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Comparison of diagnostic accuracy of QuantiFERON-TB Gold Plus and T-SPOT.TB in the diagnosis of active tuberculosis in febrile patients

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Abstract

Objective: This study aimed to compare the accuracy of QuantiFERON-TB Gold Plus (QFT-Plus) and T-SPOT.TB for diagnosing active tuberculosis (ATB) in febrile patients, to explore influencing factors of positive results and to verify the potential value of QFT-Plus in the identification of ATB and latent tuberculosis infection (LTBI).

Methods: A total of 240 febrile patients with ATB ($n = 80$) and non-ATB ($n = 160$) were recruited to assess the accuracy of QFT-Plus and T-SPOT.TB for diagnosing ATB. Multivariable logistic regression was used to analyze the influencing factors of positive results.

Results: The proportion of indeterminate results (ITRS) in QFT-Plus and T-SPOT.TB were 3.3% and 0%, respectively. The consistency between the results of the QFT-Plus and T-SPOT.TB was substantial. The area under the receiver operating characteristic curve (AUROC) of the QFT-Plus and T-SPOT.TB for diagnosing ATB was 0.792 and 0.849 ($p = 0.070$), respectively. The sensitivity of differentiating ATB from non-ATB was 92.2% in QFT-Plus versus 95.0% in T-SPOT.TB. The influencing factors of T-SPOT.TB positive result were male (odds ratio (OR) = 2.33, 95% confidence interval (CI) 1.27–4.26, $p = 0.006$), evidence of previous TB (OR 11.36, 95% CI 4.62–27.94, $p < 0.001$), while male (OR = 3.17, 95% CI 1.73–5.84, $p < 0.001$), evidence of previous TB (OR = 7.58, 95% CI 3.60–15.98, $p < 0.001$), and use of immunosuppressant (OR = 0.49, 95% CI 0.260.94, $p = 0.030$) were influencing factors for QFT-Plus positive result. There was no significant difference in QFT-Plus in differentiating ATB from LTBI in febrile patients.

Conclusion: There was no significant difference between QFT-Plus and T-SPOT.TB for diagnosing ATB in febrile patients. QFT-Plus is prone to ITRS. The influencing factors including males, evidence of the previous TB, and use of immunosuppressant should be considered when interpreting positive results.

KEYWORDS
active tuberculosis, diagnostic accuracy, febrile, QFT-Plus, T-SPOT.TB

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1 | INTRODUCTION

Tuberculosis (TB) is a chronic disease caused by *Mycobacterium tuberculosis* (Mtb) infection, potentially affecting various organs and tissues. According to the World Health Organization (WHO), there were approximately 9.87 million incident cases and 1.28 million death cases in 2020. China, in particular, is one of the 30 high TB burden countries, accounting for 8.5% (0.84/9.87 million) of global new cases alone.[1]

The etiology of febrile patients is complex, and fever is one of the most common clinical manifestations of TB. Discriminating ATB from non-ATB in febrile patients remains challenging when etiological evidence is absent. According to WHO's global tuberculosis report, only 55% of pulmonary TB is diagnosed microbiologically in China.[1] Including extrapulmonary TB, less than 30% of the patients diagnosed with ATB were microbiologically confirmed in general hospitals.[2] Therefore, how accurately identifying ATB in clinically febrile patients in a timely way is crucial for precision treatment and disease control. At present, there are two Interferon-gamma release assays (IGRAs) used for the diagnosis of Mtb infection: QuantiFERON-TB Gold In-Tube (QFT-GIT) and T-SPOT.TB. QFT-GIT measures the concentration of Interferon-gamma (IFN-γ) via an Enzyme-linked Immunosorbent Assay (ELISA) using a specific volume of whole blood. In comparison, T-SPOT.TB measures the frequencies of IFN-γ-secreting cells via Enzyme-linked Immunospot Assay (ELISPOT).[3] IGRAs can assist in the diagnosis of ATB when etiological evidence is not available. However, it cannot differentiate ATB from LTBI, causing its limited application in high TB burden areas.[4,5]

