# To assess diagnostic utility of pleural fluid adenosine deaminase (ADA), interferon gamma (IFN), lymphocyte/neutrophil ratio (L/N) and its combination in differentiating tubercular and non-tubercular exudative pleural effusion

**Varuna Jethani[1,*], Girish Sindhwani[2], Vinit Mehrotra[3], Aarti Kotwal[4]**

[1]Assistant Professor, [2-4]Professor, [1,2]Dept. of Pulmonary Medicine, [3]Dept. of Biochemistry, [4]Dept. of Microbiology, [1,3,4]Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, [2]All India Institute of Medical Sciences, Rishikesh, India

***Corresponding Author: Varuna Jethani**
Email: varuna1212@rediffmail.com

## Abstract

**Background:** One of the common extrapulmonary manifestation of tuberculosis (TB) is pleural effusion. The conventional culture suffers from lack of sensitivity. Many pleural fluid markers have been evaluated but none has been proved to be ideal in diagnosing tubercular pleural effusion. We have studied Adenosine deaminase (ADA), Interferon gamma (IFN), lymphocyte/neutrophil (L/N) ratio and their combination for diagnosis of tubercular pleural effusion.

**Methodology:** All consecutive patients with pleural effusion were subjected to thoracentesis and segregated into transudative and exudative using Light`s criteria. Patients with exudative pleural effusion were enrolled and divided into two groups. Group-I comprised of patients with tubercular etiology, Group-II- non-tubercular etiology. 45 patients were selected for each group. ADA, IFN, L/N ratio in pleural fluid of these patients were measured. The sensitivity, specificity and predictive values were calculated.

**Results:** The sensitivity, specificity, positive predictive value, negative predictive value of ADA, IFN, L/N ratio were 88.89%, 99.85%, 86.96%, 99.87%; 97.78%, 97.78%, 97.78%, 97.78%; 88.89%,17.78%, 51.95%, 61.54% respectively. Combination of ADA, IFN, L/N ratio provided the highest specificity that is 100%.

**Conclusions:** Pleural fluid ADA, IFN were found to be useful in differentiating tubercular from non-tubercular patients. Combination of ADA, IFN, L/N was found to be most beneficial.

**Keywords:** ADA; sExtrapulmonary tuberculosis; Pleural effusion.

## Introduction

One of the commonest extrapulmonary manifestations of tuberculosis (TB) is pleural effusion [1]. Detecting the organism on smear or culture being considered as gold standard but lack of sensitivity and culture also takes long duration to result such that acid fast bacilli (AFB) staining is positive in just 10 to 25% of the cases, while culture for AFB is positive in under 25% of the cases [2].

For diagnosing tubercular pleural effusion numerous pleural fluid markers have been assessed, however none has been observed to be perfect as they lack in sensitivity or specificity, difficulty in performing or availability etc. Some of these markers include serum CA 125, pleural alkaline phosphatase, pleural fluid Procalcitonin concentration, pleural fluid Interleukin-1, pleural Tumor necrosis factor, pleural fluid PCR, cell count (total and differential count), biochemical tests [Adenosine deaminase (ADA)], and microbiological tests [Ziehl-Neelsen (ZN) stain, culture] and T cell products (Interferon gamma) etc. [3]. We have studied ADA, Interferon gamma (IFN), lymphocyte/neutrophil (L/N) ratio and their combination for diagnosis of tubercular pleural effusion. Present study is the first of its kind from Indian subcontinent.

## Materials and Methods

The present study was an observational study to differentiate between tubercular and non-tubercular pleural effusion using ADA, IFN, L/N ratio. The study was conducted in the Department of Pulmonary Medicine at a tertiary care teaching institute of North India after approval from institutional ethical committee. Every sequential patient with pleural effusion was subjected to thoracentesis. Patients were additionally isolated into transudative and exudative in view of Light`s criteria. Patients with exudative pleural effusion were isolated into two groups, Group-I included patients of tubercular etiology and Group-II comprised of patients of non-tubercular etiology. Forty five patients were taken in each group. A written informed consent was taken from study subjects. Inclusion and exclusion criteria which were used while selecting patients are:

**Inclusion Criteria:**

1. Age > 20 years
2. All consecutive patients diagnosed with exudative pleural effusion

**Exclusion Criteria:**

Patients with (on pleural fluid aspiration)

1. Pyothorax
2. Hemothorax
3. Transudative pleural effusion

Immunocompromised (Human Immunodeficiency virus - positive)

**Diagnosis of Tubercular Pleural Effusion** (Group-I) was based on at least one of the following criteria:

1. Pleural fluid positive for AFB smear/ culture.
2. Sputum for AFB positive by smear/ culture.
3. Histopathology positive for TB (presence of granulomas or AFB) in the pleural biopsy specimen.
4. Patients with clinical features suggestive of TB and favourable response to anti-tubercular treatment were also retrospectively included in the study.

