



## **Diagnostic utility of pleural fluid ADA and serum CA 125 in exudative pleural effusion and co-relation with histopathology: thoracoscopic pleural biopsy experience at a tertiary care centre in North India**

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### **Abstract**

*Pleural effusion is a common respiratory illness. The two most common causes of massive exudative pleural effusion in India are tuberculosis (TB) and malignancy (mainly lung cancer). The confirmatory diagnosis of TB requires the presence of acid fast bacilli or growth of *Mycobacterium tuberculosis* in pleural fluid culture. Similarly, the conclusive diagnosis of malignancy requires histopathological evidence of cancer in pleural biopsy. Obtaining a thoracoscopic pleural biopsy is not possible or feasible in all patients with massive pleural effusion. Pleural fluid or serum markers can provide diagnostic clues in such patients. We assessed the diagnostic efficacy of pleural fluid ADA and serum CA-125 in 101 patients with massive exudative pleural effusion and compared it with histopathological examination of thoracoscopic pleural biopsy. We found that patients with malignancy were significantly older and had predominantly red coloured pleural fluid. Pleural fluid ADA was significantly higher in tuberculous effusion while serum CA-125 was significantly higher in malignant effusion. The sensitivity of ADA (for a cut off value of 45 U/L) for the diagnosis of TB pleural effusion was 79.6% while sensitivity of serum CA-125 for the cut-off value of 35 U/mL for the diagnosis of malignancy was 80.9%. We concluded that pleural fluid ADA and serum CA-125 can be used as a surrogate markers for tuberculous and malignant pleural effusion respectively.*

**Keywords:** Pleural effusion, ADA, CA 125.

### **Introduction**

Collection of abnormal amount of fluid in the pleural space is known as pleural effusion. It occurs due to an imbalance between pleural capillary hydrostatic pressure and plasma oncotic pressure. Pleural effusion can be either exudative (protein rich) or transudative (protein poor).

Among exudative causes of pleural effusion, tuberculosis (TB) and malignancy are the most prevalent. TB pleural effusion is the second most common form of extra-pulmonary TB, while one-fourth patients with pleural effusion in hospital settings are a result of cancer.<sup>1</sup>

India is not only a TB endemic country, but the incidence of lung cancer is also high, considering the number of tobacco smokers in the country. It is very important to differentiate between TB and malignancy because of difference in treatment and prognosis. A number of malignant effusions are misdiagnosed as TB and vice-versa. The facility of thoracoscopy is not available everywhere and histopathological evidence of disease is not possible in every case of exudative effusion, considering the high burden of disease.

The diagnosis of tuberculous pleural effusion can be established conclusively either by the presence of acid fast bacilli (AFB) in pleural fluid or growth of mycobacterium tuberculosis (Mtb) in fluid culture. But, pleural fluid acid fast bacilli are found in less than 5% patients and growth of Mtb in pleural fluid culture has a low sensitivity ranging from 24 to 58%.<sup>2</sup> Also, lung cancer patients with a metastatic pleural effusion are stage IV disease with a poor survival ranging from 3-12 months. In some patients with a poor performance status and advanced disease, it is not feasible to perform aggressive investigations such as thoracoscopy to establish a histological diagnosis. Also, some patients are not willing for invasive procedures.

Blood or pleural fluid markers for TB and malignancy are primary investigations which can provide a provisional diagnosis and guide treatment. ADA is the enzyme required for conversion of adenine to inosine. Its levels are elevated in TB, rheumatoid pleuritis and empyema. The sensitivity and specificity of ADA in the diagnosis of tuberculous pleural effusion is 92%.<sup>3</sup> The two isoforms of ADA enzyme are ADA1; which is found in tissues and red blood cells, and ADA2 found in monocytes and macrophages. This enzyme is required for proliferation and differentiation of lymphocytes (mainly T lymphocytes) and maturation of monocytes to macrophages. Thus, ADA is a marker of cell mediated immunity and its levels are increased in infections such as TB, which affect the manocyte-macrophage system.<sup>4</sup> The

most commonly used cut-off value for pleural fluid ADA is 40 U/L.<sup>5</sup>

CA-125 is a high molecular weight glycoprotein, expressed by a large number of epithelial ovarian cancers. The presence of CA-125 antigen is detected by OC 125 antibody, as reported by Bast et al for the first time.<sup>6</sup> CA-125 is either expressed as a membrane bound protein at cell surface or released in body fluids in soluble form. The soluble CA-125 can be measured through antibodies. Other malignancies with elevated levels of CA-125 are carcinoma breast, mesothelioma, Non Hodgkins lymphoma and gastric carcinoma, among others. Increased levels are also found in benign conditions such as pregnancy, endometriosis, liver disease, congestive heart failure and TB.<sup>7</sup> CA 125 can be measured in both serum and pleural fluid. Topalak et al studied serum and serosal fluid CA-125 levels in 133 patients and found increased serum CA-125 in 87% of patients with pleural effusion. They concluded that high serum CA-125 levels were associated with presence of serosal fluids, irrespective of the origin of fluid.<sup>8</sup>