QFT-Plus has two distinct TB antigen tubes: TB Antigen Tube 1 (TB1) and TB Antigen Tube 2 (TB2). Both tubes contain peptide antigens from the MTB-complex-associated antigens, ESAT-6 and CFP-10. Both the TB1 tube and TB2 tubes contain peptides from ESAT-6 and CFP-10 that are designed to elicit cell-mediated immune (CMI) responses from $CD4^+$ T cells; the TB2 tube contains an additional set of peptides targeted to the induction of CMI responses from $CD8^+$ T cells. Previous studies have shown that the antigen-driven expansion of Mtb-specific $CD8^+$ T cells response can be more frequently detected in individuals with ATB and LTBI,[6,7] and even associate with TB disease progression.[8] This further improves the efficiency of Mtb infection diagnosis and may apply to differentiate ATB from LTBI. However, the ATB group in previous studies only included microbiologically confirmed pulmonary tuberculosis, and the LTBI group only included healthy individuals or nonimmunosuppressive patients with few complications, leading to the probable overestimation of diagnostic accuracy. Yet, few head-to-head comparative trials are comparing QFT-Plus and T-SPOT.TB for the accuracy in diagnosing ATB.[9–11] Even more, febrile patients who may be confused with ATB in clinical practice had not been reported.

Accordingly, it is of great importance to compare QFT-Plus and T-SPOT.TB for diagnosing ATB in febrile patients in high TB burden countries, to explore the influencing factors of positive results, and to verify the potential value of QFT-Plus in the identification of ATB and LTBI.

2 | PATIENTS AND METHODS

2.1 | Study design and participants

This study applied a case-control study design. Febrile patients (age ≥18 years old) diagnosed with ATB either microbiologically or clinically in Peking Union Medical College Hospital (PUMCH) and Beijing Chest Hospital from April 2020 to July 2021 were enrolled into the case group, while those excluded from ATB during the same period were enrolled into the control group (Tables 1 and 2).

Participants' demographic information and laboratory examination were gathered. After the collection of peripheral blood, QFT-Plus and T-SPOT.TB were tested simultaneously to evaluate the consistency of the results and the sensitivity, specificity, predictive value (PV), and likelihood ratio (LR) for diagnosing ATB were compared. In addition, the influencing factors of positive results were identified, and the potential value of QFT-Plus in the identification of ATB and LTBI was verified (Figure 1).

TABLE 1 The inclusion and exclusion criteria for the ATB and non-ATB group

Diagnostic category	Inclusion criteria	Exclusion criteria
ATB	Microbiologically confirmed ATB: (a) Positive results of Mtb culture/GeneXpert/PCR/ acid-fast staining of sputum or other samples AND (b) clinical manifestations (fever, cough, chest pain, night sweats, and weight loss, etc.) AND (c) imaging characteristics suggesting ATB AND (d) untreated or had received ≤14 days of anti-tuberculosis treatment.	Patients with hematologic malignancies, HIV infection, during pregnancy or breastfeeding
	Clinical diagnosed ATB: (a) Clinical manifestations (fever, cough, chest pain, night sweats, and weight loss, etc.) AND (b) laboratory results and Imaging characteristics suggesting ATB. AND (c) Appropriate response to anti-TB therapy.	
Non-ATB	(a) Clinical manifestations (fever) AND (b) laboratory results and Imaging characteristics not suggesting ATB AND (c) the diagnosis was confirmed and the treatment was effective.	

ATB, active tuberculosis; Mtb, *Mycobacterium tuberculosis*.

