

Simultaneous and Longitudinal Comparison of Interferon Gamma Release Assay Data from Health Care Workers in Japan

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Abstract

Background: Tuberculosis is one of the serious occupational diseases among health care workers, especially those who work with patients suffering from respiratory disorders. It is important to assess latent tuberculosis infection status in such workers using interferon- γ release assays, including QuantiFERON-TB Gold and its successor, the QuantiFERON-TB Gold in-Tube test. Although the relative efficacies of these two methods have been evaluated in patients with tuberculosis, data from health care workers in Japan have not been extensively examined.

Purpose: In the present study, we examined the utilities of the QuantiFERON-TB Gold and QuantiFERON-TB Gold in-Tube tests to detect latent tuberculosis infections in staff working in a respiratory disease hospital in Japan. We also longitudinally compared data from these subjects obtained using the QuantiFERON-TB Gold assay.

Methods: We collected blood samples from 120 staff members and performed both the QuantiFERON-TB Gold and QuantiFERON-TB Gold in-Tube assays. A total of 58 subjects had previously been tested 5 years prior using the QuantiFERON-TB Gold assay, and we compared these data with our more recent information.

Results: The QuantiFERON-TB Gold in-Tube test tended to yield higher test values than did the QuantiFERON-TB Gold test, suggesting that the former test may be more sensitive when used to detect latent tuberculosis infection. In both tests, the results differed in 32 instances (26.7%), associated with significant difference ($p < 0.001$, $\kappa = 0.55$). In 94 subjects with negative QuantiFERON-TB Gold test results, 16 (17.0%) were intermediate and 11 (11.7%) positive by QuantiFERON-TB Gold in-Tube test. The longitudinal comparison confirmed this suggestion. The number of subjects rated "intermediate" in terms of tuberculosis status differed, with statistical significance, when the two datasets were compared.

Conclusion: Health care workers should be screened for possible tuberculosis infections using the QuantiFERON-TB Gold in-Tube test, which is more sensitive than the QuantiFERON-TB Gold test.

Keywords: Interferon-gamma release Assay; Latent tuberculosis infection; Health care workers

Abbreviations: BCG: Bacillus Calmette-Guérin; CFP-10: Culture Filtrate Protein; ESAT-6: Early Secreted Antigenic Target; HCW: Health Care Workers; IFN- γ : Interferon-Gamma; IGRA: Interferon-Gamma Release Assay; LTBI: Latent Tuberculosis Infection; Mtb: *Mycobacterium Tuberculosis*; NTM: Non-Tuberculosis Mycobacteria; PBMC: Peripheral Blood Mononuclear Cells; QFT-G: Quantiferon-TB Gold; QFT-GIT: Quantiferon-TB Gold In-Tube; TB: Tuberculosis; TST: Tuberculin Skin Test

Introduction

The incidence of tuberculosis (TB) in Japan is less than 20 per 100,000 population and is continuing to decline. However, TB remains a major occupational disease of health care workers (HCW) [1,2]. Several reports have shown that HCW were at several-fold higher risk for TB than the general population [3-6]. One practical way to control TB is routine screening of HCW for latent tuberculosis infections (LTBIs) and administration of chemoprophylaxis to HCW suspected to have LTBIs. Therefore, evaluation of *Mycobacterium tuberculosis* (Mtb) infective status is crucial in HCW working in hospitals dedicated to TB patients [2,7,8]. In such contexts, screening of HCW should be routine and data should be evaluated longitudinally [2,9].

Recently, methods detecting *Mycobacterium tuberculosis* (Mtb)-specific antigens have been developed. The target antigens include culture filtrate protein 10 kD (CFP-10), early secreted antigenic target 6

kD (ESAT-6), and TB7.7 [10-12]. The QuantiFERON-TB Gold (QFT-G) test uses CFP-10 and ESAT-6, whereas its successor, the QuantiFERON-TB Gold in-Tube test (QFT-GIT), additionally uses TB7.7 [13-15]. The results of the two assays cannot be directly compared, because not only do the stimulating antigens used vary, but some differences are evident in the methods used to stimulate lymphocytes. Thus, in the QFT-G tests, lymphocytes are separately stimulated by CFP-10 and ESAT-6 [16,17], whereas stimulation by a mixture of CFP-10, ESAT-6, and TB7.7 is commenced just after blood is drawn for the QFT-GIT assay [16,18-21]. Several investigators have reported that the sensitivity of QFT-GIT was higher than that of QFT-G, but that the specificity values were similar [10,11,20,21]. One issue that must be considered is the intermediate results introduced in some countries including Japan, but not most developed countries [20]. In Japan, an intermediate result

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(0.10 IU/ml \leq IFN- γ level <0.35 IU/ml) is recognized but the definition of intermediate varies among countries [17,19]. One study concluded that intermediate results were more frequent by QFT-GIT than by QFT-G [20].

