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ORIGINAL ARTICLE

Tissue Xpert™ MTB/Rif assay is of limited use in diagnosing peritoneal tuberculosis in patients with exudative ascites

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Abstract
Background Xpert™ MTB/Rif is a multiplex hemi-nested real-time PCR-based assay to detect presence of *M. tuberculosis* within 2 hours of sample collection. The present study aimed at assessing efficacy of Xpert™ MTB/Rif assay for diagnosing peritoneal tuberculosis.
Methods Patients with exudative ascites, fluid negative for acid-fast bacilli on auramine O fluorescence staining and unyielding fluid cytology for malignant cells, were included. Ultrasound-guided omental biopsy samples were obtained in all. Xpert™ MTB/Rif assay on tissue samples was assessed against a composite "reference" standard for diagnosis of peritoneal tuberculosis, defined as presence of any of the three-culture showing *M tuberculosis*, granulomatous inflammation on histology or resolution of ascites with 2 months of antitubercular therapy.
Results During January 2012–July 2013, 28 patients (age:43 ±15 years; mean±SD; male:20) were recruited. Serum ascitic albumin gradient was <1.1 in all except in four patients with underlying cirrhosis. Twenty-one of the 28 patients had peritoneal TB as diagnosed by composite reference standard (histology:18; culture:4; treatment response:3). Seven patients (25 %) had an alternative diagnosis (metastatic carcinoma 2, adenocarcinoma 2, mesothelioma 2, and systemic lupus erythematous 1). Xpert™ MTB/Rif assay was positive in 4/21 patients with peritoneal tuberculosis and in none of the 7 patients with alternative diagnosis. Thus, sensitivity, specificity, positive, and negative predictive values for tissue Xpert™ MTB/Rif assay in diagnosing peritoneal tuberculosis were 19 % (95 % C.I: 6 % to 42 %), 100 % (95 % C.I: 59 % to 100 %), 100 % (40 % to 100 %), and 29 % (95 % C.I: 13 % to 51 %), respectively.
Interpretation and conclusion Tissue Xpert™ MTB/Rif assay was of limited use in diagnosing peritoneal tuberculosis.

Keywords Granuloma · *M. tuberculosis* · Omental biopsy · Xpert™ MTB/Rif assay

Introduction

Diagnosis of extrapulmonary tuberculosis remains a challenge to the physician. Peritoneal tuberculosis is the sixth most frequent type of extrapulmonary involvement [1]. Usual diagnostic methods like smear microscopy and culture have very low yield, presumably secondary to paucibacillary nature of the specimen [2]. Ascitic fluid culture for *M. tuberculosis* (MTB) has shown a sensitivity of 8 % to 16 % [2, 3]. Diagnostic laparoscopy with peritoneal biopsy is considered as a gold standard for diagnosis, but is often limited due to time, costs, and potential complications.

Xpert™ MTB/Rif assay is a rapid automated PCR-based method which is endorsed by the World Health Organization for the diagnosis of pulmonary tuberculosis [4]. Due to the ease, speed and accuracy of the results, this test is well-suited for a low resource setting and even as a point of care facility [5]. Xpert™ MTB/Rif assay has a sensitivity and specificity of

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88 % and 98 %, respectively, for culture-confirmed cases of pulmonary tuberculosis [6].

There are only few studies evaluating the performance of Xpert™ MTB/Rif assay in extrapulmonary tuberculosis with varied results [7–9]. This study aimed to evaluate the diagnostic utility of Xpert™ MTB/Rif assay in the setting of exudative ascites to diagnose peritoneal tuberculosis.

Methods

This study was conducted from January 2012–July 2013, among out-patients attending the liver clinic of Christian Medical College, Vellore. All patients with exudative ascites (lymphocytic ascites and ascitic fluid protein content of >2.5 g/dL) [10], underwent clinical and relevant laboratory evaluation to analyze the etiology of ascites. As a part of diagnostic evaluation, all patients underwent either an ultrasonography or CT scan of the abdomen. These patients were subjected to diagnostic paracentesis on at least three different occasions for acid-fast bacilli (AFB) (Auramine O fluorescence) stain and malignant cells (Papanicolaou) stain. Patients who were negative on AFB smear microscopy and malignant cells were then subjected to ultrasound-guided omental biopsy. The sequence of investigations in these patients is depicted in Fig. 1. Patients who were not willing or not fit for the procedure were excluded from the study (Table 1).

The multiple bits obtained during omental biopsy were processed for AFB smear microscopy, culture on Lowenstein–Jensen (LJ) medium [11] or by automated liquid culture system mycobacterial growth indicator tube (MGIT) system [12], histological examination, and molecular diagnosis by Xpert™ MTB/Rif assay. Drug susceptibility testing was also done on culture-positive isolates either by 1 % proportion method on LJ medium or by MGIT method as per standard protocol. Patients, in whom the diagnosis was not confirmed despite omental biopsy, were initiated on empirical treatment trial with conventional four-drug antituberculous therapy and clinical response noted with a follow up of at least 2 months.

