

# Is GeneXpert MTB/RIF assay reliable for the Diagnosis of Pleural Tuberculosis?

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## Author's Contribution

<sup>1</sup> Conception of study

<sup>1,3</sup> Experimentation/Study conduction

<sup>4,5,6</sup> Analysis/Interpretation/Discussion

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## Abstract

**Objective:** To determine the accurate ability to diagnose the pleural fluid GeneXpert MTB/RIF assessment in the suspected pleural tuberculosis patients considered as gold standard the pleural biopsy.

**Materials & Methods:** This study was a validation cross-sectional survey and it was conducted in the pulmonology department of Pakistan Institute of Medical Sciences, Islamabad from 1<sup>st</sup> July to 31<sup>st</sup> December 2018 after approval of IRB. Participants with suspected pathology  $\geq$  18 years with exudative pleural effusion were included in the study. By implementing the process of the standard technique, closed needle pleural biopsy was executed by means of ABRAM'S needle. The collected samples of patients were sent to the pathology department of the Pakistan Institute of Medical Sciences for histopathology. Pleural fluid was referred to the National Institute of Health Islamabad for GeneXpert MTB/RIF assessment on the alike day and reports were assembled. All findings were entered in a structured Proforma. Data was entered in SPSS version 20 and analyzed.

**Results:** A total of 180 patients were included; 65.6% of patients were male. When the sensitivity of pleural fluid GeneXpert MTB/RIF examination was calculated it was 10.4% whereas, the calculated specificity was 72.8%. While +ve predictive value of GeneXpert was 22.2% and the -ve predictive value was 52.1%. The likelihood ratio was 8.25 and the ROC curve also showed similar values.

**Conclusion:** The sensitivity of pleural fluid GeneXpert MTB/RIF assay is very low and specificity is moderate for diagnosis of pleural tuberculosis when compared with pleural biopsy.

**Keywords:** Extra pulmonary tuberculosis; GeneXpert; Pleural biopsy; Tuberculosis.

## Introduction

Tuberculosis (TB), especially in developing countries is still a major health problem worldwide about 1.6 million patients were reported dead due to this pathology and 10 million new confirmed patients were recorded in 2017.<sup>1</sup> Pulmonary tuberculosis is the major and commonly present sign of TB, whereas lower respiratory tract. Tuberculosis ranges from 15-40% of reported cases,<sup>2</sup> but its prevalence rises to 50% in patients with positive HIV tests. Solovic et al. reported extra-pulmonary TB in the European Union from 4-48%.<sup>3</sup> One of the commonest forms of extra-pulmonary TB is pleural TB.<sup>4</sup> As this form is paucibacillary, the diagnosis is usually challenging, invasive, time-consuming, and expensive procedures such as pleural biopsy and thoracoscopy are used to confirm the diagnosis.<sup>5</sup> Adenosine deaminase (ADA) level in the pleural fluid has been widely studied<sup>6</sup>, interferon-gamma has been also used as a marker to diagnose pleural Tuberculosis with joint specificity and sensitivity of almost up to 100%, but it is unable to make difference between active tuberculosis and latent tuberculosis TB.<sup>7</sup>

After developing symptoms well-timed diagnosis of pleural tuberculosis is an urgent need for the reduction of associated comorbidities and mortality rate. Some new lab investigations such as nucleic acid amplification are considered a definite and rapid diagnostic tool for pulmonary tuberculosis. In recent times a fully automated, quantitative real-time PCR that is called Xpert MTB/RIF assay, was introduced and approved by World Health Organization in highly endemic areas, it can identify mycobacterium within 2 hours and also detects rifampicin resistance.<sup>8</sup> GeneXpert MTB/RIF assay is an emerging invention with high specificity and sensitivity and also with quick result generation in pulmonary TB.<sup>9</sup>

Presently there are few studies regarding the usage of Xpert MTB/RIF assay in the identification of pleural TB with a small sample size having low sensitivity and high specificity.<sup>10</sup> A study showed a sensitivity of 22.5% and specificity of 98%.<sup>11</sup> Different studies have shown sensitivity between 8% to 63% and specificity around 96.6% to 100%.<sup>5,7</sup> In endemic areas like Pakistan many patients with TB present with tuberculous pleural effusion, the reluctance of patients, and lack of a number of experts in performing blind pleural biopsy make GeneXpert MTB/RIF assay a good alternative for rapid diagnosis. Therefore, we planned this study to estimate the ability to correctly diagnose the pleural fluid GeneXpert MTB/RIF assay

in patients who with suspected of pleural tuberculosis considered as the gold standard for the pleural biopsy in our population

