Original Article

Spectrum of Pleural Fluid Cytology in a Tertiary Care Hospital: A Prospective Observational Study

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Abstract

Background: Pleural effusion (PE) is a frequent clinical finding with a wide spectrum of underlying etiologies, including infections, malignancies, and systemic disorders. Cytological examination of pleural fluid remains a valuable first-line diagnostic tool, offering rapid, minimally invasive, and cost-effective results. The adoption of standardized reporting frameworks such as The International System for Reporting Serous Fluid Cytopathology (ISRSFC/TIS) enhances diagnostic clarity and consistency.

Methods: This was a prospective observational study conducted at a tertiary care hospital. A total of 155 pleural fluid samples were evaluated using cytological analysis and classified according to the TIS system. Patient demographics, sample adequacy, fluid appearance, predominant cellular patterns, and final cytological diagnoses were recorded and interpreted.

Results: Out of 155 samples, mean age of patients was 49.93 years, with male predominance (62.6%). Straw-colored fluid was the most common macroscopic presentation (41.9%). Cytological, chronic inflammation with lymphocyte dominance was observed in 68.4% of cases. Malignant cells were identified in three cases (1.9%). Using the TIS classification, 92.9% of samples were categorized as Category II: Negative for Malignancy (NFM), facilitating standardized reporting system.

Conclusion: Cytological examination of pleural fluid remains a valuable initial diagnostic tool for evaluating serous effusions. Incorporating the TIS system enhances diagnostic accuracy and consistency, thereby supporting timely clinical decision-making and improved patient outcomes.

Keywords: The International System for Reporting Serous Fluid Cytopathology; Pleural effusion cytology; Pleural fluid; Risk of malignancy

Introduction

Pleural fluid, a serous secretion lining the pleural cavity, plays a vital role in respiration by reducing friction between pleural membranes and preventing lung collapse. It helps maintain negative intrapleural pressure, essential for effective breathing. Under physiological conditions, pleural fluid is continuously produced and reabsorbed, but this balance can be disrupted by various conditions, resulting in an abnormal accumulation of fluid within the pleural space—referred to as pleural effusion (PE). [1] A chest X-ray is commonly used to detect pleural effusion, typically presenting as a uniform opacity with indistinct margins. Computed tomography (CT) offers better visualization of parenchymal and mediastinal abnormalities often missed on radiographs. Recently, ultrasonography (USG) has become a preferred tool, demonstrating greater sensitivity, while being non-invasive, radiation-free, and suitable for repeat evaluations. [2, 3]

PE are broadly categorized into transudative and exudative types, distinguished using light's criteria. A PE is considered exudative if any one of the following is met: pleural fluid protein/serum protein ratio $>$ 0.5, pleural fluid LDH/serum LDH

ratio > 0.6, or pleural fluid LDH exceeds two-thirds of the upper limit of normal serum LDH. Transudative effusions commonly result from congestive heart failure, followed by hepatic hydrothorax, nephrotic syndrome, and hypoproteinemia. In contrast, exudative effusions are associated with tuberculosis, parapneumonic infections, viral illnesses, and malignancies, with other causes including pulmonary embolism, hypothyroidism, pancreatitis, connective tissue disorders, esophageal rupture (Boerhaave's syndrome), collagen vascular diseases, chylothorax, and hemothorax.[2, 4]

Malignant pleural effusion (MPE) involves the detection of malignant cells in pleural fluid or the parietal pleura, indicating advanced disease with a poor prognosis. MPE can be primary, arising from aggressive pleural neoplasms like malignant mesothelioma, or secondary, due to metastatic spread from cancers such as lung and breast carcinoma. As PE often presents early, timely recognition and treatment are crucial. Cytological analysis offers a safe, rapid, and accessible first-line diagnostic tool. [5, 6]

In non-neoplastic conditions, the fluid predominantly contains mesothelial and inflammatory cells. Mesothelial cells, which line the pleural cavity, are metabolically active and play a central role in maintaining pleural homeostasis. Upon injury, they proliferate and migrate to repair damaged areas. Morphologically, they may appear as flat cells with elongated nuclei or as cuboidal/columnar cells with spherical basal nuclei and a fuzzy luminal surface under light microscopy. [7]

