



## Original article

### Importance of absence of mesothelial cells and other useful parameters in the diagnosis of tubercular pleural effusion: Our experience in a tertiary care hospital

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#### ABSTRACT

**BACKGROUND:** Tuberculosis (TB) is a major public health problem in developing countries as well as across the world. In developing country like India, cytological examination of pleural fluid plays a major role in early diagnosis of tuberculosis as it is rapid, easy and cost effective. **MATERIALS AND METHODS:** The present study was conducted in the Department of Pathology, Rajiv Gandhi Super Speciality Hospital, Delhi. It includes 64 samples of pleural fluid of the patients who had biopsy proven tuberculous pleurisy. Samples were processed and underwent cytological examination. The physical and biochemical parameters were correlated with cytological findings. Ziehl Neelsen stain (ZN stain) for acid-fast bacilli was also done. **RESULTS:** Absence or paucity of mesothelial cells with predominance of lymphocytes in a pleural effusion directs towards tubercular etiology. It was suggested that presence of numerous mesothelial cells in a pleural aspirate almost excludes the diagnosis of tuberculosis. In current study three cases (4.6%) showed numerous mesothelial cells, while 11 cases (17.2%) showed only occasional mesothelial cells. Adenosine deaminase (ADA) is a useful marker in the diagnosis towards tuberculous pleural effusion. Our study demonstrated (87.5%) patients had ADA level >40 IU/L. The lymphocyte/neutrophil (L/N) ratio together with the ADA value favours diagnosis of tubercular effusion. 53 patients had value of lymphocyte /neutrophil ratio  $\geq 0.75$ . **CONCLUSION:** In a predominantly lymphocyte rich pleural fluid, the absence or minimal presence of mesothelial cells is highly suggestive of TB. When cytological examination is combined with other parameters like ADA and L/N ratio, it further enhances the possibility of accurate diagnosis of tuberculous pleural effusion. Cytological examination of pleural fluid can be used for early diagnosis of tuberculosis as well as can be well correlated with the other ancillary techniques. As compared with pleural biopsy, it is less painful, inexpensive as well as minimally invasive.

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## 1. Introduction

Tuberculosis (TB) is an old disease which has been affecting human beings since ages. Evidence from studies of human skeletons proved its existence among ancient civilization. TB is an airborne disease caused by *Mycobacterium tuberculosis*. Globally, approximately ten million new cases of tuberculosis were detected in 2017. In the same year, tuberculosis was responsible for estimated 1.3 million deaths among HIV negative patients and 300,000 deaths in HIV positive patients. Among HIV positive patients, tuberculosis is the most common opportunistic infection and cause of death. Majority of the affected individuals were adult aged 15 years and above (90%). A small percentage of patients

with tuberculosis have a concomitant HIV infection; majority of these are present in Africa and rest two thirds were in these eight countries, i.e. India (27%), China (9%), Indonesia (8%), Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). [1]

Tubercular pleural effusion can be due to primary or reactivated disease. Involvement of pleura is common in primary pulmonary TB. In pulmonary tuberculosis, the cause of pleural effusion is not due to the direct involvement of pleura but rupture of sub pleural focus. Identifying tuberculosis in pleural fluid is a challenge because the diagnosis of tuberculosis depends on the presence of acid-fast bacilli, which can be identified either by light microscopy or by culture and in only few cases of pleural effusion special stain for acid fast bacilli (AFB) or culture is positive.

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The proposed reasons for the low efficacy of the above methods are insufficient sample volume, non-uniform distribution of microorganisms as well as presence of inhibitors which may affect the amplification based studies. For investigation of pleural effusion, both pleural fluid aspiration with pleural biopsy is advised along with other necessary immunologic, biochemical, bacteriologic or cytogenetic studies.

