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ORIGINAL ARTICLE

Performance Evaluation of IGRA-ELISA and T-SPOT.TB for Diagnosing Tuberculosis Infection

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SUMMARY

Background: **To explore the application value of IGRA-ELISA in the diagnosis of tuberculosis.**
Methods: **A total of 68 tuberculosis and 58 other pulmonary disease case samples were obtained. All the samples were tested by IGRA-ELISA and T-SPOT.TB assay in parallel. The consistency of IGRA-ELISA and T-SPOT.TB in the diagnosis of TB was analyzed. Five different methods for the diagnosis of TB were assayed: IGRA-ELISA, T-SPOT.TB, AFB staining, TB-Ab, and PPD. For the different PPD positive degrees, IGRA-ELISA and T-SPOT.TB positive rates were calculated. AFB staining positive and negative samples were analyzed by IGRA-ELISA, T-SPOT.TB, TB-Ab, and PPD. Positive rates, sensitivity, specificity, PPV, NPV and accuracy values of the five different detection methods were compared.**
Results: **There was good consistency between IGRA-ELISA and T-SPOT.TB in the diagnosis of TB and other pulmonary diseases. Compared with T-SPOT.TB, there was a significant correlation between the absorbance value of IGRA-ELISA and the number of ESAT-6 or CFP-10-specific SFCs ($r = 0.902$, $p < 0.001$; $r = 0.901$, $p < 0.001$). There was a significant difference in the positive rates among the above five different detection methods in the TB group and non-TB group ($p < 0.001$). For the different PPD positive degrees, there were highly significant differences in the positive rates of IGRA-ELISA and T-SPOT.TB in non-TB group; no similar trend was observed in the TB group. No significant differences in sensitivity, specificity, PPV, NPV, accuracy, LR+ and LR- were observed between IGRA-ELISA and T-SPOT.TB. The positive rates of IGRA-ELISA and T-SPOT.TB in the TB group were significantly higher than that of AFB staining, TB-Ab, and PPD ($p < 0.05$). IGRA-ELISA and T-SPOT.TB combined with AFB staining could further improve the sensitivity of tuberculosis detection without reducing its specificity. The AUC of IGRA-ELISA, ESAT-6, CFP-10, and T-SPOT.TB were 0.923, 0.893, 0.937, and 0.919, respectively.**
Conclusions: **There was good correlation and consistency between the IGRA-ELISA and T-SPOT.TB in the diagnosis of TB. The sensitivity and accuracy of IGRA-ELISA were significantly better than those of AFB staining, TB-Ab, and PPD. IGRA-ELISA combined with AFB staining could further improve the diagnosis of tuberculosis.**
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KEY WORDS

tuberculosis, interferon-γ release assay, enzyme-linked immunosorbent assay, T-SPOT.TB test, diagnosis

LIST OF ABBREVIATIONS

TB - tuberculosis
WHO - World Health Organization
T-SPOT.TB - T cell spot test of tuberculosis

IFN - interferon
IGRA-ELISA - interferon-gamma release enzyme linked immunosorbent assay
ELISPOT - enzyme linked immunospot
PBMCs - peripheral blood mononuclear cells
PHA - phytohemagglutinin
ESAT-6 - early secreted antigenic target-6
CFP-10 - culture filtrate protein-10
SFCs - spot-forming cells
PPD - purified protein derivative
TB-Ab - tuberculosis antibody
AFB - acid fast bacilli
PPV - positive predictive value
NPV - negative predictive value
Acc - analytic accuracy
+LR - positive likelihood ratio
-LR - negative likelihood ratio
AUC - area under the curve
BCG - bacillus calmette guerin

