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Clinical Evaluation of Two Interferon-Gamma Release Assays for Diagnostic Tests of Tuberculosis Infection in a Tertiary Hospital: Clinical Evaluation of Two IGRAs for TB Infection

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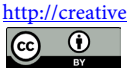

Abstract

Background: The aim of this study was to evaluate the usefulness of two interferon-gamma release assays (IGRAs) (QuantiFERON-TB Plus (QFT-plus) and T-SPOT.TB assay) for patients suspected of having tuberculosis (TB) infection as supportive methods for diagnosing TB. Patients and Methods: The subjects consisted of 45 patients who required clinical differentiation of TB disease from June 2019 to August 2023. The final clinical diagnoses were: 14 patients with active TB disease, 4 with latent TB infection (LTBI), 17 with old (cured) TB disease, and 10 with pulmonary nontuberculous mycobacterial (NTM) disease. We used the two IGRAs for these patients and evaluated the data according to the manufacturer's guidelines for interpretation or FDA-approved cut-offs. Results: Among the total of 14 patients with active TB disease (mean age: 64 years old, male: 9, and female: 5), a positive response was noted in 10 patients (71%) on QFT-plus and 9 (64%) on T-SPOT.TB. Four patients with a negative response on QFT-plus and T-SPOT.TB were elderly or cancer patients with lymphocytopenia or hypoalbuminemia. All four patients with LTBI showed a positive response (100%) on both QFT-plus and T-SPOT.TB. Among the seventeen patients with old (cured) TB disease, a positive response was noted in 8 patients (47%) on QFT-plus and 9 (53%) on T-SPOT.TB. All patients with pulmonary NTM disease showed a negative response on both QFT-plus and T-SPOT.TB. Conclusions: A false-negative response on QFT-plus as well as T-SPOT.TB was recognized in elderly patients and patients with an immunosuppressed condition, and half of patients with old (cured) TB showed no negative conversion after the completion of treatment through this study. Although it was recently reported that the positive re-

sponse rate on QFT-plus of patients with active TB disease was high, we consider it necessary to be careful in diagnosing TB infection using IGRAs for patients with severe underlying diseases in a tertiary hospital based on the results.

Keywords

QFT-Plus, T-SPOT.TB, Diagnosis of Tuberculosis, Tertiary Hospital

1. Introduction

Two interferon-gamma release assays (IGRAs) are commonly used to detect TB infection in Japan: One is QuantiFERON-TB Plus (QFT-plus: QIAGEN, Hilden, Germany) and the other is T-SPOT.TB (T-SPOT: Oxford Immunotec, Abingdon, UK). QFT-plus is an enzyme-linked immunosorbent assay (ELISA)-based method and a fourth-generation QFT replacing QuantiFERON-TB Gold In-Tube (QFT-GIT). QFT-plus has TB1 and TB2 tubes. The TB2 tube can elicit IFN-γ production by both CD4 and CD8 lymphocyte responses [1] [2]. Therefore, it can broaden the immune response to the *Mycobacterium tuberculosis* (MTB) antigen, improving sensitivity to detect TB infection among immunocompromised patients with HIV, the elderly, and renal transplant recipients [3]-[5]. T-SPOT.TB is based on the enzyme-linked immunospot (ELISPOT) method, which measures the number of IFN-γ-producing T-cells stimulated by MTB-specific antigens.

Several studies have investigated the accuracy of IGRAs (QFT or T-SPOT.TB) for the diagnosis of TB infection. The frequency of indeterminate results of IGRAs in patients with active TB disease ranged from 1to 20% [6]-[9] and that of false-negative results ranged from 17 to 19% [10]-[12]. Moreover, because the production of IFN-γ is a part of the protective immune response against TB infection, it is difficult to understand the dynamics of the protective immune response against TB infection through IGRA results.

Because there have been few reports on the clinical evaluation of diagnostic tests for TB infection (IGRAs such as QFT or T-SPOT.TB) in tertiary hospitals that many elderly patients or patients receiving immunosuppressive treatment have visited, we prospectively elucidated the accuracy of IGRAs (QFT-plus and T-SPOT.TB) as diagnostic tests of TB infection in our tertiary hospital. We also compared the quantitative values of the IFN-γ response among patients with active TB disease, LTBI, old (cured) TB disease, and pulmonary nontuberculous mycobacterial (NTM) disease in order to confirm whether it is useful to differentiate active TB disease from other time-phases of TB infection.

