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TUBERCULOSIS

Comparison between the diagnostic accuracy of Xpert MTB/Rif assay and culture for pleural tuberculosis using tissue biopsy

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ABSTRACT

Background: Early diagnosis of pleural tuberculosis is difficult as it is a paucibacillary disease and a combination of tests is required to diagnose it, which have varied diagnostic accuracy and increase the cost. The aim of this study was to evaluate the diagnostic performance of the Xpert MTB/Rif assay on thoracoscopic pleural biopsy specimens.

Methods: A total of 201 patients with exudative pleural effusion and normal lung parenchyma were included in the study. All patients underwent thoracoscopic pleural biopsy under local anaesthesia. Biopsy samples were sent for Xpert MTB/Rif assay and culture, along with histopathology. Chronic granulomatous inflammation on histopathology and response to antituberculous treatment was taken as the reference standard for diagnosis of tuberculous pleurisy.

Results: Of the 198 patients included in the final analysis, 134 had pleural tuberculosis. The sensitivity of the Xpert assay was 52.2% and specificity was 100%, and that of pleural biopsy cultures were 41% and 100% respectively.

Conclusion: The sensitivity and specificity of Xpert MTB/Rif assay scores were high, hence escalating the speed of diagnosis and imparting marked impact on patient outcomes. The Xpert MTB/Rif assay is a potential game changer in diagnosing pleural tuberculosis.

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Pleural tissue Xpert MTB/Rif assay has a high diagnostic yield for pleural tuberculosis when good-quality biopsy samples are taken by medical thoracoscopy. It speeds up the diagnosis of pleural TB and has a major impact on patients' outcomes. http://bit.ly/2GtuHJZ

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Introduction

Pakistan is ranked fifth among 22 countries with a high burden of tuberculosis (TB). Approximately 525 000 new cases of TB are diagnosed each year from the region. TB carries a high mortality rate of 1.1 in 100 000 of the population [1]. Globally, Pakistan stands fourth among high prevalence countries with multidrug resistance [2].

After lymph node disease, pleural TB is the most common form of extrapulmonary TB accounting for 30% of cases [3]. Pleural effusion is caused by a type IV hypersensitivity reaction due to mycobacterial antigens in the pleural space [4]. At present, diagnosis of TB pleural effusion depends on invasive and time-consuming tests like detection of *Mycobacterium tuberculosis* in pleural fluid or pleura by culture, or the presence of caseous granulomas in pleural tissue histopathology [5].

The Xpert MTB/Rif assay is a fully automated cartridge-based test endorsed by the World Health Organization in 2010 for the rapid detection of TB and rifampicin resistance. Its high cost is balanced by a fast turnaround time of approximately 2 h, its high diagnostic accuracy, less risk of biohazards and the need for minimal training to run this sophisticated test [6]. A meta-analysis showed a pooled sensitivity and specificity of 90% and 98% respectively of Xpert for pulmonary TB [7].

There is much literature on the efficacy of the Xpert assay on nonrespiratory samples showing promising results [8–11]. However, its use in pleural biopsy has not been evaluated.

The aim of this study was to assess the diagnostic performance of the Xpert assay on thoracoscopic pleural biopsy specimens.

Methods

Patients were recruited from the Department of Pulmonology, Jinnah Postgraduate Medical Center, Karachi, from January 2016 till December 2017. All consecutive patients aged ⩾18 years with exudative lymphocytic pleural effusion and normal lung parenchyma were included in the study. Patients with prior history of TB, or who were on antituberculous treatment, were not included. Patients with positive HIV screening were also excluded. A total of 201 patients were enrolled in the study and underwent thoracoscopy under local anaesthesia (figure 1) to obtain samples of pleural tissue. Figures 2 and 3 show

