Use of adenosine deaminase measurements and QuantiFERON in the rapid diagnosis of tuberculous peritonitis

Mohammad A. Saleh,1 Enas Hammad,2 Mahmoud M. Ramadan,3 Azima Abd El-Rahman4 and Asmaa F. Enein5

1Department of Medical Microbiology and Immunology, Damietta Faculty of Medicine, Al-Azhar University, Egypt
2Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt
3Department of Cardiology, Faculty of Medicine, Mansoura University, Egypt
4Department of Thoracic Medicine, Faculty of Medicine, Mansoura University, Egypt
5Department of Internal Medicine, Faculty of Medicine, Mansoura University, Egypt

Peritoneal tuberculosis (TB) is a considerable problem in certain developing nations. Current diagnostic tests for peritoneal TB are difficult and time-consuming. This study aimed to determine the effectiveness of an adenosine deaminase (ADA) assay and the QuantiFERON-Gold (QFT-G) assay in the rapid diagnosis of TB peritonitis. Forty-one patients with a presumptive diagnosis of TB peritonitis with ascites were admitted to Mansoura University Hospital and included in the study. Ascitic fluid and blood samples were collected from each patient. Fluid samples were examined biochemically (protein concentration), cytologically (white blood cell count) and microbiologically (Ziehl–Neelsen stain and TB culture in Löwenstein–Jensen media), and ADA levels were determined using colorimetry. Interferon-γ levels in whole-blood samples were measured using the QFT-G assay. Fourteen (34 %) patients received a final clinical diagnosis of TB peritonitis; these patients were subclassified as definite (positive culture for Mycobacterium tuberculosis; eight patients), highly probable (four patients) and probable (two patients) for TB peritonitis. Of the 14 patients with a final clinical diagnosis of TB peritonitis, 3 (21 %) tested positive using an acid-fast bacilli smear, which showed a sensitivity of 21 % and a specificity of 100 %. A receiver operating characteristic curve showed that a cut-off value of 35 IU l⁻¹ for the ADA level produced the best results as a diagnostic test for TB peritonitis, yielding the following parameter values: sensitivity 100 %, specificity 92.6 %, positive predictive value (PPV) 87.5 % and negative predictive value (NPV) 100 %. The QFT-G assay yielded the following values: sensitivity 92.9 %, specificity 100 %, PPV 100 % and NPV 96.4 %. The ADA and QFT-G assays might be used to rapidly diagnose TB peritonitis and initiate prompt treatment while waiting for a final diagnosis using the standard culture approach.

INTRODUCTION

Tuberculosis (TB) remains a public health challenge with nearly 2 billion persons (~29 % of the world’s population) exposed to Mycobacterium tuberculosis annually and 8 million new cases of TB diagnosed each year, resulting in 2 million deaths (WHO, 2006). Although most persons present with pulmonary symptoms, presentation with extrapulmonary symptoms is also possible. About 10–15 % of patients who are immunocompetent and 50–70 % of patients with AIDS present with extra-pulmonary symptoms. Peritoneal TB is currently the sixth most common location for extra-pulmonary TB, and cases of peritoneal TB can be expected to increase with the increasing incidence of TB worldwide (Riquelme et al., 2006).

Peritoneal TB usually results from the reactivation of latent TB in peritoneal foci that were established after haematogenous spread from a primary lung focus (Mehta et al., 1991). Peritoneal TB is manifested clinically as ascites of insidious onset, abdominal pain and fever (Chow et al., 2002). Although an uncommon disease, the non-specific
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symptoms associated with TB peritonitis and its challenging clinical course can interfere with a definitive diagnosis, and TB peritonitis is often confused with other intra-abdominal diseases. Delayed diagnosis increases the morbidity and mortality of TB peritonitis (Akin et al., 2000; Sanai & Bzeizi, 2005).

Current diagnostic tests for peritoneal TB are difficult and time-consuming. A diagnosis of peritoneal TB requires histological confirmation of caseous granulomas. Bacteriological confirmation can be done using ascitic fluid-derived acid-fast bacilli (AFB) smears as well as cultures for M. tuberculosis. Cultures for M. tuberculosis require 4 weeks of culture time, and AFB smears are too insensitive to meet current needs (Inadomi et al., 2001). A laparoscopy-guided biopsy can be used to obtain a rapid diagnosis of TB peritonitis; however, this method is associated with risks related to anaesthesia and potential injury and bleeding (Vogel et al., 2008). The development and evaluation of new diagnostic strategies are thus priorities for TB control programmes. New methods with high specificity and sensitivity for the rapid diagnosis of extra-pulmonary TB would improve clinical outcomes for patients with extrapulmonary TB and should accelerate the application of public health control measures (Portillo-Gómez et al., 2000).