2. Materials and Methods

1) Study population

Forty-five patients were prospectively enrolled from June 2019 to August 2023

in this study. They were diagnosed at Kawasaki Medical School Hospital (1070 beds, tertiary medical institution with emergency ward) from June 2019 and consisted of 14 patients with active TB disease (MTB smear and/or culture-positive results or PCR-positive for MTB from any clinical specimens), 4 patients with LTBI (they had a contact history with active TB patients but no clinical symptoms or abnormal findings on CT), 17 patients with old (cured) TB disease (they had a past history of TB disease and received antituberculous treatment within 1 to 30 years (average: 6.9 years)), and 10 patients with pulmonary NTM disease (causative microorganisms: *Mycobacterium avium* 5 and *Mycobacterium intracellulare*) (they satisfied the diagnostic criteria proposed by the American Thoracic Society (ATS) [13]). As inclusion criteria, all patients except those with LTBI required the differentiation of pulmonary TB clinically. Patients with an obvious history of TB disease were excluded from patients with LTBI and pulmonary NTM disease to avoid the influence on the results of IGRAs.

This study was approved by the Ethical Committee of Kawasaki Medical School (No. 3520-01) and performed in accordance with the Declaration of Helsinki Informed consent was obtained from all patients in this study.

2) IGRAs (QFT-plus and T-SPOT.TB)

Blood samples for QFT-plus and T-SPOT.TB were collected at the same time. QFT-plus assays were conducted according to the manufacturer's instructions and interpreted according to the manufacturer's guidelines [14]. T-SPOT.TB samples were transported to a commercial laboratory (SRL, Ltd., Tokyo, Japan) within 32 hours following phlebotomy. After arrival, T-cell Xtend reagent was added to the samples and the assay was performed according to the manufacturer's instructions [15]. The assay results were interpreted based on the US Food and Drug Administration-approved cutoffs: ≥8 spots were interpreted as a positive result, 5 - 7 as a borderline result, and ≤4 spots as a negative result. When the spot count in the positive control was <20 and/or the spot count in Nil was ≥10, the result was interpreted as invalid.

3) Statistical analysis

The data are reported as percentages for categorical variables (clinical findings and clinical evaluation of IGRAs) and as medians ± standard deviation (S.D.) or medians with interquartile ranges for continuous variables (clinical findings and the results of IGRAs of four groups). The chi-square test was used for categorical variables. The Mann-Whitney U test was carried out to calculate the difference between individual groups (active TB group, LTBI group, old (cured) Tb group, and NTM group) and group medians (>2) were compared using ANOVA. The positive response rate was defined as the number of positive cases as a percentage of the total number of cases tested excluding the indeterminate or invalid results, and borderline results were judged as negative. Statistical analysis was performed using Stat Flex version 7 software (Artec, Japan, 2020). A p-value of <0.05 was considered significant.

3. Results

The clinical characteristics of the study subjects are presented in Table 1. A total of 45 patients were enrolled during the study period. They consisted of 14 patients with active TB (active TB group), 4 with LTBI (LTBI group), 17 with old (cured) TB (old TB group), and 10 with pulmonary NTM disease (NTM group). There were no patients with human immunodeficiency virus (HIV) or receiving immunosuppressive treatment among the study subjects. In the LTBI group, IGRAs were performed from two to three months after contact screening with active TB patients. In the old TB group, the median period after the onset of active TB disease was 7.6 years (range: 1 - 30 years). The age was younger in the latent TB group than in the other groups. The patients in the active TB group showed significant lymphocytopenia, hypoproteinemia, and hypoalbuminemia in the laboratory findings compared with the other groups.

Clinical evaluation of QFT-plus and T-SPOT.TB excluding one case showed an indeterminate result of T-SPOT.TB, in Figure 1 and Figure 2. Positive responses to QFT-plus were noted in 10 of 14 patients (71%) in the active TB group, 4 of 4 patients (100%) in the latent TB group, 9 of 17 patients (53%) in

Table 1. Clinical characteristics of four groups of patients with active TB disease, latent TB infection, old TB disease, and nontuberculous mycobacterial disease.

Clinical findings	Active TB group (n = 14)	Latent TB group (n = 4)	Old TB group (n = 17)	NTM group (n = 10)
Age (Median ± S.D.)	70.0 ± 21.4	62.5 ± 24.6*	73.0 ± 16.0	73.0 ± 10.6
Sex (Male : Female)	9:5	2:2	13:4*	5:5
Smoking history (+)	7 (5%)	2 (50%)	14 (82%)	5 (50%)
Underlying disease (+) (with repetition)	11 (79%)	3 (75%)	17 (100%)	7 (70%)
Respiratory disease (+)	3 (21%)	1 (25%)	17 (100%)	1 (10%)
Healed tuberculosis	1	0	17	0
COPD	1	0	1	1
Others	2	1	6	0
Non-respiratory disease (+)	10 (71%)	3	11 (65%)	7 (70%)
Malignant disease	4	1	4	2
Diabetes mellitus	3	2	3	0
Renal disease	1	0	3	0
Others	4	3	4	6
Immunosuppressive treatment	0	0	0	0
Laboratory findings (Median ± S.D.)				
Lymphocytes (/ML)	1370 ± 681*	1630 ± 219	1550 ± 499	1580 ± 378
Total protein (g/dL)	6.6 ± 1.2*	6.9 ± 0.6	7.4 ± 0.6	7.1 ± 0.4
Albumin (g/dL)	3.3 ± 0.8*	4.1 ± 0.2	4.0 ± 1.0	3.8 ± 0.4

*<0.05; COPD: chronic obstructive pulmonary disease.

