Comparison of Tuberculin Skin Testing and T-SPOT.TB for Diagnosis of Latent and Active Tuberculosis

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SUMMARY: The T-SPOT.TB test does not cross-react with Bacille Calmette-Guérin or most non-tuberculosis mycobacterium species, and is based on IFN-γ responses to Mycobacterium tuberculosis-specific antigens. The objective of this study was to compare tuberculin skin test (TST) with T-SPOT.TB results used in the diagnosis of active tuberculosis (TB) as well as latent tuberculosis infection (LTBI). A total of 136 subjects participated in three different groups (47 patients with active pulmonary TB, 47 healthy persons without M. tuberculosis exposure, and 42 hospital members with a history of close contact with active TB patients). The T-SPOT.TB sensitivity (83.0%) and the negative predictive value (NPV) (82.6%) in the diagnosis of active TB were significantly higher than those of TST. The sensitivity and NPV of the TST were 38.3 and 60.8%, respectively. The T-SPOT.TB specificity (80.9%) and positive predictive value (81.3%) were lower than those of TST (95.7 and 90.0%, respectively). The performance of T-SPOT.TB and TST for diagnosing LTBI was the same (54.8%). T-SPOT.TB was superior in terms of sensitivity (83.0%); TST detected only 18, whereas T-SPOT.TB test detected 39 out of 47 patients with active TB. T-SPOT.TB is thought to have better performance than TST due to false-negative results in diagnosing active TB. However, it is considered that large prospective longitudinal studies are needed for diagnosing LTBI.

INTRODUCTION

Tuberculosis (TB) is still the most common deadly infectious disease worldwide. The Global TB Control Report released in 2007 by the World Health Organization (WHO) reflects that there are 8.8 million new TB cases in the world, and 3.9 million smear-positive patients. It is estimated that the global TB incidence and prevalence rates have reached 136 and 217 per 100,000 population, respectively (1).

Diagnosis and treatment of patients with latent tuberculosis infection (LTBI), who are at high risk of progression to active TB, are very important in the control of TB, especially throughout the developing world due to the specific socioeconomic factors, and there is growing need for new rapid and accurate diagnostic methods in order to achieve higher sensitivity and specificity compared to traditional methods of microscopic sputum examination and culture. Although the finding of tubercle bacilli is necessary for the diagnosis of active TB, there is no “gold standard” test for diagnosis of LTBI (2). Pulmonary TB can be diagnosed by its symptoms, chest radiography, and sputum smear microscopy as well as by cultivation of Mycobacterium tuberculosis, which is considered to be the gold standard. Microscopy of direct smears for acid-fast staining (AFS) is the fastest, the cheapest, and the most commonly used method for diagnosis of TB. However, AFS of sputum has a sensitivity of only 50 to 60%. Culture of mycobacteria is the reference method for detection of tubercle bacilli, but mycobacterial culture usually requires 6 to 8 weeks to be interpretable. In England, data have shown that 68% of patients with pulmonary TB and 49% of those with extra pulmonary TB are detected by the culture method. These percentages detected by the culture method are lower for children (3–5).

Detection of LTBI is still based on the tuberculin skin test (TST); the diagnosis of LTBI by the degree of positivity of TST is limited, both operationally and logistically, in populations vaccinated with Bacille Calmette-Guérin (BCG) or sensitized by non-tuberculosis mycobacteria (NTM), and by its low sensitivity in persons with suppressed immune systems who are at the greatest risk of progression.

Although the new tests based on the release of interferon (IFN)-γ from M. tuberculosis-specific T cells for TB infection could improve the diagnosis of TB, studies regarding the sensitivity and specificity of these tests have shown discrepancies.

Another new approach for diagnosing both active and latent TB follows the availability of the T-SPOT.TB test, which measures the in vitro production of IFN-γ by blood mononuclear cells in response to M. tuberculosis-specific antigens (2). The enzyme-linked immunospot assay (ELISPOT) has been shown to be more sensitive and more specific than TST (6).