The cytological examination of pleural fluid remains an economical, rapid, safe, and sensitive diagnostic method for evaluating inflammatory and infectious conditions, and plays a pivotal role in cancer detection, staging, and prognosis.[8, 9, 10, 11] Despite its value, cytological interpretation is often subjective, contributing to interobserver variability among pathologists.[12, 13] The lack of standardized diagnostic frameworks has historically led to inconsistencies in classification and reporting. [14, 15] To mitigate these challenges, the International System for Reporting Serous Fluid Cytopathology (TIS) was proposed to introduce a uniform structure that enhances diagnostic accuracy and reproducibility.[16, 17, 18, 19] By categorizing effusions using defined criteria and malignancy risk estimates, TIS improves communication between pathologists and clinicians, thereby facilitating informed clinical decision-making and effective patient management.[20, 21]

Accordingly, this study aims to analyze the cytomorphological spectrum of pleural fluid samples in a tertiary care hospital, identifying the predominant causes of pleural effusion, and re-classify cases using the TIS framework to improve diagnostic accuracy and standardization.

Materials and Methods

This was a prospective observational study conducted over a period of two years in the Department of Pathology at a tertiary care center located in Ambala, Haryana, India. A total of 155 consecutive patients presenting with PE were included in the study. Their samples were clinically or radiologically diagnosed referred from both the inpatient and outpatient departments of the tertiary care center for cytological evaluation. Samples were obtained under strict aseptic conditions and immediately forwarded to the Cytopathology Laboratory for examination. Gross features—including color, clarity, and volume—were documented at the time of receiving. To preserve cellular morphology, specimens were fixed in 50% ethanol and processed without delay. The fluid samples were centrifuged at 3,000 rpm for 5 minutes using a remi R-8C plus centrifuge. From the resultant sediment, 3–4 smears were prepared: one air-dried smear for May-Grünwald Giemsa (MGG) staining and two alcohol-fixed smears for Papanicolaou (PAP) and Hematoxylin & Eosin (H&E) staining. Additional direct smears were made as and when required.

All smears were mounted using standard glass slides and dibutylphthalate polystyrene xylene. Cellular morphology and staining characteristics were documented according to departmental standard operating procedures.

Cytological Categorization Samples were interpreted and classified following the ISRSFC into five diagnostic categories:

1. Non-diagnostic (ND): Specimens with not enough cellular elements for a cytological interpretation.

2. Negative for Malignancy (NFM): Specimens with cellular changes completely lacking evidence of mesothelial or nonmesothelial malignancy.

3. Atypia of Undetermined Significance (AUS): Specimens showing limited cellular (nuclear) and/or architectural atypia (e.g., papillary clusters or pseudo-glandular formations).

4. Suspicious for Malignancy (SFM): Specimens showing features suspicious but not definitively diagnostic for malignancy.

5. Malignant (MAL): Specimens include those with definitive findings and/or supportive studies indicating mesothelial or nonmesothelial malignancies.

Clinical and cytological data were systematically recorded on predefined forms. Statistics were performed using Microsoft Excel and SPSS software. All percentages were rounded to one decimal place for consistency and clarity. As a result, the total may not add up to exactly 100% due to rounding. All procedures performed in this study were approved by the Institutional Ethics Committee of Maharishi Markandeshwar Institute of Medical Science and Research (MMIMSR),

Mullana, Ambala (Project No.: IEC 3348, Date: 08-03-2023) in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Results

