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Correlation between the tuberculin skin test and T-SPOT.TB in patients with suspected tuberculosis infection: A pilot study

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Abstract. T-SPOT.TB is a novel screening method for *Mycobacterium tuberculosis* infection. However, it is controversial whether T-SPOT.TB should become an alternative method to the tuberculin skin test (TST) for screening *M. tuberculosis* infections. The present study aimed to evaluate this issue based on the retrospective analysis of clinical cases. TST and T-SPOT.TB tests were used on patients with suspected *M. tuberculosis* infection on admission. Demographic data and clinical information, including previous history of *M. tuberculosis* infection, were collected. A total of 118 patients were included in the analysis, among whom 30 (25.4%) were diagnosed with active *M. tuberculosis* infection, and seven patients (5.9%) were currently receiving immunosuppressive treatment. The overall sensitivity and specificity of the TST were 76.7 and 77.3%, respectively, while they were 88.3 and 68.1%, respectively, for the T-SPOT.TB test. Patients with large TST indurations had a higher number of gamma interferon-producing T cells among peripheral blood mononuclear cells compared with those of TST-negative patients. In conclusion, the T-SPOT.TB test had a higher sensitivity than the TST, but the difference was not statistically significant. Neither the T-SPOT.TB test nor the TST was sufficiently accurate to detect active *M. tuberculosis* infection.

Introduction

Mycobacterium tuberculosis (TB) infection remains a large global health problem. In 2017, an estimated 10.0 million individuals developed TB. TB is now the 10th leading cause of death worldwide and the leading cause of mortality from a single infectious agent. China is one of 30 high TB-burden countries (1). To reach the goal of TB elimination, individuals with active TB require rapid identification and treatment. Microscopy, growth in culture and molecular tests are the gold standards for the diagnosis of active TB, as they directly indicate the presence of actual TB bacilli or their DNA (1,2). However, not all cases of TB infection may be bacteriologically confirmed. For patients with a negative acid-resistant bacillus sputum-smear test, diagnosis and treatment decisions may be challenging.

The tuberculin skin test (TST) has been widely used for detecting latent TB infection (LTBI) and active TB for almost a century. The important advantages of the TST include its low cost and convenience. However, the TST result may be influenced by prior Bacillus Calmette-Guerin (BCG) vaccination and infection with non-tuberculous mycobacteria (NTM) (3). In recent years, several commercially available interferon-γ release assays (IGRAs) have been developed as an alternative screening approach for TB infection. These tests, including the T-SPOT.TB test, QuantiFERON-TB Gold or QuantiFERON-TB Gold In-Tube target unique and specific *M. tuberculosis* proteins that are not present in BCG or in most environmental mycobacteria (3). Several meta-analyses have indicated a relatively enhanced sensitivity and specificity of IGRAs over the TST in identifying TB infection. However, neither the IGRAs nor the TST exhibited ideal stability (4-6).

To assess the value of these two methods, the present retrospective analysis was performed. The performance of the T-SPOT.TB in detecting active TB was compared with that of the TST and the comparison between these two detection methods was determined.

Materials and methods

Participants and data collection. A retrospective analysis was performed on patients diagnosed at the Respiratory Department of Ningbo First Hospital (Ningbo, China) between October 2016 and 2017. A total of 118 patients who were suspected of active TB infection on admission were included in the analysis. Each patient was subjected to the TST as well as the T-SPOT.TB test. The patients' demographics and clinical information, including previous history of TB, were collected.

Definitions and diagnoses. Final diagnoses were made considering all clinical, radiological, microbiological and pathological information. Patients who had clinical, bacteriological and/or

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radiographic evidence of active TB infection were defined as active TB cases of the following varieties: i) Pulmonary TB: *M. tuberculosis* was cultured from sputum, bronchial specimens or patients whose data met the definition of a clinical case of TB (7). For clinical cases of TB, chest radiographic findings were defined as the presence of cavities, branching linear lesions, multiple centrilobular nodules or lobular consolidation upon high resolution CT (8,9). Lesions that mostly appeared as calcified nodules or fibrotic scars were not considered to be indicative of active TB infection. For patients with lesions that suggested active TB but who had a negative bacteriologic status, broad-spectrum antibiotics were given for one week. If the lesions significantly improved, a diagnosis of active TB was ruled out. All clinical cases were followed up for at least 3 months for further confirmation. ii) Pleural TB: *M. tuberculosis* was detected in the pleural fluid or a tissue biopsy, or exudative pleural effusion exhibited predominant lymphocytosis, high protein, low carcinoembryonic antigen (<5 ng/ml) and high adenosine deaminase (≥40 IU/l) (7,10). iii) Lymph node TB: *M. tuberculosis* was detected in lymph node tissue.

