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ORIGINAL ARTICLE

MTB detection frequency using GeneXpert MTB/RIF ultra assay on pleural tissue in the evaluation of exudative lymphocytic effusion

Muhammad Abrar, Faisal Faiyaz Zuberi, Jawad Asad, Raj Kumar, Muniba Aslam, Zafarullah

Ojha Institute of Chest Diseases, Dow University of Health Sciences, Karachi Pakistan

ABSTRACT

Background: Pleural tuberculosis is a paucibacillary disease, which makes the diagnosis of tuberculous pleural effusion particularly challenging. The study aimed to establish the accuracy of GeneXpert MTB / RIF Ultra in the diagnosis of *Mycobacterium tuberculosis* in pleural biopsy samples.

Material and Methods: It was a cross-sectional observational study, done at the Department of Pulmonology, Ojha Institute of Chest Disease, Dow University of Health Sciences, Karachi, following institutional ethical approval. Adult patients with exudative lymphocytic pleural effusion were enrolled by consecutive sampling. Biopsy samples of pleura have been collected through medical thoracoscopy or image-guided methods and examined using GeneXpert MTB/RIF Ultra. The data had been analyzed with SPSS version 25, and effect modifiers were stratified; $p < 0.05$ was regarded as significant.

Results: A total of 130 patients were evaluated. Mean age was 47.3 ± 16.8 years. In 88 (67.7%) patients, histopathological findings were suggestive of tuberculosis. GeneXpert MTB/RIF Ultra assay showed a sensitivity of 36.4%, specificity of 90.5%, PPV of 88.9% and NPV of 40.4%. Stratification analysis revealed significant correlations of GeneXpert positivity with age ($p = 0.041$), effusion laterality ($p = 0.028$) and lymphocyte predominance ($p = 0.019$).

Conclusion: GeneXpert MTB/RIF Ultra has high specificity and low sensitivity in pleural biopsy samples. The high positive predictive value of tuberculous pleura however, justifies its use as a confirmatory test in suspected tuberculous pleural effusion.

Keywords: Biopsy, Molecular diagnostic techniques, Pleural, Pleural effusion, Tuberculosis

BACKGROUND

Tuberculosis remains a major global public health issue. Extrapulmonary tuberculosis (EPTB) imposes a significant disease burden, and one of the most common EPTB is tuberculous pleurisy (TP). It typically presents as exudative lymphocytic pleural effusion.[1] The diagnosis of TP is challenging, and the core cause of this difficulty is the low bacterial load of the infection, as well as lack of specificity in clinical imaging features.[2]

Conventional methods for diagnosing TP, such as pleural effusion analysis and biochemical testing, have insufficient sensitivity.[3] Histopathology and pleural biopsy, while accurate, are highly invasive and difficult to implement as routine clinical practice, which has led to a long-standing gap in the timely and accurate diagnosis of TP.[4]

Molecular diagnostic technologies for tuberculosis, represented by Xpert MTB/RIF and Xpert MTB/RIF Ultra, can rapidly detect pathogenic bacteria and drug resistance, significantly improving diagnostic efficiency.[5] However, their performance is affected by sample type, and they show low yield in extrapulmonary samples such as pleural fluid.[6]

Multiple published studies show that Xpert MTB/RIF Ultra, used for the diagnosis of TP, generally has high specificity but variable sensitivity.[7] The detection performance of GeneXpert testing on pleural biopsy specimens however is superior to that on body fluids. Due to which global tuberculosis prevention and control strategies emphasize the importance of rapid molecular diagnosis in TP.[8]

Xpert MTB/RIF have moderate diagnostic sensitivity for TP, so they can only serve as an auxiliary diagnostic tool and cannot be used independently to confirm a diagnosis.[9] The new Xpert MTB/RIF Ultra assay can raise the early detection rate of extrapulmonary tuberculosis, is compatible with multiple sample types, supports non-invasive sampling, and expands the range of applicable scenarios for clinical diagnosis.[10]

Correspondence: Dr. Muhammad Abrar, Resident Pulmonology, Department of Pulmonology, Ojha Institute of Chest Diseases, Karachi Pakistan

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We aim to evaluate the detection frequency of Mycobacterium tuberculosis by Xpert MTB/RIF Ultra and to find its sensitivity and specificity in TP.

