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Clinical Evaluation of IP-10 and MIG for the Diagnosis of Active Tuberculosis Disease

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Abstract

Background: The aim of this study was to evaluate the clinical potential of IP-10 and MIG as biomarkers of tuberculosis (TB) infection adding a comparison with IGRAs (QFT and T-SPOT.TB).

Materials and Methods: The subjects consisted of 52 patients with active TB disease and 86 patients with non-TB disease. We measured two IGRAs using peripheral blood (PB), and IP-10 and MIG using the supernatant from whole blood stimulated with MTB (Mycobacterium tuberculosis)-specific antigens.

Results: In the patient group with active TB disease, while the positive response rates of QFT and T-SPOT.TB were 81% and 87%, that of IP-10 using the supernatant was 88% and that of MIG was 85%. In the patient group with non-TB disease, the positive response rate of QFT was 13%, and that of T-SPOT.TB was 14%, IP-10 using the supernatant was 14%, and that of MIG was 14%. The IP-10 and MIG levels of the patients with active TB disease using the supernatant were significantly higher than those of the patients with non-TB disease. The combination of four diagnostic methods using the supernatant increased the positive response rate to 94%.

Conclusion: IP-10 and MIG using a supernatant stimulated with MTB-specific antigens showed similar results to IGRAs. Therefore, these tests can be used as alternatives for the diagnosis of active TB disease.

Keywords: *Mycobacterium tuberculosis* (MTB) disease; Interferon-γ release assay (IGRA); Interferon-γ inducible protein-10 (IP-10); Monocyte induced interferon-γ (MIG)

Introduction

It has been reported that over nine million new tuberculosis (TB) patients were diagnosed worldwide and 1.5 million TB patients died in 2013 [1]. However, TB diagnosis mainly depends on the detection of mycobacteria by acid-fast staining and/or fluorescent microscopy, as well as bacterial culture. Sputum smear microscopy has low sensitivity and because sputum culture takes 4-8 weeks, it leads to a delay in diagnosis and treatment. Nucleic acid amplification has been developed with over 90% sensitivity for the identification of *Mycobacterium tuberculosis* (MTB) from clinical samples [2,3].

Interferon-γ release assays (IGRAs) have been introduced into clinical practice for the diagnosis of MTB infection and current evidence suggests that these two tests measure the interferon-γ (IFN-γ) release of activated T cells isolated from the patient's peripheral blood: QuantiFERON-TB Gold In-Tube (QFT) as an enzyme-linked immunosorbent assay (ELISA) and T-SPOT.TB as an enzyme-linked immunospot assay (ELISPOT). However, previous reports suggested that patients with advanced HIV and a severe immunosuppressive state have an increased proportion of negative and/or indeterminate results [4,5]. The detection rate of IGRAs can be enhanced by measuring alternative or additional biomarkers for IFN-γ [6-12]. Among these many potential biomarkers, Ruhwald et al. indicated that the interferon-γ inducible protein-10 (IP-10) response to MTB-

specific antigens was useful as a diagnostic marker for TB infection [12]. IP-10 is a CXC chemokine mainly produced by monocytes and T cells. IP-10 is elevated in the plasma samples of TB patients and produced in a highly antigen-dependent manner following infection by MTB [9]. IP-10 can be induced in monocytes and macrophages present in whole blood by antigens and mitogen stimulation in inflammatory and infectious diseases, including TB [13-16]. On the other hand, monocyte-induced interferon-γ (MIGis also strongly induced by MTB-specific antigen stimulation and release shows a high degree of correlation with IFN-γ and IP-10 [17,18]. Chung et al. reported that an MTB-specific antigen stimulated assay of MIG may be a more useful marker in the diagnosis of active TB disease than IP-10 [19]. However, as far as we know, there are few reports about the clinical usefulness of other cytokines, or chemokines except IGRAs, in the differential diagnosis of active TB disease and other respiratory diseases in Japan.