Adenosine deaminase (ADA) is a purine-degrading enzyme that catalyses the deamination of adenosine in an irreversible manner, resulting in the production of inosine as a by-product. ADA is widely distributed in tissues and body fluids, and levels of ADA can be used to differentiate T cells from B cells; ADA levels are 10–12 times higher in T cells than in B cells. ADA levels vary with the proliferative status and the maturity of cells (Dwivedi et al., 1990). ADA levels in body fluids can be measured rapidly, and they might provide an alternative for the diagnosis of TB (Giusti, 1974). ADA activity is increased in effusions resulting from infection, several other bacterial infections, rheumatological disease and lymphoproliferative disorders (Pettersson et al., 1984). Nowadays, the use of the automated method of determining ADA activity in pleural and cerebrospinal fluid samples has been validated as an alternative to the conventional modified Giusti method (Feres et al., 2008).

The QuantiFERON-TB (QFT) assay uses an ELISA to measure the release of interferon-γ (IFN-γ) after in vitro stimulation of whole-blood samples by the purified protein derivative from M. tuberculosis (Ruiz-Manzano et al., 2008). The second-generation assay QuantiFERON-TB Gold (QFT-G) also measures the release of IFN-γ after in vitro stimulation of samples with specific region of difference 1 (RD1) antigens from M. tuberculosis: early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10). The QuantiFERON-TB Gold in-tube assay is a new-generation test that includes an additional M. tuberculosis antigen, TB7.7, encoded by a phage-inserted region, RD11 (Lalvani & Millington, 2008). All of the antigens used in the QFT tests are present in the M. tuberculosis complex but not in the vaccine against TB or in any of the non-TB mycobacteria, except Mycobacterium kansasi, Mycobacterium marinum and Mycobacterium szulagi; therefore, the QFT assays can be used to discriminate between persons infected with M. tuberculosis and persons vaccinated against TB or exposed to non-TB mycobacteria (Andersen et al., 2000).

The current study aimed to determine the effectiveness of an ADA assay and the QFT-G assay as rapid methods for the diagnosis of TB peritonitis.

**METHODS**

**Study population.** This study was conducted from February 2008 to January 2010. Forty-one patients with a presumptive diagnosis of TB peritonitis with ascites were admitted to Mansoura University Hospital and included in the study. All patients were free from HIV infection. Each patient received a full history, clinical examination and several radiological tests, including abdominal ultrasound and computed tomography. A final clinical diagnosis of TB peritonitis was based on the following criteria, put forth by Portillo-Gómez et al. (2000): (A) fever, ascites and abdominal pain for more than 6 weeks; (B) M. tuberculosis-positive culture from ascitic fluid; (C) ascitic fluid showing characteristics of exudate fluid with cell counts of 150–4000 mm⁻³ (predominantly lymphocytes) and protein concentrations ≥ 2.5 mg dl⁻¹; (D) abdominal computed tomography showing high-density ascites and abdominal lymphadenopathy, and radiological findings of pleural effusion or evidence of pulmonary TB; (E) positive AFB smear and evidence of infection with M. tuberculosis elsewhere in the body. Patients were categorized as: ‘definite TB peritonitis’ when criteria A and B were met; ‘highly probable TB peritonitis’ when criterion A and any two of criteria C, D and E were met; and ‘probable TB peritonitis’ when criterion A and any one of criteria C, D or E were met. Patients with a presumptive clinical diagnosis of TB but who did not meet criteria B, C, D or E were classified as ‘definitely not TB peritonitis’, and they received other final clinical diagnoses.

**Collection of samples.** A peritoneal tap was used to collect 1 litre of ascitic fluid from each patient. Fluid samples were examined biochemically (by measuring protein content), cytologically (by counting white blood cells) and microbiologically [by Ziehl–Neelsen (ZN) stain and TB culture on Löwenstein–Jensen media], and ADA levels were determined using colorimetry. Whole-blood samples were collected from patients and used to measure levels of IFN-γ with the QFT-G test.

**Microbiological examination.** The ascitic fluid specimens were left for sedimentation, followed by centrifugation at 3000 g for 30 min at 5 °C. One-half of the sediment was used for ZN staining of AFB smears according to the manufacturer’s instructions (Emmyonya Biotech) and culture for M. tuberculosis. The remaining sediment was used for the ADA assay. A mean of 0.1 ml sediment was inoculated onto each of two Löwenstein–Jensen slants (BD Diagnostic Systems). Cultures were incubated at 37 °C for 8 weeks and were inspected two times each week for the growth of mycobacteria (Nolte & Metchock, 1995).