TABLE 2 Non-ATB group diseases detail

Disease classification (n = 160)	Diagnosis
Infectious diseases (44)	Bacterial infectious diseases (24)
	Virus infective diseases (13)
	Atypical pathogen infection (7) Mycoplasma pneumonia (2) Nontuberculous mycobacterial lung disease (1) Pulmonary nocardiasis (1) Typhoid (1) Pneumocystis jirovecii pneumonia (1) Brucellosis (1)
Noninfectious inflammatory diseases (82)	Autoimmune diseases (77)
	Systemic autoinflammatory diseases (5)
Tumor diseases (14)	Solid tumor (14)
Endocrine and metabolic diseases (4)	Polycystic ovary syndrome (2) Subacute thyroiditis (1) Climacteric syndrome (1)
Others (16)	Drug fever (5) Idiopathic constrictive pericarditis (2) Chronic granulomatous disease (3) Pulmonary embolism (1) Physiological fever (1) Pulmonary alveolus proteinosis (1) Metabolic myopathies (1) Organizing pneumonia (1) Kikuchi-Fujimoto disease (1)

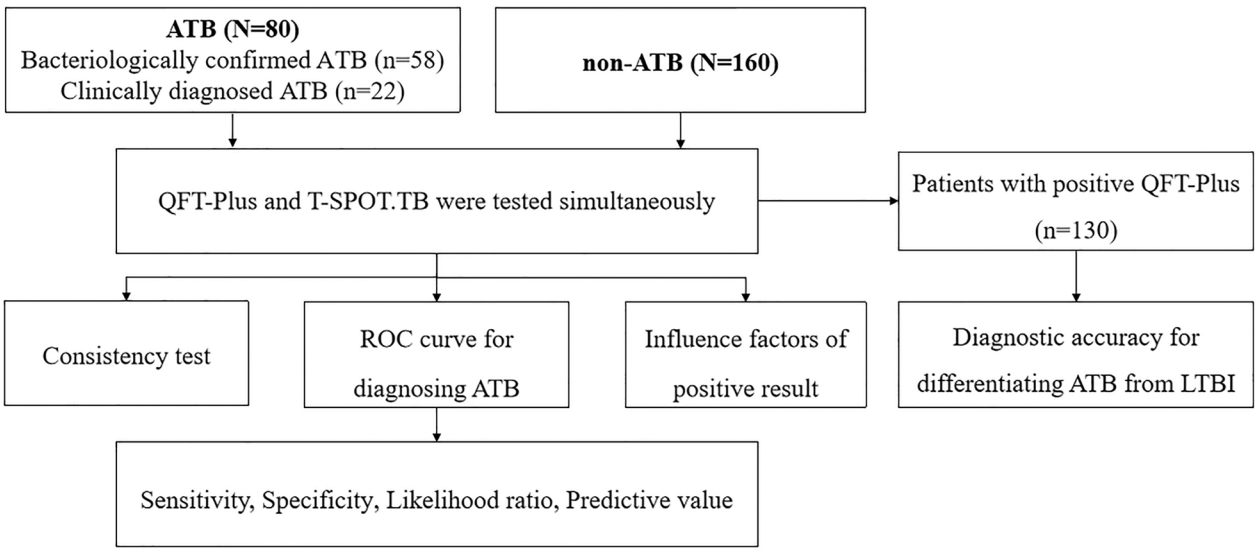

FIGURE 1 Study flow chart

TABLE 3 Demographic characteristics and laboratory examination of the subjected patients

Characteristics	ATB ($n = 80$)		Non-ATB ($n = 160$)
	Microbiologically confirmed ($n = 58$)	Clinical diagnosed ($n = 22$)	
Male sex, *N* (%)	37 (63.8)	13 (54.2)	75 (46.9)
Age (years), median, IQR	50 (31, 62)	55 (26, 67)	51 (38, 64)
Evidence of previous TB, *N* (%)	43 (86)	10 (45.5)	27 (16.9)
Glucocorticoid/immunosuppressive/ biological agents, *N* (%)	6 (10.3)	6 (27.3)	67 (41.9)
Comorbidity, *N* (%)	13 (21.2)	5 (22.7)	96 (60)
Diabetes	10 (17.2)	4 (18.2)	12 (7.5)
Solid tumor	0	0	14 (8.8)
Renal failure	0	0	2 (1.3)
Autoimmune disease	3 (5.2)	1 (4.5)	75 (46.9)
Blood test			
White blood cell count, 10^9/L (IQR)	6.14 (5.01, 8.59)	6.58 (4.80, 8.80)	7.03 (5.19, 9.85)
Neutrophil count, 10^9/L (IQR)	4.39 (3.32, 6.08)	4.50 (2.92, 4.50)	4.64 (3.26, 7.07)
Lymphocyte count, 109/L (IQR)	1.25 (0.78, 1.72)	1.48 (1.17, 2.01)	1.54 (1.06, 2.06)
Hemoglobin, g/L (IQR)	120 (104, 135)	117 (101, 140)	116 (97, 135)
Platelet count, 109/L (IQR)	307 (224, 367)	296 (229, 391)	228 (166, 311)