**Diagnosis of Non-tubercular Pleural Effusion** (Group-II) was based on either of the following:

1. Malignant pleural effusion by either cytology or histology positive for malignancy
2. Parapneumonic effusion by clinical features suggesting of pneumonia and pleural fluid negative for presence of bacteria.
3. Any other etiology confirmed by its specific marker e.g. pleural fluid positive for amylase etc.
4. No evidence of TB (defined by criteria for group-I)

ADA, IFN, L/N ratio were measured of both these groups. ADA was measured by the help of DXC 800 (Beckman Coulter) where One unit of ADA is characterized as the measure of ADA that produces one micromole of inosine from adenosine per min at $37^0$C. IFN was detected by ELISA kit. (Gene probe Diaclone) The sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated to differentiate between tubercular and non-tubercular pleural effusion.

**Data Management and Statistical Analysis**

Data was analyzed by using statistical software SPSS version 22. Quantitative data was expressed in terms of Mean ± SD. Mann – Whitney U test and independent t test was used to compare mean of two groups. ROC curve was used to calculate cut-off values of all parameters. Sensitivity, specificity, PPV and NPV were calculated at a specific cut off for ADA, IFN, L/N ratio. P< 0.05 was considered as statistical significant.

## Results

A total of 90 patients were analysed, of which 67 were males and 23 females. Accordingly to etiological diagnosis, the distribution of patients was as follows: 45 patients with tubercular pleural effusion confirmed as by above mentioned criteria, rest 45 patients with exudative pleural effusion due to malignancy (36) (confirmed by either cytology or histopathology of any intrathoracic specimen), pancreatitis (5) (based on history, investigations {amylase,}), parapneumonic effusion (4) (patients with clinical features suggesting of pneumonia along with pleural effusion, which was negative for presence of bacteria) as shown in Fig. 1.

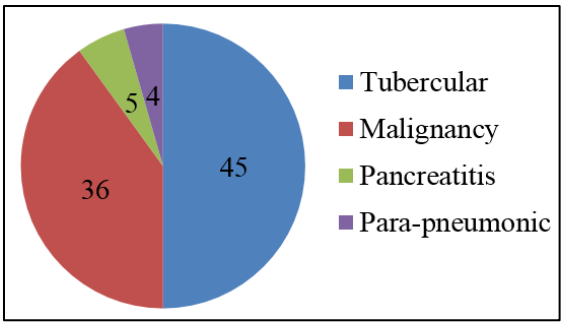


**Fig. 1: Distribution of cases according to etiology (n=90)**

The ADA, IFN, Lymphocyte count were calculated of both the groups and the mean values are mentioned in Table 1.

**Table 1: Mean values of ADA, Lymphocyte count and Interferon-gamma in group-I and group-II (n= 45)**

| Mean values of ADA, lymphocyte count and interferon-gamma in group-I and group-II | | | | |
|---|---|---|---|---|
| | **Group** | **Mean±Std. Deviation** | **Std. Error Mean** | **P- value** |
| ADA (IU/L) | I | 69.73 ±39.980 | 5.960 | 0.001* |
| | II | 25.00 ±16.671 | 2.485 | |
| Lymphocyte Count (%) | I | 77.56 ±21.345 | 3.182 | 0.032# |
| | II | 67.20 ±23.620 | 3.521 | |
| Interferon Gamma (pg/ml) | I | 377.18 ±67.656 | 10.086 | 0.0001* |
| | II | 28.60 ±62.276 | 9.284 | |

*Mann-Whitney - U test, #Independent t test

Mean values of ADA, IFN, lymphocyte count in tubercular pleural effusion cases was 69.73, 377.18 and 77.56 respectively and in pleural effusion due to other causes was 25, 28.60 and 67.20 respectively. The intergroup difference of IFN was found to be the most statistically significant among all parameters ($p < 0.0001$). In present study we used a cut off of 40 IU/L for ADA, 200 pg/ml for IFN and 0.75 for L/N ratio, based on previous studies and also calculated by using ROC curve as seen in Fig. 2. According to ROC curve, the sensitivity was 88.89%, specificity was 84.44% respectively with value of ADA above 38.36 IU/L, IFN above 115pg/ml, sensitivity was 97.78%, and specificity was 97.78% and for lymphocyte count above70 sensitivity was 68.89% and specificity of 57.78%respectively.

