Int J Clin Exp Pathol 2017;10(3):3885-3894
www.ijcep.com /ISSN:1936-2625/IJCEP0046269

Original Article

Application of interferon-γ release assay in extrapulmonary tuberculosis diagnosis, T-lymphocyte regulation, and efficacy evaluation in Northwest China

Zhao Liu[1*], Qi Zhang[2*], Qian Li[1], Longzhi Wang[3], Leipeng Ren[1]

Departments of [1]Thoracic Surgery, [2]Medicine, [3]Science & Education, Xi'an Chest Hospital (Former Xi'an Tuberculosis Hospital), Xi'an, Shaanxi, China. [*]Equal contributors.

Received December 10, 2016; Accepted December 27, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: Although the interferon-γ release assay (IGRA) has become a widely accepted means for the diagnosis of tuberculosis (TB), there are limited data on IGRA performance in the diagnosis of extrapulmonary TB in Northwest China. Using a retrospective cohort design, we enrolled 156 patients with confirmed extrapulmonary tuberculosis. The sensitivity and specificity of TSOPT.TB as well as the area under the receiver operating characteristic curve were analyzed, to compare changes of TSOPT.TB values during treatment and its correlation with T-lymphocyte subsets. The pooled sensitivity and specificity of the TB-IGRA were 71.3% and 65.9%, respectively, and the optimum area under the curve (AUC) was 0.644. Of these, the AUC of IGRA, PPD, and TB antibody of tuberculous empyema obtained by TSPOT.TB were 0.738, 0.664, and 0.634, respectively. The AUC of IGRA, PPD, and TB antibody of lymphatic tuberculosis were 0.726, 0.747, and 0.647, respectively. The AUC of IGRA, PPD, and TB antibody of spinal, bone, and joint tuberculosis were 0.670, 0.588, and 0.642, respectively, while the AUC of IGRA, PPD, and TB antibody of other tuberculosis were 0.786, 0.690, and 0.644, respectively. All of the results were better than those obtained by conventional experimental methods. After antituberculosis treatment, the positive rate and concentration of TSOPT.TB after drug withdrawal were significantly decreased ($P<0.05$). TSPOT.TB can still be a good test for the diagnosis of extrapulmonary tuberculosis. Changes in the TSPOT.TB test after antituberculosis treatment can be an auxiliary indicator of the therapeutic effect and drug withdrawal.

Keywords: IFN-γ release assay, extrapulmonary tuberculosis, T-lymphocyte, diagnosis, therapeutic effect

Introduction

In recent years, the incidence of tuberculosis (TB) is still steadily rising worldwide [1]. Extrapulmonary tuberculosis occurs outside the lung and is a multi-site, multi-system, and multi-species disease. Meanwhile, since lack of specific manifestation to describe extrapulmonary tuberculosis, the diagnosis of it requires comprehensive judgment with extensive clinical experience and auxiliary measures, which leads to diagnostic difficulty [5, 6]. The current methods for diagnosis mainly depend on imaging and histology [4, 7]. Since extrapulmonary tuberculosis lesions are hardly to observe and it is very difficult to obtain pathogenic specimens, there is a lack of gold standard for diagnosis. The current methods always result in misdiagnosis, missed diagnosis, and delayed diagnosis, which would impede timely and effective treatment [2, 3]. In addition, a good indicator to evaluate the therapeutic effect of extrapulmonary tuberculosis after the treatment is lacking [3]. Therefore, the development of a rapid, accurate, and reliable auxiliary examination to enable the early diagnosis of extrapulmonary tuberculosis and evaluation of its therapeutic effect is urgently needed.

In the past 10 years, the interferon-γ (IFN-γ) release assay (IGRA) was developed to distinguish patients suffering from TB. The IGRA measures the immune response to *Mycobacterium tuberculosis* antigens (early secreted antigenic target 6 kDa, or ESAT-6 and culture filtrate protein 10 kDa or CFP-10) *in vitro* [4]. Currently, two commercial IGRAs are recommended and available for diagnosing TB in the

Table 1. Diagnostic data for extrapulmonary tuberculosis

Diagnostic basis	Cases	Male to female ratio	Average age	Lymphatic TB	Other TBs	Tuberculous empyema	Spinal bone and joint TB
Clinical manifestations + bacteriology	33	1.6:1	36.7±15.7	10	7	8	8
Clinical manifestations + histopathology	93	1.3:1	34.6±14.6	27	15	25	26
Clinical manifestations + imaging + diagnostic treatment	30	0.9:1	37.5±16.7	9	5	8	8

