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Original Research Paper

SHOULD PLEURAL FLUID CBNAAT BE DONE TO ALL SUSPECTED CASES OF TUBERCULOUS PLEURITIS……?

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ABSTRACT:

Introduction: Molecular tests are rapid and specific for mycobacteria but expansive and have lower sensitivity in pleural fluid because of paucibacillary nature of diseases. We did this study to analyse whether a molecular test should be done in all suspected cases of tubercular pleural effusion or the use of these tests should be restricted to the patients with diagnostic dilemma only. **Methods**: Total 116 patients were enrolled for the study out of which thoracocentesis were performed in 104 patients and 63 patients were started on Anti-tubercular therapy on the basis of disease defining criteria. A total of 61 patients who showed either complete clinic radiological resolution at the end of therapy and/or microbiologically confirmed tuberculous effusions, were finally analysed. **Results**: Out of these 61 patients, biochemical and cytological analysis of 31 (50.8%) effusions were conclusive while the remaining 30 (49.2%) had inconclusive analysis for tuberculosis. Among inconclusive effusion (30), seven were microbiologically confirmed for tuberculosis including four CBNAAT positive pleural fluids. In these four patients, one patient had sputum CBNAAT positive. That means only three patients were additionally added to tubercular pleural diffusion by exclusively pleural fluid CBNAAT. The sensitivity of CBNAAT was 15.25% in our study. **Conclusion**: Pleural fluid CBNAAT should be done only in case of diagnostic dilemma, not as 'always to do test' for the diagnosis of tubercular pleural effusion.

INTRODUCTION:

The diagnosis of tubercular pleural effusion (TPE) is established either by pleural fluid (PF) analysis or by a pleural biopsy. Obtaining pleural fluid is a less invasive procedure and is preferred over pleural biopsy. Even in thoracoscopic guided pleural biopsy, tuberculosis (TB) may present as non-specific pleuritis. [1] Mycobacterial culture is although the gold standard test for the diagnosis of TPE, it is not considered as the investigation of choice in all suspected cases because of a long waiting time and lower sensitivity in pleural fluid. Nowadays we are privileged with new investigations like rapid culture methods and molecular tests for the diagnosis of TB. These tests are not only the rapid test but also have a good specificity for mycobacteria.[2] In cartridge based nucleic acid test (CBNAAT), the results are available within two hours and even the chances of cross contamination are nil. [2-3] There are two issues with these tests in pleural fluid: first is their high cost and second their lower sensitivity in pleural fluid because of paucibacillary nature of diseases.[4] Biochemical and cytological analysis of pleural fluid has been used for the diagnosis of TPE for a long time with variable diagnostic value. These tests are quick and cheap with acceptable diagnostic values. The present study was done with the aim to evaluate whether a molecular test should be done as a 'always to do test' in all cases or the uses of these tests should be restricted to the patients with diagnostic dilemma in pleural fluid analysis especially in resource limited, high TB burden countries.