Therefore, we evaluated the clinical potential of two chemokines (IP-10 and MIG) related to IFN-γ using the supernatant from whole blood stimulated with MTB-specific antigens for the differential diagnosis of active TB disease with other diseases compared with two IGRAs (QFT and T-SPOT.TB) in Japan.

Materials and Methods

Study subjects

In this study, we enrolled 52 patients with active TB disease (29 with pulmonary TB, 6 with TB lymphadenopathy, 6 with miliary TB, 4 with

pulmonary TB+TB pleuritis, 2 with TB pleuritis, 2 with pulmonary TB +TB meningitis, 1 with pulmonary TB+bronchial TB, 1 with pulmonary TB+TB lymphadenopathy, 1 with TB meningitis), 58 with nontuberculous mycobacterial (NTM) disease, and 28 with other respiratory diseases (8 with lung cancer, 4 with pulmonary abscess, 4 with bronchiectasis, 3 with pulmonary mycosis, 3 with sinobronchitis syndrome, 2 with organizing pneumonia, 2 with pulmonary nocardia, 1 with sarcoidosis and 1 with pneumoconiosis). The definite diagnosis of active TB disease was based on smear and/or culture-positive results or being PCR-positive for MTB from any clinical specimen. All patients with NTM disease satisfied the diagnostic criteria proposed by the American Thoracic Society (ATS) [20] and the causative microorganisms consisted of *Mycobacterium avium* in 27, *M. intracellulare* in 22, *M. kansasii* in 5, *M. abscessus* in 2, and *M. avium +M. intraellulare* in 2, respectively. Other respiratory diseases were confirmed in terms of histological diagnosis from the specimens using bronchoscopy.

This study was approved by the Ethical committee of Kawasaki Medical School and informed consent was obtained from all participants. All patients were examined between 2010 and 2014 and diagnosed at Kawasaki Medical School Hospital.

QFT (QuantiFERON TB gold In-tube)

A heparinized blood sample was collected before the initiation of the treatment from each patient by vein puncture and aliquots were used for two IGRA tests, IP-10, and MIG. QFT was performed according to the recommendations of the manufacturer (Cellestis Ltd., Carnegie, Australia). The judgment was performed according to the guideline proposed by the centers for control and prevention (CDC) for using QFT [21].

T-SPOT.TB

T-SPOT.TB was performed according to the recommendations of the manufacturer (Oxford Ltd., Oxfordshire, UK) and the test result was judged by UK guidelines for T-SPOT.TB to diagnose TB published by NCCCC [22].

IP-10 and MIG assays

The levels of IP-10 and MIG were measured using the supernatant acquired from the QFT assay which was stimulated with saline, mitogen or TB-specific antigens (ESAT-6, CFP-10 and TB 7.7). An ELISA development kit (R&D Systems Inc, MN, USA) was used to detect IP-10 release and MIG release according to the manufacturer's instructions.

Statistical analysis

The Mann-Whitney U test was carried out to calculate the differences between individual groups (for example, the TB disease group and the NTM disease group, the TB disease group and other disease group and the NTM disease group and other disease group) and group medians (>2) were compared using the ANOVA test. The test concordance rate was assessed using the kappa (κ) statistic. The diagnostic accuracies of the tests for IP-10 and MIG were evaluated using a receiving operating characteristic (ROC) curve. The ROC analysis was performed according to the literature [23,24]. The cut-off

value was estimated at various sensitivities and specificities and determined at the maximum sensitivity+ 1-Specificity. A p-value of <0.05 was considered significant. Data analysis was performed using the Stat Flex version 6 software (Artec, Japan, 2013).

