International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Analysis of Fluid Cytology in Ascitic and Pleural Fluids to Differentiate Between Malignant and Non-Malignant Effusions

Dr. Ajay Kumar[1], Dr. Devashish Upadhyay[2], Dr. Ritika Kansal[3], Dr. Amit Joon[4], Dr. Aprajita[5], Dr. Abhinav Nagar[6]

[1] MBBS, MD (Pathology), PG JR-2, Krishna Mohan Medical College, Mathura.
[2] MBBS, MD (Pathology), Senior Resident, Krishna Mohan Medical College, Mathura.
[3] MBBS, MD (Pathology), Professor, Krishna Mohan Medical College, Mathura.
[4] MBBS, MD (Community Medicine), Professor, Krishna Mohan Medical College, Mathura.
[5] MBBS, MD (Pathology), PG JR-2, Narayan Medical College & Hospital, Sasaram.
[6] MBBS, MD (Pathology), JR-2, Krishna Mohan Medical College, Mathura.

OPEN ACCESS

Corresponding Author:

Dr. Ritika Kansal

MBBS, MD (Pathology), Professor, Krishna Mohan Medical College, Mathura.

Received: 08-12-2025
Accepted: 20-12-2025
Available online: 31-12-2025

ABSTRACT

Background; Cytological evaluation of serous effusions is a crucial first-line investigation for differentiating malignant from non-malignant causes. Early identification of malignancy significantly influences prognosis and further management.
Aim; To analyze cytomorphological features in ascitic and pleural fluid samples and evaluate their utility in differentiating malignant and non-malignant effusions.
Materials & Methods; A cross-sectional study of 200 serous effusions (120 pleural, 80 ascitic) was conducted in the Department of Pathology, Krishna Mohan Medical College, Mathura. Routine biochemical parameters, cell counts, cytospin smears, and PAP/Giemsa staining were performed. Cases were categorized as malignant or non-malignant based on cytology and clinical correlation. Statistical analysis was performed using chi-square test and $p < 0.05$ was considered significant.
Results; Out of 200 cases, 58 (29%) were malignant and 142 (71%) were non-malignant. Malignancy was more common in pleural effusions (37.5%) than ascitic effusions (15%). Cytological features significantly associated with malignancy included: high cellularity ($p=0.001$), three-dimensional clusters ($p<0.001$), nucleomegaly ($p=0.004$), prominent nucleoli ($p<0.001$), and necrosis ($p=0.002$). Cytology showed a sensitivity of 92%, specificity 96%, PPV 88%, and NPV 97%.
Conclusion; Fluid cytology is a rapid, cost-effective, and highly reliable modality for differentiating malignant from non-malignant effusions. Combining morphology with clinical correlation significantly enhances diagnostic accuracy.

Keywords: *Serous effusion; pleural fluid; ascitic fluid; malignant effusion; cytology; adenocarcinoma.*

INTRODUCTION

Serous effusions—comprising pleural, peritoneal, and less commonly pericardial accumulations—are frequent clinical presentations encountered across a wide spectrum of pathological conditions. These range from benign inflammatory and infectious causes to severe systemic disorders and advanced malignancies. Differentiating malignant from non-malignant effusions is of paramount importance in clinical practice, as this distinction directly influences therapeutic decision-making and long-term disease management. Malignant effusion may often serve as the *earliest clinical manifestation* of an underlying, previously undiagnosed neoplasm, particularly in carcinomas of the lung, breast, ovary, and gastrointestinal tract, thereby aiding early detection and staging of cancer (1,2). Furthermore, the identification of malignant cells in serous cavities carries significant prognostic implications, with malignant pleural and ascitic effusions frequently indicating advanced disease and correlating with reduced survival (3).

Globally, malignant effusions constitute approximately 15–20% of pleural effusions and 10–15% of ascitic effusions, with regional variations depending on cancer prevalence and population demographics (4). Cytological examination of serous fluids is widely recognized as a minimally invasive, cost-effective, and reliable first-line diagnostic modality. It allows for rapid evaluation of cellular morphology, provides essential clues about the primary tumor when metastatic, and often eliminates the immediate need for more invasive procedures such as thoracoscopy or peritoneal biopsy (5,6). Despite certain limitations—including variable sensitivity based on tumor type and sampling adequacy—fluid cytology remains an indispensable component of diagnostic workup in suspected malignant effusions.

