INTRODUCTION

The World Health Organization (WHO) estimates that one-third of the world’s population is infected with Mycobacterium tuberculosis (1). For global control of tuberculosis (TB), identification and treatment of people with latent TB infection (LTBI), who are at elevated risk of progressing to active TB, and they develop more severe forms of the disease with increased fatality (2). Thus, isoniazid preventive therapy (IPT) is recommended for subjects infected with M. tuberculosis. IPT reduced the risk for contracting TB by more than 90% in patients who completed a full course of therapy (3). The identification of subjects who will receive IPT is mainly determined by examining the subjects’ risk factors for contracting TB and the results of the tuberculin skin test (TST). Until recently, detection of TB infection relied solely on the TST. However, the target for IPT is complicated by the fact that the TST does not reliably diagnose LTBI in individuals who were vaccinated with Bacille Calmette-Guérin (BCG) or who were exposed to non-tuberculous mycobacteria, because this assay is based on enumeration of interferon-γ-secreting T cells in response to Mycobacterium tuberculosis-specific antigens. We compared the TST with T-SPOT.TB with respect to different TST cut-off points in healthy unexposed BCG-vaccinated schoolchildren. A total of 209 children between 6 and 10 years of age with a TST induration of 0 (n = 50), 10-14 (n = 45), 15-19 (n = 95) and ≥20 mm (n = 19) were enrolled. Among TST-positive subjects, only 26 (23%) were positive with T-SPOT.TB, and T-SPOT.TB was positive in 4, 7, 20 and 42% of children with TST indurations of 0, 10-14, 15-19 and ≥20 mm, respectively. We suggest that confirmation of a positive TST by the interferon-γ-based test would reduce unnecessary preventive therapy significantly in healthy unexposed BCG-vaccinated children.

Original Article

Comparison of Positive Tuberculin Skin Test with an Interferon-γ-Based Assay in Unexposed Children

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SUMMARY: A false-positive tuberculin skin test (TST) may be a result of T-cell sensitivity due to Bacille Calmette-Guérin (BCG) vaccination or exposure to non-tuberculosis mycobacteria, thus leading to unnecessary isoniazid preventive therapy, especially in low-risk populations. Unlike TST, T-SPOT.TB is not confounded by BCG vaccination or exposure to most of the other non-tuberculosis mycobacteria, because this assay is based on enumeration of interferon-γ-secreting T cells in response to Mycobacterium tuberculosis-specific antigens. We compared the TST with T-SPOT.TB with respect to different TST cut-off points in healthy unexposed BCG-vaccinated schoolchildren. A total of 209 children between 6 and 10 years of age with a TST induration of 0 (n = 50), 10-14 (n = 45), 15-19 (n = 95) and ≥20 mm (n = 19) were enrolled. Among TST-positive subjects, only 26 (23%) were positive with T-SPOT.TB, and T-SPOT.TB was positive in 4, 7, 20 and 42% of children with TST indurations of 0, 10-14, 15-19 and ≥20 mm, respectively. We suggest that confirmation of a positive TST by the interferon-γ-based test would reduce unnecessary preventive therapy significantly in healthy unexposed BCG-vaccinated children.

METHODS

Subjects: The study was conducted at the Marmara University School of Medicine, Pediatric Infectious Diseases Research Laboratory. Until recently (2006), the Turkish Ministry of Health recommended BCG vaccination at 2-3 months of age and a booster vaccination in the first year of primary school, at 6-7 years of age based on TST screening. If the TST induration was <10 mm in diameter, a second BCG dose was given, and when the TST induration was >14 mm in diameter, children were evaluated for active TB disease (10). In this study, children were recruited from nine primary schools during the school TST screening before the second
dose of BCG vaccination in Kadiköy, one of the largest towns near Istanbul, Turkey, where the prevalence of TB disease is 40/100,000.

