Usefulness of QuantiFERON TB-2G, a Diagnostic Method for Latent Tuberculosis Infection, in a Contact Investigation of Health Care Workers

Yoshihiro Kobashi¹, Yasushi Obase¹, Minoru Fukuda¹, Kouichiro Yoshida¹, Naoyuki Miyashita¹, Masashi Fujii² and Mikio Oka¹

Abstract

Objective To evaluate the usefulness of QuantiFERON TB-2G (QFT-2G) for detecting latent tuberculosis infection (LTBI) in a contact investigation of health care workers.

Methods The investigated subjects were 190 subjects among the health care workers who were examined between January 2005 and June 2006. Background information, including a past history of tuberculosis (TB) or tuberculin skin test (TST) or BCG vaccination, and contact score (infectivity×contact duration) were investigated. The TST and QFT-2G test were performed on all subjects.

Results In 109 subjects with a negative TST history, the TST results were converted to positive in 38 subjects. While the TST was positive in 48 subjects (25%), the QFT-2G test was positive in only five subjects (3%). The correlation of the QFT-2G with TST results was not significant. There was no relationship between contact score and the TST result. Twenty-nine subjects had TST positive responses (22%), but there were no QFT-2G positive responses in subjects with a mild contact score. Sixteen subjects had TST positive responses (31%), but two subjects showed positive QFT-2G results (4%) in the moderate contact score group. However, the positive response rate of the TST and QFT-2G test was the same percentage in the severe contact score group (33%).

Conclusion The QFT-2G test showed a significant relationship with the contact score when compared with the TST. If the subjects with LTBI in the moderate contact score group were selectively excluded, the contact investigation in the mild contact score group may not be necessary. If there would have been many subjects with the QFT-2G positive responses in the moderate contact score group, we think that the QFT-2G test must be performed even in the mild contact score group.

Key words: latent tuberculosis infection, tuberculosis skin test, quantiFERON TB-2G, contact score, health care worker

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Introduction

Tuberculosis (TB) is a major infectious cause of morbidity and mortality throughout the world. Tuberculosis (TB) case rates continue to decline in most industrialized countries, but persons with latent tuberculosis infection (LTBI) constitute a vast pool of individuals who may develop TB, particularly when the immune response is suppressed. When patients with active TB are detected in a community tertiary hospital without a special TB ward, nosocomial infection among health care workers and other inpatients becomes a major problem. In such a situation, it is important to isolate health care workers with LTBI from the index case showing active TB to prevent the spread of nosocomial infection. Accurate diagnosis and treatment of LTBI, therefore, have become increasingly important goals of TB control to prevent nosocomial infection of TB (1).
and even though it is not recommended, as a diagnostic tool of patients with active TB, for the screening of LTBI, a population (3). Despite these limitations, TST is routinely performed to determine whether either test was superior in detecting LTBI has many well known limitations. The sensitivity of the tuberculin skin test (TST) is low for the diagnosis of active TB infections. Specificity is limited by cross-reactivity of the purified protein derivatives (PPD) with the Bacillus Calmette-Guerin (BCG) vaccine and with most nontuberculous mycobacteria (2). Moreover, the sensitivity of TST is low in immunosuppressed patients in whom the risk of progression to TB is high. As a result, a significant prevalence of false-negative results is produced among such a population (3). Despite these limitations, TST is routinely used in hospital clinical practice including contact investigation of patients with active TB, for the screening of LTBI, and even though it is not recommended, as a diagnostic tool for active TB (4), it enters the diagnostic algorithm of patients who have clinical signs suggestive of active TB.

In subjects with LTBI, memory T cells produce IFN-γ in response to Mycobacterium tuberculosis antigens. A major scientific advance has been the identification of the 6-kD M. tuberculosis early-secreted antigenic target protein (ESAT-6) and the 10-kD culture filtrate protein (CFP-10), which are absent from BCG and most environmental mycobacteria (5, 6).

