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ORIGINAL ARTICLE

Evaluation of a lateral flow assay–based IFN-γ release assay as a point-of-care test for the diagnosis of latent tuberculosis infection

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Abstract

Introduction We compared the performance of a fluorescence lateral flow assay (ichroma™ IGRA-TB) with the QuantiFERON-TB Gold PLUS (QFT-PLUS) for the diagnosis of latent tuberculosis infection (LTBI) in patients with immune-mediated inflammatory diseases (IMID) prior to receiving biologics therapy.

Method The comparability of the ichroma™ IGRA-TB assay with the QFT-PLUS assay for the diagnosis of LTBI was determined in prospectively enrolled patients with IMID prior to receiving biologics between August 2018 and October 2019. To determine the best cut-off value of the ichroma™ IGRA-TB, an ROC curve analysis was performed.

Results Patients with IMID (n = 145) had inflammatory bowel disease (n = 83; 57.2%), rheumatoid arthritis (n = 44; 30.3%), or spondyloarthropathy (n = 18; 12.4%). The median age was 40.5 (interquartile range: 27.0–56.0), 72 (49.7%) were men, and 140 (96.6%) received BCG vaccination. With the manufacturer-recommended cut-off values, 11 (7.6%) and 20 (13.8%) patients showed positive results with the ichroma™ IGRA-TB and QFT-PLUS tests, respectively. The overall agreement between the two tests was 91.0% with a Cohen's kappa value of 0.535 (95% confidence interval: 0.317–0.754). ROC curve analysis of the QFT-PLUS results showed that a cut-off value of > 0.21 IU/mL would improve the performance of the ichroma™ IGRA-TB. Using the new cut-off value, the concordance rate was improved to 93.1% with a Cohen's kappa value of 0.668 (95% confidence interval: 0.478–0.858).

Conclusions The ichroma™ IGRA-TB could be used as a point-of-care test for LTBI screening in IMID patients before starting biologics, especially in resource-limited settings.

Key Points
- *The ichroma™ IGRA-TB is an automated fluorescence lateral flow assay–based IGRA.*
- *The test has advantages like short turn-around time, low-cost, and ease of use.*
- *The ichroma™ IGRA-TB showed high agreement with the QuantiFERON-TB Gold In-Tube in patients with chronic immune-mediated inflammatory diseases before starting biologics.*

Keywords Immune-mediated inflammatory diseases · Interferon-gamma release tests · Latent tuberculosis · Point-of-care testing

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Introduction

Tuberculosis (TB) is a major global health concern and is one of the top 10 causes of death worldwide, with approximately 10 million global incident cases each year [1]. Latent tuberculosis infection (LTBI) is a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no clinical manifestation of active TB [2]. About a quarter of the global population is assumed to be infected with TB [3]. However, the exact global burden is unknown due to the lack of gold standard tests for TB infection.

Approximately 10% of individuals with TB infection will develop active TB disease over the course of their lives, usually within the first 5 years after the initial infection [4–6]. The risk for active TB after infection depends on several factors, with the most important one being immunologic status [2]. Particularly, patients with chronic immune-mediated inflammatory diseases (IMIDs) treated with biologic agents such as tumor necrosis factor-alpha (TNF-α) inhibitors have a higher risk for developing active TB [7–10]. Therefore, the World Health Organization (WHO) recommends that people undergoing anti-TNF treatment should be systematically tested and treated for LTBI [11].

The diagnosis of LTBI is indirectly made by evaluating the immune response against *M. tuberculosis* using tuberculin skin tests (TSTs) or interferon-gamma release assays (IGRAs) [11, 12]. However, TSTs have low specificity in populations vaccinated with BCG [13–16] and low sensitivity in immunosuppressed individuals [17, 18]. South Korea has a mandatory BCG vaccination program and an intermediate prevalence of TB (77 per 100,000 people in 2016) [19]; as such, IGRAs have been more commonly used than TSTs for the diagnosis of LTBI in South Korea.

The QuantiFERON-TB Gold In-Tube (QFT-GIT, Qiagen, Hilden, Germany) and the T-SPOT.TB (Oxford Immunotec, Abingdon, UK) are the two main commercially available IGRAs. The QuantiFERON-TB Gold PLUS (QFT-PLUS, Qiagen, Abingdon, UK) was introduced as a new-generation QFT-GIT and shows similar accuracies to the QFT-GIT and higher $CD8^+$ T cell responses to recent TB exposure than to remote exposure [20–26].

