Original Article

Role of Demonstration of Acid Fast Bacilli in Pleural Effusions - A one year study

Monika Kalyan[1,*], Anita Chaudhary[1], Mukesh Saini[2]

[1]Department of Pathology, Government Medical College, Patiala, India
[2]Department of Paediatrics, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, India

***Correspondence:** monika.kalyan.39@gmail.com

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Abstract

Background: Pleural effusion results from excess fluid production or decreased absorption or both. Tuberculous (TB) pleural effusion is one of the most common forms of extrapulmonary TB.
Materials and Methods: The present study included 158 cases of pleural effusions which were processed and stained with Giemsa, Papanicolaou stain (PAP), Hematoxylin and Eosin stain (H&E) and Ziehl–Neelsen (ZN) stain for Acid fast bacilli (AFB). All samples were categorized according to Indian Academy of Cytopathologists (IAC) guidelines 2019.
Results: Out of 158 cases, only one case showed positivity for acid-fast bacilli (AFB), which is consistent with the known low detection rate of AFB on microscopy. An exception to this low detection rate has been reported in patients with Human Immunodeficiency Virus (HIV) infection and in cases of tuberculous empyema, where previous studies have shown a higher yield of acid-fast bacilli on pleural fluid microscopy, with AFB positivity reported in more than 20% of such cases.
Conclusion: However, cytological examination plays an important supportive role in the diagnosis of tuberculous pleural effusion by identifying lymphocyte-predominant effusions, and demonstration of acid-fast bacilli on Ziehl–Neelsen staining, although infrequent, provides confirmatory evidence. Hence, cytological examination, when interpreted alongside clinical, radiological, and microbiological findings, contributes to improving the diagnostic accuracy of tuberculous pleural effusion.

Keywords: acid-fast bacilli; pleural fluid; ziehl–neelsen staining

Introduction

Serous effusions represent the abnormal accumulation of fluid within body cavities such as the pleural, peritoneal, and pericardial spaces and invariably indicate an underlying pathological process. These effusions constitute an important diagnostic specimen in routine clinical practice, including infectious, inflammatory, and neoplastic conditions.[1] Cytopathological examination of effusion fluids plays a crucial role in identifying the underlying etiology and guiding further clinical management.

The diagnostic yield of effusion cytology is highly dependent on adherence to proper techniques of sample collection, transportation, processing, and staining. Meticulous handling of specimens is essential to obtain adequately preserved and well-stained smears, thereby ensuring accurate cytological interpretation and minimizing diagnostic errors.[2] To achieve

uniformity in reporting and improve diagnostic consistency, the Indian Academy of Cytopathologists (IAC) has formulated guidelines for the collection, processing, interpretation, and reporting of serous effusion samples, which are widely followed in routine cytopathology practice.[1]

Pleural effusion is one of the most commonly encountered serous effusions and may result from a wide spectrum of conditions, including infections, malignancies, inflammatory disorders, cardiac failure, hepatic cirrhosis, and systemic diseases.[3] In tuberculosis-endemic regions, tuberculous pleural effusion is a major cause of exudative pleural effusions and represents one of the most common forms of extrapulmonary tuberculosis.[3, 4] Importantly, detection of *Mycobacterium tuberculosis* in sputum does not necessarily confirm tuberculous pleuritis, as pleural involvement represents a distinct extrapulmonary manifestation and may occur even in the absence of active pulmonary disease.[4, 5]

Cytologically, tuberculous pleural effusion typically presents as a lymphocyte-predominant exudate. A lymphocyte-predominant pleural effusion is generally defined as one in which lymphocytes constitute more than 50–75% of the total nucleated cells.[6, 7] However, direct demonstration of acid-fast bacilli (AFB) in pleural fluid is uncommon due to the paucibacillary nature of the disease. Microscopy for AFB identifies *M. tuberculosis* in fewer than 10% of pleural fluid samples in most cases.[8] Higher detection rates, exceeding 20% of pleural fluid samples, have been reported in specific clinical settings such as patients with HIV infection and in cases of tuberculous empyema, as documented in earlier studies.[9]

Despite its low sensitivity, Ziehl–Neelsen staining for AFB remains a simple, cost-effective, and highly specific diagnostic method. Demonstration of AFB in pleural fluid, although infrequent, provides definitive evidence of tuberculous etiology and has important implications for early diagnosis and timely initiation of appropriate therapy, particularly in resource-limited settings.[8, 9]

Aims and Objectives

Aims: To study pleural effusion samples received in the Department of Pathology and classify them according to the Indian Academy of Cytopathologists (IAC) guidelines, 2019.