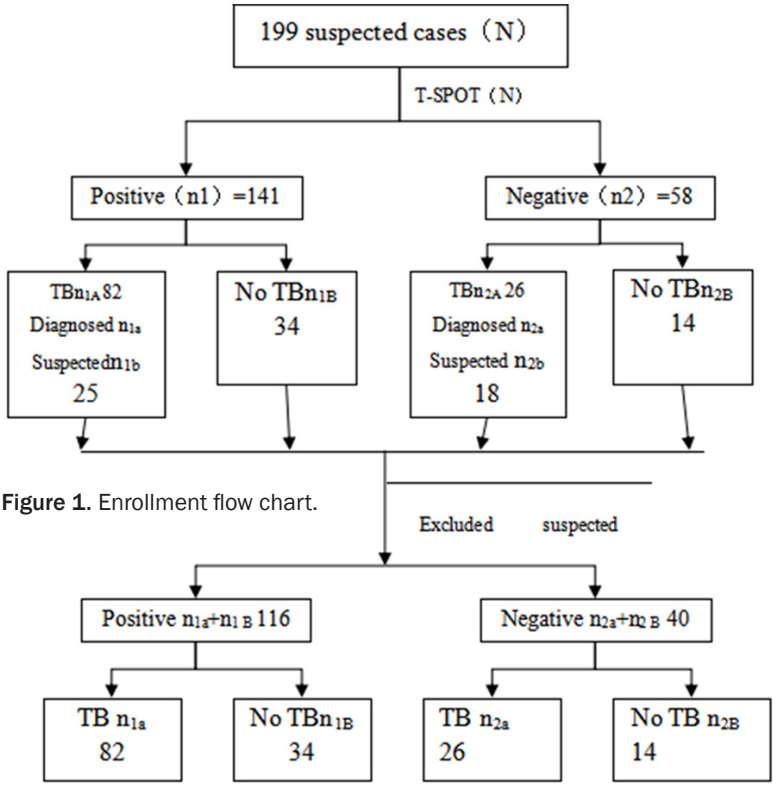

Figure 1. Enrollment flow chart.

clinic: an enzyme-linked immunospot assay TSPOT.TB assay and an enzyme-linked immunosorbant assay QuantiFERON-TB Gold In-Tube assay (QFT-IT) [5]. At present, a large number of studies have been performed to evaluate the application value of TB-IGRA in the diagnosis of tuberculosis and evaluation of its antituberculosis treatment effect [4, 6]. However, the current studies are focused in developed countries with a low prevalence of TB, and only small part of studies evaluated the application value of TB-IGRA in epidemiological screening and clinical diagnosis. In China, there are approximately one million newly diagnosed cases of TB annually. Meanwhile, there is a large population with inactive tuberculosis infection in areas with a high prevalence of TB, such as India, Indonesia, China, and other developing countries [7].

In China, large-sample studies of the application of TB-IGRA in the diagnosis of extrapulmonary tuberculosis have been less frequently reported [8]. As for the value of TB-IGRA as an auxiliary indicator for diagnosing extrapulmonary tuberculosis, the correlation between T-lymphocytes and TSPOT.TB expression, and evaluations of the therapeutic effect have been less frequently studied in China, this study is necessary. We intended to perform a TB-IGRA test of peripheral blood to investigate its diagnostic value in patients with extrapulmonary tuberculosis at different sites and investigate the correlation between T-lymphocytes and TSPOT.TB expression as well as the change of TSPOT.TB spot value after antituberculosis treatment to provide guidance for early diagnosis, immune regulation, and treatment.

Patients and methods

Patients

A total of 199 outpatient and hospitalized patients with suspected extrapulmonary tuberculosis in the Xi'an Chest Hospital (formerly the Xi'an Tuberculosis Hospital) between April 2012 and October 2014 were included. The cases were excluded if they did not meet the criteria of clinical symptoms (low fever, fatigue, night sweats, etc.), bacteriological culture (*M. tuberculosis* was cultured from postoperative granulation tissue or sanies), histopathological diagnosis (chronic granulomatous inflammation and necrosis, etc.), and effectiveness of antituberculosis treatment (symptoms had improved after antituberculosis treatment compared with those before admission), then a total of 156 patients (75 men, 81 women) were

confirmed to suffer from extrapulmonary tuberculosis. Extrapulmonary tuberculosis included tuberculous empyema, spinal, bone, and joint tuberculosis, lymphatic tuberculosis, and others (**Table 1**). Patients with tuberculosis were included according to the clinical manifestations, histopathologic diagnosis, bacterial culture findings, imaging findings, and other auxiliary diagnosis. The basic information, history of exposure to tuberculosis, obsolete pulmonary tuberculosis on a chest film, immune suppression, and other disease histories of the enrolled patients were collected and recorded (**Figure 1**). This study was approved by the Institutional Review Board of the hospital. Signed informed-consent documents were obtained from all study participants.

