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Pleural Fluid Adenosine Deaminase, C -Reactive Protein levels and Lymphocyte/ Neutrophil Count Ratio in Differentiating Tubercular and Non-Tubercular Pleural Effusion

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ABSTRACT

Introduction: Pleural effusion is a typical extrapulmonary cause of Tuberculosis (TB). The routine culture experiences an absence of affectability. Numerous markers in pleural fluid are assessed to analyze tubercular pleural fluid, yet no one is perfect. We have contemplated Adenosine deaminase (ADA), protein (CRP C - responsive) and Lymphocyte/Neutrophil (L/N) ratio in amalgamation for the determination of pleural effusion of tubercular origin .

Material & Methods: All patients presented with pleural effusion were put through thoracentesis and differentiated into transudative and exudative using Light's criteria. Patients with exudative pleural effusion aetiology were further bisected into two groups with a sample size of 30 patients. Group I consisted of patients with tubercular cause, and Group II comprised other than tubercular. ADA, CRP and L/N ratio of these subjects were estimated in pleural fluid. The sensitivity, specificity and predictive values were calculated .

Results: The ADA, CRP, and L/N ratio's sensitivity, specificity, positive predictive value, and negative predictive value were, respectively, 83.3%, 90.0%, 89.29%, 84.37%, 76.67%, 90.0%, 88.46%, 79.41%, 96.67%, 43.33%, 63.04%, and 92.86%. The conjunction of ADA and CRP exhibited the highest specificity for pleural effusion caused by Tuberculosis; however, both ADA and CRP showed comparable specificity on their own.

Conclusions: Diagnosing tubercular from non-tubercular individuals was made easier with the help of Pleural fluid ADA and CRP. Combining ADA, CRP, and L/N ratio does not offer any significant advantages beyond just combining ADA and CRP.

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Introduction

Among the extrapulmonary manifestations of Tuberculosis, pleural effusion is typical reason (1). Extrapulmonary involvement in Tuberculosis is typically connected with substantial morbidity and mortality. With the rise in the patients with HIV, the presentation of Tuberculosis has changed with an increased proportion of cases with disseminated and extrapulmonary dis-ease (2). Though common, making its diagnosis is extremely difficult (3).

The gold standard for diagnosis is finding the Mycobacterium on a smear or in a culture, but these methods have limitations in terms of sensitivity and duration. Only in 10 to 25% of patients, acid-fast bacilli (AFB) staining is positive, while cultures in less than 25% of cases AFB can be grown (4).

Numerous pleural fluid markers have been examined for diagnosis of tubercular pleural effusion, but none have been shown to be perfect due to their low sensitivity or specificity, complexity in use, or limited availability. Adenosine deaminase (ADA), Ziehl-Neelsen (ZN) stain, culture, serum CA 125, pleural alkaline phosphatase, pleural fluid Procalcitonin concentration, pleural fluid Interleukin-1, pleural tumour necrosis factor, pleural fluid PCR, total and differential count, and Interferon-gamma are a few of these markers (5).

Numerous studies have assessed ADA, CRP, and lymphocyte/neutrophil count ratio for the identification of cause of tubercular pleural effusion. In the current study, we attempted to evaluate these three criteria individually and collectively to diagnose tubercular pleural effusion.

Methods and Material

The present study was observational study to comprehend between tubercular and non-tubercular pleural effusion employing ADA, CRP and lymphocyte/neutrophil ratio. The study was conducted after taking consent from the ethical committee of our institute (SRHU/ INT /2017-84).

The study was conducted in the Department of Respiratory Medicine at a tertiary care teaching institute. All consecutive patients with pleural effusion were subjected to drainage of fluid.

Using light criteria, above patients were further segregated into transudative and exudative (1). Only patients with pleural effusion of exudative origin were enrolled in the study. They were enrolled after written and informed consent.

