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Diagnostic performance of whole-blood interferon-γ assay and enzyme-linked immunospot assay for active tuberculosis☆,☆☆

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Abstract

The aim of this study was to compare the diagnostic performance of 2 interferon-γ release assays, an enzyme-linked immunospot assay (T-SPOT.*TB*; Oxford Immunotec Ltd., Oxford, UK) and the QuantiFERON-TB Gold in-Tube assay (QFT-GIT; Cellestis Ltd., Carnegie, Australia), in patients with suspected active tuberculosis (TB). From October 2009 to October 2011, a total of 200 patients with suspected TB were enrolled. Clinical and microbiological characteristics of the patients were collected and blood samples were obtained for T-SPOT.*TB* and QFT-GIT assays. Among the 200 subjects, 98 (49%) had culture-confirmed TB, 18 (9%) had probable TB, and the remaining 84 (42%) subjects did not have TB. The sensitivity, specificity, positive predictive value, and negative predictive value for active TB diagnosis by the T SPOT. *TB* were 83%, 71%, 81%, and 75%, respectively. For QFT-GIT, the sensitivity, specificity, positive predictive value, and negative predictive value for active TB diagnosis were 66%, 76%, 80%, and 62%, respectively. The QFT-GIT assay resulted in more indeterminate and false-negative results than the T-SPOT.*TB* assay, especially in immunocompromised patients. In conclusion, T-SPOT.*TB* had a higher sensitivity and resulted in fewer indeterminate results than the QFT-GIT assay for diagnosing active TB.

Keywords: Active tuberculosis; Interferon-γ release assays; T-SPOT.*TB*; QFT-GIT; Rapid diagnosis

1. Introduction

Despite recent advances in global control efforts, tuberculosis (TB) remains one of the most serious challenges to public health (WHO, 2009). In 2008, there were an estimated 9.4 million new cases of active TB infection (140 per 100,000 population) (WHO, 2009). In Taiwan, there were 14,265 newly diagnosed TB cases in 2008, with an incidence of 62.0 cases per 100,000 population (CDC, Taiwan, 2009). Conventional diagnostic tests, including microscopic examination for acid-fast bacilli, mycobacterial culture, and pathological examinations, can be helpful in the diagnosis of TB, but are both time consuming and insensitive. Alternative and rapid diagnostic modalities are urgently needed, and immune-based blood assays, which do not require a specimen of the affected organ for microbiological examinations, may serve as adjunct diagnostic tools.

Recently, interferon-γ (IFN-γ) release assays (IGRAs) have been developed for the screening of TB infection. These immunodiagnostic tests, namely, the whole-blood interferon-γ enzyme-linked immunosorbent assay (QuantiFERON-TB Gold [QFT-G], Cellestis Ltd., Victoria, Australia) and the enzyme-linked immunospot assay (T-SPOT.*TB*, Oxford Immunotec, Oxfordshire, UK), can quantitatively measure IFN-γ production by lymphocytes specific to *Mycobacterium tuberculosis*-specific immunodominant antigens (early secretory antigenic target 6 [ESAT-6] and culture filtrate protein 10 [CFP-10]) that are encoded by the

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RD1 region of the pathogen. Another commercially available IGRA, namely, the QuantiFERON-TB Gold in-tube assay (QFT-GIT; Cellestis Ltd., Carnegie, Australia), is able to measure IFN-γ production specific to the immunodominant antigen TB7.7, ESAT-6 and CFP-10. All of these 3 assays have shown promising results in the detection of latent TB infection (LTBI) (Menzies et al., 2007) and have been approved by the Food and Drug Administration and are recommended by the Centers for Disease Control and Prevention for diagnosing latent tuberculosis (Mazurek et al., 2010). Several studies have demonstrated that interferon-γ release assays may be useful tools in the diagnosis of active tuberculosis (Kang et al., 2007; Kim et al., 2007, 2009; Lai et al., 2009a,b; Lee et al., 2009; Sester et al., 2011; Wang et al., 2007); however, few studies have compared the effectiveness of the QFT-GIT with that of the T-SPOT.*TB* assay in diagnosing active TB in patients clinically suspected of having TB (Adetifa et al., 2007; Chee et al., 2008; Diel et al., 2010; Domínguez et al., 2008; Sester et al., 2011).