TST measures hypersensitivity responses to purified protein derivative (PPD), a crude mixture of antigens shared by M. tuberculosis, Mycobacterium bovis BCG, and several NTM. TST has been, until recently, the only available tool for TB diagnosis: however, it provides low sensitivity and specificity and does not reliably distinguish LTBI from prior immunization with BCG, or from infection with environmental mycobacteria (7). Compared with M. tuberculosis H37Rv, M. bovis, and BCG strains, 16 region of difference (RD) genes were identified. It is absent from M. bovis BCG strains and

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The RD1 genomic region of *M. tuberculosis* has three major T-cell antigens, i.e., PPE68, ESAT-6, and CFP-10 (8,9). The application of these antigens to the whole blood IFN-γ assay (QuantIFERON-TB) is considered to allow for specific and sensitive diagnosis of *M. tuberculosis* infection in a relatively simple and rapid test format (10).

In the present study, we compared the T-SPOT.TB test to TST used in the diagnosis of active TB as well as LTBI. Rapid determination of TB infection by T-SPOT.TB accelerates the diagnosis of TB, enabling early treatment initiation. In addition, determination of persons with LTBI helps to prevent the risk of progression to active TB.

### PATIENTS AND METHODS

**Participants:** In this study, a total of 136 participants were screened using TST and a new ESAT-6/CFP-10 peptide-based IFN-γ assay, T-SPOT.TB, during the 1-year period from December 2007 to December 2008. Subjects were enrolled into one of three groups: Group 1 consisted of patients with active pulmonary TB diagnosed by clinical examination, chest X-ray, AFS, and culture at the Atatürk Chest Diseases & Thoracic Surgery Center and who had not received anti-TB treatment (n = 47); Group 2 consisted of persons who had no identifiable risk of *M. tuberculosis* infection (n = 47); Group 3 were contacted health workers at a TB clinic and some TB patients’ relatives accompanying them (n = 42).

The study was approved by the local ethics committee before research began. All participating subjects gave written, informed consent and were questioned regarding BCG vaccination and their history of exposure to TB patients and other TB risk factors such as having an immunosuppressive condition (i.e., human immunodeficiency virus [HIV], leukemia, lymphoma, diabetes mellitus, or renal failure).

**TST:** TST was performed using 0.1 ml of 5 TU (a standard dose of 5 tuberculin units) of PPD-S (BD-NCIPD Ltd., Sofia, Bulgaria), with the Mantoux method according to a standard protocol. Indurations were measured by the ballpoint method 72 h later, and TST results were interpreted according to the induration diameters (11–13).

In persons who were vaccinated with BCG, 0–5 mm induration measurements were classified as negative, but a 6–14 mm induration zone was attributed to BCG, and ≥15 mm was considered positive and assessed as infection. In those who were not vaccinated with BCG, a 0–5 mm induration zone was accepted as negative, and an induration measurement of ≥10 mm was classified as positive.

**T-SPOT.TB test:** This test was performed according to the manufacturer’s instructions (Oxford Immunotec, Oxford, UK). Eight milliliters of peripheral venous blood was collected from all the subjects in the study and processed within 4 h. For the T-SPOT.TB test, 250,000 peripheral blood mononuclear cells (for per well)/100 µl AIM-V medium were pipetted into four wells (nil–negative control, mitogen-positive control, and the two specific antigens, ESAT-6 and CFP-10) per person from a 96-well microtiter plate precoated with monoclonal antibodies directed against IFN-γ. After incubation for 16–20 h at 37°C in a CO2 incubator, the plate was washed with phosphate-buffered saline (PBS) solution and incubated with conjugate. The reaction was stopped following substrate (BCIP/NBT plus). After drying, the number of IFN-γ-releasing T cells in each well was scored either positive or negative. The specific antigen-stimulated wells with at least 6 spots more than the negative control (where the negative control had fewer than or equal to 5 spots) were considered positive. If the negative control well had 6 to 10 spots, it was accepted as positive when two specific antigen-stimulated wells contained at least twice as many spots as the negative control well (6,14,15).

**Statistical analyses:** Information from the questionnaires as well as TST and T-SPOT.TB assay results were entered into the computer using SPSS (Statistical Package for Social Sciences, Chicago, Ill., USA) version 15.0 and were analyzed.

