Section: Respiratory Medicine

Original Research Article

PLEURAL FLUID GENEXPERT/ CBNAAT IN THE DIAGNOSIS OF TUBERCULAR PLEURAL EFFUSION

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Abstract

Background: The burden of Tuberculosis in our country remains high. Rapid detection of the disease helps in preventing its spread and also limiting complications. The diagnostic findings for diagnosing tuberculous pleural effusion is caseating granulomas in pleural biopsy or microscopic detection of TB bacilli in pleural fluid or growth of Mycobacterium in pleural biopsy culture. Unfortunately, the culture takes 8 to 12 weeks to report the results and even longer to do drug sensitivity to diagnose drug resistance. **Materials and Methods:** An observational study on 89 biopsy-proven and its culture proven cases of tuberculous pleural effusion was conducted to check for the utility of pleural fluid CBNAAT/ Genexpert for early diagnosis from Dec. 2020 to Oct. 2022. **Results:** The majority of 36(40%) patients belonged to the 46-60 years of age group. Majority of 81(91%) patients had pleural fluid ADA levels >40IU. The majority of 75(84%) patients wre negative for pleural fluid AFB on ZN microscopy. In the majority of patients 77(86%) MTB was not detected by the GENEXPERT test. All 89(100%) patients had typical granulomatous changes on biopsy and all 89(100%) patients were positive on pleural biopsy Culture. But we found that GeneXpert had a sensitivity of 13.4% and a specificity of 100% which is statistically significant ($p < 0.05$). **Conclusion:** We have found Gene Xpert insufficient in diagnosing Tubercular pleural effusion as a standalone test.

INTRODUCTION

In our country where Tuberculosis is endemic, TB remains the most common cause of Pleural effusion. Tuberculous pleural effusion is the second most common cause of extrapulmonary tuberculosis after TB Lymphadenitis.[1] Tuberculous pleural effusions can occur due to reactivation disease or it can be primary tuberculous effusion.[2] In adults, most commonly they occur due to the reactivation of disease.[3] in children, most commonly they occur as a result of primary disease.[4]

Tuberculous pleural effusion may occur because of delayed hypersensitivity reaction to mycobacteria or mycobacterial antigens in the pleural cavity in sensitized people.[5] or by rupture of a subpleural focus of tuberculous infection into the pleural space.[6]

In some patients without apparent parenchymal involvement, the pleural disease may rarely, develop due to hematogenous spread following primary focus.

Tuberculous pleural effusion can present with constitutional symptoms of fever, cough, weight loss, and a pleuritic type of chest pain. Such patients also happen to be malnourished and have a history of exposure to tuberculosis or have a past history of tuberculosis. The diagnosis of tuberculous pleural effusions can be made by demonstrating Mycobacterium tuberculosis in the pleural biopsy or pleural fluid; either via ZN stain or Culture or by GeneXpert. In 2011, WHO recommended pleural fluid Genexpert in the diagnosis of tuberculous pleural effusions. In Pulmonary tuberculosis, the use of Genexpert was indeed promising but was not useful in extrapulmonary tuberculosis. Hence, we have undertaken this study, to study the utility of Genexpert in cases of pleural effusions for an early diagnosis of Tuberculosis.

MATERIALS AND METHODS

This was an observational study done in a tertiary care center in Pune from Dec. 2020 to Oct. 2022. 89 patients with pleural biopsy-proven tuberculosis and growth of AFB on culture of pleural biopsy specimen were used as study population and a pleural fluid Genexpert, ZN stain and Adenosine deaminase levels were also used to diagnose tuberculous pleural effusion.

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Study Participants: 89 patients with biopsy results showing granulomatous lesions and culture positive for AFB were taken for the study. Patients with minimal pleural effusions and bleeding diathesis/tendencies were excluded from the study.

Study Design: On the first visit the patients with pleural effusion on HRCT were subjected to thoracocentesis followed by pleural biopsy. Pleural fluid samples were sent for Genexpert, microscopy [ZN stain] and Adenosine deaminase levels. Pleural biopsy specimen was sent for HPE and Mycobacterial culture.

Sample Size

The Z- test was used to calculate the sample size. The level of confidence used is 95% with a Z value associated with an alpha of 1.96. 101 consecutive patients with HRCT suggestive of pleural effusion were taken, but 12 patients had to be excluded due to minimal pleural effusion and, bleeding diathesis.