Results

A total of 138 patients were enrolled during the study period. They consisted of 52 patients with active TB disease (TB group), 58 with NTM disease (NTM group) and 28 with other respiratory diseases (Other respiratory disease group). The clinical characteristics of patients in the three groups are shown in Table 1. Although there were no significant differences in terms of the sex and underlying disease, there were significant differences in terms of smoking history in the NTM group compared to the other groups. On the other hand, the patients in the TB group showed significant lymphocytopenia, hypoproteinemia and hypoalbuminemia in the laboratory findings compared to those of other groups.

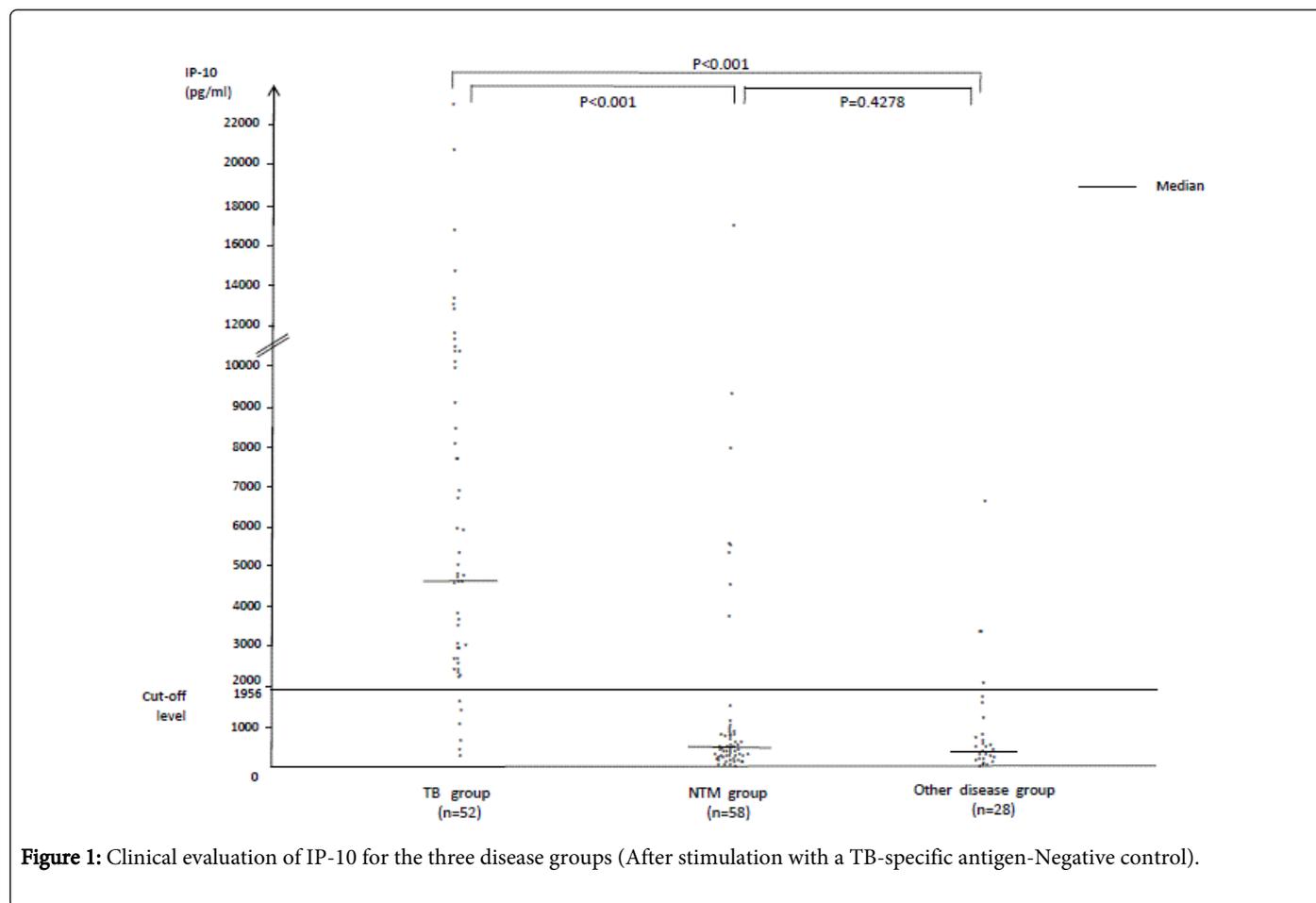|	TB group (n=52)	NTM group (n=58)	Other disease group (n=28)
Age (Median ± S.D.)	72.5 ± 18.5	69.5 ± 13.9	70.5 ± 18.5
Sex (Male : Female)	30:22:00	23:35	17:11
Smoking history (+)	30 (58%)	20 (34%)	14 (50%)
Underlying disease (+) (with repetition)	43 (83%)	39 (67%)	24 (86%)
Respiratory disease (+)	12 (23%)	10 (17%)	4 (14%)
Healed TB disease(+)	2	3	1
Malignant disease (+)	8	6	3
Chronic renal failure (+)	1	0	0
Diabetes mellitus (+)	4	7	5
HIV (+)	1	0	0
Laboratory finding (median ± S.D.)			
Lymphocyte (/μl)	1084 ± 743	1435 ± 651	1525 ± 594
Total protein (g/dl)	6.8 ± 0.8	7.3 ± 0.5	7.1 ± 0.5
Albumin (g/dl)	3.3 ± 0.8	4.0 ± 0.5	3.8 ± 0.5