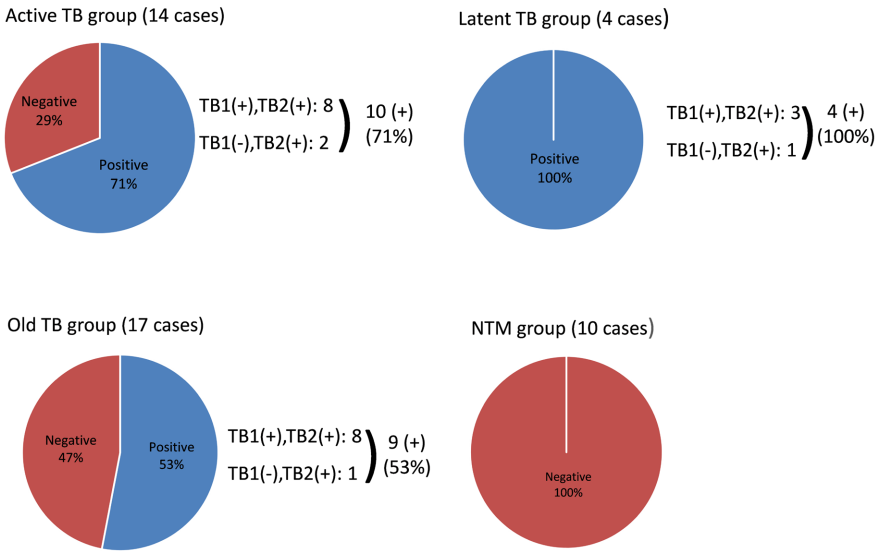

Figure 1. Clinical evaluation of QFT-plus for four groups of patients with active TB disease, latent TB infection, old TB disease, and nontuberculous mycobacterial disease.

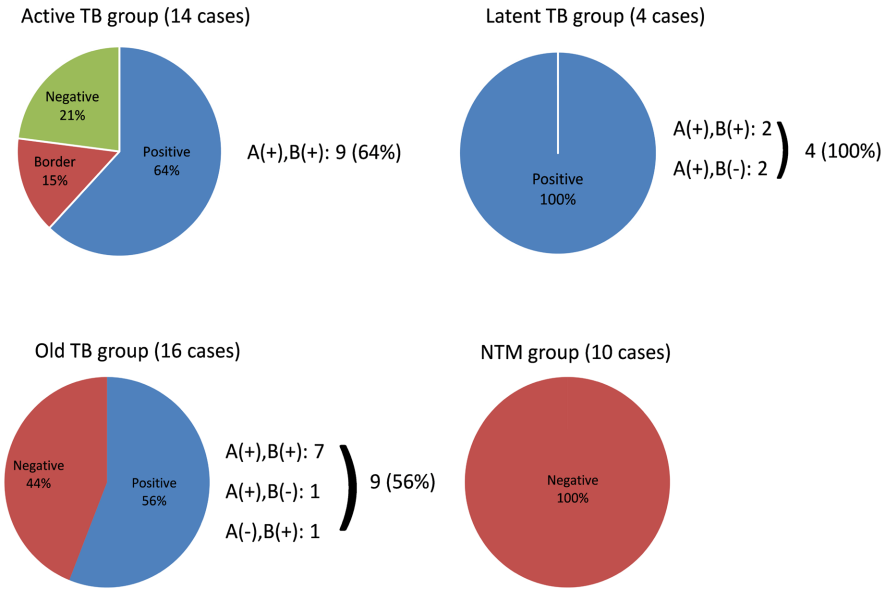

Figure 2. Clinical evaluation of T-SPOT.TB for four groups of patients with active TB disease, latent TB infection, old TB disease, and nontuberculous mycobacterial disease.

the old TB group, and 0 of 10 patients (0%) in the NTM group. On the other hand, positive responses to T-SPOT.TB were noted in 8 of 14 patients (64%) in the active TB group, 4 of 4 patients (100%) in the latent TB group, 9 of 16 patients (56%) in the old TB group, and 0 of 10 patients (0%) in the NTM group. Borderline results of T-SPOT.TB were noted in 2 of 14 patients (14%) in the active TB group.