MATERIAL AND METHODS

This was a cross-sectional observational study in the Department of Pulmonology, Ojha Institute of Chest Disease, Dow University of Health Sciences, Karachi, from 10st July 2025 – 30th December 2025, receiving approval of the Institutional Review Board (IRB No: IRB-4004/DUHS/Approval/2025/249 dated 2nd Jul 2026). All the human subjects were studied following the principles of the Declaration of Helsinki and informed consent was signed by every participant, who was enrolled in the study.

A sample size of 130 patients was determined based on the recent prospective study of (Cartridge-Based Nucleic Acid Amplification Test) CBNAAT on pleural biopsy samples in patients with unexplained pleural effusion, which reported sufficient diagnostic performance with less than 150 participants [11]. The sample size was determined using the OpenEpi online sample size calculator with the following assumptions; anticipated sensitivity of 36%, confidence level of 95% and margin of error of 5%, which gave the minimum required sample size of 124 patients; hence 130 patients were used to accommodate potential sources of data loss and enhance precision of the study.

Consecutive recruitment of adult patients aged 18-60 years of either gender with exudative lymphocytic pleural effusion were included. Exudative effusion was defined according to Light's criteria.[12]

To achieve homogeneity of the study population, patients were excluded in case they had already undergone anti-tuberculosis therapy, transudative pleural effusion, known malignancy, and other immunocompromising states. Furthermore, the patients with negative GeneXpert MTB/RIF Ultra on pleural fluid without any subsequent tissue examination were not taken as definite and were considered only when they had pleural biopsy to verify. The thorough clinical assessment with history taking, physical examination, and initial laboratory studies (complete blood count, serum protein, and lactate dehydrogenase) were conducted. Radiological evaluation, including the chest X-ray and, when necessary, the computed tomography of the thorax, was conducted to describe the effusion.

Diagnostic thoracentesis was done in aseptic conditions. The pleural fluid was examined for protein, lactate dehydrogenase and differential count of leukocytes to ascertain exudative lymphocytic effusion according to Lights criteria. Although pleural fluid evaluation was done in the first place, unmatched cases were subjected to pleural biopsy. Pleural tissue was either acquired through medical thoracoscopy or percutaneous pleural biopsy through image guidance based on clinical indication and anatomy. Six pleural tissue samples were collected in each patient and of these:

- Two samples were placed in normal saline for GeneXpert MTB/RIF Ultra
- Four samples were impregnated in formalin to be examined using histopathology.

For GeneXpert MTB/RIF Ultra all biopsy samples were taken to the microbiology laboratory in DNAase/ RNAase-free containers. The assay was carried out as per manufacturer recommendations: homogenized tissue was combined with sample reagent in a 2:1 ratio, incubated at room temperature and subjected in the GeneXpert system to automated detection of *Mycobacterium tuberculosis* complex and rifampicin resistance. The results were reported as positive, negative, or invalid; the invalid results were repeated once. We will compare the findings with the histopathological examination of the pleural tissue. The data were documented on a structured proforma with demographic, clinical, radiological and laboratory parameters.

The data were processed through SPSS (Statistical Package for the Social Sciences) version 25. Descriptive statistics were used, and categorical variables were given in forms of frequencies and percentages, and continuous variables were shown as means standard deviation. The diagnostic performance of the GeneXpert assay was determined by determining the sensitivity, specificity, positive predictive value and negative predictive value based on histopathology as the reference standard. The paramount effect modifiers including age, gender, and pleural fluid were stratified and post-stratified chi-square tests were done, and a p-value 0.05 or less was regarded as statistically significant.

RESULTS

A total of 130 patients were included in the study. The mean age was 47.3 ± 16.8 years. The most common presenting symptom was dyspnea observed in 101 (77.7%) patients, followed by fever in 96 (73.8%) and cough in 88 (67.7%), whereas weight loss was the least

common symptom, reported in 64 (49.2%) patients. Symptoms lasting more than one month were noted in 76 (58.5%) participants, while 54 (41.5%) had symptom duration ≤1 month (Table-I).