INTRODUCTION

TB remains the leading single microbial illness globally, with one-third of the world's population infected with *Mycobacterium tuberculosis* complex. Over 90% of the worldwide burden of tuberculosis is in low-income and middle-income countries. China is one of the countries with a high incidence of TB in the world. According to the 2018 WHO global TB report [1], there were about 10 million new patients and yearly there are 1.57 million deaths worldwide, and about 5% - 10% infected people will develop into active TB throughout their lives. At present, the laboratory methods of diagnosis of tuberculosis are mainly molecular biology, bacteriology, and immunology. However, the sensitivity and specificity of the above-mentioned methods cannot meet the requirements of clinical diagnosis and treatment. Accurate diagnosis is a fundamental component of TB care. Rapid diagnostic tests help to ensure early detection and prompt treatment. Therefore, it is more and more urgent to develop a fast, highly sensitive and specific technology to change this situation [2,3]. *Mycobacterium tuberculosis*-specific antigen can sensitize T cells to release IFN-γ, and T-SPOT.TB and IGRA-ELISA both showed high sensitivity and specificity in clinical detection [3]. T-SPOT.TB is a single immune effect ELISPOT to detect the infection state of a body to *Mycobacterium tuberculosis*; IGRA-ELISA measures T-cell release of IFN-γ following stimulation by antigens that are unique to *M. tuberculosis* including ESAT-6 and CFP-10, which are encoded by genes located within the region of difference 1 segment of the *M. tuberculosis* genome.

In this study, a total of 68 TB and 58 other pulmonary disease case samples were examined by IGRA-ELISA and T-SPOT.TB. The objective of this study was to compare the two experimental methods and evaluate the application value of IGRA-ELISA in the diagnosis of tuberculosis.

MATERIALS AND METHODS

From September 2017 to February 2018, a total of 126 hospitalized patients at the Affiliated Chaohu Hospital of Anhui Medical University were enrolled in the study. Ethical approval was granted by Anhui Medical University Ethics Committee. Of these patients, 68 were diagnosed as tuberculosis (TB group), 53 were male and 15 were female. Fifty-eight cases with other pulmonary diseases (non-TB group), 41 were male and 17 were female. The median age of the TB group and non-TB group was 47.5 and 64.5 years (range: 16 - 90 years), respectively.

Diagnosis of TB

Definite TB was diagnosed on the basis of *Mycobacterium tuberculosis* identification by sputum acid-fast bacilli stain, culture, and/or NAAT from clinical specimens or the presence of clinical manifestations, radiological imaging consistent with the disease, or histological findings and no improvement after a full course of antibiotics, followed by clinical improvement with anti-TB treatment.

T-SPOT®.TB

T-SPOT.*TB* IGRA was performed according to the manufacturer's instructions (Oxford Immunotec, Abingdon, UK). Interpretation of the results was made as follows: the number of SFCs in each well was counted by naked eye. A positive result was defined as ≥ 6 SFCs counted in the antigen ESAT-6 or CFP-10 well after subtraction of the number of SFCs in the negative control well where the negative control had ≤ 5 spots. The total number of SFCs in the ESAT-6 or CFP-10 well was at least twice the number of SFCs in the negative control well where the negative control had ≥ 6 spots; indeterminate test ≤ 6 SFCs for both antigens and ≤ 20 SFCs in the positive control section.

IGRA-ELISA

The IGRA-ELISA was detected by following the manufacturer's instructions (Beijing WanTai Biological Pharmacy Enterprise Co., Ltd.). The absorbance value of 450 nm in each tube was detected according to the instructions.

Tuberculin PPD

Tuberculin PPD skin test was performed using the Mantoux method. Injection of 0.1 mL PPD (5 IU, Beijing Xiangrui Biological Products Co., Ltd.) was administered intradermally on the volar surface of the forearm. The transverse redness and average diameter of the induration was measured 48 - 96 hours after injection. The results were as follows: PPD was considered negative (-) when the induration was less than 5 mm, PPD reaction was defined as weak positive (+) if the indura-

tion diameter was 5 - 9 mm, the result was considered to be positive (++) if the induration diameter was 10 - 15 mm, an induration diameter more than 15 mm was considered strong positive (+++), the local occurrence of blisters, necrosis or lymphatic inflammation was considered indicative of a strong positive reaction (++++).

TB-Ab test

The detection of Mycobacterium TB-Ab was performed with plasma samples. Assays and interpretation of results was done strictly according to the manufacturers' instructions (Shanghai Upper Bio-Tech Pharma Co., Ltd.). TB-Ab test was defined as positive (+) when the quality control area showed red, and red spots appeared in the middle of reaction holes. TB-Ab test was considered negative (-) if the quality control area showed red, and there were no red spots in the reaction pores or only traces.

Acid fast staining

Samples were stained with acid fast staining using Ziehl-Neelsen staining method (Zhuhai Beisuo Biological Technology Co., Ltd.) according to the manufacturer's instructions. Results was interpreted and graded as AFB -, 0/300 fields; +/-, 1 - 8/300 fields; 1+, 3 - 9/100 fields; 2+, 1 - 9/10 fields; 3+, 1 - 9/1 field; 4+, > 10 strips/1 field.