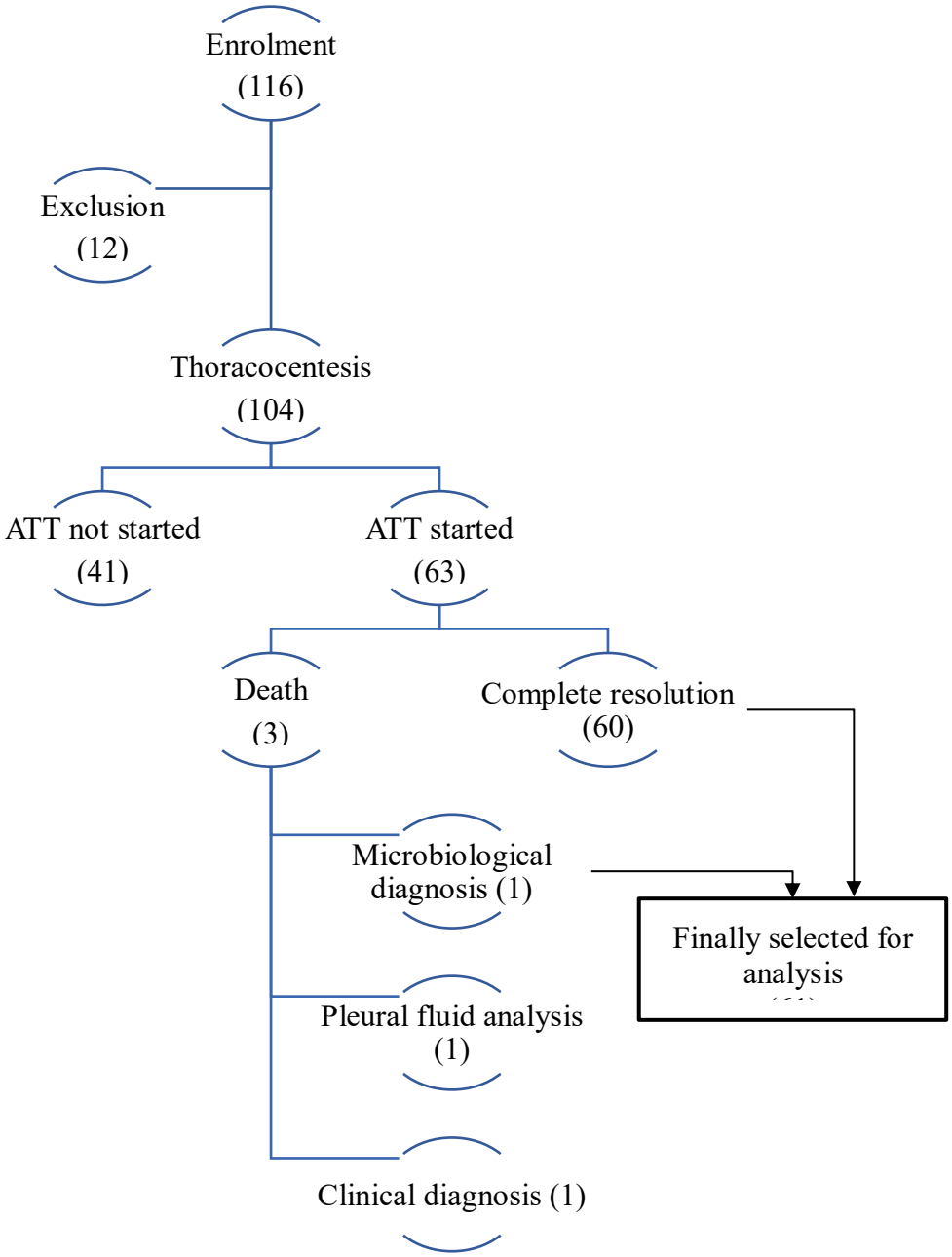
MATERIAL AND METHODS:

This prospective observational study was done during 2021-22 in Pacific Medical College and Hospital, Udaipur, Rajasthan with prior approval of the institutional ethical committee. Patients above 12 years of age of either gender who were presented with pleural effusion, either admitted or attended the outdoor unit of our hospital were enrolled for the study. The written and informed consent was taken

