Original Article

Study of diagnostic utility of Xpert MTB/Rif test on pleural fluid in the evaluation of patients presenting with Pleural Tuberculosis in Nepal

Deebya Raj Mishra[1], Narendra Bhatta[1], Madhab Lamsal[2], Narayan Bhattarai[3], Robin Maskey[4], Niharika Shah[5]

Departments of [1]Pulmonary, [2]Biochemistry, Critical Care and Sleep Medicine, [3]Microbiology, [4]Internal Medicine and [5]Pathology, B.P. Koirala Institute of Health Sciences, Dharan, Nepal

Abstract

Introduction: Existing tests for the diagnosis of pleural tuberculosis (TB) have major limitations in terms of accuracy, time to diagnosis and drug resistance testing. A test which can diagnose pleural TB and detect resistance, like Xpert MTB/Rif, would be optimal for rapid diagnosis and treatment. **Methods:** A prospective observational study was done in a tertiary care hospital in Eastern Nepal. Fifty-one patients with clinic-radiologic suspicion of pleural TB were included. The results of pleural fluid Xpert MTB/Rif were compared with two Composite Reference Standards. Composite Reference Standard-1 consisted of positive pleural fluid smear, positive culture, positive histology of pleural biopsy, and positive sputum results. Composite Reference Standard-2 included those with Composite Reference Standard-1 and those with high ADA values (>40 U/l) with response to anti-tubercular treatment at 8 weeks of follow-up. **Results:** Thirty-six patients were diagnosed as Pleural TB. Nine fulfilled Composite Reference Standard-1. Pleural fluid Xpert MTB/Rif was positive in five cases with Composite Reference Standard-1 and nine cases with Composite Reference Standard-2. The sensitivity, specificity, positive predictive value and negative predictive value with reference to Composite Reference Standard-1 were 55.56%, 88.10%, 50%, and 90.24%, respectively. Using Composite Reference Standard-2 as reference, sensitivity, specificity, positive predictive value and negative predictive value were 25%, 93.33%, 90%, and 34.15%, respectively. Two cases were diagnosed Xpert Rif resistant on pleural fluid. **Conclusion:** Due to low sensitivity, the Xpert MTB/Rif test cannot be recommended as initial test of diagnosis in a high prevalence setting. At the same time its clinical utility lies in testing of patients suspected to have drug-resistant pleural tuberculosis.

Keywords: Pleura, tuberculosis, Xpert MTB/Rif

Introduction

In the context of pleural effusion, where, diagnosis of tubercular pleural effusion remains especially challenging because of low number of *Mycobacterium tuberculosis* (MTB) bacilli present, Xpert MTB/Rif might play an important role in providing rapid molecular diagnostic assessment of suspected tubercular pleural effusion (TB pleural effusion). It seems logical to conduct local contextual studies that are focused on utility of Xpert MTB/Rif for use in TB pleural effusion, given the expanding distribution and utilization of this simple to use technology in TB endemic regions, many of which previously relied solely on conventional criteria to guide patient management. A recent systematic review concluded that Xpert MTB/Rif assay has a high potential for

Address for correspondence: Dr. Deebya Raj Mishra, Associate Professor, Department of Pulmonary, Critical Care and Sleep Medicine, B.P. Koirala Institute of Health Sciences, Dharan, Nepal.
E-mail: deebyaraj@gmail.com

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confirming TP diagnosis and differentiating TP from non-TB diseases using pleural fluid samples.[1] Wider availability of Xpert MTB/Rif opens up the possibility of use of this test on pleural fluid in primary care along with routine examinations.

Methods

The study was prospective observational and approved by the local institutional review board. All the adult patients presenting to the division of Pulmonary, Critical Care, and Sleep Medicine with symptoms suspected of TB pleural effusion, including cough, fever, night sweats, loss of weight, hemoptysis and chest pain, and features consistent with a pleural effusion on chest X-ray, were prospectively recruited over a one-year period from July 2015 till June 2016. A detailed socio-demographic data for every patient was collected and information was recorded in structured proforma. Patients were requested to participate in the study with assurance that consenting or refusing to participate in the study would have no effect on the treatment they receive. Clinical care and treatment were as per the standard clinical practice for the patient of pleural effusion followed in the institution. It was expected that patients would not be exposed to any risks beyond those normally encountered during routine clinical care of the patients with respiratory diseases.