Objectives:

To cytologically evaluate and categorize 158 pleural effusion samples as per the Indian Academy of Cytopathologists (IAC) guidelines, 2019.

To assess the positivity rate of Ziehl–Neelsen staining for acid-fast bacilli (AFB) in pleural effusion samples.

Material and Methods

This was a hospital-based prospective observational study conducted over a period of one year, from 1st January to 31st December 2020, in the Department of Pathology, Government Medical College, Patiala, Punjab. The study included all pleural fluid samples received consecutively, in accordance with the objectives of the study.

A total of 158 pleural effusion samples received during the study period were included. Samples from patients of all age groups and both sexes were evaluated. Only adequately collected pleural fluid specimens with sufficient volume for cytological analysis were included in the study. Poorly preserved or grossly inadequate samples were excluded.

Pleural fluid was collected by thoracocentesis under aseptic precautions by the treating clinician. The procedure was performed using a wide-bore needle after administration of local anesthesia. The collected fluid was immediately transferred to sterile containers and promptly transported to the cytopathology laboratory to avoid cellular degeneration.

On receipt in the laboratory, the pleural fluid samples were grossly examined for volume, color, and appearance. The samples were then centrifuged, and smears were prepared from the sediment. Multiple smears were made from each sample to ensure adequate cellular representation.

The prepared smears were air-dried and alcohol-fixed as per standard cytological protocols. The smears were stained using the Giemsa stain for overall cellular morphology, Papanicolaou (PAP) stain for nuclear and cytoplasmic details, Hematoxylin and Eosin (H&E) stain for architectural assessment and Ziehl–Neelsen (ZN) stain (20% sulfuric acid) for detection of acid-fast bacilli (AFB).

All stained smears were examined under light microscopy. The pleural effusion samples were classified according to the Indian Academy of Cytopathologists (IAC) guidelines, 2019, into appropriate diagnostic categories. Special emphasis was placed on identifying inflammatory patterns, lymphocyte predominance, and the presence or absence of acid-fast bacilli.

Informed consent was obtained from all patients prior to the procedure. Patient confidentiality was maintained throughout the study, and the data were used solely for academic and research purposes. The study was approved by the Institutional Ethical Committee with IEC No. BFUHS/2K20p-RS/847.

The data obtained from the study were compiled and analyzed using descriptive statistics only. Categorical variables were summarized as frequencies and percentages. No inferential statistical tests were applied, as the study was primarily descriptive in nature. Data entry and analysis were performed using Microsoft Excel 2013 spreadsheet.

Results

During the one-year study period, a total of 300 serous effusion samples were received in the Department of Pathology. Of these, 158 samples (52.7%) were pleural effusions, which constituted the study material and were further analyzed.

Table 1: Total number of fluids during the study period (n=300).

Type of fluid	No. Of cases
Pleural Fluid	158
Peritoneal Fluid	48
Ascitic Fluid	94
Pericardial Fluid	0
Total	300

All 158 pleural fluid samples were examined cytologically and categorized according to the Indian Academy of Cytopathologists (IAC) guidelines, 2019. The distribution of cases across the diagnostic categories is summarized in Table 2.

Table 2: Categorization of the pleural fluids was done according to the IAC guidelines, 2019 and is as follows.

Report Category	Cytopathology Diagnosis	Number of Samples
1	Unsatisfactory for evaluation	39
2	No malignant cells detected	
	Benign changes seen	
	Reactive mesothelial cells	
	Inflammatory cells seen	
	Lymphocyte-rich effusion	
	Specific infections	
	Microfilaria, Tuberculosis, fungal infection, Hydatid cyst, any other	111
3	Atypical cells, Not Otherwise Specified	3
4	Atypical cells, Suspicious for malignancy	3
5	Malignant cells seen	2

Category 1 (Unsatisfactory for evaluation): 39 cases

Category 2 (No malignant cells detected): 111 cases

These included reactive mesothelial cells and inflammatory effusions, with a predominance of lymphocytes in the majority of cases

Category 3 (Atypical cells, not otherwise specified): 3 cases

Category 4 (Atypical cells, suspicious for malignancy): 3 cases

Category 5 (Malignant): 2 cases

Thus, Category 2 effusions constituted the largest proportion of pleural fluid samples in the present study.

All pleural fluid samples were subjected to Ziehl–Neelsen (ZN) staining for detection of acid-fast bacilli (AFB). Only one case out of 158 pleural effusion samples showed AFB positivity, corresponding to an overall positivity rate of 0.63%.