Pleural fluid is examined for its physical, chemical, and cytological properties. Cytology is not a substitute for biopsy, but it is equally reliable in situations where biopsy is difficult and not possible. The predominance of lymphocytes in tubercular effusion is well known, however the paucity or absence of mesothelial cells hints towards tubercular pleural effusion. The technique of sampling of pleural fluid is not only diagnostic, but also has therapeutic importance. The aim of the study is to identify the useful and contributory parameters for the diagnosis of tubercular pleural effusion.

## MATERIALS AND METHODS

The study was conducted in the Department of Pathology of Rajiv Gandhi Super Speciality Hospital, Delhi over a period of four years, i.e. April 2016 – 2019. All the samples with pleural effusion, which were histologically proven to be tuberculous pleurisy, were included in the study.

### Pleural fluid examination:

1. Physical Examination: Done under these headings.
  - a) Volume, colour, appearance, coagulum
  - i) Appearance of fluids: Transudates are clear, straw colour and do not clot on standing
  - ii) Exudate occurs in pneumonia and cancer Bloody fluid could be due to traumatic tap or malignancy Milky fluid due to chylothorax
2. Chemical examination:
  - a. Protein: Transudate when  $<3$  gm/dl, Exudate when  $>3$  gm/dl
  - b. Glucose: Glucose was determined and by glucose oxidase method. If  $<40$  mg/dl: Low glucose levels are found in empyema, tuberculous pleurisy, infection and malignancy
3. Cytological examination:

Detailed cytological examination of pleural fluid was done after pleural tapping. The physical and biochemical findings were correlated with each other. Neubauer chamber was charged with the sample received and total leucocyte count (TLC) as well as differential leucocyte count (DLC) were done. Pleural fluid was centrifuged at 1000 RPM for 10 minutes and smears were made. One slide was prepared as direct smear and 2 slides were made from the centrifuged sediment. All the slides were stained with Giemsa stain

for the assessment of cytomorphology of the cells. Slides were mounted with DPX and examined under low power (10x) as well as high power objective (40x). Detailed cytological examination of pleural fluid was done after pleural tapping. The physical and biochemical findings were correlated with each other. Neubauer chamber was charged with the sample received and total leucocyte count (TLC) as well as differential leucocyte count (DLC) were done. Pleural fluid was centrifuged at 1000 RPM for 10 minutes and smears were made. One slide was prepared as direct smear and 2 slides were made from the centrifuged sediment. All the slides were stained with Giemsa stain for the assessment of cytomorphology of the cells. Slides were mounted with DPX and examined under low power (10x) as well as high power objective (40x).

3. Special stain for AFB: Ziehl Neelsen stain was done.

## RESULTS

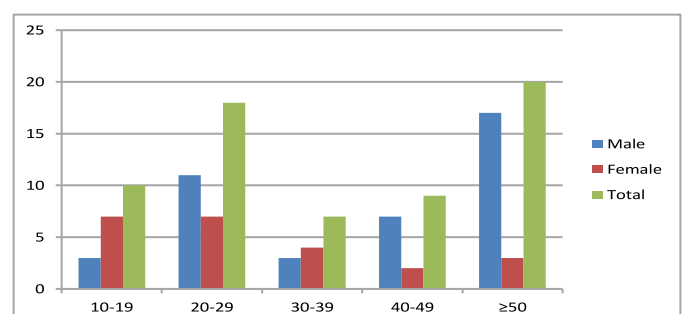
Total of 64 cases were included in the current study with age ranging from 15 years to 95 years of age. A male preponderance was noted with the ratio of male to female being 1.78:1. Out of 64 cases, 41 (64.1%) patients were male and 23 (35.9%) were females. The mean age for men was  $41.6 \pm 34.1$  years whereas mean age for women was  $32.2 \pm 40.5$  years. [Graph 1, Table 1]

The presence of high protein levels ( $\geq 3$  gm/dl) in pleural fluid was found in majority of cases. It was noted that 36 patients (56.3%) had elevated protein value more than 5 gm/dl. The glucose level ranged from 7.6 to 229 mg/dl. In 14 cases (25.1%), the value of glucose was reduced i.e., less than 60 mg/dl. A single case had elevated glucose level (229 mg/dl). Rest of the cases show glucose levels within the normal range

Adenosine Deaminase (ADA) analysis is a sensitive marker for tubercular pleural effusion. In current study, 56 patients (87.5%) had ADA values more than 40 IU/L and 08 patients (12.5%) had ADA value less than 40 IU/L.