The study protocol was submitted for ethical approval to Institutional Review Committee (IRC) and ethical clearance to conduct the study was obtained. Patient consent was obtained in each of the cases.

An attempt was made to reach a definitive etiological diagnosis based on the integration of information from various diagnostic modalities. Treating physician made clinical care and treatment decisions and treatment was based upon standard practice of treatment for patients presenting with chronic respiratory disease in our hospital.

At screening, consenting patients underwent a thoracentesis during which pleural fluid for analysis was obtained.

The following tests were done in all the patients;

i. Sputum examination for Acid Fast Bacilli (AFB)
ii. Pleural fluid examination for AFB
iii. Pleural fluid Adenosine Deaminase (ADA)
iv. Xpert MTB/Rif test on the pleural fluid
v. Pleural fluid culture: Though originally planned for all the patients, it could only be done in a few due to unavailability at various time points during the study.

A pleural biopsy procedure to obtain pleural tissue for culture and histopathology was performed if the initial tests for TB were negative, the ADA value was less than 40 U/L and the effusion was moderate to large in size. Two composite reference standards (CRS) were used for the diagnosis of TB [Figure 1].

The first **CRS (CRS-1)** included confirmed TB if

1. Acid fast bacilli (AFB) was identified on microscopic evaluation of pleural tissue or fluid,
2. Culture from pleural tissue or fluid was positive for MTB,
3. Histopathology of pleural tissue identified granulomas,
4. MTB was identified in any other sample (e.g., sputum, bronchoalveolar lavage) from the same patient by smear examination or Xpert MTB/Rif.

The second **CRS (CRS-2)** included

1. CRS - 1 and/or
2. Patients classified as TB cases if the pleural fluid was found to have ADA levels greater than 40 U/L, in the absence of any other diagnosis to explain the pleural effusion, and with response to anti-tubercular therapy at 8 weeks of follow-up.

TB pleural effusion was ruled out if either histopathology or cytology was diagnostic for malignancy, or both pleural tissue culture and histopathology showed no evidence of TB, TB was not identified from any other sample and ADA was less than 40 U/l. The sensitivity and specificity of Xpert MTB/Rif as performed on pleural fluid and pleural tissue was calculated using the two reference standards. The analysis and reporting followed the Standards for the Reporting Diagnostic Accuracy.[2]

Statistical analysis

The data were entered, edited, and coded in Microsoft Excel. The data were exported to SPSS version 20.0. The clinico-epidemiological data of the patients were analyzed descriptively. For comparing discrete variables Chi-Square test was used, while for continuous variables t-test was used. "p" value of < 0.05 was considered statistically significant. For the performance of the diagnostic test, Sensitivity, Specificity,

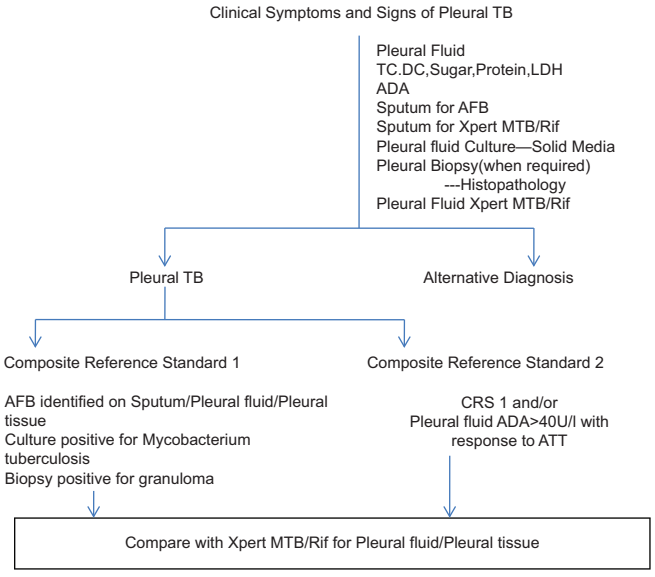

Figure 1: Flow Chart of the Study Design and Patient Enrollment Procedure

positive predictive value and the negative predictive value were calculated.