The median levels of IFN-γ using QFT-plus were as follows (median ± S.D.): 0.7 ± 1.3 IU/mL inTB1 and 1.2 ± 1.6 IU/mL in TB2 of the active TB group, 1.2 ± 1.2 IU/mL in TB1 and 1.6 ± 1.6 IU/mL in TB2 of the latent TB group, 0.2 ± 2.4

IU/mL in TB1 and 0.4 ± 3.0 IU/mL in TB2 of the old TB group, and <0.05 IU/mL in TB1 and <0.05 IU/mL in TB2 of the NTM group. Although they were significantly lower in the NTM group than in the other three groups, there were no significant differences among the active TB, latent TB and old TB groups (Figure 3). Concerning the results of T-SPOT.TB, excluding one case that showed an indeterminate result, the median levels of T-SPOT.TB were as follows (median ± S.D.): 19.0 ± 26.7 spot in panel A and 8.0 ± 36.6 spot in panel B for the active TB group, 22.0 ± 10.6 spot in panel A and 9.5 ± 5.5 spot in panel B for the latent TB group, 14.0 ± 29.6 spot in panel A and 12.0 ± 72.0 spot in panel B for the old TB group, and 0 ± 0.7 spot in panel A and 0 ± 0.7 spot in panel B for the NTM group. Although they were significantly lower in the NTM group than in the other three groups, there were no significant differences among active TB, latent TB, and old TB groups (Figure 4).

Among the 14 patients with active TB disease, one patient showed discordant results between QFT-plus and T-SPOT. This patient showed a false-negative response to QFT-plus, and we present her clinical findings in Table 2. The clinical findings of four patients with active TB disease who showed false-negative results for both QFT-plus and T-SPOT.TB tests (two patients showed borderline results for T-SPOT.TB) are shown in Table 3 and Table 4. They were all elderly patients and had many underlying diseases including malignant diseases. They also showed severe lymphocytopenia and hypoalbuminemia due to the underlying diseases. The concordance rates of QFT-plus and T-SPOT.TB tests were 93%

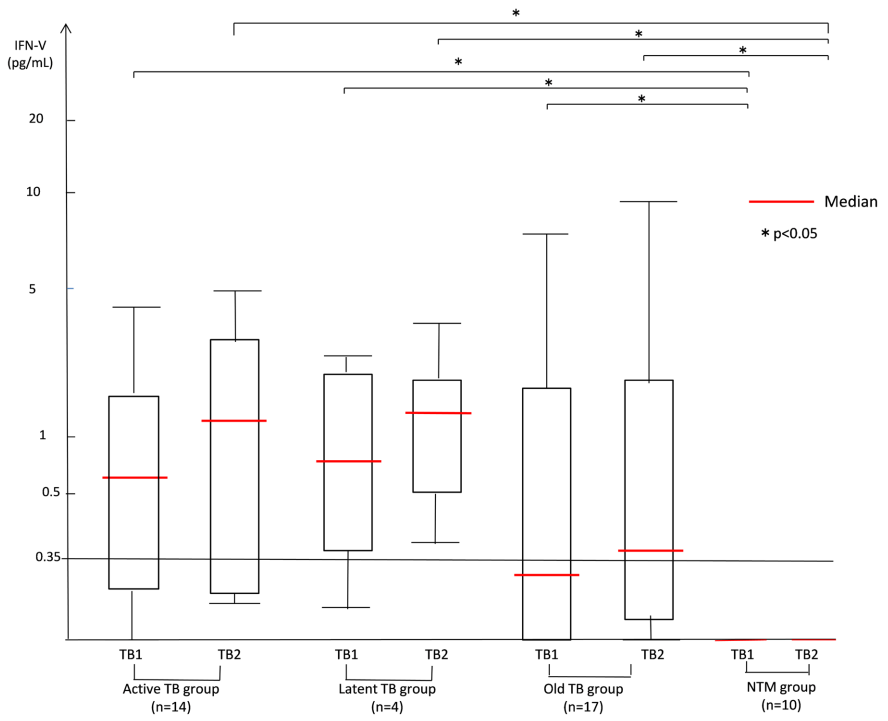

Figure 3. Comparison of the results of QFT-plus for four groups of patients with active TB disease, latent TB infection, old TB disease, and nontuberculous mycobacterial disease.

(13/14) in the active TB group, 100% (4/4) in the latent TB group, 88% (15/17) in the old TB group, and 100% (10/10) in the NTM group. There was good agreement (42/45; 93%) between QFT-plus and T-SPOT.TB test results among all patients included in this study.

