Short communication

Diagnosis of latent tuberculosis by ELISPOT assay and tuberculin skin test

Diagnostic de tuberculose par test ELISPOT et intradermoréaction à la tuberculine

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Abstract

Objective. – To determine the prevalence of latent tuberculosis infection (LTBI) in college students.

Patients and methods. – Four hundred and twenty newly admitted college students were enrolled. The Enzyme-Linked ImmunoSpot assay (ELISPOT) was used. Overall, 171 students with ELISPOT assay+/TST+ were monitored for three years to detect active TB disease.

Results. – The overall positive rate of ELISPOT assay was 40.7% among TST+ students. The ELISPOT positive rates were 36.9%, 45.4%, and 64.3% in groups of TST induration of 10–14 mm, 15–20 mm, and ≥ 20 mm, respectively, with a significant difference ($\chi^2 = 10.136$, $P < 0.01$) but no significant difference between BCG scar and no scar (41.2% vs. 38.8%; $P > 0.05$). None of the 171 untreated students contracted active TB within the three-year monitoring period.

Conclusion. – The LTBI rate might be overestimated by TST compared with interferon-γ release assays. On the basis of a close monitoring, few students developed active TB despite a positive result to the TST and ELISPOT assay.

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Keywords: Latent tuberculosis; Interferon-γ; Tuberculin skin test

Résumé

Objectif. – Mesurer la prévalence des infections tuberculeuses latentes (ITL) chez les étudiants.

Patients et méthodes. – Quatre cent vingt étudiants inclus avec test ELISPOT ; 171 étudiants avec résultat ELISPOT positif et intradermoréaction à la tuberculine positive (IDRT) suivis pendant trois ans.

Résultats. – Le taux de positifs ELISPOT était de 40,7 % chez les étudiants avec IDRT+. Les taux de positivité ELISPOT étaient respectivement de 36,9 %, 45,4 % et 64,3 % chez les patients avec une induration de 10–14 mm, 15–20 mm et ≥ 20 mm suite à une IDRT ($\chi^2 = 10,136$, $p < 0,01$). Aucune différence significative entre les groupes avec cicatrice de BCG et sans cicatrice (41,2 % contre 38,8 % ; $p > 0,05$). Aucun des 171 étudiants non traités n'a contracté de tuberculose maladie pendant la période de suivi.

Conclusion. – Notre taux d'ITL par IDRT est peut-être surestimé par rapport au taux obtenu par la détection d'IFNg. Très peu d'étudiants ont contracté une tuberculose maladie malgré une IDRT+ et un ELISPOT+.

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Mots clés : Tuberculose latente ; Interféron-γ ; Intradermoréaction à la tuberculine

1. Introduction

China has the world's second largest tuberculosis (TB) prevalence, with 550 million (44.5% in 2002) individuals latently infected by *Mycobacterium tuberculosis* [1,2]. Individuals

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presenting with latent tuberculosis infection (LTBI) are at higher risk of contracting active TB, especially in the first two years of the infection [3]. Identifying the pathogen and initiating a preventive treatment in LTBI patients are key elements for TB control. However, current detection methods lack the appropriate specificity and sensitivity required to diagnose LTBI. The authors of recent studies recommended a two-step approach to detect LTBI with an initial tuberculin skin test (TST), followed by interferon-gamma release assays (IGRAs). This strategy may be more cost-effective and appropriate in high TB-burden countries [4,5].

Two kits for IGRAs have recently been marketed: QuantiFERON-TB Gold In-Tube (QFG-IT, Cellestis Ltd, Carnegie, Australia) and T-SPOT.TB (Oxford Immunotec, Abingdon, UK). They both use early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) peptides as specific antigens. However, the use of peptides increases the cost of the kit, making it unaffordable for people living in developing countries. A fusion protein of ESAT-6 and CFP-10 (rESAT-6/CFP-10) used as the stimulus may be equivalent to overlapping peptides and would offer a cheaper and more realistic alternative for large-scale use and field testing [6,7]. College students in China are considered at high risk of TB transmission [8,9]. We aimed to measure the prevalence of LTBI among Chinese college students as well as the potential incidence of active TB in ELISPOT+/TST+ students, using the rESAT-6/CFP-10 ELISPOT assay. Patients with a positive ELISPOT test were followed for a three-year period to measure the incidence of subsequent active TB.

