

A Prospective Study of Clinico-Pathological Spectrum of Cytology of Pleural and Peritoneal Fluids at Tertiary Health Care Hospital

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Received: 01-06-2026 / Revised: 02-07-2026 / Accepted: 01-08-2026

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Conflict of interest: Nil

Abstract:

Background: Cytological examination of serous effusions is a simple, minimally invasive, and cost-effective diagnostic modality that aids in distinguishing benign from malignant conditions and in identifying infectious and inflammatory etiologies. Tuberculosis and malignancy remain leading causes of pleural and peritoneal effusions in low- and middle-income, tuberculosis-endemic settings.

Objectives: To study and evaluate the current trends in cytological evaluation of serous effusions for various pathological conditions in a tertiary health care hospital, and to analyse their frequency in relation to diagnosis.

Methods: A prospective observational diagnostic test evaluation study was conducted in the Department of Pathology of a tertiary care teaching hospital over 18 months (March 2023–August 2024). A total of 150 pleural and peritoneal fluid samples fulfilling inclusion criteria were processed by both conventional smear and cytopspin techniques and stained with Haematoxylin and Eosin and Giemsa. Demographic, clinical, fluid gross characteristics and cytological findings were recorded, and associations with final clinical diagnosis were analysed using chi-square tests.

Results: Of 150 effusions, 134 (89.3%) were pleural and 16 (10.7%) peritoneal. Most patients were 20–40 years (40.7%) and male (76%). Clear, pale-yellow fluids predominated (80.7% clear; 63.3% pale yellow). Overall, 92.0% were negative for malignancy, 6.7% positive, and 1.3% suspicious. Tuberculosis was the commonest diagnosis (39.3%), followed by bacterial pneumonia (20.0%) and viral pneumonia (13.3%); malignancy accounted for 8.0% of cases. Atypical cells were seen exclusively in malignant effusions and showed perfect specificity for malignancy; high cellularity and age >60 years were also independent predictors of malignancy. Significant associations were noted between clinical diagnosis and type of fluid, fluid appearance, predominant cell type, and cytological category ($p < 0.05$).

Conclusion: In this tuberculosis-endemic setting, lymphocyte-predominant pleural effusions were strongly associated with tuberculous etiology, whereas neutrophil-rich effusions correlated with acute bacterial infections and empyema. The presence of atypical cells and high cellularity were robust predictors of malignancy.

Keywords: Pleural Effusion, Cytology, Malignancy, Tuberculosis.

DOI: 10.25258/ijcpr.18.8.45

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Introduction

Serous cavities, including pleural and peritoneal spaces, are lined by a single layer of mesothelial cells and contain a small amount of lubricating fluid necessary for organ motion and protection. [1] Under physiological conditions, fluid formation and absorption are tightly regulated according to Starling forces and lymphatic drainage, and any imbalance results in effusion formation. Effusions can arise from a wide spectrum of pathological processes, including congestive heart failure, chronic liver disease, renal disorders, infections, autoimmune conditions and primary or metastatic malignancies. [2]

Cytological examination of serous effusions has evolved into an important diagnostic armamentarium in pathology because it is relatively non-invasive, rapid, and inexpensive, and can be performed repeatedly for disease monitoring. [3] Pleural fluid cytology is particularly useful in the diagnosis of malignant pleural effusion and also provides valuable information regarding infectious and inflammatory conditions. Similarly, analysis of peritoneal (ascitic) fluid assists in differentiating transudative effusions of cirrhosis and nephrotic syndrome from exudative effusions due to peritoneal carcinomatosis, tuberculosis or other inflammatory etiologies. [4] [5]

Several studies from India and other low- and middle-income countries have highlighted tuberculosis and malignancy as major causes of pleural effusion, with tuberculosis often predominating in younger populations. [6,7] Retrospective cytomorphological series of body fluids have also documented the high yield of cytology for malignant effusions and the predominance of lymphocytic exudates in tubercular effusions. [8]

Despite the widespread use of effusion cytology, diagnostic challenges persist in differentiating reactive mesothelial cells from malignant cells and in characterising effusions with overlapping clinical presentations. [9,10] Ancillary techniques such as cell block preparation, immunocytochemistry and molecular assays have improved sensitivity for malignancy but may not always be available in resource-constrained settings. Therefore, systematic evaluation of cytomorphological patterns and their clinicopathological correlations in local settings remains essential for optimising diagnostic pathways.

Against this background, the present study was undertaken in a tertiary care teaching hospital to evaluate pleural and peritoneal effusions using conventional smear and cytopspin techniques and to correlate fluid characteristics and cytological patterns with underlying clinical diagnoses, with particular emphasis on infectious and malignant etiologies.