Tests can now detect T cells that produce IFN-γ in response to ESAT-6 and CFP-10 using ELISA to measure IFN-γ concentrations in supernatants (QuantiFERON-TB Gold; Cellestis Ltd., Victoria, Australia) or the enzyme-linked immunospot assay (ELISPOT) to detect individual IFN-γ-producing T cells (T SPOT-TB; Oxford Immunotec, Oxford, UK) (7–9). The Food and Drug Administration (FDA) has approved the QuantiFERON-TB Gold test and is evaluating the T SPOT-TB test, which has been approved in Europe. These tests are positive in most subjects with a high likelihood of LTBI and are negative in BCG-vaccinated subjects with a low likelihood of LTBI (10).

In school or occupational TB outbreaks in Japan, QuantiFERON TB-2G (QFT-2G) results in Japan have correlated significantly better than TST test results with the degree of exposure to the index case (11, 12), suggesting that the QFT-2G test is a better marker of LTBI than TST. However, there have been few reports regarding the usefulness of the QFT-2G test for contact investigation between patients with active TB (smear positive for acid-fast bacilli) and health care workers in a community tertiary hospital without a specialty TB ward.

We compared the ability of the QFT-2G test and TST to detect LTBI in a clinical setting (a community hospital) where contact with TB patients were evaluated. Although there is no gold standard for identifying LTBI, the percentage of infected contacts is considered to be higher if the patient with TB has a positive sputum smear for acid-fast bacilli (13). The frequency of LTBI also rises in those with an increasing duration and proximity of contact with the patient (14, 15). Therefore, we compared the QFT-2G test and the TST with this contact score (infectivity×contact duration) to determine whether either test was superior in detecting LTBI.

Study subjects

This study was approved by the Kawasaki Medical School Ethics Committee and all subjects provided written, informed consent. Contact investigations were performed according to the manuals of Kawasaki Medical School Hospital. All contact subjects completed the TST, QFT-TB and underwent chest radiography three months after contact with the index cases. We enrolled 190 medical staff members of both Kawasaki Medical School Hospital (1,072 beds) and Kurashiki Central Hospital (1,570 beds) who had had recent contact history with three cases of smear and culture positive pulmonary TB evaluated at Kawasaki Medical School Hospital and with one case of smear and culture positive pulmonary TB evaluated at Kurashiki Central Hospital between January 2005 and July 2006. All subjects had negative serological findings for human immunodeficiency virus (HIV) and absence of obvious risk factors for that disease. We excluded persons with a history of tuberculosis or prior exposure to a patient with TB.

Interview and calculation of contact score

Based on a review of the literature regarding factors that affect the transmission of TB (13–16), we derived a method of calculating a contact score before analysis of study data. All contacts were interviewed using a standard questionnaire to determine the contact time with the index case. Information was obtained regarding subject's gender, age, occupation, past history of any underlying disease including TB, clinical symptoms such as respiratory symptoms (cough, sputum) and general symptoms (fever, general fatigue, weight loss), history of Bacillus Calmette-Guerin (BCG) vaccination, history of the TST, time spent with the index case, and mask equipment used. Contact scores were then calculated using two variables that are critical risk factors for infection: the time spent with the patient with pulmonary TB, and the infectivity of the patient. Regarding the infectivity of the index cases, we divided patients into four groups (1~4 points) according to the ATS classification of the sputum smear acid-fast bacilli examination (17): 1; (-): 0/300 per high power fields, 2; (1+): 1~9/300 per high power fields, 3; (2+): ≥10/100 per high power fields, 4; (3+): ≥10/1 per high power field. Contact score=infectivity×time (hours) of exposure. We divided subjects into three groups based on “contact score” (mild; 0~99; moderate; 100-199; severe; 200~). This score qualified the duration of exposure to the index case, as well as the degree of infectiousness of the index case.

Sample collection and TST

A heparinized blood sample was collected from each subject by vein puncture for whole blood IFN-γ assay. Blood

Methods

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was collected before administration of the Mantoux TST. For TST, 0.1 ml of tuberculin PPD (Nippon BCG Manufacturing, Tokyo, Japan; equivalent to about 3 TU of PPD-S) was injected intradermally into the volar aspect of the forearm and the transverse erythema and induration diameter were measured 48 hours later.

