Original Article

Comparison of 2 interferon-γ assays and Roche Cobas Amplicor *Mycobacterium tuberculosis* assay for rapid diagnosis of tuberculosis among patients with suspected tuberculosis in Taiwan

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Background and purpose: Two commercial interferon-γ (IFN-γ) assays, which are commonly used for diagnosing latent tuberculosis (TB), are also useful for diagnosis of active TB. In this study, the IFN-γ assays and polymerase chain reaction (PCR) for diagnosis of TB were compared.

Methods: A prospective comparison of the performance of 2 commercial IFN-γ assays — QuantiFERON®-TB Gold (QFT-G) and T-SPOT®.*TB* (T SPOT) — and PCR using the Roche Cobas Amplicor *Mycobacterium tuberculosis* (RCA-TB) assay for the rapid diagnosis of TB was conducted from January 2007 to December 2007 at a university-affiliated hospital in Taiwan.

Results: Of 187 patients enrolled in the study, results from both T SPOT and QFT-G were available for 154, including 109 patients with active TB and 45 with no TB. The sensitivity of T SPOT (89.0%) was higher than that of QFT-G (71.4%). RCA-TB had the highest sensitivity (90.2%) and specificity (100%), but was usually performed in patients with positive acid-fast bacilli smear test. In patients with extrapulmonary TB, T SPOT had a high diagnostic value (sensitivity, 81.3%). A significant discordance between the 2 IFN-γ assays was also noted. IFN-γ assays provided a more rapid diagnosis for tuberculosis than the conventional culture method (mean ± standard deviation, 8.23 ± 12.86 days; $p < 0.001$).

Conclusions: Use of IFN-γ may shorten the time to diagnosis of TB, especially for smear-negative patients and those with extrapulmonary disease.

Key words: Interferon-gamma; Tuberculosis

Introduction

Tuberculosis (TB) is the most important infectious disease worldwide [1,2]. In 2007, the incidence and mortality rates of TB in Taiwan were 63.5 and 3.1 per 100,000 population, respectively [3]. Successful control of TB depends on prompt detection of *Mycobacterium tuberculosis* (MTB) in clinical specimens and timely diagnosis of patients with clinically

suspected TB. Two commercial interferon-γ (IFN-γ) assays, QuantiFERON®-TB Gold (QFT-G; Cellestis Ltd, Carnegie, Australia) and T-SPOT®.*TB* (T SPOT; Oxford Immunotec, Oxford, UK), that are commonly used for diagnosing latent TB are also useful for diagnosis of active TB [4]. These assays use early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) as MTB-specific stimulants on enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot assay (ELISPOT), respectively. Several studies have demonstrated their high sensitivity and specificity for TB diagnosis [5-7]. The IFN-γ assay performance is also related to background

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disease, prevalence of human immunodeficiency virus (HIV) infection, immune status, and non-tuberculous mycobacteria (NTM) infection [8-13]. New diagnostic methods that use nucleic acid amplification and detection provide quick and specific results for identifying the MTB complex, but the reported sensitivity of this technique is highly variable [14,15].

This study directly compared the 2 commercial IFN-γ assays with a commercial polymerase chain reaction (PCR) method (Roche Cobas Amplicor *Mycobacterium tuberculosis* Assay [RCA-TB]; Roche Diagnostics, Grenzach-Whylen, Germany) for the rapid diagnosis of TB in Taiwan.

Methods

Patients and setting

This prospective study was conducted from January 2007 to December 2007 at a university hospital with 2500 beds in northern Taiwan, and was approved by the Institutional Review Board of the hospital. All patients who were suspected of having TB were selected from the infection clinic or ward. Patients were classified as having suspected TB if they had fever or respiratory symptoms (cough, dyspnea, or hemoptysis) for ≥2 weeks and compatible radiographic findings, including fibroexudative or fibrotic lesions over the upper lung, pulmonary nodules with or without cavitation, multiple patches of alveolar infiltrates, miliary shadowing, and pleural effusion with lymphocytotic and exudative characteristics [4,16]. Patients with no clinical or microbiologic evidence of MTB infection and those with no exposure to TB were classified as the non-TB group.

