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ORIGINAL ARTICLE

QuantiFERON TB-2G test for patients with active tuberculosis stratified by age groups

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Abstract
We evaluated the usefulness of the QuantiFERON TB-2G (QFT-2G) test and the tuberculin skin test (TST) in patients with active tuberculosis (TB) disease stratified by age in 10-year increments. Although the positive rate on TST was over 80% in younger patients aged ≤9 years, it decreased to 55% in patients of aged 70–79 years and 33% in patients aged over 80 years. However, the positive rate on QFT-2G test was over 80% for the age groups between 10–19 and 60–69 years, excluding younger patients aged ≤9 years. Furthermore, the rate was 79% in patients aged 70–79 years and 75% in patients over 80 years of age. The positive response rate of the QFT-2G test was significantly higher than that of the TST in patients over 50 years of age. The indeterminate result of the QFT-2G test increased with age and it is suggested that this result is concerned with the severity of underlying diseases in patients with active TB disease. Although the positive rate on QFT-2G test decreased with age in adults, it is thought to be a useful supportive diagnostic method for active TB disease compared to the TST, except in younger patients aged ≤9 years old.

Introduction

The availability of Mycobacterium tuberculosis (MTB) antigen-specific interferon (IFN)-γ release assays (IGRAs) has been a significant advance in the field of tuberculosis (TB) diagnosis [1–4]. Two commercial IFN-γ release assays have become available in the last few years: the QuantiFERON TB-Gold (QFT-G) assay (Cellestis Ltd, Carnegie, Victoria, Australia) with an enzyme-linked immunosorbent assay used to measure IFN-γ concentrations in supernatants, and T-SPOT.TB (Oxford Immunotec, Oxford, UK) with an enzyme-linked immunospot assay used to detect individual T cells producing IFN-γ [5,6]. These tests demonstrate a positive result for most patients with active TB disease. Of these tests, although the QuantiFERON TB-2G (QFT-2G) test was first used commercially in Japan in April 2005 for the diagnosis of TB, the T-SPOT.TB test and QuantiFERON TB-Gold In-Tube (QFT Gold In-Tube) test have not yet been approved for use in Japan. Therefore, in this study we performed QFT-2G testing for patients with active TB disease and compared the findings with those of the tuberculin skin test (TST).

Although there have been many reports [7–10] on the clinical usefulness of the QFT-2G test for patients with TB infection or latent TB infection (LTBI), it has been reported that care is needed when TB infection is diagnosed in the elderly or paediatric patients (<5 years old). Recently, several reports on the usefulness of IGRAs for elderly patients and young paediatric patients have been published [11–13]. However, there have been few studies performing a detailed analysis of clinical effectiveness based on age stratification of patients with active TB disease. Therefore, we evaluated the TST and QFT-2G test responses in patients with active TB disease stratified by 10-year increments to determine whether the QFT-2G test result in

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individual age groups is feasible for diagnosing TB infection.

Materials and methods

Two hundred seventy patients with active TB disease, confirmed by positive results of cultures of sputum, bronchoalveolar lavage fluid (BALF), pleural fluid, peritoneal fluid or lymph node tissue samples, were prospectively enrolled between January 2005 and December 2008. This study was approved by the ethics committee of each institution. The patients with active TB disease received their diagnosis at Kawasaki Medical School Hospital (1072 beds), Kawasaki Medical School Kawasaki Hospital (650 beds), Kurashiki Central Hospital (1570 beds), Kurashiki Daiichi Hospital (192 beds) and Asahigaoka Hospital (90 beds). We obtained written informed consent from all patients in this study. All patients demonstrated either negative response on serological tests for HIV or an absence of obvious risk factors for HIV infection. Subjects were stratified by age in 10-year increments (0–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79 and 80– years), forming 9 subgroups. From patients in individual groups with active TB disease, we collected demographic data regarding (1) history of previous TB infection or anti-tuberculous treatment, (2) other underlying diseases (i.e. respiratory disease such as healed pulmonary TB or chronic obstructive pulmonary disease (COPD), and non-respiratory diseases such as malignant disease including leukaemia, diabetes mellitus, renal failure with haemodialysis, collagen vascular disease, neurological disease, gastrointestinal disease, cardiovascular disease, and HIV infection), and (3) the receipt of immunosuppressive drugs within 3 months before enrolment into this study. Data regarding any previous Mantoux TST results and BCG vaccination inoculation, as well as data on laboratory findings (i.e. white blood cell count, lymphocyte count, total protein concentration, and albumin concentration) and radiological findings (i.e., portion, extension of lesion and cavity) were collected at the time of enrolment. Sputum or other appropriate respiratory samples were collected from all patients and culture samples were obtained for the detection of mycobacteria.