Pleural fluid analysis demonstrated exudative effusion with a mean pleural fluid protein level of 4.8 ± 0.9 g/dL and mean LDH level of 512 ± 168 IU/L. Lymphocyte predominance was present in 118 (90.8%) patients. (Table-II).

Histopathological examination demonstrated caseating granulomatous inflammation in 88 (67.7%) patients, while 42 (32.3%) patients showed no histopathological features suggestive of tuberculosis. GeneXpert MTB/RIF Ultra yielded positive results in 36 (27.7%) patients and negative results in 94 (72.3%) patients. Among the GeneXpert-positive cases, 32 patients had histopathological findings suggestive of tuberculosis, whereas 4 patients lacked such findings. Among the GeneXpert-negative cases, 56 patients showed histopathological features suggestive of tuberculosis and 38 did not (Table-III).

The GeneXpert MTB/RIF Ultra assay demonstrated a sensitivity of 36.4% and specificity of 90.5%. The positive predictive value was 88.9%, while the negative predictive value was 40.4% (Table-IV). This table presents the 2 × 2 diagnostic contingency analysis of GeneXpert MTB/RIF Ultra against histopathological confirmation of tuberculosis, along with key measures of test performance.

Stratification analysis demonstrated statistically significant associations between GeneXpert positivity and age ($p = 0.041$), effusion laterality ($p = 0.028$), and lymphocyte predominance ($p = 0.019$). Patients aged >40 years showed GeneXpert positivity in 22 (27.5%) cases, while 14 (29.2%) patients aged ≤40 years tested positive. Unilateral pleural effusion was associated with higher GeneXpert positivity [34 (29.6%)] compared with bilateral effusion [2 (15.4%)]. Similarly, lymphocyte-predominant pleural fluid showed higher positivity [35 (29.7%)] than non-lymphocytic effusions [1 (8.3%)]. No statistically significant association was observed between gender and GeneXpert positivity ($p = 0.312$) (Table-IV).

Table-I: Baseline demographic and clinical characteristics of study participants (n = 130)

Variable	Category	Frequency n (%)
Age (years)	Mean ± SD	47.3 ± 16.8
Gender	Male	78 (60.0%)
	Female	52 (40.0%)
Common Symptoms	Fever	96 (73.8%)
	Cough	88 (67.7%)
	Dyspnea	101 (77.7%)
	Weight loss	64 (49.2%)
Duration of symptoms	≤1 month	54 (41.5%)
	>1 month	76 (58.5%)

Table-II: Pleural Fluid and Radiological Characteristics of Study Participants (n = 130)

Variable	Category	Frequency n (%)
Pleural Effusion Side	Right	69 (53.1%)
	Left	48 (36.9%)
	Bilateral	13 (10.0%)
Pleural Fluid Protein (g/dL)	Mean ± SD	4.8 ± 0.9
Pleural Fluid LDH (IU/L)	Mean ± SD	512 ± 168
Lymphocyte Predominance	Yes	118 (90.8%)

Table-III: Diagnostic yield of GeneXpert MTB/RIF ultra-compared with histopathological findings suggestive of tuberculosis (n = 130).

Test Outcome	Histopathology Suggestive of TB n (%)	Histopathology Not Suggestive of TB n (%)	Total n (%)
GeneXpert Positive	32 (24.6%)	4 (3.1%)	36 (27.7%)
GeneXpert Negative	56 (43.1%)	38 (29.2%)	94 (72.3%)
Total	88 (67.7%)	42 (32.3%)	130 (100%)

Table-IV: 2 × 2 Diagnostic contingency table and performance of GeneXpert MTB/RIF ultra compared with histopathology (n = 130).

