Adapted T Cell Interferon-Gamma Release Assay for the Diagnosis of Pleural Tuberculosis

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Key Words

Pleurisy · QuantiFERON · T cell IFN- γ release assay · Tuberculosis

Abstract

Background: Better and more rapid tests are needed for the diagnosis of tuberculous pleural effusion (TPE), given the known limitations of conventional diagnostic tests. **Objectives:** To estimate diagnostic accuracy of the QuantiFERON-TB Gold In-Tube (QFT-GIT) test (and its components) using data-derived cutoffs in pleural fluid. **Methods:** The QFT-GIT test was performed on whole blood and pleural fluid from 43 patients with TPE and 29 control subjects (non-TPE). To achieve the objective, QFT-GIT test, estimating likelihood ratios and receiver operating curve analysis were performed. **Results:** The sensitivity and specificity using the QFT-GIT for the diagnosis of TPE were 48.8% and 79.3%, respectively, in pleural fluid. The best cutoff points for tuberculosis (TB) antigen, nil and TB antigen minus nil results were estimated at 0.70, 0.90 and 0.30 IU/ml, respectively. Area under the curve of TB antigen IFN- γ response was 0.86 (CI: 0.76–0.93), nil tube was 0.80 (CI: 0.69–0.89) and TB antigen minus nil tube was 0.82 (CI: 0.72–0.90). When the best cutoff scores of the nil

tubes were set at this value, the results of a likelihood ratio of a positive and a negative test were 9.44 (7.4–12.0) and 0.37 (0.09–1.5), respectively. The percentages of indeterminate results in pleural fluid among the TPE cases were 42% (most of them caused by high nil IFN- γ values) using the QFT-GIT test. **Conclusion:** QFT-GIT test or its components have poor accuracy in the diagnosis of TPE, largely because of a high number of indeterminate results due to high background IFN- γ production in the TPE.

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Introduction

Tuberculous pleural effusion (TPE) is one of the most common forms of extrapulmonary tuberculosis (TB); TB is also the major cause of exudative pleural effusion in areas of high TB prevalence [1, 2].

A diagnosis of TPE depends on the demonstration of tubercle bacilli in clinical samples. Unfortunately, TPE fluid usually contains a low number of mycobacteria, and the diagnostic sensitivity of both direct microscopy and pleural fluid cultures is relatively low [3–5]. Alternatively, the diagnosis can be established by the identification of

caseous tubercle granulomas in pleural tissue. However, collecting pleural tissue, which has imperfect diagnostic value, requires a more invasive procedure [4–6].

A variety of biological markers such as adenosine deaminase activity (ADA) and IFN- γ have been investigated in pleural fluid as indicators for the early diagnosis of TPE [8–10]. The predominance of T helper 1 (Th1) immunity in TPE is demonstrated by significantly higher levels of Th1 cells and IFN- γ in pleural fluid than in peripheral blood [11, 12]. A compartmentalization of TB-specific IFN- γ -producing cells is a key component of the host response to *Mycobacterium tuberculosis* in the pleural cavity [13–15]. However, both ADA and IFN- γ are nonspecific inflammatory markers. Furthermore, despite strong evidence of their accuracy, cost and technical considerations such as the lack of simple, standardized kits have limited their clinical use in the majority of patients with TPE, especially in developing countries [3, 8].

In the last decade, a new type of blood test, the IFN- γ release assay (IGRA), has been developed [16–18]. Two different IGRAs are commercially available: the T-SPOT TB assay (Oxford Immunotec, Oxford, UK), which is an ELISPOT assay, and the QuantiFERON-TB Gold (QFT-G)/QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis, Chadstone, Vic., Australia) test, which is an ELISA utilizing whole blood [18–20]. Although IGRAs are becoming the standard for diagnosing latent TB infection (LTBI), there are limited data regarding the diagnosis of active TB, especially TPE [20]. In TPE there is compartmentalization of high levels of TB-specific IFN- γ -producing cells in the pleural cavity, these higher levels of Th1 cells lead to high levels of IFN- γ in pleural fluid [12, 15]. Henceforth, the low cutoffs of QFT-GIT recommended for blood does not work for the pleural fluid and further studies with validation of QFT-GIT in TPE are required.

