Is the T-Cell-Based Interferon-Gamma Releasing Assay Feasible for Diagnosis of Latent Tuberculosis Infection in an Intermediate Tuberculosis-Burden Country?

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SUMMARY: The diagnosis of active and latent tuberculosis infection (LTBI) remains a challenge, especially in light of the fact that the tuberculin skin test (TST), which has been used to diagnose LTBI for over a century, has many well-known drawbacks. This study aimed to compare the diagnostic performance of the T-cell-based interferon-γ releasing assay (IGRA) T-SPOT.TB with the TST for the diagnosis of LTBI in an intermediate tuberculosis (TB)-burden country with high BCG coverage. For this purpose, a total of 91 participants, including culture-confirmed TB patients, healthy contacts known to have been exposed to Mycobacterium tuberculosis, and healthy volunteers, selected from a BCG-vaccinated population were recruited. The sensitivities of the T-SPOT.TB and TST were 79.3 and 25.8%, and the specificities were 75.9 and 56.7%, respectively. The negative- and positive-predictive values for T-SPOT.TB and TST were 78.6 and 76.7% and 42.5 and 38.1%, respectively. The diagnostic performance of the TST in LTBI diagnosis is therefore severely diminished in BCG-vaccinated populations, with the sensitivity and specificity of the T-SPOT.TB assay being markedly higher. IGRA have been reported to have higher diagnostic sensitivity and specificity in low TB-incidence settings than those seen here. Further larger scale studies in high and intermediate TB-incidence settings are therefore warranted.

INTRODUCTION

The tuberculin skin test (TST)—the most widely used test to diagnose latent tuberculosis infection (LTBI)—has many well-known limitations, especially low sensitivity (influenced by immune status) and specificity (due to Bacille-Calmette-Guérin [BCG] vaccination and environmental mycobacterial exposure) (1,2). The discovery of interferon (IFN)-γ releasing assays (IGRAs) therefore appeared to be a promising alternative to the TST (3). IGRAs include proteins that are more specific for Mycobacterium tuberculosis than purified protein derivative (PPD), which are encoded by genes located in the region of difference 1 (RD1) of the M. tuberculosis genome. These genes are not shared with BCG substrains or most environmental mycobacteria (except for Mycobacterium kansasi, Mycobacterium szulgai, Mycobacterium marinum, and Mycobacterium flavescens) (4,5).

Although several studies to evaluate the performance of IGRA using early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) proteins have been published, most were carried out in low tuberculosis (TB)-incidence settings with low BCG coverage. This study was therefore designed to compare the sensitivity and specificity of the T-SPOT.TB test—an ELISPOT-based IFN-γ assay—with the TST for the diagnosis of LTBI in Turkey, which is an area with high BCG coverage and an intermediate burden of active TB (incidence rate of 28.5/100,000/year for 2007) (6).

MATERIALS AND METHODS

Study sample: We prospectively recruited 97 patients admitted to Baskent University School of Medicine, and referred from Ataturk Training and Research Center for Chest Diseases and Thoracic Surgery and several TB dispensaries in Ankara. Six participants were excluded from the analysis: TST results were not available for four participants, one had a prior LTBI treatment history, and one healthy control subject developed active TB during follow-up. The study protocol was approved by the Baskent University School of Medicine Ethics Committee and conformed to the standards defined in the Helsinki Declaration. All participants provided written, informed consent.

Study participants were classified into three groups. Culture-confirmed active pulmonary TB (PTB) patients (who had received either no therapy or less than 4 weeks of therapy at the time of venipuncture) were consecutively enrolled as Group I. Group II consisted of healthy adults who had come into close contact with smear-positive PTB patients, and the third group (Group III) included healthy controls with no history of TB or exposure. Patients with a history of solid organ/bone marrow transplant, chronic renal/liver failure, cancer,
diabetes mellitus, treatment with immunosuppressive medication, and patients younger than 18 years of age were excluded from the study.