Previous studies have compared CA-125 values in patients with diverse malignancies, both pulmonary and extra-pulmonary. We have enrolled patients with lung cancer only. We have measured pleural fluid ADA and serum CA-125 values in 101 patients with massive, exudative pleural effusion and compared it with histopathological proven diagnosis of TB and malignancy through thoracoscopic pleural biopsy to evaluate for diagnostic utility of these two tests.

### Aims and Objectives

1. To assess the diagnostic utility of pleural fluid ADA and serum CA-125 in exudative pleural effusion.
2. To evaluate association between age, gender and pleural fluid colour and values of pleural fluid ADA and serum CA-125 in exudative pleural effusions.

## Methods and Materials

This was a cross sectional, observational study conducted at the respiratory centre of a tertiary care hospital in Uttarakhand. Patients presenting with unilateral massive pleural effusions were included in the study after an informed consent. Diagnostic thoracentesis was performed for each patient. 50 ml of fluid was aspirated and sent for routine biochemical, cytological and microbiological examination. Pleural fluid ADA was measured using ERBA-CHEM-7 colorimetric assay. Serum samples for CA-125 were collected and measured using dry chemistry auto-analyzer: VITROS-5600. Patients with transudative effusion (pleural fluid protein<3.0g/dl), empyema, unstable cardiac disease or coagulopathy were excluded from the study. Pleural fluid colour was noted in the first sitting itself as either straw coloured or red. Patients having slight blood tinged fluid due to traumatic tap were included in straw coloured group. All the patients underwent thoracoscopy via a rigid thoracoscope under conscious sedation and local anaesthesia. Biopsy was taken from the parietal pleura under direct vision and sent for histopathological examination and cultures. Malignant pleural effusion was an effusion due to histologically confirmed lung carcinoma, irrespective of cell type. TB pleural effusion was confirmed either through presence of AFB in pleural fluid, growth of Mtb in culture of fluid or presence of caseating, epithelioid cell granulomas in histological sample.

## Statistical Analysis

The data was analysed using SPSS software. Chi square test was used for comparison of proportions. For continuous variables, Levene's test for equality of variances was used as independent sample test. For a two-tailed test, a p value of less than 0.05 was considered statistically significant. The values of true positives (TP), true negative (TN), false positive (FP) and false negative (FN) were counted and diagnostic efficacy calculated as under:

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$$

$$\text{Specificity} = \text{TN} / (\text{FP} + \text{TN})$$

$$\text{Positive predictive value: PPV} = \text{TP} / (\text{TP} + \text{FP})$$

$$\text{Negative predictive value: NPV} = \text{TN} / (\text{TN} + \text{FN})$$

## Results

Out of 101 patients, 59 patients (58.4%) had tuberculous effusion while 42 patients (41.5%) had malignant pleural effusion. In our study, 62 (61.4%) patients were male while 39 (38.6%) were female. Among males, TB was found to be more common (64.5%) while among females, malignant effusion was more common (51.28%). On applying Chi square test to compare gender with histopathological diagnosis, the association was not found to be significant ( $p=0.117$ ;  $df=1$ ).

On analysing pleural fluid colour, 65 (64.4%) patients had hemorrhagic/ red pleural fluid while 36 (35.6%) patients had straw coloured pleural fluid. Among the two groups, 49.2% patients of malignant effusions had red coloured pleural fluid while 50.7% of TB effusions had red coloured pleural fluid. Among straw coloured fluid, 72.2% were diagnosed as TB, while 27.7% were diagnosed as malignant effusion. On applying Chi-square test, significant association was found between pleural fluid colour and histopathological diagnosis. ( $p=0.036$ ;  $df=1$ ).