In the subgroup analysis of patients, active TB was defined as either 'culture-confirmed TB' (culture-positive for MTB) or 'clinical TB'. Patients were diagnosed as having clinical TB if there was a histological diagnosis (granulomatous inflammation and caseous necrosis) of a biopsy specimen or there were symptoms and signs of active TB in a patient who responded clinically and radiologically to a full course of anti-TB treatment.

Patients were classified as immunocompromised if they had an immunosuppressive condition, which included treatment with immunosuppressive drugs (post-transplantation, anticancer chemotherapy), hemodialysis, hematological malignancy (leukemia, lymphoma), or diabetes mellitus. The results of serological tests for HIV were evaluated.

The dates of initial evaluation for TB, performance of IFN-γ assays and PCR, and species confirmation of positive cultures were recorded.

Interferon-γ assays

Peripheral blood was used for T SPOT and QFT-G assays. The T SPOT assay was performed according to the manufacturer's instructions. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood using Ficoll-Hypaque density gradient centrifugation, washed, resuspended, and counted. PBMCs (250,000/well) were added to wells coated with a mouse monoclonal antibody to IFN-γ, and containing antigen (ESAT-6 or CFP-10), mitogen (phytohemaglutinin [PHA] as a positive control) or nothing (as background control wells). Plates were washed after 16 to 18 h of incubation, and 50 µL of conjugate reagent containing biotinylated anti–IFN-γ monoclonal antibody was then added followed by incubation for 1 h. The plates were then washed and chromogenic alkaline phosphatase substrate was added. After 7 min, the plates were washed and dried. Spots were counted manually using a hand-held magnifying glass. The number of spots in the background control wells was subtracted from the number in the test wells. A response was considered positive if the number of spots per test well was ≥6 (when the background control had a count of 0 to 5) or at least twice the value found in the background control wells (when the background control's count was 6 to 10) [6,17].

For QFT-G assay, the IFN-γ value of the negative control for each subject was subtracted from the values obtained from the antigen-stimulated samples. The result was considered positive if the response to the specific antigen was ≥0.35 IU/mL, regardless of the value of the positive control; negative if the response to the specific antigen was <0.35 IU/mL and the IFN-γ of the positive control was ≥0.5 IU/mL; and indeterminate if both antigen-stimulated samples were <0.35 IU/mL, and the value of the positive control was <0.5 IU/mL [5].

Roche Cobas Amplicor *Mycobacterium tuberculosis* assay

Specimens from infectious sites were used for the RCA-TB assay. All specimens were processed as previously described [18]. Briefly, a respiratory specimen was processed by adding an equal volume of sodium hydroxide–citrate–*N*-acetyl-L-cysteine at room temperature for 15 min. After centrifugation, the

precipitate was resuspended in 1 mL of phosphate-buffered saline (pH, 7.4).

The RCA-TB was used for PCR processing. A portion (0.1 mL) of the sample obtained after liquefaction was washed and further processed by alkaline lysis with the reagents provided in the sample preparation kits, as recommended by the manufacturer. After adding 50 μL of sample to 50 μL of the master mix, PCR amplification tubes were dosed and the amplification ring was transferred into the RCA-TB amplification system. One positive and 1 negative control per vessel ring (12 vessels) provided with the kit were included in each run. The RCA-TB automates the amplification and detection procedure for PCR. Discrimination between positive and negative results was performed by the RCA-TB software with the cutoff set to an optical density at 650 nm of 0.35 [19].

The T SPOT, QFT-G, and RCA-TB assays were performed by well-trained permanent technicians who were blinded to the final diagnoses of the patients.

Conventional isolation and identification for mycobacteria

Smears of the processed specimens for acid-fast bacilli were stained with auramine-rhodamine fluorochrome and examined by standard procedures [20]. Fluorochrome stain–positive smears were confirmed by the Kinyoun staining method [20]. Cultures were performed by inoculating 0.5 mL of sediment onto Middlebrook 7H11 selective agar with antimicrobials (Remel Inc., Lenexa, KS, USA) [21] and by the fluorometric BACTEC technique (BACTEC MGIT 960 system; Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). Identification of MTB

was performed using conventional biochemical identification methods as previously described [20].