Although QFT-GIT and QFT-PLUS are useful, they are both based on the ELISA technique and thus entail labor-intense and time-consuming steps. To overcome these drawbacks, an automated fluorescence lateral flow assay (LFA)–based IGRA was developed in South Korea. Along with the self-developed TB-specific antigen pool and tube (TB tube), the LFA-based IGRA was developed into a commercial kit, namely the ichroma™ IGRA-TB (Boditech, Chuncheon, South Korea). The test uses an automated fluorescence LFA cartridge to detect interferon-gamma (IFN-γ) and a sandwich immunodetection method in which the dried detector and captor antibodies in the cartridge bind to IFN-γ in a sample, form a triplet antigen-antibody complex, and migrate onto the nitrocellulose matrix to be captured by the immobilized biotin-streptavidin system on the test strip.

This new assay is easy to use and requires less than 30 min to obtain the results. Therefore, the ichroma™ IGRA-TB test may be a useful method as a point-of-care test for diagnosing TB infection in low-income countries. In a previous study reported by Hur et al. [27], the LFA-based IGRA using fluorescence showed excellent agreement with the QFT-GIT in healthy subjects. In this study, we determined the comparability of the ichroma™ IGRA-TB test with the QFT-PLUS test for screening LTBI in patients with IMIDs before receiving biologic agents.

Patients and methods

Study participants and ethics

Inclusion criteria were patients who required biologic agents for their underlying IMIDs including Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), and spondyloarthropathy (SpA). Individuals with active TB were excluded.

Between August 2018 and October 2019, we prospectively enrolled 152 adult patients (age > 18 years) with IMIDs before starting on biologic agents. All patients were recruited from the Department of Rheumatology and the Department of Gastroenterology at Asan Medical Center, a tertiary referral center in Seoul, South Korea. All patients underwent both the QFT-PLUS and ichroma™ IGRA-TB test for the diagnosis LTBI. We obtained clinical data including age, sex, BCG vaccination status, underlying disease, and current medications including immunosuppressants of the patients at study inclusion.

Immunosuppressants were defined as the equivalent of ≥ 15 mg/day of prednisone for one month or longer, azathioprine, 6-mercaptopurine, methotrexate, and biologic agents including infliximab, adalimumab, and vedolizumab. Chest imaging studies including chest X-rays or chest computed tomography were assessed for signs of active TB infection in all patients.

This study was approved by the Institutional Review Board of Asan Medical Center (IRB No.: 2017-1303). All participants provided written informed consent.

QFT-PLUS and ichroma™ IGRA-TB procedures

The QFT-PLUS and ichroma™ IGRA-TB tests were performed according to the respective manufacturer's instructions. For the QFT-PLUS test, heparinized blood samples were collected in the Nil tube, TB antigen tubes (TB1 tube and TB2 tube), and Mitogen tube; for ichroma™ IGRA-TB,

the blood samples were collected in the Nil tube, TB antigen tube (TB tube), and Mitogen tube. After shaking 10 times, each tube was incubated at 37°C for 16–24 h and centrifuged for 15 min at 2000–3000 RCF (g).

For the QFT-PLUS test, IFN-γ levels (IU/mL) in four plasma samples from each patient were measured by ELISA. For the ichroma™ IGRA-TB test, three plasma samples from each patient were loaded onto the tube rack in the fluorescence LFA cartridge, and the fluorescence-labeled IFN-γ levels (IU/mL) were measured and calculated.

IFN-γ values in TB1, TB2, TB, and Mitogen tubes were subtracted from the values in the Nil tube and adjusted; the adjusted values were expressed as TB1-Nil, TB2-Nil, TB-Nil, and Mitogen-Nil. The results of both tests were considered positive when the TB1-Nil, TB2-Nil, or TB-Nil values were ≥ 0.35 IU/mL and ≥ 25% of the Nil value. In both tests, results showing a Nil value > 8.0 IU/mL or Mitogen value < 0.5 IU/mL were considered as indeterminate. In this study, two invalids and four indeterminate ichroma™ IGRA-TB results were excluded from the analysis of both tests.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics for Windows, version 22.0 (IBM Inc., Armonk, NY, USA). and MedCalc Statistical Software version 19.4.1 (MedCalc Software Bvba, Ostend, Belgium). Continuous variables were compared by the Mann-Whitney *U* test or Kruskal-Wallis test, and categorical variables were compared using the chi-squared test or Fisher's exact test. Continuous variables are presented as medians with interquartile ranges (IQRs), and categorical data are presented as frequencies and percentages.

