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Sensitivity of GeneXpert in pleural biopsy specimens in diagnosing tuberculosis in undiagnosed exudative pleural effusion

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Abstract

Pleural effusion is among the most common forms of paucibacillary extrapulmonary tuberculosis. Diagnosis is often clinical or based on elevated pleural fluid adenosine deaminase (ADA) levels. However, diagnostic uncertainty arises when clinical suspicion remains high despite low ADA levels, especially in patients already on empirical anti-tubercular therapy, which reduces bacillary load. The aim of this study is to evaluate the diagnostic utility of the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in pleural biopsy samples with histopathologically confirmed tuberculosis. In this prospective study, 260 patients with undiagnosed pleural effusion who underwent pleural biopsy *via* thoracoscopy or ultrasound guidance were included. Histopathological examination identified 90 patients with pleural tuberculosis (study group) and 170 with non-tubercular etiology (control group). CBNAAT results from pleural biopsy specimens were analyzed to determine diagnostic performance. This study found the sensitivity, specificity, positive predictive value, and negative predictive value of CBNAAT for detecting pleural tuberculosis were 23.3% (21/90), 98.2% (167/170), 87.5% (21/24), and 70.8% (167/236), respectively. Sensitivity was higher (40%) in patients with pleural fluid ADA >40 IU/L compared to those with ADA <40 IU/L (15%). Notably, 4 of 90 patients (4.4%) in the study group were found to have rifampicin-resistant tuberculosis—these patients were on first-line anti-TB treatment at the time of biopsy. The conclusion of this study was that CBNAAT demonstrates low sensitivity but high specificity for diagnosing pleural tuberculosis from pleural biopsy samples. It is a valuable tool not only for early microbiological confirmation but also for detecting rifampicin resistance.

Key words: undiagnosed pleural effusion, pleural tuberculosis, CBNAAT in pleural biopsy.

Introduction

Tuberculosis (TB) remains the most common cause of exudative pleural effusion in endemic regions such as India. The diagnosis of tuberculous pleural effusion (TPE) is often presumptive and is primarily based on the presence of a lymphocytic predominant, straw-coloured exudative effusion with elevated adenosine deaminase (ADA) levels, typically above 40 U/L. ADA has a reported sensitivity of approximately 90% and specificity of 85% for the diagnosis of TPE in high-prevalence settings [1,2].

However, in cases where clinical suspicion persists despite inconclusive fluid analysis, histopathological confirmation through pleural biopsy becomes essential. This can be performed under image guidance (ultrasound or CT) or via thoracoscopy, depending on available resources. Histology often reveals necrotizing (caseating) granulomas, which are characteristic of TB [3].

Bacteriological confirmation of TPE using conventional microbiological tools—such as smear microscopy, culture, and nucleic acid amplification tests like CBNAAT (Cartridge-Based Nucleic Acid Amplification Test)—is notoriously difficult due to the paucibacillary nature of the disease. Sensitivity of smear microscopy is typically <10%, culture ranges from 24–50%, and CBNAAT on pleural fluid demonstrates a sensitivity of approximately 20–30% [4-6]. Despite the low yield, CBNAAT offers the added advantage of rapid detection and concurrent assessment of rifampicin resistance, which is critical for guiding therapy [7].

Interestingly, even patients with ADA levels below the traditional cutoff may still have tuberculous effusions. Studies suggest that up to 10–20% of TPE cases may present with ADA <40 U/L and still be confirmed as TB on pleural biopsy [8].

Given the limitations of pleural fluid-based diagnostics, we aimed to evaluate the diagnostic performance of CBNAAT when applied to pleural biopsy specimens in cases of histologically confirmed tuberculous pleuritis. This approach could offer a faster diagnostic alternative to histopathology and simultaneously provide vital drug susceptibility information.

Aims and objectives

Aim: to evaluate the diagnostic performance of CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) on pleural biopsy specimens in patients with histologically confirmed tuberculous pleural effusion.

Objectives: i) to assess the sensitivity and specificity of CBNAAT performed on pleural biopsy tissue in diagnosing tuberculous pleural effusion; ii) to determine the proportion of cases in which CBNAAT detects rifampicin resistance from pleural biopsy specimens; iii) to evaluate the time to diagnosis using CBNAAT versus conventional histopathological examination.

