Diagnostic Performance of the QuantiFERON-TB Gold In-Tube Assay and Factors Associated With Nonpositive Results in Patients With Miliary Tuberculosis

Chang Ho Kim,1 Jae Kwang Lim,2 Seung Soo Yoo,1 Shin Yup Lee,1 Seung Ick Cha,1 Jae Yong Park,1 and Jaehee Lee1
Departments of 1Internal Medicine, and 2Radiology, School of Medicine, Kyungpook National University, Daegu, South Korea

The QuantiFERON-TB Gold In-Tube (QFT-GIT) assay provides suboptimal diagnostic performance in patients with miliary tuberculosis. QFT-GIT results should be carefully interpreted, particularly in patients suspected of having miliary tuberculosis with severe lymphocytopenia or an extent of ground glass opacity (GGO) >50% on chest computed tomography (CT). Diagnostic performance of the QFT-GIT assay was evaluated in 44 patients with miliary tuberculosis. Among these individuals, 30 (68%) had true-positive QFT-GIT results. Severe lymphocytopenia and an extent of GGO >50% on chest CT were independent risk factors for nonpositive QFT-GIT results.

Keywords: miliary tuberculosis; interferon-gamma release assay; QuantiFERON-TB Gold In-Tube assay.

A recently developed in vitro immunodiagnostic test, the interferon-gamma release assay (IGRA), has produced results superior to those of tuberculin skin tests for the detection of tuberculosis infection [1]. The usefulness of IGRA for making a rapid diagnosis of active tuberculosis has been investigated in clinical settings that involved patients with pulmonary and extrapulmonary tuberculosis. Results of these investigations showed that IGRA sensitivity varies according to method subtype, clinical status of the patient, and site of infection [1–3].

Recently, the T-SPOT.TB (Oxford Immunotec, Abingdon, UK) assay was found to produce promising results for patients with miliary tuberculosis [4]. However, data on the clinical application of the QuantiFERON-TB Gold In-Tube assay (QFT-GIT; Cellestis Ltd., Victoria, Australia) for detecting miliary tuberculosis are sparse and fragmented. Little is known about the diagnostic performance of the QFT-GIT test in patients with miliary tuberculosis. In the present study, we prospectively evaluated the diagnostic performance of the QFT-GIT test in a relatively large cohort of miliary tuberculosis patients. In addition, the risk factors associated with nonpositive QFT-GIT results for these patients were identified.

METHODS

Adult patients aged ≥20 years with suspected cases of miliary tuberculosis were prospectively recruited between September 2009 and July 2013 at the Kyungpook National University Hospital, a 1400-bed tertiary hospital in Daegu, South Korea, a country with an intermediate tuberculosis burden, where the incidence rate of tuberculosis was 109/100 000 population in 2011 [5]. The QFT-GIT test was performed in accordance with the manufacturer’s instructions [6]. Results were calculated using QFT software, which is provided by the manufacturer. All QFT-GIT tests were performed within 5 days after hospital presentation.

The diagnosis of miliary tuberculosis was made when 1 of the following criteria was met in addition to the appearance of miliary nodules on chest radiograph or computed tomography (CT): positive Mycobacterium tuberculosis culture from clinical specimens or pathological findings of chronic granulomatous inflammation with positive tuberculosis polymerase chain reaction results for biopsied lung tissue. Details regarding patient demographics, immunosuppressive conditions, body mass index, laboratory and microbiological data, and radiographic findings were also collected. Patients with immunosuppressive conditions were defined as individuals with underlying diseases such as malignancies, advanced liver cirrhosis, end-stage renal disease, or human immunodeficiency virus (HIV) infection; patients receiving immunosuppressive treatment; or patients who had undergone solid organ transplantation.

One board-certified radiologist and 2 board-certified pulmonologists who were blinded to the clinical data independently interpreted the chest radiographs and conventional chest CT scans with 2.5 mm collimation. CT findings were evaluated as
follows: preexisting active tuberculosis lesions, cavities, pleural effusion, mediastinal lymphadenopathy, and the extent of ground glass opacity (GGO). The extent of GGO on chest CT scans was rated as follows: none, within half of the entire lung field (≤50%), and beyond half of the entire lung field (>50%) [7]. Final decisions on the findings were reached by consensus.

RESULTS

Diagnostic Performance of the QFT-GIT Test

A total of 44 patients with miliary tuberculosis were included in the final analysis. Among these individuals, 42 (95%) were diagnosed according to positive M. tuberculosis culture and 2 (5%) were diagnosed based on histological results. The mean age was 64 years and 17 (39%) males were included. Thirty (68%) of the 44 patients had true-positive QFT-GIT results (Table 1). Of the remaining 14 patients, 7 (16%) had false-negative and 7 (16%) had indeterminate results; all were classified as nonpositive QFT-GIT results in order to calculate the actual sensitivity for routine clinical practice. Thus, the sensitivity of the QFT-GIT test for miliary tuberculosis was 68%.

Risk Factors for Non-positive QFT-GIT Results

Results of the univariate analysis of risk factors for nonpositive QFT-GIT results in patients with miliary tuberculosis are shown in Table 1. The baseline characteristics, including mean age, gender ratio, body mass index, and frequency of patients with immunosuppressive conditions, were not significantly different between patients with true-positive and nonpositive QFT-GIT results. Five patients had immunosuppressive conditions including HIV infection (n = 2), end-stage renal disease (n = 2), and a history of immunosuppressive therapy (n = 1). One patient with end-stage renal disease produced false-negative QFT-GIT results, while the remaining individuals with immunosuppressive conditions had true-positive results. The frequencies of patients with severe lymphocytopenia (<500/µL) were significantly greater and the mean level of C-reactive protein in the serum was significantly higher in patients with nonpositive QFT-GIT results than those with positive QFT-GIT results. The frequencies of patients with thrombocytopenia, the number of acid-fast bacilli smear-positive sputum samples, and the mean white blood cell counts, lymphocyte counts, erythrocyte sedimentation rate, and serum albumin levels were not significantly different between individuals with positive and nonpositive results.