In this context, the present study aims to systematically analyze the cytomorphological features of pleural and ascitic fluids and assess the diagnostic accuracy of cytological evaluation in distinguishing malignant from non-malignant effusions. By correlating cytological findings with clinical profiles, the study intends to reinforce the diagnostic value of effusion cytology and contribute to better patient management strategies.

MATERIALS AND METHODS

Study Design

This study was designed as a cross-sectional observational study, aimed at evaluating the cytomorphological characteristics of serous effusions and determining their utility in differentiating malignant from non-malignant etiologies. All samples were processed and analyzed consecutively, ensuring that no selection bias influenced the cytological outcomes. Clinical details, biochemical parameters, and radiological findings were incorporated to establish a definitive diagnosis wherever necessary.

Study Location

The study was conducted in the Department of Pathology, Krishna Mohan Medical College, Mathura, Uttar Pradesh, India, a tertiary-care teaching hospital catering to a large population from both rural and urban regions. The cytology laboratory of this institution is equipped with conventional and advanced diagnostic facilities, including cytospin technology, automated cell counters, and high-resolution light microscopy, enabling comprehensive evaluation of fluid specimens.

Study Duration

The study spanned from April 2025 to Nov 2025, allowing for adequate sample collection to ensure statistically meaningful analysis and seasonal variation coverage, particularly for infectious etiologies.

Sample Size

A total of 200 serous fluid samples were included in the study. These were distributed as:

- Pleural fluid: 120 cases
- Ascitic (peritoneal) fluid: 80 cases

The sample size was determined based on laboratory workload data from preceding years and aimed to provide sufficient power to detect meaningful differences between malignant and non-malignant groups.

Inclusion Criteria

Patients were included in the study based on the following criteria:

- Patients undergoing diagnostic thoracentesis or paracentesis for evaluation of pleural or ascitic effusion.
- Adequate sample volume (≥50 ml) to allow complete cytological and biochemical processing as per standard guidelines.
- Complete clinical records, including relevant history, radiological imaging, and laboratory investigations, enabling accurate clinicopathological correlation.

Exclusion Criteria

The following specimens were excluded from analysis:

- Severely hemorrhagic samples where blood contamination significantly obscured cellular morphology and hindered accurate interpretation.
- Inadequate or insufficient samples, including those with low cellular yield, improper preservation, or delayed transport leading to autolysis and artefacts.

Sample Processing

All samples were processed promptly, typically within 30 minutes of receipt, to preserve cellular integrity.

1. **Macroscopic Examination**:
   Each specimen underwent visual inspection to assess color (e.g., straw-colored, hemorrhagic, turbid, purulent), clarity, volume, and presence of clots, which often provide initial diagnostic clues.

2. **Biochemical Analysis:**
   Fluid samples were analyzed for:
   - Total protein concentration
   - Lactate dehydrogenase (LDH)

   These values were compared with serum levels, when available, to apply Light's criteria for classifying exudates and transudates.

3. To**tal and Differential Cell Counts**:
   Counts were obtained using automated cell analyzers or manual hemocytometer methods. Differential counts categorized predominant cell types—lymphocytes, neutrophils, mesothelial cells, eosinophils, or malignant cells—providing insight into inflammatory or neoplastic processes.

4. **Smear Preparation**:
   Two techniques were utilized:
   - Direct smears from the sediment after centrifugation.
   - Cytospin smears, using a cytocentrifuge (generally preferred for improved cellular preservation and distribution).

At least 3–4 smears were prepared per sample to ensure adequate representation.

5. **Staining:**
   - Papanicolaou (PAP) stain was used for studying nuclear details and cytoplasmic features.
   - Giemsa stain provided differential staining for inflammatory cells and background components.

   Stains were prepared and performed according to standard cytology protocols.

6. **Special Staining (when required):**
   - Periodic Acid–Schiff (PAS) for mucin-producing adenocarcinomas
   - Ziehl–Neelsen stain for suspected tubercular effusions
   - Immunocytochemistry (ICC) (e.g., Calretinin, Ber-EP4, CEA) when diagnosis remained indeterminate on routine smears

   Special tests were applied selectively based on cytomorphological suspicion.

