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Accuracy of polimerase chain reaction for the diagnosis of pleural tuberculosis

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Summary
Introduction: Polymerase chain reaction (PCR)-based techniques to detect *Mycobacterium tuberculosis* DNA in respiratory specimens have been increasingly used to diagnose pulmonary tuberculosis. Their use in non-respiratory specimens to diagnose extrapulmonary tuberculosis is, however, controversial. In this study, we estimated the accuracy of three in-country commercialized PCR-based diagnostic techniques in pleural fluid samples for the diagnosis of pleural tuberculosis.
Methods: Patients underwent thoracenthesis for diagnosis purposes; pleural fluid aliquots were frozen and subsequently submitted to two real time PCR tests (COBAS®TAQMAN®MTB and Xpert®MTB/Rif) and one conventional PCR test (Detect-TB®). Two different reference standards were considered: probable tuberculosis (based on clinical grounds) and confirmed tuberculosis (bacteriologically or histologically).
Results: Ninety-three patients were included, of whom 65 with pleural tuberculosis, 35 of them confirmed. Sensitivities were 29% for COBAS®TAQMAN®MTB, 3% for Xpert®MTB/Rif and 3% for Detect-TB®; specificities were 86%, 100% and 97% respectively, considering confirmed

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tuberculosis. Considering all cases, sensitivities were 16%, 3% and 2%, and specificities, 86%, 100%, and 97%.
Discussion: Compared to the 95% sensitivity of adenosine deaminase, the most sensitive test for pleural tuberculosis, the sensitivities of the three PCR-based tests were very low. We conclude that at present, there is no major place for such tests in routine clinical use.

Introduction

Tuberculosis is still a leading cause of death worldwide, and Brazil is one of the 22 countries with the highest burden of the disease [1]. Pleural tuberculosis is the second most common form of the disease [2,3], and its diagnosis remains a challenge [4]. Sensitivity of smears for acid-fast bacilli (AFB) is extremely low (<5%) [4–6], cultures have a long delay and also low sensitivity (<60%) [7–10]. Histopathological examination of the pleural tissue is the most sensitive diagnostic test (80–85%) [4,6,10,11]; however, it requires a pleural biopsy, a procedure that increases risks and costs [11]. The adenosine deaminase (ADA) enzyme is another pleural fluid marker of tuberculosis. Despite its high sensitivity (56–100%) [12–14], ADA activity reflects only a non-specific immunologic response [11]. Previous studies showed a poor positive predictive value of ADA for tuberculosis diagnosis in low-tuberculosis incidence settings [12,14]. Despite these limitations, in practice, ADA, AFB and cultures of pleural fluid as well as pleural biopsies have been recommended as the reference tests for diagnosing pleural tuberculosis [4,11,12,14,15].

After a century of stagnation regarding new technologies for the diagnosis of tuberculosis, new molecular-based technologies were approved for the detection of *Mycobacterium tuberculosis* DNA in respiratory specimens in the past two decades, and automated systems, such as the Xpert®MTB/Rif, commercially available in the last couple of years, are being rapidly incorporated for the diagnosis of pulmonary tuberculosis in high-burden countries [16–18]. However, their use in extrapulmonary samples is still controversial [19]. In-house PCR-based tests in pleural fluid have a high specificity (98%), but low and heterogenous sensitivity (43–77%), but automated systems were less studied [20]. In order to study the usefulness of PCR technique in the diagnosis of pleural tuberculosis in routine practice, in the present study, we aimed to evaluate the accuracy of three commercially available tests: two real time PCR tests (COBAS®TAQMAN®MTB and Xpert®MTB/Rif) and one conventional PCR test (Detect-TB®).

Methods

From September 2007 to March 2011, all patients with a pleural effusion needing a thoracenthesis for diagnostic purposes hospitalized in the 7th ward (an Internal Medicine Unit) of *Hospital Geral da Santa Casa da Misericórdia do Rio de Janeiro* were eligible. Adults (>18 years old) were invited to participate and those who signed an informed consent were prospectively included. Patients were excluded if they had bleeding disorders contraindicating thoracenthesis, if the fluid volume was insufficient for storage or if a final diagnosis could not be ascertained.

In this pragmatic study, diagnosis and management were carried out according to the clinicians' practice and Brazilian Guidelines [21]. Consent was obtained for the experimental (PCR) techniques only. Pleural fluid was forwarded for biochemical (protein, glucose and ADA level), for cytometric (total white cells, mononuclear, neutrophils), for bacteriological (AFB smears and *M. tuberculosis* culture in liquid media – BACTEC mycobacterial growth indicator tube [MGIT] 960 System, BD) and sporadically, Gram stain and culture for pyogenic bacteria evaluation. Pleural tissue, obtained with a Cope needle, was forwarded for histopathological analysis and for *M. tuberculosis* culture (MGIT). Aliquots were frozen at −80 °C. Spontaneous or induced sputum specimens were also forwarded for AFB and culture, when available (data not shown). According to the Brazilian Guidelines [21], the diagnosis of confirmed tuberculosis was made if any specimen was positive for AFB or culture, or if granuloma with or without caseous necrosis was present on a biopsy. A clinical diagnosis of probable tuberculosis was made if patients had symptoms compatible with tuberculosis (fever, night sweats and weight loss), an exudative pleural fluid or an ADA level > 40 IU/L and if they had clinical improvement after antituberculous therapy. Patients were clinically managed according to these results.

For the three PCR techniques, samples were simultaneously unfrozen in 2012 and processed according to the manufacturer's recommendations (except for the freezing step) by two experienced lab technicians (EFSSKO and SEM), blinded to the clinical results. In brief, COBAS®TAQMAN®MTB amplifies and detects the *rRNA 16S* gene sequence [22–24], Xpert MTB®/Rif automatically amplifies and detects the *rpo* β gene [25], and the conventional Detect-TB® amplifies and detects the *IS 6110* insertion [26].

To assess the similarity between groups of participants Wilcoxon non-parametric test was used for medians. Proportions were compared using the Fischer's exact test.

Sensitivities, specificities and their 95% confidence interval (CI) were calculated for each test using (i) the confirmed cases as the reference standard and (ii) all cases (confirmed and probable) as the second reference standard, according to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) initiative recommendations [27].

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