They were divided into two groups, Group-I comprised of patients with tubercular etiology whereas Group-II consisted of patients with non-tubercular etiology. Each group had thirty patients. All patients more than 20 years of age and having exudative pleural effusion were included in the study. While all patients with Pyothorax or Hemothorax or Transudative pleural effusion or Immunocompromised (HIV-positive) status were excluded from the study Patients were categorized as pleural effusion of tubercular variety if their pleural fluid is positive for AFB in smear/ culture or Sputum is positive for AFB by smear/ culture or parietal pleural biopsy is positive. Patients who had symptoms and clinical or radiological features of tuberculosis and had good outcome on antitubercular treatment were included in tubercular group too.

Group II included the patients who had a diagnosis other than tuberculosis, which consisted of malignancy, parapneumonic effusion or any other etiology.

ADA was calculated using DXC 800 (Beckman Coulter). The CRP was detected by ELISA kit (Gene probe Diaclone).

Regular microscopy was used to estimate the L/N ratio. To distinguish in both groups, the sensitivity, specificity, Positive predictive value (PPV), and Negative predictive value (NPV) were computed.

Data management and statistical analysis

SPSS version 22 was used for statistical analysis. Quantitative information was presented as Mean SD. The means of the two groups were compared using an independent-t test.

The cut-off values for all parameters were determined using the ROC curve. At a certain cut-off for ADA, CRP, and L/N ratio, sensitivity, specificity, PPV, and NPV have been determined. Statistics were judged significant at P-value less than 0.05.

Results

There were 60 participants in the study, 48 of whom were men and 12 were women. The examination population (60 patients) was divided into the two groups according to the etiological determination as follows: 30 patients had tubercular pleural effusion that was confirmed by the consideration criteria, and the remaining 30 patients had exudative pleural effusions due to malignancy (n=28) and parapneumonic effusion (n=2) as shown in table 1.

Calculation of ADA, CRP and L/N ratio was done in both the groups and the mean values are mentioned in Table2.

Mean values of ADA, L/N ratio and CRP in cases of tubercular pleural effusion was 55.78, 8.37 and 61.95 respectively and in pleural effusion due to other causes was 24.07, 1.54 and 25.98 respectively. Mann Whitney U test was applied to find if there is any significant difference between Group I and Group II. The calculated values were U= 399.5, 383.5, 410, hence it could be inferred that no group found to be statistically significant at the level of $p<0.05$.

In our present study, using the published literature, we used a cut off of 40 IU/L for ADA, 50mg/l for CRP, 0.75 for L/N ratio and the sensitivity, specificity, PPV and NPV were calculated as mentioned in Table 3.

Table 1. Distribution of subjects according to etiology (n=60).

Group	Disease	No. of Cases
Tubercular	Tuberculosis	30
Non Tubercular	a. Malignancy b. Para pneumonic effusion	28 02
Total		60

Table 2. Mean values of ADA, L/N and CRP in group-I and group-II.

	Group	Mean ± Std. Deviation	Std. Error Mean	U Test	P-value
ADA (IU/L)	I	55.78±18.08	3.30	399.5	0.45
	II	24.07±15.24	2.78		
L/N	I	8.37±6.75	1.25	383.5	0.32
	II	1.54±2.34	0.45		
CRP (mg/l)	I	61.95±14.94	2.73	410	0.56
	II	25.98±23.75	4.33		

Mann Whitney U test and p value <0.05 statistically significant.
Abbreviations: ADA: Adenosine deaminase, **L/N :** Lymphocyte/Neutrophil ratio.
CRP: C - responsive protein.

Table 3. Sensitivity, Specificity, PPV, NPV of ADA, CRP, L/N ratio (n=60).

	ADA (Using cut off 40IU/L)	CRP (Using cut off 50mg/l)	Lymphocyte/ Neutrophil Ratio (using a cutoff 0.75)
Sensitivity	83.33%	76.67%	96.67%
Specificity	90.0%	90.0%	43.33%
PPV	89.29%	88.46%	63.04%
NPV	84.37%	79.41%	92.86%

Abbreviations: **ADA:** Adenosine deaminase, **L/N :** Lymphocyte/Neutrophil ratio.
CRP: C - responsive protein.

In case of ADA the sensitivity, specificity ,PPV, NPV were 83.3 %, 90.0% , 89.29%, 84.37 % respectively, for CRP were 76.67%, 90.0%, 88.46%, 79.41% respectively, and for L/N ratio it was 96.67%, 43.33%, 63.04%, 92.86% respectively. The specificity of CRP and ADA individually was found to be similar.

