

Interferon-Gamma Release Assay is Not Appropriate for the Diagnosis of Active Tuberculosis in High-Burden Tuberculosis Settings: A Retrospective Multicenter Investigation

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Abstract

Background: Interferon-gamma release assay (IGRA) has been used in latent tuberculosis (TB) infection and TB diagnosis, but the results from different high TB-endemic countries are different. The aim of this study was to investigate the value of IGRA in the diagnosis of active pulmonary TB (PTB) in China.

Methods: We conducted a large-scale retrospective multicenter investigation to further evaluate the role of IGRA in the diagnosis of active PTB in high TB-epidemic populations and the factors affecting the performance of the assay. All patients who underwent valid T-SPOT.TB assays from December 2012 to November 2015 in six large-scale specialized TB hospitals in China and met the study criteria were retrospectively evaluated. Patients were divided into three groups: Group 1, sputum culture-positive PTB patients, confirmed by positive *Mycobacterium tuberculosis* sputum culture; Group 2, sputum culture-negative PTB patients; and Group 3, non-TB respiratory diseases. The medical records of all patients were collected. Chi-square tests and Fisher's exact test were used to compare categorical data. Multivariable logistic analyses were performed to evaluate the relationship between the results of T-SPOT in TB patients and other factors.

Results: A total of 3082 patients for whom complete information was available were included in the investigation, including 905 sputum culture-positive PTB cases, 914 sputum culture-negative PTB cases, and 1263 non-TB respiratory disease cases. The positive rate of T-SPOT.TB was 93.3% in the culture-positive PTB group and 86.1% in the culture-negative PTB group. In the non-PTB group, the positive rate of T-SPOT.TB was 43.6%. The positive rate of T-SPOT.TB in the culture-positive PTB group was significantly higher than that in the culture-negative PTB group ($\chi^2 = 25.118, P < 0.01$), which in turn was significantly higher than that in the non-TB group ($\chi^2 = 566.116, P < 0.01$). The overall results were as follows: sensitivity, 89.7%; specificity, 56.37%; positive predictive value, 74.75%; negative predictive value, 79.11%; and accuracy, 76.02%.

Conclusions: High false-positive rates of T-SPOT.TB assays in the non-TB group limit the usefulness as a single test to diagnose active TB in China. We highly recommend that IGRAs not be used for the diagnosis of active TB in high-burden TB settings.

Key words: Active Tuberculosis; Diagnosis; Interferon-Gamma Release Assay

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INTRODUCTION

Tuberculosis (TB) is a major health problem throughout the world, especially in developing countries. According to the World Health Organization (WHO), worldwide, 10.4 million people are estimated to have fallen ill with TB in 2015, and TB killed 1.8 million people (1.4 million human immunodeficiency virus [HIV]-negative and 0.4 million HIV-positive people).^[1] TB is also a serious problem in China. The WHO has estimated an incidence of 66/100,000 TB cases in China in 2015.^[1]

The rapid detection of mycobacteria and successful treatment of infectious patients is important for controlling and preventing TB. Chest X-rays are often used in pulmonary TB (PTB) screening and have the advantages of being simple and inexpensive. However, in smear-negative PTB, many cases show atypical or nonspecific patterns and are difficult to differentiate from other pulmonary diseases.^[2] Smear microscopy has low sensitivity. Culture of mycobacteria requires several weeks to obtain the results and has low sensitivity.

A recent breakthrough in the diagnosis of TB and latent TB infection is the introduction of interferon-gamma release assays (IGRAs), in which the production of interferon-gamma (IFN- γ) in response to *Mycobacterium tuberculosis* (MTB)-specific antigens is measured. There are currently two types of commercial IGRAs available: the QuantiFERON-TB Gold In-Tube test and the T-SPOT.TB blood test. The sensitivities of the IGRAs were reported to be high in detecting active TB patients in low TB-endemic countries in previous studies.^[3,4] However, a recent study conducted in Poland did not show that a combination of IGRA and TST might be a step forward in the diagnosis of culture-negative TB cases.^[5] The results from high TB-endemic countries were also different. Some studies indicated that the T-SPOT.TB assay is a promising diagnostic test for active PTB,^[6-8] but other studies showed that IGRA was insufficient for the diagnosis of PTB.^[9,10] In this study, we conducted a large retrospective multicenter investigation in China to further evaluate the use of IGRA in the diagnosis of active PTB in high TB-epidemic populations and the factors affecting the performance of the assay.