When chest CT findings were compared, distribution of the extent of GGO was significantly different between the 2 groups (P = .005). Only 2 (7%) of 30 patients with positive QFT-GIT results had an extent of GGO >50%, while 7 (50%) of 14 patients with nonpositive QFT-GIT results showed an extent of GGO >50%. Other findings, including the frequency of preexisting active lung lesions and cavities on chest CT scans, were not significantly different between the groups. Key features identified by univariate analysis were further analyzed with a multivariate logistic regression test. The frequencies of severe lymphocytopenia (odds ratio [OR], 6.017; 95% confidence interval [CI], 1.128–32.110; P = .036) and an extent of GGO >50% (OR, 21.628; 95% CI, 2.109–221.801; P = .010) were independently associated with an increased risk of nonpositive QFT-GIT results.

DISCUSSION

The site of infection affects the sensitivity of IGRA assays. Lymph node tuberculosis is associated with relatively high sensitivity compared with other types of extrapulmonary tuberculosis [2,3]. When the diagnostic yields of the QuantiFERON-TB Gold test (a previous version of the QFT-GIT assay) for patients with extrapulmonary tuberculosis were evaluated, only a few individuals (<8 cases) with miliary tuberculosis were included [8,9]. In our investigation, which was performed in patients with miliary tuberculosis, the QFT-GIT assay was found to have modest sensitivity (68%). This was at the lower end of the range of sensitivities for diagnosing active cases of tuberculosis reported by previous studies [1]. In addition, our results are apparently different from those in a recent publication that showed a sensitivity of T-SPOT.TB of 93% in 43 patients with miliary tuberculosis [4]. Such discrepancies may be related to differences in the assay methods as well as the clinical status of the study populations including disease severity, which was not mentioned in the previous T-SPOT.TB study [4]. To further explore this issue, a prospective study with head-to-head comparisons between the QFT-GIT and T-SPOT.TB assays in a miliary tuberculosis patient population should be conducted.

Lymphocytopenia can be acquired in several ways including advanced tuberculosis, use of immunosuppressive agents, HIV infection, malignancy, and malnutrition [10]. These respective predisposing conditions are also well-known risk factors for false-negative or indeterminate QFT-GIT results. More advanced conditions may result in more severe cases of lymphocytopenia. The effects of underlying conditions, which can vary between individuals and during the clinical course, would be ultimately reflected in lymphocyte counts. Therefore, this parameter may be a good objective marker that indicates the strength of cellular immunity and the risk of poor QFT-GIT assay performance. In particular, lymphocytopenia can cause a decrease in the production of interferon-gamma and produce indeterminate QFT-GIT results due to lower mitogen levels [11]. In the present study, 4 of 8 patients with severe lymphocytopenia and nonpositive QFT-GIT results had indeterminate QFT-GIT findings due to positive control failure.

The present study revealed that an extent of GGO >50% was an independent risk factor for nonpositive results. In patients...
with miliary tuberculosis, GGO was reported as a major radiological finding on high-resolution CT scans in addition to miliary nodules [7]. GGO varies in extent and distribution compared with miliary nodules that are uniformly distributed throughout both lungs. In patients with pulmonary tuberculosis, disease severity is radiologically classified by the extent of lung involvement (mild, moderate, or advanced). However, since miliary tuberculosis involves both lungs, the radiological classification of disease severity using this method is not practical. Extensive GGO may be indicative of more severe inflammation [12]. Additionally, individuals with acute respiratory failure or acute respiratory distress syndrome (ARDS) due to miliary tuberculosis were found to have diffuse areas of GGO [13, 14]. It has been shown that interferon-gamma secretion is inversely correlated to disease severity in patients with tuberculosis [15, 16]. Thus, miliary tuberculosis patients with an extent of GGO >50% are likely to have low levels of interferon-gamma secretion, resulting in nonpositive QFT-GIT results. In our study, 7 (78%) of 9 patients with an extent of GGO >50% had nonpositive QFT-GIT results compared with 2 (13%) of 15 patients without GGO.

Extensive GGO as an independent risk factor for nonpositive QFT-GIT results may have a more negative impact in clinical practice. Miliary tuberculosis should be considered for the differential diagnosis of ARDS with unclear etiology. Radiological findings of miliary nodules are often the first clue suggestive of miliary tuberculosis. However, extensive GGO makes the radiographic diagnosis of miliary tuberculosis difficult since the miliary nodules are superimposed on more diffuse, less structured areas of GGO. Furthermore, our results suggest that extensive...
GGO is likely to negatively affect an adjunctive role of immunological diagnosis of tuberculosis infection in these patients. Thus, the diagnosis and treatment of miliary tuberculosis in patients with extreme GGO may be potentially delayed.

To the best of our knowledge, this is the first study to investigate the diagnostic performance of the QFT-GIT test in a selected population of patients with miliary tuberculosis. Taken together, our results indicate that the QFT-GIT assay provides a suboptimal diagnostic performance for patients with miliary tuberculosis. QFT-GIT results should be carefully interpreted for patients suspected of having miliary tuberculosis accompanied by severe lymphocytopenia or an extent of GGO >50% on chest CT.

**Note**

**Financial support.** This work was supported by a Biomedical Research Institute grant from Kyungpook National University Hospital.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**