We assessed the utility of the QFT-GIT test as a diagnostic tool for distinguishing between TPE and non-TPE. We calculated the cutoff values for evaluating the results of the QFT-GIT test in pleural fluid. We also evaluated a modified algorithm for the interpretation of the QFT-GIT test in pleural fluid.

Subjects and Methods

Subjects

Patients who were supposed to have exudative pleural effusions and a medical history compatible with TPE presenting to the Department of Chest Diseases at the University Hospital of Dicle and the Diyarbakir Chest Hospital (Diyarbakir, Turkey), and the Department of Chest Diseases at Yedikule Teaching Hospital for

Chest Diseases and Thoracic Surgery (Istanbul, Turkey) between March 2008 and May 2009 were prospectively enrolled. This study was approved by the ethics committee, and all patients provided informed consent.

Patients receiving TB treatment, those who were taking immunosuppressive therapy and those younger than 15 years of age were excluded from the study.

Demographic information and patient medical history were recorded on a standard questionnaire. The patients were also investigated for any lifetime self-reported history of TB or history of TB contact, BCG vaccination, history of intravenous drug use and other immunodeficiency conditions. A physical examination, chest X-ray as well as sputum, pleura and blood examinations were performed. The tuberculin skin test was not performed routinely. Blood and pleural fluid for diagnostic analysis were obtained in parallel from all patients before initiation of anti-TB therapy.

Thoracentesis and pleural biopsies were obtained according to the clinical practices of the hospital. The QFT-GIT results were not available to the clinicians and did not influence the classification of patients or decisions concerning treatment, and the laboratory personnel were unaware of the clinical diagnoses.

Patients with TB were classified according to Losi et al. [15]. At least one of the following criteria had to be met to diagnose TPE: (1) smear and/or culture, or polymerase chain reaction positivity for *M. tuberculosis* in a pleural specimen, and/or granuloma on pleural biopsy with or without caseous necrosis; this group was named the confirmed TPE group; (2) increased ADA value (>40 IU/l) with lymphocyte predominance in young patients who had to have a positive treatment response to a full course of antituberculous therapy, and had to have no alternative diagnosis of pleurisy other than TB; this group was named probable TPE.

Patients were defined as not having TPE (non-TPE, controls) if an alternative diagnosis of pleurisy was established. Malignant pleural effusion (MPE) was diagnosed when there was positive pleural fluid cytology and/or positive pleural biopsy histology. Parapneumonic effusion or pleural empyema was diagnosed in patients who had (1) grossly purulent pleural effusion, (2) the presence of microorganisms in pleural fluid or (3) signs and symptoms of pneumonia accompanied by pleural effusion, which resolved following antibiotic treatment and/or local pleural drainage. Pleural fluids were diagnosed as transudate according to the criteria of Light [1]. The specific etiology of transudative effusions was based on clinical and laboratory data. Other causes for effusions were ascertained clinically.

QFT-GIT Testing

The QFT-GIT test was performed in two steps. Whole blood and pleural fluid were collected into each of the three QFT-GIT collection tubes, consisting of a negative control (nil) tube, a TB antigen (ESAT-6, CFP-10 and TB 7.7) tube and a mitogen control (phytohemagglutinin) tube. The tubes were incubated as soon as possible, within 12 h of collection. After a 16- to 20-hour incubation at 37°C, the tubes were centrifuged, and the supernatants were stored at -70°C until the ELISA was performed. The QFT-GIT test results for both blood and pleural fluid were interpreted according to the instructions for blood, as validated by the manufacturer [21]. The results of the test were recorded as positive, negative or indeterminate. The test was positive if the TB antigen minus nil value was ≥ 0.35 IU/ml. The nil control had to be

