Differential Leucocyte Count of Pleural Fluid and Its Diagnostic Value

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Abstract
Establishment of aetiology of pleural effusion is difficult even with all available facilities. In 20% cases of pleural effusion the etiological diagnosis cannot be made. So in those cases the differential leucocyte count of pleural fluid is helpful. Therefore the present study is undertaken with the aim to do the differential leucocyte count of pleural fluid and to see its diagnostics help. 100 patients from the Pulmonary Medicine Department of a referral centre of Odisha are taken as study group. Out of them 50 patients were suffering from tuberculosis, 30 patients were suffering from malignancy and rest 20% were suffering from other chest diseases. 10 ml of pleural fluid was collected in a test tube with anticoagulant from these patients for differential leucocyte count. The fluid was centrifuged and a smear was done. The smear was stained with Leishman’s stain and differential count was done. The observed value was compared with the standard value. In 70% of cases with +ve AFB and radiological findings there was >85% increase of the lymphocytes. In 30% of cases with –ve AFB and radiological findings there was >50% increase in lymphocytes. There was also an increase in neutrophils in exudate fluids indicating active inflammation. It was concluded in our study that the differential leucocyte count of pleural fluid is an important investigation which provide more insight to etiology mainly for tuberculosis and malignancy.

Keywords: Pleural fluid, Leucocytes, Differential Count

Introduction
The pleura is the serous membrane that covers the lung parenchyma, the mediastinum, the diaphragm and the rib case. Normally a few ml, i.e. 5-15ml (0.1 to 2 ml/kg of body weight) of pleural fluid present between the parietal and visceral pleura that acts as a lubricant during respiratory movement. When fluid is collected in excess of physiological
amount, it is known as pleural effusion. The information available from examination of pleural fluid is invaluable. If the fluid in the pleural space is detected on radiological examination it is abnormal. It may be transudative or exudative. The first step in the evaluation of patients with pleural effusion is to determine whether the effusion is a transudate or an exudate. An effusion is exudative if it meets any of the following three Light's criteria:\(^{1,2,3,4}\):

a. the ratio of pleural fluid protein to serum protein is greater than 0.5
b. the pleural fluid lactate dehydrogenase (LDH) to serum LDH ratio is greater than 0.6
c. pleural fluid LDH is greater than two thirds of the upper limit of normal for serum LDH

In most instances analysis of the pleural fluid yields valuable diagnostic information or definitely establishes the cause of the pleural effusion. When pleural fluid is detected clinically or radiologically an effort should be made to determine its aetiology. Though specific tests like culture sensitivity, smear for A.F.B, Tuberculin test/x-ray, biopsy/scanning, ultrasonogram, MRI are available, they are expensive, cumbersome/ time consuming, not conclusive. Even in some cases of pleural effusion aetiology cannot be established. So the present study was done with the aim to do the differential leukocyte count of pleural fluid in pleural effusion cases to see its diagnostic importance & to compare our findings with other authors.

Material & Methods

100 patients of different age & sex having pleural effusion were taken in the present study selected from the Pulmonary Medicine department of a tertiary hospital of Odisha. Pleural fluid was collected from these patients with all aseptic measures through diagnostic thoracentesis. 10 ml. of pleural fluid was taken in an anti-coagulated tube and centrifuged for 5 min at the rate of 3000 rpm within one hour of collection. Then resuspending the bottom cells in 0.5 ml of supernatant fluid, the smear was done after thorough mixing of cells with supernatant. The smear was stained with leishman’s stain and the differential leucocyte count was done and interpreted. Total leucocyte count of pleural fluid was also done in these subjects. This work was approved by the IEC.

Results

The normal differential count, total WBC count and differential leucocyte count are shown in Table 1 to 3. The pleural fluid smear having different pictures are shown in Fig. 1, 2, and 3.