Statistical analysis

Sensitivity, specificity, positive predictive value, and negative predictive value for the diagnosis of TB were calculated for each diagnostic test. Differences in test performances were analyzed using the chi-squared test and paired t test. Concordances between tests were assessed using the κ coefficient (κ > 0.75, excellent agreement; κ < 0.4, poor agreement; κ ≥ 0.4 and ≤ 0.75, fair to good agreement). Analyses were performed using the Statistical Package for the Social Sciences for Windows (Version 13.0; SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

Of 187 patients screened for TB, 130 had active TB and 57 had no TB. 154 patients had both T SPOT and QFT-G assays performed, and the remaining 33 patients had either T SPOT or QFT-G performed. Analysis was done for the 154 patients who had both tests performed. Of these 154 patients, the RCA-TB assay was performed for 103 patients, most of whom (97 patients) had acid-fast bacilli smear–positive specimens. The mean age of the 154 study patients was 55.0 years (range, 1-92 years) and 57.8% of patients were men. The demographic and clinical characteristics of the 154 patients are summarized in Table 1. The active TB group consisted of 109 patients, including 91 (83.5%) with culture-confirmed TB and 18 (16.5%) with clinical TB (6 were biopsy proved). Of the 109 patients with active TB, 93 (85.3%) had pulmonary TB and

Table 1. Characteristics of 154 patients with active tuberculosis or without tuberculosis who received both QuantiFERON®-TB Gold and T-SPOT®.TB tests.

Characteristic	Active tuberculosis (n = 109) No. (%)	No tuberculosis (n = 45) No. (%)
Age (years) [mean ± SD]	53.6 ± 20.4	58.4 ± 20.0
Sex		
Male[a]	69 (63.3)	20 (44.4)
Female	40 (36.7)	25 (55.6)
Underlying condition		
Hemodialysis	2 (1.8)	2 (4.4)
Immunosuppressive drug use	1 (0.9)	2 (4.4)
Diabetes mellitus	20 (18.3)	6 (13.3)
Malignancy	21 (19.3)	8 (17.8)
HIV infection	3 (2.8)	1 (2.2)

[a]$p < 0.05$.

Abbreviations: SD = standard deviation; HIV = human immunodeficiency virus.

Table 2. Comparison of results of 2 interferon-γ assays and polymerase chain reaction in patients with active tuberculosis or without tuberculosis.

Assay	Active tuberculosis (n = 109)		No tuberculosis (n = 45)		Sensitivity (%)	Specificity (%)	Predictive value (%)	
	Assay-positive	Assay-negative	Assay-positive	Assay-negative			Positive	Negative
T-SPOT®.*TB*	97	12	11	34	89.0	75.6	89.8	73.9
QuantiFERON®-TB Gold[a]	70	28	7	30	71.4	81.1	90.9	51.7
Roche Cobas Amplicor *Mycobacterium tuberculosis*	74	8	0	21	90.2	100	100	72.4

[a]Excludes 11 indeterminate results in the active tuberculosis group and 8 indeterminate results in the non-tuberculosis group.

16 (14.7%) had extrapulmonary disease (2 had TB pleurisy, 3 had TB meningitis, 2 had urinary tract TB, 1 had liver TB, 3 had TB lymphadenitis, 1 had TB of the spine, and 4 had soft tissue TB).

Forty patients (36.7%) with active TB had immunocompromised conditions, including diabetes mellitus, immunosuppressive drug use, malignancy, chronic renal failure treated with hemodialysis, and HIV infection.