METHODS

Ethics approval

This was an observational retrospective study and the three diagnostic tests were already used in clinical practice. Given that the medical information of patients was recorded anonymously by case history, which would not bring any risk to the participants, the Ethics Committee of Beijing Chest Hospital, Capital Medical University approved this retrospective study, with a waiver of informed consent from the patients.

Study subjects

China is a high TB-burden country. In 2012, the incidence of TB in the study hospital situated provinces were between

40/100,000 and 181/100,000.^[11] All patients who underwent valid T-SPOT.TB assays from December 2012 to November 2015 in six large-scale specialized TB hospitals in China and met the study criteria were retrospectively evaluated. The six hospitals with a capacity of 3000 beds are situated in the south, north, east, and center of China. At each study hospital, trained health workers extracted data from the computer database of medical records of inpatients. Records were collected in terms of age, gender, contact with TB, vaccination with Bacillus Calmette-Guérin (BCG), albumin, body mass index (BMI), smoking and alcohol intake, presenting complaints, sputum smear and culture, range of TB disease, lung cavity and its range, course of TB, history of TB treatment, comorbidity, etc. Only patients with complete information were included in the investigation. The prevalence of HIV infection is very low in China, and all cases had negative results on serological tests for HIV. All patients had not had immune diseases or received immunosuppressant before. All patients had not extrapulmonary TB. Patients were divided into the following three groups: Group 1, sputum culture-positive PTB patients, confirmed by positive MTB sputum culture; Group 2, sputum culture-negative PTB patients diagnosed on the basis of typical clinical symptoms, typical features on radiographs, and proper responses to anti-TB treatment (sputum smear-positive and smear-negative cases were included); and Group 3, non-TB respiratory diseases, including pneumonia, lung cancer, pulmonary interstitial fibrosis, chronic obstructive pulmonary disease, and bronchiectasis. In China, PTB has generally been diagnosed by traditional methods that rely on clinical symptoms together with the results of bacteriology methods (including sputum smear microscopy and bacterial culture) and X-ray examination (diagnostic criteria for TB WS-288-2008).

T-SPOT.TB assay

The T-SPOT.TB test is an *in vitro* diagnostic test that detects the effector T-cells in human whole blood by capturing IFN- γ in the vicinity of the T-cells that are responding to stimulation with MTB-specific antigens such as 6 kDa early secretory antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10). The T-SPOT.TB test (Oxford Immunotec Ltd., UK) was performed using peripheral blood mononuclear cells (PBMCs) separated from heparinized blood samples according to the manufacturer's instructions. Briefly, PBMCs were isolated and incubated with two antigens (ESAT-6 and CFP-10). The procedure was performed in plates precoated with anti-IFN- γ antibodies at 37°C for 16–20 h. After application of alkaline phosphatase-conjugated secondary antibody and chromogenic substrate, the number of spot-forming cells (million PBMCs) in each well was automatically counted with a CTL ELISPOT system. The results were interpreted as recommended by the test kit manufacturer.^[12]

Statistical analysis

Frequencies and percentages were used to describe the categorical data, and continuous variables were presented as the mean or median. Chi-square tests and Fisher's exact test were used to compare categorical data. Sensitivity,

specificity, positive predictive value (PPV), negative predictive value (NPV), and analytic accuracy (Acc) were calculated for each TB patient group. The analysis was performed on data from patients with culture-positive or culture-negative active TB and cases with nonmycobacterial lung diseases. Confidence intervals (95% CIs) were estimated according to the binomial distribution. Multivariable logistic analyses were performed to evaluate the relationship between the results of T-SPOT.TB in TB patients and other factors. Odds ratios (ORs) and 95% CIs for risk were calculated. $P < 0.05$ was considered statistically significant. Logistic regression models should be used with a minimum of 10 events per predictor variable by a Monte Carlo study.^[13] All statistical analyses were performed with SPSS software (version 13.0, SPSS Inc., Chicago, USA).