### RESULTS

In the present study, of 136 recruited participants, 47 (34.6%) were patients with active pulmonary TB (Group 1), 47 (34.6%) were controls (Group 2), and 42 (30.9%) were close-contact (Group 3). The dispersions of BCG vaccinated and non-vaccinated participants according to groups and tests are shown in Table 1. The median age of all the groups, composed of 54 (39.7%) women and 82 (60.3%) men, was 40.04 ± 12.81 (range, 17–79). Thirteen persons (9.6%) were BCG non-vaccinated, and 123 persons (90.4%) were vaccinated with BCG. All participants had negative test results for HIV.

The TST induration of 101 individuals (74.3%) was greater than or equal to 10 mm, while that of 39 individuals (28.7%) was greater than or equal to 15 mm. The entire group 1 (47 individuals) had positive culture results for TB. All the 89 participants of Group 2 and Group 3 had negative culture results for TB. Figure 1 shows the dispersion of three different groups according to the TST and T-SPOT.TB assay results.

The positive response rate in Group 1 for the T-SPOT.TB test was 83.0%; Group 2, 19.1%; Group 3, 54.8%. For the

![Fig. 1. Overall performance of T-SPOT.TB and TST tests in 3 groups including patients with active TB, controls, and close contacts.](image-url)
TST test, the positive response rate in Group 1 was 38.3%; in Group 2, 4.3%; and in Group 3, the same as the T-SPOT.TB test (54.8%).

Validity tests for the TST and T-SPOT.TB tests were assessed by using culture results as a reference method, but the contact group was excluded for validity tests. The cut-off value for TST test positivity was equal to or greater than 15 mm for cases who had a BCG history, and equal to or greater than 10 mm for cases who had no BCG history. According to this evaluation method, the specificity of the T-SPOT.TB test (80.9%) and positive predictive value (PPV) (81.3%) were lower than those of TST (95.7 and 90.0%, respectively) (Table 2). But, in terms of the BCG non-vaccinated subjects, it was observed that the TST specificity (66.7%) and PPV (83.3%) were lower than those seen for the subjects with BCG.

The sensitivity of T-SPOT.TB (83.0%) and negative predictive value (NPV) (82.6%) were higher than those of TST. There was poor concordance between T-SPOT.TB and TST ($\kappa = 0.24$, $P = 0.004$).

**DISCUSSION**

In 2007, the Department of Tuberculosis Control stated in a case report from Turkey that the TB incidence was 26 per 100,000 population (16). The goal of the TB control program is to reduce TB disease by rapid diagnosis.

The ability of TST to detect clinically diagnosed TB cases is limited, both operationally and logistically, in populations vaccinated with BCG or sensitized by NTM, and by its low sensitivity in case of underlying immunodeficiency.

Rapid tests for diagnosing latent and active TB are very important for TB control in the community. ELISA and ELISPOT (blood testing for an IFN-γ production) tests have shown very promising results for diagnosing active and latent TB in recent years (10,17–20). In particular, the use of the ELISPOT technique has recently been shown to be highly sensitive and specific for detecting latent and active TB (21).

The T-SPOT.TB test measures the in vitro production of IFN-γ by blood mononuclear cells in response to *M. tuberculosis*-specific antigens. This test has been evaluated by the Food and Drug Administration for the diagnosis of LTBI. Mori et al. have shown that the whole blood IFN-γ assay using CFP-10 and ESAT-6 is highly specific and sensitive for *M. tuberculosis* infection and is unaffected by the BCG vaccination status (10).

Recently, more studies have demonstrated that the T-SPOT.TB test may play an important role in diagnosing LTBI, and that the specificity of this test is statistically higher than that of TST, particularly in BCG vaccinated populations, while its sensitivity is at least equivalent to that of TST and, in certain studies, superior to T-SPOT.TB (2). In Khan’s study, it has been found that T-SPOT.TB has a higher sensitivity than TST in immunocompetent people with LTBI, and in patients with active TB, including those with impaired cellular immunity at high risk of false-negative TST results (5).

