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ORIGINAL ARTICLE

Clinical evaluation of QuantiFERON TB-2G test for immunocompromised patients

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ABSTRACT

Introduction: We compared the usefulness of tuberculin skin test (TST) and QuantiFERON TB-2G (QFT-TB) test in immunocompromised patients.
Materials: The subjects were 252 immunocompromised patients who were clinically suspected of tuberculosis (TB) infection between April 2005 and December 2006.
Results: Regarding the underlying diseases, 74 had malignant diseases, 72 were undergoing immunosuppressive treatment, 52 had diabetes mellitus, 50 had chronic renal failure, and four had HIV infection. While the positive rate of the QFT-TB test for the diagnosis of TB infection (TB disease or latent tuberculosis infection) was 78.1%, that of TST for TB infection was 50.0%. The QFT-TB test was significantly better than TST. However, thirty-two patients (13%) had an indeterminate QFT-TB result. Indeterminate findings were significantly more frequent in patients receiving immunosuppressive treatment (28%), especially with lymphocytopenia in the peripheral blood, than in those who had other underlying diseases. While the result of TST positive and QFT-TB test negative were recognized in immunocompromised patients with BCG vaccination or nontuberculous mycobacterial (NTM) disease, the result of TST negative and QFT-TB test positive were recognized in immunocompromised patients with a past history of TB infection.
Conclusions: We concluded that the QFT-TB test is a more useful diagnostic method for TB infection than TST for immunocompromised patients suspected of TB disease. However, because the results of the QFT-TB test show an indeterminate response for patients receiving immunosuppressive treatment, especially for those with lymphocytopenia due to severe underlying diseases, care must be taken in the interpretation of the QFT-TB test for these patients.

Key words: Immunocompromised patient, QuantiFERON TB-2G (QFT-TB), Tuberculin skin test (TST)

Introduction

In Japan, the incidence of tuberculosis (TB) is intermediate (35 per 100,000) and has recently been decreasing. However, the aging of the population and the increased use of immunosuppressive treatments (e.g., cancer chemotherapy and immunomodulatory agents) highlight the need for additional strategies to maintain and improve TB control (1). Early diagnosis of infectious cases and treatment of these immunocompromised patients infected with *Mycobacterium tuberculosis* (MTB) are important strategies for

reducing the incidence of TB in industrialized countries [1,2]. The specificity of the tuberculin skin test (TST) is limited by cross-reactivity of the purified protein derivative (PPD) with Bacille Calmette-Guerin (BCG) vaccine and with most nontuberculous mycobacteria (NTM) [3]. Its sensitivity is also low in immunocompromised patients in whom the risk of progression to TB is high [2]. Despite these limitations, TST is routinely used in hospital clinical practice to screen for LTBI [4].

The two commercialized ex vivo interferon-γ (IFN-γ) assays, QuantiFERON TB-2G (QFT-TB) (Cellestis Limited, Carnegie, Victoria, Australia) and T SPOT-TB (Oxford Immunotec, Oxford, UK), use early secretory antigenic target 6 (ESAT-6) and culture filtrate protein (CFP-10) as MTB-specific stimulants on ELISA and enzyme-linked immunospot assay (ELISPOT), respectively [5-8]. The US Food and Drug Administration have approved the QFT-TB test and is evaluating the T SPOT-TB test, which has been approved for use in Europe. These tests demonstrate a positive result for most individuals with a high likelihood of TB infection (TB disease or LTBI) and a negative result for BCG-vaccinated individuals with a low likelihood of TB infection. Of these tests, the QFT-TB was first used commercially in Japan in April 2005 for the diagnosis of TB infection. Although there have been several reports investigating whether QFT-TB test and T-SPOT.TB test would be useful in immunocompromised patients [9-11], there have been only one report investigating the usefulness of new test separately in individual important underlying disease [11]. Therefore, we prospectively report on the results of an analysis in which the QFT-TB test was routinely performed on many consecutive immunocompromised patients separated into individual underlying disease in several community hospitals. This study evaluated the feasibility and performance of the QFT-TB test compared with those of TST for immunocompromised patients in a hospital-based population including the interpretation of concordant and discordant results of both tests.