**QFT-2G test**

The QFT-2G test was performed according to the manufacturer’s recommendations (Cellestis, Carnegie, Australia). Briefly, the test consisted of a negative control (nil well, i.e., whole blood without antigens or mitogen), a positive control (mitogen well, i.e., whole blood stimulated with the mitogen phytohemagglutinin (PHA)) and two sample wells, i.e., whole blood stimulated with either of the *M. tuberculosis*-specific antigens Early Secretory Antigen Target 6 (ESAT-6) or Culture Filtrate Protein 10 (CFP-10). Whole blood specimens were incubated for 18 hours (overnight) at 37°C in a humidified atmosphere. The IFN-γ level of the nil well was considered background and was subtracted from the results of the mitogen well and the antigen-stimulated wells. The test result was considered positive if the concentration of IFN-γ in the sample well after stimulation with ESAT-6 and/or CFP-10 was greater than or equal to 0.35 IU/ml, irrespective of the result for the positive-control well (after subtraction of the value of the nil well). It was considered negative if the control concentration of IFN-γ was less than 0.35 IU/ml and if the IFN-γ level of the positive control (after subtraction of the value of the nil well) was greater than or equal to 0.5 IU/ml. The test result was considered indeterminate if the IFN-γ level was less than 0.35 IU/ml in both antigen wells and less than 0.5 IU/ml in the positive control well.

The QFT-2G test was performed on all investigated subjects three months after contact with the index cases. Otherwise, we performed the QFT-2G test again for positive subjects two months, four months, six months, nine months, and twelve months after prophylactic administration of antituberculous drugs (isoniazid 400 mg/day).

**Statistical analysis**

Information from the questionnaires, TST results, and whole blood IFN-γ assay results were entered into Excel 2000 (Microsoft, Redmond, WA) and transferred to Santa version 7.0 (Santa, College Station, TX) for statistical analysis. The contact score as an ordinal variable was used for trend tests. To determine whether the effect of the contact score on test result (positive or negative) differed with the test type (TST or QFT-2G), generalized estimating equations were used to perform a repeated-measures logistic regression with test results as the dependent variable, “subject” as a random effect, and independent terms for the contact score, test type, and the interaction between the contact score and test type. Generalized estimating equations were also used to evaluate the effect of the test (QFT-2G vs. TST) on the test result (positive or negative). Fisher’s exact test or χ² test of association were examined using the Tukey-Kramer approach. All analyses were performed with Santa version 7.0. A probability level less than 0.05 was considered significant for all tests.

**Results**

**Demographics of contact investigators**

Blood samples were obtained from 190 subjects. Data are reported for all these subjects and all had evaluable results. Forty-six subjects were male and one hundred forty-four subjects were female. The average age of the 190 subjects was 30.6 ± 8.5 (mean ± SD) years old. All of the subjects were health care workers and included 68 doctors, 118 nurses, and 4 pharmacologists. Two subjects (1%) had clinical symptoms at the time of contact investigation. One hundred forty-eight subjects (78%) had a history of BCG vaccination. One hundred twelve subjects (59%) received the first vaccination at ages three to six years old, 78 (41%) at ages seven to twelve years old. The past history of TST was positive (≥10 mm maximum length of erythema) in 81 subjects (43%) and negative in 109 (57%) (<9 mm maximum length of erythema). None of the subjects had used mask equipment during contact with the index cases.

**Index cases with pulmonary TB appeared in a community tertiary hospital after admission**

There were four index cases in this study. Three of four patients were elderly (over 80 years old) and all had nonrespiratory underlying diseases. All four patients had clinical symptoms such as fever and moderate or severe cough and 5~24 days was required to obtain an appropriate diagnosis after admission. Sputum smear acid-fast bacilli examinations showing infectivity presented as (2+)~(3+). Regarding the radiological findings, there were no cavitory lesions or bronchiectatic lesions, but there were inhomogeneous infiltration shadows in all cases and it was difficult to distinguish these findings from pneumonia. Final clinical diagnosis was pulmonary TB in three patients, pulmonary TB + tuberculous pleuritis in one.