Xpert™ MTB/Rif assay Xpert™ MTB/Rif assay is a multiplex, nested real-time PCR with species-specific primers allowing amplification of the MTB *rpo*B core region [6]. A series of molecular beacons is used to simultaneously detect the presence of MTB and to diagnose rifampicin resistance as a surrogate marker for multidrug-resistant disease. Species-specific primers allow amplification of the MTB *rpo*B core region [13]. Nested PCR is used in order to increase the sensitivity of the assay. At the end of the real-time PCR, the Xpert™ MTB/Rif assay identifies the presence of MTB in the sample and also *rpo*B gene, a surrogate marker for multidrug-resistant tuberculosis [14].

Diagnosis of peritoneal tuberculosis (reference standard) We considered the diagnosis of peritoneal tuberculosis if any one of the following criteria were met:

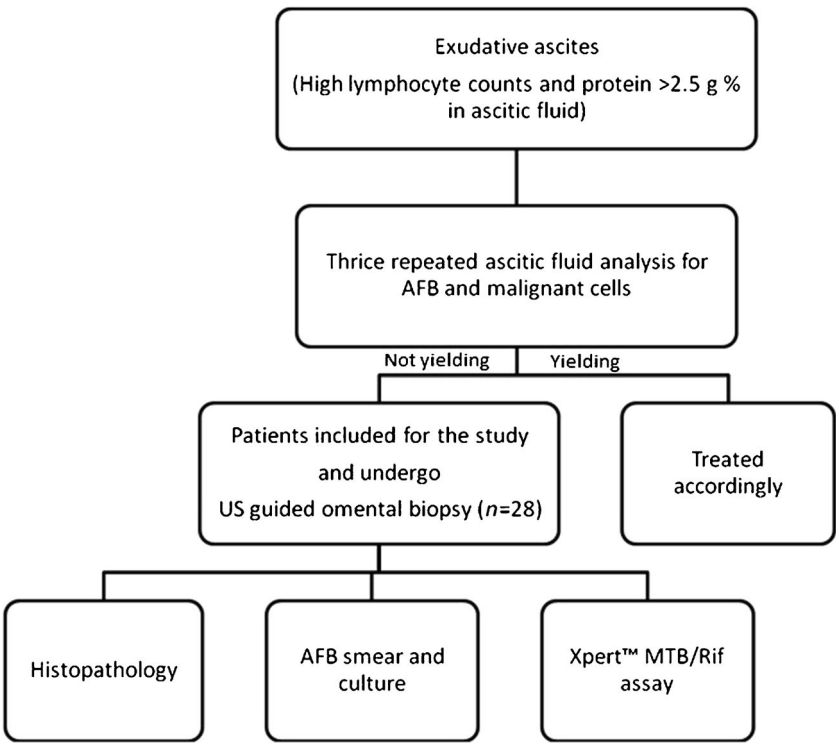

Fig. 1 Flow chart depicting the inclusion of study patients and their investigation protocol

Table 1 Diagnostic performance of Xpert™ MTB/Rif assay against a composite reference standard (microbiology, histopathology, and treatment response) in diagnosing peritoneal tuberculosis among patients with exudative ascites

		Reference standard[a]	
		Positive for tuberculosis	Negative for tuberculosis
Xpert™ MTB/Rif assay positive	Positive for *M. tuberculosis*	4	0
	Negative for *M. tuberculosis*	17	7

AFB acid-fast bacilli, *US* ultrasound

[a] Of the 21 patients with reference standard positive, four had *M.tuberculosis* on culture (microbiology), 18 had granulomatous inflammation with response to therapy (histology), and three had response to therapy alone (treatment response). All patients positive on microbiology also had histology suggestive of granulomatous inflammation

1. Microbiology The tissue smear or culture demonstrating MTB
2. Histology Granulomatous inflammation on histopathology and treatment response (as in 3)
3. Treatment response Exudative ascites with non-yielding microbiology and histopathology, but disappearance of ascites with 2 months of antituberculous therapy.

The results were expressed as numbers (percentage) or mean±standard deviation (SD). The sensitivity, specificity, and predictive values of tissue Xpert™ MTB/Rif assay were calculated against the reference standard previously defined. The study was approved by the institutional ethics committee.

Results

Twenty-eight patients (age: 43±15 years; mean±SD; male:female:: 20:8) were enrolled in the study. Four of these patients had underlying cirrhosis. All patients had exudative ascites with non-yielding result on thrice-repeated ascitic fluid analysis for AFB (by smear) and malignant cells (Pap smear). All study patients thus underwent omental biopsy. The serum ascitic albumin gradient was <1.1 in all except in the four patients with underlying cirrhosis.

Diagnosis of the study patients

Of the 28 study patients, 21 patients were diagnosed to have peritoneal tuberculosis. Of these 21 patients, 18 had granulomatous inflammation on histology and four had positive culture (all had concomitant granulomatous inflammation on histology). Of the 18 patients with granulomatous inflammation, 17 had both discrete and confluent granulomas with foci of necrosis in six and with acid-fast bacilli in three. One patient had only discrete granulomas with no necrosis or acid-fast bacilli. On follow up, all 18 patients showed response to antitubercular therapy in the form of resolution of ascites.

