HRCT and Whole-Blood Interferon- γ Assay for the Rapid Diagnosis of Smear-Negative Pulmonary Tuberculosis

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Key Words

Tuberculosis · Interferon- γ · High-resolution CT · Polymerase chain reaction

Abstract

Background: Early diagnosis of active pulmonary tuberculosis (PTB) is critical for TB control, and difficult in patients with smear-negative sputum. **Objective:** We wanted to evaluate the usefulness of clinical findings, high-resolution computed tomography (HRCT), interferon- γ -releasing assay (IGRA) and polymerase chain reaction (PCR) of sputum in the diagnosis of smear-negative PTB. **Methods:** From June 2006 to September 2008, 178 patients with suspected PTB on the basis of clinical and radiological findings visited our institute. After excluding smear-positive cases ($n = 77$) and cases with an inconclusive diagnosis ($n = 17$), we studied 84 patients. Their clinical records, HRCT, sputum TB-PCR assay and IGRA results were retrospectively evaluated. A QuantiFERON-TB Gold (QFT-G; Cellestis Ltd., Carnegie, Vic., Australia) assay was used for the IGRA. **Results:** Active PTB was diagnosed in 40 (48%) of 84 patients; lack of sputum and young age were significantly associated with an increased risk of PTB. The sensitivities of sputum PCR assay, IGRA, and HRCT were 43.2, 84.4 and 80.0%, respectively, and the specificities were 97.7,

82.9 and 70.5%, respectively. Among the 38 patients suspected of having PTB based on HRCT, 24 patients showed positive results on the IGRA, and 23 of these were diagnosed with active PTB. Among the 35 patients suggested not to have TB based on HRCT, 25 showed negative results on the IGRA, and 23 (92%) of these were diagnosed as not to have TB. **Conclusion:** The combined results of HRCT and the IGRA could help decision-making for early initiation of treatment in smear-negative patients.

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Introduction

Tuberculosis (TB) is a major health problem around the world, with an estimated 9.2 million new cases of TB occurring in 2006 (139 per 100,000) [1]. The most powerful strategy for a TB control program is the prompt diagnosis and successful treatment of patients with active, contagious disease. Therefore, early diagnosis of active pulmonary TB (PTB) is critical for TB control.

Active TB can be confirmed only with a positive bacteriological examination. The acid-fast bacilli smear of respiratory specimens, including sputum and induced sputum, is the essential modality for the prompt diagno-

sis of PTB. However, the acid-fast bacilli smear has poor sensitivity (between 30 and 70% depending on how the test is implemented) [2]. Although the culture of tuberculous bacilli is more sensitive (80–85%), culture results usually require 3–8 weeks [3–5]. Bronchoscopy has been regarded as an effective alternative method in smear-negative cases [6]. However, the availability of this technique may be limited in most settings because bronchoscopy is an invasive procedure that needs expertise. Thus, in TB-endemic countries, the diagnosis of patients with smear-negative TB is complicated and often delayed.

High-resolution computed tomography (HRCT) provides information on the extent and distribution of PTB [7, 8] and can help distinguish active from inactive disease [9]. However, because of the smaller mycobacterium burden present in smear-negative disease, such patients have clinical and radiological findings different from those with smear-positive TB [10, 11]. The typical radiographic pattern of reactivation TB seen in smear-positive patients, i.e., apical or upper lobe infiltrates or cavities, is not likely to be present in patients with smear-negative disease [10, 11]. Although the sputum polymerase chain reaction (PCR) assay can be used as an adjunctive diagnostic method in the diagnosis of active PTB, it has low sensitivity in smear-negative cases [10–12].

Immunodiagnostic tests based on *Mycobacterium tuberculosis*-specific antigens have led to the development of a new specific diagnostic test of infection with TB [13, 14]. These assays measure T-cell-induced interferon- γ (IFN- γ) responses to TB-specific peptide derived from the early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These tests show considerable promise and have excellent specificity for diagnosing latent TB infection [15]. Several studies have evaluated the usefulness of this assay for the diagnosis of active PTB [16–18], but the assay has limited ability to differentiate active and latent TB infection.

Here, we wanted to compare the diagnostic value of clinical findings, chest HRCT, sputum TB-PCR, and IFN- γ -releasing assay (IGRA) in the diagnosis of active PTB in smear-negative cases suspected of having active PTB in an intermediate TB burden country.

