The diagnostic efficiency of QuantiFERON®-Gold test in the diagnosis of tuberculous pleurisy

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ABSTRACT
Background: Diagnosis of tuberculous pleurisy is difficult and better diagnostic tools are needed. Interferon gamma release assays (IGRAs) are in vitro immunologic diagnostic tests used to identify Mycobacterium TB infections. They cannot differentiate between latent and active infections. As IGRA tests have recently been approved for the differential diagnosis of active TB, the diagnostic accuracy of the latest generation of IGRA were assessed to detect tuberculous pleurisy in this study.

Methods: The QuantiFERON®-Gold (QFT-G) test was used in pleural fluid from 100 immunocompetent patients (23 patients for the tuberculous group and 77 patients for the non-tuberculous group). Clinical data were recorded. Adenosine deaminase activity (ADA) analysis and TB culture were performed on pleural fluid.

Results: The QFT-G in pleural fluid was positive in 10 (43.5%) patients and indeterminate in 13 (56.5%) patients in the tuberculous pleurisy group. There was not a single patient with a negative test result in the tuberculous pleurisy group. The ADA levels were detected as 46.2 ± 12.6 in patients with tuberculous pleurisy and 18.6 ± 39.8 in patients with non-tuberculous pleurisy. The sensitivity, specificity, positive predictive value and negative predictive value of QFT-G in pleural fluid for tuberculous pleurisy were 43.5%, 54.5%, 30.3% and 100%; and of ADA in pleural fluid (>40 IU/ml) for tuberculous pleurisy the results were 82.6%, 96.1%, 90.5% and 92.5% respectively.

Conclusion: While the value of the QFT-G test in exclusion of tuberculous pleurisy was found to be higher in this study, its other diagnostic efficiency values were detected to be low. It is recommended that a new cut-off value be established while diagnosing active TB in prospective clinical studies and that it is also essential to do the same for the studies in various regions with high and low prevalence of TB.

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Introduction
Tuberculous pleurisy is the most common cause of exudative pleural effusion and it is also one of the most common forms of extrapulmonary tuberculosis (TB) in Turkey [1]. Definitive TB diagnosis is based on the detection of Mycobacterium tuberculosis (MTB) in the culture of pleural fluid or pleural biopsy specimens, which usually takes 4–6 weeks. Supportive evidence includes the demonstration of classical tuberculous granulomas in the pleura, elevated adenosine deaminase
activity (ADA) and interferon gamma (IFN-γ) levels in pleural fluids [2]. As a result of the increase in T-lymphocytes in pleural fluid, the diagnosis of tuberculous pleurisy in the studies that focus on the usages of lymphokines released from lymphocytes have shown an increase in recent years. The most emphasized of these lymphokines is the IFN-γ released from activated CD4+ T-lymphocytes [3,4]. In 2010, the Centers for Disease Control and Prevention (CDC) updated its guidelines for testing TB infection concluding that "IGRAs [Interferon gamma release assays] may be used instead of a tuberculin skin test as an aid in diagnosing MTB infection[5]." This prospective study was aimed at determining the role of the QFT-G-IT test in the diagnosis of tuberculous pleurisy.

Materials and methods

Patients presenting with pleural effusion admitted to the Dr. SuatSeren Education and Research Hospital for Chest Diseases and Thoracic Surgery, Izmir, Turkey, in the period January 2009–October 2009 were recruited into the study. The patients were categorized as ‘Tuberculous’ pleurisy (study group, Acid-Fast Bacillus [AFB] microscopy or culture-positive pleural fluid or tissue and/or specific histology for TB), and ‘non-tuberculous’ pleurisy when diagnosed as malignancy or another non-tuberculous condition (control group). Thoracocentesis and pleural biopsies were obtained according to clinical practice at the hospital. Ethical approval and signed informed consent for the study was obtained.

Quantiferon®-Gold assay: 1 ml pleural fluid, provided by the manufacturer (Cellestis Ltd., Victoria, Australia) was added to each of the tuberculous antigens, positive mitogen control (phytohemagglutinin [PHA]) and Nil control and mixed well and incubated at 37 0C for 20 h. The tubes were centrifuged and 500 μl of the supernatants was harvested and stored at −70 0C until the IFN-γ was measured in an ELISA reader. The IFN-γ concentrations (IU/ml) were calculated by the ‘QFT-G-IT analysis Software.’ The test was positive if the tuberculous antigen minus Nil value was ≥0.35 IU/ml. The Nil control was ≤8 IU/ml and PHA (positive control) minus Nil was ≥0.5 IU/ml or tuberculous antigen minus Nil value was ≥0.35 IU/ml and ≥25% of the Nil for the subject to have a valid QFT-G test.