Methods

IGRA assay: TSPOT.TB (Oxford, Britain), human lymphocyte separation medium and RPMI Media 1640 cell culture medium were purchased from MD Pacific Biotechnology Co., Ltd., Tianjin, China. The procedures were as following: 8 mL of whole blood and 8 mL of peripheral blood were collected separately (fasting was not necessary) in tubes with anti-coagulated with heparin, then were preserved at room temperature for less than 4 h, and sent for testing. The whole blood was subjected to gradient centrifugation to isolate peripheral blood mononuclear cells (PBMCs). After activation, PBMCs were added to a plate that was pre-coated with anti-IFN-γ antibody, 100 μL of the cell solution for each well, four wells for each sample, and a concentration of 250,000 cells in each well. No antigen was applied to a negative control well, and 5 μL of hemagglutinin (PHA) was added to the positive control well, while antigen A (ESAT-6) and antigen B (CFP-10) were added to the experimental well separately for a final concentration of 5 μg/mL. The lip was then covered and incubated in a 5% CO_2 incubator at 37°C for 16-24 h. On the second day, the plate was rinsed, antibodies were added and incubated for 1 h. After washing, avidin was added and incubated for 1 h and then the chromogenic agent was added. ELISPOT was used to count the cells after the plate was dried. At last, the results were read. Positive results were determined referring to the following criteria: when the spot count of the blank control well was 0-5, the spot count of experimental well A or B spot count of blank control ≥6; when the spot count of the blank control well was 6-10, the spot count of antigen A or B was ≥2 times of that of the blank control well. (2) Cases with a spot count that failed to meet the results described in (1) were considered as negative results.

Tuberculin skin test (TST): Patients underwent a TST with five units of PPD of tuberculin, and their conditions of skin redness, callus diameter, diabrosis, and blister within 72 h were recorded. Positive criteria: skin callus >10 mm or diabrosis/blister.

TB antibody test: Serum *M. tuberculosis* antibody immunoglobulin G was rapidly detected in cases of suspected tuberculosis. Cases were identified as positive results if a test line and red control line appeared simultaneously in the reaction plate but determined as negative if only a red control line appeared.

T-lymphocyte subset analysis: A FACS Calibur Cytometer (Becton Dickinson, USA) and Multi-TEST IMK Kit were used for cell analysis. CD3FITC/CD8PE/CD45PerCP/CD4APC (10 μL) and 3FITC/CD16PE+56PE/CD45PerCP/CD19-APC (10 μL) were separately added to the bottom of a flow cytometry tube, and then 100 μL of EDTA-anticoagulated blood and monoclonal antibody were separately added and mixed, placed in the dark at room temperature for 20 min. The tubes were centrifuged to remove the supernatant, rinsed once with phosphate buffered saline, and re-suspended. After fluorescent antibody staining was analyzed using the flow cytometer, the cells were mounted and tested. Finally, the ratio of CD3+ to CD3+CD4+/CD3+ cells in the T-lymphocyte subsets were analyzed using CELLQUEST software (Becton Dickinson).

Antituberculosis treatment and follow-up: The data of patients with confirmed extrapulmonary tuberculosis were recorded.

Antituberculosis treatment consisted of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for an average course of 12±3 months. Exclusion criteria: HIV-positive; immunosuppressed; age >75 years; and underlying diseases such as hypoproteinemia, immunosuppressive therapy, autoimmune diseases, chronic renal insufficiency, or lymphopenia. All