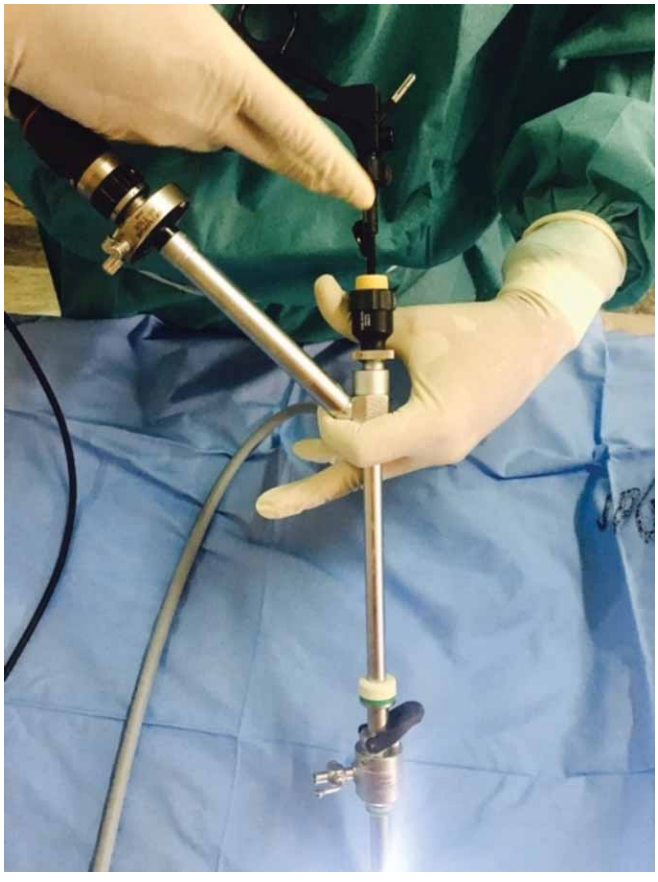

FIGURE 1 10-mm rigid thoracoscope with biopsy forceps in the working channel.

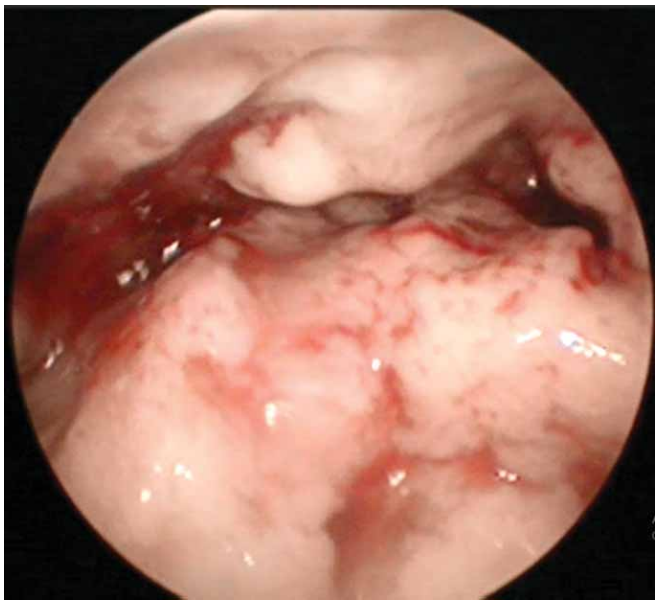

FIGURE 2 Thoracoscopic view of the pleural cavity showing thick slough.

the thoracoscopic view of the pleural cavity. Pleural biopsies were stored in 4% formalin for histopathology and saline solution for TB culture and Xpert assay. The Bactec method and solid culture medium were used for the detection of *M. tuberculosis*. Pleural tissue processing for the Xpert assay and culture was performed according to World Health Organization recommendations [6]. The Xpert MTB/RIF cartridge was used for detecting *M. tuberculosis* and rifampicin resistance.

The Xpert assay gave invalid results for one patient and two patients had contaminated culture reports, so they were not included in the final analysis.

The reference standard used for the diagnosis of TB was evidence of chronic granulomatous inflammation with caseous necrosis on histopathology and the patient's clinical response on antituberculous treatment.

Written informed consent was obtained from all study participants and the study was approved by the hospital's ethical committee.

SPSS version 23 was used for statistical analysis. Mean±SD was calculated for age. Categorical variables were reported as numbers and percentages and compared using Pearson's Chi-squared and Fisher's exact test, as appropriate. Sensitivity, specificity, positive predictive value and negative predictive value were calculated using reference standard for diagnosis of TB. A p-value <0.05 was taken as significant.

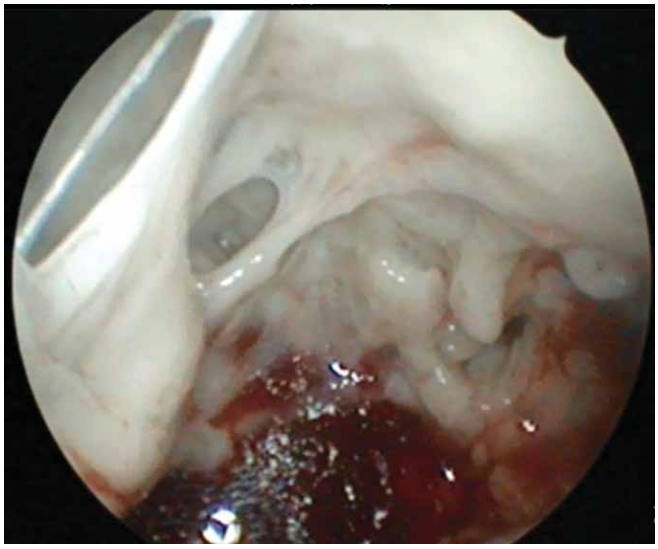

FIGURE 3 Thick slough with adhesions in a patient with chronic empyema (thoracoscopic view).