**ADA assay.** ADA levels in the ascitic fluid were determined by colorimetry (Giusti, 1974). Ascitic fluid (0.1 ml) was transferred to a test tube containing 1 ml buffer (Giusti, 1974) and the adenosine substrate. The tube was incubated at 37 °C for 1 h. Sodium hypochlorite (0.75 ml) and phenol (0.75 ml) were added to the tube, and the tube was incubated for an additional 30 min. The enzymatic reaction was detected by measuring the OD₆₅₀ using a Beckman DU640 UV/Vis spectrophotometer (Scientific Support).
QFT-G assay. The QFT-G assay (Cellestis) was conducted using blood samples that were processed according to the manufacturer’s instructions. Aliquots of whole blood were incubated with the test antigens for 16–24 h. Test kits included two synthetic peptides, ESAT-6 and CFP-10, which were used as antigens, and saline, which was used as a negative control to measure non-specific binding. After incubation, the concentration of IFN-γ was determined using ELISA via a Stat Fax 2100 Microplate Reader (MIDSCI), in accordance with the manufacturer’s directions. The amount of IFN-γ released was determined by subtracting the levels measured using the negative control from the levels measured using samples stimulated with ESAT-6 and CFP-10. The cut-off for a positive result was 0.35 IU ml⁻¹.

Statistical analysis. Data entry and analysis were accomplished using the Statistical Package for Social Science software program (version 17) and EPI Info program version 3.5.1 (CDC, Atlanta, Georgia, USA). Means, standard deviations, ranges, frequencies and percentages were calculated using descriptive statistics. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the ADA and QFT-G assays by comparing results for patients with a definitive diagnosis of TB peritonitis with those for patients with a diagnosis of non-TB peritonitis. Student’s independent samples t-test was used to compare ADA levels between the two groups of TB and non-TB peritonitis. The statistical heterogeneity of sensitivities and specificities was assessed using the chi-square test and a receiver operating characteristic plot of sensitivity versus the false-positive rate (one minus specificity).

Ethical approval. The study protocol was reviewed and approved by the local ethical committee of Damietta Faculty of Medicine at Al-Azhar University. The subjects provided informed consent before study participation.

RESULTS

A total of 41 patients [29 men (71 %)] aged 51.7 ± 9.0 years with a presumptive diagnosis of TB peritonitis were included in this study. Fourteen (34.1 %) patients received a final clinical diagnosis of TB peritonitis, and the remaining 27 (65.9 %) patients received a final clinical diagnosis of non-TB peritonitis (e.g. spontaneous bacterial peritonitis) or another diagnosis (e.g. malignancy). The 14 patients with a final clinical diagnosis of TB peritonitis (Table 1) were subcategorized as follows: 8 with definite TB peritonitis (confirmed by culture); 4 with highly probable TB peritonitis; and 2 with probable TB peritonitis. The mean age ± SD of the 14 TB peritonitis patients was 51.9 ± 10.7 years. One patient had history of pulmonary TB, and two patients had concomitant cirrhosis of the liver. Anti-TB treatment (i.e. therapeutic trial) was initiated for one patient before specimens were processed, and the patient showed clinical improvement.

Of the 14 patients with a final clinical diagnosis of TB peritonitis, the AFB smear was positive in only 3 (21.4 %) cases, and this assay showed a sensitivity of 21.4 % and a specificity of 100 %.

Ascitic fluid samples from all 14 patients who received a final clinical diagnosis of TB peritonitis had an ADA level ≥ 35 IU l⁻¹. ADA levels in ascitic fluid samples were significantly higher for patients with TB peritonitis (70.3 ± 26.3 IU l⁻¹) than for patients with non-TB peritonitis (24.1 ± 7.4 IU l⁻¹; P<0.001). This difference was observed even if data from two patients with TB peritonitis and co-existing cirrhosis of the liver were excluded from the analysis. A receiver operating characteristic curve showed that a cut-off level of 35 IU ADA l⁻¹ yielded the best result as a diagnostic test for TB peritonitis, i.e. sensitivity 100 %, specificity 92.6 %, PPV 87.5 % and NPV 100 %. Of the 27 patients with non-TB peritonitis, ascitic fluid samples from only 2 (7.4 %) patients had an ADA level above the cut-off value of 35 IU l⁻¹. These two patients were males, aged 42 and 68 years, and classified as ‘definitely not TB peritonitis’ as

Table 1. Clinical characteristics and laboratory findings for 14 patients who received a final clinical diagnosis of TB peritonitis

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/gender</th>
<th>TB history</th>
<th>Co-existing disease</th>
<th>Therapeutic trial</th>
<th>+ CT finding</th>
<th>AFB smear</th>
<th>TB culture</th>
<th>ADA level (IU l⁻¹)</th>
<th>QFT-G assay</th>
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described in Methods. Blood samples from 13 of the 14 (92.8 %) patients with a final clinical diagnosis of TB peritonitis tested positive in the QFT-G assay; the one sample that tested negative in the QFT-G assay was from a patient who tested positive for TB peritonitis by both culture for *M. tuberculosis* and the AFB smear (patient no. 7 in Table 1). This patient had an ADA level of 76 IU l⁻¹.