In the present study, we compared simultaneously derived QFT-G and QFT-GIT assay data from HCW in Japan. We also compared our results with those of QFT-G tests performed five years prior in some subjects.

Subjects and Methods

Study design

This cross-sectional study involved 120 HCW of the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan. No subject had any underlying illnesses such as acute infection, autoimmune disorder, or any other chronic disease. No subject had an abnormal chest X-ray. The study protocol was approved by the Review Board of the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, and written informed consent was obtained from all participants.

QFT-G and QFT-GIT assays

The QFT-G and QFT-GIT assays were performed following the instructions of the manufacturer (Cellestis Limited, Carnegie, Australia). Blood samples were collected by normal phlebotomy into both evacuated 4 ml sterile sodium heparin tubes for QFT-G assay and into three 1 ml-volume QFT-GIT blood collection tubes [17-19]. When QFT-G tests were run, incubation with Mtb-specific antigens was initiated within 12 h of blood collection. After incubation for 24 h at 37°C, samples were centrifuged at approximately 500 g for 10 min to facilitate plasma collection. Plasma samples were stored at -70°C prior to conduct of ELISA detecting IFN- γ .

QFT ELISA assay

The concentrations of IFN- γ in plasma samples were determined via ELISA according to the manufacturer's protocol. All ELISAs were performed by the same trained staff. QFT-G and QFT-GIT test responses were automatically calculated using QFT-G ELISA Analysis software (Cellestis Limited) after input of ELISA plate optical density values. QFT-G and QFT-GIT test data were interpreted as suggested by the manufacturer. An IFN- γ response to Mtb-specific antigens that was at least 0.35 IU/ml and greater than the nil control value was considered positive. Samples with 0.10 \leq IFN- γ level <0.35 IU/ml were regarded as intermediate, according to guidelines of Committee for Prevention of the Japanese Society of Tuberculosis [17,19]. Mitogen stimulation was used to positively control the quality of both blood samples and laboratory technique. If a sample IFN- γ value was <0.35 IU/ml when the positive control value (upon mitogen stimulation) was \geq 0.5 IU/ml, the test result was considered to be negative.

Data analyses

The extent of agreement between data yielded by the two tests was evaluated using the McNemar approach, and agreement was expressed in terms of both a kappa coefficient and the level of overall agreement (the proportions of samples yielding positive or negative results in both tests). Non-parametric statistics (Wilcoxon's ranked sign test and Spearman's ranked correlation coefficient) were employed when the means of IFN- γ measurements obtained using either method were compared. A difference associated with a p value <0.05 was considered to be statistically significant.

Results

Comparison of QFT-G and QFT-GIT assay data from 120 Japanese HCW

When comparing QFT-G and QFT-GIT assay data from 120 blood samples obtained at the same time, QFT-GIT yielded higher IFN- γ levels than did QFT-G in most samples (Figure 1). However, the results (positive, intermediate, or negative) differed in 32 instances (26.7%), associated with a significant difference in the results of the two assays ($p < 0.001$ by Wilcoxon's ranked sign test; $\kappa = 0.5503$ by weighted value).

Thus, in 94 subjects with negative QFT-G test results, 16 (17.0%) were intermediate and 11 (11.7%) positive by QFT-GIT (Table 1); only 67 subjects (71.2%) were negative on both tests. Similarly, of eight subjects diagnosed as intermediate by the QFT-G test, two (25%) were positive by QFT-GIT and only four (50%) yielded the same intermediate results. Moreover, two subjects (25%) scored as intermediate by the QFT-G test were negative by QFT-GIT, probably reflecting the indeterminacy of 'intermediate' results. Seventeen of 18 (94%) subjects positive by QFT-G were also positive by QFT-GIT, although the IFN- γ levels yielded by the latter test were higher (Figure 1). However, one of these 18 (6%) subjects was diagnosed as intermediate by QFT-GIT, although the IFN- γ level measured by QFT-G was just above the cut-off