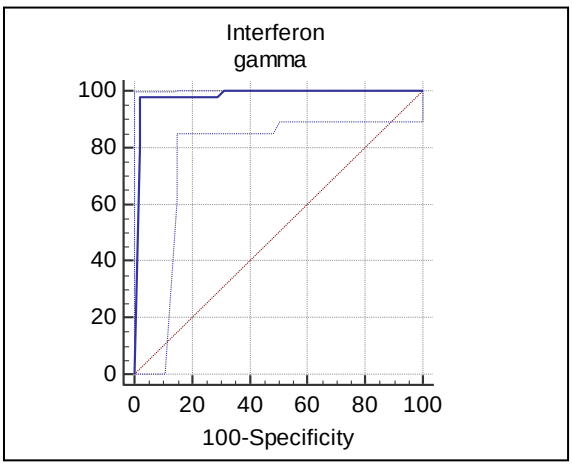


**Fig. 2: ROC curve of ADA and IFN**

| | |
|---|---|
| Youden index J | 0.7333 |
| Associatd criterion | >38.36 |
| Sensitivity | 88.89 |
| Specificity | 84.44 |
| Area under the curve | 0.901 |

| | |
|---|---|
| Youden index J | 0.9556 |
| Associated criterion | >115 |
| Sensitivity | 97.78 |
| Specificity | 97.78 |
| Area under curve | 0.98 |

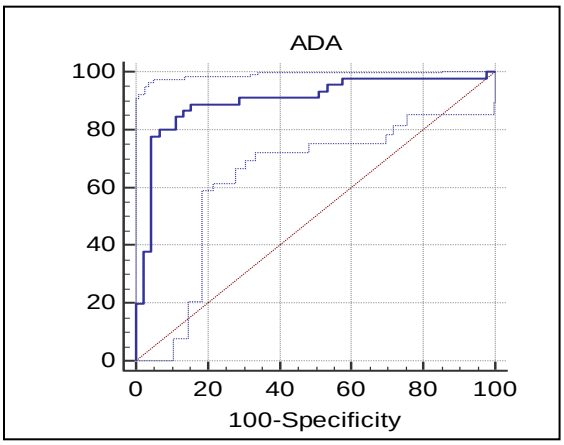


**Fig. 3**

The Sensitivity, Specificity, PPV, NPV of ADA, IFN, L/N ratio were calculated and are given in Table 2. The comparison of Sensitivity, Specificity, PPV, NPV of ADA, IFN and its combination is given in Table 3.

**Table 2: Sensitivity, specificity, PPV, NPV of ADA, IFN, L/N ratio**

| **ADA (Using cut off 40IU/L)** | | **Interferon Gamma(Using cut off 200pg/ml)** | **Lymphocyte/Neutrophil Ratio(using a cutoff 0.75)** |
|---|---|---|---|
| Sensitivity | 88.89% | 97.78% | 88.89% |
| Specificity | 99.85% | 97.78% | 17.78% |
| PPV | 86.96% | 97.78% | 51.95% |
| NPV | 99.87% | 97.78% | 61.54% |

**Table 3: Sensitivity, Specificity, PPV, NPV of ADA and IFN combination and comparison with individual parameters (n=90)**

| | **Sensitivity** | **Specificity** | **PPV** | **NPV** |
|---|---|---|---|---|
| ADA and Interferon gamma | 97.78% | 84.78% | 86.27% | 97.50% |
| ADA and lymphocyte / neutrophil ratio | 77.78% | 91.11% | 89.74% | 80.39% |
| Interferon gamma and lymphocyte / neutrophil ratio | 86.67% | 97.78% | 97.50% | 88.00% |
| ADA, Interferon gamma and lymphocyte/neutrophil ratio | 75.56% | 100.00% | 100.00% | 80.36% |
| ADA | 88.89% | 99.85% | 86.96% | 99.87% |
| Interferon gamma | 97.78% | 97.78% | 97.78% | 97.78% |
| lymphocyte/neutrophil ratio | 88.89% | 17.78% | 51.95% | 61.54% |

Thus, ADA, IFN and L/N ratio were observed to be valuable in separating tubercular from non-tubercular patients. ADA being more specific and IFN being sensitive and most interestingly the combination of ADA, IFN and L/N ratio was found to be most specific that is 100%.

## Discussion

Tubercular pleural effusion is a typical appearance of extrapulmonary TB [4]. It occurs due to rupture of sub-pleural focus exposing tubercular antigen to helper T cells resulting in delayed type hypersensitivity [5]. This mechanism is seen in both primary and reactivation cases of TB. As clearly evident, there will be either no bacteria or less bacteria present in the pleural fluid6. As discussed above, as pleural TB being a paucibacillary disease will have a low sensitivity in cases of direct microscopy (0-5%) and mycobacterial culture (25-35%) which are the gold standard

tests for diagnosing effusion. Only pleural biopsy done via thoracoscopy has high sensitivity [3].