Formulae

1) Sample Size = $\frac{Z^2 * P * (1-P)}{E^2}$

Z^2=6.64 P=0.84 E2=0.01

Gene Expert v/s Gold Standard		Status of the person as per Gold Standard	
		Has the condition	Does not have the condition
Result from Gene Expert Test	Positive	a - True Positive	b - False Positive
	Negative	c - False Negative	d - True Negative

Sample Size =	89
a =	12
b =	0
c =	77
d =	0

Sensitivity	=[a/(a+c)]×100	13.48%
Specificity	=[d/(b+d)]×100	100.00%
Positive Predictive Value (PPV)	=[a/(a+b)]×100	100.00%
Negative Predictive Value (NPV)	=[d/(c+d)]×100.	0.00%

ADA v/s Gold Standard		Status of the person as per Gold Standard	
		Has the condition	Does not have the condition
ADA	Positive	a - True Positive	b - False Positive
	Negative	c - False Negative	d - True Negative

Sample Size =	89
a =	81
b =	0
c =	8
d =	0

Sensitivity	=[a/(a+c)]×100	91.01%
Specificity	=[d/(b+d)]×100	100.00%
Positive Predictive Value (PPV)	=[a/(a+b)]×100	100.00%
Negative Predictive Value (NPV)	=[d/(c+d)]×100.	0.00%

RESULTS

We screened 89 patients with biopsy proven results suggestive of tuberculous pleural effusion.

Table 1: Distribution of patients according to their age and gender

CHARACTERISTICS	N=89	PERCENTAGE
AGE (in years)		
18-30	7	8
31-45	29	33
46-60	36	40
61-75	17	19
GENDER		
Male	66	75
Female	23	25

Table no.1 shows that majority 36(40%) patients belonged to 46-60 years of age group followed by 29 (33%) belonged to 31-45 years of age group. Majority 66(75%) patients were males.

Table 2: Distribution of patients according to their ADA levels

CHARACTERISTICS	N=89	PERCENTAGE
ADA LEVELS		
>40IU	81	91
<40IU	8	9

Table no.2 shows that majority 81(91%) patients had ADA levels >40IU and 8(9%) patients had ADA levels <40IU.

Table 3: Distribution of patients according to pleural fluid for AFB on ZN stain

CHARACTERISTICS	N=89	PERCENTAGE
AFB ZN MICROSCOPY		
Positive	14	16
Negative	75	84

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Table no.3 shows that majority 75(84%) patients were negative for AFB and 14(16%) patients were Positive for AFB on ZN smear.

Table 4: Distribution of patients according to their GENEXPERT results on pleural fluid

CHARACTERISTICS	N=89	PERCENTAGE
GENEXPERT		
MTB detected	12	14
MTB not detected	77	86

Table no.4 shows that majority 77(86%) patients MTB not detected by GENEXPERT test and in 12(14%) patients MTB was detected.

Table 5: Distribution of patients according to their BIOPSY results

CHARACTERISTICS	N=89	PERCENTAGE
BIOPSY		
Typical granulomatous	89	100
Non granulomatous	0	0
CULTURE		
Positive	89	100
Negative	0	0

Table no.5 shows that all 89(100%) patients had Typical granulomatous changes on biopsy. While, all 89(100%) patients were positive on Culture for AFB.

DISCUSSION

In the present study, we screened 89 patients with pleural biopsy-proven results suggestive of tuberculous pleural effusion and positive for Tuberculosis on Mycobacterial culture.

The majority of 36(40%) patients were 46-60 years of age followed by 29 (33%) in 31-45 years of age group. The majority 66(75%) patients were males.

In a study by Torgersen J et al (2006) the median age of patients with pulmonary tuberculosis was 45 years (range,1–103 years) and the median age for patients with extrapulmonary disease ,patients with pleural disease,and patients with non-respiratory bisease were 41 years (range,1 93 years), 45years (range, 1792 years), and 40 years (range,1–93 years), respectively.[3] Also, Diacon AH et al (2003) in a study of 51 patients were with a mean age of 34yrs(range:15–63yrs) and 59%were male.[7]

Diagnostic thoracentesis is warranted in the following circumstances--- suspected tuberculous pleural effusion in the absence of an established diagnosis of pulmonary TB (via sputum or other studies) and there is a diagnosis of pulmonary TB established (via sputum or other studies) but another aetiology for effusion (for eg malignancy) is suspected clinically.

In the present study majority of 81(91%) patients had ADA levels >40IU and 8(9%) patients had ADA levels <40IU.