GeneXpert MTB/RIF Ultra	Histopathology Positive	Histopathology Negative	Total
Positive	32 (TP)	4 (FP)	36
Negative	56 (FN)	38 (TN)	94
Total	88	42	130

DISCUSSION

In the current study, the diagnostic attribute of GeneXpert MTB/RIF Ultra was assessed among patients with exudative lymphocytic pleural effusion and found low sensitivity and high specificity to detect *Mycobacterium tuberculosis*. These results align with the renewed guidelines emphasizing the role of rapid molecular diagnostics as add-on tests, but not as isolated tests in extra pulmonary TB, especially in paucibacillary cases of TB, like pleural TB.[12]

The sensitivity in this study (36.4%) is relatively low when compared with certain past studies and the possible reason is the low bacillary load in the pleural tissues samples. A systematic review has demonstrated that Xpert Ultra has variable sensitivity in the extra pulmonary sample, and that diagnostic yield is strongly affected by the type of specimen and bacterial burden[13]. Similar results have been evident in the regional studies where GeneXpert was found to have a low sensitivity in lymph node and pleural samples but was found to be highly specific to confirmative test and not for screening modality.[14]

High specificity (90.5%) and positive predictive value (88.9%) observed in our study demonstrates that a positive GeneXpert result has a high rate of correlation with true disease. This is in line with the past comparative research findings in which GeneXpert demonstrated an outstanding rule-in sensitivity in tuberculosis despite inconclusive results using traditional diagnostic procedures like smear microscopy or culture.[15] In addition, Xpert Ultra has developed further with better detection limits to increase its sensitivity to low bacillary burdens, though sensitivity remains impaired in extra pulmonary settings.[16]

Prospective observational studies that show enhanced diagnostic accuracy of combined methods that include histopathology, biochemical markers, and molecular assays also confirm this.[18] GeneXpert MTB/RIF Ultra demonstrated low sensitivity (36.4) and failed to detect a significant proportion of histopathology-confirmed cases in the present study, which is a characteristic of paucibacillary pleural tuberculosis. Nevertheless, its high specificity and the possibility to identify *Mycobacterium tuberculosis* and rifampicin resistance in a short period of time offer valuable complementary value. The combination of Xpert MTB/RIF Ultra and histopathological examination will guarantee optimal diagnostic yield and benefit clinical decision-making. Consequently, any test should be performed simultaneously to improve the accuracy of the diagnosis and enable early and specific treatment.[18]

Recent publications also note that diagnosis of tuberculous pleural effusion must be based on multimodal approach which includes clinical, radiological, microbiological, and histopathological evidence.[19] In our case, lymphocytic effusion and related clinical manifestations prevailed, which helped to make the diagnosis, but GeneXpert served as a quick adjunctive test. It has employed histopathology as a reference standard, which provides a useful diagnostic benchmark for identifying tuberculous pleural effusion and thereby enhances the internal validity of the results The sample size is reasonably large which enhances the reliability. It is acknowledged that mycobacterial culture remains the gold standard for tuberculosis diagnosis; however, it was not utilized in this study. The limitations of our study are, the research was conducted in one center and thus, it could be said that the study could not be generalized to other populations or care environments. Second, the sensitivity of GeneXpert was probably underrated in other types of specimen by the inherent paucibacillary character of pleural tuberculosis. Third, mycobacterial culture, which remains the gold standard for tuberculosis diagnosis, was not performed, which could have provided further microbiological validation and comparison. In spite of these shortcomings, the research is an insight into the clinical diagnostic performance of GeneXpert MTB/RIF Ultra in pleural tuberculosis.

CONCLUSION

The GeneXpert MTB/ RIF Ultra assay on pleural tissue was found to be highly specific but low in sensitivity (36.4%). GeneXpert MTB/ RIF Ultra along with histopathology can serve as a prompt confirmation of TB when positive and could be utilized in clinical

decision-making, especially in the context of a setting that necessitates rapid diagnosis.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Muhammad Abrar: Substantial contributions to study design and acquisition of data, manuscript drafting and critical review for important intellectual content, final approval, accountable for all aspects of research.

Jawad Asad: Substantial contributions to acquisition of data, manuscript drafting and critical review for important intellectual content, final approval, accountable for all aspects of research.

Raj Kumar: Substantial contributions to analysis and interpretation of data, critical review of the manuscript, final approval, accountable for all aspects of research.

Muniba Aslam: Substantial contributions to concept and study design, critical review of the manuscript, final approval, accountable for all aspects of research.

Zafarullah: Substantial contributions to acquisition and interpretation of data, critical review of the manuscript, final approval, accountable for all aspects of research.

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