## Materials and Methods

This validation cross-sectional survey was approved by the Institutional Review Board of Pakistan Institute of Medical Sciences (PIMS) Islamabad, Pakistan (IRB No. PIMS/REC-203/18) and performed in accordance with the principles of the declaration of Helsinki. Written informed consents were taken from the patients. This study was piloted at the department of Pulmonology of the institute from 1st July 2018 to 31st December 2018. The calculated sample size was n=180 by using the WHO sample size calculator for observational studies,<sup>12</sup> [(the following parameters were used; the population proportion was 13.5% with CI 95% and alpha type I error (level of significance) was 5%)].<sup>2,3</sup> Patients presenting with fever or cough  $\geq$  2 weeks with exudative lymphocytic pleural effusion, age  $\geq$  18 years, without gender discrimination, were included by consecutive sampling. Patients already diagnosed or taking anti-tuberculous therapy, unwilling for the procedure, having bleeding disorders and severely ill patients were excluded from the study. Closed needle pleural biopsy of selected patients was done using ABRAM'S needle with the standard technique. Tissue taken for histopathology from patients was sent to the pathology section of the Pakistan Institute of Medical Sciences with appropriate cataloging and the results of reports were collected. Pleural fluid aspirated at the same time was also sent for GeneXpert MTB/RIF assay to the National Institute of Health Islamabad (NIH) and the results of reports were collected. A structured proforma was used to enter all findings. Socio-demographic factors like age, gender, and clinical information like history of fever, cough, etc., were also noted. For statistical analysis, the data was entered in SPSS version 20. Means and standard deviations were calculated for quantitative variables like age. Frequencies were calculated for gender. Specificity Sensitivity, negative predictive value, and positive predictive value of GeneXpert MTB/RIF assay were also assembled by using findings of pleural biopsy as the gold standard. The optimum cut-off value of GeneXpert MTB/RIF was calculated by using the trade-off between specificity and also sensitivity on the receiver-operating characteristic (ROC) curves, and the ability to correctly diagnose the disease, the GeneXpert MTB/RIF was estimated using the area under the receiver-operating curve (AUC).

## Results

A total of 180 participants were recruited for the study; 65.6% participants were male and 34.4% included were females. Also, the mean age of the participants was 47.58±16.95 years. Right-sided pleural effusion was more common, in 71.7% participants (n=129) as compared to left-sided pleural effusion 28.3% (n=51). GeneXpert was positive for MTB in 20% (n=36) participants only, it was negative in 80% (n=144). No patient was found to have rifampicin resistance. Histopathology of pleural biopsy confirmed tuberculous pleural effusion in 42.8% (n=77) patients, suggested by either in the form of caseous necrosis/granulomatous inflammation or both. GeneXpert was positive in only 10% (n=8) of patients with confirmed tuberculous pleural effusion (biopsy proven) shown in Table 1. Sensitivity was very low although specificity was more than 70% (Table 2).

**Table 1: Cross tabulation GeneXpert and pleural biopsy**

GeneXpert	Pleural Biopsy (caseous necrosis, granuloma)		Total
	Yes	No	
Detected	A = 8 (10%)	B = 28 (27%)	36
Not detected	C = 69 (90%)	D = 75 (73%)	144
Total	77	103	180

Chi square value = 7.768  
p-value = 0.005

**Sensitivity** =  $A / (A + C) \times 100$

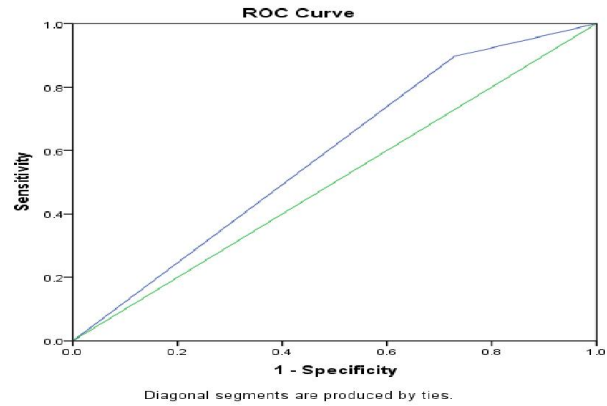
**Specificity** =  $D / (D + B) \times 100$

**Positive predictive value** =  $A / (A + B) \times 100$

**Negative predictive value** =  $D / (D + C) \times 100$

**Table 2: Pleural fluid GeneXpert MTB/RIF assay sensitivity and specificity**

Sensitivity	10.4%
Specificity	72.8%
Positive predictive value	22.2%
Negative predictive value	52.1%
Likelihood Ratio	8.25



**Figure 1: ROC and AUC curve of pleural fluid GeneXpert MTB/RIF assay sensitivity and specificity**

**Table 3: Coordinates of the curve of pleural fluid GeneXpert MTB/RIF assay sensitivity and specificity**

Positive if Greater Than or Equal To <sup>a</sup>	Sensitivity	1-Specificity
.0001	1.000	1.000
1.5000	.896	.728
3.0000	.001	.001

Area = 0.584

GeneXpert has at least one connection between the negative actual state group and the positive actual state group. The blue line curve shows the sensitivity (AUC value = 0.896).

a. The least cut-off value -1 which is the minimum observed test value and +1 is the largest cut-off value of the maximum observed test. All other observed test values are the averages of two consecutive orders.