Table 1: Clinical characteristics of the three disease groups.

In the QFT, 42 of 52 patients in the TB group showed a positive response (81%). In the patients with non-TB disease (the NTM group and other respiratory disease group), the positive response rate was 13% (10% in the NTM group and 18% in the other respiratory disease group). One of three patients with healed TB and two of five patients with pulmonary *M.kansasii* disease showed a positive QFT response in the NTM group. One patient with healed TB showed a negative QFT response in the other respiratory disease group. On the other hand, three patients in the TB group (7%) showed indeterminate responses due to a low mitogen response (Table 2).

Diagnostic method	Judgment	TB disease (n=52)	Non-TB disease (n=86)	NTM disease (n =58)	Other respiratory diseases (n=28)
QFT	Positive	42(81%)	11(13%)	6(10%)	5(18%)
	Negative	7(13%)	74(87%)	51(88%)	23(82%)
	Indeterminate	3(6%)	0	1(2%)	0
T-SPOT.TB	Positive	45(87%)	12(14%)	7(12%)	5(18%)
	Negative	6(11%)	74(86%)	51(88%)	23(82%)
	Indeterminate	1(2%)	0	0	0
IP-10	Positive	46(88%)	12(14%)	8(12%)	4(17%)
	Negative	6(12%)	74(86%)	50(88%)	24(83%)
MIG	Positive	44(85%)	12(14%)	8(12%)	4(17%)
	Negative	8(15%)	74(86%)	50(88%)	24(83%)
QFT: QuantiFERON; IP-10 : Interferon-gamma-induced protein; TB: Tuberculosis; MIG: Monokine induced by interferon-gamma; NTM: Nontuberculous mycobacteria					

Table 2: Comparison of the results of the two IGRAs tests, IP-10 and MIG, for patients with TB disease and other respiratory diseases.