The lymphocyte/neutrophil (L/N) ratio together with the ADA activity improves the sensitivity, specificity and efficacy. It had been noticed in our study that 53 patients (82.8%) had value of lymphocyte /neutrophil ratio  $\geq 0.75$ . On cytological examination, a predominance of lymphocytes was noted among all patients. Mesothelial cells were present in only 11 cases (17.2%). The current study showed the nature of exfoliation of mesothelial cells. [Table 2]

**Graph 1: Age and sex distribution**



**Table 1 : Age and sex distribution**

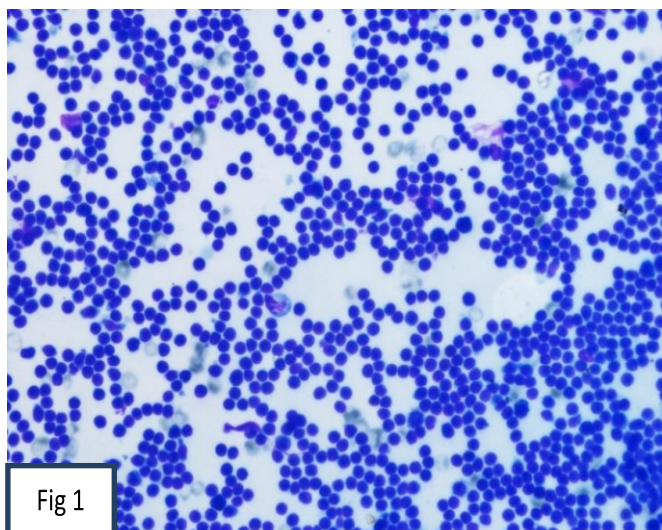
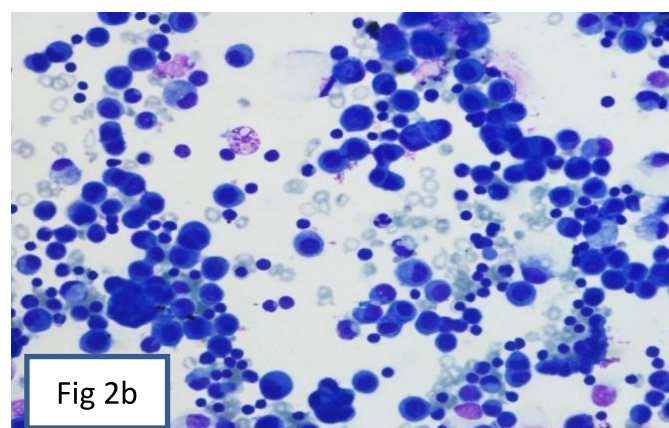
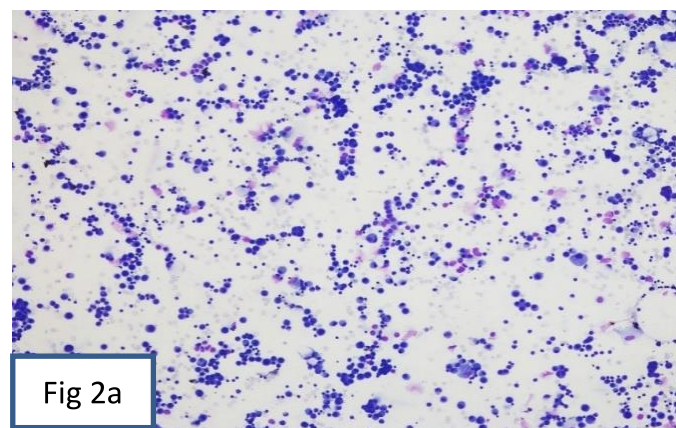
	Male (%)	Female (%)	Total (%)
<b>10-19</b>	7.3	30.4	15.6
<b>20-29</b>	26.8	30.4	28.1
<b>30-39</b>	7.3	17.4	10.9
<b>40-49</b>	17.1	8.7	14.1
<b>≥50</b>	41.5	13.0	31.3