In a study by Castro et al (2003) the pleural fluid ADA levels in 410 lymphocytic non-tuberculous pleural fluids was found to be above 40 IU/L in seven (1.7%).[8]

Liang QL et al (2008) stated that ADA is a purine-degrading enzyme found in all cells, particularly monocytes and lymphocytes, which is present in high concentrations in tuberculous effusions and can be quantified through a rapid, cost-effective assay which has a high overall diagnostic sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) for TB when tested in pleural fluid (92%, 90%, 9.03 and 0.10, respectively).[9]

Diacon AH et al (2003) found that ADA had a sensitivity of 95% with a specificity of 89% when used alone.[7]

Aljohaney A et al (2012) observed that in all HIV-infected patients regardless of CD4 counts, the sensitivity of ADA was 94% when the cut-off value of 30 u/l was used and specificity of 95%. The positive likelihood ratio was 18.9 and the negative likelihood ratio was 0.06. The sensitivity was also high at 96% when the cut-off value used was 60 u/l in HIV-infected patients. These results were comparable to HIV-uninfected patients.[10]

Baba K et (2008) in their study amongst ninety-seven tuberculous pleuritis and 40 non-tuberculous pleuritis patients using the ADA cut-off value of 30U/L, the overall sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of ADA was 94%, 95%, 19, and 0.06 respectively. ADA analysis is a sensitive marker of tuberculous pleuritis even in HIV patients with very low CD4 counts in a high TB endemic region.[11]

In a study by Sivakumar P et al (2017) of the 132 patients tested for pleural fluid ADA, 27 had confirmed pleural TB and 105 did not, with median pfADA levels of respectively 63 IU/l (interquartile range [IQR] 47-88) and 12 IU/l (IQR 7.5-22.5). ROC curve analysis determined the optimal pfADA cut-off to be 30 IU/l, which had positive and negative predictive values of respectively 60.5% and 98.9%, 96.3% sensitivity (95%CI 0.892-1.000) and 83.8% specificity (95%CI 0.768-0.909). The calculated area under the ROC curve was 0.934 (95%CI 0.893-0.975).[12]

The above studies support our findings.

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In the present study, the majority of 75(84%) pleural fluid did not detect AFB on ZN stain. 14(16%) patients pleural fluid was found to have AFB on ZN stain.

Zhai K et al (2010) observed that the combination of pleural fluid and sputum cultures in the diagnostic workup of TPE had a combined diagnostic yield of 79%.[1]

Conde and associates (2003) prospectively evaluated the diagnostic yield of mycobacterial smears and cultures in 84 patients with tuberculous pleuritis. In 10 of the 44 patients, sputum smears were positive, whereas cultures were positive in all. The sputum was positive in 35 of 64 patients (55%) who had a normal chest radiograph except for the effusion and in whom the sputum was induced. They concluded that sputum examination is underutilized in the diagnosis of tuberculous pleuritis.[13]

Aljohaney A et al (2012) observed that the Ziehl Neelsen (ZN) stain.[13,15], liquid culture using BACTEC.[14,15] and Lowenstein-Jensen (LJ) cultures consistently provided a higher yield in HIV-infected individuals compared to HIV-uninfected individuals.[10]

In the present study, in the majority of 77(86%) patients MTB was not detected by the GENEXPERT test and in 12(14%) patients MTB was detected.

Kohli M et al (2018) in a metanalysis of GeneXpert in pleural biopsy reported a pooled sensitivity and specificity of 30.5% and 97.4%, respectively.[14]

Denkinger CM et al (2014) observed that 14 studies (841 samples in total, 92 culture positive) evaluated Xpert in pleural fluid versus culture. Xpert sensitivity varied widely (0–100%). [15]

Trajman A et al (2014) stated that ADA had the highest sensitivity, followed by histopathological examination.[16]

Chakraborty A et al (2019) found that the mean ADA levels in pleural fluid was 61.7 U/L ± 16.2 (SD). Pleural fluid CBNAAT was positive for Mycobacterium tuberculosis (MTB) in 24 patients (32%). Sputum smear for acid-fast bacilli (AFB) was positive in 3 (4%) patients, whereas in sputum CBNAAT MTB was detected in 8 (10.6%) persons. Association between pleural fluid ADA, lymphocyte count and CBNAAT positivity was evaluated by Student T-test. There was a significant association between higher ADA levels and CBNAAT (p-value = 0.001). They concluded that pleural fluid CBNAAT, owing to its low sensitivity, should not be included in the diagnostic protocol of Tuberculous pleural effusion in high-prevalence areas.[17]