RESULTS

Demographic characteristics of the three groups

A total of 3082 patients for whom there was complete information were included in the investigation. The sample population included 905 sputum culture-positive PTB cases, 914 sputum culture-negative PTB cases, and 1263 non-TB respiratory disease cases. Demographic characteristics of the three groups are presented in Table 1.

Clinical characteristics of the tuberculosis patients

Clinical characteristics of the TB patients are summarized in Table 2. The rates of cough, productive cough, fever, sweat, and weight-loss in the culture-positive PTB group were higher than those in the culture-negative PTB group ($P < 0.05$). The ranges of TB disease and cavity were more extensive in the culture-positive PTB group than that in the culture-negative PTB group ($P < 0.01$). The frequency of diabetes in the culture-positive PTB group was higher than that in the culture-negative PTB group ($P < 0.01$).

Diagnostic performance of T-SPOT.TB

The positive rates of TSPOT.TB were 93.3% (95% CI: 91.7–94.9%), 86.1% (95% CI: 83.7–88.5%), 43.6% (95% CI: 40.9–46.3%) in the culture-positive PTB group,

culture-negative PTB group, and non-TB group, respectively. The positive rate of T-SPOT.TB in the culture-positive PTB group was higher than that in the culture-negative PTB group or in the non-TB group ($P < 0.01$). The positive rate of T-SPOT.TB in the culture-negative PTB group was higher than that in the non-TB group ($P < 0.01$). The overall sensitivity, specificity, PPV, NPV, and Acc were 89.7% (95% CI: 88.2–91.0%), 56.4% (53.6–59.1%), 74.8% (95% CI: 72.9–76.6%), 79.1% (95% CI: 76.3–81.7%), and 76.0% (74.5–77.5%), respectively.

Risk factors associated with positive T-SPOT.TB results

Risk factors associated with positive T-SPOT.TB results are presented in Table 3. The sensitivity in the older patients was lower than that in nonolder patients ($P < 0.01$). The sensitivity in patients with a BMI < 18.5 kg/m² was higher than that in patients with a BMI ≥ 18.5 kg/m² ($P = 0.02$). The sensitivity in the smear-positive patients was higher than that in the smear-negative patients ($P < 0.01$). The sensitivity in the patients with comorbidity was higher than that in noncomorbidity patients ($P = 0.017$). The sensitivity in the patients with a record of contact with TB was higher than that in noncontact with TB patients ($P = 0.049$). The sensitivity of T-SPOT.TB in the patients with a record of vaccination with BCG was higher than that in noncontact with TB patients ($P = 0.040$).

Association between T-SPOT.TB result and other factors in tuberculosis patients

In multivariate logistic regression analysis, the affecting factors of T-SPOT.TB results in TB patients included gender (OR, 0.714; 95% CI, 0.515–0.989; $P = 0.043$), age (OR, 0.691; 95% CI, 0.556–0.859, $P < 0.01$), BMI (OR, 0.942; 95% CI, 0.900–0.987, $P = 0.012$), sputum culture (OR, 1.929; 95% CI, 1.271–2.927, $P = 0.002$), and contact with TB (OR, 2.635; 95% CI, 1.037–6.695, $P = 0.042$) [Table 4]. Females, age of > 65 years, a BMI ≥ 18.5 kg/m², sputum culture-negative, and noncontact of TB were demonstrated to be independent factors associated with negative test results.

