

# Diagnostic Accuracy of Ada at Various Cut off Limit in Tubercular Pleural Effusion

Dr. Shubhra Tiwari<sup>1</sup>, Dr. Rakhi Negi<sup>2</sup>, Dr. Pratibha Misra<sup>3</sup>, Dr. Arpitha<sup>4</sup>, Dr. Prashant Patil<sup>5</sup>

<sup>1</sup> Junior Resident, Department of Biochemistry, AFMC Pune, Maharashtra (India)

<sup>2</sup> Professor, Department of Biochemistry, AFMC Pune, Maharashtra (India)

<sup>3</sup> Professor & HoD, Department of Lab Sciences 153 GH,

<sup>4</sup> Classified Specialist & HoD, Department of Pathology, AICTS Pune,

<sup>5</sup> Graded Specialist, Department of Microbiology, AICTS Pune

**Abstract:** *Background:* Tuberculosis (TB), is a bacterial disease caused by *Mycobacterium tuberculosis*. The spectrum of extrapulmonary TB (EPTB) includes pleural fluid, ascitic fluid, intestine, spine etc. Diagnosis of TB can be done using Ziehl - Neelsen (ZN) staining and Polymerase Chain Reaction (PCR), but these methods do not have enough sensitivity and specificity. The gold standard test for diagnosing TB is mycobacterium culture, but it takes six weeks to isolate which can lead to a delay in diagnosis. Estimating Adenosine deaminase (ADA) is a simple, low - cost, quick aid for diagnosing tubercular pleural effusion. *Methodology:* We measured ADA in pleural fluid in 60 patients in a tertiary care hospital by using a Robonik biochemistry semi - auto analyser. Other investigations like - ZN staining, culture, and cytological analysis are also performed on patients. A cross - sectional study is done to know the sensitivity and specificity of ADA at various cut off ranging from <40, 40 - 60, 60 - 80 and >80IU/L in diagnosing tubercular pleural effusion. Also, correlation is done with predominant leucocyte count and LDH/ADA ratio. *Results:* Pleural fluid ADA level <40 IU/L has a high sensitivity for ruling out the disease. Sensitivity and specificity are found to moderate in the range of 40 - 80IU/L and high at the ADA level of >80 IU/L. *Conclusion:* Though ADA is an established marker for the diagnosis of tubercular pleural effusion, the cut - off with high sensitivity and specificity remains unclear. So, we aim to study the sensitivity and specificity of ADA at various cut - off levels and the utility of the LDH/ADA ratio in the diagnosis of tubercular pleural effusion.

**Keywords:** ADA, tubercular pleural effusion, sensitivity and specificity

## 1. Introduction

Tuberculosis (TB) is a bacterial disease caused by *Mycobacterium tuberculosis*. Being one of the main health problems, early detection and treatment are of utmost importance to reduce the burden of the disease. The most obvious impact of COVID - 19 is a large global drop in reported and newly diagnosed cases of TB. This fall in 2021 is 18% compared to 2019, a decline back to the level of 2012, which is approximately 10 million people who developed TB in 2020 but were not reported or diagnosed. 16 countries accounted for 93% of this reduction, with India, Indonesia and the Philippines being the worst affected (1). The prevalence of TB in India is 316 per one lakh in 2021 as per a survey conducted by the Ministry of Health and Family Welfare under the National Tuberculosis Elimination Programme (2). The spectrum of Extrapulmonary TB (EPTB) includes pleural fluid, ascitic fluid, intestine, spine etc. Pleural effusion refers to the accumulation of fluid in pleural space caused by an imbalance between the formation and absorption of pleural fluid (3). The etiology of pleural effusion is vast and depends on the type of pleural effusion - Transudative or exudative. The most common etiology for pleural effusion is Tuberculosis (TB), a bacterial disease caused by *Mycobacterium tuberculosis*. For a definite diagnosis of TB microbiological tests available are sputum microscopy, culture and the use of Cartridge - based Nucleic Acid Amplification Tests (CB - NAAT). Extrapulmonary TB is more difficult to diagnose (4). The definitive diagnosis of EPTB is made upon demonstration of tubercle bacillus

within the specimens like pleural fluid, ascitic fluid, pericardial fluid, Cerebrospinal Fluid (CSF) or pleural biopsy specimen (5). The gold standard test for diagnosing TB is mycobacterium culture, but it takes six weeks to isolate which can lead to a delay in diagnosis, sensitivity to the anti - tubercular drug will further take six weeks (5). There is a need for a test which is simple, fast and reliable and easy to perform.