The annual incidence of TB infection was estimated to be 1.51% in primary school children in Edirne, which is a neighboring city of Istanbul (11, 12). Between March 2006 and June 2006, a total of 18,344 schoolchildren in Kadiköy, Istanbul were evaluated as part of a national school TB screening program. Among them, 14,833 children (80%) underwent TST, of whom 12,102 (81%) had at least one BCG vaccination scar. Out of 14,833 children, 1,979 (14%) had a TST induration size of ≥11 mm. The TST positivity rate was found to be 14% in the entire schoolchildren population of Kadiköy. Children with known TB exposure and/or having a history of IPT were excluded from the study.

To evaluate the false positivity rate of certain TST cut-off points in a low-risk population, we enrolled children with a TST induration >9 mm for whom caregivers gave informed consent to participate in the study. We also enrolled children who were TST non-reactors (TST induration of 0 mm) so we could evaluate whether 0 mm induration occurs because of energy or technical error during TST application or because of a test reading error, since most of the enrolled children were BCG vaccinated. All children had blood drawn for T-SPOT.TB during the TST screening.

Ethical approval for the study was obtained from the Marmara University School of Medicine Ethics Committee. All participants’ caregivers provided written informed consent for study participation.

Demographic details, clinical history and current medications were recorded. Because there is no official vaccination record system in Turkey and most of the parents do not keep their child’s vaccination records, we assumed that the presence of a BCG vaccination scar was an indication of BCG vaccination. Children with a positive TST or T-SPOT.TB underwent collection of a detailed clinical history, physical examination and chest X-ray. Anyone living in the house of a child with a positive TST or T-SPOT.TB was offered a chest X-ray for the investigation of active TB. After the exclusion of active TB disease, all children with a positive TST or T-SPOT.TB were offered IPT for 6 months.

**TST:** All patients underwent TST, which was performed on the volar aspect of the forearm by the Mantoux method using 0.1 mL (5 tuberculin units) of PPD Tuberculin tween 80 (BB-NCIPD Ltd., Sofia, Bulgaria). The tests were administered and read by the study pediatricians (DT, OT). Induration was measured after 48-72 h using the ballpoint pen method. TST response was scored as positive if the induration size was 15 mm or more, irrespective of BCG scar status (13).

**T-SPOT.TB assay:** The tests were done according to the manufacturer’s instructions by the study pediatrician (AS), who had previously performed hundreds of RD1-ELISpot assays (14). Peripheral blood mononuclear cells were separated from blood by the Ficoll-centrifuge method and plated (at 2.5 X 10^5/well) with 50 μl of media, phytohemagglutinin (PHA) or peptides from ESAT-6 and CFP-10. These cells were then transferred onto anti-IFN-γ antibody pre-coated plates for overnight incubation at 37°C in 5% CO₂. After incubation, the wells were washed and a conjugate solution was added to each well, and then the plates were incubated at 2-8°C for 60 min. The wells were then washed again and incubated with substrate solution at room temperature for 7 min, before a final wash with distilled water. Spot-forming units (SFUs) were counted using an automated ELISpot counter (AID-GmbH, Strassberg, Germany). The test was deemed valid if the positive control well had more than 20 SFUs and the negative control well had fewer than 10 SFUs. The test positivity criteria were determined according to the official control well SFUs. If the positive control well had 0-5 SFUs, the test was accepted as positive if either or both of the ESAT-6 or CFP-10 wells contained a mean of at least 6 SFUs more than the negative control. If the negative control contained ≥6 SFUs, the test was accepted as positive when the antigen wells contained at least twice the number of SFUs as were in the negative well.