Three patients were diagnosed on the basis of resolution of ascites with empirical antituberculous therapy.

Seven patients (25 %) had an alternative diagnoses on omental biopsy (metastatic carcinoma: 2, adenocarcinoma: 2, mesothelioma: 2 and systemic lupus erythematous (SLE): 1). SLE was diagnosed on the basis of serositis as evidenced by pericardial and pleural effusion and serology.

Performance of Xpert™ MTB/Rif assay in diagnosing peritoneal tuberculosis

Xpert™ MTB/Rif assay was positive in four of the 21 patients (19 %) diagnosed with peritoneal tuberculosis. Two of these four patients were culture-positive. Xpert was negative in all seven patients with alternative diagnoses. Indeterminate result was not noted in this test. Thus, sensitivity, specificity, positive, and negative predictive values for tissue Xpert assay in diagnosing peritoneal tuberculosis were 19 % (95 % C.I: 6 % to 42 %), 100 % (95 % C.I: 59 % to 100 %), 100 % (95 % C.I: 40 % to 100 %), and 29 % (95 % C.I: 13 % to 51 %), respectively.

Relative performance of culture and Xpert™ MTB/Rif assay in diagnosing peritoneal tuberculosis

Culture was positive in four of the 21 patients (19 %) diagnosed with peritoneal tuberculosis, and two of them found to be multidrug-resistant. Two of these four patients were Xpert™ MTB/Rif assay positive. Granulomatous inflammation was demonstrated in all of these four patients.

Rifampicin resistance was detected in two patients with Xpert™ MTB/Rif assay (one multidrug resistance and other was indeterminate for Rifampicin resistance). One of these patients was also multidrug-resistant by MGIT method and was subsequently treated according to DOTS-Plus regimen [15]. The other patient was cirrhotic and expired shortly after diagnosis due to variceal bleed.

Discussion

In a patient with exudative ascites, if routine non-invasive tests and ascitic fluid analyses do not yield a diagnosis, laparoscopy and peritoneal biopsy remains the gold standard. In this study, we performed ultrasound-guided omental biopsy as a cheaper and less invasive alternative. Omental biopsy yielded diagnosis of peritoneal tuberculosis in 18 of the 28 study patients.

Response to antitubercular therapy was noted in all 18 patients in the form of resolution of ascites.

We have employed a composite reference standard comprising histopathology, microbiology (smear and culture), and treatment response for the diagnosis of peritoneal tuberculosis. This remains a valid clinical practice in India, due to high endemicity and the pauci-bacillary nature of the illness [16, 17].

Ascitic fluid analysis (smear and culture) for MTB are only seldom helpful in these patients, and thus, we decided to use only omental biopsy tissue for our test and reference analysis. Xpert™ MTB/Rif assay had a 100 % specificity (C.I: 59 % to 100 %) and positive predictive value, but a low sensitivity (19 %) and negative predictive value (29 %) for diagnosing peritoneal tuberculosis. High specificity gives credence to positive results, but low sensitivity, even on omental tissue, argues against routine use of this test in diagnostic algorithm of these patients. Interestingly, sensitivity of Xpert™ MTB/Rif assay was the same as that of omental tissue culture for MTB. The estimates are based on a limited number of study patients, and these need to be further validated in larger samples.

There are very few studies regarding extrapulmonary tuberculosis with varying sensitivity and specificity with different samples [7–9]. In a study by Hillemann et al. [7], sensitivity of Xpert™ MTB/Rif assay in culture-positive extrapulmonary tissue specimens was approximately 70 %. Other studies have shown good sensitivity and specificity from various extrapulmonary sites when it was compared with culture-positive specimens [8, 9]. In our study, Xpert™ MTB/Rif assay positivity was 50 % (two positive out of four culture-positive specimen) in culture-positive specimen. Sensitivity was very low in patients with histopathology-proven peritoneal tuberculosis. Laparoscopy-assisted peritoneal biopsy may increase the microbiological yield for MTB, but this was not done in this study. Ascitic fluid adenosine deaminase (ADA) was not done in the study. Sharma et al. [18], in their study exploring the efficacy of Xpert™ MTB/Rif assay in multiple extrapulmonary sites against culture and composite reference standard, found a low sensitivity in fluids (including ascitic fluid) as compared to the abscess and lymph node. Low sensitivity for peritoneal tuberculosis observed in this study has also been seen in specimens like pleural tissue and may be secondary to the pauci-bacillary nature of the specimen [8].

Based on the study results (albeit with limited sample size), we conclude tissue Xpert™ MTB/Rif assay, though specific, cannot be recommended for use in diagnosing peritoneal tuberculosis.

Compliance with ethical standards The study was performed in a manner to conform with the Helsinki Declaration of 1975, as revised in 2000 and 2008, concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

Conflict of interest CB, JSM, DB, SBS, SG, BR, AG, and CE declare that they have no competing interests.

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