Diagnosis of tubercular pleural effusion is made by isolation of mycobacterium from a pleural biopsy specimen or pleural fluid or identifying caseating granuloma in the histology of biopsy, but these procedures are difficult to perform, plus for definitive diagnosis, both histological and microbiological examination of three pleural biopsy specimen is required (6).

Adenosine Deaminase (ADA) is a purine degrading enzyme involved in mononuclear phagocyte maturation, it catalysis deamination reaction from adenosine to inosine. Two isoforms of ADA have been identified, ADA - 1 and ADA - 2. ADA - 1 is mainly present in RBC and other tissues, and ADA - 2 is present only in monocyte and macrophages. It acts in the proliferation and differentiation of T - lymphocytes. It can be a good indicator of active cellular immunity. Due to its presence in body fluids, it is being used as a surrogate marker for the diagnosis of TB. The levels increase in TB due to the stimulation of T - cells by mycobacterial antigens (7).

It has been seen to accumulate in the pleural fluid of TB patients and can be used as a useful marker to predict TB pleural effusion with high sensitivity and specificity (6) . It is a simple, low - cost, quick aid for diagnosing tubercular pleural effusion (5) .

This study aimed to compare the sensitivity and specificity of pleural ADA at different levels, LDH: ADA ratio as diagnostic methods for tubercular pleural effusion, using the receiver operating characteristic curve (ROC) curve for different levels of ADA and LDH: ADA ratio, in a tertiary care hospital.

## 2. Methodology

We have conducted a hospital - based observational study, in a tertiary care hospital. A total of 60 patients with suspected TB pleural effusion having symptoms such as pleuritic chest pain, dry cough, weight loss, dyspnoea, and evening rise of temperature, were selected irrespective of age and sex. The procedure was explained to the patients and their informed consent was obtained. Patient data such as age and sex were noted.

### Sample Collection

We collected pleural fluid from 60 patients with pleural effusion suspected of tubercular pleural effusion. 30 - 40mL of pleural fluid was collected in a sterile, leak - proof, screw - capped universal sterile container using a standard thoracentesis procedure. The sample was sent for biochemical testing along with ADA and LDH measurements. The sample was also sent for ZN staining and culture and sensitivity.

Different biochemical parameters like protein, albumin, glucose, cholesterol, LDH and ADA were estimated. ADA was measured by using a Robonik biochemistry semi - auto analyzer with a colorimetric - based enzymatic Adenosine Deaminase assay kit from Erba. Other biochemical parameters were estimated using Siemens Dimension EXL200.

Other investigations like - cytological analysis were also performed on pleural fluid. Detailed clinical history and other investigation details like chest X - ray, and Ultrasonography findings were obtained.

A cross - sectional study was done to know the sensitivity and specificity of ADA at various cut off ranging from >40 IU/L, >60 IU/L, and >80IU/L in diagnosing tubercular pleural effusion. Also, the correlation was done with predominant leucocyte count and LDH: ADA ratio.

### Statistical Analysis

Data were entered into a Microsoft excel document. The sensitivity and specificity were calculated manually. ROC curve was obtained using GraphPad Prism software for ADA, LDH: ADA ratio and used to determine the optimal cut - off point for the diagnosis.

## 3. Results

A total of 60 patients between the age of 15 years to 75 years were analyzed, with a mean age being 36 years. There were 11 females and 49 males. Maximum patients were between the age group of 31 to 50 years with male predominance (80%) [Table - 1].

**Table 1:** Age and sex distribution of the 60 participants

Age distribution (years)	Male	Female	Total
11 to 20	8	3	11
21 to 30	10	1	11
31 to 40	14	1	15
41 to 50	12	2	14
51 to 60	2	3	5
61 to 70	1	1	2
71 to 80	2	0	2
Total	49	11	60

Out of 60 cases, 41 cases were reported positive for culture for TB pleural effusion, while 18 were negative culture report.