**Table 2: Nature of mesothelial exfoliation**

Mesothelial cells	No.	%
<b>Absent</b>	50	78.1
<b>Occasional</b>	11	17.1
<b>Marked</b>	3	4.6

**Table 3: Comparison of similar studies**

Authors	Year	No. of cases	Mesothelial cells
Springggs and Boddington et al	1960	65	1
Yam et al	1967	49	0
Light et al	1973	14	0
S. Huwwitz et al	1980	76	14
Jamal I et al	2018	100	0
Present study	2019	64	11

**Figure 1: Lymphocytes rich tubercular pleural effusion (400X)****Figure 2a & 2b: Marked mesothelial exfoliation showing predominantly mesothelial cells with few lymphocytes and macrophages (100X, 400X)**

### Discussion

The cytological examination of body fluid is a complete diagnostic modality, which points towards the etiology of effusion. Cytological examination has a great significance in early diagnosis as it aids in early initiation of treatment and a better prognosis. In the present study, most cases of pleural effusion were noted among patients younger than 40 years of age (54.6%) which is comparable with the study of Porcel JM and Baumann MH et al. In our study, a male predominance was noted with male to female ratio of 1.78:1. Various other studies also have showed similar findings.

Most of the cytological smears in our study revealed predominance of lymphocytes. There was paucity or nearly absence of mesothelial cells with presence of other cells including neutrophils, eosinophils and macrophages in few numbers. It had been seen that neutrophil count was low in pleural effusion supported by study in accordance with Burgess LJ et al. The percentage of eosinophils were found to be reduced and they were present only in 14 patients (21.9%). It had been noted that the



significant increase in numbers of eosinophils, i.e. more than 10% rule out the diagnosis of tuberculous pleuritis. Along with these cells, macrophages were also present in majority of the cases but were very few in numbers.

Mesothelial cells are usually polygonal and are about 20 µm in diameter. They appear in sheets as well as scattered singly, separated from each other by clear gaps or "windows". The cells generally are round to oval with centrally placed nuclei and one to two visible nucleoli. The cells have a delicate, sharply demarcated, cyanophilic or eosinophilic cytoplasm. Occasionally, a central fold may appear in the nucleus.

It had been noted that there was paucity of mesothelial cells in tuberculous pleural effusion and presence of mesothelial cells could be the exclusion criteria for tuberculosis. Study done by AI Spriggs and co-worker explained that the absence of mesothelial cells suggested towards tubercular pleural effusion. Another study also supported the fact that the absence of mesothelial cells is secondary to extensive chronic inflammation. In both conditions, pleural layer prevents the exfoliation of mesothelial cells into the pleural space. Presence of numerous mesothelial cells could be possible in acute phase of inflammatory process. Multiple studies supported that the absence or paucity of mesothelial cells favoured tuberculous pleural effusion. [Table 3] Presence of mesothelial cell exfoliation was noted and graded as absent, occasional and marked. When only scattered mesothelial cells present, it was graded as 'occasional' and as 'marked' when numerous, in small groups, clusters or reactive forms of mesothelial cells were observed. Study done by S Hurwitz and colleagues found that among 85 cases, majority of patients 83.5% showed absence of mesothelial cells, 15.3% had minimal exfoliation of mesothelial cells and 1.2% had marked exfoliation of mesothelial cells. We found that in our study, 03 cases (4.6%) had numerous mesothelial cells whose HIV status were not known, 11 cases (17.1%) had occasional exfoliation of mesothelial cells and 50 cases (78.1%) had absence of mesothelial cells.