2.2 | Laboratory measurements

2.2.1 | QFT-Plus

Following QFT-Plus (Qiagen, Hilden, Germany) manufacturer's instructions, 1 ml blood was collected by venipuncture into each QFT-Plus blood collection tube (Nil tube, TB1 tube, TB2 tube, Mitogen tube). Blood was incubated at 37°C ± 1°C as soon as possible within 6 h of collection. Following a 16–24 h incubation period, the tubes were centrifuged 3000 × *g* for 15 min and the plasma was removed. Dynex-DS2 automated ELISA system was used for operation and QFT-Plus v2.71 software for analysis.

2.2.2 | T-SPOT.TB

Following T-SPOT.TB (Oxford Immunotec Ltd, Oxford, UK) manufacturer's instructions, 4 ml of peripheral venous blood were obtained from subjects into heparin lithium-anticoagulant tubes and tested within 6 h of collection. T-SPOT.TB utilized AIM-V (GIBCO™ AIM V Medium liquid, Invitrogen, USA) as nil control, PHA as the positive control, and ESAT-6 and CFP-10 as specific antigens. Peripheral blood mononuclear cells (PBMCs) obtained from each subject were seeded (2.5 × 105 per well) on a plate precoated with the antibody against IFN-γ. Plates were incubated for 16–18 h at 37°C in 5% carbon dioxide. After incubation, wells were developed with a conjugate against the antibody used and an enzyme-substrate. Spot-forming cells (SFCs) were counted with an automated ELISpot reader (AID-ispot, Strassberg, Germany).

Laboratory staff who conducted the assays were all blinded to patients' clinical data. The result criteria for each IGRA were presented in Supplementary Table S1.

2.3 | Statistical analysis

Statistical analysis was performed using IBM-SPSS 26.0 package program. Whether the numeric variable data followed a normal distribution was checked by the Kolmogorov–Smirnov test. The variables with normal distribution were expressed as the mean ± standard deviation, whereas the variables with nonnormal distribution were expressed as the median and interquartile range (IQR). The enumeration data were presented as percentages and 95% confidence intervals (95% CI). Comparisons of continuous variables were performed using the *t*-test and Mann–Whitney *U* test for normal and nonnormal data, respectively. Categorical data were compared using the chi-squared test. The agreement between the two assays was evaluated using Cohen's kappa coefficient. ROC curve was used to compare the accuracy for diagnosing ATB and assess the accuracy of QFT-Plus for differentiating ATB from LTBI, Multivariable logistic regression model was used to explore the influencing factors of positive results (backward LR, entry 0.10, removal 0.05). The sensitivity, specificity, PV, LR, and 95% CI were calculated by medcalc software (MedCalc Software Ltd., Ostend, Belgium). $p < 0.05$ (two-sided) was considered to be statistically significant.