Interferon-γ assays and Roche Cobas Amplicor *Mycobacterium tuberculosis* assay

Among the 154 patients, 19 (17.4%) had indeterminate QFT-G results. There were no indeterminate T SPOT results. After excluding the indeterminate results, the sensitivity of T SPOT (89.0%) was higher than that of QFT-G (71.4%; Table 2), but this difference was not significant. QFT-G (81.1%) had higher specificity than T SPOT (75.6%), but this difference was not significant. In patients with culture-confirmed TB, T SPOT sensitivity (95.0%) was higher than QFT-G sensitivity (75.0%). QFT-G had poor sensitivity (55.6%) when used for patients with clinical TB (T SPOT sensitivity, 83.3%). For immunocompromised patients, the sensitivity of T SPOT (91.7%) was higher than that for QFT-G (69.4%). Comparison of QFT-G and T SPOT did not reach statistical significance due to the relatively small number of patients.

RCA-TB was performed for 103 patients (specimens: sputum, 92; biopsies, 9; cerebrospinal fluid, 1; urine, 1). The sensitivity of RCA-TB was 90.2% and the specificity was 100%. The QFT-G sensitivity for these 103 patients was lower (74.7%), but this difference was not significant; T SPOT sensitivity was 91.5%. QFT-G had higher specificity (83.3%) than T SPOT (71.4%) for these 103 patients. For the 51 patients who did not receive the RCA-TB test, 40 (78.4%) had acid-fast smear–negative results. T SPOT had higher sensitivity (81.5%) than QFT-G (60.9%) for diagnosing TB in these 51 patients, but this difference was not significant. For immunocompromised patients, the sensitivity of RCA-TB was 87.1%.

For the 16 patients with extrapulmonary TB who had both T SPOT and QFT-G performed, the sensitivity was 81.3% and 60.0%, respectively. For the 12 patients with a clinical diagnosis of TB, T SPOT and QFT-G sensitivities were 83.3% and 50.0%, respectively.

The mean ± standard deviation (SD) time from initial suspicion of TB to culture or biopsy confirmation for patients with active TB was 21.99 ± 33.78 days. The average time from initial suspicion of TB to performing IFN-γ assays that showed positive results was significantly shorter (13.76 ± 33.56 days; $p < 0.001$). For extrapulmonary disease, the duration of diagnosis using IFN-γ assays was also decreased (mean ± SD, 10.7 ± 23.4 days) compared with methods using culture or biopsy confirmation.

Discordance among test results

After excluding the 19 patients with indeterminate QFT-G results, significant discordance (McNemar test, $p < 0.001$) was found between the T SPOT and QFT-G tests. The κ values between T SPOT and QFT-G were 71.2% (κ = 0.377).

Among the 98 patients with active TB, 29 (29.6%) had discordant results between T SPOT and QFT-G. Most of these patients (86.2%) were QFT-G–negative/T SPOT–positive and 4 (13.8%) were QFT-G–positive/T SPOT–negative, suggesting that T SPOT was more sensitive than QFT-G for diagnosing MTB infection.

Among the 37 TB patients without TB (excluding 8 indeterminate QFT-G results), 4 (10.8%) were QFT-G–positive/T SPOT–positive, suggesting that they might have been infected with MTB or another ESAT-6– or CFP-10–containing NTM. Of the 37 patients,

10 (27.0%) had discordant results between T SPOT and QFT-G; 7 were T SPOT–positive/QFT-G–negative (including 1 with *Mycobacterium gordonae*, 1 with *Mycobacterium abscessus*, and 2 with *Mycobacterium avium* complex infection) and 3 were T SPOT–negative/QFT-G–positive (including 1 with *Mycobacterium fortuitum* infection). The discordance between the T SPOT and QFT-G results for patients without TB was not statistically significant.

Discussion

For rapid diagnosis of active TB, nucleic acid amplification techniques have high specificity but variable sensitivity, and are especially poor for smear-negative and extrapulmonary TB [14,22,23]. These techniques provide rapid differentiation between MTB complex and NTM among smear-positive specimens. There is some debate, which has been widely addressed in 2 recent publications, regarding the use of IFN-γ release assays for diagnosis of active TB [24,25]. The authors concluded that IFN-γ release assays are useful for diagnosis of latent TB, but not for active TB, with a low specificity, particularly in areas with a high prevalence of latent TB [12,13,24,25]. However, for patients with pulmonary lesions with positive acid-fast bacilli respiratory secretions, these assays could be useful to differentiate patients with TB from those with colonization or infection with NTM, particularly in areas with a high incidence of respiratory colonization or infections with NTM [4,12,26,27].