**Contact score and TST positive rate or QFT-2G positive rate**

We selected TST positive response as (≥30 mm) because Shigeto et al indicated the possibility of TB infection was high in health care workers showing erythema of 30 mm or more despite previous BCG vaccination (18). The relationship between the contact score and TST positive (≥30 mm) and QFT-2G positive rates is shown in Table 1. There were no QFT-TB positive responses (0/130) in subjects with a mild contact score. Two subjects showed QFT-2G positive results in the group with a moderate contact score. We could extract the health care workers with LTBI in the group with a moderate contact score selectively by using the QFT-2G test compared to TST through this study. Although the TST
Table 1. The Relationship between Contact Score and TST Positive Rate or QFT-2G Positive Rate

<table>
<thead>
<tr>
<th>Contact score</th>
<th>TST positive (30mm≤ )</th>
<th>QFT-2G positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ~ 99</td>
<td>29/130 (22%)</td>
<td>0/130 (0%)</td>
</tr>
<tr>
<td>100 ~ 199</td>
<td>16/51 (31%)</td>
<td>2/51 (4%)</td>
</tr>
<tr>
<td>200 ~</td>
<td>3/9 (33%)</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>48/190 (25%)</td>
<td>5/190 (3%)</td>
</tr>
</tbody>
</table>

* : p < 0.05

TST: Tuberculin skin test, QFT-2G: QuantiFERON TB-2G

positive rate was unchanged with increase in the contact score, the QFT-2G positive rate was significantly increased in the group with a severe contact score compared to that in the groups with a mild or moderate contact score. In addition, the overall TST positive rate (25%) was significantly higher than the overall QFT-2G positive rate (3%). There were no indeterminate QFT-2G results in this study.

Maximum length of erythema for TST and QFT-2G results

The distribution of the maximum length of erythema for the TST and QFT-2G test is shown in Fig. 1. Although three of five subjects showed a QFT-2G positive response among the group of subjects with a TST positive response (≥30 mm), the remaining two subjects were recognized among the group of subjects with a TST positive response (10~29 mm). There was no relationship between the maximum length of erythema for TST and the results of QFT-2G.

Clinical findings of contact subjects with a positive response for QFT-2G

The clinical findings of contact subjects with a positive response for QFT-2G are shown in Table 2. Three subjects were nurses and two were doctors. Although one of these subjects had no BCG vaccination history and no TST positive history, she also converted from negative to positive TST reaction after contact with the index case. A preventive antituberculous drug (isoniazid: 400 mg/day for six months) was administered to the five subjects who had positive QFT-2G responses. Subsequently, there was no active TB infection in any of these subjects. Regarding the QFT-2G transitional change during treatment, two of the four subjects converted from positive to negative response on QFT-2G six and nine months, respectively, after the initiation of treatment.

Discussion

An accurate diagnosis of latent tuberculosis infection (LTBI) among health care workers is important to prevent nosocomial infection with *M. tuberculosis*. Especially, because health care workers cannot avoid contact with index cases of active TB in a tertiary community hospital, we always attach infectious risk of TB to health care workers who may have LTBI. In such hospitals, there may be many immunocompromised patients with risk factors such as immunosuppressive therapy, HIV infection, cancer, and chronic renal failure associated with progression to active TB disease (19-21). The high prevalence of BCG vaccination in Japan has significantly reduced the usefulness of TST because of the known cross-reactivity of PPD with the BCG vaccine, which generates overuse of prophylactic drug administration. Recently, a new *in vitro* test, QuantiFERON-TB Gold (QFT-2G, Cellestis Limited, Carnegie, Australia), received final approval from the U.S. Food and Drug Administration (FDA) as an aid in diagnosing LTBI (22). The QFT-2G test was commercialized for the detection of LTBI in Japan in April 2005. As previously reported in other studies (10, 23-25), this test is not influenced by BCG-vaccination status. Therefore, we adopted the QFT-2G test for contact investigation of health care workers when active TB patients appeared in tertiary community hospitals. Subsequently, we were able to reduce the indication for chemoprophylaxis from 25% based on TST results to 3% based on QFT-2G test results. Finally, although prophylactic antituberculous treatment (INH) was performed for five subjects with a positive
QFT-2G response, there was no progression to active TB in any cases. The QFT-2G test is technically feasible to perform and is likely to be a more accurate method of diagnosing LTBI than the TST. The improved specificity of the QFT-2G test will reduce the number of false-positive diagnoses of LTBI in BCG-vaccinated individuals.