T-SPOT.TB: After obtaining informed consent, 8 mL of blood was drawn from each subject by venipuncture and the T-SPOT.TB test performed within 8 h in accordance with the manufacturer’s recommendations (Oxford Immunotec, Oxford, UK) (7). Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation. They were then resuspended and counted using a hemocytometer. The PBMCs (2.5 \times 10^6/well) were incubated overnight at 37°C, 5% CO2 with nil control, mitogen, ESAT-6, and CFP-10 antigens separately in IFN-γ pre-coated ELISPOT plates. T-SPOT.TB plates were scored manually using a magnifying glass as well as with an automated ELISPOT counter (AID, Strassburg, Germany). Scoring was performed according to the manufacturer’s instructions (7). Test plates were scored by two researchers with experience of evaluating ELISPOT plates, both of whom were blinded to the patient data.

TST: Tuberculin skin testing was performed following the Mantoux method using 0.1 mL (5 TU) of PPD (PPD Tuberculin Tween 80; BB-NCLPD, Sofia, Bulgaria). Induration was measured after 48 to 72 h. BCG vaccination is mandatory in Turkey, therefore following BCG vaccination, a TST induration of 15 mm rather than 10 mm is recommended to diagnose LTBI (8). Test results were interpreted according to Turkish National Tuberculosis Guidelines, and TST responses were scored as positive if the induration diameter was 15 mm or greater (9).

Statistical analyses: Statistical analyses were performed using the SPSS software package for Windows (Statistical Product and Service Solutions, version 17.0; SSPS, Chicago, Ill., USA). All parameters were expressed as mean values ± standard derivation (SD) and median (min-max). One-way analysis of variance, a Student t test, and the Mann-Whitney U test were used to compare the continuous variables between groups, whereas the chi-square test was used to compare the categoric variables between groups. Multivariate logistic regression models were used to identify variables significantly associated with positive TST and T-SPOT.TB results. Concordance between the two tests was assessed using kappa (κ) statistics. A P value less than 0.05 was considered significant. IGRA sensitivity and specificity assessments are hampered by the lack of confirmatory tests to diagnose LTBI. In our study, the sensitivity was estimated by analyzing the proportion of positive T-SPOT.TB results among subjects with culture-confirmed TB (Group I). Likewise, the specificity was estimated by analyzing the proportion of negative T-SPOT.TB results among subjects who were very unlikely to have LTBI according to their medical records and self-reports (Group III) (10).

RESULTS

A total of 91 subjects, who underwent both TST and T-SPOT.TB test, were included in the final analysis. The mean age of the subjects was 36.7 ± 13.7 years, and the BCG vaccination rate was 93.4% for the group as a whole. In the subgroup analysis, PTB patients were found to have a lower vaccination rate and lower tuberculin reaction size than the other groups (P < 0.05). Overall, the TST was positive in 23 (46%) of the T-SPOT.TB-negative subjects (n = 50) and negative in 6 (15.8%) of the T-SPOT.TB-positive patients (n = 38). The level of agreement between the two tests was poor (47.7%, κ = 0.065). The sensitivity and specificity of TST for different cut-off values and T-SPOT.TB tests are shown in Table 1. T-SPOT.TB plates were scored both manually by two researchers and by an automated ELISPOT reader. Intraobserver agreement (first researcher versus second researcher) was 96% (κ = 0.92; P < 0.05), and concordance between the two interpretations (manual scoring versus automated ELISPOT reader) was 85.8%, with good agreement (κ = 0.73; P < 0.05). Three tests (3.3%), two of which had more than 10 spots in the nil-control well and the other of which was attributed to insufficient response to mitogen control, gave indeterminate results (ITR) with the T-SPOT.TB assay. These ITR samples were not retested. In contrast, there were no differences in quantitative ESAT-6 and/or CFP-10 specific T-lymphocyte responses among the study subgroups as regards their ability to discriminate active TB, LTBI, and no previous exposure to M. tuberculosis (data not shown).