Table 1: Demographic characteristics of the three groups

Characteristics	Sputum culture positive (n = 905)	Sputum culture negative (n = 914)	Non-TB (n = 1263)
Male, n (%)	640 (70.7)	608 (66.5)	822 (65.1)
Age (years)			
Mean	45.41	43.05	53.81
Range (minimum–maximum)	12–88	6–88	10–88
BMI (kg/m ²)*			
Median	20.76	20.83	22.47
P ₂₅ –P ₇₅	18.37–22.86	18.82–23.11	20.32–24.57
Decreased albumin, n (%)	294 (32.5)	181 (19.8)	227 (18.0)
Contact of TB, n (%)	52 (5.7)	54 (5.9)	43 (3.4)
Vaccination of BCG, n (%)	527 (58.2)	424 (46.4)	777 (61.5)
Smoking, n (%)	307 (33.9)	280 (30.6)	378 (29.9)
Alcohol, n (%)	158 (17.3)	187 (20.7)	263 (20.8)

*The BMI is the weight in kilograms divided by the square of the height in meters. BMI: Body mass index; TB: Tuberculosis; BCG: Bacillus Calmette–Guérin.

Table 2: Clinical characteristics of the TB patients

Characteristics	Sputum culture positive (n = 905)	Sputum culture negative (n = 914)	χ^2	P
Presenting complaints, n (%)				
Cough	850 (93.9)	729 (79.8)	79.646	<0.01
Productive cough	807 (89.2)	650 (71.1)	92.997	<0.01
Fever	392 (43.3)	350 (38.3)	4.748	0.029
Hemoptysis	150 (16.6)	153 (16.7)	0.009	0.925
Sweat	306 (33.8)	203 (22.2)	30.375	<0.01
Weight loss	259 (28.6)	141 (15.4)	46.133	<0.01
AFB positive, n (%)	767 (84.8)	194 (21.2)	736.41	<0.01
Range of TB disease, n (%)				
≤3 lung fields	479 (52.9)	647 (70.8)	61.503	<0.01
≥4 lung fields	426 (47.1)	267 (29.2)		
Lung cavity and its range, n (%)				
No cavity	416 (46.0)	678 (74.2)	152.192	<0.01
≤3 lung fields	448 (49.5)	222 (24.3)		
≥4 lung fields	41 (4.5)	14 (1.5)		
Duration of TB, n (%)				
<1 month	129 (14.3)	230 (25.2)	34.169	<0.01
≥1 month	776 (85.7)	684 (74.8)		
History of TB treatment, n (%)				
New treatment	683 (75.5)	749 (81.9)	11.393	0.01
Retreated	222 (24.5)	165 (18.1)		
With comorbidity, n (%)				
Diabetes	141 (15.6)	66 (7.2)	31.507	<0.01
COPD	25 (2.8)	27 (3.0)	0.060	0.806
Silicosis	4 (0.4)	4 (0.4)	–	0.999*
Bronchiectasis	36 (4.0)	23 (2.5)	3.095	0.079
Asthma	1 (0.1)	1 (0.1)	–	0.999*
Heart disease	9 (1.0)	3 (0.3)	3.080	0.079
Respiratory failure [†]	4 (0.4)	0	–	0.061*
Liver injury	98 (10.9)	105 (11.5)	0.192	0.661

*Determined by Fisher's exact test. Not indicated: Determined by Pearson's Chi-square test. [†]Respiratory failure was defined as PaO₂ lower than 60 by arterial blood gas analysis. TB: Tuberculosis; AFB: Acid-fast bacilli; COPD: Chronic obstructive pulmonary disease. –: Not applicable.

DISCUSSION

IGRAs were developed for the indirect or immunologic diagnosis of MTB infection. With their relatively high sensitivity and specificity, IGRAs have been widely used to diagnose the infection under national guidelines in many developed countries, such as the USA, UK, and Japan.^[14] In most developing countries, including China, the clinical utilization of IGRAs for diagnosing active TB is not recommended, due to insufficient evidence of their performance in high TB-burden settings. Nevertheless, many private health-care providers in high-burden countries are using IGRAs for the diagnosis of active TB,^[14] and many investigators continue to recommend their use for active TB.^[15-17] Thus, there is a growing concern about the inappropriate use of IGRAs for the diagnosis of active TB in high-burden settings, particularly when used to “rule-in” disease.^[18] The aim of this study, performed in a country with a high prevalence of TB, was to determine the performance of the T-SPOT.TB test for diagnosing TB in routine clinical practice.

