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Prevalence and influencing factors of the high nil-control spot count in T-SPOT.*TB*: A matched case-control study

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ABSTRACT

Background: T-SPOT.*TB* may yield indeterminate results, including high nil responses and insufficient mitogen responses. We explored the incidence and risk factors of high nil responses.

Methods: A 1:1 matched case-control study of patients who underwent T-SPOT.*TB* tests in Peking Union Medical College Hospital from Jan 1, 2015 to Apr 30, 2017 was conducted. High nil responses were defined as > 10 spots in negative control wells. Cases and controls were matched based on when the tests were performed. Demographic, clinical and laboratory data were obtained from the Medical Record System.

Results: A total of 644 out of 36,316 (1.76%, 95% CI: 1.63%–1.90%) patients presented with high nil responses (280 cases and 280 controls were enrolled). Multivariate analysis revealed that male (OR = 1.882, 95% CI: 1.222–2.899), Behcet's disease (OR = 7.764, 95% CI: 1.714–35.167), heavy use of corticosteroids within a month (OR = 0.357, 95% CI: 0.138–0.921, for > 1000 mg group) and hypoalbuminemia (OR = 0.385, 95% CI: 0.241–0.615) are significantly associated with high nil responses.

Conclusions: High nil responses in T-SPOT.*TB* assays are quite rare. Male gender and Behcet's disease are suggested as independent risk factors, while recent excessive use of corticosteroids and hypoalbuminemia seem to be independent protective factors.

1. Introduction

Globally, *Mycobacterium tuberculosis* (MTB) remains a major cause of morbidity and mortality [1,2]. It is estimated that one-third of the world's population is latently infected with MTB [3]. Although people with latent MTB infection (LTBI) do not show any symptoms and are not infectious, they serve as the reservoir from which future cases of active TB will emerge [2,4,5]. The lifetime risk of developing active TB for a person with LTBI is approximately 5 to 15% [5]. Preventive treatment, which must be based on a sensitive diagnosis, can avert the reactivation of tuberculosis and has become a new priority action for the End TB Strategy proposed and approved by the WHO [1,4].

There are no perfect methods available for the diagnosis of LTBI. The tuberculin skin test (TST) and the interferon-γ release assays (IGRAs), which detect host sensitization to MTB antigens and thus indirectly measure TB infection, are widely used [2,5]. By detecting IFN-γ secreted by T cells in response to specific MTB antigens, IGRAs (e.g., the QuantiFERON-TB (QFT) Gold In-Tube assay and the T-SPOT.*TB* assay) allow a rapid diagnosis with a similar sensitivity and a superior specificity than TST, independent of BCG-vaccination status [2,6]. Nevertheless, IGRAs, unlike TST, sometimes yield indeterminate results (ITRs), limiting their clinical utility [2]. Studies reported a higher sensitivity and less ITRs with the T-SPOT test than the QFT method, which may be attributed to the use of a fixed number of washed peripheral blood mononuclear cells (PBMCs) in the T-SPOT assay [2,7].

ITRs have been reported with a frequency of 0–5.4% in the general population and 3.5–33% in immunocompromised patients for T-SPOT.*TB* [8–13]. Age, underlying diseases, immunosuppressive treatment, C-reactive protein (CRP), $CD4^+$ T cell count and the season during which samples are collected have been suggested as potential influencing factors of the ITRs [6,8,10–12,14]. However, the data was limited due to the biases resulting from small sample size, various definitions of ITRs and incomplete data collection. In addition, ITRs arise from two test observations in T-SPOT.*TB*: a high nil-control spot count

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or an insufficient response to the mitogen in positive control wells [15]. The host factors pertinent to these two observations are probably varied, but there are few studies exploring them separately. According to experience at our center, inadequate positive responses are much less frequent than high nil responses.

2. Methods

2.1. Study design and participants

We conducted a 1:1 matched case-control, retrospective study of patients for whom a T-SPOT.*TB* test was performed in a tertiary hospital, the Peking Union Medical College (PUMCH) from Jan 1, 2015 to Apr 30, 2017. T-SPOT.*TB* was mainly performed when patients were suspected of TB infection or planned to use immunosuppressive drugs. Patients with high nil responses and available clinical or laboratory data in the Electronic Medical Record System were included as eligible case patients. The high nil response was defined as when the background number of spots in the negative control well was in excess of ten [15]. HIV-positive patients were excluded from this study. When a patient experienced high nil responses multiple times in repeated T-SPOT.*TB* assays, only the earliest indeterminate result was included in the comparative analysis.

The control group consisted of HIV-negative patients with spot-forming cells (SFCs) in nil control wells ≤ 10. On a daily basis, all patients undergoing T-SPOT.*TB* tests were allocated a number in chronological order. The matched control for each case was selected from those patients who underwent T-SPOT.*TB* later on the same day using the following criteria: 1) with normal nil responses (SFCs ≤ 10); 2) with available clinical and laboratory data in the Electronic Medical Record System; 3) with a sequential allocated number closest to the case; 4) patients who had already been enrolled in the case-control analysis model were excluded. If no eligible controls were acquired, the same selection strategy was utilized among patients with T-SPOT.*TB* performed earlier on the same day. Following this schema, unnecessary variation in materials and environmental factors between cases and controls was avoided.