The pleural fluid was also sent for analysis of ADA using a commercial colorimetric assay kit (Diazyme General Atomics, California) with a cutoff value for a positive test of 40 IU/L.

Statistics

The statistical analysis was performed by SPSS 14 (SPSS Inc., Chicago, Illinois, USA). IFN-γ and ADA responses were shown as median and ranges. The results were analysed through exact Mann–Whitney U and Kruskal–Wallis tests (including post hoc Mann–Whitney tests with Bonferroni correction for a group of three post hoc comparisons) for a comparison between the tuberculous and non-tuberculous groups. Also, IFN-γ responses within the tuberculous group were analysed through exact paired sample Wilcoxon tests. The Spearman correlation coefficient was computed regarding the associations between continuous variables. A p-value of <0.05 was considered to signify a statistically significant difference. The sensitivity, specificity, positive predictive value and negative predictive value of QFT-G and ADA tests for tuberculous pleurisy were calculated.

Results

A total of 100 patients (23 patients for the tuberculous group and 77 patients for the non-tuberculous group) were enrolled in the study. The patients’ characteristics were presented in Table 1. The median age was 57.3 ± 17.1 (18–99) and male to female ratio was 80/20. The end diagnosis of patients in the non-tuberculous group were malignant pleurisy, non-specific pleurisy, paraneumonic pleurisy, paraneoplastic pleurisy, conjunctive heart and kidney failure related to transudate and empyema in 30 (30%), 17 (17%), 14 (14%), 10 (10%), 7 (7%) and 3 (3%) of patients, respectively.

The QFT-G test results and mean ADA levels for the ‘tuberculous’ and ‘non-tuberculous’ cases are as given in Table 2. The QFT-G test was positive in 10 (43.5%) patients and indeterminate in 13 (56.5%) patients in the tuberculous pleurisy group. There was not a single patient with a negative test result in the tuberculous pleurisy group. The ADA levels were detected as 45.0 (28–88) in patients with tuberculous pleurisy and 13.5 (1–223) in patients with non-tuberculous pleurisy.

The diagnostic efficacy of pleural fluid QFT-G and ADA tests in patients with tuberculous pleurisy groups were presented in Table 3. The sensitivity, specificity, positive predictive value and negative predictive value of QFT-G test for tuberculous pleurisy were 43.5%, 54.5%, 30.3% and 100%, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of ADA (>40 IU/ml) for tuberculous pleurisy were 82.6%, 96.1%, 90.5% and 92.5%.

The mean ADA level was less than 40 IU/ml in all patients with non-tuberculous pleural effusion. However, the mean ADA levels were higher than 41 IU/ml in 19 (82.6%) patients and lower than 40 IU/ml in 4 (17.4%) patients with tuberculous pleural effusion.

Discussion

The diagnosis of tuberculous pleurisy is generally difficult and the acid fast bacilli (AFB) microscopy method rarely detects the tubercle bacilli, whereas the culture is positive in about 40% of cases, but it requires 2–6 weeks to grow. Histology of pleural biopsies could offer a sensitivity of up to 80% in immunocompetent patients. However, this procedure requires greater expertise, is more invasive, and is subject to sampling error. Due to these limitations of conventional tests, plus the delay of several weeks for mycobacterial culture results, newer rapid tests and biomarkers such as polymerase chain reaction, ADA and IFN-γ in pleural fluid have been evaluated [4,6–8].

ADA, released from active lymphocytes, macrophages and neutrophils, is a non-specific marker of inflammation. A meta-analysis concludes that ADA and its isoenzymes in pleural fluid is used as a marker of tuberculous pleurisy. However, empyema, rheumatoid pleurisy and malignancy may
give false positive results [9–11]. Villegas et al. found the sensitivity, specificity, and negative predictive value of ADA (value \( P \geq 45.5 \) IU/ml) for tuberculous pleurisy were 88.1%, 85.7% and 88.2% respectively [7]. The sensitivity, specificity, positive predictive value and negative predictive value of ADA (>40 IU/ml) for tuberculous pleurisy were 82.6%, 96.1%, 90.5% and 92.5% respectively.

IFN-\( \gamma \) usage in tuberculous pleurisy diagnosis was described by Ribera, in 1988 [12]. Poyraz et al. compared the 15 patients with tuberculous pleural effusion and 20 patients with malignant pleural effusion, and they reported the IFN-\( \gamma \) sensitivity and specificity were 87%, 95% for tuberculous pleural effusion were 82.6%, 96.1%, 90.5% and 92.5% respectively.

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IGRAs in the differential diagnosis of active TB [17]. Several systematic reviews and meta-analyses have recently been published, specifically on the diagnostic accuracy of IGRAs in active TB [18–20]. Strong heterogeneity between study populations is a major limitation of these meta-analyses, and most studies have had small sample sizes.