To our knowledge, this multicenter study that included 1819 TB patients and 1263 non-TB patients is the largest to date evaluating the performance of the T-SPOT.TB test for

diagnosing TB in high-burden settings. The diagnosis of TB is problematic for the clinician as only 50% of patients with active disease have microbiologically confirmed TB disease. A negative IGRA may be a convenient “rule-out” test for TB if the diagnostic sensitivity of the assay is sufficiently high, for example, nearly 95%.^[19] In our study, the sensitivity (89.66%) and NPV (79.11%) of the T-SPOT.TB test suggest that T-SPOT.TB does not have good rule-out value for active TB in high TB-burden settings, like China. Furthermore, the low specificity (56.37%) and PPV (74.75%) limit its usefulness to rule-in active TB in these settings, where the prevalence of latent tuberculosis infection (LTBI) is considerable. In China, IGRA is currently being used for diagnosis of active TB and for differentiating between TB and other diseases. However, the positive rate of T-SPOT.TB found that here was 43.6% in the non-TB group. Such a high false-positive rate makes it necessary to reconsider the value and scope of T-SPOT.TB in clinical practice.

The overall sensitivity of the T-SPOT.TB test in our study was 89.66% in all the PTB patients, which increased to 93.26% in culture-positive TB patients. Similar sensitivities for the diagnosis of active TB were demonstrated in the

Table 3: Risk factors associated with positive T-SPOT.TB results, n = 1819

TB patients	T-SPOT positive (n)	Sensitivity (%)	χ^2	P
Gender				
Male (n = 1248)	1131	90.6	3.957	0.047
Female (n = 571)	500	87.6		
Age				
Older (>65 years) (n = 293)	245	83.6	13.781	<0.01
Nonolder (\leq 65 years) (n = 1526)	1386	90.8		
BMI				
BMI <18.5 kg/m ² (n = 434)	402	92.6	5.397	0.020
BMI \geq 18.5 kg/m ² (n = 1385)	1229	88.7		
Albumin				
Normal (n = 1344)	1209	90.0	0.469	0.493
Decreased (n = 475)	422	88.8		
Culture				
Positive (n = 905)	844	93.3	25.118	<0.01
Negative (n = 914)	787	86.1		
Smear				
Positive (n = 961)	889	92.5	17.771	<0.01
Negative (n = 858)	742	86.5		
Range of disease				
\geq 3 lung fields (n = 1126)	1012	89.9	0.142	0.706
\geq 4 lung fields (n = 693)	619	89.3		
Lung cavity				
Yes (n = 725)	662	91.3	3.523	0.061
No (n = 1094)	969	88.6		
Range of cavity				
\leq 3 lung fields (n = 1764)	1581	89.6	0.095	0.758
\geq 4 lung fields (n = 55)	50	90.9		
Course of TB				
<1 month (n = 359)	330	91.9	2.459	0.117
\geq 1 month (n = 1460)	1301	89.1		
History of TB treatment				
New treatment (n = 1432)	1293	90.3	2.870	0.090
Retreated (n = 387)	338	87.3		
With comorbidity				
Yes (n = 943)	830	88.0	5.736	0.017
No (n = 876)	801	91.4		
Contact of TB				
Yes (n = 106)	101	95.3	3.834	0.049
No (n = 1713)	1530	89.3		
Vaccination of BCG				
Yes (n = 951)	866	91.1	4.199	0.040
No (n = 868)	765	88.1		
Smoking				
Yes (n = 587)	533	90.8	1.207	0.272
No (n = 1232)	1098	89.1		
Alcohol				
Yes (n = 345)	304	88.1	1.102	0.294
No (n = 1474)	1327	90.0		

BMI: Body mass index; TB: Tuberculosis; BCG: Bacillus Calmette–Guérin.

studies performed in China by Zhang *et al.* (94.7%),^[20] Feng *et al.* (94.7%),^[7] and Liu *et al.* (93.2%),^[21] and in one study from India (90.6%).^[9] From the ten reported studies evaluating T-SPOT.TB in China, the combined sensitivity was 88%.^[22] However, the sensitivity in our study was higher than the reported range in the earlier studies from other highly

endemic countries, for example, 76% in South Africa,^[23] 78.7% in Gambia,^[24] and 74% in Zambia.^[25] Previous investigations identified several factors that may affect the sensitivity of IGRA. HIV is one of the factors,^[26] and in most of the studies conducted in other high-burden countries, HIV-positive patients were included.^[24,25] Exclusion of HIV