Customary demonstrative tests for pleural TB incorporate microscopic examination of the pleural fluid for AFB, sputum or pleural tissue, mycobacterial culture of pleural fluid and pleural tissue histopathological examination for granulomatous inflammation.

Various alternative novel tests have been evaluated to differentiate between tubercular and non-tubercular pleural effusion. They include pleural fluid ADA, serum CA 125, pleural IFN, pleural alkaline phosphatase, pleural fluid Procalcitonin concentration, pleural fluid Interleukin-1, pleural Tumor necrosis factor, pleural fluid PCR etc. The evaluation of efficacy of alternative novel tests is usually done against gold standard that is mycobacterial culture which has low sensitivity as discussed above. The results from these tests should be interpreted cautiously taking the complete clinical and other supportive laboratory parameters and should not be followed blindly.

We, in our study, planned to evaluate the efficacy of ADA, IFN, L/N ratio individually and in combination, to diagnose tubercular pleural effusion. Elevated levels of ADA, IFN, L/N ratio in tuberculous pleural effusion have been noted by several authors but the combination of these markers have not been adequately evaluated in past.

ADA is released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation. It can be raised in both tubercular and non-tubercular pleural effusion. The ADA2 isoenzyme predominant in tubercular pleural effusion, released from monocytes and macrophages is the dominating supporter of aggregate ADA activity [7]. With a cut –off of 40 U/l, in our study, ADA was found to have sensitivity and specificity of around 89% and 99.8% respectively which is quiet similar to various other studies as shown in Table 4. Comparison of sensitivity and specificity of ADA in tubercular pleural fluid at various cut off values with previous major studies

**Table 4: ADA in tubercular pleural effusion**

| Authors | n (no. of subjects) | Cut Off Value (IU/L) | Sensitivity | Specificity |
|---|---|---|---|---|
| Jayalakshmi et al., [8] | 50 | > 40 | 83.87% | 78.94% |
| Sharma S.K et al., [10] | 75 | >35 | 83.3% | 66.6% |
| Krenke R et al., [9] | 94 | >40.3 | 100% | 93.9% |
| Maria V.V et a., [11] | 140 | >45.5 | 88.1% | 85.7% |
| Lesley J et a., [12] | 303 | >50 | 91% | 81% |
| Present study | 90 | >40 | 88.89% | 99.85% |

IFN is also released from lymphocytes which are stimulated by the tuberculoprotein causing delayed type hypersensitivity. Hence its value is also highly raised in lymphocytic predominant tubercular effusions [13]. It was found to be more specific and sensitive than interleukin (IL)-IL-18, 12p40, or immunosuppressive acidic protein.

Various studies have used different cut- off values for IFN which could be due to different techniques of processing and measuring IFN[13] In our study, we used cut off of 200 pg/ml and found that measurement of IFN is highly sensitive and specific and the sensitivity, specificity of L/N ratio using a cut of 0.75 it was found to be 88.89%, 17.78% respectively. Table 5 shows comparison of our results of IFN with various studies.

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

| IFN in tubercular pleural effusion | | | | |
|---|---|---|---|---|
| **Authors** | **n (no. of subjects)** | **Cut Off Value(pg/ml)** | **Sensitivity** | **Specificity** |
| Wongtim. S et al., [14] | 66 | 240 | 94.9% | 96.3% |
| Krenke et al., [9] | 94 | 75 | 100% | 98.5% |
| Krenke et al., [15] | 90 | 100 | 100% | 98.5% |
| Present study | 90 | 200 | 97.8% | 97.8% |

Subsequently, the test of symptomatic productivity in various conditions of prevalence may be tended to utilizing blends of these quick strategies on pleural liquid. ADA, IFN and L/N ratio measurement are simple; we believe that the results of this investigation show that separately these strategies can offer a methods for getting analytic proficiency and differentiate between tubercular and non-tubercular pleural effusion, but on combining these test the specificity is increased.

Maria V [11] did a study on evaluation of PCR, ADA, and IFN in pleural fluid for the differential diagnosis of pleural tuberculosis in 140 cases of pleural effusion they used

a cut off >45.5 U/L for ADA and 0.8U/ml was used as sensitivity for IFN assay detection. IFN levels in combination with ADA activity displayed the specificity of 83.8% and sensitivity of 89.7%, whereas in our study the combination of ADA and IFN has specificity of 84.78% and sensitivity of 97.78.