Because there is no gold standard test for the diagnosis of LTBI, comparability between the QFT-PLUS and ichroma™ IGRA-TB tests was evaluated by calculating the overall percentage of concordant results and Cohen's kappa coefficient. Kappa values of < 0.40, 0.40 to < 0.60, 0.60 to < 0.80, and > 0.80 were interpreted as fair, moderate, substantial, and near-perfect agreement, respectively [28].

For the quantitative analysis of both tests, IFN-γ values of the TB antigen tubes were compared by the Wilcoxon signed-rank test. The overall concordance between the two tests was evaluated by calculation of the intraclass correlation coefficient (ICC). A Bland-Altman plot was used to analyze the agreement between the two tests with mean differences between the measurements, which represent the estimated bias and 95% upper and lower limits of the agreement. Results with IFN-γ values of > 10 IU/mL in the QFT-PLUS test were excluded from quantitative analysis because the ELISA cannot accurately measure values > 10 IU/mL.

To improve the performance agreement between the two tests and set a new optimal cut-off value of the ichroma™ IGRA-TB test, an ROC curve analysis was performed assuming the positive result of the reference method—the QFT-PLUS test—as the true LTBI. The area under the curve (AUC) was reported with a 95% confidence interval (CI). Two-tailed *p* values < 0.05 were considered statistically significant.

Results

Clinical characteristics of the enrolled patients

Of the enrolled 152 patients, seven patients were excluded due to active TB (n = 1) or invalid/indeterminate ichroma™ IGRA-TB results (n = 6). As a result, 145 patients were included in the final analysis. Among them, 83 (57.2%) patients had inflammatory bowel disease (IBD) including CD and UC, 44 (30.3%) had RA, and 18 (12.4%) had SpA (ankylosing spondylitis, n = 17; psoriatic arthritis, n = 1).

The baseline clinical characteristics of the 145 patients are presented in Table 1. The median age was 40.5 years (IQR: 27.0–56.0) and 72 (49.7%) were men; 140 (96.6%) received BCG vaccination, 140 (96.6%) had no contact history with a patient with TB, seven (4.8%) had received anti-TB treatment, three (2.1%) had diabetes mellitus, eight (5.5%) had hypertension, one (0.7%) had undergone kidney transplantation, and 94 (64.8%) were taking immunosuppressants. Most of the baseline clinical variables such as age, sex, comorbidity, and baseline medications were significantly different according to the underlying IMIDs.

Comparison between QFT-PLUS and ichroma™ IGRA-TB tests

Based on each manufacturer-recommended cut-off values, 11 (7.6%) and 20 (13.8%) patients showed positive IFN-γ responses with the ichroma™ IGRA-TB and QFT-PLUS test, respectively. In both tests, the positive rates were the highest in the RA group (range, 15.9–27.3%) and the lowest in the IBD group (range, 2.4–6.0%) (Table 2).

The ichroma™ IGRA-TB and QFT-PLUS tests showed a moderate degree of agreement, with an overall concordance rate of 91.0% (132/145) and a kappa value of 0.535 (95% CI: 0.317–0.754). The IBD group, RA group, and the SpA group had overall agreement rates of 96.4%, 82.2%, and 83.3%, respectively, and kappa values of 0.556, 0.539, and 0.308, respectively (Table 3).