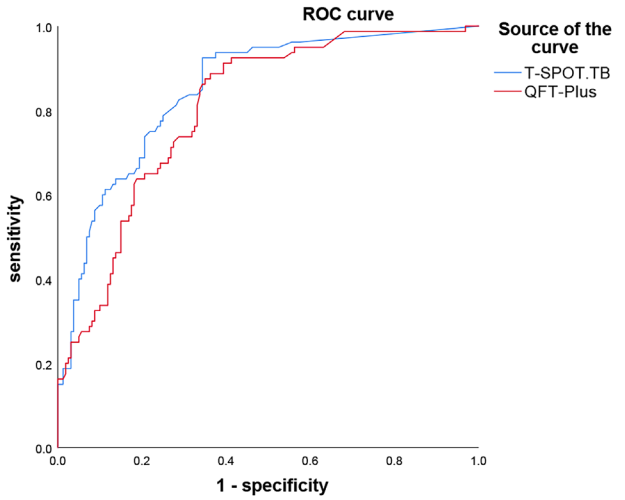

FIGURE 2 ROC curve of the accuracy of QFT-Plus and T-SPOT.TB for diagnosing ATB

3 | RESULTS

3.1 | Baseline characteristics

A total of 240 febrile patients were enrolled; of the 80 patients with ATB, 72.5% (58/80) were microbiologically confirmed and 160 patients were non-ATB. The demographic characteristics and laboratory examination of the study subjects are shown in Table 3.

3.2 | Comparison of accuracy of QFT-Plus and T-SPOT.TB for diagnosing ATB

In 240 febrile patients, the ITRS of QFT-Plus and T-SPOT were 3.3% (8/240) and 0%, respectively. The consistency between QFT-Plus and T-SPOT.TB was substantial (kappa = 0.61, $p < 0.001$). After comparison, QFT-Plus selected TB2-Nil results to be the optimal test variable (Supplementary Figure S1) while T-SPOT.TB selected the sum of frequencies of SFCs (ESAT-6 and CFP-10) as the test variable, and ROC curves were plotted to assess the diagnostic accuracy (Figure 2). The AUROC of QFT-Plus and T-SPOT.TB for diagnosing ATB was 0.792 (95% CI 0.734–0.851) and 0.849 (95% CI 0.799–0.900) ($p = 0.07$), respectively. The sensitivity, specificity, PV, and LR of the two assays for diagnosing ATB are shown in Table 4. In 58 febrile patients with microbiologically confirmed ATB, the sensitivity of QFT-Plus and T-SPOT.TB were 93.1% versus 96.6%, respectively.

3.3 | The accuracy of QFT-Plus TB2-TB1 for differentiating ATB from LTBI

Among 130 febrile patients with positive results of QFT-Plus, 71 patients were diagnosed as ATB, 59 patients were defined as LTBI. The median level of TB2-TB1 in patients with ATB and LTBI were 0.33

TABLE 4 The accuracy of QFT-Plus and T-SPOT.TB for diagnosing ATB

IGRAs	Cut-off value	Sen% (95% CI)	Spe% (95% CI)	PV+% (95% CI)	PV−% (95% CI)	LR+ (95% CI)	LR− (95% CI)
QFT-Plus	0.33 IU/ml	92.2 (83.8, 97.1)	61.9 (53.8, 69.6)	54.6 (45.7, 63.4)	94.1 (87.6, 97.8)	2.42 (1.96, 2.99)	0.13 (0.06, 0.27)
T-SPOT.TB	68 SFC/106 PBMC	95.0 (87.7, 98.6)	52.5 (44.5, 60.4)	50.0 (41.8, 58.2)	95.5 (88.8, 98.8)	2.00 (1.69, 2.37)	0.10 (0.04, 0.25)

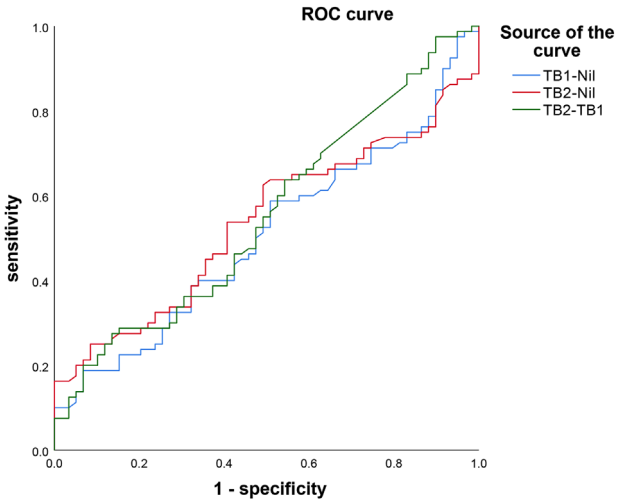

FIGURE 3 ROC curve of the accuracy of QFT-Plus for differentiating ATB from LTBI. TB1-Nil indicates Mtb-specific CD4+T cells IFN-γ release level. TB2-Nil indicates Mtb-specific CD4+T cells and CD8+T cells IFN-γ release level. TB2-TB1 indicates Mtb-specific CD8+T cells IFN-γ release level