Samples from all 27 (100 %) patients with a final diagnosis of non-TB peritonitis tested negative in the QFT-G assay. The values for the parameters of the QFT-G assay were as follows: sensitivity 92.9 %, specificity 100 %, PPV 100 % and NPV 96.4 %.

**DISCUSSION**

The diagnosis of TB peritonitis is often delayed because of the non-specific clinical symptoms associated with this disease resembling those of other diseases. The development of techniques that are rapid and less invasive and that have high sensitivities, specificities, PPVs and NPVs would aid in the diagnosis of TB peritonitis and the swift initiation of treatment (Shawar et al., 1993; Sathar et al., 1995).

ZN staining of ascitic fluid for the detection of *M. tuberculosis* is often difficult because at least 5000 bacilli (ml specimen)⁻¹ are required to detect *M. tuberculosis* in stained smears (Yeager et al., 1967; Hobby et al., 1973; Dineeen et al., 1976). In the present work, AFB smears were positive for only 3 of 14 (21.4 %) patients with a final clinical diagnosis of TB peritonitis, thus underpinning the insufficiency of this diagnostic technique. Further, Sathar et al. (1995) found that ZN-stained AFB smears yielded negative results in all patients in their study. In contrast, Portillo-Gómez et al. (2000) found that ZN-stained AFB smears were positive for six of seven (85.7 %) patients tested. The specificity of ZN staining was very high (100 %) in our study.

In the current study, cultures from 1 litre samples of ascitic fluid were positive for *M. tuberculosis* for 8 of 14 (57.1 %) patients with a final clinical diagnosis of TB peritonitis. By contrast, Inadomi et al. (2001) found that cultures from small samples of ascitic fluid yielded an *M. tuberculosis* detection rate of <20.5 %. Menzies et al. (1985) found that ascitic fluid cultures yielded a 66 % *M. tuberculosis* detection rate, although the volume of ascitic fluid that was used for culture was not specified. In other studies, cultures from large volumes of ascitic fluid yielded *M. tuberculosis* detection rates of 63 % (Geake et al., 1981) and 83 % (Singh et al., 1969; Martinez-Vazquez et al., 1986).

The ADA assay has been recommended for use as a diagnostic test for TB peritonitis (Ruiz-Manzano et al., 2008). Also, ADA assay in biological fluids has been used as a tool for the differential diagnosis of different forms of extrapulmonary TB, such as pleuritis, synovitis, meningitis, TB of female genitals and peripheral lymph nodes, and uveitis (Titarenko et al., 2006). In pleural fluid, cut-off values of ADA vary from 33 to 48 U l⁻¹, with sensitivity higher than 80 % and specificity near 100 % (El Jahiri et al., 2006; Liang et al., 2008). In cerebrospinal fluid, a value of 7 U l⁻¹ can discriminate negative and positive cases with a good sensitivity and specificity. The data from the literature show that 50 U l⁻¹ in pericardial fluid is a reliable threshold for TB diagnosis (El Jahiri et al., 2006). Synovial fluid ADA levels higher than 31 U l⁻¹ were highly correlated with a diagnosis of TB arthritis, with a high sensitivity and specificity (Foocharoen et al., 2011).

Previous studies have used arbitrary diagnostic cut-off values for ADA levels, which decreases the clinical relevance of the results (Dineeen et al., 1976). In the present study, high levels of ADA (≥ 35 IU l⁻¹) in ascitic fluid were diagnostic of TB peritonitis, and the ADA assay showed high sensitivity (100 %) and specificity (92.6 %). Previous studies reported that the ADA assay has a sensitivity of 100 % and specificities in the range of 92–100 % for the diagnosis of TB peritonitis (Voigt et al., 1989; Fernandez-Rodriguez et al., 1991; Gupta et al., 1992; Brant et al., 1995). In contrast, Martinez-Vazquez et al. (1986) found that the ADA assay was not specific for the diagnosis of TB peritonitis, and other studies showed that the sensitivity of the ADA assay was significantly lower for patients with underlying liver cirrhosis (Sathar et al., 1995; Hillebrand et al., 1996), a finding not observed in our current study. Although ADA levels were elevated in other infectious or inflammatory disorders, definite cut-off values were associated only with TB peritonitis. This was demonstrated in one study in which, out of 264 patients with ascites, 50 had ascites caused by TB and all of them had ADA values ≥ 39 IU l⁻¹. Only 6 patients with an ADA value over this cut-off point had other diagnoses, but ADA values ≥ 56 IU l⁻¹ were associated only with TB peritonitis (Riquelme et al., 2006). It was confirmed that ADA levels in non-TB effusions seldom exceed the cut-off set for TB effusions (Jiménez Castro et al., 2003).