Figure 1: Clinical evaluation of IP-10 for the three disease groups (After stimulation with a TB-specific antigen-Negative control).
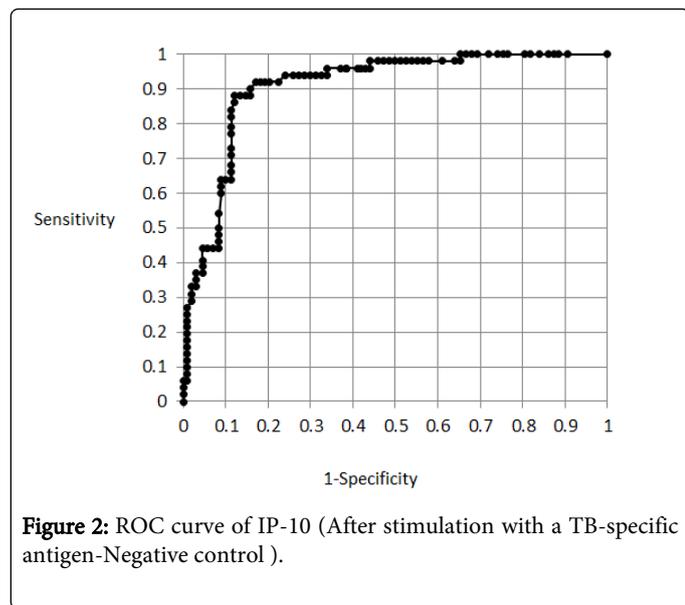
In the T-SPOT.TB, 45 of 52 patients in the TB group had a positive response (87%). In non-TB disease patients, the positive response rate was 14% (12% in the NTM group and 18% in the other respiratory disease group). One of three patients with healed TB and two of five

patients with pulmonary *M. kansasii* disease showed a T-SPOT.TB positive response in the NTM group. One patient with healed TB showed a T-SPOT.TB negative response in the other respiratory disease group. One patient in the TB group (2%) showed an indeterminate response due to the mitogen response (Table 2).
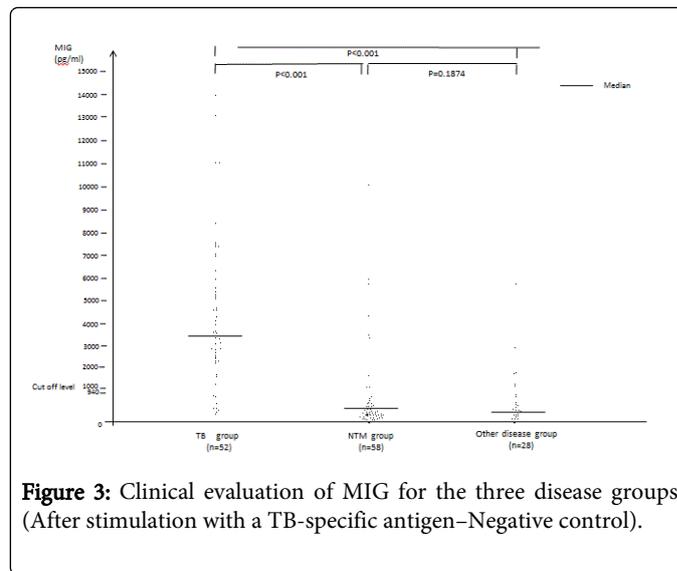
The median levels of IP-10 stimulated by MTB-specific antigen were as follows (median ± S.D); 4,700 ± 5,066 pg/ml in the TB group, 560 ± 2.778 pg/ml in the NTM group and 400 ± 1,417 pg/ml in the other respiratory disease group, respectively. They were significantly higher (p<0.001) in the TB group than the other two groups (Figure 1).

The diagnostic performance of IP-10 in the experiment using the MTB-specific antigen was first assessed with the ROC curve (Figure 2). The patients in the TB group were used as the true positives and non-TB patients consisting of patients with NTM disease and other respiratory diseases were the true negatives. The AUC of IP-10 was 0.907 (95%CI: 0.882-0.932). The optimal cut-off value for IP-10 was determined to be 1,965 pg/ml by ROC analysis. When the levels stimulated by MTB-specific antigen- negative control levels were estimated over the optical cut-off values, these patients were regarded as having a positive response, and when they were estimated below the cut-off values, they were regarded as having a negative response. While the positive response rate of IP-10 was 88% in the patients in the TB group, it was only 12% in those in the non-TB group (12% in the NTM group and 17% in the other respiratory disease group). The positive response rate of IP-10 was significantly higher in the patients in the TB group than the other two patient groups (p<0.001) (Table 2).