Materials and Methods

This is Prospective, Observational study done in a university hospital in north India. Duration of study: 1st November 2021 to 31st October 2024. Ethical Approval: Ethical approval of this study had taken from institutional ethical committee (IEC) (Ref. code: V-PGTSC-11A/P20).

Study population and sample derivation

This prospective observational study included all consecutive eligible patients with undiagnosed exudative pleural effusion who underwent pleural biopsy (thoracoscopic or ultrasound-guided) at our centre during the study period. Patients were enrolled according to predefined inclusion and exclusion criteria, without a pre-fixed numerical target, in order to maximise the number of pleural biopsy samples available for evaluation and to allow precise estimation of the diagnostic performance of CBNAAT on pleural tissue. For analysis, patients were subsequently classified into two groups based on histopathological examination of the pleural biopsy: those with features consistent with tuberculous pleuritis (study group) and those with non-tubercular etiologies (control group).

Inclusion criteria

- Age > 18 years for both male and females
- Undiagnosed pleural effusion (defined as exudative pleural effusions as per Light's criteria with Gene X pert and 2 times cytology negative for malignancy).

Exclusion criteria

- Patients who are not fit for performing thoracoscopy or USG guided pleural biopsy as in the following cases: -
    - Those with severe uncorrected hypoxemia despite continuous oxygen administration.
    - Patients with unstable hemodynamics.
    - Patients with coagulation defects.
- Pregnant female patients.
- Patients who did not give consent for thoracoscopy/ USG guided biopsy.

Methods

All enrolled patients were subjected history, examination and routine blood investigations like Complete Hemogram, Kidney function test, liver function test, Viral markers, coagulation profile, radiology like chest x ray, Computed tomogram of thorax etc.

All eligible patient were screened under ultrasound for pleural anatomy like maximum collection of fluid, pleural nodule, mass etc. If suitable site could be identified using USG,

guided biopsy was taken, otherwise thoracoscopy was performed. Also when USG guided biopsy was inconclusive, thoracoscopy was performed in such cases.

Biopsy sample sent for histopathological examination and CBNAAT. The CBNAAT test was done by Gene Xpert Ultra Cartridge.

Biopsy specimen demonstrating features of tuberculosis in histopathological examination were kept in Group A (Study Group) and those patients who were non tubercular in histopathology were kept in Group B (Control Group). Pleural biopsy specimens were examined by an experienced histopathologist. Tuberculous pleural effusion (Group A) was defined by the presence of necrotising (caseating) granulomas and/or epithelioid cell granulomas with or without Langhans-type giant cells in the appropriate clinical and radiological context, after exclusion of alternative granulomatous diseases such as sarcoidosis and fungal infections and of malignancy based on the clinical scenario. Cases showing only non-specific chronic inflammatory changes without granuloma or caseation were classified as non-tubercular (Group B). Diagnostic abilities of CBNAAT such as sensitivity, specificity, Positive predictive value (PPV) and negative predictive value (NPV) were calculated by comparing both group.

Statistical analysis

All the data were collected, tabulated and analysed using the statistical package for the social sciences (SPSS version 25). The results were presented as mean ± SD or percentage. Differences in categorical data were compared using the chi-square test of Fisher exact test. Paired data were compared using student T test. A p value of < 0.05 was considered as statistically significant.

Results

A total of 260 eligible patients with undiagnosed pleural effusion underwent pleural biopsy. Out of 260 patients, 90 patients were found to have tuberculosis on histopathological examination (Group A) and 170 patients had a Non-Tubercular etiology of pleural effusion on histopathological examination (Group B). Of these, 190 (73.1%) underwent thoracoscopic biopsy, while 70 (26.9%) underwent ultrasound-guided pleural biopsy.