Table 4: Multivariable logistic regression analysis of the association of T-SPOT.TB and clinical characteristics

Variables	T-SPOT positive, <i>n</i> (%)	T-SPOT negative, <i>n</i> (%)	Univariate analysis: <i>P</i>	Multivariate logistic regression	
				<i>OR</i> (95% <i>CI</i>)	<i>P</i>
Gender					
Male	1131 (90.6)	117 (9.4)	0.047	0.714 (0.515–0.989)	0.043
Female	500 (87.6)	71 (12.4)			
Age group					
Age <45 years	841 (91.8)	75 (8.2)	<0.01	0.691 (0.556–0.859)	0.001
45 years ≤ Age ≤65 years	546 (89.4)	65 (10.6)			
Age >65 years	244 (83.6)	48 (16.4)			
BMI					
BMI <18.5 kg/m ²	402 (92.6)	32 (7.4)	0.020	0.942 (0.900–0.987)	0.012
BMI ≥18.5 kg/m ²	1229 (88.7)	156 (11.3)			
Albumin					
Normal	1209 (90.0)	135 (10.0)	0.493		
Decreased	422 (88.8)	53 (11.2)			
Culture					
Positive	844 (93.3)	61 (6.7)	<0.01	1.929 (1.271–2.927)	0.002
Negative	787 (86.1)	127 (13.9)			
Smear					
Positive	889 (92.5)	72 (7.5)	<0.01	1.501 (0.987–2.284)	0.058
Negative	742 (86.5)	116 (13.5)			
Range of disease					
≤3 lung fields	1042 (89.9)	114 (10.1)	0.706		
≥4 lung fields	619 (89.3)	74 (10.7)			
Lung cavity					
Yes	662 (91.3)	63 (8.7)	0.061	0.994 (0.696–1.420)	0.975
No	969 (88.6)	125 (11.4)			
Range of cavity					
≤3 lung fields	1581 (89.6)	183 (10.4)	0.758		
≥4 lung fields	50 (90.9)	5 (9.1)			
Course of TB					
<1 month	330 (91.9)	29 (8.1)	0.117	0.684 (0.443–1.057)	0.087
≥1 month	1301 (89.1)	159 (10.9)			
History of TB treatment					
New treatment	1293 (90.3)	139 (9.7)	0.090	0.791 (0.547–1.146)	0.216
Retreated	338 (87.3)	49 (12.7)			
With comorbidity					
Yes	830 (88.0)	113 (12.0)	0.017	0.786 (0.563–1.096)	0.155
No	801 (91.4)	75 (8.6)			
Type of comorbidity					
Diabetes	188 (11.5)	19 (10.1)	0.561		
COPD	47 (2.9)	5 (2.7)	0.863		
Silicosis	7 (0.4)	1 (0.5)	0.583*		
Bronchiectasis	49 (3.0)	10 (5.3)	0.090	0.514 (0.244–1.080)	0.079
Asthma	1 (0.1)	1 (0.5)	0.196*	0.145 (0.008–2.655)	0.193
Heart disease	9 (0.6)	3 (1.6)	0.119*	0.401 (0.101–1.592)	0.194
Liver injury	185 (11.4)	18 (9.7)	0.486		
Respiratory failure	4 (0.2)	0	0.999*		
Contact of TB					
Yes	101 (95.3)	5 (4.7)	0.049*	2.635 (1.037–6.695)	0.042
No	1530 (89.3)	183 (10.7)			
Vaccination of BCG					
Yes	866 (91.1)	85 (8.9)	0.040	0.978 (0.703–1.363)	0.897
No	765 (88.1)	103 (11.9)			

Contd...

Table 4: Contd...