Results

Patient enrollment and study results as per the study design

During the study period of one year, we enrolled 51 cases of pleural effusion which were clinico-radiologically suspected to be tubercular in origin [Figure 2].

On further investigation and treatment response, 36 cases were categorized as having Tubercular Pleural Effusion. Among them, nine cases fulfilled the CRS-1 criteria, whereas 27 others had a pleural fluid ADA value of more than 40 U/l with response to ATT at eight week follow up duration. 15 patients with pleural effusion had an alternative diagnosis. Five of these patients had pneumonia associated effusion, two were proven to have malignancy and one patient remained undiagnosed at the eight weeks follow-up period. Seven patients had a pleural fluid ADA value of less than 40 U/l, however, they responded to a trial of ATT. They were grouped under pleural effusion due to other diseases as the CRS criteria did not include this group.

In patients with TB pleural effusion (n = 36), pleural fluid smear was positive only in 8% (3/36) of the cases while sputum smear was positive in 5% (2/36) of the cases. In both these cases, sputum Xpert MTB/Rif was also positive. Pleural biopsy was only done in selected cases which did not have any investigation suggestive of TB, including a high ADA level and had moderate to large size of the effusion. Histological evidence suggestive of granuloma was seen in 28% of the cases where pleural biopsy could be done. Though pleural fluid culture was initially planned in all the cases, due to technical reasons, it could be done in only 15 cases and all of them yielded no growth at 12 weeks reading.

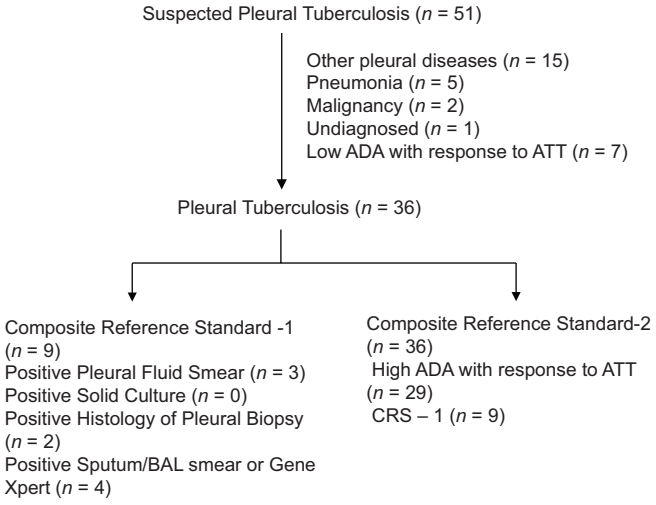

Figure 2: Flow Chart of the Study Design and Patient Enrollment

Performance status of Pleural fluid Xpert MTB/Rif

Pleural fluid Xpert MTB/Rif was done in all (51/51) the cases. In the cases of pleural effusion diagnosed as CRS-1, pleural fluid Xpert MTB/Rif was positive in five cases. Pleural fluid Xpert MTB/Rif was positive in all three cases which also had pleural fluid smear positive. Interestingly, two cases with Xpert MTB/Rif positive in the pleural fluid were Rifampicin (Rif) Resistant. In one of those cases, sputum Xpert MTB/Rif also revealed Rif Resistant status whereas in the other patient sputum Xpert MTB/Rif was negative but pleural fluid smear was positive. In the group with pleural TB diagnosed only on the basis of high ADA with clinical response to ATT at 8 weeks period, pleural fluid Xpert MTB/Rif was positive in four of the cases. Performance status of pleural fluid Xpert MTB/Rif is illustrated in Table 1.