Statistical analysis

All statistical analyses were carried out using SPSS software package version 17.0. The related data was presented as medians (interquartile ranges), Mann-Whitney *U* rank test and McNemar test were used for comparisons where appropriate. The concordance between IGRA-ELISA and T-SPOT.TB was evaluated using proportion agreement and Kappa statistic. Differences in categorical variables were compared using the χ^2 test. The sensitivity, specificity, PPV, NPV, Acc, +LR and -LR of the above-mentioned assays were calculated. The AUC of IGRA-ELISA and T-SPOT.TB was analyzed to evaluate the diagnostic efficiency for tuberculosis. All statistical analyses were two-tailed, and a p-value < 0.05 indicated that the difference was statistically significant.

RESULTS

The consistency of the two tests

The blood samples of 126 patients were tested using both T-SPOT.TB and IGRA-ELISA. For 68 TB cases, there was moderate consistency between the two tests (Kappa = 0.485, $p < 0.001$). For 58 non-TB cases, there was excellent consistency between the two tests with a kappa value of 0.811 ($p < 0.001$). Among the 126 cases, the consistency of the two assay methods was good (Kappa = 0.871, $p < 0.001$) (Table 1).

Agreement between IGRA-ELISA and T-SPOT.TB assays

For IGRA-ELISA method, there was a highly significant difference in the absorbance value between the TB group and non-TB group ($p < 0.001$). For T-SPOT.TB assay, a significant difference was found in the numbers of ESAT-6 and/or CFP-10-specific SFCs between the TB group and non-TB group (all $p < 0.001$). There was a positive correlation between the absorbance value of IGRA-ELISA and the number of ESAT-6 and CFP-10-specific SFCs ($r = 0.902$, $p < 0.001$; $r = 0.901$, $p < 0.001$). The results of this study revealed a significant correlation between the number of ESAT-6-specific SFCs and CFP-10-specific SFCs ($r = 0.820$, $p < 0.001$) (Table 2).

Analysis of positive test results

The positive rates of IGRA-ELISA, T-SPOT.TB, AFB staining, TB-Ab, and PPD in the pulmonary tuberculosis group were all significantly higher than those in other pulmonary disease groups (all $p < 0.001$) (Table 3).

Comparison of IGRA-ELISA and T-SPOT.TB positive rates for the positive degree of PPD

In the TB group, no significant differences were found in the positive rates of IGRA-ELISA and T-SPOT.TB for the positive degree of PPD ($p > 0.05$). In the non-TB group, the positive rates of IGRA-ELISA and T-SPOT.TB showed a significant difference for the positive degree of PPD ($p < 0.01$). The positive rates of IGRA-ELISA and T-SPOT.TB increased with the increase of positive level of PPD (Table 4).

Sensitivity of different detection methods in the diagnosis of TB with AFB staining negative

In the TB group, IGRA-ELISA showed no difference in diagnostic sensitivity between AFB staining positive and negative ($p > 0.05$). T-SPOT.TB, TB-Ab, and PPD showed the same results ($p > 0.05$). The detection sensitivity of IGRA-ELISA, T-SPOT.TB, TB-Ab, and PPD in the AFB staining negative TB group were all significantly higher than those in the non-TB group ($p < 0.01$) (Table 5).

Comparison of positive rates of the different detection methods

The positive rates of IGRA-ELISA and T-SPOT.TB in the TB group were significantly higher than those of AFB staining, TB-Ab, and PPD ($p < 0.05$). There was no significant difference among the positive rates of IGRA-ELISA, T-SPOT.TB, and PPD in non-TB group ($p > 0.05$). Significant differences were found in positive rates of IGRA-ELISA as compared to AFB staining and TB-Ab (Table 6).

Sensitivity and specificity estimates of different detection methods in the diagnosis of TB

The sensitivity of IGRA-ELISA and T-SPOT.TB in the TB group was significantly higher than that of the other

Table 1. Comparison of the consistency of IGRA-ELISA and T-SPOT.TB in TB and non-TB groups.

IGRA-ELISA	T-SPOT.TB				Total
	TB group		non-TB group		
	positive	negative	positive	negative	
Positive	65	1	18	3	87
Negative	1	1	2	35	39
Total	66	2	20	38	126

Table 2. Comparison of absorbance values and the number of SFCs detected by IGRA-ELISA and T-SPOT.TB in TB and non-TB groups.