TABLE 1 Aetiology of pleural effusion

Tuberculosis [#]	134 (67.67%)
Other	64 (32.32%)
Acute on chronic inflammation	14 (7.07%)
Malignancy	23 (11.61%)
Nonspecific inflammation	27 (13.63%)

[#]: tuberculosis was diagnosed if there was evidence of chronic granulomatous inflammation with caseous necrosis on histopathology and based on the patient's clinical response on antituberculous treatment.

TABLE 2 Diagnostic performance of mycobacterial culture and the Xpert assay on pleural tissue specimens

Diagnostic test	Result	Tuberculosis	Nontuberculous	Sensitivity	Specificity	PPV	NPV	p-value
Xpert assay	Positive	70	0	52.2%	100%	100%	50%	<0.001
	Negative	64	64					
Mycobacterial culture	Positive	55	0	41%	100%	100%	44.8%	<0.001
	Negative	79	64					

PPV: positive predictive value; NPV: negative predictive value.

Results

Using pleural tissue histopathology and response to antituberculous treatment as a diagnostic reference standard, pleural TB was diagnosed in 134 patients, out of a total of 198 participants. The remaining 64 patients had pleural effusion secondary to a nontuberculous aetiology (table 1). The mean age of the study participants was 36.56±16.59 years. The male to female ratio was 13:9 (117, 59.1% male; 81, 40.9% female). The Xpert assay detected *M. tuberculosis* in 70 pleural tissue specimens. Out of these 70 Xpert-positive cases, 55 had a positive pleural tissue acid-fast bacillus culture. Rifampicin resistance was detected by Xpert in seven patients. Out of these, six patients were diagnosed with multidrug-resistant TB and one with rifampicin monoresistance on culture. The overall diagnostic performance of the Xpert assay and mycobacterial culture on pleural tissue is shown in table 2.

Discussion

There is a huge body of literature showing promising results of the Xpert assay for the diagnosis of pulmonary TB and the efficacy of the Xpert assay for the detection of extrapulmonary TB using samples from body fluids, lymph nodes and other biopsy specimens [12–15].

Very limited data exist with contrasting results on the performance of the Xpert assay for the detection of *M. tuberculosis* in pleural tissue samples.

Our study reveals that the Xpert assay has high diagnostic yield in pleural tissues biopsied by thoracoscopy under local anaesthesia; 52.2% of TB cases were detected by the Xpert assay. Furthermore, it is also evident from the results that the Xpert assay is more sensitive and specific in diagnosing pleural tuberculosis than pleural tissue *M. tuberculosis* cultures which has a sensitivity of 41%. Our result contradicts those of Christopher *et al.* [16] who suggested in their study that the Xpert assay has limited utility in diagnosing pleural TB as no case was detected by the assay.

The comparatively low sensitivity of culture (41%) than that of the Xpert assay (52.2%) in our study may be explained by the fact that specimens from extrapulmonary sites have a low bacillary burden and *M. tuberculosis* has the propensity to form clusters, leading to an unequal distribution of the organism [17]. Also, the Xpert assay detects DNA of bacteria whether live or dead [18], therefore samples can be detected as positive by the Xpert assay and negative by culture.

The Xpert assay detected rifampicin resistance in seven patients. Rapidly determining MDR status of the patient is of utmost importance in decreasing mortality and halting the spread of this deadly organism. Conventional culture and drug sensitivity testing take around 1.5–2 months from inoculation [19].

Keeping in mind that we took large pleural tissue biopsies with a thoracoscope, most of the positive cases had a low or very low burden of *M. tuberculosis*. As the disease is paucibacillary, this emphasises that sample quality is a crucial factor for using the Xpert assay on pleural tissue [20]. Furthermore, a

next-generation assay, the Xpert MTB/RIF Ultra assay (Ultra) is now available that is more sensitive but less specific than the Xpert assay. Ultra has a limit of detection of 16 bacterial CFU·mL^{-1} (compared with 114 CFU·mL^{-1} for Xpert). The accuracy of rifampicin resistance detection is also improved as Ultra is based on melting-temperature-based analysis instead of real-time PCR [21].

This study was not designed to evaluate the efficacy of the Xpert assay for detecting drug resistance. Further studies are needed in this regard. Future studies may focus on increasing the sensitivity and specificity of Xpert by improving sample collection and processing methods, and development of improved diagnostic algorithms, including rapid diagnostic tests.

Conclusion

Usually multiple tests are required to diagnose pleural TB. This increases the cost per patient and often there is a delay in diagnosis. The sensitivity and specificity of the Xpert assay scores was high, hence escalating the speed of diagnosis and imparting a marked impact on patient outcomes. The Xpert assay is a potential game changer in diagnosing pleural TB.

Conflict of interest: None declared.

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