This single AFB-positive case belonged to Category 2B (benign inflammatory effusion) as per the IAC classification and showed dense inflammatory infiltrate with a predominance of neutrophils. The patient was HIV-negative, and there was no evidence of tuberculous empyema.

Accordingly, the statement "ZN positive cases amongst Category 2B pleural fluid cases is 1%" has been corrected, as the correct proportion based on the total number of pleural effusion samples analyzed is 0.63% (1/158 cases).

Discussion

The present one-year observational study was conducted in the Department of Pathology, Government Medical College, Patiala, and focused on the cytological evaluation of 158 pleural effusion samples, classified according to the Indian

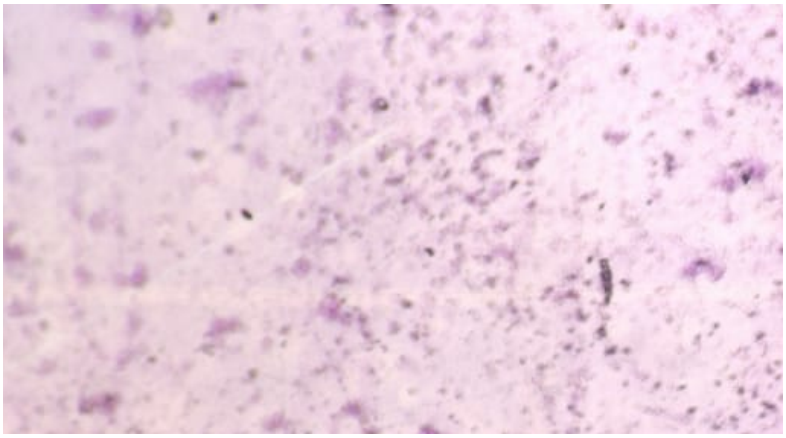

Figure 1: Smear shows debris only: category 1 - unsatisfactory for evaluation as per IAC guidelines 2019 (MGG; X100).

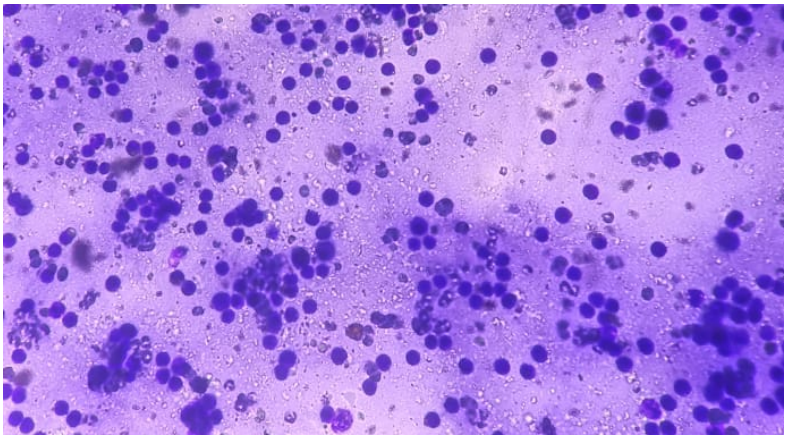

Figure 2: Shows numerous lymphocytes: category 2 (MGG; X100).

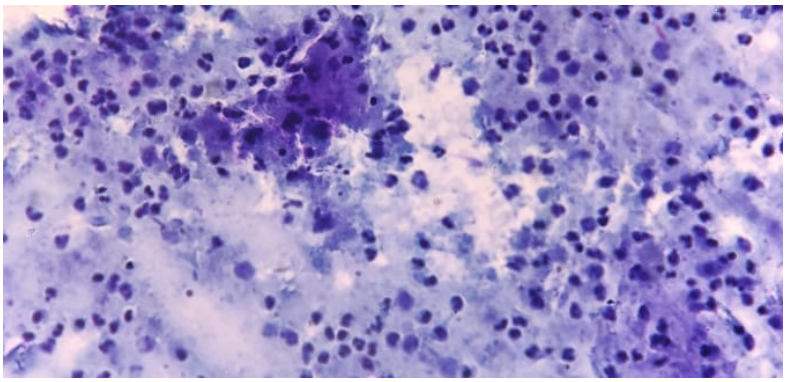

Figure 3: Showing numerous neutrophils: category 2 (MGG; X100).

Academy of Cytopathologists (IAC) guidelines, 2019. The primary objective was to assess the utility of routine cytology and Ziehl–Neelsen (ZN) staining in the detection of acid-fast bacilli (AFB) in pleural effusions.