Methods

Study Population

The study was approved by the Ethics Committee of Kawasaki Medical School. Two hundred sixty-four immunocompromised patients (over 16 years old) with underlying diseases who were clinically suspected of TB disease were prospectively enrolled in this study between April 2005 and December 2006. The patients suspected of TB disease demonstrated the appearance of new lesion in the lung field, pleural effusion, or lymphadenopathy on chest radiograph or chest computed tomography (CT) during follow-up of the underlying disease. Twelve patients were excluded because there were

no obvious findings suggesting TB disease on chest computed tomography (CT). The remaining two hundred fifty-two immunocompromised patients (204 inpatients and 48 outpatients) were finally analyzed in this study. Most of these patients had the underlying disease on admission for which they consulted the following hospitals: Kawasaki Medical School Hospital, Kawasaki Medical School Kawasaki Hospital, Kurashiki Central Hospital, Kurashiki Daiichi Hospital, and Asahigaoka Hospital. We obtained written, informed consent from all participants in this study. All patients except for the four with HIV infection had negative results on serological tests for HIV or had no obvious risk factors for HIV infection. Demographic, clinical, radiological, and microbiological data were collected for all patients. Collected data included any history of previous TB disease and risk factors for TB (i.e., malignant diseases including leukemia, immunosuppressive treatments such as systemic immunosuppressive drugs or anti-tumor necrosis factor alpha (TNF-α) agents within the past three months, diabetes mellitus, chronic renal failure with hemodialysis, and HIV infection with anti-HIV treatment). The diagnosis of TB disease was definitively confirmed by culture of sputum, bronchoalveolar lavage fluid (BALF), or pleural fluid samples found to be positive for MTB microbiologically. In Japan, inoculation with the BCG vaccination is first administered during infancy. Thereafter, if the response to TST is negative at the time of entrance of junior high school, BCG vaccination is administered again until conversion of the TST to positive. Information regarding any previous Mantoux TST results and BCG vaccination, as well as information about clinical and laboratory findings, was collected from each patient at the time of enrollment. Sputum or other appropriate respiratory samples were collected from all patients, and culture samples were obtained for the detection of MTB.

Sample collection and TST

A heparinized blood sample was collected from individual patients by vein puncture for whole-blood IFN-γ assay. Blood samples were collected before administration of the Mantoux TST. For the TST, 0.1ml of tuberculin PPD (purified protein derivative) (Nippon BCG; equivalent to 3 tuberculin unit (TU) of PPD-S) was injected intradermally into the volar aspect of the forearm, and the transverse induration diameter was measured 48 hours later. The TST results were interpreted according to the level of risk, as reported in current guidelines [12].

QuantiFERON TB-2G (QFT-TB) test

The QFT-TB test was performed according to the recommendations of the manufacturer (Cellestis). Briefly, the test consisted of a negative control (a nil well; i.e., whole blood without antigens or mitogen), a positive control (a mitogen well; i.e., whole

blood stimulated with the mitogen photohemaggulutinin), and two sample wells (i.e., whole blood stimulated with either ESAT-6 or CFP-10). Whole blood specimens were incubated for 18 hours (overnight) at 37□ in a humidified atmosphere. The IFN-γ level of the nil well was considered the background value and was subtracted from the values for the mitogen well and the antigen-stimulated wells. The test result was considered positive and suspected of TB infection if the IFN-γ level in the sample well after stimulation with ESAT-6 and/or CFP-10 was □0.35 IU/ml (after subtraction of the value for the nil well), irrespective of the result for the positive control well. The test result was considered negative and difficult to diagnose TB infection if the IFN-γ level was <0.35 IU/ml and if the IFN-γ level of the positive control well (after subtraction of the value for the nil well) was □0.5 IU/ml. The test result was considered indeterminate and impossible to interpret the result if the IFN-γ level was <0.35 IU/ml in both antigen wells and <0.5 IU/ml in the positive control well or the IFN-γ level was below half of negative control well in both antigen wells and >0.7 IU/ml in the negative control well. This judgment was performed according to the guidelines proposed by the Centers for Disease Control and Prevention for using the QFT-TB test [13].