**RESULTS**

Between March 2006 and June 2006 a total of 1,775 children were screened by TST in nine randomly selected primary schools, and among them 1,331 (76%) underwent TST, of whom 1,137 (85%) had at least one BCG scar. Of 1,331 children, 939 (70%), 256 (29%) and 139 (11%) had a TST induration size of 0-5, 6-10 and ≥11 mm, respectively. TB infection prevalence diagnosed by TST was found to be 11% in our study population. Among 1,331 children, 209 healthy schoolchildren without a known history of TB contact from 6 to 10 years of age (mean 8.4 ± 0.68 years), of whom 112 (54%) were female and 97 (46%) were male, were enrolled for the T-SPOT.TB assay in addition to the TST. Among them, 188 (90%) had at least one BCG scar. Of 209 children 50, 45, 95 and 19 had a TST induration size of 0, 10-14, 15-19 and ≥20 mm, respectively. Five (2.0%) T-SPOT.TB assays were considered invalid: all showed no response compared to the positive control. Among them one had a TST induration of 0 mm, and the remaining 4 had TST indurations of ≥14 mm. Of the 111 TST-positive subjects, only 26 (23%) were positive according to the T-SPOT.TB assay. However, T-SPOT.TB was found to be positive in 5 (5%) of 97 children with negative TST. Of 50 children with a TST induration size of 0 mm, only 2 (4%) had a positive T-SPOT.TB. On the other hand T-SPOT.TB was positive in 7, 20 and 42% of children with a TST induration of 10-14, 15-19 and ≥20 mm, respectively, denoting that increasing TST induration increases the likelihood of the detection of true infection (Table 1). None of the children with a positive TST or T-SPOT.TB or their household contacts revealed active TB after investigation of subjects by physical examination and chest X-ray.

**DISCUSSION**

Despite the recommendations of major policymaking organizations, routine TST screening of children before entering school is often practiced in some parts of the world and in our country (10,15,16). In Turkey, the TST is routinely

![Table 1. Comparative result of the T-SPOT.TB test according to the TST cut-off points](image-url)
applied to all children when they begin primary school, before the second dose of the BCG vaccination. Children with positive TST are further investigated for active TB disease by physical examination and chest-X ray, and those without active disease are offered IPT for 6 months, as recommended by international guidelines (10,13). However, IPT takes a long time and includes a risk of liver toxicity, so it is important to identify TB infection accurately. The TST remains one of the most widely used diagnostic tests for the determination of LTBI worldwide, but the test's many drawbacks limit its diagnostic value in LTBI among BCG-vaccinated subjects.

Our study population was composed of BCG-vaccinated children (90%), and the interpretation of the TST in a BCG-vaccinated individual often poses a dilemma. For those reasons different cut-off points are used to interpret TST results in clinical practice, to minimize the effect of BCG vaccination and NTM infection on TST results. Identification of the best cut-off point for LTBI is difficult, since there is no gold standard for LTBI. Previous studies have shown post-vaccination BCG-induced tuberculin reactivity to range from no induration to reaction of 19 mm, and positive skin tests with indurations of >15 mm are more likely to be the result of TB infection than of BCG vaccination in subjects without a known TB contact (17,18). Many other investigators also demonstrated that TST reactions among BCG-vaccinated children in the 5 - 9, 10 - 14 and 15 - 19 mm induration range may be attributable to BCG vaccination (19,20). Therefore, interpretation of TST indurations between 10 and 14 mm and especially between 15 and 19 mm in BCG-vaccinated children should be evaluated very carefully. There is more likelihood of children with reaction sizes in this range harboring TB infection in the presence of a history of contact with a case of TB. On the other hand, such reactions among the BCG-vaccinated children, in the absence of contact history or suggestive symptoms of disease are more likely to be attributable to BCG vaccination than to true TB infection. Chadha et al. found an induration size of 19 mm to be the upper limit for BCG-induced tuberculin sensitivity (18). Therefore, they concluded that all reactions above 19 mm in size should be considered to be due to natural infection with tubercle bacille, irrespective of the BCG vaccination status.

In the present study, by using the T-SPOT.TB assay as a reference test for LTBI, we evaluated the accuracy of TST induration of 0, 10 - 14, 15 - 19 or ≥20 mm as indicators of TB infection. Among 111 children with positive TST (induration ≥15 mm), which is thought to be mostly consistent with *M. tuberculosis* infection, 85 (77%) had a negative T-SPOT.TB. This finding suggests that among the TST-positive subjects, 77% of TST reactivity may be false-positive, which may be a consequence of BCG vaccination or exposure to NTM. Even if we use a large TST induration criterion (≥20 mm), only 8 (42%) were confirmed as infected by T-SPOT.TB.