Specificity depends highly on the definition of the control group. Pai et al. reported a pooled specificity of 99% among non-BCG-vaccinated and 96% among BCG-vaccinated low-risk groups [21]. According to a recent meta-analysis that was not restricted to studies on specificity to low-risk groups [20], the specificity of QFT-G was only 0.79 (95% CI 0.75–0.82).

Sensitivity in these studies was also found to be highly dependent on the study population, notably local TB prevalence, and ranged from 0.58 in a high-prevalence country [23] to 1.00 in a low-prevalence country [20] when QFT-G was assessed. Diel et al. found a pooled sensitivity of 0.84 (95% CI 0.81–0.87) when he included only developed countries [20], which was consistent with the results published by Sester et al. (0.77, 95% CI 0.75–0.80) [19]. Vesenbeckh et al. assessed the diagnostic accuracy of the latest generation IGRA for detection of active TB in a low-incidence area in Germany. Their consecutive case series included 61 HIV negative, MTB culture-positive patients, as well as 234 control patients. In 11/61 patients with active TB (18.0%), the test result was <0.35 IU/mL, resulting in a sensitivity of 0.82. They recommended establishing a new cut-off value for the differential diagnosis of active TB assessed by prospective clinical studies and in various regions with high and low prevalence of TB [22].

Baba et al. evaluated the utility of the QFT-G test as a diagnostic tool of tuberculous pleurisy in a high TB and HIV endemic area. The QFT-G test in pleural fluid was positive in 27% and 56% of the ‘confirmed TB’ and ‘probable TB’ cases, respectively, whereas the corresponding sensitivities in blood were 58% and 83%. They concluded that the commercial QFT-G test in blood could prove useful in the diagnosis of tuberculous pleurisy in the HIV-positive population in order to urge initiation of TB therapy. Although the QFT-G test in pleural fluid in certain cases could contribute to the TB diagnosis in HIV patients with low CD4 cell counts, they did not support the routine use of this test in pleural fluid at this stage [23].

The QFT-G test is technically more complicated and more expensive than established biomarkers, and its diagnostic performance for active pleural TB is highly variable between studies and settings [24]. As well as complying with large volume testing and showing flexibility in analysis, some studies concerning the QFT-G test show that the high background IFN-γ level in the negative control tube precludes the usage of pleural fluid that was not additionally processed [25]. In antigen-based tests such as ELISA, false positive and negative results can be obtained owing to the antigen/antibody excess or with other proteins causing a cross-reaction. There are some researchers who suggest diluting in order to preclude the cases in which the test is indeterminate as a result of antigen/antibody excess. Hence, if a false positive result is obtained because the sample contains foreign protein, since it decreases in number after diluting, there is no non-specific attachment and the result turns into negative. In one study, the results of the first test performed on a TB group showed 5 samples to be positive and 4 samples to be indeterminate as they consisted of unreadably high values. After diluting the pleural fluid in a 1:1 ratio, the indetermined results were found to be positive (prezon incident-antigen excess). The sensitivity, specificity, positive predictive value and negative predictive value of QFT-G for tuberculous pleurisy were 64.3%, 100%, 100% and 80.7%, respectively [3]. In the present study, QFT-G test results were indeterminate in 13 patients and positive in 10 patients in the tuberculous pleurisy group.

There was not a single patient whose test result was negative in the tuberculous pleurisy group. It was determined that the sensitivity, specificity, positive predictive value and negative predictive value of QFT-G for tuberculous pleurisy was 43.5%, 54.5%, 30.3% and 100%, respectively.

In order for the QFT-G test to become useful in the diagnosis of tuberculous pleurisy, which test was designed to detect latent TB infection, the result should be positive in the analysis performed with serum in patients such as those with TB infection, and in patients with pleural effusion such as non-TB related malignancy. On the other hand, tests performed with pleural fluid, the results should be negative. This finding should give rise to thought that the lymphocytes sensitized with TB remain in peripheral blood, yet are not present in pleural fluid. However, this hypothesis has not yet been proven. Unless otherwise proven, whether or not such analyses in the diagnosis of pleural effusion will make additional contributions of more than just a free measurement of IFN-γ in pleural fluid will remain a controversial topic [25].

While the value of the QFT-GIT test in exclusion of tuberculous pleurisy was found to be higher in the present study, its other diagnostic efficiency values were detected to be low. Although the diagnostic sensitivities of both IGRAs were higher than that of tuberculin skin tests, they were still not high enough to use as a test to rule out TB. Also, positive evidence for the use of IGRAs in compartments other than blood will require more independent and carefully designed prospective studies.

Conflict of interest

The authors had no conflicts of interest to declare in relation to this article.

References