Statistical Analysis

Information from the questionnaires, TST results, and whole blood IFN-γassay results were entered into Excel 2000 software (Microsoft) and then transferred to Santa software, version 7.0 (Santa), for statistical analyses. Statistical analyses were performed to assess (1) the feasibility and performance of the QFT-TB test compared with those of TST, (2) the proportion of QFT-TB tests with an indeterminate result and the associated risk factors, (3) the concordance and discordance between the QFT-TB and TST results, and (4) the positive rate of the QFT-TB and TST results in patients with a final diagnosis of active TB disease. The analysis of concordance between the QFT-TB and TST results was calculated using κ-value. Both the QFT-TB and TST results were compared using the χ^2 test. Ninety-five percent CIs for the positive rate of both tests were calculated using the Wilson score method [14].

RESULTS

Two hundred fifty-two patients who were clinically suspected of TB disease were tested with the TST and the QFT-TB test between April 2005 and December 2006. Their demographic and clinical characteristics are shown in Table 1. Regarding underlying diseases among these patients, 74 patients had malignant diseases (all of these patients had advanced cancer without surgical treatment) including leukemia (12 patients during the follow-up period including patients receiving anti-cancer therapy), 72 were

undergoing immunosuppressive treatment for underlying diseases (52 were receiving the systemic steroid prednisone, 10 were receiving anti-TNF-α agents, 10 were receiving the systemic steroid prednisone plus other immunosuppressive agents), 52 had diabetes mellitus (all patients were receiving oral hypoglycemic agents or insulin), 50 had chronic renal failure (all patients were receiving hemodialysis), and four had HIV infection (all patients were receiving anti-HIV treatment). The mean age of all immunocompromised patients was 62.0 years old and there were 156 males and 96 females. One hundred fifty two patients (60.3%) had a past history of BCG vaccination and 24 (9.5%) had a past history of healed pulmonary TB. The incidence of TB disease was 12.6% in the overall patient group. On separation of patients by underlying immunocompromised diseases, there were no significant differences, but in laboratory findings, the peripheral lymphocyte count and CD4 lymphocyte count were significantly lower in patients with immunosuppressive treatment than in those with other underlying diseases. There were 31 patients with bed-ridden status (12.3%) and there were no significant differences between individual groups with any underlying disease.

Among the 252 immunocompromised patients who underwent the QFT-TB test, 32 (12.6%) patients had an indeterminate result. All of these 32 showed a positive-control failure of the QFT-TB result (IFN-γ: <0.5 IU/ml). On univariate analysis (not shown in this article), patients undergoing immunosuppressive treatments had the highest and most significant proportion of indeterminate QFT-TB results (odds ratio 3.64, p=0.0008) compared with other patients with immunocompromised diseases (malignant diseases (odds ratio 2.28, p=0.201), diabetes mellitus (odds ratio 1.38, p=0.780), chronic renal failure (odds ratio 1.45, p=0.695) and HIV infection). On multivariate analysis (Table 2), patients undergoing immunosuppressive treatments also had similar and the highest and most significant proportion of indeterminate QFT-TB results. The QFT-TB test produced a significantly higher proportion of indeterminate results in patients with a negative TST result (24 of 150, 16.0%), compared with that in TST-positive patients (8 of 102, 7.8%) ($p<0.05$). The distribution between the TST results and the QFT-TB test results in all immunocompromised patients is shown in Table 3. Indeterminate QFT-TB test results were significantly more frequent in patients with a TST negative result than in those with a TST positive result ($p<0.05$).

Among all patients tested with the QFT-TB test and the TST, 32 patients (12.6%) were diagnosed with TB disease because they were culture positive for MTB. The QFT-TB test and TST results of these patients are shown in Table 4. The positive response rate for QFT-TB test of 32 patients with TB disease (25 of 32, 78.1%) was significantly higher than that for TST (16 of 32, 50.0%) ($p<0.05$).