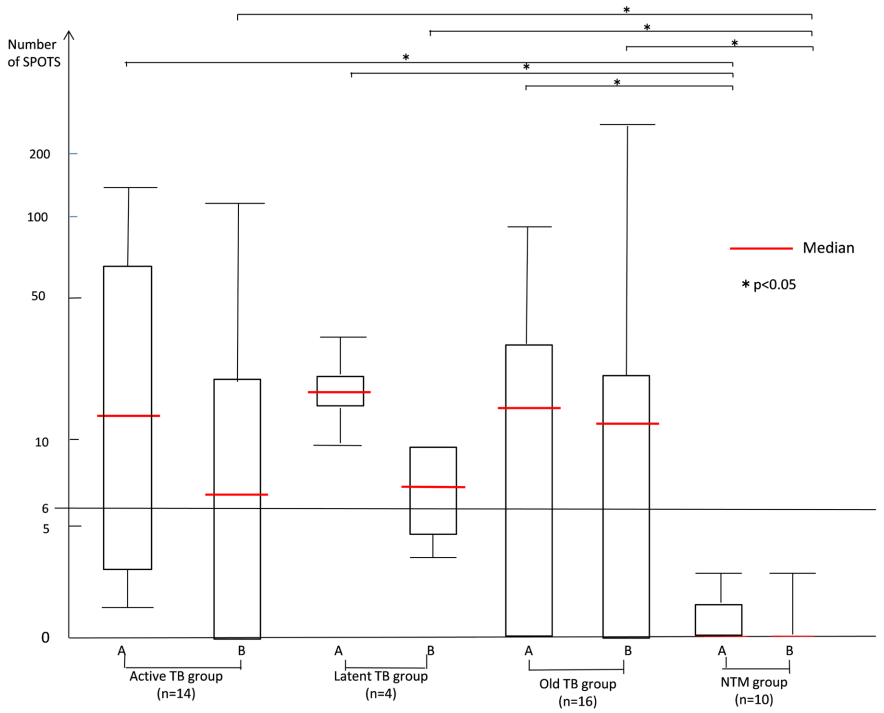

Figure 4. Comparison of the results of T-SPOT.TB excluding cases showing indeterminate results for four groups of patients with active TB disease, latent TB infection, old TB disease, and nontuberculous mycobacterial disease.

Table 2. Clinical findings of cases showing a false-negative response to only T-SPOT.TB in patients in the active TB group.

Case	Age, Sex	Smoking history	Underlying disease	Diagnosis on admission	Laboratory findings			
					QFT-plus	WBC (/uL)	Lymphocytes (/uL)	Total protein (g/dL)	Albumin (g/dL)
1	72, F	(−)	Diabetes mellitus	Pulmonary tuberculosis susp.	Positive TB1:0.41 TB2:0.60	4600	1440	6.5	3.0

Duration until definite diagnosis	Radiological findings (Portion, cavity, extension)	Diagnostic method	Microbiological findings				Final Diagnosis	Treatment Outcome	
			Smear	Culture	DDH	PCR			
1 week	Left Cavity (−) 1	BALF Puncture fluid	(−)	(+) (Resistance (−))	(+)	(+)	Pulmonary tuberculosis Knee joint tuberculosis	(+)	Survival (4 years)

DDH: DNA-DNA hybridization, PCR: Polymerase chain reaction; Extension: 1, within one-third of unilateral lung field; 2, within unilateral lung field; 3, over unilateral lung field.

Table 3. Clinical findings of cases showing a false-negative response to QFT-plus in patients in the active TB group.

Case	Age, Sex	Smoking history	Underlying disease	Diagnosis on admission	Laboratory findings				
					T-SPOT.TB	WBC (/μL)	Lymphocytes (/μL)	Total protein (g/dL)	Albumin (g/dL)
1	94, M	(+)	Pancreatic cancer COPD	Pulmonary tuberculosis susp.	Borderline	6540	720	5.7	2.6
2	72, M	(+)	Bladder cancer Colon cancer ope (Lung meta) Gastric cancer ope	Tuberculous pleuritis susp.	Borderline	7680	820	6.2	2.3
3	92, M	(+)	Cerebral infarction Angina pectoris Prostatic cancer COPD	Pneumonia susp.	Negative	11670	320	5.9	2.9
4	75, F	(−)	Pulmonary vein thrombosis Cerebral infarction Pulmonary NTM disease	ARDS	Negative	24200	240	5.4	2.4

COPD: Chronic obstructive pulmonary disease, ARDS: Adult respiratory distress syndrome; NTM: Nontuberculous mycobacteria, WBC: White blood cells.

Table 4. Clinical findings of cases showing a false-negative response to T-SPOT.TB in patients in the active TB group.

Case	Duration until definite diagnosis	Radiological findings (Portion, cavity, extension)	Diagnostic method	Microbiological findings				Final diagnosis	Treatment	Outcome
				Smear	Culture	DDH	PCR			
1	1 week	Bilateral Cavity (−) 2	Sputum	(+)	(+) (Resistance (−))	(+)	(+)	Pulmonary tuberculosis	(+)	Death (1 month later)
2	1 month	Right Cavity (−) Pleuritis	Pleural effusion	(−)	(+) (Resistance (−))	(+)	(+)	Tuberculous pleurisy	(+)	Survival (2 years)
3	5 days	Bilateral Cavity (+) 3	Sputum	(+)	(+) (Resistance (−))	(+)	(+)	Pulmonary tuberculosis	(+)	Survival (1 year)
4	2.5 months	Bilateral Cavity (−) 2	Sputum	(−)	(+) (Resistance (−))	(+)	(+)	Pulmonary tuberculosis	(+)	Survival (6 months)

DDH: DNA-DNA hybridization, PCR: Polymerase chain reaction; Extension: 1, within one-third of unilateral lung field; 2, within unilateral lung field; 3, over unilateral lung field.