2. Study population and methods

The study was approved by the ethics committee of the Beijing Chest Hospital. We also obtained the written informed consent of each patient included in the study. A total of 420 newly admitted students in a college of Changping District of Beijing, China, with a positive TST were prospectively enrolled in September 2010 (mean age ± SD, 19 ± 2 years; range 15–28; 48.3% of men). A BCG-vaccination scar was observed in 331 students (78.8%). The chest X-ray and clinical evaluation of each participant suggested that none of them had active TB. Participants were routinely examined and received an intradermal injection of 0.1 mL of 5 IU purified protein derivative (PPD) in the left forearm. The diameters of both axes of skin induration were measured and recorded by a certified physician 72 hours after antigen injection. A positive result was defined as a TST induration of ≥ 10 mm or as local blisters, necrosis, and lymphangitis.

Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation from 6 mL heparinized blood sample and then placed into three wells (2.0×10^5 cells per well) of a pre-coated ELISPOT plate (product code: 3420-4AST-4, Mabtech, Swedish): no antigen (negative control); phytohemagglutinin (PHA positive control) at 5 μg/mL; rCFP-10/ESAT-6 fusion protein antigen (provided by the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) 10 μg/mL at 37 °C in 5% CO_2 for 20 hours.

The number of spot-forming cells (SFCs) in each well was automatically counted with a CTL-ImmunoSpot® S5 Versa Analyzer (Cellular Technology Ltd., Shaker Heights, OH). The tests were scored as positive if the test well contained at least five more SFUs than the negative control well or had at least twice as many SFUs as the negative control well.

Results were expressed as mean ± standard deviation (SD) if continuous variables were normally distributed or as a median (interquartile range [IQR]). The comparisons between the TST and ELISPOT assay were analyzed by Chi^2 test or by Fisher's exact test. Continuous variables were compared using Student's *t*-test or Mann–Whitney nonparametric test when appropriate. A *P*-value < 0.05 was considered statistically significant.

3. Results

The ELISPOT results of 420 students, including 335 with a BCG scar (79.8%), were available for analysis. We observed a significant difference in terms of BCG scar rate between a TST induration of 10–15 mm and a TST induration ≥ 15 mm: 83.7% for 10–15 mm and 70.4% for ≥ 15 mm ($\chi^2 = 9.838$, $P = 0.002$) (Fig. 1). The overall positive rate to the ELISPOT assay was 40.7% (171/420; 95% CI [33.9–47.5]). We performed a stratification of the students based on the presence of a BCG scar and observed that the positivity to the ELISPOT assay was not significantly associated with the presence of a BCG scar (BCG scar vs. no scar, 41.2% vs. 38.8%; $P = 0.394$). Positive results to the ELISPOT assay accounted for 36.9%, 45.4%, and 64.3% in groups of TST induration of 10–14 mm, 15–20 mm, and ≥ 20 mm, respectively ($\chi^2 = 10.136$, $P = 0.006$) (Fig. 2). We observed a significant difference in the mean values of SFCs: the median number of SFCs was $4/2 \times 10^5$ (3 to $16/2 \times 10^5$), $5/2 \times 10^5$ (3 to $21/2 \times 10^5$), and $8/2 \times 10^5$ (3 to $31//2 \times 10^5$) in groups of TST induration of 10–14 mm, 15–20 mm, and ≥ 20 mm, respectively ($P = 0.020$) (Table 1).

A total of 171 untreated students with ELISPOT assay+/TST+ received health education and were asked to comply with a yearly chest X-ray examination to exclude any
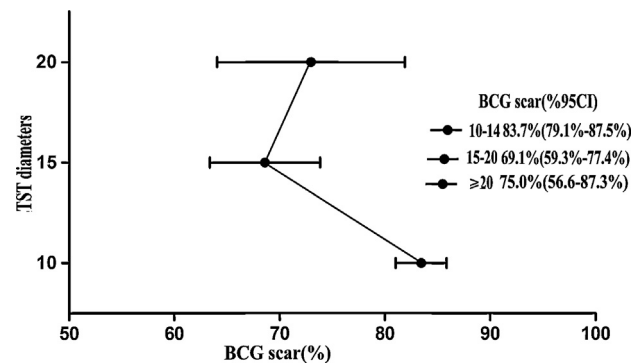

Fig. 1. BCG-vaccination scar in groups of various TST diameters. The circles and lines represent the BCG scar rates and 95% CIs, respectively. On the basis of a Chi^2 analysis, the BCG scar rates between TST diameters of 10–15 mm and ≥ 15 mm were significantly different (*P* = 0.002).
Cicatrices laissées par le vaccin BCG au sein de groupes présentant différents diamètres d'induration.