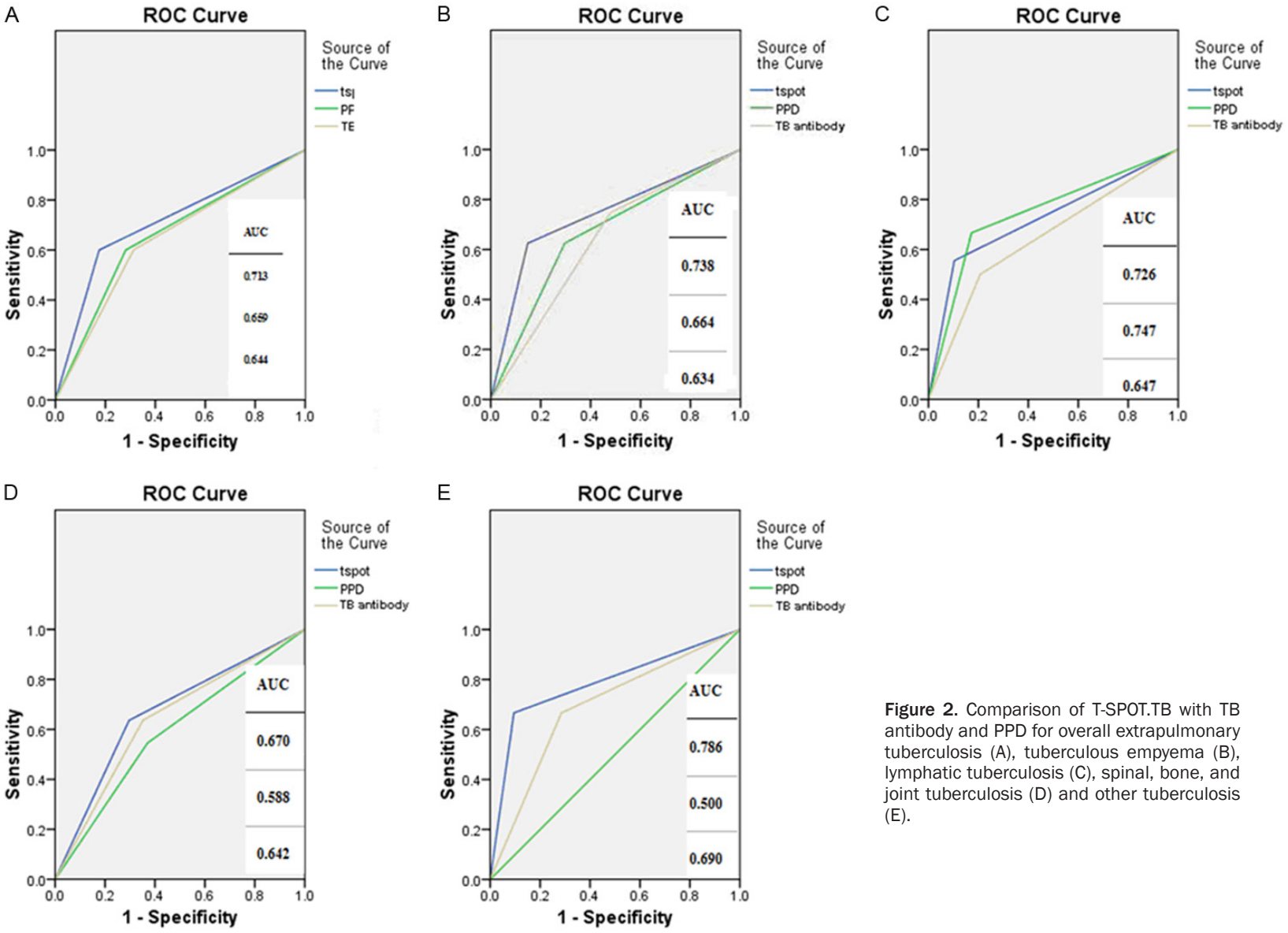

Figure 2. Comparison of T-SPOT.TB with TB antibody and PPD for overall extrapulmonary tuberculosis (A), tuberculous empyema (B), lymphatic tuberculosis (C), spinal, bone, and joint tuberculosis (D) and other tuberculosis (E).

Table 2. Correlation between CD3+ cell and TSPOT.TB value

Descriptive statistics			
	Mean	Standard deviation	N
T	1.6194	.63069	106
Zscore: T-SPOT initial	.0000000	1.00000000	106
Correlation			
		T	Zscore: T-SPOT initial
T	Pearson correlation	1	-.130
	Significance (two-sided)		.184
	N	106	106
Zscore: T-SPOT initial	Pearson correlation	-.130	1
	Significance (two-sided)	.184	
	N	106	106

P>0.05: No significant correlation.

of the patients underwent review at 1 month before drug withdrawal, and the T-spot values were compared with those before antituberculosis treatment. Meanwhile, the patients were followed up for 1 year after drug withdrawal to observe the recurrence and observe and analyze the therapeutic effect.

Statistical analysis: Statistical analyses were performed using SPSS17.0 software. For the count data, the area under the receiver operating characteristic (ROC) curve (AUC) was used to analyze the merits of several diagnostic methods for TB, and the Pearson correlation coefficient was used to analyze the correlation between T-lymphocyte and TB-TSPOT expression level, while values of $P<0.05$ were considered as statistically significant.

Results

Overall test

Among the 156 patients diagnosed with extrapulmonary tuberculosis, the AUC of TSPOT.TB, PPD, and TB antibody were 0.713, 0.659, and 0.644, respectively (**Figure 2A**). For different tuberculosis types, the AUC values obtained by these 3 methods were different. The AUC of TSPOT.TB, PPD, and TB antibody of tuberculous empyema were 0.738, 0.664, and 0.634, respectively (**Figure 2B**). The AUC of TSPOT.TB, PPD, and TB antibody of lymphatic tuberculosis were 0.726, 0.747, and 0.647, respectively (**Figure 2C**). The AUC of TSPOT.TB, PPD, and TB antibody of spinal, bone, and joint tuberculosis were 0.670, 0.588, and 0.642, respectively (**Figure 2D**). The AUC of TSPOT.TB, PPD, and TB antibody of other tuberculosis types were 0.786, 0.690, and 0.644, respectively (**Figure 2E**).

T-lymphocyte analysis

TB-TSPOT and T-lymphocyte analysis were performed in patients underwent antituberculosis treatment. The correlation between CD3+ cells and CD3+CD4+/CD3+ ratio with TB.TSPOT expression level (load capacity of TB.TSPOT in patients with extrapulmonary tuberculosis [9]) were analyzed using the Pearson method and then standardized using the Z-score method. The results revealed that the CD3+CD4+/CD3+ ratio was moderately and negatively correlated with TSPOT.TB value (two-sided) with a correlation coefficient of -0.195 ($|r|<0.4$) ($P<0.05$) (**Table 2**), while the CD3+ cells were not significantly correlated with TB.TSPOT value ($P>0.05$) (**Table 3**).