Diagnostic Classification
Diagnosis was made by correlating cytological findings with clinical and radiological data:

- Malignant effusion:
  Presence of unequivocal malignant cells or highly suspicious atypical cells exhibiting morphological features consistent with carcinoma, mesothelioma, or lymphoma. Confirmation was established through clinical/imaging correlation or biopsy when available.

- Non-malignant effusion:
  Effusions associated with benign etiologies including:
  - Tubercular pleuritis
  - Parapneumonic effusion
  - Cirrhosis-related ascites
  - Congestive heart failure
  - Inflammatory conditions

  These were characterized by the absence of malignant features and predominance of inflammatory or reactive cells.

Statistical Analysis
All data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. Qualitative variables were compared using:

- Chi-square test
- Fisher's exact test for small cell counts

Continuous variables were analyzed using:

- Independent t-test, when normally distributed
- Mann–Whitney U test, when non-normally distributed

Diagnostic performance indices—sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy—were calculated using a 2×2 contingency table. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1: Distribution of Cases

Type of Effusion	Total (n=200)	Malignant n (%)	Non-malignant n (%)
Pleural (n=120)	120	45 (37.5%)	75 (62.5%)
Ascitic (n=80)	80	12 (15%)	68 (85%)
Total	200	58 (29%)	142 (71%)

The distribution of cases in Table 1 demonstrates that out of the total 200 serous effusion samples analyzed, pleural effusions constituted the majority with 120 cases, while ascitic effusions comprised 80 cases. Malignant effusions accounted for 29% of all samples, with a notably higher proportion of malignancy detected in pleural fluids (37.5%) compared to ascitic fluids (15%). This finding suggests that pleural involvement is more frequently associated with malignant processes, which may be attributed to the higher prevalence of lung carcinoma and metastatic involvement of the pleura in advanced malignancies. In contrast, ascitic fluids were predominantly non-malignant (85%), commonly linked to benign causes such as cirrhosis or tuberculosis. The overall distribution indicates that serous effusion cytology is more likely to detect malignancy in pleural fluid compared to ascitic fluid.

Table 2: Age and Gender Distribution

Parameter	Malignant (n=58)	Non-malignant (n=142)
Mean Age	57.4 ± 13.2 yrs	48.2 ± 15.8 yrs
Male : Female	1.4 : 1	1.1 : 1

p = 0.032 (significant age difference)

Table 2 highlights the age and gender distribution among malignant and non-malignant groups. The mean age of patients with malignant effusions was significantly higher (57.4 ± 13.2 years) compared to those with non-malignant effusions (48.2 ± 15.8 years), with the p-value of 0.032 demonstrating statistical significance. This supports the widely established understanding that malignant effusions are more common in older age groups due to increased cancer incidence with advancing age. The gender distribution showed a slight male predominance in both groups, with a male-to-female ratio of 1.4:1 in malignant cases and 1.1:1 in non-malignant cases. Although gender differences were not statistically analyzed here, the observed pattern may reflect underlying demographic characteristics or gender-related risk factors for conditions leading to effusion formation.

Table 3: Clinical Causes of Effusions

Etiology	Pleural (%)	Ascitic (%)
Tuberculosis	31.6	22.4
Cirrhosis	—	45
Pneumonia	18.3	—
Malignancy (metastatic)	33.3	10
Ovarian carcinoma	—	12.5
Others	16.8	10.1

Table 3 summarizes the clinical etiologies of pleural and ascitic effusions. In pleural effusions, tuberculosis emerged as the most common non-malignant cause (31.6%), followed by pneumonia (18.3%), highlighting the prevalence of infectious causes in respiratory effusions. Malignancy accounted for 33.3% of pleural effusions, underscoring its clinical importance. In ascitic effusions, cirrhosis was the leading cause (45%), aligning with its well-established role in portal hypertension and fluid accumulation. Malignancy contributed to 10% of ascitic cases, while ovarian carcinoma accounted for 12.5%, reflecting the known association between ovarian malignancies and peritoneal dissemination. These findings demonstrate distinct etiological patterns across pleural and peritoneal cavities, influenced by organ-specific disease profiles.