**Table 2:** Distribution of cases among various ADA cut - off

ADA levels	<40 IU/L	>40IU/L	>60 IU/L	>80 IU/L
Total cases having ADA value more than cut off	16.6 % (n=10)	83.3% (n=50)	60% (n=36)	33.3% (n=20)
Total positive by ZN stain	00	45% (n=27)	45% (n=27)	28.3% (n=17)
Total positive by culture	3.3% (n=2)	66.7% (n=40)	50% (n=30)	30% (n=18)
Total positive by both ZN stain and culture	3.3% (n=2)	70% (n=42)	53.3% (n=32)	30% (n=18)

The maximum level of ADA in pleural effusion was 265 IU/L while the minimum was 15.65 IU/L with the mean value of 86.61 IU/L [Table - 3].

**Table 3:** Range of ADA levels.

	In positive cases	In negative cases	In total cases
Maximum (IU/L)	265	169	265
Minimum (IU/L)	15.78	15.65	15.65
Mean	140.39	92.33	140.33

### Analysis of Pleural ADA levels

Using the ROC curve of pleural ADA level [Fig - 1], it was found that for the cut - off value of >40 IU/L, sensitivity and specificity are 95.2% and 44.45%, respectively [Table - 4]. The sensitivity and specificity at a cut - off of >60 IU/L are 71% and 66.67%, respectively. At ADA level >80 IU/L sensitivity and specificity are 56.25% and 88.8% respectively.

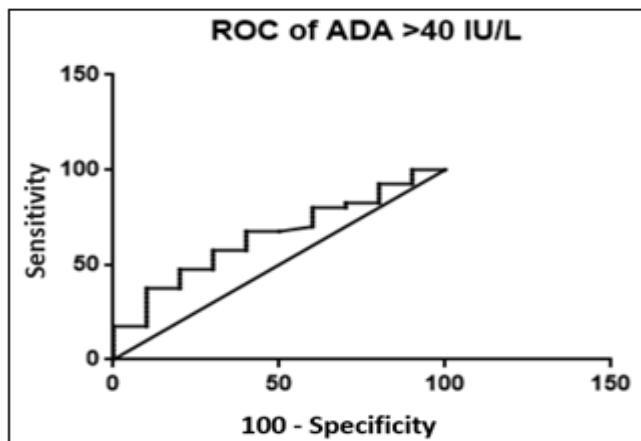


Figure 1: ROC curve of pleural ADA levels

Table 4: Statistical analysis of ADA level

Variables	Values
Area under the ROC curve (AUC)	0.65
Standard error	0.091
Associated criterion	>40 IU/L
Sensitivity	95.2%
Specificity	44.45%

**Analysis of pleural LDH levels**

The maximum level of LDH in pleural effusion was 1646 IU/L, and the minimum was 59 IU/L [Table - 5]

Table 5: Range of LDH level

	In positive cases	In negative cases	In total cases
Maximum (IU/L)	1646	865	1646
Minimum (IU/L)	78	59	59
Mean	862	462	852.5

Using the ROC curve of pleural LDH level [Fig - 3], for the cut - off value of >150IU/L, the sensitivity and specificity are 72.72% and 60%, respectively [Table - 6].

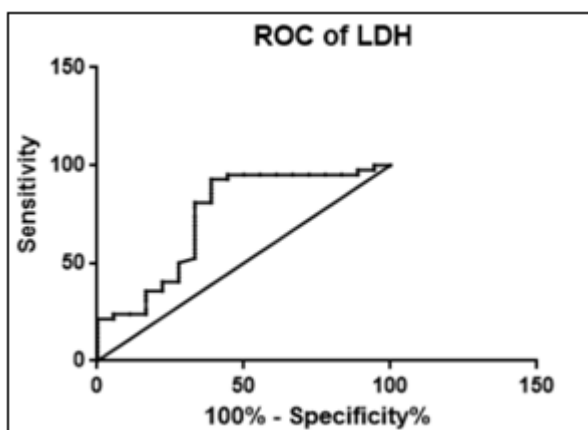


Figure 3: ROC curve for LDH

Table 6: Statistical analysis of LDH level

Variables	Values
Area under the ROC curve (AUC)	0.74
Standard error	0.078
Associated criterion	>150 IU/L
Sensitivity	72.72%
Specificity	60%

**Analysis of Pleural LDH: ADA ratio**

The maximum ratio of LDH: ADA ratio in pleural effusion level was 32.59, and the minimum ratio was 1.61 with a mean value of 7.72 [Table - 7]

Table 7: Range of LDH: ADA ratio

	In positive cases	In negative cases	In total cases
Maximum (IU/L)	32.59	16.17	32.59
Minimum (IU/L)	1.61	1.77	1.61
Mean	17.1	8.97	17.1

Using the ROC curve of pleural LDH: ADA ratio was plotted [Fig - 4], and no significant discrimination power was found.