4. Discussion

We evaluated the accuracy of diagnostic tests for TB infection (IGRAs: QFT-plus and T-SPOT.TB) in our tertiary hospital, which many elderly patients or patients receiving immunosuppressive treatment have visited, to elucidate quanti-

tative values of the IFN-γ response for patients with active TB, LTBI, old TB, and pulmonary NTM disease.

The positive response of patients with active TB disease showed a lower rate (71% on QFT-plus and 64% on T-SPOT.TB) compared with recently published reports of systematic reviews [16]-[19]. As a reason, false-negative responses to QFT-plus as well as T-SPOT.TB were recognized in elderly patients and patients with an immunosuppressed condition due to malignant disease. All patients with false-negative responses presented with lymphocytopenia (<1000/μL) and hypoalbuminemia (<3.0 g/dL) in the laboratory findings at the time of definite diagnosis. The frequency of false-negative results of IGRAs for patients with active TB disease ranged from 17% - 19% in recent studies, being significantly correlated with: elderly, female, acid-fast bacilli smear-negative, HIV co-infection, and inflammatory disease [10]-[12]. In order to overcome the false-negative results of IGRAs, we need to discover new specific biomarkers of active TB disease other than IFN-γ. For example, the measurement of IFN-γ-induced protein 10 (IP-10) or MIG using QFT supernatants was useful for the prediction of TB infection in patients with borderline QFT results (≥0.15 and <0.35 IU/mL) to reduce the false-negative results of IGRAs [20]. Concerning the comparison of IFN-γ concentrations of TB1 and TB2 in patients with active TB disease, that of TB2 was higher than that of TB1, but there was no significant difference. Pourakbari *et al.* also reported that higher IFN-γrelease in TB2 in the diagnosis of active TB was noted in the majority of patients with active TB disease compared with TB1, being consistent with the results of this study [18].

Regarding evaluation of the identification of active TB, LTBI, old TB, and NTM groups, there were no significant differences excluding between the NTM group and the other three groups. Although IFN-γ release in active TB and LTBI groups was higher than that in the old TB group, there were no significant differences between individual groups. Actually, because the production of IFN-γ is partly involved in the immune response against TB infection, it is difficult to identify the dynamics of immune responses against TB infection through IGRA results. In fact, IFN-γ levels using IGRAs were higher in patients with active TB than in those with LTBI, but many patients in both groups showed overlapping IFN-γ levels [21] [22]. To distinguish between active TB and LTBI, Mamishi *et al.* reported that interleukin-2 (IL-2) release stimulated by TB-specific antigens (ESAT-6, CFP-10, and TB7.7) was significantly higher in patients with LTBI compared with patients with active TB. IL-2 is a potential biomarker for discrimination between active TB and LTBI instead of IFN-γ *[23]*. In a previously reported meta-analysis, the sensitivity of IL-2 following stimulation with TB antigen of QFT-GIT for diagnosing LTBI ranged from 43 to 100%, and the specificity of this biomarker ranged from 89% to 100% [24]. Akashi *et al.* reported that IL-1ra in the QFT-GIT supernatant may be a good biomarker for the discrimination of active TB and LTBI [25]. To date, because there have been no large-scale studies on many biomarkers that can discriminate between active TB

and LTBI, it is necessary to identify new biomarkers that may be useful for the differentiation of active TB and LTBI.

Regarding the transitional change of IGRAs after the initiation of antituberculous treatment, Pourakbari *et al.* reported that the positive rate of IGRAs (QFT-GIT and T-SPOT.TB) following antituberculous treatment was estimated at 76% and there was no difference on comparing with the positive rate of IGRAs before the initiation of treatment, which was 76% [26]. Although the median period after the onset of active TB disease was 7.6 years in patients in the old TB group, the positive rate of QFT-plus was 53% and that of T-SPOT.TB was 56%, with no significant difference between the estimation of QFT-plus and T-SPOT.TB regarding both the positive rate and median levels in the old TB group and those in the active TB group. Because the immune response of IFN-γ continues after the end of antituberculous treatment, it is not always useful to judge cured (old) TB infection by IGRAs, and so the development of new biomarkers instead of IFN-γ that are be able to discriminate active TB and cured (old) TB is eagerly anticipated.

This study had several limitations. First, the small sample size in each group, especially in the LTBI group, wasa major limitation. Therefore, there might not be significant differences in IFN-γ levels for TB-specific antigens among three groups (active TB, LTBI, and old TB groups). The number of patients with LTBI visiting our hospital has recently decreased. Secondly, because this study was performed with patients with various severe underlying diseases in a tertiary hospital-based population suspected of having active TB disease, the positive response rates of IGRAs might be lower than in other previous studies. However, it is noteworthy that this study assessed such high-risk populations. Thirdly, because this study was geographically restricted to a small area of Japan, we should perform a large-scale nationwide study in Japan in the future.