In the present study, pleural effusions constituted 52.7% of all serous effusions received during the study period (Table 1), emphasizing the clinical importance of pleural fluid cytology in routine diagnostic practice. On categorization as per IAC guidelines (Table 2), the majority of pleural effusion samples belonged to Category 2 (benign, no malignant cells detected), accounting for 111 cases, followed by Category 1 (unsatisfactory) and a small number of atypical and malignant cases. This distribution is comparable to previously published studies where benign inflammatory effusions form the largest diagnostic category.

A substantial proportion of Category 2 effusions showed lymphocyte predominance, a cytological pattern frequently associated with tuberculous pleural effusion in tuberculosis-endemic regions. Although tuberculosis is recognized as a major cause of lymphocyte-predominant exudative pleural effusions, the exact number of lymphocyte-predominant cases was not separately quantified in the present study, as the analysis was performed according to broad IAC diagnostic categories rather

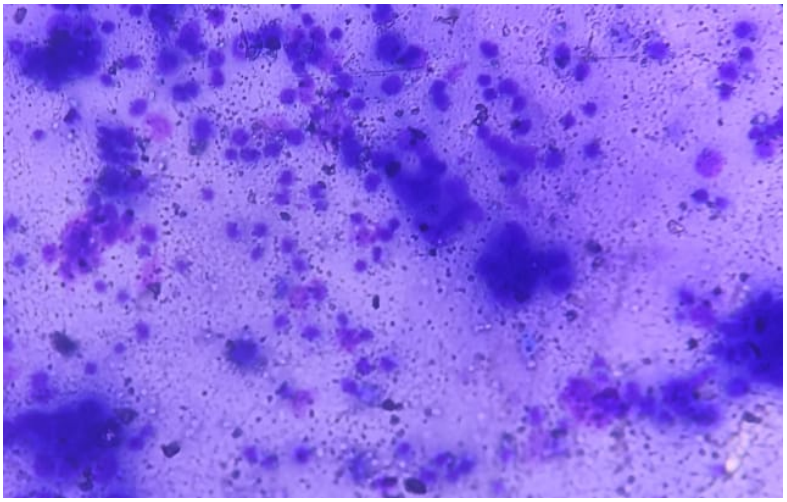

Figure 4: Smear shows scattered atypical cells - category 3 (MGG; X400).

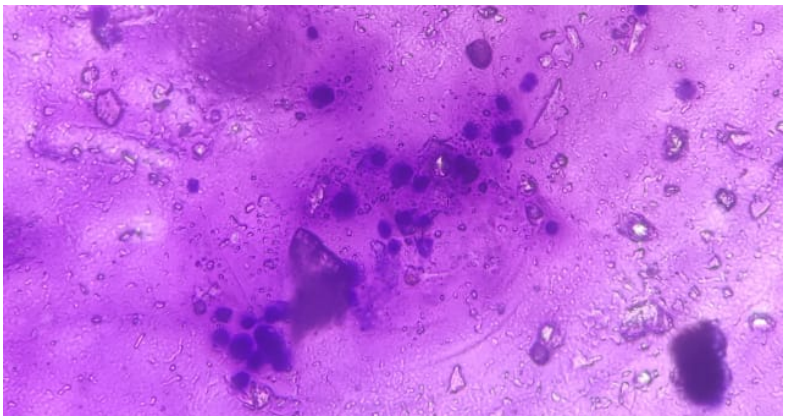

Figure 5: Smears show cells scattered and small groups exhibiting altered N/C ratio and hyperchromatic nuclei: category 5 (MGG; 400X).

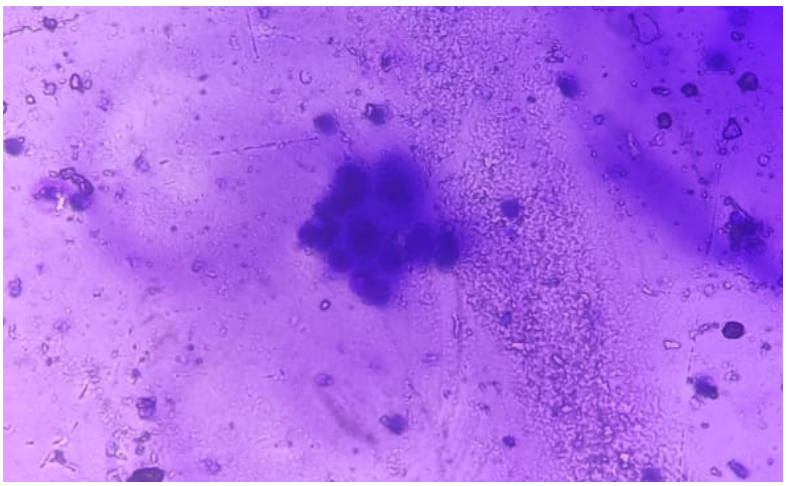

Figure 6: Cellular smear shows hard edge cell balls with hyperchromatic nuclei: category 5 (MGG; X400).

than detailed differential cell counts. This represents a limitation of the study.