Methodology

Study Design and Setting: This was a prospective observational comparative diagnostic test evaluation study conducted in the Department of Pathology, GMERS Medical College and Civil Hospital, Sola, Ahmedabad, a tertiary care teaching hospital in western India, over a period of 18 months from March 2023 to August 2024.

Study Population and Sample Size: All pleural and peritoneal fluid samples received in the cytology laboratory during the study period and fulfilling eligibility criteria were included. A total of 150 unique effusion samples (134 pleural and 16 peritoneal) from 150 patients constituted the final study cohort. Repeated samples from the same patient, cerebrospinal, pericardial, synovial and other body fluids were excluded.

Inclusion Criteria

- All pleural and peritoneal effusion samples received with adequate volume and clinical information.

Exclusion Criteria

- Inadequate sample quantity.
- Delay >2 hours between collection and

processing or suboptimally preserved specimens.

- Body fluids other than pleural and peritoneal fluids.

Ethical Considerations: The study protocol was reviewed and approved by the Institutional Ethics Committee prior to commencement. Written informed consent was obtained as per institutional policy for diagnostic procedures and use of anonymised data for research.

Specimen Collection and Handling: Pleural effusions were collected by thoracentesis and peritoneal effusions by paracentesis under aseptic precautions by the treating clinicians. Fluid samples were collected in clean, dry, labelled sterile containers; where required, heparinised bottles were used to prevent clotting in accordance with standard recommendations. Delayed samples were stored at 2–6 °C until processing to preserve cellular morphology.

Gross Examination: Each sample was subjected to macroscopic assessment for volume, colour (pale yellow, yellow, reddish, milky, etc.) and appearance (clear vs turbid). Gross characteristics were recorded and later correlated with clinical diagnosis.

Cytological Preparation: Each specimen was processed by both conventional smear and cytopspin techniques.

Conventional Smear Technique: Approximately 5 mL of fluid was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded, and the cell-rich sediment was used to prepare at least two smears. One smear was air-dried and stained with Giemsa, while the other was fixed in alcohol and stained with Haematoxylin and Eosin (H&E). Smears were mounted with DPX.

Cytopspin Technique: Using a Cyto-Tek cytocentrifuge, 0.5 mL of well-mixed fluid was placed in a cytopspin funnel with filter paper and centrifuged at 2500 rpm for 10 minutes to obtain a monolayer of cells in a restricted area of the slide. Two cytopspin smears were prepared per case: one alcohol-fixed for H&E and one air-dried for Giemsa staining.

Microscopic Evaluation: All conventional and cytopspin smears were examined systematically under light microscopy by experienced cytopathologists. Cellularity was graded as scanty, moderate or high. The predominant cell population was categorised as lymphocytes, neutrophils or atypical cells (suspicious/malignant). Background features (necrosis, proteinaceous material, haemorrhage) and mesothelial/reactive changes were also noted.

Cytological diagnoses were classified into three categories, following widely accepted reporting

conventions:

- Negative for malignant cells (benign/reactive effusions).
- Positive for malignant cells.
- Suspicious for malignancy (atypical cells insufficient for definitive malignant diagnosis).

Clinical Data and Final Diagnosis: Demographic data (age, sex), clinical features and provisional diagnoses were obtained from requisition forms and clinical records. Final clinical diagnoses (e.g. tuberculosis, bacterial pneumonia, viral pneumonia, empyema, cirrhosis, nephrotic syndrome, malignancy, acute infection, cardiovascular disease) were based on composite assessment by treating teams, including radiology, microbiology, biochemical parameters, histopathology and follow-up, as available.

Statistical Analysis: Data were entered in a spreadsheet and analysed using standard statistical software. Categorical variables (e.g. type of fluid, appearance, predominant cell type, cytological category, clinical diagnosis) were summarised as frequencies and percentages. Associations between categorical variables were tested using the chi-square test. Odds ratios (ORs) and adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were calculated to evaluate predictors of malignancy and diagnostic patterns, where appropriate. A p-value <0.05 was considered statistically significant.

Results

Demographic Profile: A total of 150 patients were included. The majority (40.7%) were aged 20–40 years, followed by 34.7% in the 40–60-year group and 20.0% in the 60–80-year group; only 0.7% were >80 years. There was a marked male predominance with 114 males (76.0%) and 36 females (24.0%). There was no statistically significant association between gender and type of fluid (pleural vs peritoneal).