The median levels of MIG stimulated by MTB-specific antigen were as follows (median ± S.D); 3,540 ± 3,138 pg/ml in the TB group, 555 ± 1,786 pg/ml in the NTM group and 345 ± 974 pg/ml in the other respiratory disease group, respectively. They were significantly higher (p<0.001) in the TB group than the other two groups (Figure 3).


Figure 3: Clinical evaluation of MIG for the three disease groups (After stimulation with a TB-specific antigen–Negative control).

The diagnostic performance of MIG in the experiment using the MTB-specific antigen was assessed with the ROC curve (Figure 4). The AUC of MIG was 0.889 (95%CI: 0.860-0.918). The optimal cut-off value for MIG was determined to be 940 pg/ml by the ROC analysis. While the positive response rate of MIG was 85% in the TB group patients, it was only 14% in the non-TB group (12% in the NTM group and 17% in the other respiratory disease group). The positive response rate of MIG was significantly higher in the TB group patients than the other two patient groups (p<0.001) (Table 2).

Figure 2: ROC curve of IP-10 (After stimulation with a TB-specific antigen-Negative control).
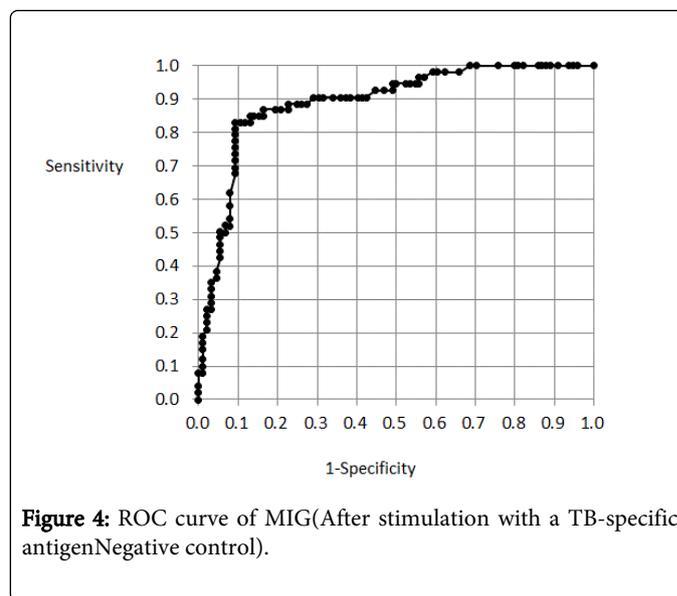
Figure 4: ROC curve of MIG(After stimulation with a TB-specific antigenNegative control).

The median level of IP-10 stimulated by MTB-specific antigen in patients that showed QFT positive responses (n=42) was 6,250±4,964 pg/ml and that in those with QFT-negative responses (n=7) was 1,400 ± 830 pg/ml in the TB disease group. The median level of IP-10 stimulated by MTB-specific antigen in QFT-positive patients (n=11) was 4,500 ± 2,505 pg/ml and that in those with a QFT-negative response (n=74) was 425 ± 1,985 pg/ml in the non-TB disease group. There were significant differences in the IP-10 levels stimulated by MTB-specific antigen between QFT-positive and negative responses in both disease groups (p<0.001).

The median level of MIG stimulated by MTB-specific antigen in patients showing QFT-positive responses (n=42) was 4,100 ± 3,081 pg/ml and that in those who showed QFT-negative responses (n=7) was 560 ± 903 pg/ml in the TB disease group. The median level of MIG stimulated by MTB-specific antigen in patients who showed QFT-positive responses (n=11) was 2,800 ± 1,964 pg/ml and that in those showed QFT-negative responses (n=74) was 455 ± 1,285 pg/ml. There were significant differences in the MIG levels stimulated by MBT-specific antigen between QFT-positive and -negative response in both disease groups.