Friedrich SO et al (2011) found the sensitivity and specificity of the Xpert assay in the pleural fluid were 25% and 100%, respectively.[18]

Pleural fluid ADA level may help to make a diagnosis of tuberculous pleural effusion, in cases where the AFB smear and cultures are negative.[1,2,5] Raised pleural fluid ADA levels can occur in other conditions other than TB infection, including malignancy. Some studies suggest that an ADA level >45 to 60 units/L is 100 per cent sensitive and up to 97 per cent specific for tuberculous pleural effusion.[19]

The diagnostic use of ADA levels can be affected by the local prevalence of TB. In low-prevalence regions, it's useful in its negative predictive value. In one study from London between 2009 to 2015, patients having lymphocytic predominant pleural effusion and pleural fluid ADA level <40 U/L were unlikely to have tuberculous pleural effusion (negative predictive value 98 percent).[12] In contrast, in high-prevalence regions, tuberculous pleural effusion cannot be ruled out in the presence of lymphocytic predominant pleural effusion by any level of ADA in pleural fluid.[48] The diagnostic possibility of tuberculous pleural effusion is high in patients with ADA levels >70 U/L. Although, if the ADA level is indeterminate (40 to 70 U/L), further investigation depends upon the pretest probability of TB.[20] Cases with lymphocytic predominant pleural effusion and with a low ADA level (<40 U/L) should be considered for a pleural biopsy to assess for tuberculous pleurisy or an alternative pathology. HIV status can alter the diagnostic accuracy of pleural fluid ADA levels. Amongst patients with HIV, one study suggested an ADA cut-off level of 60 units/L in patients with HIV infection and provided a sensitivity and specificity of 95 and 96 percent, respectively.[21]

The results of our study further have further consolidated the findings that GeneXpert is not a useful tool.

The present study shows that all 89(100%) patients had typical granulomatous changes on biopsy and all 89(100%) pleural biopsy samples grew Mycobacterium Tuberculosis.

Valdés L et al (1998) studied 248 patients with tuberculous pleuritis. The needle biopsy of the pleura showed granulomas in 198 patients (80%), the AFB stain of the biopsy was positive in 64 (25.8%) and the culture of the biopsy tissue was positive in 140 (56%). In this study at least one of the three tests was positive in 227 (91%).[19]

Kirsch CM (1997) found that 60% of patients had pleural biopsy culture positive for Mycobacterium tuberculosis and 80% had diagnostic histology. Overall pleural biopsy sensitivity (histology and culture) for tuberculous pleurisy was 87%.[22]

Aljohaney A et al (2012) found that histopathological examination of pleural biopsy was of diagnostic utility in HIV-infected patients where granulomatous inflammation or caseous necrosis was detected in 52%–92%.[10]

Pleural fluid AFB culture − Pleural fluid AFB cultures are positive in lesser than 20 to 30 percent of cases without HIV infection.[23] Cases with HIV infection may present with a higher burden of bacilli than patients without HIV; in patients with HIV infection with CD4 count less than 200 cells/mm3,

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AFB cultures are positive in approximately 50 percent of cases.[52-54]

Pleural biopsy - should be sent for AFB smear, culture and histopathological evaluation. Positive culture enables organism identification and drug susceptibility testing. Histopathology may show granulomas and/or acid-fast bacilli. The presence of caseating granulomas on histologic examination is diagnostic of tuberculous pleural effusion. Although Noncaseating granulomas may also be observed; occasionally, these can be seen in other disorders too.

In cases without HIV infection, the diagnostic yield of pleural biopsy is 60 to 95 percent. The culture of pleural biopsy material is positive in 40 to 80 percent of cases, and histology shows granulomas in 50 to 97 percent of cases.[23]

CONCLUSION

The present study was conducted in an endemic region hence a good sample quality was obtained. No cases were lost to follow-up over the period of 1 year. Genexpert reports of all patients were acquired before the initiation of Antitubercular drugs hence not interrupting with results and hence keeping the number of false negatives to the bare minimum. Our study has also shed light on the fact that even though Adenosine Deaminase levels are having high sensitivity (91%) and high specificity (100%) its levels are also elevated in other common conditions. GeneXpert was not of much utility in the diagnosis of Tuberculous pleural effusion. And hence developing another test with higher sensitivity is the need of the hour.

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