis, based on a positive culture result for *M. tuberculosis* from respiratory samples [7], were prospectively enrolled to the study at the Saitama Medical University Hospital (Saitama, Japan) between June 6, 2011 and August 31, 2014. Patients were treated with the anti-TB drugs: isoniazid (INH), rifampicin (RFP), ethambutol (EB) and pyrazinamid (PZA). Treatment regimens for TB treatment were based on established guidelines [8] according to the drug susceptibility result and patients' tolerance.

Ethical consideration

The research protocol for the study was approved by the institutional review boards of Saitama Medical University Hospital (institutional review board [IRB] number 11-005, 6 June 2011).Informed consent was obtained from participants before enrollment to the study.

QuantiFERON®-TB Gold In-Tube

Blood samples for the QFT-GIT were collected from active TB patients. The QFT-GIT test was performed according to the manufacturer's instructions. The test results with an IFN-γ level ≥ 0.35 IU/ml in TB antigens (greater than the nil control value) were considered positive, and those with < 0.10 IU/ml were considered negative. Those with IFN-γ levels between 0.10 and 0.35 were considered indeterminate. Using those cutoffs, the sensitivity and specificity of the QFT-GIT test were reported at 84% and 99%, respectively [9].

Results

Fourteen patients with active pulmonary TB, culture-confirmed *M. tuberculosis* isolated from respiratory samples, were enrolled to this study (Table 1). Subsequently they were treated with anti-TB drugs [8] and blood samples were collected for QFT-GIT at the time of diagnosis

***Corresponding author:** Takashi Hirama, Division of Respirology, Toronto General Hospital, Toronto, Ontario, Canada; Tel:1- 416-340-4996; Fax: 1- 416-340-4996; Email: mycobacteriumtuberculosis@hotmail.com

Received September 19, 2016; **Accepted** October 13, 2016; **Published** October 19, 2016

Citation: Hirama T, Minezaki S, Kanazawa M, Nakamura H, Nagata M, et al. (2016) The Quantiferon®-TB Gold In-Tube Assay Detects Interferon-Γ Release Responses to *Mycobacterium Tuberculosis* Antigens for Extended Periods of Time. J Mycobac Dis 6: 225. doi: 10.4172/2161-1068.1000225

Age at diagnosis (year)	
Mean (min-max)	49.7 (18-76)
Body weight (kg)	
Mean +/-SD	53.4 +/-7.9
Sex	
Male/Female	6/8
Smoking status (no.[%])	
Current/Former	7 (50.0%)
Never	7 (50.0%)
Alcohol user (no.[%])	
Addicted	0 (0%)
Occasional/None	14 (100%)
Underlying condition (no.[%])	
Chronic lung disorder	6 (42.9%)
Chronic other organ dysfunciton	2 (14.3%)
Neoplastic disease	1 (7.1%)
Immunosuppresive therapy	2 (14.3%)
Diabites	1 (7.1%)
Psychiatric disorder	1 (7.1%)
Previous anti-tuberculosis treatment (no.[%])	
Yes/No	1 (7.1%)
Sputum test results (no.[%])	
AFB smear positive	4 (28.6%)
Culture positive	14 (100%)
Drug susceptibility test (no.[%])	
INH resistance	1 (7.1%)
RFP resistance	0 (0%)
Other resistance	2 (14.3%)
Radiological feature (no.[%])	
Limited in upper field	7 (50.0%)
Bilateral lung	4 (28.6%)
Cavities	3 (21.4%)
Pleural effusion	3 (21.4%)
Miliary	1 (7.1%)
Extratpulmonary tuberculosis	1 (7.1%)
Initial drug regimen (no.[%])	
INH, RFP, PZA, EB	9 (64.3%)
INH, RFP, EB	4 (28.6%)
RFP, PZA, EB, SM	1 (7.1%)

Table 1: Characteristics of pulmonary tuberculosis patients enrolled in the study (n=14) AFB, acid-fast bacilli; INH, isoniazid; RFP, rifampicin; PZA, pyrazinamide; EB, ethambutol SM, streptomycin. Data were at the time of diagnosis.