There were 13 cases of discordant results: 11 cases had negative results in ichroma™ IGRA-TB and positive results in QFT-PLUS, and two cases had positive results in

Table 1 Baseline characteristics of the 145 patients for biologic agents with both valid ichroma™ IGRA-TB and QFT-PLUS results

	Total (n = 145)	IBD (n = 83)	RA (n = 44)	SpA (n = 18)	p value
Age, yr (interquartile range)	40.5 (27.0–56.0)	31.0 (24.0–40.0)	59.5 (52.0–67.5)	40.5 (27.0–51.0)	< 0.001
Male	72 (49.7%)	51 (61.4%)	9 (20.5%)	12 (66.7%)	< 0.001
BCG vaccination					0.013
Yes	140 (96.6%)	83 (100%)	40 (90.9%)	17 (94.4%)	
Unknown	5 (3.4%)	0	4 (9.1%)	1 (5.6%)	
Previous contact with a TB patient					0.110
None	140 (96.6%)	82 (98.8%)	42 (95.5%)	16 (88.9%)	
Yes	5 (3.4%)	1 (1.2%)	2 (4.5%)	2 (11.1%)	
Previous history of TB treatment	7 (4.8%)	4 (4.8%)	2 (4.5%)	1 (5.6%)	> 0.99
Comorbidity					
Diabetes mellitus	3 (2.1%)	0	2 (4.5%)	1 (5.6%)	0.102
Hypertension	8 (5.5%)	1 (1.2%)	5 (11.4%)	2 (11.1%)	0.019
Kidney transplantation	1 (0.7)	0 (0)	0 (0)	1 (5.6%)	0.124
Medications at baseline					
Glucocorticoid	50 (34.5%)	19 (22.9%)	29 (65.9%)	2 (11.1%)	< 0.001
Purine analogues*	51 (35.2%)	49 (59.0%)	2 (4.5%)	0	< 0.001
Methotrexate	29 (20%)	2 (2.4%)	27 (61.4%)	0	< 0.001
Leflunomide	3 (2.1%)	0	3 (6.8%)	0	0.067
Biologics	22 (15.2%)	19 (22.9%)	0	3 (16.7%)	0.003
Tacrolimus	2 (1.4%)	0	1 (2.3%)	1 (5.6%)	0.091
Mycophenolate mofetil	1 (0.7%)	0	0	1 (5.6%)	0.124
Immunosuppressants† at baseline	94 (64.8%)	61 (73.5%)	29 (65.9%)	4 (22.2%)	< 0.001

IGRA interferon-gamma release assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold PLUS, *IBD* inflammatory bowel disease including Crohn's disease and ulcerative colitis, *RA* rheumatoid arthritis, *SpA* spondyloarthropathy including ankylosing spondylitis and psoriatic arthritis

*Including azathioprine and 6-mercaptopurine

† Including purine analogues, methotrexate, leflunomide, biologics, tacrolimus, and mycophenolate mofetil

ichroma™ IGRA-TB and negative results in QFT PLUS. Except for one case with positive ichroma™ IGRA-TB and negative QFT PLUS results, the 12 patients with discordant results had TB-Nil IFN-γ levels of less than 1.0 IU/mL. None of the discordant cases developed active TB during follow-up. Statistical analysis could not be performed in discordant cases because of the small number of patients. The clinical characteristics of the 13 patients with discordant results are summarized in Table 4.

Quantitative analysis and cut-off value determination in ichroma™ IGRA-TB test

Three patients with TB1-Nil or TB2-Nil IFN-γ levels of > 10 IU/mL were excluded from the quantitative analysis. In the whole patient cohort, the median (IQR) IFN-γ levels of TB-Nil (0.04 [−0.02–0.12]), TB1-Nil (0.01 [0.0–0.1]), and TB2-Nil (0.01 [−0.01–0.07]) were not significantly different (p = 0.318). The ICC values showed a moderate correlation

Table 2 Proportion of patients with positive results in each test

	ichroma™ IGRA-TB	QFT-PLUS (TB1 or TB2)	QTF-PLUS (TB1)	QTF-PLUS (TB2)
All (n = 145)	11 (7.6%)	20 (13.8%)	19 (13.1%)	20 (13.8%)
IBD (n = 83)	2 (2.4%)	5 (6.0%)	5 (6.0%)	5 (6.0%)
RA (n = 44)	7 (15.9%)	12 (27.3%)	11 (25.0%)	12 (27.3%)
SpA (n = 18)	2 (11.1%)	3 (16.7%)	3 (16.7%)	3 (16.7%)