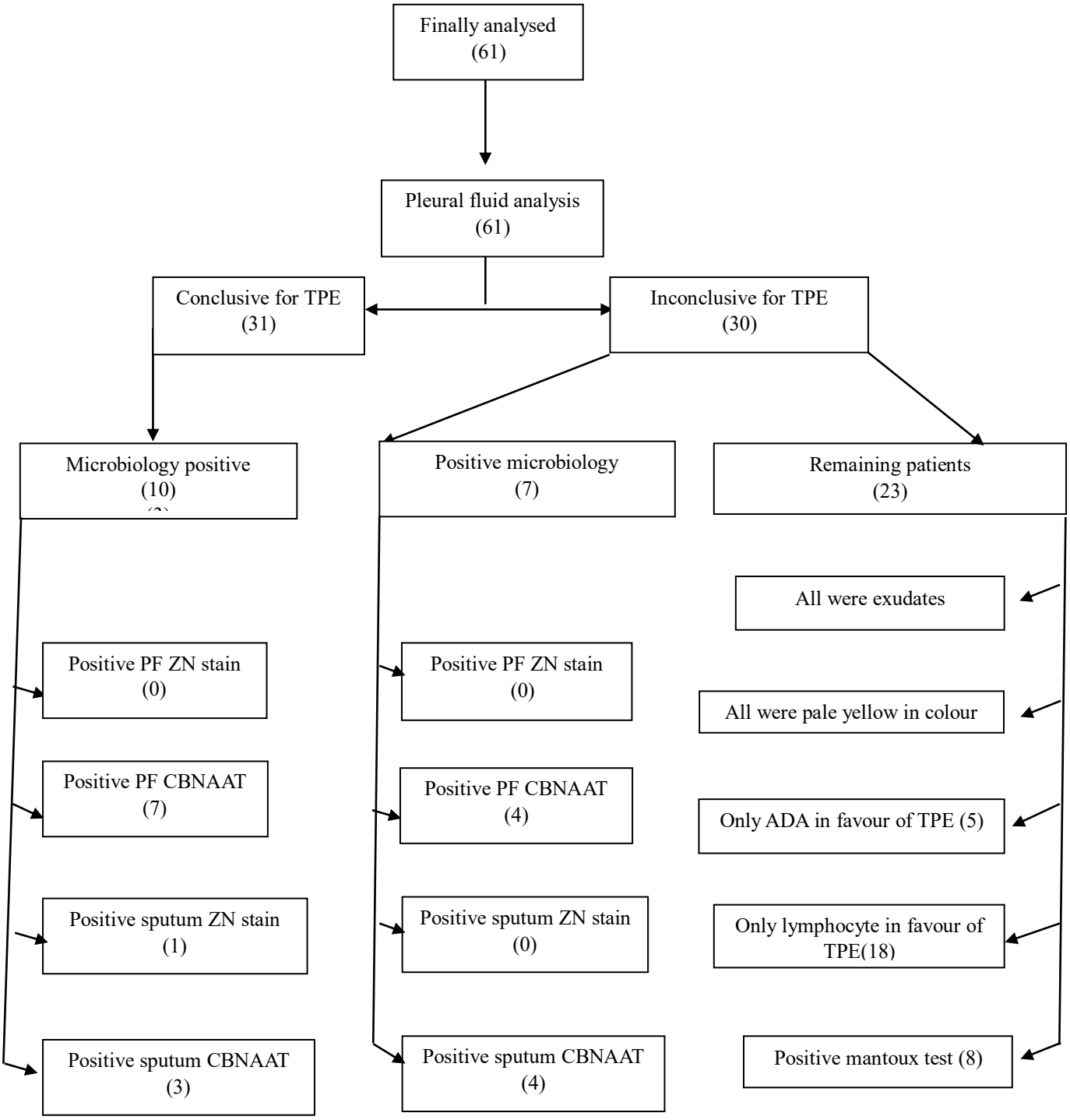
from each patient after explanation of study protocol and their willingness to participate in the study. Microbiologically confirmed cases of pulmonary tuberculosis with co-existing pleural effusion were also included in the study. Haemorrhagic pleural effusions and contraindications for thoracentesis were the exclusions. Total 116 patients were screened on the basis of clinical suspicion of pleural effusion. Detailed clinical history taken from all enrolled patients especially regarding clinical features of active TB, any previous history of TB treatment and any co morbid conditions. The clinical history was followed by systemic examination and chest radiograph. The patients whose clinical examination and chest radiograph suggested a pleural effusion were the subjects for thoracentesis and PF analysis. The PF analysis included pH, adenosine deaminase (ADA), protein, sugar, total and differential cells count, Zeihl neelsen (ZN) stain and CBNAAT. We also advised sputum ZN stain and CBNAAT, serum total protein and Mantoux test (MT) to all patients. Thoracentesis was performed in 104 patients and the aspirated fluids were sent immediately to the laboratory for analysis.