Group I contained 31 culture-confirmed (M. tuberculosis) active TB subjects. All patients had PTB, and three also had extrapulmonary involvement. Furthermore, 26 (84%) of these patients were smear-positive, 28 (90%) had consolidation, 8 (26%) had cavitation, and 6 (19%) had both cavitation and consolidation in chest X-rays. Two patients had miliary TB. The mean TST induration was 11.8 ± 5.6 mm, and 84% of the group was BCG-vaccinated. The TST and T-SPOT.TB test results for active TB patients are shown in Table 2. The TST gave false-negative results in 23 of 31 patients, whereas the T-SPOT.TB test gave a negative result in 6 of 31 active TB patients. All of these T-SPOT.TB-negative TB patients were positive with TST, with one having miliary TB, one smear-positive pulmonary and genitourinary system TB, and the remaining four having smear-positive PTB. Of the two miliary TB patients, one was T-SPOT.TB-negative while the other was T-SPOT.TB-positive.

Group II consisted of 30 healthy contacts who were at high risk of M. tuberculosis infection due to their history of exposure to active PTB patients. At the time of screening, no signs of active TB were detected in any of these subjects by clinical examination or chest X-ray. The participants were screened for 1 year, and none went on to develop active TB during that period. A total of 60% (n = 18) of these healthy contacts had a positive TST while 26.6% (n = 8) were T-SPOT.TB-positive (Table 2). Multivariate logistic regression models were

<table>
<thead>
<tr>
<th>TST</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 15 mm</td>
<td>25.8 (12.5–44.9)</td>
<td>56.7 (37.6–74.0)</td>
<td>38.1</td>
<td>42.5</td>
</tr>
<tr>
<td>≥ 10 mm</td>
<td>83.9 (65.5–93.9)</td>
<td>13.3 (4.35–31.6)</td>
<td>50</td>
<td>44.4</td>
</tr>
<tr>
<td>T-SPOT.TB</td>
<td>79.3 (59.7–91.2)</td>
<td>75.9 (56.0–88.9)</td>
<td>76.7</td>
<td>78.6</td>
</tr>
</tbody>
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TST, tuberculin skin test; PPV, positive-predictive value; NPV, negative-predictive value; CI, confidence interval.
used to identify the relation between risk factors such as age, sex, BCG vaccination status, exposure time to the index case with TST, and T-SPOT.TB results in healthy contacts. Positive results were significantly associated with increased exposure time (≥ 400 h) to the index case (OR, 11.3; 95% CI, 1.6–78.3, *P* = 0.01) for the TST.

Group III included 30 healthy subjects with no known history of exposure to *M. tuberculosis* infection. Thirteen (43%) of these healthy control subjects had positive TST results, whereas 7 (23.2%) had positive T-SPOT.TB tests (4 [13%] of whom were both TST- and T-SPOT.TB-positive). Three patients who had a negative tuberculin reaction were T-SPOT.TB-positive (Table 2).

### Discussion

We present the results of a prospective trial to compare the T-SPOT.TB assay and the TST in participants exposed to *M. tuberculosis*, active TB patients, and healthy controls in a high BCG-coverage population. In agreement with many previous studies (3,11), the sensitivity and specificity of the T-SPOT.TB assay (79.3 and 75.9%, respectively) were found to be higher than those for TST (25.8 and 56.7%, respectively, for TST ≥ 15 mm; 84 and 13%, respectively, for TST ≥ 10 mm). In a recent meta-analysis, Pai et al. reported that the estimated pooled sensitivity of the TST was 77% (0.71–0.82%), whereas the specificity in BCG-vaccinated populations was low, with a pooled estimate of 59% (0.46–0.73%) (3). The sensitivity of TST increased, and the specificity is decreased, in our study if an induration of TST ≥ 10 mm was considered as positive. These results are consistent with previous studies, which reported that an increase in TST sensitivity is associated with a loss in specificity for the diagnosis of LTBI (12,13). These findings suggest that the low specificity of the TST, which mainly arises due to the BCG vaccination, limits the reliability of this test in settings with a high BCG coverage, such as Turkey.