Thus, amalgamating ADA and CRP, we observed highest specificity to detect pleural effusion due to tuberculosis. L/N ratio alone was found to be the most sensitive test while ADA & CRP being most specific.

In present study we calculated cut off for ADA, CRP and L/N ratio by using ROC curve as seen in Figure 1, 2& 3.

According to ROC curve, the sensitivity was 83.33%, specificity was 93.60% respectively with value of ADA above 40 IU/L, value of CRP above 50mg/l, sensitivity was 100%, specificity was 90% respectively while for L/N above 0.75 was found to be significant, with sensitivity of 90% and specificity of 76.7% respectively.

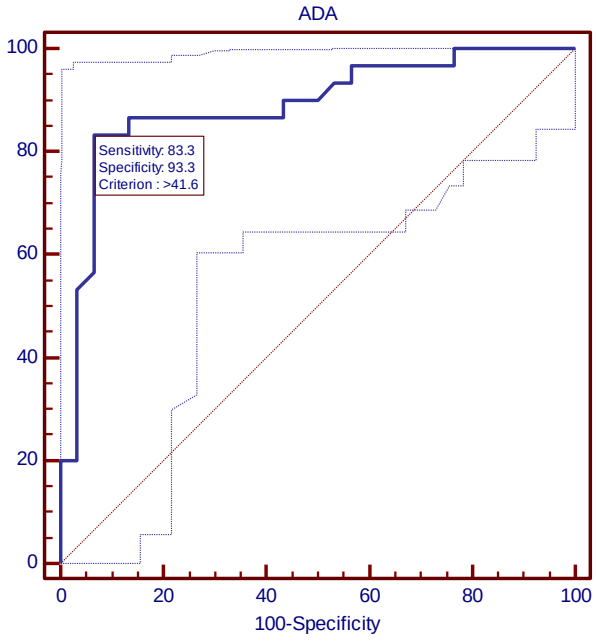

Figure 1. ROC curve for ADA in pleural fluid showing specificity on x- axis and sensitivity on y-axis showing sensitivity 83.3 and specificity as 93.3 at criteria 41.6. for diagnosing tubercular effusion.

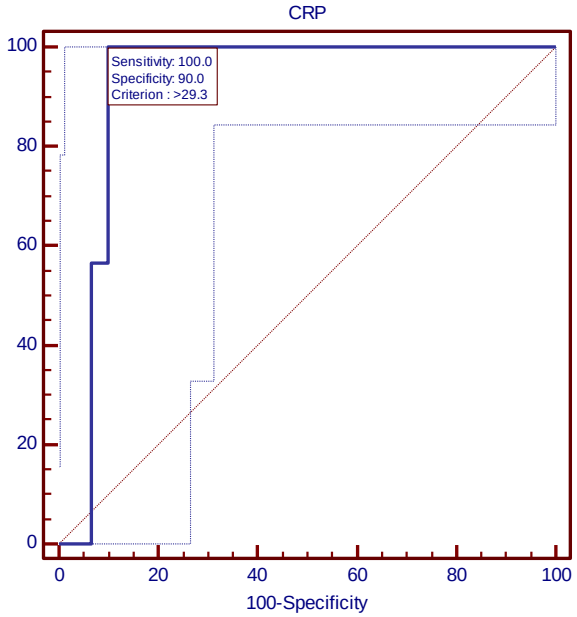

Figure 2. ROC curve for CRP in pleural fluid showing specificity on x- axis and sensitivity on y-axis showing sensitivity of 100%, specificity of 90%taking criteria more than 29 for diagnosing tubercular effusion.