Table 1. IFN- γ responses (TB antigen, mitogen and nil) with the QFT-GIT test of blood and pleural fluid samples

	Non-TPE group (n = 29)	TPE group (n = 43)	p value
Blood, IU/ml			
Negative control, nil	0.11 (0.07–0.16)	0.22 (0.12–0.43)	0.001
TB antigen	0.22 (0.12–0.89)	1.68 (0.76–8.16)	<0.001
Positive control, mitogen	7.6 (1.9–21.3)	7.07 (2.24–11.59)	0.42
Pleural fluid, IU/ml			
Negative control, nil	0.23 (0.10–0.37)	3.24 (0.48–8.39)	<0.001
TB antigen	0.22 (0.12–0.48)	9.09 (2.17–12.51)	<0.001
TB antigen minus nil	0.01 (0–0.13)	1.82 (0.28–5.83)	<0.001
Positive control, mitogen	4.89 (0.96–21.36)	11.41 (4.82–12.95)	0.57

Data are medians (with interquartile ranges in parentheses). p values are for comparisons between TPE and non-TPE groups.

≤ 8 IU/ml and positive control minus nil had to be ≥ 0.5 IU/ml or TB antigen minus nil value had to be ≥ 0.35 IU/ml and $\geq 25\%$ of the nil for the subject to have a valid QFT-GIT test.

Statistical Analysis

Statistical values are expressed as the means $\pm$ standard deviation for normally distributed data. The medians and interquartile ranges are given for skewed data. Categorical data were compared using Pearson's χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared using nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test) when the data were not normally distributed. Discriminative properties of the nil IFN- γ , TB antigen IFN- γ and TB antigen minus nil IFN- γ results were evaluated using receiver operating characteristic (ROC) curves analysis and calculated area under the curve (AUC). For each ROC curve, a cutoff point was determined as the value of IFN- γ that maximized the sum of the sensitivity and specificity for diagnosing TPE. A p value ≤ 0.05 was considered statistically significant. Analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Ill., USA).

Results

Non-TPE conditions were diagnosed in 29 patients, and TPE was diagnosed in 43 patients. The non-TPE group included 11 patients with MPE, 8 patients with parapneumonic and 10 patients with miscellaneous underlying conditions [2 patients with post-bypass surgery syndrome, 3 with transudative effusion caused by heart failure, 2 with pulmonary thromboembolism, 1 with rheumatoid pleural effusion and 2 with chronic fibrinose pleuritis (they did not develop signs or symptoms of TB after 12 months)]. The MPE group consisted of 6 patients with lung cancer, 4 with malignant pleural mesothelio-

ma and 1 with breast carcinoma. The TPE diagnosis was based on clinical data in 26 patients (probable TPE group) and microbiological or pathological results in 17 patients (confirmed TPE group).

The mean age of the patients was 41.1 ± 22.4 years. The non-TPE cases (53.3 ± 19.4 years) were older than the patients with TPE (32.9 ± 20.7 years, $p < 0.0001$). There were 47 (65%) males, and 54% of the patients had received a BCG vaccination. Diabetes mellitus was reported in 6 (8.3%) patients. None of the patients reported a history of TB and had never taken TB treatment. Twelve (16.7%) had a history of contact with contagious TB patients. There were no statistically significant differences in gender, diabetes, BCG status or contact history between the TPE and non-TPE groups.

The IFN- γ concentrations in the negative control samples were significantly higher in the TPE group than in the non-TPE group, in both pleural fluid and blood (table 1). There were no statistically significant differences in the IFN- γ results (both nil IFN- γ and induced IFN- γ results) between the probable and confirmed TPE groups, for pleural fluid or blood. There were no statistically significant differences among subgroups (PPE, MPE and others) of the non-TPE group (data not shown).