This study prospectively compared 2 commercial ELISA- and ELISPOT-based IFN-γ assays with PCR for the diagnosis of TB in Taiwan. Several previous studies comparing ELISA- and ELISPOT-based IFN-γ assays showed that T SPOT sensitivity was approximately 78% to 96% [10,17,28]. This study found that T SPOT sensitivity (89.0%) was high for the diagnosis of TB in Taiwan, as shown in a previous study performed in Taiwan [4]. QFT-G sensitivity (71.4%) was low and within the previously reported range (64% to 89%) [5,29]. Due to the low sensitivity of QFT-G, some reports have suggested that it should not be used alone to exclude active TB [29].

Although early TB diagnosis is important for TB control and favorable outcome, the present microbiological diagnostic techniques are insensitive, slow, and time consuming. The results from this study show that combining IFN-γ assays and PCR shortens the duration from clinical suspicion to definitive diagnosis of TB and the start of antituberculous drugs. This study also shows that IFN-γ assays can provide rapid and accurate diagnosis, especially for extrapulmonary disease, for which definitive culture is difficult, and for clinically diagnosed disease.

IFN-γ assays are now used for diagnosing latent TB infection [12,13]. T SPOT and QFT-G use similar antigens (ESAT-6 and CFP-10), but they differ in the specimens used (whole blood versus mononuclear cells), so it is easier to perform QFT-G. The commercially available PCR test approved by the United States Food and Drug Administration (FDA), RCA-TB, has excellent performance when used for testing smear-positive specimens (sensitivity, >95%; specificity, 100%) [30]. On the basis of these data, the FDA recommended the use of PCR only for smear-positive respiratory specimens from patients who had not received antituberculous drugs for 7 or more days or within the previous 12 months [31].

Combining IFN-γ assays and PCR also provided rapid detection of NTM infection. Among the 187 patients in this study, 125 (66.8%) were acid-fast bacilli smear positive and 19 (15.2%) had NTM infection. It takes time for culture to differentiate MTB and NTM species. PCR can rapidly differentiate TB from NTM. However, this method is affected by anti-TB treatment [31]. IFN-γ assays are not affected by anti-TB treatment, but using ESAT-6 and CFP-10 as antigens may result in false-positive findings for some NTM infections [32,33]. When IFN-γ assays and PCR are performed together, the specificity for NTM infection can reach 100%.

Consistent with other studies [10,34,35], this study found considerable discordance between the 2 IFN-γ assays. The results of this study are in agreement with those of others [10,34,35] that most of the discordance is due to QFT-G–negative and T SPOT–positive combinations. Some of the discordance may be related to test formats and the inclusion of an additional antigen for QFT-G, but these effects remain poorly understood.

IFN-γ assays can yield indeterminate results. One study found that this was common (21%) when using QFT-G assay and most of the patients were immunocompromised [34]. In the present study, 19 of 154 patients (17.4%) had indeterminate QFT-G results, and 9 of the patients were immunocompromised.

The present study had several limitations. First, the number of patients without TB was small. This may have influenced the findings regarding specificity

of the IFN-γ assays. The specificity results in this study were not as good as in previous reports [4,12]. Second, underestimation of the sensitivities of the IFN-γ assays is possible due to the fact that some patients with active TB were diagnosed clinically rather than by the standard criterion of bacteriological confirmation. However, even when the analysis was restricted to culture-confirmed TB, there were no significant differences in the comparative results among the tests.

In conclusion, T SPOT had higher sensitivity than QFT-G for diagnosing TB, but the difference was not significant. A combination of IFN-γ assay and PCR may provide rapid and accurate diagnosis of TB, especially for patients for whom definitive culture is difficult and when there is a high prevalence of NTM in the clinical setting.

Acknowledgment

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