Distribution and Characteristics of Effusions: Pleural effusions comprised 134 (89.3%) of cases, while peritoneal effusions accounted for 16 (10.7%), indicating clear predominance of pleural involvement in this cohort. Regarding colour, pale-yellow fluids were most common (63.3%), followed by reddish (27.3%) and yellow (9.3%) fluids. Most effusions were clear (80.7%), whereas 19.3% were turbid. Clear fluids were more frequent in tuberculosis, cirrhosis and cardiovascular causes, while turbidity was commonly observed in empyema, acute infections and viral pneumonia.

Cellularity was moderate in 126 (84.0%) cases, high in 14 (9.3%) and scanty in 10 (6.7%). High cellularity was significantly associated with malignancy on multivariable analysis.

Predominant Cell Patterns: Lymphocytes were the predominant cell type in 95 cases (63.3%), neutrophils in 43 (28.7%) and atypical cells in 12 (8.0%). Lymphocyte-predominant effusions were strongly associated with tuberculosis and viral pneumonia, whereas neutrophil-predominant effusions were associated with acute bacterial infections, including bacterial pneumonia and empyema. Atypical cells were seen exclusively in malignant effusions, with no occurrence in non-malignant conditions.

Cytological Diagnostic Categories: Out of 150 effusions, 138 (92.0%) were reported as negative for malignant cells, 10 (6.7%) as positive for malignant cells and 2 (1.3%) as suspicious for malignancy. All cases with clinical final diagnosis of malignancy had either positive (10/12; 83.3%) or suspicious (2/12; 16.7%) cytology, while all non-malignant clinical diagnoses had negative cytology, resulting in high specificity without false positives in this dataset.

Clinical Diagnoses and Clinicopathological Correlations: Tuberculosis was the most common final diagnosis, observed in 59 patients (39.3%), followed by bacterial pneumonia in 30 (20.0%), viral pneumonia in 20 (13.3%), malignancy in 12 (8.0%), empyema in 10 (6.7%), acute infection in 8 (5.3%), cirrhosis in 6 (4.0%), cardiovascular disease in 3 (2.0%) and nephrotic syndrome in 2 (1.3%). Tuberculosis accounted for 44.0% of pleural effusions.

A highly significant association was found between type of fluid and clinical diagnosis ($\chi^2 \approx 72.3$, $p < 0.001$). Acute infections and cirrhosis were largely confined to peritoneal fluids, whereas tuberculosis, pneumonia (bacterial and viral), empyema and most malignancies presented as pleural effusions.

Fluid appearance (clear vs turbid) also showed significant association with clinical diagnosis ($\chi^2 \approx 32.2$, $p < 0.001$). Tuberculosis, cirrhosis, cardiovascular disease and most bacterial pneumonia cases presented with clear fluids, while empyema and viral pneumonia had a higher proportion of turbid fluids.

Predominant cell type displayed a strong correlation with diagnosis ($\chi^2 \approx 250.4$, $p = 0.002$). All malignancies ($n = 12$) showed atypical cells as the predominant population; tuberculosis and viral pneumonia were uniformly lymphocyte-predominant, and bacterial pneumonia, empyema and acute infections were predominantly neutrophilic. Similarly, cytological category (negative, positive, suspicious for malignancy) had a statistically significant association with the final diagnosis ($\chi^2 \approx 126.5$, $p = 0.003$), with all malignant cases clustered in the positive/suspicious group.