TST and T-SPOT.TB. A TST was performed following standard procedures. A total of 0.1 ml of purified protein derivate (Chengdu Institute of Biological Products Co., Ltd.) was injected intradermally into the inner side of the forearm, and the transverse induration was measured in mm after 48-72 h by trained nurses. An induration of ≥10 mm (or ≥5 mm in immunosuppressed individuals) was classified as a positive result. The T-SPOT.TB assays (Beijing Wan Tai Bio-Pharmaceutical Co., Ltd) were performed and interpreted according to the manufacturer's specifications (11).

Statistical analysis. Continuous variables are expressed as the mean and standard deviation. Frequencies were calculated for demographic and clinical data. Comparisons between different groups were performed using a Student's t-test or least-significant difference (LSD) test. The LSD test was performed following one-way analysis of variance. Fisher's exact or χ^2 tests were used for univariate analyses. In each analysis, $P<0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS for Windows, version 22.0 (IBM Corp.) and GraphPad Prism 5.0 (GraphPad Software, Inc.).

Results

Characteristics of the study population. A total of 118 patients were included in the analysis and 70 (59.3%) of them were female. The median age was 56.5 years (range, 18-95 years). One patient (0.8%) was confirmed as HIV-positive and seven patients (5.9%) were currently receiving immunosuppressive treatment. A total of 10 patients (8.5%) had previously been diagnosed with TB and had received anti-TB treatment. BCG scars were present in 88 patients (74.6%). Active TB infection was diagnosed in 30 patients (25.4%) and 15 of them had pulmonary TB. A total of 88 patients (74.6%) were diagnosed with non-TB conditions. Pneumonia was the most common disease among those non-TB cases. Concerning the laboratory data, TB patients had a lower lymphocyte ratio than that in the non-TB group. There were no significant differences between the $CD4^+$ lymphocyte ratio and the $CD8^+$ lymphocyte ratio between the two groups (Table I).

Performance of T-SPOT.TB and TST in active TB. Of all of the 118 patients, the TST results were positive for 43 patients (36%), 23 of whom were diagnosed with active TB; the TST results were negative for 75 patients (64%), 7 of whom were diagnosed with active TB. The overall sensitivity and specificity of the TST were 76.7 and 77.3%, respectively. In the T-SPOT.TB test, 53 patients (45%) had positive results, 25 of whom were diagnosed with active TB; 65 patients (55%) had negative results, 5 of whom were diagnosed with active TB. The overall sensitivity and specificity of the T-SPOT.TB test were 88.3 and 68.1%, respectively. However, no significant difference was observed between T-SPOT.TB and TST (Table II).

The accuracy of the TST and T-SPOT.TB test for pulmonary and extrapulmonary TB (EPTB) was calculated separately. For pulmonary TB, the sensitivity of the TST was 80.0%, and the specificity was 70.0%. The sensitivity and specificity of the T-SPOT.TB test was 80.0 and 60.2%, respectively. For the EPTB, the TST sensitivity was 73.3% and the specificity was 68.9%, while the sensitivity and specificity of the T-SPOT.TB were 86.7 and 61.2%, respectively. No significant difference was noted in the above results between the TST and the T-SPOT.TB test. The negative predictive value (NPVs) of the TST and T-SPOT.TB test was higher than the respective positive predictive value (PPVs) (Table II).

Association between the TST spot size and T-SPOT.TB results. There was a trend toward an increased likelihood of T-SPOT.TB positivity with increased TST spot size. Patients with a large TST size (>20 mm) had a higher number of gamma interferon-producing T cells among their peripheral blood mononuclear cells (PBMCs) than that of TST-negative patients (spot size, <10 mm; 240.2±155.6 vs. 101.4±129.1; spot-forming cells/10^6 PBMCs, P=0.008). However, no such differences were observed among other stratified data based on the TST size (Table III and Fig. 1).

Discussion

The major results of the present study were as follows: i) The T-SPOT.TB test had a higher sensitivity than the TST, but the specificity and PPV were comparatively lower than those of the TST. However, none of the above results were statistically significant; ii) the NPVs of the TST and the T-SPOT.TB test were much higher than the PPVs; and iii) increased TST spot size is associated with a trend toward increased rates of T-SPOT.TB positivity.