IU/ml (IQR, 0–1.25) and 0.17 IU/ml (IQR, 0–0.71), respectively (p = 0.136). ROC curves were plotted to assess the accuracy of the TB1, TB2, TB2-TB1 for differentiating ATB from LTBI, and the AUROC were 0.562 (95% CI 0.463–0.661, p = 0.223), 0.604 (95% CI 0.507–0.701, p = 0.041), and 0.576 (95% CI 0.477–0.674, p = 0.138), respectively (Figure 3).

3.4 | Influencing factors associated with QFT-Plus and T-SPOT.TB positive results

The differences in gender, age, use of glucocorticoid/immunosuppressive/biological agents, evidence of the previous TB, blood routine test, alanine transaminase (ALT), creatinine, high sensitivity c-reactive protein (hsCRP) were compared between IGRAs positive and negative groups. Variables that showed a p < 0.1 were entered into the multivariable logistic regressions model. The results showed the influencing factors of T-SPOT.TB positive result were male (OR 2.33, 95% CI 1.27–4.26, p = 0.006) and evidence of previous TB (OR 11.36, 95% CI 4.62–27.94, p < 0.001) (Table 5), while the influencing factors of QFT- Plus positive result were male (OR 3.17, 95% CI 1.73–5.84, p < 0.001), evidence of previous TB (OR 7.58, 95% CI 3.60–15.98, p < 0.001), and use of glucocorticoid/immunosuppressive/biological agents (OR 0.49, 95% CI 0.26–0.94, p = 0.030) (Table 6).

4 | DISCUSSION

The diagnosis of ATB still is a challenge in clinical practice. Fever is one of the most common clinical manifestations of TB, and febrile patients can interfere with the judgment of clinical physicians to a great extent. As the gold standard, Mtb culture usually takes more than 2 weeks to get a confirmatory result. Acid-fast staining has low sensitivity considering nontuberculosis mycobacterium (NTM) infection cannot be distinguished. Moreover, recent Bacillus Calmette Guerin (BCG) vaccination may cause a false-positive TST result. On the other hand, imaging characteristics have low specificity of TB since they usually overlap with other pulmonary diseases. While PCR and Xpert Mtb/RIF require high levels of Mtb complex in biopsy specimens, IGRAs can greatly assist the diagnosis of ATB when etiological evidence is not available and the results are not confounded by BCG vaccination and are less likely to be confounded by exposure to NTM.

As the new generation of QFT, QFT-Plus contains peptides able to specifically stimulate IFN-γ production by $CD8^+$ T cells to improve the sensitivity of Mtb infection detection, providing the possibility to differentiate ATB from LTBI. Recent studies show that $CD8^+$ T cells play impartial roles in the onset of ATB in recently TB-exposed adults, children, and HIV-infected patients.[8,12] Previous studies have shown that the IFN-γ released by tuberculosis-specified $CD8^+$ T cells is more frequently detected in patients with ATB and LTBI, especially ATB.[6,7,13] In a meta-analysis that evaluated the sensitivity of QFT-GIT and T-SPOT.TB in diagnosing ATB, the pooled sensitivity was 81% for QFT-GIT and 92% for T-SPOT.TB.[14] Another meta-analysis containing 15 studies from 8 countries and regions showed the pooled sensitivity for QFT-Plus was 94%.[15] In our study, the accuracy of QFT-Plus and T-SPOT.TB was similar to those of previous studies. In patients with microbiologically confirmed ATB, the sensitivity of QFT-Plus and T-SPOT.TB were 93.1% and 96.6%, respectively. In addition, a recent study from Japan that included 99 microbiologically confirmed ATB patients demonstrated the sensitivity of QFT-Plus and T-SPOT.TB were 98.9% and 96.9%, respectively, which were much higher than those of previous studies. This might attribute to all enrolled participants being microbiologically confirmed ATB patients under nonimmunosuppressive status.