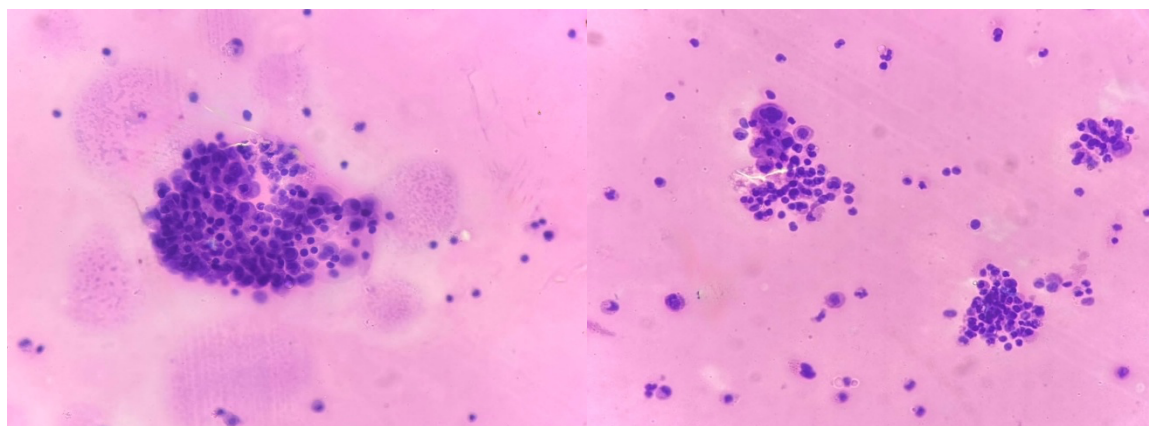


Figure 1: Atypical cells in malignant peritoneal effusion

Table 1: Association Between Clinical Diagnosis and Cytological Findings

Clinical Diagnosis	Negative Malignant (n=138)	for Cells	Positive Malignant (n=10)	for Cells	Suspicious for Malignancy (n=2)	Total (n=150)
Acute infection	8		0		0	8
Bacterial Pneumonia	30		0		0	30
Cardiovascular disease	3		0		0	3
Cirrhosis	6		0		0	6
Empyema	10		0		0	10
Malignancy	0		10		2	12
Nephrotic syndrome	2		0		0	2
Tuberculosis	59		0		0	59
Viral Pneumonia	20		0		0	20
Total	138		10		2	150
$\chi^2 = 126.465, p = 0.003$						

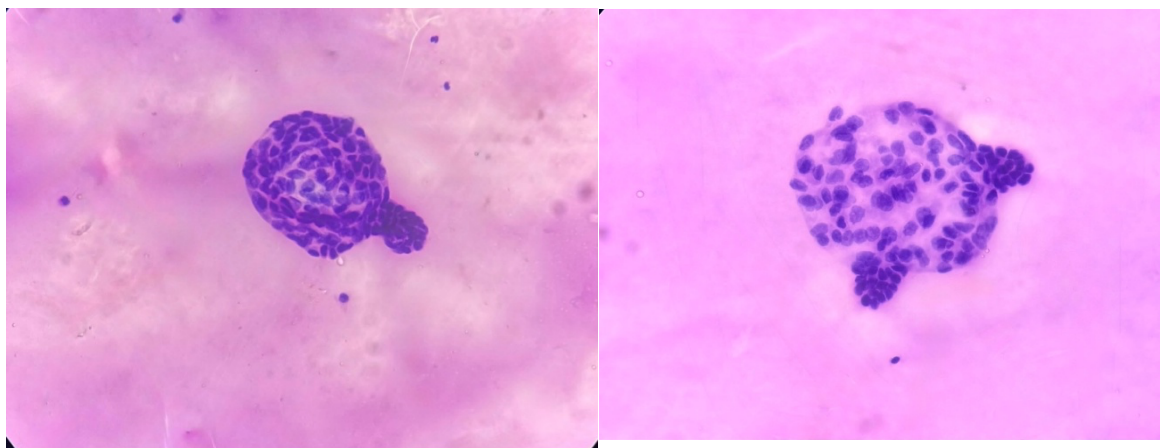


Figure 2: Atypical cells in malignant pleural effusion

Predictors of Malignancy: On bivariate and multivariable analysis, the presence of atypical cells was the single most powerful predictor of malignancy, with an odds ratio approaching infinity due to the absence of atypical cells in non-malignant effusions. After adjustment for age, gender, fluid type and cellularity, atypical cells remained a very

strong independent predictor (aOR >400, $p < 0.001$). Age >60 years (aOR ≈ 3.8, $p \approx 0.024$) and high cellularity (aOR ≈ 5.6, $p \approx 0.002$) were also significantly associated with malignant effusions, whereas male gender and pleural vs peritoneal site were not independently significant predictors.

Table 2: Adjusted Odds Ratios (aOR) for Malignancy Prediction

Predictor	aOR (95% CI)	p-value	AUROC Contribution
Atypical cells	487.3 (58.2- ∞)*	<0.001	0.42
Age >60 years	3.8 (1.2-12.1)	0.024	0.15
Pleural fluid	2.1 (0.7-6.3)	0.18	0.08
High cellularity	5.6 (1.9-16.8)	0.002	0.22
Male gender	1.4 (0.5-3.9)	0.52	-

(aOR: adjusted odds ratio; ∞ : non-finite due to near perfect prediction; AUROC: area under receiver operating characteristic curve)

Lymphocytic predominance showed perfect discrimination for tuberculous effusions in this dataset and was significantly associated with viral pneumonia and cirrhosis compared to acute bacterial infections. Neutrophilic predominance was strongly associated with bacterial pneumonia and empyema and virtually absent in tuberculosis, providing high discriminatory value between these infectious etiologies.