Patients and Methods

Study Setting and Subjects

In this retrospective evaluation, we reviewed the clinical records, HRCT findings, sputum TB-PCR assay results, and IGRA results of all patients with suspected TB seen at our institute from June 2006 to September 2008. A patient with suspected TB was

defined as an individual with clinical or radiographic evidence consistent with active TB. We excluded patients who were smear positive or who had an inconclusive diagnosis owing to loss of follow-up.

From June 2006 to September 2008, 178 patients suspected of having PTB visited our institute. After excluding smear-positive cases ($n = 77$) and cases with an inconclusive diagnosis ($n = 17$), we retrospectively evaluated the diagnostic accuracy of chest CT, sputum TB-PCR assay results, and whole-blood IFN- γ assay results in 84 patients. The recorded clinical information on these patients included age, gender, cough, sputum, hemoptysis, weight loss, night sweat, fever and chest pain. The Institutional Review Board of the Yong San Hospital, Seoul, Korea, approved this study.

Diagnostic Definition

Active PTB was diagnosed when (1) *M. tuberculosis* was cultured; (2) a caseating granuloma was found in the lung tissue by transthoracic needle biopsy and showed appropriate response to treatment; or (3) clinical findings were compatible with TB, no clinical improvement was seen with empirical antibiotics, and treatment with anti-TB medication resulted in clinico-radiological improvement. All subjects without culture-confirmed TB were followed for at least 4 months, to evaluate changes in their diagnostic categorization. A final diagnosis of 'non-TB' was accepted when an alternative diagnosis was reached, the symptoms resolved rapidly, and anti-TB therapy was not commenced.

HRCT, Sputum TB-PCR and IGRA

Two radiologists who were blinded to the clinical history and final diagnosis in each case retrospectively interpreted the CT scans and evaluated the following CT findings: (a) air-space consolidation of varying degrees with or without internal necrosis, (b) cavities, (c) ill-defined centrilobular air-space nodules that indicate endobronchial spread of infection, (d) tree-in-bud pattern, (e) hilar/mediastinal lymph node enlargement with central necrosis and (f) mass. After reviewing the CT findings, the two observers reached a final diagnosis by consensus.

For the sputum TB-PCR assay, sputum samples were digested and decontaminated by the sodium dodecyl sulfate-NaOH method, followed by centrifugation. PCR testing for tuberculosis was performed using an Amplicor kit and a Cobas Amplicor analyzer (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.

A QuantiFeron-TB Gold (QFT-G; Cellestis Ltd., Carnegie, Vic., Australia) assay was used for the IGRA. The QFT-G assay was performed in 2 stages according to the manufacturer's instructions. First, 1 ml of heparinized whole blood was incubated with aliquots of antigen-free control and antigens ESAT-6, CFP-10, or phytohemagglutinin for 16–24 h at 37°C in a carbon dioxide incubator. Then, after overnight incubation, 200 μ l of plasma was removed from each well and the concentration of IFN- γ was determined using the assay kit according to the manufacturer's instructions. A positive result on the QFT-G assay was defined as ≥ 0.35 IU; either of ESAT-6 or CFP-10.

Statistical Methods

Univariate comparisons between active PTB and non-TB patients were performed using Fisher's Exact Test for categorical variables and the Mann-Whitney test for continuous variables

where appropriate. All tests of significance were two sided; $p \leq 0.05$ was considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio of a positive test result (LR+), and likelihood ratio of a negative test result (LR-) for the diagnosis of active PTB disease were calculated for each diagnostic test.

Table 1. Demographic and clinical characteristics of the 84 patients with suspected active pulmonary TB and negative sputum smear

Characteristic	Data
Median age ¹ , years	57 (17–95)
Male/female gender, n	47/37
History of TB	18 (21%)
Final diagnosis, n	84
Active pulmonary TB	40 (48%)
Culture confirmed, n	29
Biopsy confirmed, n	4
Clinical diagnosis, n	7
Pneumonia	20 (24%)
Lung cancer	6 (7%)
Interstitial lung disease	4 (5%)
Nontuberculous mycobacterium	3 (4%)
Aspergilloma	2 (2%)
Sequelae of previous infection	2 (2%)
Others ²	7 (8%)

Data are presented as numbers and percentages unless otherwise indicated.

¹ Range is shown in parentheses. ² Anthracofibrosis, leptospirosis, pulmonary edema, angiodysplasia, atelectasis, bronchiectasis. TB = Tuberculosis.