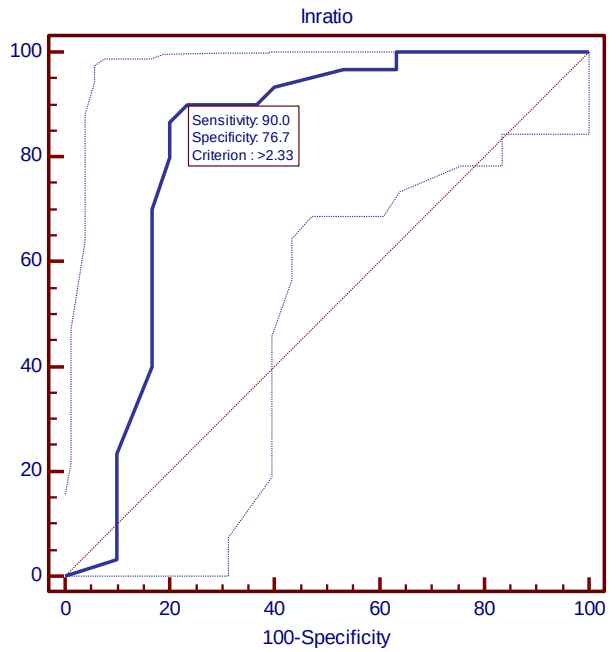

Figure 3. ROC curve for L/N ratio in pleural fluid showing specificity on x- axis and sensitivity on y- axis showing sensitivity of 90% and specificity of 76.7% at criterion more than 2.3 for diagnosing tubercular effusion.

Discussion

Among the extrapulmonary manifestations of tuberculosis, pleural effusion is an important cause. Tubercular pleural effusion is a paucibacillary disease. The conventional laboratory methods viz, Z-N staining and direct ex-amination of pleural fluid require high bacterial load (10,000bacilli/ml) and hence have a low sensitivity (0 to 1%) (15), although bacterial culture re-quires minimum of 10 to 100 viable bacilli and is time consuming (requires 2 to 6 weeks) it is more sensitive (11 to 50%) (16). Pleural biopsy specimens have higher sensitivity to detect tuberculosis either by culture (39 to 79%) or histological examination (71 to 80%). However, the procedure is more invasive, subject to sampling error and requires greater expertise (17).

Thus, dependence on pleural biopsy has led to the evaluation of alternative strategies to diagnose tubercular pleural effusion.

To differentiate tubercular from other causes of pleural effusion various pa-rameters like pleural fluid ADA, CRP, Ca 125, Interferon gamma, Alkaline phosphatase, Procalcitonin concentration, Interleukin-1, Tumor necrosis factor, and pleural fluid PCR have been studied. These tests are tested against culture which is the gold standard. However in pleural fluid culture it has low sensitivity. These tests are also not fool proof. The results need to be interpreted in context with clinical condition of patient.

Out of the above tests we, in our study, planned to evaluate the efficacy of ADA, CRP and L/N ratio alone and in combination, to diagnose tubercular pleural effusion (Table 4). Several authors have mentioned about the utility of elevated levels of ADA, CRP and L/N ratio in tuberculous pleural effusion but to the best of our knowledge, the combination of these tests have not been studied.

ADA is a nonspecific marker of inflammation. Activated lymphocytes, macrophages and neutrophils produce it. It can be raised in both tubercular and non-tubercular pleural effusion. However in tubercular effusion, ADA 2 is predominant (18). In a meta-analysis it was discussed that ADA levels more than 70 are quite diagnostic of tuberculosis. (19). In our study, we used cut off of 40 U/L and it showed a good sensitivity and specificity. Similar result is shown in studies as described in Table 5.

CRP is a non-specific acute phase protein which is produced by liver (20). IL6 and TNF-alpha act as triggers for its production (21, 22). We used cut off of 50 U/L which again showed good sensitivity and specificity. Similar result is shown in studies as described in Table 6.

In tubercular pleural fluid, cell counts are usually in range of 100 to 5000 per millilitre with more than 90% lymphocyte in two thirds of the cases (23). The probability of parapneumonic effusion, pulmonary infarction, pancreatitis or other acute inflammatory conditions of the pleura is in-creased by the predominance of polymorphonuclear leukocytes in pleural fluid. However lymphocytes predominate in malignant and tubercular pleural effusions (24). We used cut off of 0.75 which again showed good sensitivity and specificity. Similar result is shown in studies as described in Table 3.