The commercially available QFT-GIT test has been validated for use with blood. We performed ROC analysis to find the best cutoff value for discriminating the TPE group from the non-TPE group and calculated the AUC (fig. 1). The best cutoff point for TB antigen, nil and TB antigen minus nil results were estimated at 0.70, 0.90 and 0.30 IU/ml, respectively. AUC of TB antigen IFN- γ response was 0.86 (95% CI 0.76–0.93, $p = 0.0001$), nil

Table 2. The sensitivity, specificity, diagnostic accuracy and likelihood ratios when the best cutoff scores are used for nil, TB antigen and TB antigen minus nil results

	Sensitivity	Specificity	Accuracy	PPV	NPV	Lr+	Lr-
Nil	65.12 (49.1–79.0)	93.10 (77.2–99.2)	76.4 (66.6–86.2)	93.3 (84.4–99)	64.3 (49.8–78.8)	9.44 (7.4–12.0)	0.37 (0.09–1.5)
TB antigen	86.05 (72.1–94.7)	86.21 (68.3–96.1)	86.1 (78.1–94.1)	90.2 (81.2–99.3)	80.6 (66.7–94.6)	6.24 (5.2–7.5)	0.16 (0.05–0.5)
TB antigen minus nil	74.42 (58.8–86.5)	89.66 (72.6–97.8)	80.6 (71.4–89.7)	91.4 (82.2–99)	70.3 (55.5–85.0)	7.19 (5.8–8.9)	0.29 (0.09–0.9)

Lr+ = Likelihood ratio of a positive test; Lr- = likelihood ratio of a negative test.

Fig. 1. ROC analysis showing IFN- γ responses (nil tubes, TB antigen-coated tubes and TB antigen minus nil tube results) with the QuantiFERON-TB Gold In-Tube test of pleural fluid samples. AUC of nil tube IFN- γ response was 0.80 (95% CI 0.69–0.89, $p = 0.0001$). The ROC curve of TB antigen passed closer to the upper left corner than nil tubes and TB antigen minus nil tube results. There were no statistically significant differences of the AUC among different tubes.

tube was 0.80 (95% CI 0.69–0.89, $p = 0.0001$) and TB antigen minus nil tube was 0.82 (95% CI 0.72–0.90, $p = 0.0001$). There was no statistically significant difference in the AUC among different tubes. With the best cutoff scores set at this values, the results of sensitivity, specificity, diagnostic accuracy, likelihood ratio of a positive test and likelihood ratio of a negative test are shown in table 2.

Indeterminate test results using QFT-GIT were found in 18 patients (42%) with TPE in pleural fluid. In 83% of them (15/18 patients), these results were due to high nil value (>8 IU/ml). Since high background positivity of IFN- γ (high nil values) was found in pleural fluid samples of patients with TPE, we proposed an adapted formula for evaluating the QFT-GIT test results in pleural fluid (table 3). When using the adapted QFT-GIT formula in pleural fluid, there were only 5 indeterminate results and all of them were due to low mitogen response. Indeterminate results were seen in 3 patients (10%) with non-TPE in pleural fluid using both QFT-GIT test and adapted QFT-GIT. All 3 indeterminate results were due to low mitogen response. Indeterminate results in blood were caused by inadequate blood mitogen IFN- γ responses in both TPE and non-TPE cases.

The QFT-GIT assay was positive in 21 of 43 patients with TPE (sensitivity: 48.8%) and negative in 23 of 29 non-TPE patients (specificity: 79.3%). When we used the adapted QFT-GIT algorithm, sensitivity and specificity were 88.4 and 75.9%, respectively. However, if the indeterminate results were excluded from the analyses, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio of a positive test and likelihood ratio of a negative test of the adapted QFT-GIT assay for the diagnosis of TPE were 100, 84.6, 90.5, 100, 6.5 and 0.0, respectively. Both QFT-GIT and adapted QFT-GIT results in pleural fluid are shown in table 4, and diagnostic values in table 5. In addition, QFT-GIT results in blood are shown in table 5.