Results

Demographic Characteristics

The baseline characteristics of the 84 patients are summarized in table 1. All participants were human immunodeficiency virus (HIV) negative. Two patients had risk factors related to immunosuppression: one was undergoing chemotherapy for a solid tumor, and the other had end-stage renal disease. Active PTB was diagnosed in 40 patients (48%). Of the 40 patients, 29 (73%) had a positive TB culture, 4 (10%) were confirmed by biopsy, and 7 (18%) were diagnosed clinically. The clinical parameters are described in table 2. Lack of sputum and young age were significantly associated with increased risk for active PTB.

Diagnostic Validity of HRCT, Sputum TB-PCR and the QFT-G Assay

The HRCT findings are summarized in table 3. Findings of cavities, noncalcified nodules and the tree-in-bud pattern were significantly associated with increased risk for PTB. Table 4 summarizes the diagnostic accuracy. Of the 45 patients suspected of having active PTB, 32 (71%) were correctly diagnosed with PTB. Of the 39 patients assumed to be non-TB cases, 31 (79%) were correctly excluded. The sensitivity, specificity, PPV, NPV, LR+, and LR- of HRCT for the diagnosis of PTB were 80.0, 70.5, 71.1, and 79.5%, 2.71 and 0.26, respectively.

PCR assay results were available for 80 of the 84 patients. Three of the 4 patients who did not undergo PCR were diagnosed with active TB by transthoracic needle biopsy, and the other was diagnosed as non-TB. Seven-

Table 2. Comparison of the clinical findings in sputum smear-negative TB patients and non-TB patients with suspected TB

Characteristics	Active TB (n = 40)	Non-TB (n = 44)	p value	OR ¹
Median age ²	40 (23–78)	63 (17–95)	0.002	
Male/female	19/21	28/16	0.14	1.93 (0.81–4.63)
Symptoms				
Cough	22 (55%)	32 (73%)	0.09	0.46 (0.18–1.14)
Sputum	19 (48%)	32 (73%)	0.02	0.34 (0.14–0.84)
Fever	12 (30%)	13 (30%)	0.96	1.02 (0.40–2.61)
Hemoptysis	8 (20%)	13 (30%)	0.31	0.60 (0.22–1.64)
Chest pain	7 (18%)	3 (7%)	0.13	2.90 (0.70–12.09)
Weight loss	4 (10%)	4 (9%)	0.89	1.11 (0.26–4.77)
Night sweat	3 (8%)	2 (5%)	0.57	1.70 (0.27–10.75)

Data are presented as numbers and percentages unless otherwise indicated.

¹ 95% confidence intervals is shown in parentheses. ² Range is shown in parentheses. TB = Tuberculosis; OR = odds ratio.

Table 3. Multivariate regression analysis of CT findings in sputum smear-negative TB patients and non-TB patients with suspected TB

Parameter	Active TB (n = 40)	Non-TB (n = 44)	p value	OR ¹
Mass	1 (3%)	5 (11%)	0.205	0.2 (0.02–1.791)
Consolidation	19 (48%)	24 (55%)	0.662	0.75 (0.32–1.78)
Cavity	17 (43%)	5 (11%)	0.002	5.77 (1.88–17.71)
Nodule	30 (75%)	10 (23%)	<0.001	10.20 (3.74–27.86)
Mediastinal LAP	4 (10%)	2 (5%)	0.418	2.33 (0.40–13.49)
TIB	16 (40%)	3 (7%)	<0.001	9.11 (2.41–34.52)

Data are presented as number (%) unless otherwise indicated. CT = Computed tomography; LAP = lymphadenopathy; OD = odds ratio; TB = tuberculosis; TIB = tree-in-bud pattern. ¹ 95% confidence intervals is shown in parentheses.

Table 4. Diagnostic accuracy of HRCT findings, TB-PCR assay results, and QFT-G assay results in smear-negative TB

Diagnostic method	Sensitivity ¹ , %	Specificity ¹ , %	PPV ¹ , %	NPV ¹ , %	LR+ ¹	LR- ¹
HRCT	80.0 (63.9–90.4)	70.5 (54.6–82.8)	71.1 (55.5–83.2)	79.5 (61.1–90.1)	2.71 (1.67–4.38)	0.26 (0.14–0.48)
TB-PCR	43.2 (27.5–60.4)	97.7 (86.2–99.9)	94.1 (69.2–99.7)	66.7 (53.6–77.7)	18.59 (2.59–133.59)	0.58 (0.44–0.77)
QFT-G	84.4 (66.5–94.1)	82.9 (67.4–92.3)	79.4 (61.6–90.7)	87.2 (71.8–95.2)	4.94 (2.48–9.86)	0.19 (0.08–0.43)

¹ 95% confidence intervals is shown in parentheses. HRCT = High-resolution computed tomography; TB = tuberculosis; PCR = polymerase chain reaction; QFT-G = QuantiFeron-TB Gold; PPV = positive predictive value; NPV = negative predictive value; LR+ = likelihood ratio of a positive test result; LR- = likelihood ratio of a negative test result.

teen patients showed a positive PCR result, and 16 of these were diagnosed with active PTB. The sensitivity, specificity, PPV, NPV, LR+, and LR- of the PCR assay for the diagnosis of active PTB were 43.2, 97.7, 94.1, and 66.7%, 18.59 and 0.58, respectively.