Sample collection and TST

Each patient in each age group had a heparinized blood sample collected by venipuncture for performing a whole-blood IFN-γ assay. Blood samples were collected before the administration of the Mantoux TST. For TST, 0.1 ml of tuberculin purified protein derivative (Nippon BCG Manufacturing, Tokyo, Japan) (equivalent to 3 tuberculin units of purified protein derivative solution) was injected intradermally into the volar aspect of the forearm and the transverse induration diameter was measured 48 h later. The results of the test were interpreted by hospital staff based on the patient's degree of risk, according to current guidelines [14]; a lower cut-off of 5 mm for a positive test result was used for each patient.

QFT-2G test

The QFT-2G test was performed according to the recommendations of the manufacturer and the test result was judged according to the guidelines of the Centers for Disease Control and Prevention [15]. The test result was considered positive if the IFN-γ level in the sample well after stimulation with ESAT-6 and/or CFP-10 was $\geq$0.35 IU/ml, irrespective of the result for the positive control well. The test was considered negative if the IFN-γ level was $<$0.35 IU/ml and the IFN-γ level of the positive control well was $\geq$0.5 IU/ml. The test result was considered indeterminate if the IFN-γ level was $<$0.35 IU/ml in both antigen wells and $<$0.5 IU/ml in the positive control well.

Statistical analysis

Information from the questionnaires and TST and QFT-2G test results were subjected to statistical analyses. Statistical analyses were performed to assess each of the 9 age groups for the following: (1) the feasibility and performance of the QFT-2G test compared with those of TST, (2) the proportion of QFT-2G tests with a positive result and the associated factors, (3) the concordance and discordance between TST and QFT-2G test results, and (4) a comparison of positive and indeterminate response rate between TST and QFT-2G test. The analysis of concordance between TST and QFT-2G test results was calculated using the κ-value. TST and QFT-2G test results were compared by Chi-square test. The Wilson score method was used to calculate 95% confidence intervals for the positive response rate of both tests [16].

Results

The clinical characteristics and laboratory findings of patients with active TB disease stratified into 10-year increments are shown in Table I. While there were many elderly patients aged over 80 years (48 patients), there were 10 patients aged $\leq$9 years. With regard to underlying diseases, smoking history

Table I. Clinical characteristics and laboratory findings of patients with active TB disease stratified by age in 10-year increments.

	Age									
Characteristic	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–	Total
Subjects	10	15	25	27	36	39	37	33	48	270
Male/female	6/4	10/5	18/7	19/8	28/8	30/9	29/8	26/7	39/9	205/65
Smoker	0	0	14 (56)	18 (67)	26 (72)	28 (72)	28 (76)	25 (76)	37 (77)	176 (65)
Alcohol abuse	0	0	1 (4)	5 (19)	9 (25)	11 (28)	10 (27)	7 (21)	5 (10)	48 (18)
Underlying disease	2 (20)	2 (13)	8 (32)	12 (44)	21 (53)	28 (72)	31 (84)	30 (91)	46 (96)	180 (67)
Respiratory disease	0	0	3 (12)	5 (19)	9 (25)	13 (33)	15 (41)	16 (48)	23 (48)	84 (31)
Non-respiratory disease	2 (20)	2 (13)	5 (20)	7 (25)	12 (33)	15 (39)	16 (43)	14 (43)	23 (48)	97 (36)
Immunosuppressive treatment[a]	1 (10)	0	0	3 (11)	4 (11)	5 (13)	6 (16)	5 (15)	6 (13)	30 (11)
History of BCG vaccination	5 (50)	8 (53)	16 (64)	18 (67)	23 (64)	24 (62)	23 (62)	21 (64)	29 (60)	167 (62)
Laboratory finding										
WBC count (cells/μl)	4018±510	4023±526	3996±516	4011±528	3972±520	3855±526	3774±508	3610±502	3325±486	3860±501
Lymphocyte count (cells/μl)	1460±125	1353±104	1072±85	707±61	701±60	680±57	635±58	576±52	552±50	684±56
Total protein (g/dl)	6.9±1.4	7.0±1.5	6.9±1.4	6.9±1.5	6.8±1.3	6.6±1.2	6.5±1.2	6.3±1.2	6.2±1.1	6.6±1.2
Albumin (g/dl)	3.6±0.8	3.7±0.9	3.6±0.8	3.6±0.9	3.6±0.7	3.4±0.7	3.3±0.7	3.1±0.6	2.9±0.6	3.4±0.7