Group	n	IGRA-ELISA	T-SPOT.TB	
			ESAT-6	CFP-10
TB group	68	262.10 (131.93, 400.65)	51.5 (23.3, 116.5)	63.0 (22.8, 159.0)
non-TB group	58	6.35 (0.18, 68.58)	1.0 (0, 10.0)	0.0 (0, 3.0)
Z value		-8.160	-7.644	-8.521
p-value		< 0.001	< 0.001	< 0.001

Table 3. Comparison of different detection methods for diagnosis of pulmonary tuberculosis.

Group	IGRA-ELISA		T-SPOT.TB		AFB staining		TB-Ab		PPD	
	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
TB group	66	2	66	2	39	29	20	48	45	23
non-TB group	21	37	20	38	0	58	2	56	19	39
χ^2	54.234		56.567		48.176		14.641		13.986	
p-value	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

Table 4. Positive rates in comparison between IGRA-ELISA and T-SPOT.TB for the positive degree of PPD.

PPD	IGRA-ELISA		T-SPOT.TB	
	negative n, (%)	positive n, (%)	negative n, (%)	positive n, (%)
TB group	n = 68			
Negative	2 (8.70)	21 (91.30)	2 (8.70)	21 (91.30)
Positive (1+)	0 (0)	9 (100.00)	0 (0)	9 (100.00)
Positive (2+)	0 (0)	18 (100.00)	0 (0)	18 (100.00)
Positive (3+)	0 (0)	18 (100.00)	0 (0)	18 (100.00)
χ^2	4.032		4.032	
p-value	0.258		0.258	
non-TB group	n = 58			
Negative	32 (82.05)	7 (17.95)	31 (79.49)	8 (20.51)
Positive (1+)	4 (57.14)	3 (42.86)	4 (57.14)	3 (42.86)
Positive (2+)	1 (10.00)	9 (90.00)	3 (30.00)	7 (70.00)
Positive (3+)	0 (0)	2 (100.00)	0 (0)	2 (100.00)
χ^2	21.815		12.970	
p-value	0.000		0.005	

Table 5. Diagnostic value of different detection methods for pulmonary tuberculosis with AFB staining negative.

Test	IGRA-ELISA		T-SPOT.TB		TB-Ab		PPD	
	positive n, (%)	negative n, (%)	positive n, (%)	negative n, (%)	positive n, (%)	negative n, (%)	positive n, (%)	negative n, (%)
TB group	n = 68							
Positive AFB staining	37 (94.87)	2 (5.13)	38 (97.44)	1 (2.56)	12 (30.77)	27 (69.23)	25 (64.10)	14 (35.90)
Negative AFB staining	29 (100.00)	0 (0)	28 (96.55)	1 (3.45)	8 (27.59)	21 (72.41)	20 (68.97)	9 (31.03)
χ^2	-		-		0.081		0.176	
*p-value	0.504		1.000		0.776		0.675	
non-TB group	n = 58							
Negative AFB staining	21 (36.21)	37 (63.79)	20 (34.48)	38 (65.52)	2 (3.45)	56 (96.55)	19 (32.76)	39 (67.24)
χ^2	32.190		30.115		8.827		10.248	
#p-value	0.000		0.000		0.003		0.001	

* p - positive rate comparison of IGRA-ELISA, T-SPOT.TB, TB-Ab, and PPD between positive AFB staining and negative AFB staining TB patients.
# p - positive rate comparison of IGRA-ELISA, T-SPOT.TB, TB-Ab and PPD between negative AFB staining TB patients and non-TB patients.

Table 6. Comparison of positive rate for the different detection methods.