A total of 155 pleural fluid samples were examined during the study period. Cytological evaluation was performed using conventional staining methods, and all cases were categorized according to the ISRSFC after assessment of cellular adequacy and cytomorphological features. The study population had a mean age of 49.93 years, with a notable male predominance observed: 62.6% (n=97) males and 37.4% (n=58) females, resulting in a male-to-female ratio of 1.67:1. Patient ages ranged from 14 to 96 years. Most samples, 97.4% (n=151), were adequate for evaluation, while 2.6% (n=4) were non-diagnostic due to poor preservation and degenerated morphology and reported as Non-Diagnostic (ND) - Category I. Macroscopically, straw-colored fluid was the most common appearance, 41.9% (n=65), predominantly associated with benign cytology. Hemorrhagic effusions were noted in 12.9% (n=20) samples, out of which three were malignant; the remaining hemorrhagic samples were attributable to traumatic taps or infectious etiologies and were categorized as Negative for Malignancy (Category II) under TIS. Overall, the Negative for Malignancy (NFM) – Category II group constituted the vast majority of cases, 92.9% (n=144), most commonly exhibiting chronic lymphocytic inflammation, 68.4% (n=106), along with reactive mesothelial changes in several samples. Only 1.9% (n=3) of cases were classified as Malignant (MAL)- Category V. The first two were with known primary, the first one was a known case of breast carcinoma, and the second presented with a lung mass. The third one demonstrated malignant plasmacytid morphology and was subsequently confirmed as non-secretory multiple myeloma on follow-up.

Discussion

This study analyzed the cytological spectrum of pleural effusions in a tertiary care setting, with a focus on diagnostic categorization through the TIS. Conventional cytological evaluation was complemented by TIS-based classification to enhance reporting uniformity and facilitate diagnostic interpretation.

The comparison of age incidence across various studies highlights both the diversity and consistency in the affected population's age profile for pleural effusion cases. In the present study, the ages of the patients varied from 14 to 96 years, with a mean age of 49.93 years. This aligns closely with findings from studies like Shilpi Jha MD et al[17] and Sachin Kolte et al[22], where the mean ages were 49.64 years and 47.4 years, respectively. Similarly, Lekha MB et al [23] reported a comparable mean age of 48.5 years, with a slightly wider age range of 7–93 years. In contrast, the study by Lorna Pairman et al.[24] noted a significantly higher mean age of 69.8 years and a narrower age range, 31–93 years, indicating a predominantly older population. Similar demographic patterns were observed by Gupta et al[25], who studied 1,000 cases and found comparable age-group predominance. Notably, in our study, 20% of the cases belonged to the 61–70-year age group, showing the increased susceptibility in older populations.

In the present study, males constituted 62.6% of the cases, while females accounted for 37.4%, resulting in a sex ratio of 1.67:1. This is consistent with observations from Lekha MB et al[23] and Shilpy Jha MD et al[17], where males comprised 60% and 64.4% of cases, respectively, and their ratios were 1.5:1 and 1.8:1, respectively. Therefore, this indicates that pleural effusion is more prevalent in males, which can be attributed to exposure to smoking, occupational hazards, and various systemic conditions. However, a study by Sachin Kolte et al[22] reported a nearly balanced gender distribution, with males accounting for 47.3% and females for 50.3%, yielding a male-to-female ratio of approximately 0.96:1.

Pleural fluid appearance is not diagnostic on its own, but it may offer preliminary context when considered alongside cytological findings. In our study, straw-colored effusions were mostly benign, while hemorrhagic samples showed varied causes including trauma, infection, and malignancy. However, these associations were inconsistent, and fluid appearance alone was not helpful in reliably predicting cytological outcomes. This contrasts with the findings of Ozcakar et al [26], who also reported no significant link between fluid appearance and malignant cell detection. Our data further emphasize the limited diagnostic value of macroscopic evaluation, supporting its role only as a supplementary observation within broader cytological assessment.

The comparison of benign and malignant pleural effusions across various studies reveals significant differences in their distribution. The present study demonstrates 92.9% of PE as benign and 1.9% as malignant. Our study shows concordance with Lekha MB et al [23] with benign cases 88.33% and malignant cases 6.66%. But there were two studies reported by Priya Dharmalingam et al[27] and Lorna Pairman et al[24] which showed discordance with malignant cases, 28.2% and 25.7%, respectively. The differences could stem from various factors, including patient selection criteria, geographic variations in disease prevalence, or the methodologies and diagnostic techniques employed. For instance, the higher benign effusion rate in the present study may indicate effective early screening, intervention, and a small population of study. The findings underscore the importance of identifying benign causes of PE, which can prevent unnecessary invasive procedures and guide appropriate clinical management.