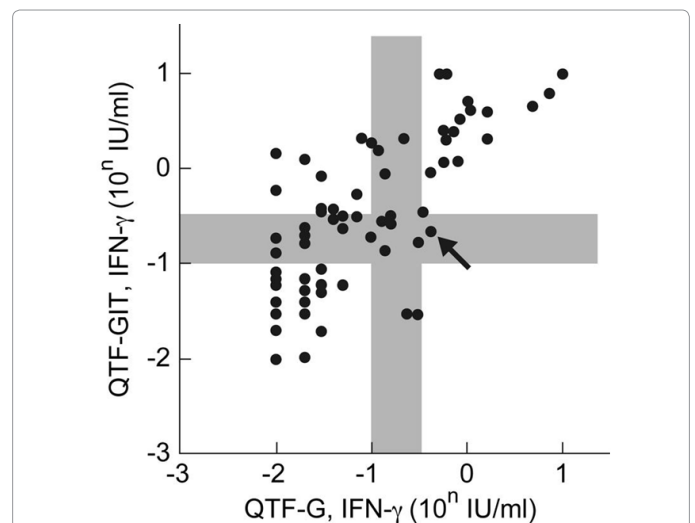
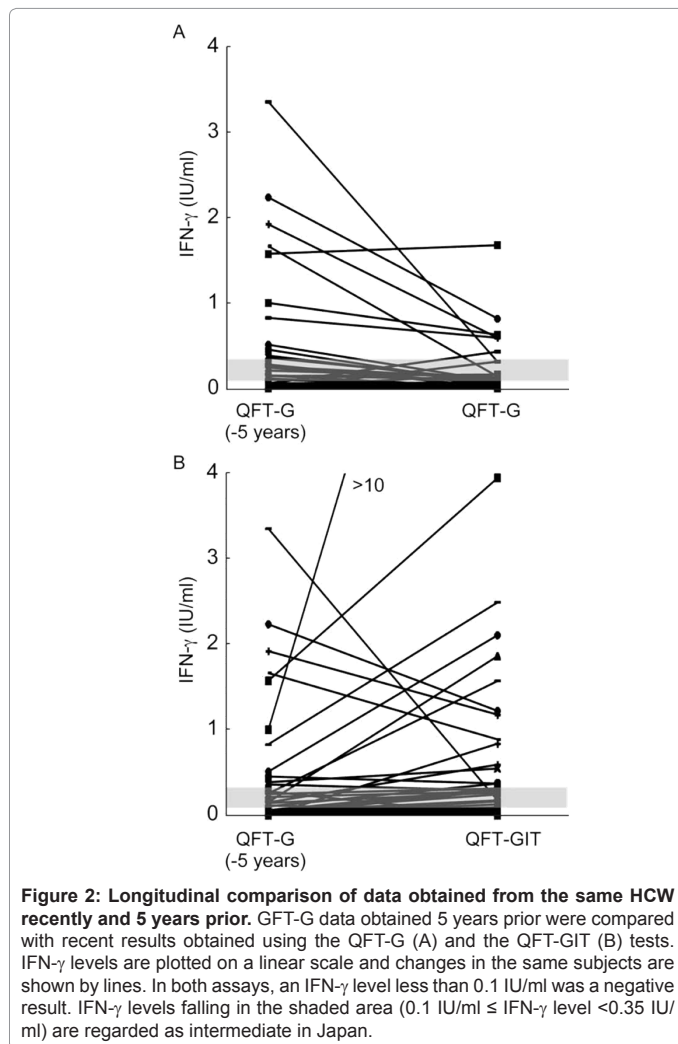


Figure 1: Comparison of two simultaneous IGRAs. Blood samples from 120 HCW were simultaneously examined using two different IGRAs. IFN- γ concentrations are shown. In both assays, a level of IFN- γ less than 0.1 IU/ml constituted a negative result. IFN- γ levels falling in the shaded area (0.1 IU/ml \leq IFN- γ level <0.35 IU/ml) are regarded as intermediate in Japan. An arrow indicates one case that yielded an intermediate QFT-GIT test result, but who was positive by QFT-G, although the IFN- γ level was just above the (lower) positive cut-off value in the latter test.

		QFT-GIT			Total
		(-)	(\pm)	(+)	
QFT-G	(-)	67 (71.2%)	16 (17.0%)	11 (11.7%)	94 (100%)
	(\pm)	2 (25%)	4 (50%)	2 (25%)	8 (100%)
	(+)	0 (0%)	1 (6%)	17 (94%)	18 (100%)
Total		69	21	30	120

(+): IFN- γ level > 0.35 IU/ml, (\pm): 0.1 \leq IFN- γ level < 0.35, (-): IFN- γ level < 0.1 IU/ml

Table 1: Comparison of simultaneous QFT-G and QFT-GIT assays.



value (arrow in Figure 1). These results show that the QFT-GIT is more sensitive when used to detect latent Mtb infections and that the assays yield different results, with statistical significance.