Method	TB group				non-TB group			
	positive number	positive rate (%)	χ^2	p-value	positive number	positive rate (%)	χ^2	p-value
IGRA-ELISA	68 (66)	97.06	-	-	58 (21)	36.21	-	-
T-SPOT.TB	68 (66)	97.06	0.000	1.000	58 (20)	34.48	0.038	0.846
AFB staining	68 (39)	57.35	30.459	0.000	58 (0)	0	25.642	0.000
TB-Ab	68 (20)	29.41	66.925	0.000	58 (2)	3.45	19.577	0.000
PPD	68 (45)	66.18	21.613	0.000	58 (19)	32.76	0.153	0.696
IGRA-ELISA + AFB staining	68 (68)	100.00	0.507	0.476	58 (21)	36.21	0.000	1.000
IGRA-ELISA + TB-Ab	68 (67)	98.53	0.000	1.000	58 (23)	39.66	0.146	0.702
T-SPOT.TB + AFB staining	68 (67)	98.53	0.000	1.000	58 (20)	34.48	0.038	0.846
T-SPOT.TB + TB-Ab	68 (67)	98.53	0.000	1.000	58 (22)	37.93	0.037	0.848

three methods ($p < 0.001$). The specificity of AFB staining and TB-Ab in the non-TB group was significantly higher than that of the other three methods ($p < 0.001$). IGRA-ELISA and T-SPOT.TB combined with AFB staining could further improve the sensitivity of tuberculosis detection without reducing its specificity (Table 7). There were significant differences between AFB staining and IGRA-ELISA, T-SPOT.TB, and PPD ($p < 0.01$). Among the five methods, the highest positive predictive value was AFB staining, followed by TB-Ab, and the lowest was PPD in 126 cases of pulmonary disease. The highest negative predictive value and accuracy of the five methods for the diagnosis of tuberculosis were T-SPOT.TB followed by IGRA-ELISA. There was no statistical difference between the two methods ($p > 0.05$). IGRA-ELISA and T-SPOT.TB combined AFB staining could further improve the positive predictive value, negative predictive value, and accuracy of tuberculosis detection increase the positive likelihood ratio and reduce the negative likelihood ratio.

Table 7. Sensitivity and specificity estimates of different detection methods in the diagnosis of TB.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR+	LR-
IGRA-ELISA	97.06	63.79	75.86	94.87	81.75	2.68	0.05
T-SPOT.TB	97.06	65.52	76.74	95.00	82.54	2.81	0.04
AFB staining	57.35	100.00	100.00	66.67	76.98	-	0.43
TB-Ab	29.41	96.55	90.91	53.85	60.32	8.53	0.73
PPD	66.18	67.24	70.31	62.90	66.67	2.02	0.50
IGRA-ELISA + AFB staining	100.00	63.79	76.40	100.00	83.33	2.76	0
IGRA-ELISA + TB-Ab	98.53	60.34	74.44	97.22	80.95	2.48	0.02
T-SPOT.TB + AFB staining	98.53	65.52	77.01	97.44	83.33	2.86	0.02
T-SPOT.TB + TB-Ab	98.53	62.07	75.28	97.30	81.75	2.60	0.02

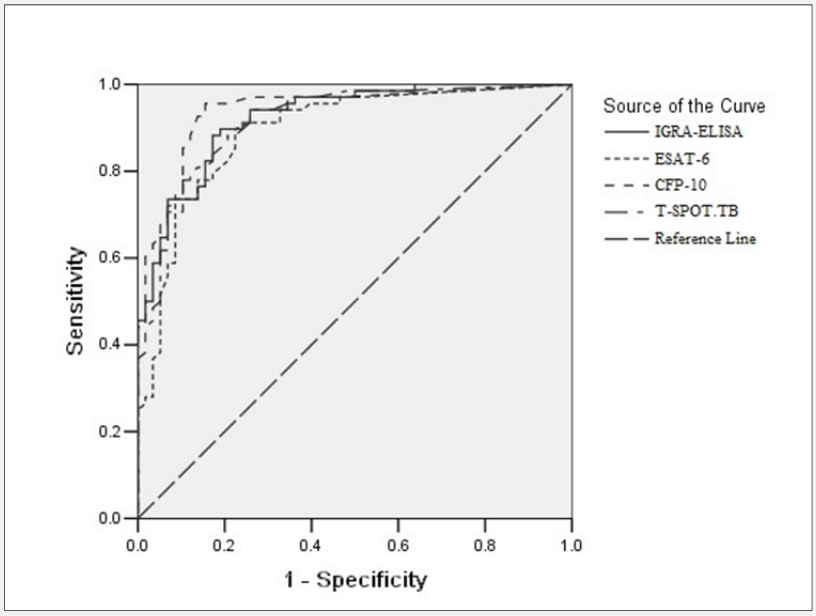

Figure 1. ROC curves analysis of IGRA-ELISA and T-SPOT.TB for diagnosing pulmonary tuberculosis.