Table. No 1 Normal Differential count in pleural fluid.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Name of Cells</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Macrophage</td>
<td>60-75</td>
</tr>
<tr>
<td>2</td>
<td>Lymphocyte</td>
<td>20-40</td>
</tr>
<tr>
<td>3</td>
<td>Nutrophil</td>
<td>1-5</td>
</tr>
<tr>
<td>4</td>
<td>Eosinophil</td>
<td>0-2</td>
</tr>
<tr>
<td>5</td>
<td>Basophil</td>
<td>0-1</td>
</tr>
<tr>
<td>6</td>
<td>Mesothelial Cells</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Table. No 2 Total WBC count in study group (n=100).

<table>
<thead>
<tr>
<th>TWBC / mm3 of pleural fluid</th>
<th>No. of cases</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1000</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>&lt; 1000</td>
<td>06</td>
<td>06</td>
</tr>
</tbody>
</table>

Table. No 3 Differential Leucocyte count in study group (n=100).

<table>
<thead>
<tr>
<th>Name of Leucocytes</th>
<th>% of Leucocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=36</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>10-12</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>85-90</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0-1</td>
</tr>
<tr>
<td>Monocyte</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Fig 1. Pleural fluid smear with Neutrophil predominance in Tubercular effusion
Discussion

36 patients having >85% lymphocytes in pleural fluid correlated with their clinical signs and symptoms and radiological findings. They are found to be tubercular effusion. 40 patients having 50 to 70% lymphocytes in pleural fluid are suggestive of tubercular/ malignancy. Out of this 21 cases showing the clinical signs and symptoms of T.B but were radiologically –ve / responding to anti T.B treatment. But ADA values of >60U/L confirmed the diagnosis of T.B. In general 7 percent of acute tuberculous pleurisy and 20 percent of malignant pleural effusions, a neutrophilic fluid predominance can be seen.

Neutrophils dominate in pleural fluid resulting from acute inflammation such as pneumonia, early tuberculosis, pancreatitis. In infective conditions of pleura the neutrophils undergo degeneration, nucleus blurred and no longer stains purple. The cytoplasm shows toxic granules. Evans and Karnovsky found that phagocytosis is associated with a markedly increased lactic acid production, related to a TPN-linked LDH. Actively phagocytising polymorphonuclear leukocytes or macrophages were seen in exudative effusions.

Basophilic pleural effusion are uncommon. Basophil count is always < 2. Basophil count more than 10 was was seen in Leukaemic pleural effusion.

The pleural fluid eosinophilia is commonly due to either air or blood in pleural space. If neither air nor blood is present in pleural space & eosinophil is >10 then it is due to asbestos related pleural effusion and parasite disease such as amoebiasis or ascariasis.

Lymphocytosis in an exudative pleural effusion has diagnostic importance. 100% lymphocytes in pleural fluid is due to pleural effusion of Chronic Lymphatic Leukaemia (CLL). Lymphocytosis > 85 % are mostly tubercular. When Lymphocytes is 50 to 70%, patients probably have tuberculous pleuritis / a malignant disease. To distinguish tuberculosis from malignancy the help of other marker such as ADA (adenine deaminase) test is useful. As per the Year Book 2005 On Pulmonary Diseases if there is lymphocytosis in pleural fluid and ADA is more than 60 U/L (normal-40U/L) it is tubercular.

Conclusion

Lymphocytosis in pleural fluid has diagnostic significance for tuberculosis and malignancy. Differential leukocyte count of pleural fluid is an important investigation which provides more insight into aetiology of pleural effusion. Our investigation has positive support. So DLC is a simple cheaper test and can easily be done in rural areas where the facilities are not available for other investigations. Mesothelial cells and malignant cells may be included in differential count for better interpretation of result. Differential count of peripheral blood & differential count of pleural fluid may be compared in the same patient having pleural effusion. Simultaneous immunologic, microbiologic, lipid studies are essential to support the
investigation further. Where controversy arises the help of other markers like ADA & gama-interferon, pleural biopsy, bronchoscopy & helical CT.

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References

Abbreviations
WBC – White Blood Cell
LDH – Lactic dehydrogenase
AFB – Acid Fast Bacillus
MRI – Magnetic Resonance Imaging
IEC – Institutional Ethical Committee
TB – Tuberculous Bacillus
ADA – Adenine Deaminase
CT – Computerised Tomography
CLL – Complete Lymphatic Leukaemia