The overall sensitivity of the TST and T-SPOT.TB test were 76.7 and 88.3%, respectively, in the present study. The T-SPOT.TB test had a comparatively higher sensitivity than that of the TST, which was consistent with the results of certain previous meta-analyses (6,12). However, compared to most data for cohorts from developed countries (3,12), a lower specificity (68.1%) and PPV (47.2%) of the T-SPOT.TB was determined in the present study, which is more consistent with the result of one large-scale retrospective multicenter study from China (13). The accuracy of the TST and T-SPOT.

Table I. Baseline characteristics of the subjects.

Characteristic	TB (n=30)	Non-TB (n=88)	P-value
Male sex	14 (47.7)	56 (63.7)	0.102
Age, years	50.0±19.5	58.7±17.4	0.024
BMI	20.7±3.8	21.2±3.6	0.485
Smoking index	198.5±350.3	283.8±503.8	0.393
Prior treatment of TB	3 (10.0)	7 (8.0)	0.728
Current immunosuppressive treatment	4 (13.3)	3 (3.4)	0.047
BCG scar present	18 (60.0)	70 (79.5)	0.100
WBC (10^9/l)	6.7±1.9	7.3±4.0	0.404
Lymphocytes (%)	19.0±6.4	23.3±9.7	0.007
CRP (mg/dl)	20.0±22.1	27.2±48.4	0.273
$CD4^+$ T lymphocytes (%)	41.4±10.5	40.3±8.8	0.736
$CD8^+$ T lymphocytes (%)	24.1±11.1	24.6±10.3	0.900
Final diagnosis			
Pulmonary TB	14 (46.7)		
Endobronchial TB	1 (3.3)		
Pleural TB	13 (43.3)		
Lymph node TB	2 (6.7)		
Pneumonia		65 (73.9)	
Pulmonary fungal infection		4 (4.5)	
Sarcoidosis		2 (2.3)	
Lung tumor		6 (6.8)	
Others		11 (12.5)	

Values are expressed as the mean ± standard deviation or n (%). Percentages are calculated for the number in each subgroup within the same column. TB, tuberculosis; TST, tuberculin skin test; WBC, white blood cells; BMI, body mass index; BCG, Bacillus Calmette-Guerin; CRP, C-reactive protein.

Table II. Performance of T-SPOT.TB and TST in active TB.

Parameter (%)	T-SPOT.TB	TST	P-value
Overall sensitivity	83.3 (25/30)	76.7 (23/30)	0.51
Overall specificity	68.1 (60/88)	77.3 (68/88)	0.17
Pulmonary TB			
Sensitivity	80.0 (12/15)	80.0 (12/15)	1
Specificity	60.2 (62/103)	70.0 (72/103)	0.14
PPV	23.6 (12/53)	27.9 (12/43)	0.55
NPV	95.4 (62/65)	96.0 (72/75)	0.86
Extrapulmonary TB			
Sensitivity	86.7 (13/15)	73.3 (11/15)	0.41
Specificity	61.2 (63/103)	68.9 (71/103)	0.24
PPV	24.5 (13/53)	25.6 (11/43)	0.91
NPV	96.9 (63/65)	94.7 (71/75)	0.51

TB, tuberculosis; TST, tuberculin skin test; NPV, negative predictive value; PPV, positive predictive value.

TB test for EPTB was also evaluated. The specificity of the T-SPOT.TB test did not exhibit any advantage over that of the TST (61.2 vs. 68.9%), which may indicate a relatively high prevalence of LTBI in the region of residence of the present cohort. The T-SPOT.TB test and the TST are based on cellular immune responses, and they are unable to distinguish between latent TB infection and active TB (14). Therefore, the T-SPOT.TB may have limited value in detecting active TB, particularly in high TB burden settings (15,16).

It was found that the NPVs were much higher than the PPVs for the TST (91 vs. 53%) and for the T-SPOT.TB test (92 vs. 47%). This indicated that the T-SPOT.TB test and the TST may be more appropriate for ruling out active TB than for ruling it in. A previous study suggested that the combination of negative results obtained by IGRAs with the TST may enable the rapid exclusion of TB (17,18). Further studies are required to identify the optimal combined strategy for targeted screening. However, considering the low PPV of the two methods, active TB should not simply be excluded for high-risk individuals without a thorough microbiological examination of *M. tuberculosis*.