Variables	T-SPOT positive, n (%)	T-SPOT negative, n (%)	Univariate analysis: P	Multivariate logistic regression	
				OR (95% CI)	P
Smoking					
Yes	533 (90.8)	54 (9.2)	0.272		
No	1098 (89.1)	134 (10.9)			
Alcohol					
Yes	304 (88.1)	41 (11.9)	0.294		
No	1327 (90.0)	147 (10.0)			

*Determined by Fisher's exact test. Not indicated: Determined by Pearson's Chi-square test. If the $P < 0.2$ in the univariate analysis, then the affected factors were entered in the multivariate logistic regression. BMI: Body-mass index; TB: Tuberculosis; BCG: Bacillus Calmette-Guérin; COPD: Chronic obstructive pulmonary disease.

patients in our study may be one of the reasons for obtaining higher sensitivity. Furthermore, the sensitivity of IGRA reportedly decreased significantly with the age of patients^[5] and gradually decreased with the treatment duration.^[6] In our study, only 15.02% (245/1631) patients were older TB patients (≥ 65 years) and 20.72% (338/1631) of patients were retreated ones, which may be another reason for obtaining higher sensitivity.

As T-SPOT.TB tests are unable to distinguish between LTBI and active TB, the specificity of the T-SPOT.TB test depends on the prevalence of LTBI. The specificity of the T-SPOT.TB test in diagnosing active TB is known to be high ($\geq 93\%$) in low TB incidence settings,^[27] and as low as 61% in low- and middle-income countries^[28] The poor specificity (56.44%) of T-SPOT.TB test obtained in this study was expected and several factors should be considered. First, the high prevalence of LTBI, as high as 44.5% in China,^[29] inevitably decreases the specificity of T-SPOT.TB test. Second, this study was designed to evaluate the diagnostic validity of T-SPOT.TB test in routine clinical practice and thus focused on unselected patients with suspected active TB. In this setting, the diagnostic validity tends to be lower than in studies in which healthy people are enrolled as negative controls.

IGRAs are designed to detect MTB infections, whether latent or active. However, in our study, 6.74% (61/905) of the persons with culture-confirmed TB had a negative T-SPOT.TB result, comparable to 8.7% (46/528) described by Pan *et al.*^[30] and 14.4% (182/1264) described by Kwon *et al.*^[31] There are a multitude of factors that may modulate the sensitivity of IGRA including HIV coinfection, immune suppression, young or advanced age, advanced disease, malnutrition, extrapulmonary TB, disseminated TB, concomitant TB treatment, bacteria strain differences, and smoking.^[30,32] In our multivariate logistic regression analysis, gender, age, BMI, sputum culture, and contact with TB were affecting factors for the false-negative results of the T-SPOT.TB test. In addition, this study showed that the sensitivity of T-SPOT.TB for the diagnosis of TB in the culture/smear-positive group was significantly higher than that in the culture/smear-negative group, which implied that the T-SPOT.TB result may be affected by the bacterial load.

There were several limitations to our study. First, T-SPOT.TB results were analyzed in retrospect and quantitative test results were not acquired. Second, this study was performed in an area where the prevalence of LTBI is considerable. Third, this study did not include extrapulmonary TB and immunodeficient patients. Fourth, external validity is a concern, because this was a hospital-based study and may have overestimated the sensitivity of IGRA. Fifth, the retrospective study design limited us to describing the association between IGRA and affecting factors. Sixth, sputum culture-negative PTB patients were included according to clinical diagnosis, which may influence the diagnostic Acc. Additional longitudinal studies are needed to prove any causality in the associations found.

Despite these limitations, to our knowledge, this might be the multicenter large-scale investigation to evaluate the role of T-SPOT.TB in diagnosis of PTB in China. Our study demonstrated that gender, age, BMI, sputum culture, and contact of TB are factors affecting the false-negative results of T-SPOT.TB test. Inadequate sensitivity and high false-positive rates of this test limit its usefulness as a single test to rule-in or rule-out active TB in China. We highly recommend that IGRAs not be used for the diagnosis of active TB in high-burden TB settings.

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Conflicts of interest

There are no conflicts of interest.

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