Table 4. Sensitivity, Specificity, PPV, NPV of various combinations along with comparison to each parameters (n=60).

	Sensitivity	Specificity	PPV	NPV
ADA and CRP	100%	83.33%	85.71%	100%
ADA and L/N ratio	100%	36.67%	61.22%	100%
CRP and L/N ratio	100%	33.33%	60.0%	100%

Abbreviations: ADA: Adenosine deaminase, **CRP:** C-responsive protein and **(L/N) ratio:** Lymphocyte/Neutrophil

Table 5. Comparison of sensitivity and specificity of ADA in tubercular pleural fluid at various cut off values with previous major studies.

Authors	N (no. of subjects)	Cut Off Value (Iu/L)	Sensitivity	Specificity
Jayalakshmi et al (6)	50	> 40	83.87%	78.94%
Sharma S.K et al (7)	75	>35	83.3%	66.6%
Krenke R et al (8)	94	>40.3	100%	93.9%
Maria V.V et al (9)	140	>45.5	88.1 %	85.7%
Lesley J et al (10)	303	>50	91%	81%
Jethani et al (11)	90	>40	88.89%	99.85%
Present study	60	>40	83.33 %	90.0%

Table 6. Comparison of sensitivity and specificity of CRP in tubercular pleural fluid at various cut off values with previous studies

Authors	N(no. of subjects)	Cut Off Value (mg/l)	Sensitivity	Specificity
Chierakul N et al (12)	148	>30	72%	93.0%
Pachon et al (13)	144	>50	95%	95%
Yilmaz T U et al (14)	97	>30	93.7%	76.5%
Present study	60	>50	76.67%	90.0%

Very few studies have been done of ADA and L/N ratio together. We calculated the sensitivity, specificity, PPV and NPV of the above two . our results were comparable with the data of study done by Lesley J. Burgess et al (23). In our study the calculated sensitivity and specificity was 100% and 36.67% while PPV and NPV of this combination was 61.22% and 100% respectively and in comparison to study done by Lesley J. Burgess et al (23) using same cut off L/N ratio of 0.75 or greater in 303 patients the sensitivity, specificity, PPV and NPV was 88%, 95%, 95% and 88% respectively.

We also studied the combinations that is the combination of ADA and CRP and other combination being ADA, CRP and L/N ratio to calculate the sensitivity, specificity, PPV, NPV of ADA and CRP was 100%, 83.33%, 85.71%, 100% respectively and that of ADA, CRP and L/N ratio was 100%, 30%, 58.82%, 100% respectively.

For the diagnosis of tubercular pleural effusion measurement of CRP and ADA activity individually have been evaluated. Perleppe et al did a similar study to find that ADA and CRP can be used to differentiate between exudative pleural effusions (24). Our discoveries propose that joint estimation of CRP levels and ADA activity can be specially used to advance affectability in an either/or combination, or specificity, in a combination requiring the two strategies utilized, to be certain.

The current study differentiating tubercular pleural effusion from pleural effusion due to other causes shows a statistical significant association of ADA, CRP and L/N ratio levels (p value <0.001). On comparison of individual and combination test in our study, it is found that L/N ratio being more sensitive than CRP and ADA, while ADA and CRP being most specific but the combination of ADA and CRP added to the sensitivity and specificity in comparison to individual methods. The most specific combination was use of both biomarkers (ADA & CRP) which yielded specificity of 83.33%. While combination of any two or all three biomarkers yielded 100% sensitivity which is helpful in identifying tubercular pleural effusion .

In this way, in various conditions the test of demonstrative difficulty and the resulting deferred treatment inception might be used in utilizing combination of these fast

strategies on pleural fluid. ADA and CRP estimation are basic and promptly accessible. We trust that the results of the present examination show that separately and in combination, above strategies can offer viable mean of expanding demonstrative viability.

Discussion

Thus, in our study ADA and CRP were found to help in distinguishing tubercular pleural effusion and on adding these two tests the specificity in-creased. L/N ratio was found to be most sensitive while combination of ADA and CRP being the most specific investigation to diagnose tubercular pleural effusion. Combining ADA, CRP increases both the sensitivity and specificity significantly.

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