Table 1 compares the variables, namely age, gender, pleural fluid colour, pleural fluid ADA and serum CA-125 values among the two groups. The mean age for TB effusion was 49.90 years while the mean age for malignant effusion was 61.36 years and this difference was statistically significant ( $p=0.013$ ). The mean pleural fluid ADA for TB effusion was 73.43 U/L, while for malignant effusion was 29.42U/L, the difference being significant ( $p=0.048$ ). The mean serum CA-125 for TB effusion was 90.97U/mL while that for malignant effusion was 196.08U/mL. CA-125 value was also statistically higher in malignant effusions ( $p=0.011$ )

Table 2 denotes the diagnostic utility (sensitivity, specificity, PPV and NPV) of pleural fluid ADA (cut-off value taken as 40U/L) and serum CA-125 (cut off value: 35 U/mL) for the diagnosis of TB

and malignant pleural effusion respectively. As is evident from the table, ADA has a sensitivity of 79.6% in our study for the diagnosis of TB pleural

effusion while serum CA-125 has a sensitivity of 80.9% for diagnosis of malignant pleural effusion.

**Table 1** Comparison of characteristics of 101 patients with tuberculous and malignant pleural effusion

	Tuberculous effusion (n=59)	Malignant effusion (n=42)	P value
Age (mean $\pm$ SD) (years)	49.90 $\pm$ 18.13	61.36 $\pm$ 14.80	0.013
Gender [n (%)]			
Male	40 (67.8%)	22 (52.4%)	0.117
Female	19 (32.2%)	20 (47.6%)	
Pleural fluid Colour [n (%)]			
Red	33 (55.9%)	32 (76.2%)	0.036
Straw	26 (44.1%)	10 (23.8%)	
Pleural fluid ADA (mean $\pm$ SD) (U/L)	73.43 $\pm$ 48.76	29.42 $\pm$ 40.45	0.048
Serum CA 125 (mean $\pm$ SD) (U/mL)	90.97 $\pm$ 129.45	196.08 $\pm$ 310.70	0.011

**Table 2** Diagnostic efficacy of pleural fluid ADA and Serum CA-125 in massive exudative pleural effusion

	Sensitivity	Specificity	PPV	NPV
Pleural fluid ADA*	79.6%	85.7%	88.6%	75.0%
Serum CA 125 #	80.9%	77.9%	72.3%	85.1%

\* Cut off; 45 U/L

#Cut off; 35 U/mL

## Discussion

Among males, TB was found to be more common (64.5%) while among females, malignant effusion was more common (51.28%), but this association was not significant. How et al also found a similar female pre-ponderance in malignant effusions and male pre-ponderance in benign effusions.<sup>9</sup> Aoki et al found a male pre-ponderance in both TB and non-TB effusions, although their sample size was small (n=39).<sup>10</sup> Hoshy et al also found a male pre-ponderance in both TB and non-TB effusions; although this was not statistically significant.<sup>11</sup> Our respiratory centre caters predominantly to a rural population, residing in cold, hilly terrains, where both tobacco smoking and using bio-mass fuel as a cooking and heating medium is very common, especially among females. This might explain the female pre-dominance of malignant pleural effusion.

Among straw coloured fluid, 72.2% were diagnosed as TB, while 27.7% were diagnosed as malignant effusion. A significant association was found between pleural fluid colour and histopathological diagnosis. (p=0.036; df=1). In Western literature, TB is not mentioned as a cause

of hemorrhagic pleural effusion, but in our study we found a significant number of TB patients having bloody effusion (50.7%). In a retrospective study conducted by Villena et al among 715 patients with pleural effusion, 34% patients with malignancy had blood tinged pleural fluid while 50% patients had straw coloured fluid. Among 123 patients of TB pleural effusion, 74% had serous pleural fluid while 21% had blood-tinged. They stated that fluid with bloody appearance increases the probability of malignancy (p=0.04, OR=1.73) while it decreases the probability of TB (p=0.003, OR=0.15). They found that TB was an uncommon cause of hemorrhagic pleural effusion.<sup>12</sup>

As is evident from table 1, the mean age of patients with malignant effusion (61.36 $\pm$ 14.8 years) is more than the mean age of patients with TB effusion (49.9 $\pm$ 18.1 years), and this association is statistically significant (p= 0.013). El Hoshpy et al found a mean age of 44.87 $\pm$ 13.55 years for TB effusion and 47.15 $\pm$ 12.93 years for malignant effusion and there was no significant association with age.<sup>11</sup>