In the present study, a trend toward an increased likelihood of a positive T-SPOT.TB result with increased TST spot size was observed. Several studies have evaluated the association between the TST size and IGRAs result in LTBI, and the results indicated that the TST size may help identify those subjects with the highest risk of LTBI (19-21). All of these studies lack the gold standard testing for determining LTBI. The present study identified a relative concordance of a positive T-SPOT.TB result and the TST size in detecting active TB. Patients with a TST size of

Table III. Comparison between size of TST induration and T-SPOT.TB result.

		T-SPOT.TB	
TST induration (mm)	N	Positive n (%)	SFCs/10^6 PBMC, mean ± SD
<10	75	18 (24.0)	101.4±129.1
≥10	43	34 (79.1)	196.7±134.9
			[a]P=0.017
10-14	22	16 (72.7)	168.9±112.0
15-19	6	5 (83.3)	172.8±143.1
≥20	15	13 (86.7)	240.2±155.6
			[b]P=0.297

[a]P-value refers to the number of gamma interferon-producing T cells in TST-positive patients compared with TST-negative negative patients. [b]P-value refers to the differences among stratified data based on TST spot size. TST, tuberculin skin test; SFCs, spot-forming-cells; PBMC, peripheral blood mononuclear cells; SD, standard deviation.

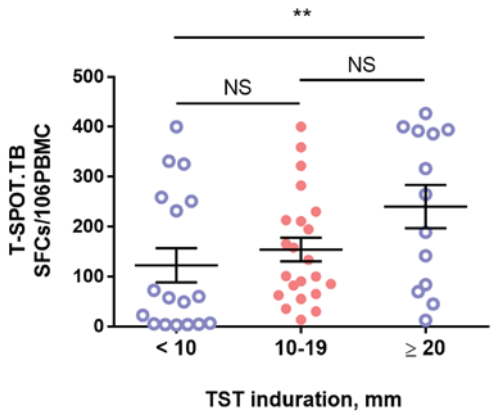

Figure 1. Correlation between the size of the TST induration and T-SPOT.TB result. **P=0.008; NS, not significant; TST, tuberculin skin test; PBMC, peripheral blood mononuclear cell; SFCs, spot-forming-cells.

>20 mm had a higher number of gamma interferon-producing T cells than those with a negative TST result, which implied that the body had a strong immune response to the exposure to TB bacilli. However, when the TST size was <20 mm, the correlation between the number of T cells producing gamma interferon among PBMCs and the size of the TST spot was not high. One possible explanation is that a positive TST test cannot differentiate between *M. tuberculosis* infection, prior BCG vaccination and exposure to NTM (22), particularly when the TST induration is <15 mm (23,24). In addition, the TST and the T-SPOT.TB test are designed to detect the presence of *M. tuberculosis*-specific T-cell responses (25) and represent indirect evidence for past or present exposure to TB bacilli. Neither a positive TST size nor T-SPOT.TB result may discriminate active TB infection from LTBI. In light of the high TB burden in China, even if the TST result is strongly positive, a diagnosis of active TB still requires further examination and comprehensive consideration.

There are certain limitations of the present study. First, as a retrospective study, the decision to perform the T-SPOT.TB test and TST depended on the physician's judgment at that time, possibly introducing a selection bias. Second, the number of cases included is limited. On the one hand, as a commercial test, the T-SPOT.TB test has been introduced at our hospital only recently, so the number of cases is not high. On the other hand, the number of patients diagnosed with active TB infection is also not large (pulmonary TB, 15 cases and extrapulmonaryTB, 15 cases). Considering these limitations, the results of the present study should be interpreted with caution. A population-based study with a sufficient sample size and follow-up is required to fully compare the performance of IGRAs and the TST in high-risk TB populations (26).

In conclusion, the T-SPOT.TB test had a higher sensitivity than the TST. An increased TST spot size was associated with a trend toward an increased rate of T-SPOT.TB positivity. However, neither the T-SPOT.TB test nor the TST was sufficiently accurate to be used for detecting active TB disease. Given the comparable performance, the selection of TST or T-SPOT.TB should rather depend on other considerations, including cost, benefits and resources.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JY, WK and NX were responsible for the acquisition, analysis and interpretation of the data and contributed to the drafting of the manuscript. XC provided statistical support and data interpretation. XH and XC made critical revisions to the manuscript for important intellectual content and performed

the final proofing. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed with the informed consent of each subject and with the approval of the local Ethics Committee of Ningbo First Hospital (Ningbo, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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