**ROC Curve:** also shows that under the curve area is below 60% (Area value = 0.584) with a value  $\leq 0.6$ . The area under the curve shows that GeneXpert is not suitable for the diagnosis of tuberculous pleural effusion.

## Discussion

We found that the sensitivity of GeneXpert MTB/RIF assay to diagnose tuberculous pleural effusion, taking pleural biopsy as the gold standard was only 10.4% which is very low, whereas specificity was 72.8%. +ve predictive value was 22.2% and -ve predictive value was 52.1%. The diagnostic accuracy of the GeneXpert MTB/RIF assay was 46.1% in our study.

Pleural TB was diagnosed in 43% of patients with exudative pleural effusion in our study based on pleural biopsy. Many pleural diseases present with exudative pleural effusion, tuberculosis is one of the common causes. Many other studies have the same findings, in a study conducted in Egypt 55.6% of patients with exudative pleural effusion were diagnosed as a case of tuberculosis based on pleural biopsy, comparable with our results.<sup>13</sup> A local study conducted by Akhter et al.,<sup>14</sup> showed that 67.7% of patients with exudative pleural effusion were having tuberculosis, nonspecific inflammation was found in 13.6% and malignancy was diagnosed in 11.6%, also comparable with our results.

Although there are lots of studies showing promising results of GeneXpert MTB/RIF assay for quick detection of mycobacterium in respiratory samples but research on the use of GeneXpert MTB/RIF assay to detect mycobacterium in non-respiratory specimens, especially in pleural fluid is very limited. Results are conflicting as well with regard to the diagnostic performance of GeneXpert MTB/RIF assay in non-respiratory specimens. Sensitivity was found at 48% for pleural TB in a study conducted by Aref et al.<sup>15</sup> Although sensitivity is not high enough to consider GeneXpert as a good test to rule out pleural TB in this study but it is high as compared to our results, it was only 10% in our study. This difference may be due to the smaller number of participants in this study. A study conducted by Li et al.<sup>16</sup> showed a sensitivity of 68.8% with an adequate sample size. These results are in contrast with our results, but there was an important difference in methodology as Li et al., used pleural tissue and we used pleural fluid to detect mycobacterium by GeneXpert which may be the reason for high sensitivity. Sensitivity was quite low in many studies where pleural fluid was used like our study supporting our findings.<sup>10,13</sup> As pleural TB is a paucibacillary infection, that may be the cause of low sensitivity of GeneXpert in pleural TB where pleural fluid is used for GeneXpert.

The specificity of GeneXpert was found very high to diagnose pleural TB in previous studies. Specificity was 100% in two Egyptian studies.<sup>13,15</sup> A Chinese study showed that specificity is 98.6%.<sup>17</sup> Specificity in our study was 72.8%, which is lower compared to previous studies. Again, this difference may be due to less sample size in previous studies; the sample size was 27, 71, and 134 respectively in these studies which is small as compared to our study. High specificity with a small sample size is not reliable. One study showed 65% specificity when compared with a

composite reference standard (CRS),<sup>16</sup> almost comparable with our results.

The positive predictive value was 22.2%, the negative predictive value was 52.1% and the area under the curve (ROC) was 58% in our study. Results from previous studies are conflicting in this regard. The positive predictive value and negative predictive value of GeneXpert were 56.4 and 75.6%, respectively in a study conducted in China,<sup>16</sup> supporting our results. The positive predictive value was 100% in a local study conducted in Karachi,<sup>14</sup> but pleural tissue was used instead of pleural fluid, which may be the cause of the high positive predictive value in this study. Another study<sup>16</sup> showed 100% +ve predictive value in contrast to our findings but -ve predictive value was 67% for pleural TB almost comparable with our findings. Conflicting results of previous studies and disappointing results from our study indicate that GeneXpert cannot be considered a good diagnostic test for pleural TB.

Our study had some limitations; we only used pleural biopsy as the gold standard. Some previous studies also used a composite reference standard (CRS) along with a pleural biopsy. Secondly, it was a single-center study. We suggest multi-center studies using a composite reference standard (CRS) along with a pleural biopsy.

## Conclusion

Our study concluded that GeneXpert MTB/RIF assay, when applied on pleural fluid, has poor sensitivity as compared to histopathological examination of pleural tissue for the diagnosis of pleural tuberculosis. However, specificity is much better as compared to sensitivity. So, we didn't recommend isolated use of pleural fluid GeneXpert MTB/RIF assay for diagnosing tuberculous pleural effusion.

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