Seventy-five of the 84 patients had QFT-G assay results, but the results in 2 cases were indeterminate [(mitogen – nil), <0.5 IU/ml]. Thus, we evaluated the diagnostic accuracy of the QFT-G assay in 73 patients. Thirty-four patients (47%) showed positive reactions and 27 (79%) of these patients were diagnosed with active PTB. Five (13%) of 39 patients who showed negative results were diagnosed with active PTB. The sensitivity, specificity, PPV, NPV, LR+ and LR- of the QFT-G assay for the diagnosis of PTB were 84.4, 82.9, 79.4, and 87.2%, 4.94 and 0.19, respectively.

Combined Result of HRCT and the QFT-G Assay

QFT-G assay results were available for 38 of the 45 patients suspected of having active TB based on HRCT. Twenty-four patients had positive QFT-G assay results,

Table 5. Cases diagnosed based on combined HRCT and QFT-G assay results

	TB (n = 32)	Non-TB (n = 41)
TB on HRCT with positive QFT-G	23	1
TB on HRCT with negative QFT-G	3	11
Non-TB on HRCT with positive QFT-G	4	6
Non-TB on HRCT with negative QFT-G	2	23

Data are presented as number of patients. HRCT = High-resolution computed tomography; QFT-G = QuantiFeron-TB Gold; TB = tuberculosis.

and 23 (96%) of these patients were diagnosed with active PTB. Of the 14 patients with negative QFT-G results, 11 (79%) were diagnosed as non-TB (table 5). Of the 39 patients thought to be non-TB cases based on HRCT, 35 had QFT-G assay results. Twenty-five patients had negative QFT-G results, and 23 (92%) of these patients were

diagnosed as non-TB. Of the 10 patients with positive QFT-G results, 4 (40%) were diagnosed with active TB (table 5).

Discussion

Smear-negative TB is a common problem in clinical practice and is the cause of transmission in at least 1 in 6 patients with pulmonary involvement [19]. However, no standard practice for diagnosis or treatment has been established in these populations. The decision to treat is usually based on clinical symptoms, sputum PCR assay results, and radiographic findings. Considering our results, these criteria have several limitations for diagnosing PTB in smear-negative populations. However, the combined results of HRCT and QFT-G were valuable for predicting active PTB.

A number of studies have evaluated the clinical criteria for the diagnosis of smear-negative TB [20–22]. Samb et al. [21] reported that chronic cough lasting longer than 3 weeks, chest pain lasting longer than 15 days, absence of sputum, and absence of shortness of breath were independent predictors of active TB. Our study also showed that lack of sputum and young age were predictors of active PTB, although only 19 of 40 patients (48%) with TB lacked expectoration. A study in San Francisco, Calif., USA, identified cough with expectoration as a negative predictor of smear-negative TB [10]. Although these parameters suggest PTB, their diagnostic value is limited. First, the clinical parameters are likely to be influenced by a number of conditions, such as prevalence of HIV, nutritional status, and incidence of TB, and therefore cannot be universalized. Second, the diagnostic accuracy was low when a scoring system was applied. Samb et al. [21] devised a scoring system based on clinical parameters, but the PPV was only 50%. Kanaya et al. [10] also developed a TB prediction scoring system, but the specificity was low for a cut-off value ≥ 1 .

Detection of *M. tuberculosis* by sputum PCR has been useful in the diagnosis of PTB [23]. PCR has a sensitivity of 86–95% and a specificity of 98–100% in smear-positive specimens. However, PCR has a significantly lower sensitivity (48–77%) in smear-negative specimens, although the specificity is still high at 98% [12, 24–27]. Our findings, 97.7% specificity and 43.2% sensitivity for PCR in smear-negative patients, are also consistent with these reported values. It may be that the mycobacterial burden is relatively low in smear-negative cases compared with smear-positive cases, resulting in a lower level of detect-

able DNA in smear-negative cases. Therefore, despite its good PPV, we conclude that PCR alone has limited efficacy in screening for the early detection of active PTB in smear-negative cases.