(1st QFT), completion of treatment (2nd QFT) and 12 months after (3rd QFT) (Figure 1). Overall, the IFN-γ values, although showing a downward trend, were far above the cutoff value to be diagnosed as a negative result even after a year of treatment. At the 1st QFT, 12 out of 14 patients (85.7%) had a positive QFT, with a mean IFN-γ level of 2.37 IU/ml. Similarly, 75% of those who completed TB treatment for either 6 months or 9 months also had a positive QFT at the 2nd QFT, in which the mean IFN-γ level was 1.74 IU/ml. One year after treatment, 88.9% of QFT still remained steadily positive with mean IFN-γ levels of 1.70 IU/ml. No patients have relapsed after completing TB treatment.

Discussion

Although a variety of studies using QFT-G and QFT-GIT have been conducted to assess the conversion ratio of IFN- γ release after initiating TB treatment, the literature focusing on the long-lasting potency of IGRAs is limited. In this paper, we have therefore examined the period of IFN-γ release assessed by QFT-GIT in response to *M. tuberculosis*

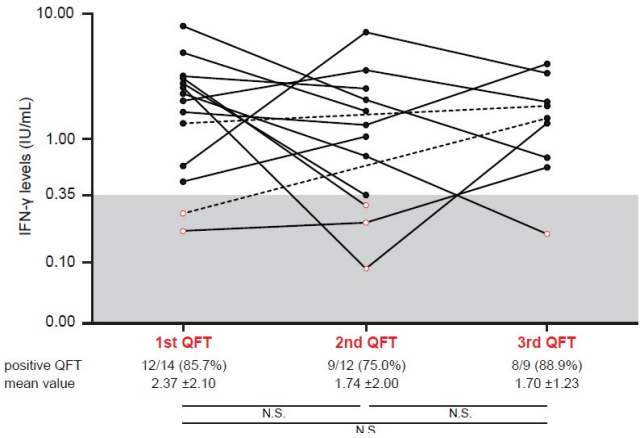

Figure 1: The change in IFN-γ level in patients with active pulmonary tuberculosis.
Blood sampling for the QFT-GIT was performed before initiating anti-TB drugs (1st QFT), after completion of treatment (2nd QFT) and 12 months after treatment (3rd QFT). Two patients who missed 2nd QFT are depicted as the dashed lines. Shaded area indicates IFN-γ levels below the cutoff value for the positive QFT 0.35 IU/ml (greater than the nil control value). Data at the bottom were means ± SD of the IFN-γ levels using Prism 6 (GraphPad Software, Inc. La Jolla, CA). Statistical analysis was calculated using unpaired t-tests (Welch's method for uneven variances). N.S. = not significant.

antigens in serially registered TB patients who were followed up until one year after treatment.

In previous studies in which the former version QFT-G was used, it was concluded that treatment of active TB disease resulted in reduced IFN-γ release in response to the *M. tuberculosis* antigens [10]. Despite a decline in IFN-γ release in IGRAs by TB treatment, some literature indicated that the conversion ratio was heterogeneous, supposedly due to different genetic characteristics and the immune competence in the host who carried TB [10]. To minimize genetic influence, only studies conducted in Japan were compared with our data. Patients with active TB who were 83% positive by QFT-G reduced to 58% after treatment, followed by 50% 6 months later [11]. Likewise, treatment of active TB disease led 76% of positive patients to 62%, which subsequently dropped to 36% in a year after [12].These data, together with our results, indicated that the incubation of lymphocytes with three *M. tuberculosis* antigens simultaneously results in apparent TB positivity for a longer period after the onset of treatment, which also demonstrates that QFT-GIT is the more sensitive examination as opposed to QFT-G.