IGRA interferon-gamma release assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold PLUS, *TB1* TB1 antigen tube, *TB2* TB2 antigen tube, *IBD* inflammatory bowel disease including Crohn's disease and ulcerative colitis, *RA* rheumatoid arthritis, *SpA* spondyloarthropathy including ankylosing spondylitis and psoriatic arthritis

Table 3 Concordance between the ichroma™ IGRA-TB test and the QFT-PLUS test

	ichroma™ IGRA-TB	QFT-PLUS		Overall agreement	Kappa (95% CI)
		Positive	Negative		
Total (n = 145)	Positive	9	2	91.0%	0.535 (0.317–0.754)
	Negative	11	123		
IBD (n = 83)	Positive	2	0	96.4%	0.556 (0.114–0.998)
	Negative	3	78		
RA (n = 44)	Positive	6	1	82.2%	0.539 (0.250–0.828)
	Negative	6	31		
SpA (n = 18)	Positive	1	1	83.3%	0.308 (-0.283–0.898)
	Negative	2	14		

IGRA interferon-gamma release assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold Plus, *CI* confidence interval, *IBD* inflammatory bowel disease including Crohn's disease and ulcerative colitis, *RA* rheumatoid arthritis, *SpA* spondyloarthropathy including ankylosing spondylitis and psoriatic arthritis

between the ichroma™ IGRA-TB and QFT-PLUS: the ICC for TB1-Nil and TB-Nil IFN-γ levels was 0.542 (95% CI: 0.362–0.671) and the ICC for TB2-Nil and TB-Nil IFN-γ levels was 0.649 (95% CI: 0.512–0.748).

Figure 1 shows the Bland-Altman difference plots representing the differences in the IFN-γ levels between the two tests against the average of two measurements. For TB1-Nil and TB-Nil, the mean difference of IFN-γ levels was 0.1 (limits of agreement: −1.6–1.8). For TB2-Nil and TB-Nil, the mean difference of IFN-γ levels was also 0.1 (limits of agreement: −1.8–1.9).

To determine an optimal cut-off value for the ichroma™ IGRA-TB test, an ROC analysis was performed based on the QFT-PLUS results in which 20 (13.8%) patients were

Table 4 Characteristics of the 13 patients with discordant results between ichroma™ IGRA-TB and QFT-PLUS tests

ichroma™ IGRA-TB result	ID	Age (year)	Sex	Previous contact with a TB patient	Occurrence of TB during follow-up	Underlying disease	Medications	IFN-γ (IU/mL)		
								ichroma™ IGRA-TB	QFT-PLUS	
								TB-Nil	TB1-Nil	TB2-Nil
Negative	7	35	M	None	None	CD	5-ASA, azathioprine	0.28	1.17	1.57
	15	48	M	None	None	UC, HTN	5-ASA	0.21	0.74	0.77
	19	79	F	None	None	RA, DM, HTN	MTX, HCQ, GC	0.01	0.27	0.46
	58	46	M	None	None	RA	MTX, SSZ, HCQ, GC, NSAID	0.77*	0.73	0.6
	106	56	F	None	None	RA	NSAID	0.28	1.58	1.86
	109	53	M	None	None	UC	5-ASA, azathioprine	0.32	1.02	0.72
	112	61	F	>2 years	None	AS	SSZ, NSAID	0.12	0.41	0.59
	128	42	M	None	None	RA	MTX, GC	0.07	0.75	1.34
	139	56	M	None	None	AS	SSZ, GC	0.13	0.78	0.72
	161	68	M	None	None	RA	-	0.34	1.69	1.18
	189	43	F	None	None	RA	MTX, GC	0.22	0.35	0.42
Positive	87	32	F	None	None	AS	SSZ, NSAID	0.87	−0.08	−0.08
	155	69	F	None	None	RA	MTX, GC	8.17	0.04	0.09

IGRA interferon-gamma release assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold Plus, *IFN-γ* interferon-gamma, *TB-Nil* TB antigen minus Nil tube interferon-gamma value, *TB1-Nil* TB1 antigen minus Nil tube interferon-gamma value, *TB2-Nil* TB2 antigen minus Nil tube interferon-gamma value, *M* male, *F* female, *UC* ulcerative colitis, *RA* rheumatoid arthritis, *AS* ankylosing spondylitis, *HTN* hypertension, *DM* diabetes mellitus, *5-ASA* 5-aminosalicylic acid, *MTX* methotrexate, *HCQ* hydroxychloroquine, *GC* glucocorticoid, *SSZ* sulfasalazine, *NSAID* non-steroidal anti-inflammatory drug