The cytological re-categorization of serous fluid findings across various studies as per TIS (Table 1) shows that the Non-Diagnostic (ND) category was assigned to four cases (2.6%). This proportion is approximately similar to our findings, except for a study conducted by Sushma Bharti et al [28], which reported 7.97% of cases as ND. This higher percentage may reflect a larger study population compared to ours.

Table 1: Distribution of patients and ROM according to the International System for Reporting Serous Fluid Cytopathology (ISRSFC).

Categories	Number	Percentage (%)
I (ND)	4	2.6
II (NFM)	144	92.9
III (AUS)	3	1.9
IV (SFM)	1	0.6
V (MAL)	3	1.9
Total	155	100

The cases were re-categorized based on the ISRSFC/TIS classification system, which was then used to distribute patients, total cases (n=155) out of which 4 (2.6%) cases were classified as I (ND), 144 (93%) cases as II (NFM), 3 cases (1.9%) as III (AUS), 1 case (0.6%) as IV (SFM) and 3 cases (1.9%) as V (MAL).

Negative for Malignancy (NFM) category was most prevalent, comprising 92.9% of cases, potentially reflecting robust diagnostic criteria. Although ROM was not directly quantified in our study, the predominance of benign categories—particularly NFM—corresponds with ROM patterns described in previous TIS-based studies.[28, 29] In our cases, NFM (Figure 1) included mostly lymphocytic-rich effusions along with many reactive mesothelial cells in the background, 68.4% (n=106). Neutrophilic-rich effusions 13.5% (n=21) were reported in infectious patients, mostly suffering from pneumonia. These cytomorphological features are consistent with those described in a one-year study by Sandeep et al.[30] Benign and reactive mesothelial cells, 11.7% (n=18), were also reported. Saravanakumar et al [31] noted similar morphological findings in their effusion cytology spectrum.

Atypia of undetermined significance (AUS) was noted in 1.9% (n=3) cases, which could not be classified as SFM or MAL. These three cases showed small clusters of atypical cells exhibiting nucleomegaly, moderate cytoplasm, and coarse chromatin (Figure 1). One of the three was marked by poor preservation, which made it more difficult to report. The other two had very less atypical cells to definitely label in higher categories.

Suspicious for malignancy (SFM) was only reported in 0.7% (n=1) cases, which were lost to follow-up in later stages. Other studies also quote similar percentages, except for Zhu et al [32], who reported 19% of cases as SFM. The major reason for this huge difference is that the number of people included in their study is much higher (n= 2326), whereas in our study, only 155 patients presented with PE in 2 years.

Malignant (MAL) cases were only 1.9% (n=3) cases, which were noted lowest in our study. This finding was slightly similar to Sushma Bharti et al [28], which was (5.94%). The highest malignant cases were noted in Zhu et al [32] (47.7%) study. In our study, three cases included were malignant secondaries, out of which the first case was an already known case of breast carcinoma, the second one presented with a lung mass and recurrent PE and the third one was first time diagnosed on PE as malignant. The cytospin smears from the first and second showed similar cytomorphological features, including the presence of tumor cells forming cannon balls and focal acinar pattern with nucleomegaly, coarse chromatin and prominent nucleoli. A few tumor giant cells with bizarre nuclear forms were also noted (Figure 1). However, the definite typing of these tumor cells on ICC was not included in the article due to cost restrained. So, on clinical and radiological basis, these two cases were reported as in the MAL-secondary category.

The third case showed the presence of many tumor cells with plasmacytoid morphology for the first time since the patient presented to our hospital. This patient was followed and later diagnosed with non–secretory multiple myeloma. These cells were large, having round eccentrically pushed nuclei, coarse chromatin, prominent eosinophilic nucleoli, and abundant basophilic cytoplasm. A few binucleate and bizarre forms are noted along with atypical mitotic figures (Figure 2).

The marked predominance of NFM (92.9%) cases supported the clinical utility of effusion cytology in distinguishing benign conditions from malignancy. Findings by Goyal et al [33] reinforce the practical utility of cytological analysis in guiding targeted management. Furthermore, consistent international patterns of TIS application [14] highlight its role in standardizing serous fluid reporting across institutions, improving reproducibility and clinical communication.