We took a decision to start ATT if one or more than one following criterion was fulfilled. [5]

1. **Microbiological diagnosis:** Confirmation of the presence of *Mycobacterium tuberculosis* in pleural fluid and/or sputum by ZN stain and/or CBNAAT.
2. **Clinical diagnosis:** Clinical presentation consistent with TB with the exclusion of other possible clinical consideration.
3. **Pleural fluid analysis:** Exudative effusions with predominant lymphocytic cytology and ADA >40 IU/L (All three tests must be positive).



Based on above criteria, anti-tubercular therapy (ATT) was started to 63 patients and these patients were kept under regular follow up till the end of therapy. The patients who showed complete clinical and radiological resolution at the end of therapy and/or microbiological evidence of TPE, were included for the analysis. Pleural fluid cytological and biochemical analysis (irrespective of microbiology) were considered favourable if all three tests (exudate, ADA> 40 IU/L and lymphocytic predominance) mentioned in criteria three were fulfilled and unfavourable in presence of one or two positive tests. The data was analysed using social sciences (SPSS) software version 22. [Flow chart 1]



Among the 63 patients, three patients died during the treatment while 60 patients showed a complete clinico-radiological resolution. The basis of diagnosis in these three patients was clinical in first, microbiological in second and PF analysis in third patient. Total 61 patients (60 with complete resolution and one death with microbiologically confirmed TPE) were taken for the final analysis.

The mean age in patients with TPE was 45.47 ± 18.94 (range: 16-79) years. The male and females were 43 (70.5%) and 18 (29.5%) respectively. The duration of symptoms was less than two weeks in the majority 45 (73.77%) of patients. The most common symptoms were chest pain (88.5%), followed by cough (78.6%), breathing difficulty (77%) and fever (52.4%). The sites of effusions were right side (62.3%), left side (34.4%) and bilateral (3.3%). We found 54 (88.5%) pale yellow colour, 6 (9.8%) pus like fluid and one clear effusion.

The mean protein was 4.42 ± 1.35 (range: 0.87-7.35) mg/dL. According to light's criteria, 59 (96.7%) effusions were classified exudates and the remaining 2 (3.3%) were transudates.[6] The mean ADA was 46.77 ± 29.05 (range: 5.5 -194) IU/L. The ADA above 40 IU/L was present in 37 (60.6%) effusions. A lymphocyte predominant effusion and LN ratio above 0.75 were present in 83.6% and 85.2% patients respectively. Microbiological evidence of mycobacteria was present in 17(27.8%) patients. Positive CBNAAT were found in 11(18.03%) pleural fluids and seven (11.47%) sputum samples. We found one pleural fluid with *rpoB* gene mutation (rifampicin resistant) in our study. [flow chart 2] [Table1]