As shown in Figure 1, the absorbance cutoff values of IGRA-ELISA and the number of ESAT-6 and CFP-10-specific SFCs of T-SPOT.TB were established by the ROC curves between the TB and non-TB group. The AUC of IGRA-ELISA, ESAT-6, CFP-10, and T-SPOT.TB were 0.923, 0.893, 0.937, and 0.919, respectively. Using IGRA-ELISA cutoff value of 14.85 could detect 97.1% sensitivity and 63.8% specificity. Using the ESAT-6 cutoff value of 5.5 could detect 94.1% sensitivity and 67.2% specificity. When CFP-10 cutoff value was 5.5, the sensitivity and specificity were 95.6% and 84.5%, respectively. When the T-SPOT.TB cutoff value was 6.0, the sensitivity and specificity were 97.1% and 65.5%, respectively.

DISCUSSION

Tuberculosis is one of the infectious diseases that seriously endanger human health. Rapid diagnosis of tuberculosis is an effective means to control tuberculosis. IGRAs are a rapid, sensitive, and specific immunodiagnostic test for tuberculosis. IGRA-ELISA is an ELISA to detect the level of IFN-gamma released by sensitized T cells stimulated by MTB-specific antigens (EAST-6 and CFP-10). T-SPOT.TB assay uses enzyme linked immune spot assay (ELISPOT) to determine the number of effector T cells that can release IFN-gamma in peripheral blood mononuclear cells stimulated by MTB-specific antigen, also known as cell detection or tuberculosis infection T cell detection. The tuberculous specific antigen EAST-6 and CFP-10 of these two methods are encoded by the same operon in the genomic RD1 region of *Mycobacterium tuberculosis*, and all of the Bacillus Calmette Guerin (BCG) loses the gene sequence, and most environmental MTBs have no RD1 region. Therefore, IGRAs can better distinguish BCG vaccination from MTB infection, making IGRAs a rapid method for assistant diagnosis of MTB infection [4,5].

In this study, IGRA-ELISA and T-SPOT.TB were used to detect 68 patients with pulmonary tuberculosis and 58 patients with other pulmonary diseases in parallel. The consistency of the two assay methods was excellent (Kappa = 0.871, $p < 0.001$), which is consistent with the previous reports [6]. Further, by detecting the absorbance value of IGRA-ELISA and the number of SFCs of T-SPOT.TB, we found that the absorbance value of IGRA-ELISA and the number of SFCs of T-SPOT.TB in tuberculosis patients were significantly higher than those in other lung diseases ($p < 0.001$), which was consistent with the previous report [7]. With the increase of the absorbance value of IGRA-ELISA, the number of SFCs (EAST-6 and CFP-10 antigens) increased. There was a correlation between the absorbance value and the number of SFCs detected by EAST-6 and CFP-10 antigens ($r = 0.855$, $p < 0.001$; $r = 0.790$, $p < 0.001$), and there also was a correlation between the number of ESAT-6-specific SFCs and CFP-10-specific SFCs ($r = 0.820$, $p < 0.001$) [8]. These data suggested an excellent concordance between these two IGRA assays. The test methods based on the same principle have the same detection efficiency, that is, the two methods have the same significance. IGRA is a good indicator for differential diagnosis of tuberculosis, especially for early and latent tuberculosis [9,10]. However, when facing a large number of samples, IGRA-ELISA has the operational advantage of no need to extract mononuclear cells and can achieve batch operation on the ELISA platform.

In 68 cases of pulmonary tuberculosis and 58 patients with other pulmonary diseases, the positive rates of IGRA-ELISA and T-SPOT.TB were 97.06% and 36.21%, 97.06%, and 34.48% respectively. The positive rates of IGRA-ELISA and T-SPOT.TB in the pulmonary tuberculosis group were significantly higher than those in the other pulmonary disease group ($p < 0.001$). It is in line with previous reports [11,12], and the sensitivity of T-SPOT.TB was a little higher than the result of Wang et al. (85%) conducted in a general hospital in western China [13] and Bae et al. (92.1%) in Seoul, Republic of Korea [14], and agree with the result (96.9%) of Takasaki et al. carried out in Tokyo, Japan [15].