Therapeutic effect evaluation

Patients first underwent TB-TSPOT testing at the initial treatment for extrapulmonary tuberculosis, and then underwent a review after receiving antituberculosis treatment for 12±3 months, i.e. 1 month before drug withdrawal. A total of 156 patients diagnosed with extrapulmonary tuberculosis were followed up, which showed that the overall descent rate, as well as those of tuberculous empyema, tuberculosis of the spine bone, and joint, lymphatic tuberculosis, and other tuberculosis were statistically significant, while the TB-IGRA positive rate was also significantly decreased ($P<0.0001$) (**Figure 3** and **Table 4**). Meanwhile, TSPOT.TB value was elevated rather than decreased in 10 patients (3 cases of spinal tuberculosis, 5 cases of tuberculous empyema, and 2 cases of lymphatic tuberculosis) after antituberculosis treatment. Furthermore, at the 1-year follow-up after drug withdrawal, 12 patients with lymphatic tuberculosis had enlarged lymph nodes and confirmed to have lymphatic tuberculosis by biopsy and positive TSPOT.TB.

Table 3. Correlation between CD3+CD4+/CD3+ and TB.TSPOT

Descriptive statistics			
	Mean	Standard deviation	N
Z score: TSPOT initial	.0000000	1.00000000	106
CD3+CD4+/CD3+	54.511	9.9938	106
Correlation			
		Zscore: TSPOT initial	Th
Z score: TSPOT initial	Pearson correlation	1	-.195*
	Significance (two-sided)		.045
	N	106	106
Th	Pearson correlation	-.195*	1
	Significance (two-sided)	.045	
	N	106	106

*Significant correlation at a level of 0.05 (two-sided).

Discussion

This study compared the effectiveness of TB-IGRA, TB antibody test, and TST test for diagnosing patients with extrapulmonary tuberculosis using the T-SPOT test (Oxford). The results revealed that, for the overall extrapulmonary tuberculosis, the AUC obtained by TB-IGRA was larger than those by the TST and TB antibody tests, which indicated that the diagnostic effectiveness of TB-IGRA was better than those of the TST and TB antibody test. In addition, in individual analysis, except that lymphatic tuberculosis appeared at an intersected area under the ROC curve, a better diagnostic effect of TB-IGRA than those of the TST and TB antibody tests in other types of tuberculosis.

TSPOT has been widely applied for tuberculosis analysis in recent years. Huang et al. used TSPOT susceptibility survey in 31 patients with extrapulmonary tuberculosis, finding that 29 of 31 patients tested as positive and the susceptibility of 93.5% [10]. Liao et al. from TaiPei Far Eastern Memorial Hospital performed T-SPOT studies in 138 cases of suspected extrapulmonary tuberculosis and found an overall sensitivity of 79.8% and specificity of 81.6%, which was significantly decreased compared with previous results of >90% [11]. In 2011, Cho et al. from Ulsan Medical College conducted a T-SPOT analysis involving 368 cases with suspected extrapulmonary tuberculosis, and concluded an overall sensitivity of 84% and specificity of only 51%, which differed significantly from previous results [9]. In 2012, Feng Y et al. from the Department of Infectious Diseases of Shanghai Huashan Hospital performed a T-SPOT study involving 226 cases of suspected active extrapulmonary tuberculosis and revealed an overall sensitivity of 94.7% and specificity of 84.1% versus a sensitivity and specificity of 93.3% and 88.9%, respectively, for the extrapulmonary tuberculosis group [12]. Fan et al. from Shanghai Pulmonary Disease Hospital published in PLoS a meta-analysis involving 1700 cases from 20 articles (including QFT-G) that analyzed the significance of the IFN-γ release assay in the diagnosis of extrapulmonary tuberculosis and found a T-SPOT sensitivity and specificity of 90% and 68%, respectively [8]. All of these findings revealed that the specificity of TSPOT assay differed widely between China and other countries, which may due to the different incidence of tuberculosis in different countries. Therefore, clinicians need to explain the laboratory results by considering the patients' disease conditions. Overall, these results suggest that TB-IGRA is still of significance in diagnosing patients with extrapulmonary tuberculosis under a background of high tuberculosis infection, which is better than the conventional laboratory examination.