Discussion

The present study demonstrates that systematic cytological evaluation of pleural and peritoneal effusions, using conventional smear and cytospin methods, provides valuable diagnostic information across a spectrum of inflammatory, infectious and malignant conditions in a tertiary care setting.

In the current study, pleural effusions (89.3%) far outnumbered peritoneal effusions (10.7%), consistent with several Indian series where pleural fluids were the predominant specimens, although the proportions vary. Ayyagari Sudha et al., in a cytological study from Hyderabad, reported pleural fluids in 49% and peritoneal in 41.39% of serous effusion specimens, reflecting a more balanced distribution than observed here, possibly due to different case-mix and referral patterns. [11,12]

Tuberculosis emerged as the leading cause of effusion (39.3% overall; 44.0% of pleural effusions), which aligns well with studies from tuberculosis-endemic regions where tuberculous pleuritis is a major etiology. [13] Another study found tuberculosis in 52% of pleural effusions, pneumonia in 25% and malignancy in around 10%, closely paralleling the pattern in the present study, though with a slightly higher malignant fraction. [14] Other body-fluid cytology series have also highlighted the dominance of lymphocyte-rich exudates in tuberculous effusions. [15]

The overall malignant effusion rate of 8.0% in this cohort is lower than the 13–26% malignancy rates reported in some Indian and international studies, likely reflecting the high burden of infectious diseases and younger age profile in the present population. Nonetheless, malignancy remains an important consideration, and cytology demonstrated excellent specificity, with all clinically malignant

cases being either cytologically positive or suspicious and no false positives among benign diagnoses. [16]

The predominance of lymphocytes in 63.3% of effusions, particularly in tuberculosis, viral pneumonia and cirrhosis, is in keeping with prior reports where lymphocyte-rich exudates were commonly associated with chronic inflammatory and granulomatous diseases. Neutrophil predominance in bacterial pneumonia and empyema mirrors classical descriptions of acute bacterial effusions and empyemas in the literature. [17]

Role of Cytology and Ancillary Techniques:

Cytological examination of body fluids is recognised as a frontline diagnostic modality for effusions because of its simplicity and the possibility of repeated sampling. However, conventional smears alone may have suboptimal sensitivity for malignancy, especially in cases with low tumour cell yield or prominent reactive mesothelial atypia. Studies comparing conventional smears with cell block preparations report higher cellularity, better architectural preservation and improved malignant cell detection with cell blocks, with sensitivity for malignancy rising from around 65–70% with smears to over 90% when cell blocks are added. [18,19]

In the present study, both conventional smear and cytospin methods were employed; however, routine cell block preparation was not incorporated. Even so, the complete concordance between clinical malignancy and positive/suspicious cytology suggests high specificity in this series. Nevertheless, in diagnostically challenging cases, particularly where reactive mesothelial cells mimic carcinoma, routine use of cell blocks and immunocytochemistry (e.g. calretinin, WT-1, Ber-EP4, MOC-31) as recommended in contemporary guidelines would likely enhance sensitivity and assist in primary site characterisation.

Strengths and Limitations: Strengths of this study include its prospective design, uniform processing of all effusions with both conventional smear and cytospin techniques, and systematic clinicopathological correlation. The study reflects real-world diagnostic practice in a large tertiary care hospital serving a tuberculosis-endemic population.

Key limitations include the single-centre nature and relatively modest sample size, particularly for peritoneal and malignant effusions, which may limit

generalisability. Histopathological confirmation was not available for all malignant cases, although clinical and radiological correlation was used to assign final diagnoses. Cell block and immunocytochemistry were not routinely performed, precluding direct comparison of diagnostic yield between techniques.

Future Directions: Future work should include larger, multicentric studies incorporating cell block techniques and immunocytochemistry to quantify incremental diagnostic yield over conventional cytology in this population. Prospective integration of biochemical markers (e.g. ADA, lactate dehydrogenase, tumour markers) and molecular diagnostics, where feasible, could further refine the diagnostic algorithm for serous effusions.

Conclusion

Cytological examination of pleural and peritoneal effusions remains a valuable, minimally invasive and cost-effective diagnostic tool in the evaluation of patients with serous effusions in tuberculosis-endemic, resource-constrained settings. In this study, pleural effusions predominated, and tuberculosis was the leading cause, characterised by lymphocytic, clear effusions. Neutrophilic effusions were strongly associated with acute bacterial infections and empyema, while the presence of atypical cells and high cellularity were robust predictors of malignant effusions. Careful interpretation of cytomorphological patterns in conjunction with clinical and radiological findings allows effective triage, guides further investigations and contributes significantly to patient management.

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