The concordance rate between the QFT-TB test and the TST was 59.5% (150 of 252) with a p-value of 0.56 (95% CI 0.32-0.68). Sixty-four patients (25.4%) had a positive TST result and a negative QFT-TB test result; of those, 52 (81.3%) were BCG vaccinated, 10 (15.6%) had a non-tuberculous mycobacterial (NTM) disease and the remaining two were unknown. Six patients (2.4%) had a positive QFT-TB result and a negative TST result. Although none of these patients had been BCG vaccinated, five of six had a past history of TB disease and one was unknown. One hundred fifty two of 252 patients (60.8%) on whom both tests were performed had been BCG vaccinated. In this subgroup, TST was positive in 96 (63.2%) and QFT-TB test was positive in 30 (19.7%) ($p<0.05$). The concordance rate between the TST and the QFT-TB test was significantly lower among BCG-vaccinated individuals than among non-BCG-vaccinated subjects (27.8% versus 62.0%) ($p<0.05$).

Five patients (15.6%) among 32 with a QFT-TB indeterminate response appeared to have TB disease. Twenty-five patients among 36 with a QFT-TB-positive response (69.4%) appeared to have TB disease. Two patients among 184 with a QFT-TB-negative response appeared to have TB disease. While 16 patients (15.6%) appeared to have TB disease among 102 with a TST-positive response, 16 patients (10.7%) among 150 with a TST-negative response also appeared to have TB disease. The incidence of TB disease was significantly higher in the QFT-TB-positive response group than in TST-positive response group ($p<0.05$).

DISCUSSION

In Japan, most patients receiving immunosuppressive treatment for systemic underlying diseases have a past history of BCG vaccination. However, it has been noted that the sensitivity of TST is not high in immunosuppressed patients in whom the risk of progression to TB is high [3]. In place of the TST test in vivo, the QFT-TB test in vitro was first used commercially for patients with TB infection in Japan in April 2005 because the QFT-TB test demonstrated a negative result for BCG-vaccinated individuals and a negative result for most nontuberculosis mycobacteria (NTM) [2, 15]. Therefore, we prospectively investigated the results of QFT-TB test being routinely performed on consecutive immunocompromised patients in several community hospitals. Although we separated the patients by individual systemic underlying diseases (malignant diseases including leukemia, those receiving immunosuppressive treatment, diabetes mellitus, chronic renal failure and HIV infection) that induce immunosuppression, there were no significant differences among the clinical characteristics (Table 1). However, while we could not detect significant differences between other immunosuppressive patients in the

laboratory findings, except for those in several patients with HIV infection, patients receiving immunosuppressive treatment showed significantly lower lymphocyte and CD4 lymphocyte counts than other immunocompromised patients.

Regarding the specificity and sensitivity of the QFT-TB test, Mori et al [6] reported a sensitivity of 89% in a selected population of patients with clinical signs suggestive of TB infection. They also reported a specificity of 98% in low-risk subjects who had been vaccinated with BCG and who were assumed to be truly free of TB. However, in the present study, the positive response rate for the QFT-TB test was relatively low; 78.1% (25 of 32 patients with TB disease). An indeterminate result for the QFT-TB test was recognized in 12.6% (32 of 252 immunocompromised patients) and it appeared most frequently in patients receiving immunosuppressive treatments who presented with lymphocytopenia (especially CD4 lymphocytopenia). In previous reports, Ferrara et al noted that the QFT-TB-positive control failed in 21% of tests performed in routine clinical diagnostic microbiology laboratories and community-based contact tracing protocols and that these indeterminate results were significantly overrepresented among patients receiving immunosuppressive treatments [9]. A similar result (indeterminate rate: 11%) has been confirmed in a prospective study on 393 individuals [16]. However, there has not been any speculation concerning the reason. In our study, a similar result was obtained and all patients showed QFT-TB-positive control failure (IFN-γ: <0.5 IU/ml) although the indeterminate result of the QFT-TB test was 12.7% similar to Ferrara's data. One reason was that lymphocytopenia (especially CD4 lymphocytopenia) predominantly appeared in patients with immunosuppressive treatment [17] (Table 1). Although the QFT-TB test depends on the elaboration of inflammatory cytokines by T cells previously sensitized to MTB-specific antigens. In the blood, mononuclear cells from peripheral blood are stimulated in vitro, and the production of IFN-γ from sensitized T lymphocytes by MTB-specific antigen is measured by ELISA in the QFT-TB test [18]. Therefore, we speculated that lymphocytopenia caused decreased production of IFN-γ and lower mitogen, ESAT-6, or CFP-10 QFT-TB levels. Secondly, immunosuppressive drugs such as corticosteroid drugs directly reduce the production of inflammatory cytokines such as IFN-γ, IL-1 and TNF-α from T lymphocytes [13,19,20]. Finally, the decrease of IFN-γ induced indeterminate results of the QFT-TB test because of the lower mitogen, ESAT-6 or CFP-10 QFT-TB levels. For these reasons, indeterminate results due to positive control failure seem to be less frequent with the T SPOT TB, which detects individual T cells producing IFN-γ using an enzyme-linked immunospot assay (ELISPOT) than the QFT-TB test [10,16,21]. This also applies to the use of the T SPOT TB in subgroups with impaired cellular immunity, with a recent report that HIV-infected subjects tested with