In addition, IGRA-ELISA, T-SPOT.TB, TB-Ab, and PPD all showed no difference in sensitivity between AFB staining positive and negative pulmonary tuberculosis (94.87% vs. 100.00%, 97.44% vs. 96.55%, 30.77% vs. 27.59%, and 64.10% vs. 68.97%, respectively, $p > 0.05$). The detection sensitivity of IGRA-ELISA, T-SPOT.TB, TB-Ab, and PPD in AFB staining negative pulmonary tuberculosis was all significantly higher than that in other pulmonary diseases (100.00% vs. 36.21%, 96.55% vs. 34.48%, 27.59% vs. 3.45%, and 68.97% vs. 32.76%, respectively, $p < 0.01$). This suggested that IGRA-ELISA and T-SPOT.TB tests had high sensitivity, are able to detect smear-negative patients effectively, and can be used for latent TB detection [4,16,17]. IGRA-ELISA and T-SPOT.TB detection in other lung diseases also have a higher positive rates, which may be due to the high prevalence of tuberculosis in China, some patients have other lung diseases combined with MTB latent infection or obsolete tuberculosis, or the presence of small extrapulmonary tuberculosis lesions that could not be detected by conventional detection methods [7]. IGRAs could not distinguish latent, active, and old tuberculosis, resulting in IGRA-ELISA and T-SPOT.TB detection specificity to be reduced, so IGRA-ELISA and T-SPOT.TB test positive could not be relied on solely to diagnose active tuberculosis.

PPD skin test is a traditional method and an important reference index for the diagnosis of MTB latent infection. However, the number of BCG vaccinations in the domestic population is large. BCG vaccinations or the presence of non-tuberculosis Mycobacteria (NTM) infection can cause false positive [17-19], and some false negative results can be found in subjects who use hormones or immunosuppressive agents [20,21]. Therefore, the sensitivity and specificity of PPD test are not high [22]. In this study, the positive rate of PPD test in 68 patients with pulmonary tuberculosis was 66.18% and that in 58 patients with other lung diseases was 32.76%. The positive rate of PPD test in the TB group was higher than that in the non-TB group ($p < 0.001$). We also compared the results of IGRA-ELISA and T-SPOT.TB for the positive degree of PPD, and no significant differences were found in the positive rates of IGRA-ELISA and T-SPOT.TB for the positive degree of PPD ($p > 0.05$) These results agreed with the findings of the study carried out by Tamašauskienė et al. [8]. In the non-TB group, IGRA-ELISA and T-SPOT.TB results showed significant differences for the positive degree of PPD ($p < 0.01$), and with the increase of positive levels of PPD test results, the positive rates of IGRA-ELISA and T-SPOT.TB increased. For the positive degree of PPD, no similar studies on IGRA tests have

been reported yet. It is plausible that the co-positive responses of other lung disease patients by PPD and IGRA assays could be due to latent tuberculosis infection (LTBI). However, evaluating the accuracy of IGRAs in diagnosing LTBI remains a problem since there is no "gold standard" for such diagnoses [4].

In addition, we also carried out AFB staining and TB-Ab detection and found that the positive rate of AFB staining in the TB group was 57.35%. Sputum smears for acid-fast bacilli is a traditional bacteriological examination. The examination is economical, does not require special equipment, and is easy to operate. Because of poor compliance with sputum, difficult standardization of sample collection, and intermittent bacterial excretion of patients, it is easy to cause a low positive rate of smears, and it is necessary to send samples repeatedly. In addition, the results could not determine whether it was MTB or NTM infection and also could not determine bacterial activity. The positive rate of TB-Ab was 29.41% in the TB group and 3.45% in non-TB group. These results are similar to the study performed by Ji et al. [16]. The reason for the low positive rate of TB-Ab test in this study may be that the kit could only detect 38kD-IgG antibody, but not IgM antibody. 38kD-IgG antibody often appears in the middle and late stage of tuberculosis, so when the patient is in the window of infection or early onset of the disease, it will cause false negative results. Pseudo-negative results also occur when the patient's immunity is low and could not produce enough tuberculosis antibodies, or when the antibody bound to the antigen and there was no free antibody. In addition, the immune response caused by *Mycobacterium tuberculosis* is that T cells can be activated by a variety of antigen epitopes, and the lack of abundant antigen epitopes provided by the kit for serum detection is also the cause of false negative detection [23, 24]. Therefore, although tuberculosis antibody detection is a simple, rapid, and highly specific method [25], it could not meet the clinical needs because of its low sensitivity [24,26].