5. Conclusion

The purpose of this study was to evaluate the usefulness of two IGRAs (QFT-plus and T-SPOT.TB) for patients suspected of having TB infection in a tertiary hospital. Although we could describe the usefulness of the two IGRAs between NTM and three other groups, we could not demonstrate the clinical usefulness among the three groups (active TB, LTBI, and old TB groups) using them. In the future, new biomarkers other than IFN-γ need to be discovered to discriminate among active TB, LTBI, and old TB. Otherwise, because false-negative responses of the two IGRAs were recognized in elderly patients and patients with immunocompromised conditions through this study, we think that it is necessary to carefully consider the diagnosis of TB infection using IGRAs in patients with severe underlying diseases in a tertiary hospital.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Petruccioli, E., Chiacchio, T., Pepponi, I., Vanini, V., Urso, R., Cuzzi, G., *et al.* (2016) Characterization of the CD4 and CD8 T-Cell Response in the QuantiFERON-TB Gold Plus Kit. *International Journal of Mycobacteriology*, **5**, S25-S26. https://doi.org/10.1016/j.ijmyco.2016.09.063

[2] Knierer, J., Gallegos Morales, E.N., Schablon, A., Nienhaus, A. and Kersten, J.F. (2017) QFT-Plus: A Plus in Variability?—Evaluation of New Generation IGRA in Serial Testing of Students with a Migration Background in Germany. *Journal of Occupational Medicine and Toxicology*, **12**, Article No. 1. https://doi.org/10.1186/s12995-016-0148-z

[3] Chiacchio, T., Petruccioli, E., Vanini, V., Cuzzi, G., Pinnetti, C., Sampaolesi, A., *et al.* (2014) Polyfunctional T-Cells and Effector Memory Phenotype Are Associated with Active TB in HIV-Infected Patients. *Journal of Infection*, **69**, 533-545. https://doi.org/10.1016/j.jinf.2014.06.009

[4] Lancioni, C., Nyendak, M., Kiguli, S., Zalwango, S., Mori, T., Mayanja-Kizza, H., *et al.* (2012) CD8+ T Cells Provide an Immunologic Signature of Tuberculosis in Young Children. *American Journal of Respiratory and Critical Care Medicine*, **185**, 206-212. https://doi.org/10.1164/rccm.201107-1355oc

[5] Rozot, V., Vigano, S., Mazza-Stalder, J., Idrizi, E., Day, C.L., Perreau, M., *et al.* (2013) *Mycobacterium tuberculosis*-Specific CD8+ T Cells Are Functionally and Phenotypically Different between Latent Infection and Active Disease. *European Journal of Immunology*, **43**, 1568-1577. https://doi.org/10.1002/eji.201243262

[6] Fabre, V., Shoham, S., Page, K.R. and Shah, M. (2014) High Proportion of Indeterminate QuantiFERON-TB Gold In-Tube Results in an Inpatient Population Is Related to Host Factors and Preanalytical Steps. *Open Forum Infectious Diseases*, **1**, ofu088. https://doi.org/10.1093/ofid/ofu088

[7] Brown, J., Kumar, K., Reading, J., Harvey, J., Murthy, S., Capocci, S., *et al.* (2017) Frequency and Significance of Indeterminate and Borderline Quantiferon Gold TB IGRA Results. *European Respiratory Journal*, **50**, Article ID: 1701267. https://doi.org/10.1183/13993003.01267-2017

[8] Bi, C., Clark, R.B., Master, R., Kapoor, H., Kroll, M.H., Salm, A.E., *et al.* (2021) Retrospective Performance Analyses of over Two Million U.S. Quantiferon Blood Sample Results. *Microbiology Spectrum*, **9**, e00096-21. https://doi.org/10.1128/spectrum.00096-21

[9] Osakabe, Y., Yamaguchi, F., Suzuki, A., Kitano, H., Hiraiwa, M., Shiratori, Y., *et al.* (2022) In-Hospital Blood Collection Increases the Rate of Indeterminate Results in Interferon-Gamma Release Assays. *Therapeutic Advances in Respiratory Disease*, **16**, 1-10. https://doi.org/10.1177/17534666221077817

[10] Santos, J.A., Duarte, R. and Nunes, C. (2020) Host Factors Associated to False Negative and Indeterminate Results in an Interferon-γ Release Assay in Patients with Active Tuberculosis. *Pulmonology*, **26**, 353-362. https://doi.org/10.1016/j.pulmoe.2019.11.001

[11] Shangguan, Y., Fang, H., Wang, S., Ji, Z., Shi, P., Feng, X., *et al.* (2020) Risk Factors for Negative T-SPOT.TB Assay Results in Patients with Confirmed Active Tuber-

culosis: A Retrospective Study. *The Journal of Infection in Developing Countries*, **14**, 1288-1295. https://doi.org/10.3855/jidc.12063

[12] Li, Q., Ren, W., Yuan, J., Guo, H., Shang, Y., Wang, W., *et al.* (2022) Significant Difference in Th1/Th2 Paradigm Induced by Tuberculosis-Specific Antigens between IGRA-Positive and IGRA-Negative Patients. *Frontiers in Immunology*, **13**, Article ID: 904308. https://doi.org/10.3389/fimmu.2022.904308

[13] Griffith, D.E., Aksamit, T., Brown-Elliott, B.A., Catanzaro, A., Daley, C., Gordin, F., *et al.* (2007) An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases. *American Journal of Respiratory and Critical Care Medicine*, **175**, 367-416. https://doi.org/10.1164/rccm.200604-571st

[14] Qiagen (2019) QuantiFERON-TB Gold Plus (QFT-Plus) Package Insert. Quiagen.