*Less than 25% of Nil value

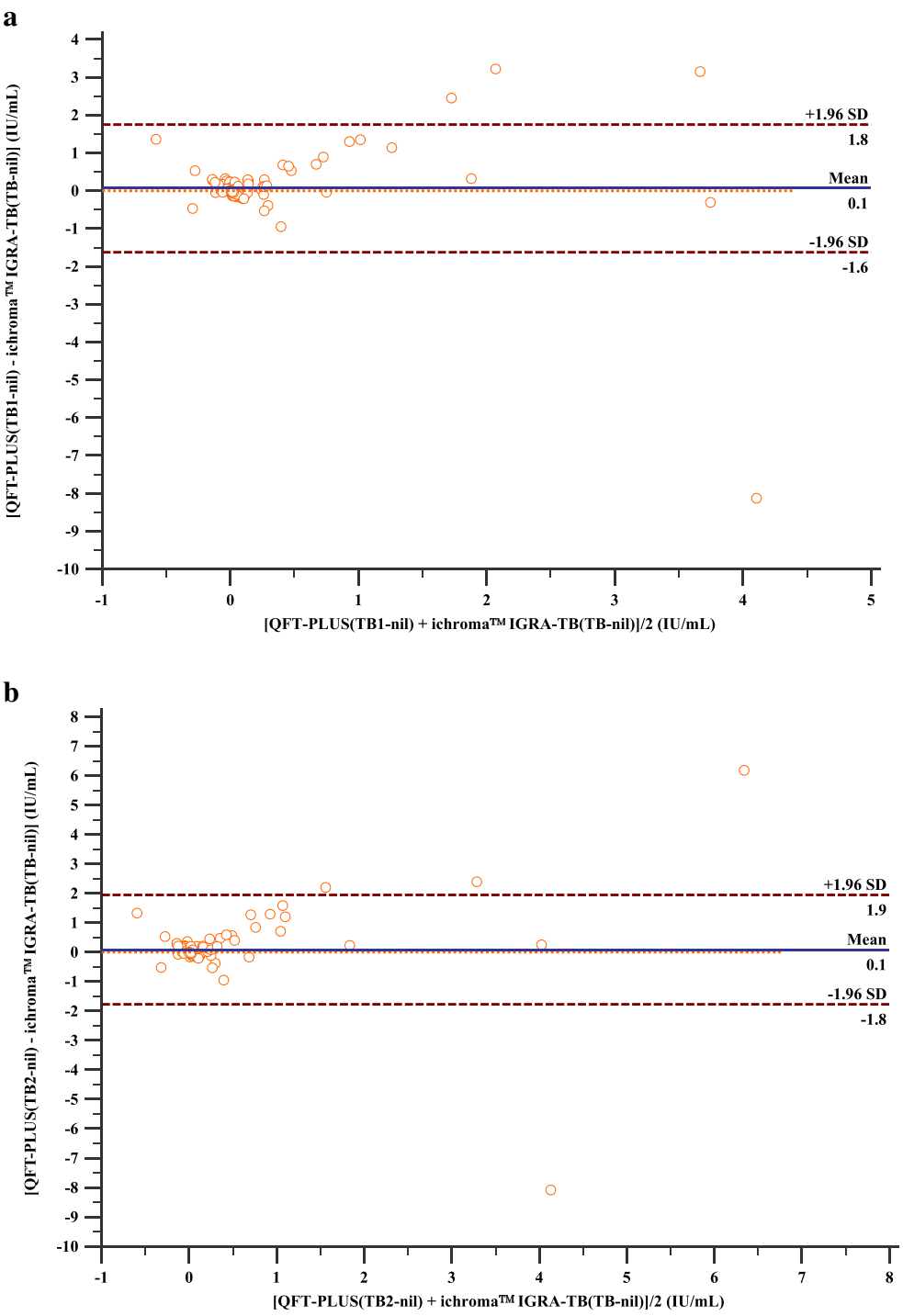

Fig. 1 Bland-Altman difference plots for IFN-γ levels measured by ichroma™ IGRA-TB and QFT-PLUS assays. (**a**) For IFN-γ levels of TB1-Nil and TB-Nil tube. (**b**) For IFN-γ levels of TB2-Nil and TB-Nil tube. *IFN-γ* interferon-gamma, *IGRA* interferon-gamma release assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold PLUS