All pleural fluid samples were subjected to Ziehl–Neelsen staining for AFB. Only one case out of 158 pleural effusion samples (0.63%) demonstrated AFB positivity. This finding is in accordance with existing literature done by Trajman et al. [10] and Agarwal et al. [11], which highlights the low sensitivity of direct microscopy in pleural fluid due to the paucibacillary nature of tuberculous pleural effusion. Previous studies have consistently reported AFB positivity rates of less than 10% on pleural fluid microscopy, with higher yields mainly observed in patients with HIV infection or tuberculous empyema. In the present study, the single AFB-positive patient was HIV-negative, reinforcing the rarity of demonstrable

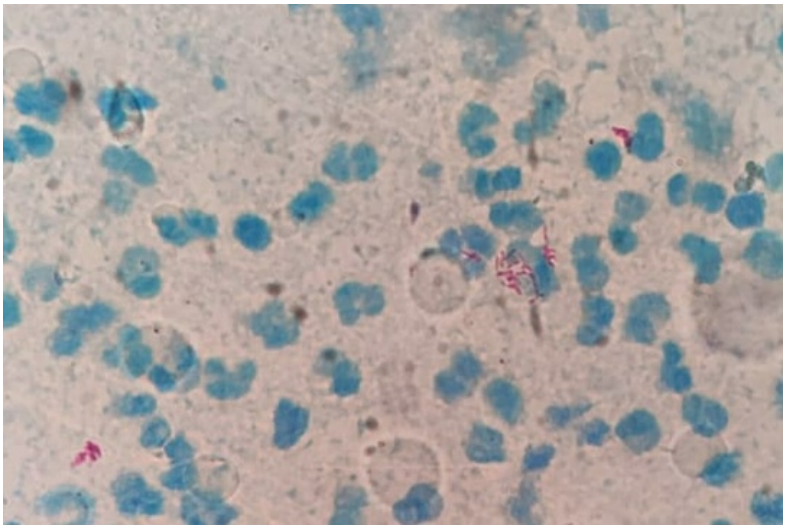

Figure 7: Smear showing rod shaped acid fast bacilli which stain bright red colored in blue background (20 percent ZN stain; X1000).

bacilli in routine pleural fluid cytology.

It is important to emphasize that no ancillary diagnostic tests, such as pleural fluid culture, adenosine deaminase (ADA) estimation, cartridge-based nucleic acid amplification test (CBNAAT), pleural biopsy, or radiological correlation, were included or analyzed as part of this study. The diagnosis and categorization were based solely on cytomorphological findings and ZN staining, and therefore conclusions regarding comparative diagnostic performance with newer modalities cannot be drawn from the present data.

The cytomorphological features observed in various IAC categories are illustrated in Figures 1–7. Figures 1–3 demonstrate benign and inflammatory effusions, including unsatisfactory smears and lymphocyte- and neutrophil-predominant effusions corresponding to Category 1 and Category 2. Figures 4–6 depict atypical and malignant effusions categorized under Categories 3 and 5, respectively, highlighting the spectrum of cytological findings encountered in pleural fluids. Figure 7 illustrates the presence of rod-shaped acid-fast bacilli on ZN staining, which represents the single confirmed case of tuberculous pleural effusion in the study. These figures support the cytological categorization and reinforce the diagnostic utility of routine staining techniques.

Despite its low sensitivity, ZN staining remains a simple, economical, and highly specific test. Demonstration of AFB in pleural fluid, although infrequent, provides definitive evidence of tuberculous etiology and facilitates early initiation of appropriate therapy, particularly in resource-limited settings where access to advanced diagnostic modalities may be restricted.

Conclusion

Pleural fluid cytology, combined with routine staining techniques, remains a valuable tool for the preliminary evaluation of pleural effusions. Although direct demonstration of acid-fast bacilli by Ziehl–Neelsen staining was observed in only one out of 158 cases (0.63%), this method provides definitive evidence of tuberculous etiology when positive. The low yield of AFB highlights the paucibacillary nature of tuberculous pleural effusions and underscores the need for cautious interpretation of negative results. Despite its limited sensitivity, ZN staining remains a simple, cost-effective, and specific diagnostic adjunct, particularly in resource-limited settings where more advanced tests may not be readily available.

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Competing Interests: None.

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