This study was approved by the Ethics Committee of PUMCH.

2.2. Data collection

2.2.1. T-SPOT.TB assays

T-SPOT.*TB* assays (Oxford Immunotec) were performed according to the standard protocol proposed by the manufacturer in all patients [15]. Four milliliters of peripheral blood was collected and specific T cell responses to RD1 encoded antigens were subsequently detected by T-SPOT.*TB* within 6 h. T-SPOT.*TB* utilized AIM-V (GIBCO™ AIM V Medium liquid, Invitrogen, US.) as nil control, PHA as positive control, and ESAT-6 and CFP-10 as specific antigens, respectively. PBMCs obtained from each subject were plated (2.5×10^5 per well) on a plate pre-coated with the antibody against interferon-γ. Plates were incubated 16-18 h at 37 °C in 5% carbon dioxide. After incubation, wells were developed with a conjugate against the antibody used and an enzyme substrate. SFCs were counted with an automated ELISpot reader (AID-ispot, Strassberg, Germany), each SFC represented an antigen-specific T cell secreting interferon-γ. All T-SPOT.*TB* assays were performed by a single senior technician and the results were interpreted by her and a researcher.

2.2.2. Demographic, clinical and laboratory data collection

Demographic data, underlying conditions and medications within 4 weeks prior to T-SPOT.*TB* assays were obtained from electronic medical records. The dosage of various kinds of glucocorticoids was standardized into the equivalent dose of the Prednisone and the cumulative dosage of corticosteroids was estimated by the following formula: (course of treatment) × ((maximum dose + minimum dose)/2) [16]. Results of blood tests performed on the same day of T-SPOT.*TB* were recorded. If not applicable, blood tests performed within a week before or after T-SPOT.*TB* were reviewed.

2.3. Statistical analyses

Continuous variables with normal distributions, which were examined by the Kolmogorov-Smirnov test, were expressed as mean ± SD, while data without normal distributions were described as median and interquartile range (IQR). Categorical variables are presented as proportions. Comparisons of continuous variables between case patients and control subjects were made with the paired sample *t*-test and the Wilcoxon signed-rank test for normal and non-normal data, respectively. Categorical data were compared using the McNemar test. A $p < .05$ was considered statistically significant. Univariate analyses of influencing factors were performed using the conditional univariate logistic regression model to calculate odds ratio (OR) with 95% confidence intervals (CI). Variables with a $p < .1$ were consecutively subjected to a conditional multivariate logistic model to evaluate the independent impact of each influencing factors on high nil responses. A stepwise procedure (backward LR) was used with a p value of < 0.05 to enter and higher than 0.1 to remove. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., USA).

3. Results

Among 36,516 patients on whom a T-SPOT.*TB* test was performed in PUMCH from Jan 1, 2015 to Apr 30, 2017, 644 (1.76%, 95% CI: 1.63%–1.90%) patients exhibited high nil responses. Of these, 280 patients with available medical records were included in the matched case-control analysis (Fig. 1). In 12 out of the 280 case patients, retesting on T-SPOT.*TB* was performed within four weeks. The second assays remained ITRs in 2 (16.7%) of these 12 subjects (data not shown).

Significant differences were detected between case patients and control participants in age and gender. Among underlying diseases, including diabetes mellitus, malignancy, rheumatic diseases and Crohn's disease, only Behcet's disease (BD) reached statistical significance between the cases and the controls. Nearly one third of participants in this study had received immunosuppressive treatment within four weeks before the T-SPOT.*TB* assays. Cumulative dosage of corticosteroids was noted to display a significant difference between the two groups, while no evidence indicated that the difference in maximum daily dose of corticosteroids or the use of immunosuppressive or biological agents was significant. Complete blood counts were performed in most patients, the data suggesting significant differences

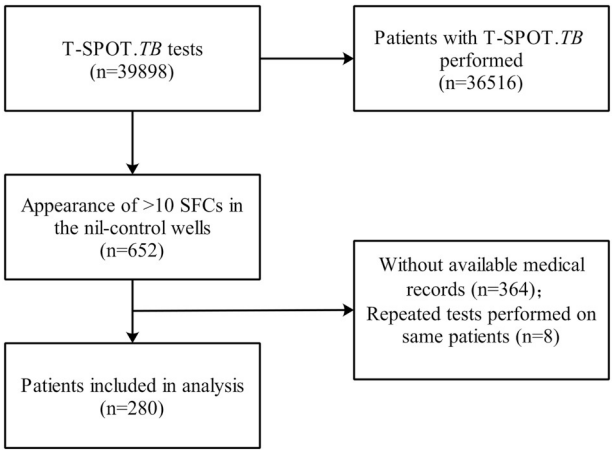

Fig. 1. Flowchart of selection of case patients with high nil responses in T-SPOT.*TB*. Abbreviations: SFCs, spot-forming cells.