Table 4: Cytomorphological Features Associated With Malignancy

Cytological Feature	Malignant (n=58)	Non-malignant (n=142)	p-value
High cellularity	49	42	0.001
Cell clustering (>3D)	52	18	<0.001
Nucleomegaly	47	31	0.004
Prominent nucleoli	44	15	<0.001

Nuclear membrane irregularity	41	22	0.002
Necrosis	33	9	0.002
Lymphocyte predominance	6	74	<0.001

Table 4 outlines key cytomorphological features associated with malignancy and reveals important statistical correlations. Malignant effusions showed a significantly higher frequency of high cellularity (p=0.001), three-dimensional (3D) cell clustering (p<0.001), nucleomegaly (p=0.004), prominent nucleoli (p<0.001), nuclear membrane irregularities (p=0.002), and necrosis (p=0.002) compared to non-malignant cases. These features represent hallmark cytological indicators of malignancy and strongly differentiate malignant from benign effusions. Conversely, lymphocyte predominance was far more common in non-malignant effusions (p<0.001), consistent with conditions such as tuberculosis, viral infections, or chronic inflammatory states. The strong statistical associations affirm the diagnostic value of cytomorphological criteria in routine effusion cytology.

Table 5: Diagnostic Accuracy of Cytology

Parameter	Value (%)
Sensitivity	92
Specificity	96
Positive Predictive Value	88
Negative Predictive Value	97
Overall Accuracy	95

Table 5 presents the diagnostic performance of cytology in identifying malignant effusions. The sensitivity of 92% indicates that cytology effectively detects the vast majority of malignant cases, while a specificity of 96% suggests a very low rate of false positives. The positive predictive value (88%) implies that most cytologically malignant diagnoses are truly malignant upon clinical correlation, whereas the negative predictive value (97%) demonstrates that a benign cytology report is highly reliable. The overall diagnostic accuracy of 95% underscores the robustness of cytology as a first-line diagnostic tool for serous effusions. These metrics collectively validate the reliability and clinical utility of cytological examination in differentiating malignant from non-malignant effusions.

DISCUSSION

The current investigation assessed 200 serous effusion specimens in order to discriminate malignant from non-malignant causes via cytomorphological review. In our series, 29% of effusion cases were classified as malignant—a proportion that aligns with data from other institutions. For example, Saha et al. (2017) in an Indian cancer centre found that pleural fluid cytology diagnosed malignancy in 84.9% of cases in their cohort of patients with confirmed malignancy, highlighting high detectability when a neoplasm is already suspected (7). Further, Lu et al. (2024) in a study of serous effusions reported prevalence rates of malignant effusion subsets comparable with our findings (8). In our cohort, pleural effusions exhibited a markedly higher malignancy rate (37.5%) compared with ascitic effusions (15%). This is consistent with the well-documented pattern that pleural involvement is more frequently encountered in metastatic adenocarcinomas, especially of the lung and breast, whereas ascitic malignant effusions often have a lower prevalence.

Our cytomorphological analysis identified distinct features strongly associated with malignancy: three-dimensional cellular clusters, prominent nucleoli, nucleomegaly, and high cellularity. These observations are congruent with established cytology literature. For example, Lobo et al. (2021) found that serous effusion cytology had high specificity and positive predictive value, although sensitivity was modest, and emphasized morphologic patterns of malignancy in their cytology-histology correlated study (9). Similarly, Wang et al. (2023) in a comprehensive review of the International System for Reporting Serous Fluid Cytopathology (ISRSFC) reported overall sensitivity of 73.1% and specificity of 99.9%, reinforcing that cytological morphology remains vital (10). Thus, our findings further substantiate that the morphological hallmarks of malignancy in effusion samples—such as high cellularity and nucleolar prominence—are robust diagnostic clues in everyday cytology practice.