In the five methods, the positive rate of IGRA-ELISA and T-SPOT.TB in the TB group was significantly higher than that of AFB staining, TB-Ab, and PPD ($p < 0.05$). IGRAs have a higher sensitivity than the other 3 methods in the diagnosis of tuberculosis [8]. The reasons are as follows: (1) IGRAs are not affected by BCG and most non-tuberculous Mycobacterium during the diagnosis and have a high sensitivity for the detection of active tuberculosis, extrapulmonary tuberculosis, and latent tuberculosis infection; (2) IGRAs diagnosis is more objective and convenient for interpretation of results. (3) IGRAs diagnosis is rapid, can be obtained in 24 to 48 hours, and no need for multiple follow-ups to patients; (4) IGRAs detection is less affected by factors, not affected by whether the patient is in the period of bacterial excretion and the amount of bacterial excretion, and also have high sensitivity and sensitivity for immunocompromised and immunosuppressed populations [27]. IGRAs can be used to detect TB infection more effectively. In addition, based on this study, a negative IGRA-ELISA or T-SPOT.TB provided an efficient way for excluding TB in some patients, preventing the unnecessary application of anti-TB drugs and adverse effects of them [7].

In this study, the specificity of acid-fast staining was the highest in 58 patients with other lung diseases, reaching 100.00%, followed by TB-Ab 96.55%, which may be related to the small number of samples, and TB-Ab can only detect IgG antibodies. The specificity of IGRA-ELISA, T-SPOT.TB, and PPD was significantly different from that of AFB staining and TB-Ab ($p < 0.05$). The specificity of IGRAs was not high, which was slightly lower than the result reported by Kim SH (74.1%) [7]. The highest negative predictive value and accuracy of the five methods for the diagnosis of tuberculosis in 126 patients with pulmonary diseases was T-SPOT.TB, followed by IGRA-ELISA, and the lowest was TB-Ab, which was only 53.85% and 60.32% ($p < 0.05$). There was no significant difference between T-SPOT.TB and IGRA-ELISA ($p > 0.05$). The five methods of 126 cases of pulmonary disease patients with the highest positive predictive value of tuberculosis diagnosis was the AFB staining, followed by TB-Ab, and the lowest was PPD with only 70.31%. There was a significant difference between AFB staining and PPD ($p < 0.05$). IGRAs detection was superior to the other three methods as a whole, with high sensitivity and negative predictive value (see Table 5), and therefore, it can be used as an effective method for clinical diagnosis and exclusion of tuberculosis. The ROC curve was used to analyze the detection efficiency of IGRA-ELISA and T-SPOT.TB. The area under IGRA-ELISA and T-SPOT.-TB curves was 0.923 and 0.919, respectively. The results showed that the two detection methods had high diagnostic efficiency for tuberculosis. In addition, IGRA-ELISA and T-SPOT.TB combined AFB staining can further improve the sensitivity, positive predictive value, negative predictive value and accuracy of tuberculosis detection without reducing the specificity of detection, while increasing the positive likelihood ratio and reducing the negative likelihood ratio. In this study, IGRA-ELISA combined with AFB staining further improved the sensitivity and accuracy of tuberculosis detection (100.00% and 83.33%, respectively). Therefore, compared with independent detection items, clinical should be combined with several detection methods to make a correct diagnosis of tuberculosis and reduce the incidence of misdiagnosis and missed diagnosis.

Our study has some limitations that could have an impact on the results. The sample size was small, caution is needed when interpreting the results. We have no further suggestion on how to choose appropriate regime for NTM cases with T-SPOT.TB or IGRA-ELISA positive, but we think the first-line anti-TB drugs should be considered.

CONCLUSION

In summary, the evidence in this current study shows that IGRA-ELISA, a recently licensed interferon-gamma release enzyme linked immunosorbent assay in China, has high sensitivity and specificity in diagnosis of active TB with essentially excellent agreement with the widely available T-SPOT.*TB* assay. Additionally, it may be useful to aid in the clinical detection and diagnosis of MTB infection in patients with smear- and/or culture-negative TB. Because IGRA-ELISA does not need to separate peripheral blood mononuclear cells, it has lower requirements for operation and equipment, and is more advantageous in batch operation. Therefore, it is more suitable for clinical popularization and application. IGRA-ELISA should be combined AFB staining to improve the accuracy and clinical application value of tuberculosis diagnosis.

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Declaration of Interest:
None of the authors have any potential conflict of interest.

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