The baseline characteristics of all 260 participants are shown in Table 1. The cohort had a slight male predominance and was predominantly in the fourth decade of life, with cough, chest pain and weight loss as the most common presenting symptoms and diabetes and hypertension as the most frequent co-morbidities. The pleural fluid characteristics of the study group (Group A) are summarized in Table 2. Most effusions were unilateral and straw-coloured, with exudative protein levels, lymphocyte predominance and moderate ADA values, and ultrasound frequently demonstrated pleural thickening and free-flowing

effusions. CBNAAT (Gene Xpert) performed on pleural biopsy specimens was positive in 21 patients, yielding an overall sensitivity of 23.3% (21/90) for diagnosing pleural tuberculosis.
Specificity, Positive Predictive value (PPV) and Negative Predictive value (NPV) of CBNAAT is 98.2 % (167/170), 87.5% (21/24) and 70.8 % (167/236), respectively, in the diagnosis of pleural tuberculosis in pleural biopsy samples (Table 3).
Among the study group of 90 patients of tuberculosis, Gene Xpert positivity was seen in 15 of 66 thoracoscopic biopsies (22.7%) and 6 of 24 ultrasound-guided biopsies (25.0%). The diagnostic yield of CBNAAT was comparable between the two biopsy methods, with no statistically significant difference ($p = 0.81$).
Gene Xpert demonstrated an overall sensitivity of 23.3% (21/90) when performed on pleural biopsy specimens. When stratified by pleural fluid ADA levels, the sensitivity was significantly higher in patients with ADA >40 IU/L (40%) compared to those with ADA <40 IU/L (15%). This difference was statistically significant, with an odds ratio of 3.715 (95% CI: 1.335–10.67) and a p-value of 0.01189, indicating that higher ADA levels are associated with an increased likelihood of CBNAAT positivity in pleural biopsy specimens. This is shown in Table 4.
Among the CBNAAT-positive study-group patients, rifampicin resistance was identified in 4 cases, as detailed in Table 5. Additionally, the mean time to result for CBNAAT was significantly shorter at 2 days, compared to 14 days for histopathology. This highlights the added advantage of CBNAAT in not only providing rapid diagnosis but also offering critical drug resistance information, which can guide early initiation of appropriate therapy.

Discussion

The study evaluated the diagnostic utility of CBNAAT (Gene Xpert) performed on pleural biopsy specimens in patients with histologically confirmed tuberculous pleural effusion. The overall sensitivity of CBNAAT on pleural tissue in our cohort was modest, whereas specificity was very high, indicating that a positive result is highly reliable in this paucibacillary form of extrapulmonary tuberculosis. These findings are in keeping with earlier work on molecular tests in pleural TB, which also report relatively low sensitivity but good specificity for tissue-based assays [9,10].
An important finding of our study is the clear influence of pleural fluid ADA on CBNAAT performance. Sensitivity increased from 15% in patients with ADA <40 IU/L to 40% in those with ADA >40 IU/L, with a statistically significant association between higher ADA levels and CBNAAT positivity on pleural biopsy [11]. This supports the concept that ADA reflects cell-mediated immune activation and, indirectly, mycobacterial burden, and that molecular tests are more likely to be positive when the immunological and bacillary load is higher. At the same time, the presence of biopsy-proven TB in patients with ADA below the

conventional cut-off in our cohort underscores the limitation of relying on ADA alone and highlights the potential role of pleural biopsy plus CBNAAT in such patients.

We also observed that CBNAAT yields were comparable between thoracoscopic and ultrasound-guided pleural biopsy approaches, with positivity rates of 22.7% and 25.0%, respectively, and no statistically significant difference between the two techniques. This suggests that, when performed skilfully, image-guided closed biopsy can provide diagnostically adequate tissue for both CBNAAT and histopathology, and may serve as a practical alternative to thoracoscopy in centres where thoracoscopy is not routinely available [12,13].

Beyond diagnostic confirmation, CBNAAT offered two clinically important advantages in this study. First, it provided simultaneous detection of rifampicin resistance, with 4 of 21 CBNAAT-positive cases showing rifampicin-resistant disease on pleural biopsy [14]. Early recognition of drug resistance is crucial in pleural TB because many patients are already on empirical first-line therapy, and delays in adjusting treatment can adversely affect outcomes and facilitate transmission of resistant strains. Second, CBNAAT significantly reduced diagnostic turnaround time compared with histopathology, with results available in a mean of 2 days versus 14 days for conventional histological reporting [15]. In patients with significant symptoms or comorbidities, this rapid availability of actionable information can be particularly valuable for clinical decision-making.