With an IGRA, false-positive results occurred in 10 of 29 (34%) blood samples, 3 of 29 (10%) with pleural fluid samples. False-positive results occurred in 4 (14%) with pleural fluid using the adapted formula. Two of these were due to high nil IFN- γ levels, and 2 were due to high antigen-induced IFN- γ . All false-positive results occurred in patients with MPE (only 1 of them had a history of exposure to *M. tuberculosis*). No false-negative result occurred with the adapted formula.

Table 3. Interpretation of QFT-GIT test results for pleural fluids

Nil value IU/ml	TB antigen minus nil IU/ml	Mitogen minus nil, IU/ml	Test results
≥0.9	any ≥0.35 and ≥25% of nil value	any any	positive
<0.9	≥0.35 and <25% of nil value	≥0.5	negative
	<0.35	≥0.5	
	<0.35 ≥0.35 and <25% of nil value	<0.5 <0.5	indeterminate

Discussion

The IGRA has been studied for use in diagnosing LTBI and TB; however, few studies have been conducted in patients with TPE [17–19]. Furthermore, commercially available IGRA were designed for use with peripheral blood and have not been validated with pleural fluid. Recent studies applying the diagnostic values of IGRA to blood and pleural fluid in the setting of TPE have shown dichotomous results [14, 15, 22–25]. IGRA sensitivities in pleural fluid of patients from low TB incidence countries (85–100%; two ELISPOT and one QFT-G study [14, 15, 22]) are reportedly higher than in those from intermediate and high TB incidence countries (40–57%; one QFT-G and two QFT-GIT studies [23–25]). Studies conducted in intermediate and high TB incidence countries have reported higher sensitivity of IGRAs in blood compared with pleural fluid [23–25].

In our study, the percentages of indeterminate results among the TPE cases were 2% using blood and 42% using pleural fluid. For most previous studies, the mitogen tube was not supplied with the IGRA, and thus negative blood results could not be distinguished from indeterminate results [22–24]. In one QFT-GIT study in which the majority of TB patients were HIV infected, indeterminate test results were common (25% of blood tests and 52% of pleural fluid tests) in TPE patients, using the method previously validated for blood [25]. They found that in pleural fluid indeterminate results were caused by high nil (negative control) IFN- γ responses in the TPE group [25]. Consistent with this, in our study, most of the indeterminate results in pleural fluid (83%) were caused by high nil (negative control) IFN- γ values, when using the method previously validated for blood. When we used the new algorithm, indeterminate results in pleural fluid were decreased from 42 to 14%. Therefore, high background nil IFN- γ levels should not be interpreted as an indeterminate result; they may be positive results.

Table 4. Results of the QFT-GIT test in patients with TPE (n = 43) and non-TPE patients (n = 29)

Test/sample	Cause of pleurisy	Negative	Positive	Indeterminate
QFT-GIT/PF	non-TPE	23 (80)	3 (10)	3 (10)
	TPE	4 (9)	21 (49)	18 (42)
Adapted QFT-GIT/PF	non-TPE	22 (76)	4 (14)	3 (10)
	TPE	0	38 (88)	5 (12)
QFT-GIT/blood	non-TPE	15 (52)	10 (34)	4 (14)
	TPE	12 (28)	30 (70)	1 (2)

Data are numbers of patients (with percentages in parentheses). PF = Pleural fluid.

High background positivity of IFN- γ (high nil values) in pleural fluid samples of patients with TPE is well known [12, 15, 25]. In fact, IFN- γ alone is highly accurate in diagnosis of TPE [26]. In light of these facts, a more interesting question could have been whether stimulated IFN- γ (results from TB antigen-coated tubes) or a difference of specific TB antigen-coated and uncoated tubes (TB antigen minus nil) is any better than nil tube results alone. When we used the optimal cutoff values obtained by ROC curve analysis, there was no statistically significant difference in the AUC among different tubes.