Table: 1. Comparison between tuberculous and nontuberculous effusions

		TPE (N=61)	Non TPE (N=41)	P values
Age (Years)		45.47± 18.94	48.39±17.45	p=0.43
Gender	Male	43 (70.5%)	25 (61%)	p=0.31
	Female	18 (29.5%)	16 (39%)	
Duration of symptoms (Wks)	< 2	45 (73.8%)	18 (43.9%)	p=0.07
	2-4	12 (19.6%)	14 (34.1%)	
	>4	4 (6.5%)	9 (21.9%)	
Symptoms	Cough	48 (78.6%)	22 (53.6%)	p=0.74
	SOB	47 (77%)	20 (48.7%)	
	Chest pain	54 (88.5%)	32 (78%)	
	Fever	32 (52.4%)	14 (34.1%)	
Site of pleural effusion	Right	38 (62.3%)	18 (44%)	p=0.008
	Left	21 (34.4%)	14 (34.1%)	
	Bilateral	2 (3.3%)	9 (21.9%)	
PF Colour and appearance	Pale yellow	54 (88.5%)	30 (73.1%)	p=0.01
	Frank pus	6 (9.8%)	3 (7.3%)	
	Clear	1 (1.6%)	6 (14.6%)	
	Reddish	0	2 (4.9%)	
PF Biochemistry	Mean Protein	4.42 ± 1.35	3.92±1.68	p=0.10
	Exudate	59 (96.7%)	32 (78%)	p<0.001
	Mean ADA	46.7±29.05	38.58±25.13	p=0.14
	ADA >40 IU/L	37 (60.7%)	15 (36.6%)	p=0.01
PF cytology	Lymphocytic effusion	51 (83.6%)	26 (63.4%)	p<0.001
	LN ratio >0.75	52 (85.2%)	26 (63.4%)	p=0.01
PF Microbiology	Positive ZN stain	1 (1.6%)	0	p=0.41
	CBNAAT	11 (18%)	0	p=0.0042

Pleural fluid analysis was in favour of TB (favourable PF analysis) in 31 (50.8%) patients and not in favour of TB (unfavourable PF analysis) in 30 (49.2%) patients. A microbiological evidence i.e., pleural fluid and/or sputum ZN stain and/or CBNAAT was found in 17 patients out of which, 10 patients had a favourable PF analysis while the remaining seven patients had unfavourable PF analysis. [Table2]

Table 2: Tuberculous effusion subgroup analysis

		PF biochemical and cytological analysis favourable for TPE (n=31)	PF biochemical and cytological analysis unfavourable for TPE (n=30)
Pleural fluid	ZN stain	0	1
	CBNAAT	7	4
Sputum	ZN stain	1	0
	CBNAAT	3	4
Positive Mantoux test		10	8

In patients who had unfavourable PF analysis (30), 23 patients had no evidence of mycobacteria in either pleural fluid or sputum. The ATT was started for these patients because of a very strong clinical suspicion and after ruling out other possibilities. All these effusions were pale yellow in colour and were classified as exudates according to light's criteria. [6] In these patients, ADA was above 40 IU/L in five patients while the remaining 18 had a lymphocytic predominant cytology. Eight patients showed a positive mantoux test among these 23 patients. The 11 patients, who had positive pleural fluid CBNAAT were analysed separately. Sputum was positive for CBNAAT in one patient while none of the patients had a positive sputum ZN stain in these patients. The mean ADA was 45.32 (range of 16-106) IU/L with seven effusions having ADA above 40 IU/L. The mean protein was 3.93 (range: 0.87-5.94) mg/dL. Nine effusions were classified as exudates and remaining two as transudates. A lymphocyte predominant effusion was present in 8 patients while a LN ratio above 0.75 was present in 9 patients. The PF analysis was favourable in seven and unfavourable in four patients. One patient form unfavourable PF analysis had sputum CBNAAT positive. That means pleural fluid CBNAAT diagnosed only three additional cases which had unfavourable PF analysis.[Table3]

Table 3: Analysis of pleural fluid CBNAAT positive patients (n=11)

Test	Present (n)	Percentages
Sputum Zn stain Positive	0	0%
Sputum CBNAAT Positive	1	9%
Favourable Lymphocyte (>50%)	8	72.7%
Favourable LN ratio (>0.75)	9	81.8%
Favourable ADA (>40 IU/L)	7	63.6%
Exudate	9	81.8%
Favourable cytology and biochemistry	7	63.6%
Unfavourable cytology and biochemistry	4	36.4%