Lymphocytes are mainly divided into T-lymphocytes (CD3+), helper T cells (CD3+CD4+) and suppressor/cytotoxic T cells (CD3+CD8+). Helper CD4+ T cells mainly coordinate the interaction of macrophages and T-lymphocytes in protective immunity against antituberculosis infection [13], which mainly involves immune response of Th1 cells and expands the immunoprotective effect by secreting various cytokines [14]. CD4+ T-lymphocytes produce and release a high concentration of IFN-γ and low concentrations of interleukin (IL)-4 and IL-5, hereby inhibiting the growth of intracellular *M. tuberculosis* [15, 16]. The principle of TB-TSPOT is that T-lymphocytes sensitized by *M. tuberculosis* tend to release Th1 cytokine IFN-γ when they are stimulated by antigens again, where a high level of IFN-γ response suggests a possibility of *M. tuberculosis* infection or sensitiza-

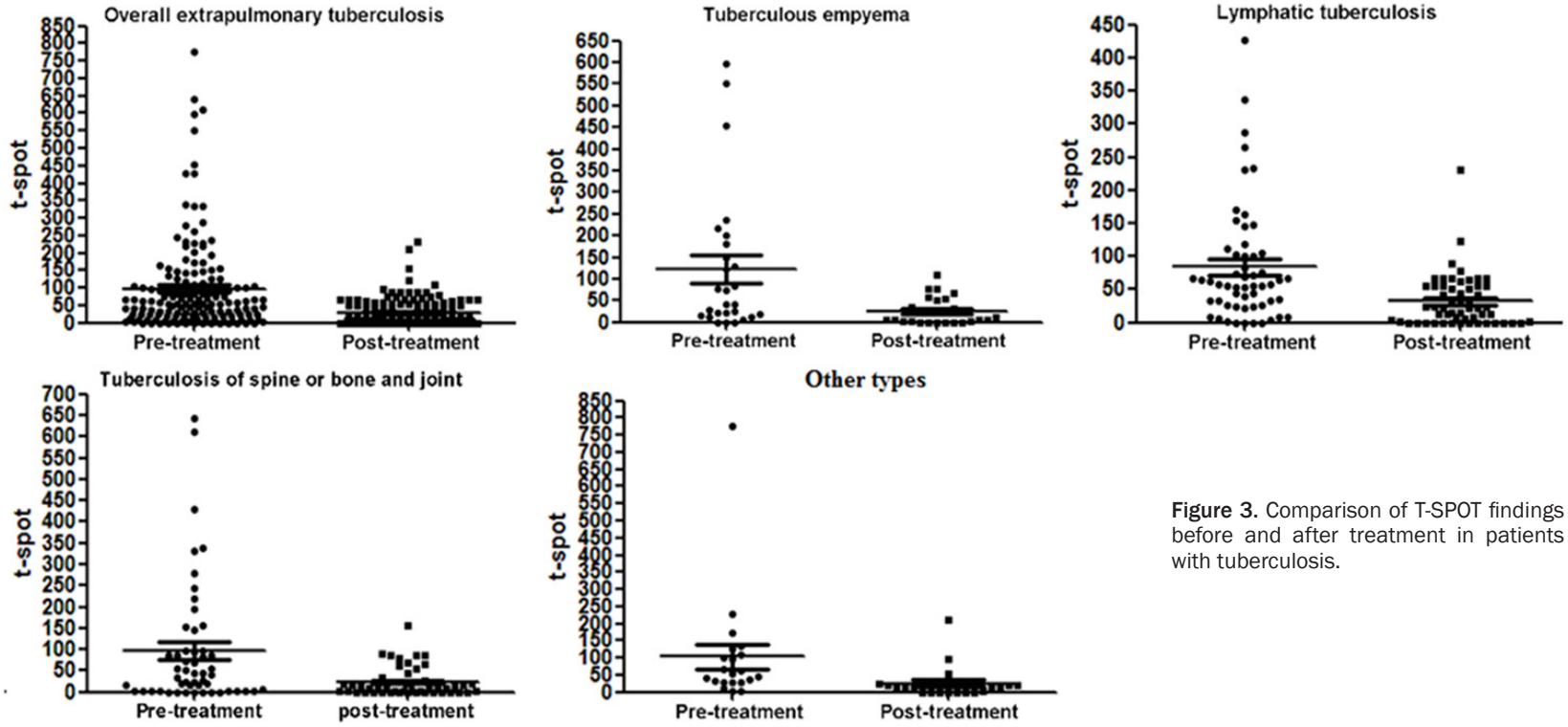

Figure 3. Comparison of T-SPOT findings before and after treatment in patients with tuberculosis.

Table 4. Comparison of T-SPOT score before and after treatment in different tuberculosis

	N		Pre-treatment	Post-treatment	Z	P
Tuberculosis of spine or bone and joint	52	25	4.00	.00	-5.436	0.0001
		50	43.50	10.00		
		75	98.00	27.25		
Other tuberculosis	21	25	27.50	.00	-4.015	0.0001
		50	55.00	14.00		
		75	117.50	22.50		
Tuberculous empyema	27	25	14.00	1.00	-3.829	0.0001
		50	42.00	12.00		
		75	181.00	50.00		
Lymphatic tuberculosis	56	25	26.00	.50	-5.784	0.0001
		50	57.50	22.00		
		75	105.25	55.00		
Overall extrapulmonary tuberculosis	156	25	18.25	.00	-9.712	0.0001
		50	54.50	14.50		
		75	117.25	44.75		

tion [17, 18]. In this study, the CD3+CD4+/CD3+ percentage was negatively correlated with TB-TSPOT expression with statistical significance. The test results first indicated that TB-TSPOT expression was associated with patient immune status and negatively correlated with CD+4 cells. Meanwhile, further studies verified that the disease would aggravate and T-SPOT.TB value would increase under a status of low immunity. Therefore, we infer that a high T-SPOT.TB value may suggest a heavy tuberculosis load in vivo and indirectly reflect patient disease. The limitation of this study was that we did not further analyze the CD4+ subsets or further study the changes of Th1, Th2, Th3, and Th17 [19], nor did we analyze the correlation between increase/decrease of suppressor/cytotoxic cells CD8+ and T-SPOT.TB and disease changes in patients.