the T SPOT TB showing only one (3%) indeterminate result, and positive control responses were not adversely affected by CD4 counts [22]. Otherwise, new IFN-γ release assay including MTB-specific antigen (TB 7.7) (QuantiFERON TB-Gold In-Tube (Cellestis Ltd, Carnegie, Australia)) [23] has recently been developed and will replace the QFT-TB 2G test in Japan as soon as possible. We had to examine immunocompromised patients with suspected of TB infection using the QFT-TB test in this study because only this test is currently available commercially in Japan. Hereafter, although Liebeschuetz et al had reported the results of ELISPOT test for immunosuppressed populations with HIV suspected of TB disease [24], we would like to carry out a prospective large scale study of immunocompromised patients with various severe underlying diseases other than HIV infection in a community hospital-based population with suspected TB infection using the T SPOT TB test instead of the QFT-TB test.

Regarding the comparison of TST and QFT-TB test results, our findings support the conclusion that QFT-TB test provides more accurate results than TST in immunocompromised patients [25]. Our findings also suggest that the QFT-TB test might have limited clinical usefulness in patients receiving immunosuppressive treatment. However, in most patients, the QFT-TB test produced a valid result although this IFN-γ blood test may raise some questions regarding the validity of a negative TST result. Namely, because TST does not have an internal positive control, the clinician cannot distinguish between a true negative result and a false-negative result. With the QFT-TB test, a proportion of false-negative test results will be scored as indeterminate, allowing the clinician to disregard such results. Naturally, there would still be a certain proportion of false-negative test results associated with a valid positive control, as we also observed. We think that the reason the discordances between TST and QFT-TB test can be mostly explained by BCG vaccination or NTM infection causing the result of TST to be positive and that of QFT-TB test to be negative and by a past history of TB infection causing the result of TST to be negative and that of QFT-TB test to be positive based on the results of this study.

In conclusion, this study demonstrated the clinical utility of the QFT-TB test compared to the TST for immunocompromised patients with TB infection. However, because the QFT-TB test result showed an indeterminate response for patients receiving immunosuppressive treatment, especially with lymphocytopenia due to severe underlying diseases, care must be taken when making a diagnosis of TB for these patients based on QFT-TB test results. In the future, we would like to increase the diagnostic rate of TB infection (TB disease or LTBI) for immunocompromised patients with underlying diseases in a community hospital-based population with suspected of TB infection by

adding other IFN-γ detecting methods (T-SPOT.TB or QuantiFERON-TB Gold In-Tube).

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Table 1 : Clinical characteristics and laboratory findings of immunocompromised patients who were suspected of TB infection (n=252)