As for QFT, in addition to factors associated with the experimental operation, it has been reported that malignancy, lymphopenia, HIV infection, hematologic malignancy, use of immunosuppressive agents, and severe tuberculosis may also be risk factors for ITRS of IGRAs due to the insufficient response to mitogen positive control.[16–18] In our study, QFT-Plus ITRS was observed in 8/240 tests (3.3%), 7 out of 8 subjects with QFT-Plus ITRS had diabetes or autoimmune disease and 1 had severe tuberculous pericarditis. ITRS was not observed in T-SPOT.TB in our study. Beffa et al.[19] systematically evaluated the factors influencing the results of the T-SPOT.TB assay. The two parameters found to significantly affect the incidence of ITRS were elderly (>75 years old) and the season during which samples were transported; immunosuppressive status was not associated with ITRS. The possible reason might be T-SPOT.TB is based on the measurement of the IFN-secreting cells in a standardized number of PBMCs via ELISPOT assay, while QFT assay uses ELISA to quantify the IFN-γ response of whole blood; the amount of IFN-γ is expected to be affected by total or T lymphocytes count in QFT assay. Immunosuppressive drugs can efficiently prevent T-cell proliferation by decreasing the expression of activation

TABLE 5 Factors associated with T-SPOT.TB-positive result

Variables	T-SPOT. TB		Univariable analysis	Multivariable analysis	
	Positive ($n = 152$)	Negative ($n = 88$)	p	p	OR (95% CI)
Male, *N* (%)	60 (39.5)	55 (62.5)	0.001	0.006	2.33 (1.27, 4.26)
Age (years), median, IQR	55 (38, 64)	50 (31, 62)	0.234		
Glucocorticoid/immunosuppressive/biological agents, *N* (%)	42 (27.6)	37 (42.0)	0.022		
Evidence of previous TB, *N* (%)	70 (46.1)	6 (6.8)	<0.001	<0.001	11.36 (4.62, 27.94)
White blood cell count, 109/L (IQR)	6.59 (5.14, 9.35)	7.01 (5.11, 9.83)	0.686		
Neutrophil count, 109/L (IQR)	4.50 (3.26, 6.54)	4.89 (2.81, 7.39)	0.482		
Lymphocyte count, 109/L (IQR)	1.46 (1.00, 2.05)	1.45 (1.01, 1.91)	0.795		
Hemoglobin, g/L (IQR)	121 (105, 138)	111 (90, 128)	0.007		
Platelet count, 109/L (IQR)	261 (195, 349)	233 (161, 331)	0.114		
ALT, U/L (IQR)	17 (11, 27)	17 (10, 32)	0.6		
Creatinine, μmol/L (IQR)	68 (56, 79)	63 (51, 83)	0.188		
hsCRP, mg/L (IQR)	14.18 (1.57, 44.76)	7.56 (2.36, 46.13)	0.553		

TABLE 6 Factors associated with QFT-Plus positive result

Variables	QFT-Plus		Univariable analysis	Multivariable analysis	
	Positive ($n = 130$)	Negative ($n = 102$)	p	p	OR (95% CI)
Male sex, *N* (%)	45 (34.6)	66 (64.7)	<0.001	<0.001	3.17 (1.73–5.84)
Age (years), median, IQR	55 (34–63)	51 (36–64)	0.758		
Glucocorticoid/immunosuppressive/biological agents, *N* (%)	29 (22.3)	45 (44.1)	<0.001	0.03	0.49 (0.26, 0.94)
Evidence of previous TB, *N* (%)	64 (49.2)	11 (10.8)	<0.001	<0.001	7.58 (3.60–15.98)
White blood cell count, 109/L (IQR)	6.37 (4.92, 9.00)	7.14 (5.47, 10.21)	0.027		
Neutrophil count, 109/L (IQR)	4.31 (3.22, 6.19)	5.09 (3.42, 7.46)	0.062		
Lymphocyte count, 109/L (IQR)	1.38 (0.97, 1.94)	1.52 (1.06, 1.99)	0.158		
Hemoglobin, g/L (IQR)	122 (106, 140)	111 (95, 131)	0.007		
Platelet count, 109/L (IQR)	261 (199, 354)	242 (166, 325)	0.052		
ALT, U/L (IQR)	16 (10, 26)	18 (12, 35)	0.091		
Creatinine, μmol /L (IQR)	66 (56, 79)	63 (53, 82)	0.274		
hsCRP, mg/L (IQR)	14.18 (1.55, 45.06)	8.62 (2.40, 45.16)	0.662		