TB-TSPOT tends to overcome the above shortcomings of PPD and TB antibody utilizing ESAT-6 and CFP-10, which lack BCG and non-tuberculous mycobacteriosis as an antigen to stimulate the infected mononuclear cells to generate a proliferative response and a large amount of IFN-γ. The central memory T-lymphocytes only produce IL-2 but are unlikely to produce IFN-γ [20], while only effector memory T-lymphocytes are able to produce and secrete a high concentration of IFN-γ [21]. Although immune response activated by TB specific protein antigen tends to proliferate a large amount of effector memory T-lymphocytes, they are produced when there exists *M. tuberculosis* in the body, and they will disappear after healing of tuberculosis [22]. Therefore, the load capacity of *M. tuberculosis* is positively correlated with the IGRAs effect, which is a good indicator to evaluate the antituberculosis effect.

In this study, 156 patients with extrapulmonary tuberculosis underwent TB-IGRA test, antituberculosis treatment and follow-up. Of these, the T-SPOT.TB value was elevated rather than decreased in 10 patients (3 cases of spinal tuberculosis, 5 cases of tuberculous empyema and 2 cases of lymphatic tuberculosis) after antituberculosis treatment, which was speculated to be due to drug-resistance since their clinical manifestations were improved. In addition, a 1-year follow-up was performed after drug-withdrawal, and 12 patients with lymphatic tuberculosis appeared enlargement of lymph nodes, and they were diagnosed with lymphatic tuberculosis by biopsy and their T-SPOT.TB appeared positive at review. After treatment, the reproduction of *M. tuberculosis* is inhibited, bacterial count is gradually reduced with antituberculosis treatment. On the contrary, for patients with a poor therapeutic effect, their effector T cells tend to elevate the IFN-γ to a high level; thus, the TB-IGRA shows a good correlation with in vivo load capacity of *M. tuberculosis*, which suggests that TB-IGRA can be an auxiliary indicator to evaluate the efficacy of antituberculosis treatment and drug withdrawal indications [23]. A larger sample size should

be used to stratify different kinds of extrapulmonary tuberculosis to confirm the diagnostic value of T-SPOT.TB for different kinds of extrapulmonary tuberculosis, investigate the correlation between T-SPOT and T-lymphocytes, and evaluate the therapeutic effect.

Conclusion

In conclusion, this study demonstrated that TSPOT.TB can still be a good basis for the diagnosis of extrapulmonary tuberculosis in China. The CD3+CD4+/CD3+ level of helper T cells in the peripheral blood tends to decrease in a high TB load (higher IFN-γ expression means a higher TB infection load in vivo) in patients with extrapulmonary tuberculosis. After antituberculosis treatment, the TSPOT.TB test revealed that the change of TSPOT.TB in the peripheral blood is significantly decreased as antituberculosis treatment progresses, which is consistent with the clinical symptoms and imaging results. Therefore, it can be considered as an auxiliary indicator of therapeutic effect and drug withdrawal indications.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qi Zhang, Department of Medicine, Xi'an Chest Hospital, Xi'an 710061, Shaanxi, China. Tel: +86-29-88852982; Fax: +86-29-88852982; E-mail: qizhang886@sina.com

References

[1] Zumla A, George A, Sharma V, Herbert RH, Oxley A, Oliver M. The WHO 2014 global tuberculosis report--further to go. Lancet Glob Health 2015; 3: e10-12.

[2] Singh N, Sreenivas V, Gupta KB, Chaudhary A, Mittal A, Varma-Basil M, Prasad R, Gakhar SK, Khuller GK, Mehta PK. Diagnosis of pulmonary and extrapulmonary tuberculosis based on detection of mycobacterial antigen 85B by immuno-PCR. Diagn Microbiol Infect Dis 2015; 83: 359-364.

[3] Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul) 2015; 78: 47-55.

[4] Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, Metcalfe JZ, Cattamanchi A, Dowdy DW, Dheda K, Banaei N. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev 2014; 27: 3-20.

[5] Whitworth HS, Scott M, Connell DW, Donges B, Lalvani A. IGRAs--the gateway to T cell based TB diagnosis. Methods 2013; 61: 52-62.