We also found that increasing TST induration increases the likelihood of detection of true infection (Table 1). Supporting our findings, Farhat et al. reported that based on 240,203 vaccinees in 24 studies reviewed, BCG vaccination in infancy caused an overall rate of 8.5 false-positive TST reactions per 100 vaccinees, with a rate of 2.6 false-positive TST reactions per 100 vaccinees for reactions of 15 mm +. They also revealed that in populations with a low prevalence of true TB infection, false-positive TST reactions due to NTM were very uncommon (21). Our results and the findings of Farhat et al. suggest that TST does not accurately identify true infection in a BCG-vaccinated population, hence resulting in a high possibility of IPT.

Wrighton-Smith and Zellweger (22) investigated the costs of screening LTBI by TST or T-SPOT.TB in a number of populations including persons recently in contact with infectious TB patients. In their study, of 267 contacts, 193 (72.3%) were positive by TST and only 74 (27.7%) were positive by T-SPOT.TB. They found that only 33.7% (65/193) of the TST-positive individuals were also positive by the T-SPOT.TB. They concluded that screening for LTBI by TST followed by confirmation with T-SPOT.TB is less costly than screening with TST alone, as it allows a reduction in the number of people who would receive IPT. Our results demonstrate that even though 55% of a low-risk population had positive TST, only 23% of the TST-positive subjects had *M. tuberculosis* specific T-cells, suggesting that BCG vaccination and/or NTM exposure could contribute to a high rate of false-positive TST results. Therefore, we suggest that positive TST results in low-risk children with no known TB contact should be confirmed by more specific and possibly sensitive assays such as T-SPOT.TB in order to avoid unnecessary IPT.

Our results also indicate that IFN-γ-based assays may be a more accurate surrogate than the TST for estimating the prevalence of LTBI in a BCG-vaccinated low-risk population, but the cost of IFN-γ-based assays is a major constraint. Among 50 TST non-reactive children (TST induration size = 0 mm), only 2 (4%) had a positive T-SPOT.TB, suggesting a high specificity of TST in a low-risk population. In several studies IFN-γ assays had a higher specificity compared to the TST (8,9). Among children with no risk factor for LTBI with TST induration size of 0 mm, a positive T-SPOT.TB assay may be a false positive rather than an indication of true TB infection. Priwitzer et al. reported a false-positive IFN-γ assay among women without increased risk for LTBI (23). Although viral infections, immunodeficiencies and steroid therapy, etc., may lead to a false-negative TST reaction, our study population had none of those conditions. Thus, we believe that non-reactive TST in healthy children with no TB contact does not need to be confirmed with more accurate IFN-γ-based assays.

In the absence of a gold-standard test for LTBI, we used T-SPOT.TB results as a surrogate reference standard for our analysis of the diagnostic accuracy of the TST. Results of a previous study showed that after completion of treatment for latent infection the frequencies of RD1-specific T cells had declined and even became negative during follow-up in some of the treated subjects (24). Mori et al. also indirectly showed that among Japanese adults the IFN-γ response determined by the Quantiferon-TB Gold assay waned with passing years (25). We cannot expect waning of IFN-γ response in our population, since none of them received LTBI treatment and they are younger than Mori's study population, leaving no opportunity to observe a time effect of IFN-γ response. The T-SPOT.TB itself is not a perfect test of TB infection, and more research is required to further validate it, but this assay already represents a significant improvement on the TST (8,9,22). The interpretation of TST results is mainly affected by the prevalence of TB infection, the level of environmental mycobacterial exposure and the prevalence or absence of BCG vaccination of the studied population. Thus, our results cannot be directly extrapolated to other populations where TB prevalence is low and BCG vaccination is not uniformly carried out.

In conclusion, we suggest that positive TST results should be confirmed by the T-SPOT.TB to avoid unnecessary IPT.
in healthy unexposed children in intermediate and low-risk countries where the BCG vaccination is still recommended.

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