confirmed to have LTBI (Fig. 2). The AUC value was 0.921 (95% CI: 0.864–0.959); according to the Youden index, the calculated cut-off value for IFN-γ positivity was > 0.20 IU/mL with a sensitivity of 80.0% (95% CI, 56.3–94.3) and a specificity of 93.6% (95% CI, 87.8–97.2). However, to further improve the specificity with a minor decrease in sensitivity, we chose a cut-off value of > 0.21 IU/mL, which resulted in a sensitivity of 75.0% (95% CI, 50.9–91.3) and a specificity of 95.0% (95% CI, 89.8–98.2).

With the new cut-off value, 14 (9.7%) and 20 (13.8%) patients showed positive IFN-γ responses in the ichroma™ IGRA-TB test and the QFT-PLUS test, respectively. The overall concordance rate between the two tests was improved from 91.0 to 93.1% (135/145) and the kappa value also

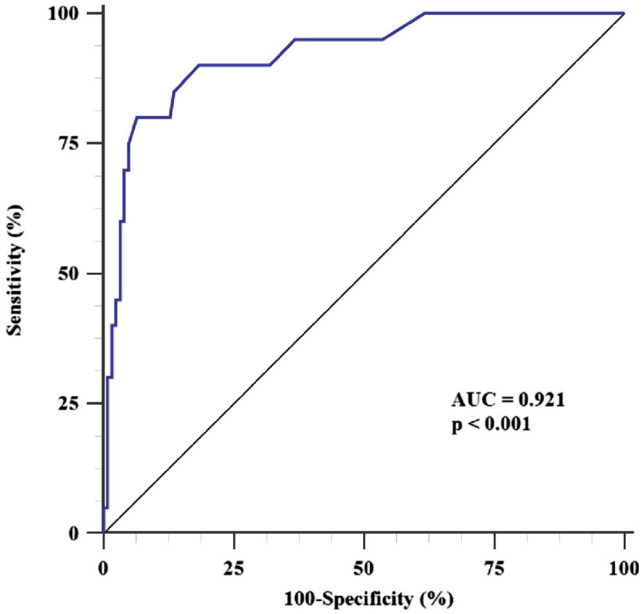

Fig. 2 ROC curve of the ichroma™ IGRA-TB for diagnosing latent tuberculosis infection based on the results of the QFT-PLUS assay. *AUC* area under the curve, *IGRA* interferon-gamma releasing assay, *TB* tuberculosis, *QFT-PLUS* QuantiFERON-TB Gold PLUS

increased from 0.535 to 0.668 (95% CI: 0.478–0.858). There were ten cases of discordant results with the cut-off value: eight cases with negative ichroma™ IGRA-TB and positive QFT-PLUS results and two cases with positive ichroma™ IGRA-TB and negative QFT PLUS results.

Discussion

In this study carried out in a country with an intermediate burden of TB, we showed that the ichroma™ IGRA-TB assay and the QFT-PLUS assay have a high agreement for the diagnosis of LTBI in IMID patients who are scheduled to undergo therapy with biologic agents such as TNF-α inhibitors. Although the test performances were slightly different according to the underlying IMIDs, which is likely due to differences in sample size, the overall agreement was high at 91.0% with a kappa value of 0.535. The ichroma™ IGRA-TB test is expected to be a useful method for LTBI screening in patients with IMIDs before the initiation of biologic therapies.

The majority (85%, 11/13) of the discrepant cases showed positive results in QFT-PLUS and negative results in ichroma™ IGRA-TB. Accordingly, the sensitivity of the ichroma™ IGRA-TB test might be lower than that of the QFT-PLUS. In another preliminary study, we measured the IFN-gamma levels using both LFA and QFT-PLUS kits in the supernatant extracted from the QFT-PLUS assay and compared the results in patients with active TB; as a result, we found there was no significant difference in the positive rates between the two methods (unpublished). Therefore, the reason for the lower sensitivity of the ichroma™ IGRA-TB test might be due to the self-developed TB-specific antigen pool or the tube itself rather than the detection method, LFA. However, in this study, the positive rates in both tests were relatively low, even though the overall concordance rate was high. Further studies with more positive cases are needed to conclusively determine the test performance. In addition, the self-developed TB-specific antigen pool may need to be upgraded to improve the test performance.