Chest radiography remains the first choice for an initial evaluation of patients with PTB [8, 28, 29], and many patients can be diagnosed without HRCT. However, in smear-negative cases, CT may enable a presumptive diagnosis of active PTB and allow anti-TB chemotherapy to begin sooner because CT is superior to chest radiography in evaluating PTB – especially its extent and distribution [8, 28–30]. Furthermore, CT can help in diagnosing PTB and in distinguishing active from inactive disease [8, 9]. Nevertheless, Matsuoka et al. [31] stated that the CT findings in smear-negative patients differ from those in smear-positive patients and suggested that CT findings are not helpful in judging smear-negative TB. Recently, Nakanishi et al. [32] reported that the tree-in-bud appearance, lobular consolidation and large nodules are significantly associated with active PTB in smear-negative PTB, but the diagnostic accuracy was not satisfactory. In our study, there were significant differences between TB and non-TB cases with respect to HRCT findings of nodules, tree-in-bud pattern and cavities, but 13 of 45 (29%) patients with suspected TB based on HRCT were diagnosed as non-TB, and 8 of 39 (21%) patients thought to be non-TB cases based on CT were diagnosed with TB. Although HRCT provides additional information for diagnosing PTB, HRCT alone has limited value for diagnosing PTB in smear-negative patients.

IGRA was approved in the United States for the diagnosis of latent TB infection. However, estimates of sensitivity and specificity of this test for TB infection have been hampered by the lack of a ‘gold standard’. Several studies have evaluated its sensitivity in active TB cases, and the high rates of positive response observed in these studies suggest a possible role for this assay in diagnosing active TB. To our disappointment, two previous studies showed low sensitivity or specificity [16, 33]. Our result showed relatively good sensitivity compared with 64% sensitivity reported by Dewan et al. [33] and good specificity compared with the 47% specificity of Kang et al. [17]. Even so, the QFT-G assay has the intrinsic inability to distinguish between latent infection and active disease. Therefore, a positive result on this test alone is not sufficient for making a diagnosis of active PTB. To overcome this limitation, we evaluated the accuracy of this test result combined with HRCT findings and found that the combination of HRCT and the QFT-G assay was useful in predicting or excluding TB in smear-negative pa-

tients. HRCT findings suggesting active PTB plus a positive QFT-G result gave a PPV of 96%. Conversely, HRCT findings suggesting non-TB and a negative QFT-G result gave a NPV of 92%. Therefore, the current findings support that the combination of HRCT and QFT-G allow for a rapid diagnosis of PTB in patients who are sputum smear negative or have no sputum production at all. However, the high false-positive rates of these tests might limit their usefulness in TB-endemic areas, where the prevalence of latent TB infection is considerable. Therefore, our results could be more valuable in the diagnosis of active PTB in areas with a lower prevalence of latent TB infection.

The present study has several limitations. First, the tuberculin skin test (TST), which is also used in clinical settings, was not evaluated in this study. However, several reports have claimed that the TST shows a high false-positive rate because South Korea has received multiple Bacille Calmette-Guérin vaccines [34, 35]. Thus, it would be of limited value for evaluating TB in South Korea. Second, as our study was a retrospective study, all participants had not undergone all tests, which might have skewed the results. Third, only 2 patients were immunocompromised, and none had HIV infection. Actually, smear-negative cases are frequently seen among immu-

nocompromised patients, notably those infected with HIV, and low accuracy can be expected in this population. A re-evaluation of our findings may be required in this population. Fourth, the use of HRCT to diagnose PTB is not clinically available worldwide. In addition, to improve the diagnostic accuracy, a well-trained radiologist or pulmonologist is essential. Radiation exposure and the costs also limit the use of HRCT in all TB-suspected patients. However, to control TB, early diagnosis is essential. Considering our results, HRCT could be an adjunctive diagnostic method in smear-negative cases. Finally, the small sample size was also limiting. Nevertheless, this is the first evaluation that compares the diagnostic accuracy of clinical findings, CT findings, QFT-G assay results, and sputum PCR assay results in the diagnosis of smear-negative TB patients in a situation in which no standard methods exist.

In summary, our study suggests that though sputum TB-PCR had good PPV, its low sensitivity limits its use in the diagnosis of active PTB. HRCT or QFT-G alone had relatively good sensitivity, but the low PPV hampered the decision of starting anti-TB medication. This study also suggests that the combined results of HRCT and QFT-G assay could help decision-making for early initiation of treatment in smear-negative patients.

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