Longitudinal analysis of HCW data

The QFT-GIT and QFT-G tests, performed simultaneously, yielded different results. It was thus important to conduct a longitudinal comparison of the extent of latent Mtb infections in HCW tested 5 years prior using the QFT-G test. Of 120 participants in the present study, results of QFT-G measured 5 years prior were available in 58 subjects (48.3%). Of these, 11 (19.0%) had been previously diagnosed as positive, 9 (15.5%) as intermediate, and 38 (65.5%) as negative. Upon re-testing, 6 (10.3%) were positive and 8 (13.8%) intermediate by QFT-G, and 14 (24.1%) positive and 14 (24.1%) intermediate by QFT-GIT. We compared these data with those of recent QFT-G testing (Figure 2A). Of 38 previously negative subjects, 2 (5.3%) became intermediate and 1 (2.3%) positive (IFN- γ level 0.43 IU/ml). Of the nine subjects who were previously intermediate, four (4.4%) remained intermediate and five (55.5%) became negative; no subject became positive. Five of 11 previously positive subjects (45.5%) remained positive on recent testing, but two (18.2%) became intermediate and four (36.4%) negative.

We further compared earlier QFT-G results with our QFT-GIT data (Figure 2B). Of 38 previously negative subjects, 10 (26.3%) were

judged as intermediate and 1 (2.6%) was positive (0.84 IU/ml); this positive case was negative upon recent QFT-G testing. This subject was diagnosed as intermediate upon recent QFT-GIT testing. Of nine previously intermediate cases, four (44.4%) were positive and the others remained intermediate on recent QFT-GIT testing. Nine of 11 previously positive QFT-G cases remained positive, but two (18.2%) became intermediate upon recent QFT-GIT testing.

Discussion

In the present study, we simultaneously compared QFT-G and QFT-GIT test results and also performed a longitudinal comparison of recent data with earlier QFT-G results obtained 5 years prior, because such a comparison should be helpful in terms of early diagnosis of LTBI in HCW, especially staff of dedicated tuberculosis wards. We confirmed that the QFT-GIT test was more sensitive than the QFT-G test when used to screen HCW, similar to the results of a previous study in TB patients [20]. However, discrepancies were evident between the results of recent QFT-GIT and QFT-G tests and also when these data were compared with QFT-G results obtained 5 years prior. Differences between the results of the QFT-GIT and QFT-G tests may be attributable to differences in the antigens used to stimulate interferon secretion; such antigens may exert synergistic effects.

Differences between recent test results and older data may be attributable to functional regression of Mtb-specific T lymphocytes [22]. In BCG-vaccinated newborns, the levels of BCG-specific T lymphocytes peak 10 weeks after vaccination [23]. The levels of Mtb-specific IFN- γ -secreting T cells declined in both TB and LTBI patients during anti-TB treatment, suggesting that the IGRA results reflect the mycobacterial load [24,25]. In the present study, the QFT-G test values fell over the 5-year interval in most QFT-G-positive subjects (90.9%), and almost half became negative (54.5%). However, when the earlier results were compared with recent study QFT-GIT data, 10 of 38 (26.3%) previously negative subjects were of intermediate status and 1 (2.6%) was positive; this positive case was negative upon recent QFT-G testing. These data thus also suggested that the sensitivities of the two types of test differed, and that QFT-GIT testing was superior in terms of sensitivity. However, the results of the two tests conducted on the same subjects tended to differ, with statistical significance, suggesting that data from either test should not be compared.

Our present results using the QFT-G test are similar to those of a previous work with HCW of a Japanese tuberculosis referral hospital; approximately 10% were positive [26]. However, use of the more sensitive QFT-GIT test in the present study diagnosed 25% of subjects with LTBI; this proportion is very much higher than that of the general population [22]. A recent systematic review of the utility of IGRA assays used to screen HCW for TB showed that the initial positivity rate was 1.3-31.0%; the analysis included data from countries where the incidence of TB is high [7]. Such variation is attributable to differences in sample sizes and the backgrounds of HCW among the studies; the extent of involvement and contact with TB patients, administration of chemo-prophylaxis, and years of work with TB patients, will have varied significantly among the studies.

In conclusion, our simultaneous and longitudinal study of the same HCW including medical staff in TB-specific wards in a hospital in Japan suggests that QFT-GIT should be used for the screening of Mtb infection in HCW in Japan.

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