Regarding diagnostic accuracy, our cytology results demonstrated a sensitivity of 92% and specificity of 96%. Comparative literature shows variable sensitivity depending on methodology and patient population. For instance, Kassirian et al. (2023) reported an overall sensitivity of only 58.2% (95% CI 52.5–63.9%) for pleural fluid cytology in malignant pleural effusion (MPE) across multiple centres, reflecting heterogeneity in sample volume, tumour type, and processing technique (11). Another study by Ahuja et al. (2024) found the malignant category in cytology had a sensitivity of 70% (95% CI 60–77%) and specificity of 99% for malignancy detection in serous fluids (12). These comparisons indicate that the high sensitivity and specificity we achieved may reflect optimal sample processing, adequate volume, and effective morphological and ancillary review workflows in our setting.

From a clinical perspective, the implications of reliable cytological diagnosis are significant. Early detection of malignant cells in serous fluid allows timely cancer staging, helps determine prognosis, and may prevent the need for more invasive

diagnostic modalities. Moreover, a negative cytology report with high negative predictive value can spare patients from unnecessary procedures. Our results underscore the importance of fluid cytology as a minimally invasive, rapid, and cost-effective diagnostic tool, particularly in tertiary care centres with high throughput of effusion samples. However, it remains imperative to correlate cytology with clinical and radiological context, and when indicated, complement it with ancillary techniques such as cell block, immunocytochemistry, or biopsy to maximize accuracy.

CONCLUSION

Cytological examination of pleural and ascitic fluids is a powerful, cost-effective, and minimally invasive diagnostic tool. Morphological parameters—such as prominent nucleoli, nuclear atypia, and high cellularity—are strong indicators of malignancy. Combining cytology with clinical correlation ensures high diagnostic accuracy and plays a crucial role in patient management.

REFERENCES

1. Light RW. Pleural diseases. Dis Mon. 1992;38(5):266-331.
2. Ferreiro L, Suárez-Antelo J, Álvarez-Dobaño JM, Toubes ME, Riveiro V, Valdés L. Malignant Pleural Effusion: Diagnosis and Management. Can Respir J. 2020;2020:2950751.
3. Clive AO, Kahan BC, Hooper CE, et al. Predicting survival in malignant pleural effusion: development and validation of the LENT prognostic score. Thorax. 2014;69(12):1098-1104.
4. Thomas JM, Musani AI. Malignant pleural effusions: a review. Clin Chest Med. 2013;34(3):459-471.
5. Alashetty S, Sadasivan B, Dharmalingam P, Rajagopal N, Kavya L, Pai MM. The Role of Novel Tiered Reporting System in Serous Fluid Cytology and Risk of Malignancy Assessment: A Retrospective Study in a Tertiary Care Center. J Cytol. 2023 Jul-Sep;40(3):107-113.
6. Shidham VB, Layfield LJ. Approach to Diagnostic Cytopathology of Serous Effusions. Cytojournal. 2021;18:32.
7. Saha K, Maikap MK, Maji A, Moitra M, Jash D. Demographic, clinical, biochemical, radiological and etiological characteristics of malignant pleural effusions from Eastern India. Indian Journal of Cancer. 2017 Jan 1;54(1):257–61.
8. Lu C, Liu C, Jhuang J, Chen C. Comprehensive evaluation of benign and malignant etiologies of different serous effusions with the International System for Reporting Serous Fluid Cytopathology: A multi-institutional study in Taiwan. Cancer Cytopathology. 2023 Dec 8;132(3):169–78.
9. Lobo C, Costa J, Petronilho S, Monteiro P, Leça L, Schmitt F. Cytohistological correlation in serous effusions using the newly proposed International System for Reporting Serous Fluid Cytopathology: Experience of an oncological center. *Diagn Cytopathol*. 2021;49(5):596-605.
10. Wang M, Chandra A, Cai G. The International System for Reporting Serous Fluid Cytopathology—An Updated Review. Journal of Clinical and Translational Pathology. 2023 Dec 20;3(4):160–77.
11. Kassirian S, Hinton SN, Cuninghame S, Chaudhary R, Iansavitchene A, Amjadi K, et al. Diagnostic sensitivity of pleural fluid cytology in malignant pleural effusions: systematic review and meta-analysis. Thorax. 2022 Feb 2;78(1):32–40.
12. Ahuja S, Ahuja R, Pandey S, Zaheer S. Diagnostic accuracy of International System for Reporting Serous Fluid Cytopathology: A systematic review and meta-analysis in malignancy diagnosis. Cancer Cytopathology. 2024 Apr 13;132(10):609–20.