We found that mean ADA levels were significantly higher in patients with TB peritonitis than in patients with non-TB peritonitis, in agreement with other studies (Gupta et al., 1992; Brant et al., 1995; Sathar et al., 1995). These findings suggest that measurement of ADA activity in ascitic fluid may be used to cost-effectively and easily diagnose TB peritonitis (Voigt et al., 1989; Fernandez-Rodriguez et al., 1991; Gupta et al., 1992). In the current work, ascitic fluid from 2 of 27 (7.4 %) patients with a final clinical diagnosis of non-TB peritonitis or another disorder had ADA levels ≥ 37 IU l⁻¹ (i.e. above the 35 IU l⁻¹ cut-off point for diagnosis of TB peritonitis). These 2 patients received a final diagnosis of subacute bacterial peritonitis in which *Klebsiella pneumoniae* was isolated, and both patients responded to antibiotic therapy.

Immunodiagnosis of TB has been considered an attractive alternative to microbiological and molecular tests (Pai et al., 2004). Kobashi et al. (2006) found that use of the QFT-G assay for the detection of TB infection in patients with active pulmonary TB yielded the following approximate
values: sensitivity 86%, specificity 94%, PPV 16.7% and NPV 96.1%. Our present study yielded the following QFT-G assay values: sensitivity 92.9%, specificity 100%, PPV 100% and NPV 96.4%. Mori et al. (2004) reported a sensitivity of 89% in a select population of patients with clinical signs suggestive of TB; they reported a specificity of 98% in low-risk patients who were assumed to be truly free of TB. Another blinded prospective study of 82 patients with clinical signs of active TB showed that the QFT-G assay was 85% sensitive for the detection of TB (Ravn et al., 2005). Our results, however, showed the highest sensitivity (92.9%), which supports the utility of the QFT-G assay in the diagnosis of TB peritonitis.

In our study, the QFT-G assay was negative for only one patient (i.e. a false-negative diagnosis) who tested positive using both the AFB smear and the culture for M. tuberculosis (i.e. a definite diagnosis of TB peritonitis). The false-negative rate using the QFT-G assay was low (7.1%) and was similar to that reported by Aguado et al. (1990). The one patient who had a false-negative diagnosis using the QFT-G assay received a final clinical diagnosis of severe lymphocytopenia. Because the QFT-G assay measures the production of IFN-γ by sensitized T cells from peripheral blood, the lymphocytopenia may have caused a reduction in IFN-γ production that resulted in the negative test result.

A question may be raised about our choice of ADA and QFT-G assay methods instead of the molecular rapid amplification-based tests (such as PCR) which detect specific regions of DNA or RNA of M. tuberculosis in body fluids and tissues with high sensitivity and specificity. In fact, reports suggest a reasonably good performance of the various PCR tests with sensitivity reaching up to 95% in smear-positive patients. However, as the ZN stain in patients with TB peritonitis is positive in only 3% of cases, these figures suggest that PCR sensitivity would be similarly very low (Sanai & Bzeizi, 2005). Moreover, other publications found that all PCR tests for detecting TB bacilli in ascitic fluid were negative even when the patient had AFB on histopathological examination (Dede et al., 2007; Miura et al., 2009). Apart from a few case reports, there is no one controlled study evaluating PCR in patients with TB peritonitis (Schwake et al., 2003). Another concern is the issue of false-positives which may result from the cross-contamination of samples or the DNA from dead bacilli (Sanai & Bzeizi, 2005).

The results of the current study showed that both the ADA and QFT-G assays can be used as rapid diagnostic tests for TB peritonitis, and these assays are associated with high sensitivity and specificity. One of the limiting factors for the use of the QFT-G assay is its high cost, which is particularly an issue in poor areas where TB is endemic. False-positive and false-negative results also might restrict the use of these assays. Nonetheless, the ADA and QFT-G assays should be considered when a rapid diagnosis of TB peritonitis is required.

REFERENCES


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