markers, and the patients who receive long-term immunosuppressant therapy may have relatively compromised immunocyte function and count.[20] Therefore, compared to QFT, T-SPOT.TB results are less influenced by lymphocyte counts and the use of immunosuppressive agents.

In our study, multivariable analyses of logistic regression showed the influencing factor of QFT-Plus and T-SPOT.TB positive results were male and evidence of the previous TB. Several explanations might exist. First of all, the gender difference in tuberculosis had been reported by WHO showing there are more male tuberculosis patients.[1] Secondly,Social pressures,smoking cigaretttes,and excessive alcohol are risk factors of developing tuberculosis,[21,22] while males have higher prevalence of smoking and drinking compared to females, maybe social pressure as well. The positive rates of Mtb infection among people with radiographic lesions suggest evidence of previous TB was higher than those without lesions[23]; this may relate to that Mtb can sustain a state of persistent replication in granulomatous.[24,25] Hence, those people are more prone to develop ATB.[26] In addition,

the results of QFT-plus may also be affected by immunosuppressive agents and glucocorticoids; it was reported that immunosuppressive agents and glucocorticoids may impair the sensitivity of the QFT test.[27,28] In our study, 74 patients (31.9%) who were treated with glucocorticoid/immunosuppressive/biological agents had a 39.2% (29/74) QFT-Plus positive rate, which was significantly lower than that of those who did not use glucocorticoid/immunosuppressive/biological agents (45/74, 60.8%) ($p < 0.001$). However, there was no significant difference in the positive rate of T-SPOT.TB between the two groups, suggesting the immunosuppressive state has little effect on the diagnostic sensitivity of T-SPOT.TB. The results were similar to the previous studies.[3,29,30]

QFT-Plus is the fourth-generation QFT assay; the newly added antigen elicited a specific CD8 T-cell response. Petruccioli et al.[13] reported the first characterization of CD4 and CD8 T-cell response to QFT-Plus. The study indicated that CD8 T-cell response is mainly due to TB2 stimulation largely associated with ATB. Lee et al.[31] demonstrated ATB had a higher specific CD8 response compared to LTBI. TB2-TB1 was higher in ATB than in LTBI in our study, but the difference did not reach statistical significance. This might be due to a great part of our patients having complex etiology composition and under immunosuppressive status.

In the present study, we compared the accuracy of QFT-Plus and T-SPOT.TB for diagnosing ATB in febrile patients in high TB burden countries, which lack domestic and foreign research. This is of great importance for ATB diagnosis in clinical practice. In addition, we investigated the influencing factors associated with QFT-Plus and T-SPOT.TB of positive results, which may help us to interpret IGRAs results and, in the end, led to the rational clinical use of the IGRAs.

This study has several limitations. First, this is a case-control study, which may overestimate the differential diagnostic accuracy. Second, in individuals with previous TB infection, the different stages of ATB they were at might influence the positive rate of IGRAs. Third, we focused on the differential diagnosis of ATB in febrile patients in this study, and the applicability of this conclusion to asymptomatic TB patients needs to be further evaluated.

In conclusion, there was no significant difference between the QFT-Plus and T-SPOT.TB in differential diagnosing ATB and non-ATB in febrile patients. QFT-Plus might be prone to ITRS. QFT-Plus results should be carefully interpreted, particularly in patients using immunosuppressive agents. The accuracy of QFT-Plus for differentiating ATB from LTBI needs to be evaluated in further studies, especially in clinical patients. Finally, the study results need to be verified by a prospective cohort study with a large sample.

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INFORMED CONSENT

All patients provided their written informed consent.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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