In the present study, we found that the sensitivity of the T-SPOT.TB test in the diagnosis of active TB is higher than that of TST, while the specificity is lower. Hence, TST may give false-negative results.

Kang et al. have shown that high NPV and high T-SPOT.TB sensitivity for the diagnosis of active TB might play a supplementary role in this test to diagnose active TB (22). Lee et al. have reported that the sensitivity of the T-SPOT.TB test is significantly higher than that of TST in the active disease group, whereas in the control group, the T-SPOT.TB test (86%) shows better specificity over the TST (82%) (23). Meier et al. have reported that the T-SPOT.TB test is a sensitive assay for detection of TB and represents a useful addition to the diagnostic algorithm available for detecting TB in low-incidence settings (21). Our results support the notion that the T-SPOT.TB test is better than TST for diagnosing active TB patients.

Similarly, the present study has indicated that the sensitivity and NPV of T-SPOT.TB are higher than those of TST in the active disease group, but contrary to study results of Lee et al., the T-SPOT.TB test showed higher positivity in the control group (23). These results led us to speculate that T-SPOT.TB could also detect LTBI in individuals of the control group due to its higher sensitivity than that of TST.

Diel et al. have compared TST with ELISPOT testing for the diagnosis of LTBI (24). In their study, there was no correlation between BCG vaccination and ELISPOT results ($\kappa = 0.04$), and they suggested that the TST cut-off should be raised from 5 to 10 mm in order to minimize the number of false-positive results for BCG vaccinated contacts. In conclusion, it was stressed that ELISPOT is superior to TST in detecting LTBI.

In the present study, the concordance between the TST and the ELISPOT was low ($\kappa = 0.24$, $P = 0.004$). We evaluated as the TST cut-off ≥15 mm for cases with BCG history and ≥10 mm for persons without BCG history. Also, in the close-contact group, the ability of T-SPOT.TB to detect LTBI was equal to that of TST, unlike Diel et al.’s finding. In this situation, if we base our assumptions only on the cost-effectiveness of LTBI diagnosis, T-SPOT.TB is superior to the TST that only one clinic visit is necessary for T-SPOT.TB compared with the two necessary for the TST. When LTBI is diagnosed accurately, it may decrease costs for those who have positive-TST due to the fact that BCG and unnecessary chest X-rays or preventive chemotherapy are excluded. However, costs might actually rise for the false-negative TB cases occurring due to immunosuppression. In various studies of cost-effectiveness, it has been shown that the T-SPOT.TB test makes no difference in outcomes when compared with using TST, and that the greater sensitivity of the T-SPOT.TB test makes it the better test in LTBI or active TB disease (25–27).

Ozekinci et al. (28) compared the T-SPOT.TB test with the TST for the diagnosis of LTBI in different groups of subjects. They concluded that, in countries where vaccination is routinely performed, the T-SPOT.TB test is a useful diagnostic test for LTBI in high-risk groups when carried out either together with the TST and/or to confirm the TST results.

In conclusion, T-SPOT.TB appears to be a useful and sensitive assay for diagnosing active TB in routine clinical prac-

### Table 2. The validity test results for TST and T-SPOT.TB

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<tr>
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<th>T-SPOT.TB (%)</th>
<th>TST (%)</th>
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</thead>
<tbody>
<tr>
<td>Reference test-spum</td>
<td>n = 94</td>
<td>n = 94</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>83.0</td>
<td>38.3</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>80.9</td>
<td>95.7</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>81.3</td>
<td>90.0</td>
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<tr>
<td>NPV (%)</td>
<td>82.6</td>
<td>60.8</td>
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1) Cut-off value of TST positivity is ≥15 mm with BCG history and ≥10 mm without BCG history; PPV, positive predictive value; NPV, negative predictive value.
tice, as well as to identify individual with LTBI. However, the low PPV restricts its usefulness in routine clinical practice in countries with a strong prevalence of LTBI. More studies are needed to compare the two tests available with regard to the diagnosis of active TB at different ages, and in immunocompromised and specialized groups of patients. We believe that T-SPOT.TB test is a useful diagnostic test for LTBI in high-risk groups together with the TST or to confirm the TST results.

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