This raises the concern that TB reinfection, although rarely experienced in the general public, could occur under specific circumstances, such as for health-care workers (HCWs) in TB wards or residents in highly TB prevalent areas [13,14,15]. IGRAs, such as QFT-GIT or T-SPOT.TB, are not only used as a diagnostic tool for latent TB infection but also implemented for the investigation of TB transmission among HCWs [2,3,4,15]. With their high sensitivity and specificity, applying QFT-GIT to screening for latent TB infections is clearly advantageous, yet it will be harder to distinguish the TB reinfection from those whose TB has previously been treated due to its long lasting potency.

Conclusion

To sum up, assessing the status of being infected with TB is easily implementable owing to the latest advancements in IGRAs, such as

QFT-GIT; however there are limitations to evaluating the infectious condition of *M. tuberculosis* in specific environments. Additionally, appraising long-lasting potency of IGRA is difficult based on our study with small sample size. Thus, further investigation with a large scale is required.

Disclosure

Authors have no conflict of interest.

Acknowledgements

We would like to thank my colleague Johnathan Canton, Ph.D., Program in Cell Biology, Hospital for Sick Children, Toronto, Canada, for proofreading of manuscript draft.

Funding information

This work was supported by the Saitama Medical University Hospital. No additional external funding received for this study.

References

1. Zwerling A, van den Hof S, Scholten J, Cobelens F, Menzies D, et al. (2012) Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review. Thorax 67: 62-70.
2. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, et al. (2010) Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. MMWR Recomm Rep 59: 1-25.
3. World Health Organization (2011) Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries: policy statement (World Health Organization).
4. Hirama T, Hagiwara K, Kanazawa M (2011) Tuberculosis screening programme using the QuantiFERON-TB Gold test and chest computed tomography for healthcare workers accidentally exposed to patients with tuberculosis. J Hosp Infect 77: 257-62.
5. Perry S, Sanchez L, Yang S, Agarwal Z, Hurst P, et al. (2008) Reproducibility of QuantiFERON-TB gold in-tube assay. Clin Vaccine Immunol 15: 425-432.
6. Matsumoto T, Hirayama Y, Hisamitsu Y, Fukumura M, Hirata A, et al. (2013) Simultaneous and Longitudinal Comparison of Interferon Gamma Release Assay Data from Health Care Workers in Japan. J Mycobact Dis 3: 3-6.
7. Diagnostic Standards and Classification of Tuberculosis in Adults and Children (2000) This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 161: 1376-1395.
8. American Thoracic Society, CDC, Infectious Diseases Society of America (2003) Treatment of tuberculosis. MMWR Recomm Rep 52: 1-77.
9. Diel R, Loaddenkemper R, Nienhaus A (2010) Evidence-based comparison of commercial Interferon?? Release assays for detecting active TB a metaanalysis. Chest 137: 952-968.
10. Clifford V, He Y, Zufferey C, Connell T, Curtis N (2015) Interferon gamma release assays for monitoring the response to treatment for tuberculosis: A systematic review. Tuberculosis 95: 639-650.
11. Kobashi Y, Mouri K, Yagi S, Obase Y, Miyashita N, et al. (2009) Transitional changes in T-cell responses to Mycobacterium tuberculosis-specific antigens during treatment. J Infect 58: 197-204.
12. Takayanagi K, Aoki M, Aman K, Mitarai S, Harada N, et al. (2011) Analysis of an interferon-gamma release assay for monitoring the efficacy of anti-tuberculosis chemotherapy. Jpn J Infect Dis 64: 133-138.
13. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, et al. (1999) Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med 341: 1174-1179.
14. Verver S, Warren RM, Beyers N, Richardson M, van der Spuy GD, et al. (2005) Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. Am J Respir Crit Care Med 171: 1430-1435.
15. McCarthy KM, Scott LE, Gous N, Tellie M, Venter WD, et al. (2015) High incidence of latent tuberculous infection among South African health workers: an urgent call for action. Int J Tuberc Lung Dis 19: 647-653.