In this study we found a significant positive association between LN ratio and pleural fluid CBNAAT. Among all exudates (n=91), the sensitivities of pleural fluid CBNAAT, ADA and lymphocytic predominance were 15.25%, 62.7% and 83% and the specificities were 100%, 59.4% and 62.6% respectively. The diagnostic accuracy of pleural fluid CBNAAT, ADA and lymphocytic predominance among exudative effusions were 45%, 65% and 76.92% respectively. When the patients with TPE were compared with the non TPE group, we found a significantly higher number of patients with ADA above 40 IU/L, lymphocytic predominant effusion and a LN ratio above 0.75 in TPE group as compared to non TPE group. [Table 4]

Table 4: Sensitivities and specificities of different pleural fluid investigations in all exudates (n=91)

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
Lymphocyte (> 50%)	83%	65.6%	81.7%	67.7%	77%
LN ratio (>0.75)	84.7%	40.6%	72.5%	59%	69.2%
CBNAAT	15.3%	100%	100%	39%	45%
ADA (>40 IU/L)	62.7%	59.4%	74%	46.3%	61.5%
ADA (>35 IU/L)	69.5%	50%	71.9%	47%	62.6%

DISCUSSION:

In this study, we found some well-known facts like chest pain as the commonest clinical presentation of pleural effusion, early clinical presentation in TPE, predominant right-side effusion and pale-yellow colour in TPE. These facts had been already established in various previous studies [7-10] In our study, we found a lower sensitivity of pleural fluid CBNAAT as compared previous studies. The sensitivity of pleural fluid CBNAAT has been reported to be as low as 19.1% and as high as 85.71% in previous studies. [11-15] The variation in sensitivities may be because of varying sample size, age of the study population, diseases defining criteria and burden of TB in study region. It is common practice of primary care physicians to use quinolones in suspected respiratory infection in our region, which may alter the course of TPE and responsible for lower sensitivity in our study. We highlight the diagnostic role of pleural fluid CBNAAT here. In our study, although pleural fluid CBNAAT was 100% specific, the sensitivity was lower than traditional biochemical and cytological PF analysis. The diagnostic accuracy was also lower as compared to traditional biochemical and cytological PF analysis. The pleural fluid CBNAAT added only three cases exclusively to the TPE group. Such a lower sensitivity and diagnostic accuracy can miss a large number of true disease cases and should not be used solely for diagnostic purposes especially in high TB burden countries. The cost of CBNAAT makes it unsuitable in resource limited countries. The second thing we emphasize here is the identification of difficult to diagnose cases of TB by CBNAAT. In this study, pleural fluid CBNAAT identified two cases of TPE among transudative effusions which had not been reported earlier. The third effusion was exudative non-lymphocytic (lymphocyte 24%) with ADA value of 22 IU/L, which seems to be an early stage of TPE. The use of CBNAAT may be justified in such difficult to diagnose cases. The use of CBNAAT should be restricted to those patients who had a strong clinical suspicion but unfavourable PF analysis especially in resource limited high TB burden countries. On the other hand, biochemical and cytological analysis of pleural fluid is cheaper, quick and more accurate with reasonable sensitivity and specificities. If we change the criteria of ADA to 35 IU/L, the PF analysis becomes favourable in 36 (59%) patients. In this study the sensitivity and specificity of ADA above 35 IU/L were found to be 69.5% and 50% respectively with diagnostic accuracy of 62.6%. We suggest large sample studies in high TB endemic countries and redefining ADA cut off in pleural fluid for the diagnosis of tuberculous pleuritis.

CONCLUSION:

The pleural fluid CBNAA should be used only in case of diagnostic dilemma not as a 'always to do test' because it provides only a little additional advantage to clinical and pleural fluid analysis in diagnosing tubercular pleural effusion. We recommend a cut off value for ADA as above 35 IU/L in pleural fluid for the diagnosis of tubercular pleural effusion.

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