The mean pleural fluid ADA for TB effusion is 73.43 U/L, while for malignant effusion is 29.42 U/L in our study. Hoshy et al reported a mean ADA of  $93.30 \pm 12.10$  U/L in TB pleural effusion ( $n=20$ ).<sup>11</sup> The mean CA-125 in TB effusion is 90.97 U/mL while that for malignant effusion is 196.08 U/mL in our study. In comparison, Hoshy et al reported a mean value of  $41.73 \pm 20.74$  and  $309.27 \pm 79.56$  of CA-125 among TB and malignant effusions respectively; and the difference was statistically significant, although they measured pleural fluid values of CA-125.<sup>11</sup> There was a significant difference between the two groups for values of ADA in TB pleural effusion and CA-125 in malignant pleural effusion. Aoki et al found no significant difference between TB pleural effusion and non TB pleural effusion for the values of ADA and serum CA-125, but pleural fluid IFN-gamma was significantly higher in patients with TB pleural effusion ( $p<0.01$ ).<sup>10</sup> Hoshy et al found that pleural fluid ADA was significantly higher in TB pleural effusion than non-TB pleural effusion (malignant and para-pneumonic).<sup>11</sup>

As is evident from table 2, ADA has a sensitivity of 79.6% and a specificity of 85.7% in our study for the diagnosis of TB pleural effusion while serum CA-125 has a sensitivity of 80.9% and a specificity of 77.9% for the diagnosis of malignant pleural effusion. Our findings are similar to a recent study in Egypt, where the sensitivity of ADA was 75% for the diagnosis of TB pleural effusion while the sensitivity of CA-125 was 74.1% for the diagnosis of malignant pleural effusion.<sup>11</sup> Aoki et al reported a sensitivity of 81.8% and 100% for ADA and CA-125 respectively.<sup>10</sup> Shalaby et al reported a mean serum CA-125 of  $159 \pm 29$  for malignant effusion and no statistically significant difference between malignant and non-malignant effusion, while mean value of pleural fluid CA-125 was  $1482 \pm 540$  and there was a statistically significant difference between malignant and non-malignant effusion.<sup>13</sup> How et al have shown that the level of pleural fluid CA-125 is higher than serum CA-

125, supporting the hypothesis of reabsorption of CA-125 from pleural fluid into the bloodstream.<sup>9</sup> In the diagnostic work up of a patient with pleural effusion, pleural fluid examination is the first step. Pleural fluid ADA has already been included in the group of routine bio-chemical investigations requested in a pleural aspiration sample as numerous studies have provided a sensitivity upto 95% for the diagnosis of TB. But, serum or pleural fluid CA-125 is not used routinely. We found a sensitivity of 80.9% for a cut off value of 35 U/mL for CA-125 for the diagnosis of malignancy. In this group of patients, values of serum CA-125 can provide supportive evidence in the favour of malignancy.

Just as pleural fluid ADA has reduced the need for invasive tests to histologically prove a diagnosis of TB, similarly serum and pleural fluid CA-125 can be used to avoid invasive tests in patients with malignant effusion. The drawbacks of using CA-125 values are that the primary site of malignancy cannot be ascertained and the cell type of metastatic lung carcinoma cannot be determined..

### **Shortcomings**

Our study had certain shortcomings. The sample size was small, so the results cannot be generalized to the whole population. The values of ADA and CA-125 were compared only for patients with TB and malignancy. Other variables such as smoking status were not taken into consideration. Also, the cell type of lung cancer was not taken into account in our study; the association of CA-125 values with various cell types (small cell, squamous cell and adenocarcinoma) would have provided more insight. Further larger scale studies are mandated to find a correlation between histological classification of lung cancer and CA 125 and ADA values.

### **Conclusion**

In our study, we found a significant association with pleural fluid colour and histo-pathological diagnosis with straw coloured fluid suggestive of TB effusion while red fluid suggestive of

malignant effusion. Also, older age was significantly associated with malignant effusion. Pleural fluid ADA was significantly elevated in TB pleural effusion and serum CA-125 was significantly elevated in malignant pleural effusion. The sensitivity of ADA (for a cut off value of 45 U/L) for the diagnosis of TB pleural effusion was 79.6% while sensitivity of serum CA-125 for the cut-off value of 35 U/mL for the diagnosis of malignancy was 80.9%. So, ADA can be used as a surrogate marker for TB effusion and serum CA-125 can be used as a surrogate marker for lung malignancy, although the results have to be interpreted in the context of clinico-radiological features.

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