In our study, the overall sensitivity of the QFT-GIT test in blood was 69.8%, compared with 48.8% in pleural fluid. This sensitivity in blood was comparable to the sensitivities of 60–90% reported in previous pulmonary and TPE studies in HIV-negative patients [15, 22–24]. These results suggest that sensitivity of QFT-GIT using blood is only moderate in TPE patients. Sensitivity of QFT-GIT using the cutoff values recommended for blood is low if pleural fluid is used instead of blood; this is due to high background levels of IFN in pleural fluid from TPE pa-

Table 5. Diagnostic values for the QFT-GIT test of pleural fluid and blood from patients with TPE (n = 43) and non-TPE patients (n = 29)

Test/sample	Sensitivity	Specificity	Accuracy	PPV	NPV
QFT-GIT/PF	48.8 (33.9–63.8)	79.3 (64.2–93.8)	61.1 (49.9–72.4)	87.5 (74.3–100)	85.2 (71.8–98.6)
Adapted QFT-GIT/PF	88.4 (78.8–98.0)	75.9 (60.3–91.4)	83.3 (74.7–91.9)	90.5 (81.6–99.4)	100.0 (100.0–100.0)
QFT-GIT/blood	69.8 (56–83.5)	51.7 (33.5–69.9)	62.5 (51.3–73.7)	75.0 (61.6–88.4)	55.6 (36.8–74.3)

Data are medians (with interquartile ranges in parentheses). PF = pleural fluid.

tients, which is not seen for non-TPE. A new interpretation for pleural fluid QFT-GIT results was therefore needed. When we used our adapted formula (modified algorithm) to interpret the QFT-GIT test results, the sensitivity and specificity were 88 and 76%, respectively, in pleural fluid. Moreover, with our adapted QFT-GIT formula, the sensitivity in pleural fluid was superior to that of previous studies (40–57%) in high TB incidence areas [22–24]. In addition, if the indeterminate results were excluded from the analyses, the sensitivity, specificity, PPV and NPV of the adapted QFT-GIT assay for the diagnosis of TPE were 100, 81, 91 and 100, respectively.

The false-positive results in the blood are more frequent than in the pleural fluid (34 vs. 14%). All false-positive results in the pleural fluid occurred in patients with MPE. Although only one patient had a history of exposure to *M. tuberculosis*, they may have had LTBI. 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. The false-positive and indeterminate QFT-GIT assay results in pleural fluid could have been managed by diluting the samples before performing ELISA following optimization experiments. Larger prospective studies are required to determine the optimal technical aspects and optimal cutoff values for the application of IGRA to the diagnosis of TPE using pleural effusion. The benefits of using the QFT system over simply measuring the IFN level in pleural fluid is currently unclear.

The diagnostic sensitivity and specificity of both ADA and ex vivo IFN- γ has been reported to be quite high in TPE [3, 7–9]. It has been reported that it had better performance than IGRA for diagnosis of TPE and is cheaper as well as simple to perform [27]. In the present study, ADA was not carried out in all patients and ex vivo IFN- γ was not measured. ADA, ex vivo (in-house) IFN- γ release assay and commercially available QFT-GIT assay could have been performed simultaneously from blood

and pleural fluid in order to compare the diagnostic performance of the three. While assessing IGRA as a tool for diagnosis of TPE, its prohibitive cost and technical difficulties like trained manpower and requirement of laboratory infrastructure should have been considered.

The main limitations of this study were the absence of a definitive diagnosis in some patients with TPE, although we did not find any difference in IFN- γ response between the TPE groups, due to the fact that consecutive patients were not enrolled. The immunity might be affected by the nutritional status and HIV status of patients, unfortunately we did not evaluate these. The present study was also limited to small numbers of patients.

Conclusion

Elevated nil IFN- γ values in pleural fluid should not be interpreted as indeterminate. The QFT-GIT test or its components have poor accuracy in diagnosis of TPE. Use of a modified algorithm may improve diagnostic accuracy, something which needs to be evaluated in future studies.

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