Despite these strengths, several limitations must be acknowledged. The overall sensitivity of CBNAAT on pleural biopsy remained modest, likely reflecting the low bacillary load and patchy distribution of mycobacteria within pleural granulomas [16]. Our primary analysis was restricted to histology-confirmed TB and non-TB pleural diseases, which allowed robust estimation of sensitivity and specificity but did not fully capture performance in unselected pleural effusions. In addition, a substantial proportion of patients had received empirical antitubercular therapy before biopsy, which could have further reduced bacillary load and negatively influenced CBNAAT yield.

Notwithstanding these limitations, the findings support the inclusion of CBNAAT testing on pleural biopsy specimens as a complementary tool in the diagnostic algorithm for suspected tuberculous pleural effusion. In patients with exudative effusions, particularly those with uncertain ADA profiles, pleural biopsy combined with CBNAAT can provide rapid microbiological confirmation and critical information on rifampicin resistance, while histopathology remains the definitive reference for diagnosis. In endemic settings where empirical treatment is common and delays in drug susceptibility testing are frequent, this combined approach may help bridge the diagnostic gap, guide timely modification of therapy and improve patient outcomes.

Conclusions

CBNAAT performed on pleural biopsy specimens demonstrates modest sensitivity but very high specificity for the diagnosis of tuberculous pleural effusion, with better performance in patients who have higher pleural fluid ADA levels. In addition to confirming disease, CBNAAT offers rapid turnaround time and early detection of rifampicin resistance, providing information that histopathology alone cannot. Pleural biopsy with CBNAAT should therefore be considered a complementary tool to histopathology in the diagnostic workup of exudative pleural effusions in endemic settings, particularly when fluid-based tests are inconclusive or drug resistance is a concern.

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Table 1. Baseline characteristics of patients.

Variables	Number/Mean
Gender (N%)	
Male Female	150 (57.7%) 110 (42.3%)
Mean Age in Years	
Male Female	42.5 38.7
Symptoms at Presentation (N%)	
Fever Cough Chest pain Night sweat Loss of appetite and weight	66 (25.4%) 179 (68.9%) 141 (54.4%) 26 (10%) 150 (57.8%)
Co-Morbidity (N%)	
Diabetes Hypertension Coronary Artery disease Hypothyroidism Connective Tissue Disease Chronic Liver disease	67 (25.6%) 55 (21.1%) 23 (8.9%) 17 (6.7%) 6 (2.2%) 6 (2.2%)
Past History of Tuberculosis (N%)	37 (14.2%)
History of Empirical Anti TB intake for current illness (N%)	156 (60%)

Table 2. Baseline pleural effusion characteristics in study group (Group A).

Side of pleural effusion(N%)	
Right Side Left Side Bilateral	48 (53.3%) 35 (38.9%) 7 (7.8%)
Colour of Fluid (N%)	
Straw colour Haemorrhagic Thin Pus Thick Pus Milky Colour	42 (46.7%) 18 (20%) 15 (16.7%) 12 (13.3%) 3 (3.3%)
Pleural Fluid Analysis	
Protein gm% Adenosine Deaminase IU/L Lymphocyte %	4.54 32.6 73%
USG Screening Prior to Procedure (N%)	
Pleural Thickening Loculations Free Fluids	65 (72.2%) 23 (25.6%) 67 (74.4%)

Table 3. Diagnostic accuracy of CBNAAT in pleural biopsy.

	Group A (HPE-tuberculosis)	Group B (HPE-non-tubercular)	Total
CBNAAT test Positive	21	03	24
CBNAAT test Negative	69	167	236
Total	90	170	

Table 4. Gene X pert sensitivity according to pleural fluid ADA in Group A (Chi Square and Exact Measures of Association).

	ADA>40 (n)	ADA<40 (n)	Total	ODDS Ratio (CI)	p-value
Gene X pert Positive	12	9	21	3.715 (1.335-10.67)	0.01189
Gene X Pert Negative	18	51	69		
Total	30	60	90		

Table 5. Rifampicin resistance status in CBNAAT-positive patients in the study group.

Total CBNAAT positive patient	Rifampicin resistance detected	Rifampicin resistance – not detected
21	4	17