**Analysis of ZN staining**

Out of 60 cases of pleural effusion 41 were positive for TB pleural effusion in culture, only 12 are found positive for ZN staining, showing AFB.

LDH has a maximum area under curve (0.74), followed by ADA (0.65) and while LDH: ADA ratio showed a low area under curve (0.51), and ADA has a maximum sensitivity of 95.2% at a cut - off of 40 IU/L and ZN staining having maximum specificity of 100%.

**Analysis of cell counts**

Out of 60 cases, 40 were having lymphocytic predominance in their pleural fluid and 17 had neutrophilic predominance while 03 samples were found hemolytic so cell counts could not be ascertained.

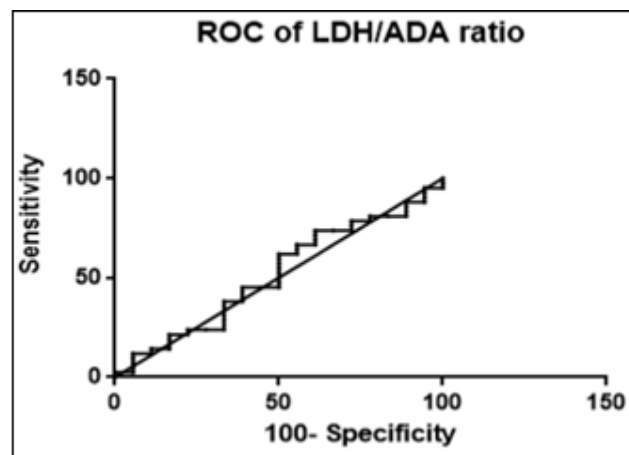


Figure 4: ROC curve of Pleural LDH: ADA ratio

**4. Discussion**

TB is a highly prevalent disease in India and other developing countries and is also one of the most infectious diseases worldwide. The spectrum of extrapulmonary TB involves many organ systems, one of the most common is tubercular pleural effusion. The common presenting features include coughing, pleuritic chest pain, breathlessness, and weight loss.

The cut - off of ADA used by the developed countries cannot be used for the diagnosis of tubercular pleural effusion in developing countries due to the high disease burden. We conducted this study to know the sensitivity and specificity of ADA at various cut - off levels for the

diagnosis of tubercular pleural effusion. We also studied the pleural fluid LDH and LDH: ADA ratio.

In our study, ADA has been shown a very good candidate biomarker in the diagnosis of tubercular pleural effusion in culture - positive cases. ROC curve analysis showed a significant diagnostic utility for ADA at cut - off levels 40 IU/L, 60 IU/L and 80 IU/L. It has been noted in previous studies that ADA cut - off of >40 IU/L especially with lymphocyte predominant pleural effusion has excellent positive predictive value in countries with a high burden of tuberculosis (8). In our study, the maximum diagnostic utility was seen in the cut - off level of 40 IU/L with a sensitivity of 95.2%. In a meta - analysis by Aggarwal et al, 65 studies with ADA threshold of 40 - 44 IU/L showed summary sensitivity and specificity of 0.93 and 0.90 respectively for tubercular pleural effusion (9).

LDH: ADA ratio does not show significant diagnostic utility in our study, however, LDH alone has shown significant diagnostic utility at a cut - off of 150 IU/L with a sensitivity of 72.72% & specificity of 60%. In a previous study by Zan et al the pleural fluid LDH: ADA ratio was shown to have only moderate diagnostic accuracy for tubercular pleural effusion (10). However, in a large cohort study by Beukes A et al, the pleural fluid LDH: ADA ratio was found to be a very useful diagnostic marker for tubercular pleural effusion. The inconclusive results in our study may be due to the small sample size.

## 5. Conclusion

Though ADA is an established marker for the diagnosis of tubercular pleural effusion, the cut - off with high sensitivity and specificity remains unclear. So, we aim to study the sensitivity and specificity of ADA at various cut - off levels and the utility of the LDH: ADA ratio in the diagnosis of tubercular pleural effusion. Further studies can be conducted with a larger population size.

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