QFT, T-SPOT.TB, IP-10, and MIG showed sensitivities of 81.0% (95%CI: 76.2-85.8%), 87.0% (95%CI: 82.5-91.5%), 87.2% (95%CI: 83.5-91.0%), and 84.6% (95%CI: 80.9%-88.3%). When the indeterminate results were excluded for sensitivity calculations, QFT and T-SPOT.TB yielded diagnostic sensitivities of 85.7% (95%CI: 81.3-90.1%) and 88.2% (95%CI: 84.2-92.2%), respectively. Although there were no significant differences among the four tests, the sensitivity of IP-10 was the highest for the differential diagnosis of TB disease. QFT, T-SPOT.TB, IP-10, and MIG showed specificities of 86.0% (95%CI: 81.7-90.3%), 86.1% (95%CI: 81.9-90.5%) and 87.0% (95%CI: 82.6-91.3%), and 85.8% (95%CI: 81.5-90.3%), respectively. There were no significant differences among the four tests in terms of specificity. On the other hand, the positive predictive values (PPV) for QFT, T-SPOT.TB, IP-10 and MIG were 79.2% (95%CI: 75.3-83.1%), 78.9% (95%CI: 75.0-82.9%), 79.2% (95%CI: 75.2-83.2%) and 78.6% (95%CI: 74.7-82.5%). The negative predictive values (NPV) for QFT, T-SPOT.TB, IP-10 and MIG were 91.4% (95%CI: 87.4-95.4%), 92.5% (95%CI: 88.5-96.5%), 92.5% (95%CI: 88.7-96.3%) and 90.2% (95%CI: 86.3-94.1%), respectively.

The concordance rates of individual tests were as follows; QFT and T-SPOT.TB were 95.5% (κ=0.907), QFT and IP-10 were 94.8% (κ=0.892), QFT and MIG were 92.5% (κ=0.891), T-SPOT.TB and IP-10 were 94.6% (κ=0.878), T-SPOT.TB and MIG were 93.4% (κ=0.864) and IP-10 and MIG were 94.2% (κ=0.862). The concordance rate of the four tests in all patients except patients with indeterminate results of two IGRAs was 89.6% (κ=0.784). All concordance rates showed high agreement. If we judged the patient positive when either of the four tests showed a positive response, the positive response rate increased to 94.2%.

Discussion

We demonstrated that IP-10 and MIG are useful immunodiagnostic markers to make a differential diagnosis of active TB disease and non-TB diseases such as NTM disease or other respiratory diseases in Japanese patients using clinical samples stimulated by MTB-specific antigens in this study. Concerning the comparison with the two IGRAs (QFT and T-SPOT.TB), although the results of IP-10 and MIG were similar to those of the two IGRAs in terms of sensitivity and specificity, the accuracy of the diagnosis of TB infection was increased when we used chemokines related to IFN-γ such as IP-10 or MIG in combination with the two IGRAs. Wang et al. also reported that both IP-10 and MIG had sensitivity and specificity comparable to IFN-γ in the detection of active TB disease [25] and that combined detection of IFN-γ, IP-10 and MIG showed significantly improved sensitivity and specificity as compared with individual cytokine and chemokine detection, as in our report.

Chemokines such as IP-10 or MIG are CXC chemokine receptor 3 (CXCR3) ligands which play an important role in the T-helper type 1 (Th1) lymphocyte pathway that is related to the pathogenesis of TB disease [26-28]. Although the release of IP-10 and MIG was increased from monocytes and granulocytes following MTB infection, this was also described in other autoimmune disorders [13-16].