Our study had the limitation of having small sample size. More number of subjects should be recruited to such study in a larger multicenter study to assess the sensitivity, specificity of these tests. Another limiting factor of our study was presence of a heterogenous group of non-tubercular etiology. Our control group had patients of malignant effusions, pancreatic effusions, parapneumonic effusion. Actual picture of each etiology could be gathered if each etiology is addressed individually.

## Conclusions

In our study ADA, IFN, L/N ratio were found to help in differentiating tubercular pleural effusion from pleural effusion due to other causes but on combining these test specificity was increased. IFN was found to be most sensitive and ADA the most specific investigation to diagnose tubercular pleural effusion.

**Abbreviations:** TB = tuberculosis, ADA= adenosine deaminase, IFN= interferon gamma, L/N ratio = lymphocyte/neutrophil ratio, AFB = acid fast bacilli, ZN= Ziehl-Neelsen, PPV = Positive predictive value, NPV= Negative predictive value

**Conflicts of Interests:** None declared.

**Acknowledgments:** Nil.

## References

1. Light RW. Useful tests on the pleural fluid in the management of patients with pleural effusions. *Curr Opin Pulm Med* 1999;5:245–249.
2. Scharer L, McClement JH. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura. *Am Rev Respir Dis* 1968;97(3):466-468.
3. Crompton GK, McHardy GF. Diseases of the respiratory system. In: Edwards CR, Boucher IA. Davidson's Principles and Practice of Medicine. 16 th edition. Edinburgh: ELBS; 1992. p. 410-412.
4. A. Trajman, M. Pai, K. Dheda, R. vanZylSmit, A.A. Zwerling, R. Joshi, S. Kalantri, P. Daley, D. Menzies. Novel tests for diagnosing tuberculous pleural effusion: what works and what does not? *Eur Respir J* 2008;31:1098–1106.
5. Barnes PF, Mistry SD, Cooper CL, Pirmez C, Rea TH, Modlin RL. Compartmentalization of a CD4+ T lymphocyte subpopulation in tuberculous pleuritis. *J Immunol* 1989;142:1114–1119.
6. Kim HJ, Lee HJ, Kwon SY. The prevalence of pulmonary parenchymal tuberculosis in patients with tuberculous pleuritis. *Chest* 2006;129:1253–1258.
7. Haque SS. Evaluation of Adenosine Deaminase (ADA) in Tuberculous Pleurisy. *Am J Med Med Sci* 2012:2(1):1-4.
8. Lingam J, Kandi S. Evaluation of pleural fluid interferon gamma as a diagnostic marker of tuberculous pleural effusion. *J NTR Univ Health Sci* 2013;2:92-95.
9. Krenke R, Safianowska A, Paplinska M, Nasilowski J, Dmowska-Sobstyl B, Bogacka-Zatorska E et al. Pleural fluid adenosine deaminase and interferon gamma as diagnostic tools in tuberculosis pleurisy. *J Physiol Pharmacol* 2008;59(Suppl 6):349-360.
10. Sharma SK, Mohan A. Eptuberculosis. *Indian J Med Res* 2004;120:316–353.
11. Villegas MV, Labrada LA, Saravia NG. Evaluation of polymerase chain reaction, adenosine deaminase, and interferon-gamma in pleural fluid for the differential diagnosis of pleural tuberculosis. *Chest* 2000;118:1355-1364.
12. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. *Chest* 1996;109(2):414-419.
13. Ribera E, Ocana I, Martinez-Vazquez JM, *et al*. High level of interferon gamma in tuberculous pleural effusion. *Chest* 1988;93:308–11.
14. Wongtim S, Silachamroon U, Ruxrungtham K, Udompanich V, Limthongkul S, Charoenlap P, et al. Interferon gamma for diagnosing tuberculous pleural effusions. *Thorax* 1999; 54:921–924.
15. Krenke R, Safianowska A, Paplinska M, Korczynski P, Dmowska-Sobstyl B, Bogacka-Zatorska E et al. Pleural fluid interferon-gamma measurement as a diagnostic tool in tuberculous pleurisy. *Pneumonol Alergol Pol* 2008;76(4):237-245.

**How to cite this article:** Jethani V, Sindhwani G, Mehrotra V, Kotwal A. To assess diagnostic utility of pleural fluid adenosine deaminase (ADA), interferon gamma (IFN), lymphocyte/neutrophil ratio (L/N) and its combination in differentiating tubercular and non-tubercular exudative pleural effusion. *Indian J Immunol Respir Med* 2019;4(1):68-72.