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Original Research Article

# Use of interferon-gamma release assay and tuberculin skin test in diagnosing tuberculosis in Lithuanian adults: A comparative analysis

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ARTICLE INFO

*Article history:*
Received 15 June 2016
Received in revised form
8 February 2017
Accepted 18 May 2017
Available online xxx

*Keywords:*
Tuberculosis
Tuberculin skin test
Interferon-gamma release assay
T SPOT TB

ABSTRACT

*Background and objective:* Lithuania belongs to the group of countries with a high-incidence of tuberculosis (TB). Some scientific studies show that the interferon-gamma release assay is more accurate and correlates more highly with TB exposure as compared to the tuberculin skin test (TST). This study aimed at comparing the efficacy between the T SPOT TB and TST for diagnosing TB among Lithuanian adults.

*Materials and methods:* Individuals with diagnosed TB, healthcare workers with known risk for TB and individuals without any known risk for TB underwent clinical examinations, interviews about their history of TB exposure and chest radiography. Then the TST and the T SPOT TB were performed on patients.

*Results:* A positive T SPOT TB was more common in the group with diagnosed TB compared to healthcare workers and the low risk for TB groups (97.5%, 36.4%, and 0%, respectively, $P < 0.01$). Positive TST results did not differ between the groups with diagnosed TB and the healthcare workers (92.5% vs. 95.5%, $P > 0.05$). Agreement between TST and T SPOT TB was poor (kappa 0.14, $P > 0.05$). T SPOT TB had higher specificity and sensitivity compared to TST (area under the ROC $0.9 \pm 0.04$, $P < 0.01$, vs. $0.5 \pm 0.06$, $P > 0.05$).

*Conclusions:* The T SPOT TB showed greater accuracy in diagnosing TB than TST did. Positive T SPOT TB result but not the TST was more common in patients with diagnosed TB.



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http://dx.doi.org/10.1016/j.medici.2017.05.003


Please cite this article in press as: Tamašauskienė L, et al. Use of interferon-gamma release assay and tuberculin skin test in diagnosing tuberculosis in Lithuanian adults: A comparative analysis. Medicina (2017), http://dx.doi.org/10.1016/j.medici.2017.05.003

## 1. Introduction

Despite the fact that the incidence of global tuberculosis (TB) has slowly decreased during the past 13 years, this disease remains widespread worldwide – 9 million incident cases of TB were reported in 2013 [1]. The World Health Organization (WHO) reports the incidence of TB in Lithuania in 2013 at 65/100,000 inhabitants [2]. This is more than 50/100,000 inhabitants, which means that Lithuania belongs to the group of high-incidence countries [3]. Moreover, Lithuania has one of the highest rates of TB drug resistance numbering 11% among new TB cases in 2013 [2,4].

Diagnosis of latent TB is very important in high-incidence countries. The definition of latent TB is the presence of immune responses to a previously acquired *Mycobacterium tuberculosis* (*M. tuberculosis*) infection without clinical evidence of active TB [5]. Individuals with latent TB are at risk for developing active TB [5]. The WHO guidelines for latent TB management in countries with high or middle-upper incomes and TB incidences of <100/100,000 inhabitants involve systematic testing for and treatment of latent TB. Testing and treatment should be applied for people living with the human immunodeficiency virus (HIV), adults and children in cases of contacts with pulmonary TB, patients for whom anti-tumor necrosis factor treatments have been initiated, patients receiving dialysis, patients preparing for organ or hematological transplantations and patients with silicosis. Such testing and treatment should also be considered for prisoners, healthcare workers, immigrants from high TB burden countries, homeless persons and illicit drug users [5].

These recommendations suggest using the interferon-gamma (IFN-gamma) release assay or the Mantoux tuberculin skin test (TST) for diagnosing latent TB [5]. The TST has been the most commonly used test in Lithuania for many years. However, some researchers propose that a previous bacille Calmette-Guerin (BCG) vaccination can influence false positive results of the TST [6–8]. Moreover, repeated TST can cause a booster effect and show false positive results [6–9]. The vaccinations among the Lithuanian population are usually with BCG (98.9% of the population) [10,11]. IFN-gamma assays use antigens absent in BCG strains, and therefore are more accurate and have higher correlation with TB exposure compared to TST [12–18]. One IFN-gamma assay – the T SPOT TB – identifies T cells secreting IFN-gamma by using an enzyme-linked immunospot (ELISPOT) assay technique [16]. The aim of this study was to compare the efficacy between T SPOT TB and TST in diagnosing TB among Lithuanian adults.

## 2. Material and methods

### 2.1. Study sample

Individuals from 18 years old with diagnosed TB, healthcare workers with a known risk for TB and individuals with no known risk for TB who attended the Hospital of Lithuanian University of Health Sciences and gave their informed consents were included in the study. The Regional Bioethics Committee of the Lithuanian University of Health Sciences approved this study.

All subjects were divided into the following three groups according to the TB-related anamnesis, clinical symptoms and presence of contacts: (1) TB group of individuals with a bacteriologically confirmed TB diagnosis (smear positive culture) ($N = 40$), (2) healthcare workers, the subjects who worked in hospitals and had contacts with TB patients but were free of TB clinical symptoms ($N = 22$) and (3) the low risk for TB group consisting of subjects with no history of contact with TB patients and no clinical symptoms of this disease ($N = 21$).

All these subjects underwent a clinical examination, an interview about their history of TB exposure and chest radiography. All these individuals were HIV negative and had previously received a BCG vaccination. The residual scar evaluated the BCG status.

### 2.2. Tuberculin skin test

Trained nurses performed the TST according to the Mantoux technique using two units of the purified protein derivative (PPD) (Copenhagen Statens Serum Institute, Denmark). A pulmonologist measured the transverse diameter of induration after 72 h. The TST reaction was considered positive when the induration was ≥10 mm [19]. The TST was divided into 4 groups according to the diameter of induration: (1) 0–4 mm, (2) 5–9 mm, (3) 10–14 mm and (4) ≥15 mm.

### 2.3. T SPOT TB

Before performing the TST, 10 mL of blood was drawn from the peripheral vein for the diagnostic test of *M. tuberculosis*-specific IFN-γ secreting T-cells (T SPOT TB, Oxford Immunotec, Oxford, UK). The T SPOT TB test was performed using fresh blood (<5 h at room temperature) according to the manufacturer's instructions. The mononuclear cells from peripheral blood were seeded at $2.5 \times 10^5$ cells/well in single-well plates, and two separate pools of overlapping peptides spanning the full length of ESAT-6 and CFP-10 proteins were used together for the negative and for the positive controls. The count of individual spots for ESAT-6 (T SPOT A) and CFP-10 (T SPOT B) was taken by using manual counting with Trypan Blue and a hemocytometer. The results were evaluated according to the manufacturer's instructions.

### 2.4. Statistical analysis

The statistical analysis was performed by using the Statistical Package for Social Sciences (SPSS) 13. Values are expressed as mean ± SEM. Methods of statistical analysis were selected after performing the Kolmogorov–Smirnov test. Consequently the Kruskal–Wallis $H$ test was applied for comparing the T SPOT A and T SPOT B with the groups of TB patients, healthcare workers and low risk for TB. Application of the Mann–Whitney $U$ test was for comparing data between two groups. The One-way ANOVA method was used to compare age and the TST diameter of induration between TB patients, high risk for TB and low risk for TB groups. The $\chi^2$ test was used for data comparisons of binary variables. The Kendall tau-b

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