Our study has a major limitation of a smaller number of cases, even though we included all patients (including OPD & IPD). There was no proper follow-up for patients reported as AUS & SFM. Due to cost restraints, no immunocytochemistry correlation with the cell block was done.

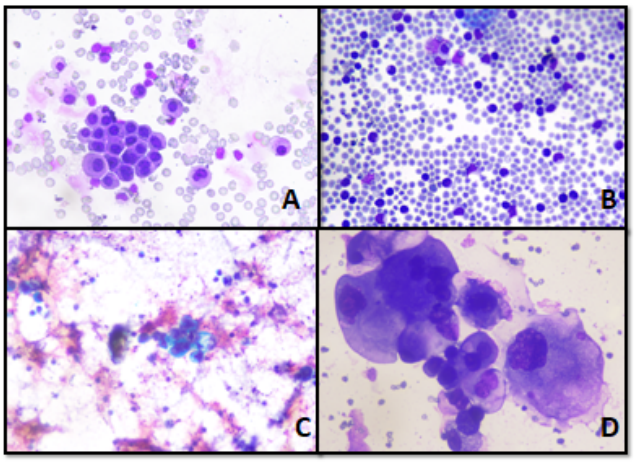

Figure 1: (A) Negative for malignancy (NFM): Benign and reactive mesothelial cells (MGG,100X). (B) Lymphocytic effusion (MGG,40X). (C) Atypia of undetermined significance (AUS): Small cluster showing nucleomegaly and coarse chromatin (PAP,100X). (D) Malignant - secondaries (MAL): Metastatic cells from breast carcinoma (MGG,400X).

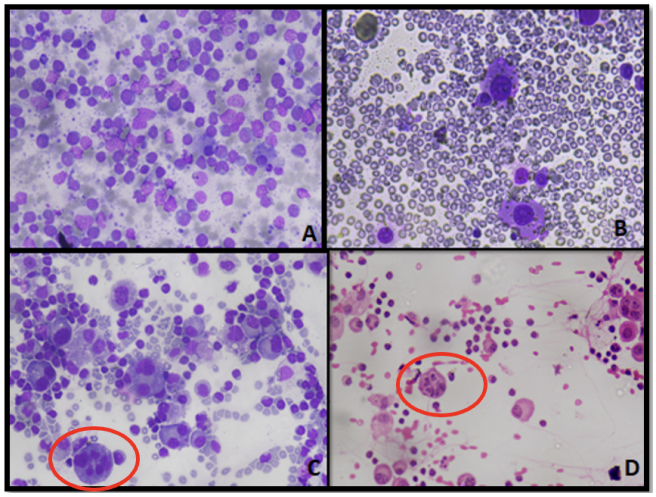

Figure 2: (A-D) Illustrating the presence and morphological spectrum of malignant cells exhibiting plasmacytoid features. (A) and (B) show key diagnostic features including eccentric nuclei and prominent nucleoli (MGG, 100X). (C) and (D) multinucleation in plasmacytoid cell (encircled) (MGG, H&E 100x).

Conclusion

Pleural fluid aspiration continues to serve as a safe, minimally invasive technique for both diagnostic clarification and therapeutic relief in effusion cases. The current study reinforces its utility by demonstrating cytological findings that are largely in agreement with previously documented literature. Cytological examination of pleural effusion remains a valuable frontline tool in pathology, offering a cost-effective and accessible method for identifying inflammatory, infectious, and malignant conditions. In this study, the majority of cases were classified as negative for malignancy, emphasizing the importance of recognizing benign etiologies such as reactive mesothelial cells in inflammatory and infectious processes, as they are mimickers of malignancy. The incorporation of the TIS enhanced diagnostic consistency and reporting accuracy, facilitating effective clinical communication and decision-making. The findings affirm the value of standardized cytological interpretation in guiding appropriate management of pleural effusions and highlight the diagnostic relevance of effusion cytology in routine pathological practice.

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