TB, tuberculosis; BCG, bacille Calmette–Guérin; WBC, white blood cell.
Values are given as the mean±standard deviation or *n* (%), unless otherwise indicated.
[a]All patients received corticosteroid therapy.

and alcohol abuse, the frequency increased with age. However, there were no significant differences between age groups with regard to gender, immunosuppressive treatment and history of bacille Calmette–Guérin (BCG) vaccination. Concerning laboratory findings, white blood cell count, lymphocyte count, total protein and albumin were significantly decreased in the older compared to the younger age groups. Lymphocyte counts in particular were below the normal range (<600 cells/μl) in those over 70 years old.

Comparison of the results of the TST and QFT-2G test among patients with active TB disease stratified into 10-year age groups is shown in Table II. The positive response rate of the QFT-2G test was higher than that of the TST in all age groups except those aged ≤ 9 years. In particular, the positive response rate of the QFT-2G test (75%) was significantly higher than that of the TST (33%). With regard to the false-negative response rate and indeterminate response rate of the QFT-2G test, although there were no significant differences among the age groups overall, both rates were significantly higher in younger patients aged ≤ 9 years and elderly patients aged >80 years compared to the other age groups in the middle range. The overall positive test response rate for both the TST and QFT-2G test in all patients was 61%, and it decreased with age. Among 270 patients with active TB disease, while 65 patients showed positive results on QFT-2G test despite negative results on TST, 5 patients demonstrated false-negative results on QFT-2G test despite positive results on TST.

Regarding the comparison of results of QFT-2G test analyzed by combined and separate responses to ESAT-6 and CFP-10 antigens among patients with active TB disease stratified into 10-year age groups, the positive response rate of both ESAT-6 and CFP-10 antigens was significantly lower than that of either ESAT-6 antigen only or CFP-10 antigen only in patients aged ≤ 9 years and in elderly patients aged >80 years. However, there were no significant differences in the positive response rate for ESAT-6 antigen only, CFP-10 antigen only and both ESAT-6 and CFP-10 antigens in the other age groups.

Discussion

Although problems about the sensitivity of the QFT-2G test in paediatric patients (<5 years old) and elderly patients have been noted previously, it has recently been reported that this test is useful for diagnosing TB disease in young children [11,12] and elderly patients over 80 years of age [13]. However, because only a few studies have performed a detailed investigation of the clinical effectiveness based on age stratification of patients with active TB disease, we evaluated the TST and QFT-2G test response in patients with active TB disease stratified into 10-year age groups. Consequently, the QFT-2G test was shown to be a useful supportive diagnostic method for TB disease compared with the TST, except in patients ≤ 9 years old. In particular, the positive response rate of the QFT-2G test was significantly higher than that of the TST in elderly patients.

Concerning the positive response rate of the QFT-2G test and TST stratified into age groups, Mori et al. [7] reported that that of the QFT-2G test was 80% (8/10 patients) and that of the TST was 16.7% (1/6 patients) in elderly patients over 80 years old with active TB disease. However, this study had a small-scale population of elderly patients with active TB disease. In our study, 48 elderly patients over 80 years of age were enrolled and the positive response rate of the QFT-2G test was significantly higher (75%) than that of TST (33%). We believe that the reason why the positive response rate of the QFT-2G test decreased with age is concerned with

Table II. Comparison of the results of TST and QFT-2G test among patients with active TB disease stratified by age in 10-year increments.