Diagnostic utility of Xpert MTB/Rif on pleural fluid using CRS-1

Using CRS-1, five cases with pleural fluid Xpert MTB/Rif were true positive, whereas the other five cases were false positive. Among the cases in which Xpert was negative, four were false negative whereas 37 were true negative. Using CRS-1 as reference criteria, the sensitivity of Xpert MTB/Rif in pleural fluid was 55.6%, whereas the specificity was 88.10% [Table 2]. The positive and the negative predictive value were 50% and 90.24%, respectively [Table 2].

Table 1: Xpert MTB/Rif results for pleural fluid in cases diagnosed as TB by protocol

	n	Xpert positive in pleural fluid
A. Tuberculosis (CRS-1)		
Positive pleural fluid smear	3	3
Positive pleural fluid culture	0	0
Histopathology with Granulomas	2	0
Positive		
Sputum Smear	2	1
Sputum Xpert MTB/Rif	1	1
BAL Xpert MTB/Rif	1	0
B. Tuberculosis (excluding CRS-1)		
High ADA with Clinical Response	27	4
C. CRS 2=A + B	36	9

Table 2: Diagnostic utility of Xpert MTB/Rif on pleural fluid using CRS-1 as reference criteria

CRS -1	TB pleural effusion	
	Present	Absent
Test		
Positive	5 (TP)	5 (FP)
Negative	4 (FN)	37 (TN)
Statistics	**Value**	**95% Confidence Interval**
Sensitivity	55.56%	21.20% to 86.30%
Specificity	88.10%	74.37% to 96.02%
Positive predictive value	50.00%	18.71% to 81.29%
Negative predictive value	90.24%	76.87% to 97.28%

Diagnostic utility of Xpert MTB/Rif on pleural fluid using CRS-2 as reference criteria

Using CRS-2 as reference criteria, nine cases with pleural fluid Xpert MTB/Rif were true positive, whereas one case was false positive; among the cases in which Xpert MTB/Rif was negative, 27 were false negative whereas 14 were true negative.

Thus the sensitivity of pleural fluid Xpert was 25%, whereas the specificity was 93.33. The positive and the negative predictive value were 90% and 34.15%, respectively [Table 3]. Because of the high specificity of 94% and a high positive predictive value of 90%, the diagnosis of pleural TB can be made with confidence when the Xpert MTB/Rif is positive in the pleural fluid.

Discussion

We have explored the position of Xpert MTB/Rif assay in relation to conventional diagnostic tools in the work-up of pleural effusion suspected with tubercular origin, highlighting the diagnostic and clinical utility in accurate and timely diagnosis of Tubercular pleural effusion among the contemporary cohort of patients presenting with pleural effusion in Nepal.

We used Composite Reference Standard rather than individual tests for the diagnosis of tubercular pleural effusion as there is no single test which provides the optimum level of Sensitivity and Specificity at the same time. CRSs are often used as a standard for comparison of test performance in detecting extrapulmonary TB, as culture is suboptimal in this case.[3] Also, the considerations of multiple clinical and lab parameters mirrors the clinical care in resource limited settings. The role of pleural fluid smear and sputum smear examination in pleural effusion is established owing to the minimal cost for such testing, though the sensitivity is low.[4,5] Pleural fluid culture is more sensitive with a yield ranging from 12 to 70%.[6] As pleural TB is more often a pauci-bacillary disease, pleural tissue histology is often done for diagnosis. The sensitivity varies from 50% to 97%.[6,7] Though the sensitivity of pleural biopsy and histology is more, the procedure has its own complications and is not routinely used in clinical practice. To mirror the clinical use, pleural biopsy in our study was limited to the patients who were pleural fluid smear and sputum smear negative, had an ADA value lower than 40 U/l and had moderate to large size of effusion.