According to the previous studies [29, 30], it has been reported that there was substantial variability with IGRA results in the borderline range of less than 1.0 IU/mL. In the present study, the majority (92.3%, 12/13) of the discrepant cases also had IFN-γ levels in the borderline range, which might be the main reason for the discrepancy between the two tests. Further studies with a large sample size are needed to evaluate the performance of the ichroma IGRA-TB™ and QFT-PLUS in the borderline range of IFN-γ levels.

In a previous study in which IFN-γ levels in QFT-GIT tubes were measured using the QFT-GIT and ichroma™ IGRA-TB assays, the IFN-γ levels were lower in the ichroma™ IGRA-TB assay and a cut-off value of > 0.21 IU/mL was thus suggested [27]. Likewise, in our current study, the mean difference of IFN-γ levels between QFT-PLUS and ichroma™ IGRA-TB assay was 0.1 IU/mL, and the optimal cut-off value of > 0.21 IU/mL was chosen based on results from the ROC analysis. When the new cut-off value was adopted, the overall concordance rate was increased to 93.1% with a kappa value of 0.668, and three discordant cases (positive in QFT-PLUS and negative in ichroma™ IGRA-TB) were changed to concordant cases (positive in both tests). Therefore, our results suggest that the manufacturer-recommended cut-off value of the ichroma™ IGRA-TB assay should be set at a lower level when screening for LTBI but not for active TB. In order to develop and validate a new cut-off value for the ichroma™ IGRA-TB, the following issues should be addressed in future studies: alpha priori power calculation before sample collection, the inclusion of a healthy control group, and re-calibration of a test introduces QC bias.

Considering the high risk of patients with IMIDs for developing active TB, LTBI screening in such patients before starting biologics such as TNF-α inhibitors is a routine clinical practice in many countries as recommended by the WHO [11]. IGRAs are commonly used to diagnose LTBIs with better sensitivity and specificity than TST, especially in countries with a high rate of BCG vaccination. Moreover, due to the lower sensitivity of TST in immunocompromised hosts, IGRAs are included in the diagnostic process for excluding LTBI in South Korea [31]. Currently, commercially available IGRAs are composed of ELISA-based tests (QFT-GIT and QFT-PLUS) and Enzyme-Linked ImmunoSpot-based tests (T-SPOT.TB). In South Korea, ELISA-based IGRAs have been more commonly used than T-SPOT.TB due to their

convenience. However, ELISA-based IGRAs are difficult to be utilized in low-resource settings because they are expensive, time-consuming, and require specialized equipment and skilled personnel. On the other hand, LFA-based IGRAs such as the ichroma™ IGRA-TB have several advantages over pre-existing IGRAs including short turn-around time (<30 min), low-cost, and ease of use without the need for skilled personnel. Therefore, in resource-limited settings, LFA-based IGRAs could be used as the point-of-care test for the rapid diagnosis of LTBI in patients with high risks for developing TB.

This study has several limitations. First, it was conducted in patients with chronic IMIDs at a single center. Therefore, the results may not be readily generalizable in other settings. Second, we were not able to evaluate the exact sensitivity and specificity of the ichroma™ IGRA-TB assay due to the lack of a gold standard test for the diagnosis of LTBI, which is an inherent limitation in studies on LTBI. Finally, due to the relatively small number of study participants, we were not able to perform statistical analysis to determine the factors influencing discordant results between the two tests.

In conclusion, our study showed a high agreement between the ichroma™ IGRA-TB assay and the QFT-PLUS test. The ichroma™ IGRA-TB assay could be used as a point-of-care screening method for the diagnosis of LTBI in patients with IMID before starting biologics, especially in resource-limited settings.

Code availability Not applicable.

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Data availability Not applicable.

Declarations

Ethics approval This study was approved by the Institutional Review Board of Asan Medical Center (IRB No.: 2017-1303). All participants provided written informed consent.

Consent to participate Obtained.

Consent to publication Obtained.

Disclosures None.

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