Underlying disease / Characteristics	Malignant disease + (n=74)	Immunosuppressive treatment $ (n=72)	DM (n=52)	CRF (n=50)	HIV infection (n=4)	Total (n=252)
Age (year ± S.D.)	63.4 ± 10.8	60.5 ± 10.4	68.2 ± 11.2	64.6 ± 10.8	42.0	62.0 ± 10.4
Male / Female	48 / 26	37 / 35	35 / 17	32 / 18	4 / 0	156 / 96
Smoker	50 (67.6%)	43 (59.7%)	36 (69.2%)	33 (66.0%)	3 (75.0%)	165 (65.5%)
Alcohol abuse	13 (17.6%)	9 (12.5%)	10 (19.2%)	8 (16.0%)	0	40 (15.9%)
Past history of TB	7 (9.5%)	5 (6.9%)	6 (11.5%)	6 (12.0%)	0	24 (9.5%)
Bed ridden status	9 (12.2%)	9 (12.5%)	6 (11.5%)	6 (12.0%)	1 (25.0%)	31 (12.3%)
BCG vaccination	45 (60.8%)	43 (59.7%)	31 (59.6%)	30 (60.0%)	3 (75.0%)	152 (60.3%)
WBC count (cells/μl ± S.D.)	3476 ± 485	2890 ± 564	3762 ± 570	3702 ± 546	2960	3480 ± 504
Lymphocyte count (cells/μl ± S.D.)	502 ± 52	380 ± 36*	576 ± 58	522 ± 54	296	496 ± 50
CD4 lymphocyte (cells/μl ± S.D.)	208 ± 31	114 ± 29*	220 ± 36	212 ± 32	40	196 ± 32
TP (g/dl ± S.D.)	6.4 ± 1.4	6.6 ± 1.6	6.8 ± 1.5	6.8 ± 1.5	6.8	6.7 ± 1.5
Alb (g/dl ± S.D.)	3.3 ± 0.7	3.5 ± 0.8	3.6 ± 0.8	3.6 ± 0.8	3.6	3.5 ± 0.8
r-Glb (g/dl ± S.D.)	1.1 ± 0.3	1.4 ± 0.4	1.2 ± 0.3	1.3 ± 0.3	1.4	1.2 ± 0.3
TB disease	10 (13.5%)	9 (12.5%)	7 (13.5%)	6 (12.0%)	0	32 (12.6%)

Note. CRF : Chronic renal failure, DM : Diabetes mellitus, BCG : Bacille Calmette–Guerin, WBC : White blood cell, TP : Total protein Alb : Albumin, r-Glb : r-globurin,

* $p < 0.05$

+ Patients with a diagnosis of malignant disease who were receiving anti-cancer therapy (n=24) and who were not receiving anti-cancer therapy (n=50) (including 12 patients with leukemia)

$ Patients receiving the systemic steroid prednisone (n=52), and anti-tumor necrosis factor alpha agents (n=10) at the time of testing with QFT-TB, and the systemic steroid prednisone plus other immunosuppressive drugs

Table 2 : Multivariate analysis of the indeterminate QFT-TB results

Underlying Disease	QFT-TB		OR (95%CI)	p value
	Indeterminate (%)[#]	Determinate (%)		
Malignant disease[†] (n=74)	8 (10.8)	66 (89.2)	2.19 (0.75 – 4.88)	0.201
Immunosuppressive$ treatment (n=72)	20 (27.8)	52 (72.2)	3.80 (2.10 – 9.54)	0.0006
DM (n=52)	2 (3.8)	50 (96.2)	1.48 (0.65 – 2.60)	0.780
CRF (n=50)	2 (4.0)	48 (96.0)	1.50 (0.68 – 2.69)	0.712
HIV infection (n=4)	0	4 (100.0)	N.D.	N.D.
Total (n=252)	32 (12.6)	220 (87.4)		

Note. 95%CI : 95% confidence interval, OR : Odds ratio, QFT-TB : QuantiFERON TB-2G, N.D. : Not done, DM : Diabetes mellitus, CRF : Chronic renal failure

[#] All indeterminate QFT-TB results were due to a low response to the mitogen PHA in the positive control well, according to the manufacturer's instructions.

[†] Patients with a diagnosis of malignant disease who were receiving anti-cancer therapy (n=18) and who were not receiving anti-cancer therapy (n=56)

$ Patients receiving the systemic steroid prednisone (n=52), and anti-tumor necrosis factor alpha agents (n=10) at the time of testing with QFT-TB, and the systemic steroid pnednisone plus other immunosuppressive drugs (n=10)

Table 3 : QFT-TB and TST results for immunocompromised patients (n=252)

TST / QFT-TB	Positive	Negative	Total
Positive	30	6	36
Negative	64	120	184
Indeterminate	8	24	32
Total	102	150	252

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

Table 4 : QFT-TB and TST results for immunocompromised patients with TB disease (n=32)

TST / QFT-TB	Positive	Negative	Total
Positive	15	10	25
Negative	0	2	2
Indeterminate	1	4	5
Total	16	16	32

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