In a previous report, Hong et al. found that not only MTB antigen-dependent IP-10 levels, but also serum IP-10 were higher in active TB than in latent TB infection (LTBI) and non-TB controls [29]. One reason for the different result may be because they included many populations without underlying diseases in the LTBI and non-TB groups, while there were many patients, including those with comorbidity diseases with NTM disease or other respiratory diseases, in our study. Recently, Guo et al. reported the diagnostic accuracy of IP-10 for TB using meta-analysis consisting of 14 studies [30]. The summary estimates for IP-10 in the diagnosis of TB were 73% for sensitivity and 83% for specificity. Finally, IP-10 may improve the accuracy of TB diagnosis when combined with other tests, but the diagnostic accuracy was moderate. The results of IP-10 should be interpreted in parallel with clinical findings and the results of other conventional tests. However, IP-10 gave good results of 87% for sensitivity and 87% for specificity in our study compared with that of Guo et al., regardless of no significant differences among the four diagnostic methods.

As for MIG, when TB antigen tube levels- nil tube levels were obtained according to the IGRAs interpretation strategy, Chung et al. reported that MIG and IFN-inducible T-cell α-chemoattractant (I-TAC) showed high sensitivity (MIG: 92.5%, I-TAC: 90.3%) and high specificity (MIG: 85.2%, I-TAC: 90.7%), respectively [19]. They demonstrated that TB-antigen-stimulated levels of MIG and I-TAC were significantly increased in the active TB group compared with non-TB pulmonary diseases and control subjects. This result was similar to our study using a supernatant stimulated by MTB-specific antigens and we could obtain a significant difference in the supernatant levels of MIG between the patients with TB disease and those with non-TB diseases.

Regarding the agreement among the two IGRAs and IP-10 and MIG stimulated by MTB specific-antigens, there was excellent agreement in the individual comparison of the four tests as described in the results. This high agreement rate can be explained by the fact that IP-10 and MIG are chemokines induced by the same IFN-γ. If we judged positive when any of the two IGRAs, IP-10 and MIG, stimulated by MTB specific-antigens showed a positive response, the positive response rate was increased to 94.2%.

Although it has been reported that two IGRAs (QFT and T-SPOT.TB) can generate false-positive results for patients with healed TB or NTM disease (the causative microorganism was *M. kansasii, M. marinum*, etc), two of five patients with pulmonary *M.kansasii* disease and one of four patients with healed TB showed false- positive results with both IP-10 and MIG, as well as the two IGRAs in this study. However, if we excluded patients with healed TB disease or pulmonary *M. kansasii* disease from this study to avoid any cross-reaction for MTB-specific antigens, there was no big influence on the sensitivity, specificity, PPV or NPV (not shown in these results). Because IP-10 and MIG are IFN-γ-related chemokines, we have to be careful with the judgment of IP-10 or MIG for these patients, as well as the two IGRAs, when a supernatant stimulated by MTB-specific antigens is used.

There are some limitations to this study. Firstly, although we used medium-scale patients with underlying disease in order to evaluate the usefulness for the diagnosis of active TB disease, the study was

geographically restricted to a small area in Japan with an intermediate TB population. We hope that a large scale nationwide study will be practically performed with immunological tests such as IGRAs in combination with IP=10 and MIG quantification in a hospital setting in Japan. Secondly, only one patient with TB disease with HIV infection was included in this study. Although IP-10 was a useful diagnostic marker of TB disease for patients with HIV infection in a previous report [31], our patient with HIV infection showed negative results for the tests with both IP-10 and MIG, as well as IGRAs. However, it was noteworthy that this study assessed a high proportion of immunosuppressed patients who were more likely to be candidates for a TB disease diagnosis. Thirdly, the cut-off levels of positive response for serum or MTB antigen-stimulated IP-10 and MIG were different from those of previous reports [27,32]. The discordance among these levels may be attributable to the different activities of TB disease and non-TB disease or the effects of ethnicity, geographic location, and technical factors.

In conclusion, IP-10 and MIG responses for the MTB specific-antigen showed similar results to IGRAs tests. Therefore, these tests can be alternatives for the diagnosis of active TB disease.

Acknowledgement

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