[15] Oxford Immunotec (2016) T-SPOT.TB UK, Oxford Immunotec.

[16] Oh, C.E., Ortiz-Brizuela, E., Bastos, M.L. and Menzies, D. (2020) Comparing the Diagnostic Performance of QuantiFERON-TB Gold Plus to Other Tests of Latent Tuberculosis Infection: A Systematic Review and Meta-Analysis. *Clinical Infectious Diseases*, **73**, e1116-e1125. https://doi.org/10.1093/cid/ciaa1822

[17] Sotgiu, G., Saderi, L., Petruccioli, E., Aliberti, S., Piana, A., Petrone, L., *et al.* (2019) Quantiferon TB Gold Plus for the Diagnosis of Tuberculosis: A Systematic Review and Meta-Analysis. *Journal of Infection*, **79**, 444-453. https://doi.org/10.1016/j.jinf.2019.08.018

[18] Pourakbari, B., Mamishi, S., Benvari, S. and Mahmoudi, S. (2019) Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon-Γ Release Assays: A Systematic Review and Meta-Analysis. *Advances in Medical Sciences*, **64**, 437-443. https://doi.org/10.1016/j.advms.2019.09.001

[19] Shafeque, A., Bigio, J., Hogan, C.A., Pai, M. and Banaei, N. (2020) Fourth-Generation QuantiFERON-TB Gold Plus: What Is the Evidence? *Journal of Clinical Microbiology*, **58**, e01950-19. https://doi.org/10.1128/jcm.01950-19

[20] Uzorka, J.W., Bossink, A.W.J., Franken, W.P.J., Thijsen, S.F.T., Leyten, E.M.S., van Haeften, A.C., *et al.* (2018) Borderline Quantiferon Results and the Distinction between Specific Responses and Test Variability. *Tuberculosis*, **111**, 102-108. https://doi.org/10.1016/j.tube.2018.06.002

[21] Higuchi, K., Harada, N., Fukazawa, K. and Mori, T. (2008) Relationship between Whole-Blood Interferon-Gamma Responses and the Risk of Active Tuberculosis. *Tuberculosis*, **88**, 244-248. https://doi.org/10.1016/j.tube.2007.11.009

[22] Kobashi, Y., Shimizu, H., Ohue, Y., Mouri, K., Obase, Y., Miyashita, N., *et al.* (2010) Comparison of T-Cell Interferon-Γ Release Assays for Mycobacterium Tuberculosis-Specific Antigens in Patients with Active and Latent Tuberculosis. *Lung*, **188**, 283-287. https://doi.org/10.1007/s00408-010-9238-3

[23] Mamishi, S., Pourakbari, B., Marjani, M., Bahador, A. and Mahmoudi, S. (2015) Discriminating between Latent and Active Tuberculosis: The Role of Interleukin-2 as Biomarker. *Journal of Infection*, **70**, 429-431. https://doi.org/10.1016/j.jinf.2014.12.011

[24] Mamishi, S., Pourakbari, B., Teymuri, M., Rubbo, P.-., Tuaillon, E., Keshtkar, A.A., *et al.* (2014) Diagnostic Accuracy of IL-2 for the Diagnosis of Latent Tuberculosis: A Systematic Review and Meta-Analysis. *European Journal of Clinical Microbiology & Infectious Diseases*, **33**, 2111-2119. https://doi.org/10.1007/s10096-014-2190-z

[25] Akashi, S., Suzukawa, M., Takeda, K., Asari, I., Kawashima, M., Ohshima, N., *et al.* (2021) IL-1RA in the Supernatant of QuantiFERON-TB Gold In-Tube and Quan-

tiFERON-TB Gold Plus Is Useful for Discriminating Active Tuberculosis from Latent Infection. *Journal of Infection and Chemotherapy*, **27**, 617-624. https://doi.org/10.1016/j.jiac.2020.11.023

[26] Pourakbari, B., Mamishi, S., Benvari, S., Sauzullo, I., Bedini, A., Valentini, P., *et al.* (2020) Can Interferon-Γ Release Assays Be Useful for Monitoring the Response to Anti-Tuberculosis Treatment? A Systematic Review and Meta-Analysis. *Archivum Immunologiae et Therapiae Experimentalis*, **68**, Article No. 4. https://doi.org/10.1007/s00005-020-00568-4