[6] Wyndham-Thomas C, Dirix V, Schepers K, Martin C, Hildebrand M, Goffard JC, Domont F, Libin M, Loyens M, Locht C, Van Vooren JP, Mascart F. Contribution of a heparin-binding haemagglutinin interferon-gamma release assay to the detection of mycobacterium tuberculosis infection in HIV-infected patients: comparison with the tuberculin skin test and the QuantiFERON-TB gold In-tube. BMC Infect Dis 2015; 15: 59.

[7] Badawi A, Sayegh S, Sallam M, Sadoun E, Al-Thani M, Alam MW, Arora P. The global relationship between the prevalence of diabetes mellitus and incidence of tuberculosis: 2000-2012. Glob J Health Sci 2015; 7: 183-191.

[8] Fan L, Chen Z, Hao XH, Hu ZY, Xiao HP. Interferon-gamma release assays for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. FEMS Immunol Med Microbiol 2012; 65: 456-466.

[9] Cho OH, Park KH, Kim SM, Park SJ, Moon SM, Chong YP, Sung H, Kim MN, Jeong JY, Lee SO, Choi SH, Woo JH, Kim YS, Kim SH. Diagnostic performance of T-SPOT.TB for extrapulmonary tuberculosis according to the site of infection. J Infect 2011; 63: 362-369.

[10] Huang Z, Xiong G, Luo Q, Jiang B, Li W, Xu X, Li J. Evaluation of the performance of the microscopic observation drug susceptibility assay for diagnosis of extrapulmonary tuberculosis in China: A preliminary study. Respirology 2014; 19: 132-137.

[11] Liao CH, Chou CH, Lai CC, Huang YT, Tan CK, Hsu HL, Hsueh PR. Diagnostic performance of an enzyme-linked immunospot assay for interferon-gamma in extrapulmonary tuberculosis varies between different sites of disease. J Infect 2009; 59: 402-408.

[12] Feng Y, Diao N, Shao L, Wu J, Zhang S, Jin J, Wang F, Weng X, Zhang Y, Zhang W. Interferon-gamma release assay performance in pulmonary and extrapulmonary tuberculosis. PLoS One 2012; 7: e32652.

[13] Kim SH, Song KH, Choi SJ, Kim HB, Kim NJ, Oh MD, Choe KW. Diagnostic usefulness of a T-cell-based assay for extrapulmonary tuberculosis in immunocompromised patients. Am J Med 2009; 122: 189-195.

[14] Yao S, Huang D, Chen CY, Halliday L, Wang RC, Chen ZW. CD4+ T cells contain early extrapulmonary tuberculosis (TB) dissemination and rapid TB progression and sustain multieffector functions of CD8+ T and CD3- lymphocytes: mechanisms of CD4+ T cell immunity. J Immunol 2014; 192: 2120-2132.

[15] Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clin Dev Immunol 2011; 2011: 814943.
[16] Schierloh P, Landoni V, Balboa L, Musella RM, Castagnino J, Moraña E, de Casado GC, Palmero D, Sasiain MC. Human pleural B-cells regulate IFN-gamma production by local T-cells and NK cells in a Mycobacterium tuberculosis-induced delayed hypersensitivity reaction. Clin Sci (Lond) 2014; 127: 391-403.
[17] Ferrara G, Losi M, Meacci M, Meccugni B, Piro R, Roversi P, Bergamini BM, D'Amico R, Marchegiano P, Rumpianesi F, Fabbri LM, Richeldi L. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2005; 172: 631-635.
[18] Lee SW, Lee CT, Yim JJ. Serial interferon-gamma release assays during treatment of active tuberculosis in young adults. BMC Infect Dis 2010; 10: 300.
[19] Kumar NP, Sridhar R, Banurekha VV, Nair D, Jawahar MS, Nutman TB, Babu S. Expansion of pathogen-specific mono- and multifunctional Th1 and Th17 cells in multi-focal tuberculous lymphadenitis. PLoS One 2013; 8: e57123.
[20] Reiley WW, Shafiani S, Wittmer ST, Tucker-Heard G, Moon JJ, Jenkins MK, Urdahl KB, Winslow GM, Woodland DL. Distinct functions of antigen-specific CD4 T cells during murine Mycobacterium tuberculosis infection. Proc Natl Acad Sci U S A 2010; 107: 19408-19413.
[21] Harari A, Vallelian F, Meylan PR, Pantaleo G. Functional heterogeneity of memory CD4 T cell responses in different conditions of antigen exposure and persistence. J Immunol 2005; 174: 1037-1045.
[22] Lee YM, Park KH, Kim SM, Park SJ, Lee SO, Choi SH, Kim YS, Woo JH, Kim SH. Risk factors for false-negative results of T-SPOT.TB and tuberculin skin test in extrapulmonary tuberculosis. Infection 2013; 41: 1089-1095.
[23] Clifford V, He Y, Zufferey C, Connell T, Curtis N. Interferon gamma release assays for monitoring the response to treatment for tuberculosis: a systematic review. Tuberculosis (Edinb) 2015; 95: 639-650.