To take on the new challenge of quantifying the risk of recent exposure to TB infection, we first derived a contact score at the point of infectivity and contact duration based on data from prospectively obtained, standardized interviews in this study. We investigated the relationship between responses to QFT-2G test and TST, and this contact score, which served as a surrogate measure for the likelihood of LTBI. The QFT-2G test results in health care workers were considered to be more strongly associated with a severe contact score than TST results in health care workers (Table 1).

In a previous report in which Sham et al reported the usefulness of the ELISPOT test for the diagnosis of LTBI (26), they adopted a contact score using three variables that are critical risk factors for infection; the relationship of the contact to the patient with TB, the infectivity of the patient, and the extent of exposure to the patient. The likelihood of a positive ERISPOT and TST increased significantly with a rising contact score. The contact score was more strongly related to ELISPOT results than to TST results, although this difference was not significant. Because there were many BCG-vaccinated subjects (78%) included in this study, the QFT-2G results were more useful for determining the risk of LTBI following exposure to the index case showing active TB disease, but TST was not useful for determining this risk. As for the items of the contact score, we selected infectivity (the degree of sputum smear positivity) and contact duration (total hours), because the contact place was restricted to the same ward for all health care workers and there was no remarkable difference in the relationship between the index case showing active TB and the health care workers. However, although it was important to consider the severity of cough in the index case or the mask equipment in the assessment of contact score, we excluded these from the assessment of contact score in this study because it was difficult to judge the degree of passive cough of each health care worker from the index case objectively and there were no health care workers using mask equipment. Subsequently, we found out that there were no QFT-2G positive responses in subjects with a mild contact score. If the contact score is mild, it may not be necessary to perform contact investigation of health care workers using QFT-2G.

In this study, although we used a cut off value of IFN-γ for positive and negative judgment according to the guidelines proposed by the CDC (22), there were no indeterminate QFT-2G results and it was not difficult to judge the QFT-2G results. However, there are several problems in making an accurate diagnosis of LTBI. Although a blood sample for QFT-2G test was collected from each subject three months after contact with the index case, it is unknown when QFT-2G test converted from negative to positive after TB infection and how long a transient positive response to the QFT-2G test continues. Therefore, we cannot establish with certainty whether five medical staff members with a positive response to the QFT-2G test in this study who were thought to have LTBI, were actually infected by the index case.

In conclusion, because the QFT-2G test appeared to be more specific than TST for the detection of LTBI in a con-
Table 2. Clinical Findings of Contact Investigations in Subjects with a Positive QFT-2G Response

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, Sex</th>
<th>Occupation</th>
<th>BCG Vaccination</th>
<th>TST history</th>
<th>TST (Induration/erythema) (mm)</th>
<th>Contact score</th>
<th>QFT-2G transitional change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30, F</td>
<td>Doctor</td>
<td>(+)</td>
<td>(+)</td>
<td>10 / 24</td>
<td>256</td>
<td>ESAT-6 (-) → (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFP-10 (1.96 → 1.08 (6 mo after last TB contact)</td>
</tr>
<tr>
<td>2</td>
<td>28, F</td>
<td>Nurse</td>
<td>(-)</td>
<td>(-)</td>
<td>20 / 40</td>
<td>182</td>
<td>ESAT-6 0.84 → 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFP-10 (-) → (-)</td>
</tr>
<tr>
<td>3</td>
<td>27, F</td>
<td>Nurse</td>
<td>(+)</td>
<td>(+)</td>
<td>15 / 26</td>
<td>284</td>
<td>ESAT-6 1.78 → 1.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFP-10 1.42 → 0.85 (4 mo after last TB contact)</td>
</tr>
<tr>
<td>4</td>
<td>24, F</td>
<td>Nurse</td>
<td>(+)</td>
<td>(+)</td>
<td>20 / 30</td>
<td>158</td>
<td>ESAT-6 0.70 → (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFP-10 (-) → (-)</td>
</tr>
<tr>
<td>5</td>
<td>33, M</td>
<td>Doctor</td>
<td>(+)</td>
<td>(+)</td>
<td>30 / 55</td>
<td>226</td>
<td>ESAT-6 1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CFP-10 (-) → (-) 2 mo after last TB contact</td>
</tr>
</tbody>
</table>

QFT-2G : QuantiFERON-TB 2G
TST : Tuberculin skin test
TB : Tuberculosis

References