There lies a difference between tuberculous and non-tuberculous pleura. Tuberculous pleura lacks mesothelial layer while non tuberculous pleura shows mesothelial cell proliferation which is histologically not seen in tuberculosis. Most probably the tuberculous process involves the whole pleural surface area. It had been concluded that the presence of numerous mesothelial cells almost excludes a diagnosis of tuberculosis. In the present study, 61 patients (95.3%) had absent or occasional presence of mesothelial cells in the pleural effusion. Study conducted by Spriggs et al

described the same as they did not find any case among 65 cases which showed increase in number of mesothelial cells more than 2.5 per 1,000 WBCs. In current study, we noted that only 03 cases among 64 cases had numerous mesothelial cells however these patient's HIV status was not known. It correlated well with the study done by other groups. Another possibility of excess mesothelial cells in pleural fluid is acute phase inflammatory process. It was also supported by the study that in serial thoracentesis, there were increased number of mesothelial cells but their HIV status was unknown. Another study depicted that there were increased number of mesothelial cells in HIV infected patient who had TB related pleural effusion.

Acid fast bacilli stain in pleural fluid is rarely diagnostic and positive only in less than 10% of cases. We found 04 AFB positive cases (6.25%) among our study group. Newer tests like PCR and nucleic acid amplification test which has high efficacy in diagnosing tuberculous pleural effusion can be used.

In our study, we found that most of the patients had protein value more than 3 g/dl while among them 36 patients (56.3%) had protein level more than 5 gm/dl. It correlated well with the study conducted by Porcel JM et al.

In our study, the glucose level of tuberculous pleural fluid ranges from 6.5 to 229 mg/dl. 23 patients (36%) had low glucose concentration (<60 mg/dl) and rest had glucose level of >60 mg/dl. Study conducted by Porcel JM and colleagues also showed elevated glucose concentration in 25% of cases.

Adenosine deaminase (ADA) is a commonly used marker in the diagnosis of tuberculous pleural effusion. It is significantly increased in tubercular effusion. The most widely accepted cut off value for pleural fluid ADA is 40 U/L. Higher levels are associated with a greater chance of a patient having TB. Persistently low level of ADA strongly excludes TB. This fact was also supported by this group. In our study, 56 patients (87.5%) had elevated ADA levels. Increased pleural fluid ADA is greatly associated with tuberculous pleuritis, however it has also been seen to be increased in other diseases, like bacterial infections, empyema, collagen vascular disease, malignancy, etc., hereby decreasing the diagnostic utility and specificity of ADA for the diagnosis of TB.

Combined approach of ADA with L:N ratio in pleural fluid gives good result. Burgess and colleagues explained that the L/N ratio ( $\geq 0.75$ ) together with the ADA activity ( $\geq 50$  U/L) improved the

sensitivity (88%), specificity (95%), PPV (95%), NPV 88% and efficiency (92%). It has been found that in case of para infective effusion, L/N ratio is found to be lower ( $\leq 0.75$ ). Similar findings were noticed in the present study with sensitivity, specificity, PPV and NPV which were 56.3%, 94.6%, 94.7%, 55.6% respectively.

## CONCLUSION

The clinical significance of a cytological report suggesting tuberculous effusion could have great impact if an opinion is also well explained about the presence and frequency of mesothelial cell. Absence of mesothelial cells irrespective of lymphocytic population in a pleural effusion hints towards tubercular etiology. If there are numerous mesothelial cells then tuberculosis is highly unlikely and reassessment and further investigation needed for the effusion. Combined approach of Fluid ADA and L:N ratio has also play a very good result in support of diagnosing tuberculous pleural effusion. Other ancillary test also has great aid in diagnosing tubercular effusion when this is combined with cytological examination.

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