The reported estimated pooled sensitivity and specificity of T-SPOT.TB in the above-mentioned meta-analysis, which included 20 studies from low-incidence countries, were 90% (0.86–0.93%) and 93% (0.86–1.00%), respectively (3). Indeed, the specificity of IGRA has been shown to approach 100% in populations at very low risk of LTBI (14). In our study, the specificity of the T-SPOT.TB assay was found to be lower than in many previous studies (3). As there is no gold standard for LTBI testing, specificity is estimated on the basis of healthy persons with a low likelihood of exposure. The lower specificity of T-SPOT.TB in this study could be a result of the high rate of LTBI in Turkey and exposure to mycobacteria other than *M. tuberculosis*.

The sensitivity of immune-based tests—both IGRA and TSTs—is mainly confounded by the immune status of the patients, and the number of heterogeneous results reported previously have been explained by the severity of TB and the effect of immunosuppression caused by various conditions (12,15–18). The sensitivity of T-SPOT.TB assay in this study was lower than that reported previously in studies in other countries. However, the value obtained is similar to that reported by Soysal et al., who estimated a T-SPOT.TB sensitivity of close to 83% in Turkey (12). The fact that 61.3% of PTB patients in our study had advanced disease (with two having miliary TB, three suffering both pulmonary and extrapulmonary involvement, and 14 with cavitary disease) could explain the lower sensitivity.

The poor agreement between TST and T-SPOT.TB can be explained by the lower sensitivity and specificity of TST in our study population. Furthermore, consistent with the published literature regarding concerns about the ITR results of IGRA (19–21), a small proportion of T-SPOT.TB tests (3.3%) yielded indeterminate results in the group as a whole. It has been shown that antigen-specific T-lymphocyte responses cannot discriminate active TB from LTBI (18). Indeed, O’Neal et al. pointed out that the negative-predictive value (NPV)—the probability that a person with a negative IGRA result is, in fact, not infected—is critical in clinical judgments regarding LTBI and that the NPV of IGRA decreases as the prevalence of LTBI increases (22). The NPV of T-SPOT.TB in that study was 79%, which means that approximately one in four patients with a negative T-SPOT.TB result might actually have had LTBI. We therefore conclude that T-SPOT.TB may be a more useful tool to exclude active TB in low endemic settings rather than intermediate and high TB-burden countries (22,23).

A significant variation in the interpretation of the results of T-SPOT.TB tests between independent observers and an automated reader has been reported previously (24). In our study, although intraobserver agreement between the two researchers was excellent, the concordance between the two interpretations (manual versus automated reader) was approximately 86%, with a good, but not excellent, agreement. This latter figure suggests a 14% mis/overdiagnosis of the study population. In light of this, we suggest that the
scoring of plates by two different methods (manually and using an automated ELISPOT reader) should be evaluated in further studies.

This study has several limitations. First of all, due to the small sample size, we were unable to evaluate the association between \textit{M. tuberculosis} exposure time and the T-SPOT.TB assay (which has been shown in many previous studies [22,23]). However, in contrast to larger TB contact tracing studies (4,21,25,26), we were able to show that TST results were closely correlated with higher cumulative exposure time (OR, 11.3; 95% CI, 1.6–78.3; \(P = 0.01\)). Second, the number of active TB patients recruited into the study was small, thus limiting our ability to evaluate the sensitivity performance of the T-SPOT.TB test. Additionally, as there is no gold standard for LTBI testing, we estimated the specificity from persons with a low likelihood of exposure (according to their medical records and self-reports), as was the case in previous IGRA-related studies. However, this meant that it was impossible to definitively rule-out \textit{M. tuberculosis} exposure, which may lead to a significant decrease in the specificity of the T-SPOT.TB assay.

In conclusion, the diagnostic sensitivity, and especially the specificity, of the TST is severely diminished in BCG-vaccinated populations. In light of this finding, it becomes clear that tests which are both more specific and more sensitive than the TST are needed to diagnose LTBI in such populations. The diagnostic performance of IGRA's has been reported to be better in low endemic settings than in Turkey. In addition, the relatively small number of participants included in this study limits the generalization of these results to the country as a whole. Further studies in high and intermediate TB-incidence settings with larger numbers of subjects are therefore needed to determine the diagnostic performance of IGRA's for the diagnosis of LTBI.

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Conflict of interest None to declare.

REFERENCES