Table 3: Diagnostic utility of Xpert MTB/Rif on pleural fluid using CRS-2 as reference criteria

CRS -2	TB pleural effusion	
	Present	**Absent**
Test		
Positive	9 (TP)	1 (FP)
Negative	27 (FN)	14 (TN)
Statistics	**Value**	**95% Confidence Interval**
Sensitivity	25.00%	12.12% to 42.20%
Specificity	93.33%	68.05% to 99.83%
Positive predictive value	90.00%	55.50% to 99.75%
Negative predictive value	34.15%	20.08% to 50.59%

Pleural fluid Xpert MTB/Rif was positive in a total of 10 cases. Of these, nine cases were diagnosed as pleural TB whereas one was diagnosed as low ADA with response to ATT. In the cases fulfilling CRS-1, pleural fluid Xpert MTB/Rif was positive in five of the nine cases. Among 27 other cases, pleural TB was diagnosed on the basis of high ADA with response to ATT at 8 weeks follow-up. In this group, pleural fluid Xpert MTB/Rif was positive in four cases.

Using CRS-1, the sensitivity of pleural fluid Xpert MTB/Rif comes out to be 55.56% with a specificity of 88.10%. However, as only 17% (9/51) cases were diagnosed as pleural TB using these criteria, the generalization of such findings would be incorrect given that the prevalence of pleural TB in all exudative samples was 41.3% at the same center.[8] Further, since cases were only included after screening for clinico-radiologic suspicion of pleural TB, we would have expected a higher prevalence of the disease with such screening. In a study where Xpert MTB/Rif was done on thoracoscopic pleural biopsy, the sensitivity of Xpert was 69.0% against the CRS data and that against MGIT 960 culture was (56.6%).[9]

Using CRS-2, a total of 36/51 (71%) cases were diagnosed as pleural TB. In this subset, sensitivity of pleural fluid Xpert MTB/Rif was 25% with a specificity of 93.33%, a positive predictive value of 90% and a negative predictive value of 34.15%. Because of the high specificity of 94% and a high positive predictive value of 90%, the diagnosis of pleural TB can be made with confidence when the Xpert MTB/Rif is positive in the pleural fluid. At the same time, Xpert MTB/Rif was unable to correctly identify 75% of pleural TB cases (low sensitivity). More worrisome is the fact that, 66% of patients could still have pleural TB even if Xpert MTB/Rif is negative (low negative predictive value). However, pleural fluid Xpert MTB/Rif was able to detect two cases with resistance to Rifampicin.

The results of our study are comparable to those of a recent meta-analysis which reported the pooled sensitivity of Xpert MTB/Rif in diagnosis of tubercular pleural effusion.[10] In the sub-analysis for pleural TB they looked at two specific groups of studies, one with culture as the reference standard and another with CRS. The group with culture as the reference standard included 14 studies and had a pooled sensitivity of 46.4% and a pooled specificity of 99.1%. Six studies used CRS as the reference standard, as in our study, and the pooled sensitivity were lower at 21.4% with a higher specificity of 100%. The poor sensitivity of Xpert MTB/Rif in pleural fluid is probably due to the pauci-bacillary nature of the disease.

However, the high specificity as also demonstrated in our study is remarkable. Also noteworthy is the detection of Rifampicin

Resistance in two of the cases in our study. In low- and middle-income countries (LMICs) where maintaining the bio-safety levels of culture labs may not always be feasible, the use of Xpert MTB/Rif in context of suspicion of drug-resistant TB might be appropriate.

This is the first study from Nepal to have studied the performance of Xpert MTB/Rif in pleural effusion using standardized study protocol. The study results clearly show that Xpert MTB/Rif has low sensitivity and high specificity for diagnosing tubercular pleural effusion.

There are certain limitations of our study inherently related to study design and lack of complete follow up of patients. Regardless of all these limitations however, our study reasonably fulfills the objective which was to evaluate the diagnostic and clinical utility of Xpert MTB/Rif testing with respect to its Sensitivity, Specificity, Positive, and Negative predictive value in the evaluation of patients presenting with Tubercular Pleural effusion in Nepal.

Conclusion

Thus, based upon these findings of our study, we conclude that Xpert MTB/Rif test in the evaluation of patients presenting with tubercular pleural effusion in Nepal although has a lower sensitivity, and therefore cannot be used as standalone initial test in high prevalence settings.

At the same time its clinical utility lies in the work-up of suspected drug-resistant TB pleural effusion along with conventional tools of pleural fluid analysis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient (s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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