	Age									
Test result	0–9 ($n=10$)	10–19 ($n=15$)	20–29 ($n=25$)	30–39 ($n=27$)	40–49 ($n=36$)	50–59 ($n=39$)	60–69 ($n=37$)	70–79 ($n=33$)	80– ($n=48$)	Total ($n=270$)
TST										
Positive	8 (80)	12 (80)	20 (80)	21 (78)	27 (75)	25 (64)	22 (59)	18 (55)	16 (33)	169 (63)
Negative	2 (20)	3 (20)	5 (20)	6 (22)	9 (25)	14 (36)	15 (41)	15 (45)	32 (67)	101 (37)
QFT-2G test										
Positive	7 (70)	13 (87)	23 (92)	25 (92)	33 (92)	34 (87)	32 (86)	26 (79)	36 (75)	229 (85)
Negative	1 (10)	1 (7)	0	1 (4)	0	2 (5)	1 (2)	2 (6)	3 (6)	11 (4)
Indeterminate	2 (20)	1 (7)	2 (8)	1 (4)	3 (8)	3 (8)	4 (11)	5 (14)	9 (19)	30 (11)
Both TST and QFT-2G test positive	7 (70)	11 (80)	20 (80)	20 (80)	27 (75)	23 (59)	22 (59)	18 (55)	16 (33)	164 (61)

TST, tuberculin skin test; QFT-2G, QuantiFERON TB-2G; TB, tuberculosis.
Values are given as the number (%), unless otherwise indicated.

lymphocytopaenia and/or hyponutrition state such as hypoproteinaemia and hypoalbuminaemia, as we have described in a previous report [13] (Table I). We used the QFT-2G test as an IGRA in this study because this test is currently the only IGRA commercially available in Japan. If lymphocytopaenia existed in patients with active TB disease, the QFT-2G test may have shown false-negative or indeterminate results because the lymphocyte count affects this diagnostic method.

Regarding the positive response rate of the QFT-2G test and TST in patients $\leq$9 years old, the positive response rate of the TST (80%) was slightly higher than that of the QFT-2G test (70%). The reason why the positive response rate of the QFT-2G test was lower than that in the other age groups was that IGRAs may revert to negative following clearance of TB infection [15] and the T lymphocytes of younger patients cannot produce sufficient IFN-γ after stimulation with Mycobacterium tuberculosis (MTB)-specific antigens because of their immature characteristics, despite preservation of lymphocyte counts and/or nutritional state in this age group. On the other hand, 5 patients demonstrated a false-negative response on QFT-2G test despite positive results on TST in this study. Although 2 of 5 patients were younger patients below 15 years of age, the remaining 3 patients were adult patients. They also had no underlying diseases and showed normal lymphocyte counts and normal nutritional status. The reason for the presenting false-negative results on QFT-2G test in the 3 adult patients may be speculated to be because of the possibility that mature lymphocytes cannot produce IFN-γ for MTB-specific antigens.

Regarding the combined and separate responses to ESAT-6 and CFP-10 antigens in the QFT-2G test, the positive response rate for both antigens in elderly patients $>$80 years old and younger patients $\leq$9 years old was significantly lower than that of those in the intermediate age groups (20–59 years old) in this study. As the reason underlying the lower positive response rate for both antigens, it was suspected that the dose of IFN-γ produced by MTB-specific antigens (ESAT-6 and/or CFP-10 protein) decreases functionally with aging in these 2 groups.

There are a few limitations in this study. Firstly, although there was an adequate sample for statistical analysis among the age groups, there were not very many patients aged $\leq$9 years with active TB disease (10 patients) compared to the numbers in the other age groups in this study. Secondly, we had to use the QFT-2G test as an IGRA in this study because the QFT-2G test is currently the only IGRA commercially available in Japan. We should also investigate newer commercial IGRAs such as T-SPOT.TB (Oxford-Immunotec) or QuantiFERON-TB Gold In Tube (Cellestis) to determine the sensitivity for patients aged $\leq$9 years and those aged $>$80 years.

In conclusion, we have demonstrated that the QFT-2G test is a feasible supportive diagnostic method compared with the TST for adult patients with active TB disease, but excluding patients $\leq$9 years old based on the present findings. However, both the QFT-2G test and TST may be performed for patients with active TB disease $\leq$9 years old. In the future, a large-scale study should be performed using the newer IGRA tests for a wide range of patients with active TB disease including patients aged $\leq$9 years and those aged $>$80 years.

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