The aim of this study was to assess the diagnostic value of the QFT-GIT and T-SPOT.*TB* assays in clinically suspected cases of active TB in Taiwan.

2. Materials and methods

2.1. Setting and patients

This study was conducted at the National Taiwan University Hospital, a 2500-bed medical center in northern Taiwan, and was approved by the hospital's institutional review board. After obtaining written informed consent, all patients with suspected active TB were prospectively enrolled from October 2009 to October 2010. Medical records were reviewed and data were collected on age, sex, underlying diseases, pathology, microbiological results, and follow-up observations. Subjects were classified as having confirmed TB if *M. tuberculosis* was recovered from a clinical specimen; as having probable TB if a biopsy specimen exhibited histologic evidence of TB infection (granulomatous inflammation and/or caseating necrosis) and if symptoms and signs of active TB were present in a patient who responded clinically and/or radiologically to a full course of anti-TB treatment according to the criteria reported by Kim et al. (2007, 2009); or as not having TB if another diagnosis was made or if there was clinical improvement without anti-TB therapy. The underlying conditions evaluated included end-stage renal disease, diabetes mellitus, AIDS or HIV, malignancy, liver cirrhosis, steroid use (daily use of 20 mg prednisolone for at least 2 weeks), autoimmune disorder, use of immunosuppressants, and history of organ transplant. Inactive malignancy was not included as an underlying disease.

2.2. Laboratory procedures

Smears of the processed specimens for acid-fast bacilli were stained with auramine–rhodamine fluorochrome and examined by standard procedures. Fluorochrome stain-positive smears were confirmed by the Kinyoun staining method. Sediment (0.5 mL) was inoculated onto Middlebrook 7H11 selective agar with antimicrobial agents (Remel Inc., Lexena, KS) and then evaluated using the fluorometric BACTEC technique (BACTEC MGIT 960 system; Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD) (Lai et al., 2008). Identification of *M. tuberculosis* and non-tuberculous mycobacteria species was performed using conventional biochemical identification methods as previously described (Lai et al., 2010).

2.3. Histopathology

For histopathologic examination, formalin-fixed and paraffin-embedded tissue blocks of biopsy specimens were stained with hematoxylin–eosin stain. Tissue sections were also subjected to Ziehl–Neelsen acid-fast staining for mycobacteria and Grocott's methenamine-silver nitrate staining for fungal organisms.

2.4. ELISPOT and QFT-GIT assays

The T-SPOT.*TB* and the QFT-GIT assays were performed using whole blood according to the manufacturers' instructions as previously described (Adetifa et al., 2007; Chee et al., 2008; Domínguez et al., 2008).

2.5. Statistical analysis

Diagnostic performance was expressed in terms of sensitivity, specificity, positive predictive value, and negative predictive value. All analyses were performed using the statistical package SPSS (version 12.0, SPSS, Chicago, IL).

3. Results

3.1. Patient characteristics

A total of 200 patients with suspected active TB were recruited during the study period. The clinical characteristics of these patients are summarized in Table 1. The mean age (standard deviation) was 57.5 (18.5) years, and 71% of patients were male. Among these patients, 98 (49%) had confirmed TB, 18 (9%) had probable TB, and the remaining 84 (42%) patients did not have TB. Diabetes mellitus was the most common underlying condition ($n = 53$, 26.5%), followed by cancer ($n = 47$, 23.5%) and usage of steroids ($n = 35$, 17.5%). Among the patients with malignancy, lung cancer ($n = 8$) was the most common type of cancer, followed by acute leukemia ($n = 5$) and lymphoma ($n = 5$). Among the 116 patients with confirmed or probable TB, 33 (28.4%) had extrapulmonary TB and 2 patients (1.7%) had disseminated TB.

3.2. Diagnostic performance of IGRAs

Of the 200 patients with suspected TB, 119 (59.5%) had positive T-SPOT.*TB* results. In addition, positive T-SPOT.