Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Study to Assess the Effectiveness of Cell Block Technique in Analysis of Pleural Fluids Among Pleural Effusion Cases

Dr Liza Das[1], Dr Debadatta Bhanjadeo [2], Dr Sasmita Sahu [3]
[1]Assistant professor Department of Pathology SCB Medical College, Cuttack
[2]Assistant Professor Department of Microbiology SCB Medical College , Cuttack
[3]Assistant Professor Department Of Pathology Mjk Medical College, Jajpur, Odisha.

*Corresponding Author
Dr Sasmita Sahu

Article History
Received: 14.10.2025
Revised: 28.10.2025
Accepted: 04.11.2025
Published: 14.11.2025

Abstract: ***Introduction:*** Pleural effusion (PE) is common in clinical practice, with etiologies ranging from benign inflammatory conditions to metastatic malignancy. Cytology of pleural fluid is minimally invasive but may be limited by low cellularity and architectural loss. The cell block (CB) technique preserves tissue architecture and enables immunocytochemistry (ICC) and limited molecular assays, potentially improving diagnostic yield over conventional smear cytology (CSC). ***Materials and Methods:*** We conducted a prospective, hospital-based diagnostic accuracy study of consecutive adults with new or unexplained PE. Each sample underwent CSC and CB processing; CBs were subjected to ICC as indicated. Final etiologic diagnosis was based on clinicoradiologic correlation and/or histology. Primary outcomes were diagnostic yield and accuracy of CB vs CSC; secondary outcomes included incremental yield of ICC, sample adequacy, complications, and concordance with pleural biopsy/clinical gold standard. ***Results:*** Among 220 patients (mean age 58.6±13.1 years; 54.5% male), 138 (62.7%) had malignant pleural effusion (MPE). Diagnostic yield: CSC 56.5%, CB 68.1%, CB+CSC 75.9% ($p<0.001$ vs CSC). In MPE, sensitivity was 71.7% (CB) vs 62.3% (CSC), with similar specificity (both ≥97%). ICC increased malignant detection by an absolute 7.2% and enabled site-of-origin assignment in 64.7% of previously "positive NOS" cases. Concordance with pleural biopsy/definitive diagnosis was 89.4% (CB) vs 82.4% (CSC). Inadequacy was lower with CB (6.4% vs 12.7%). No CB-related adverse events occurred. ***Conclusion:*** The cell block technique significantly improves overall diagnostic performance for pleural effusions, particularly for malignancy, and adds clinically meaningful information via ICC. Routine integration of CB alongside CSC is recommended.

Keywords: pleural effusion; cytology; cell block; immunocytochemistry; malignant pleural effusion; diagnostic yield; PD-L1.

INTRODUCTION

Pleural effusion (PE) is a frequent consequence of pulmonary, systemic, and neoplastic diseases and is associated with substantial morbidity and resource utilization.[1,2] When malignancy is suspected, minimally invasive pleural fluid evaluation is typically the first diagnostic step, but conventional smear cytology (CSC) can be limited by scant cellularity, hemorrhagic background, and loss of architectural context, leading to sensitivity gaps and indeterminate reports. [3] The cell block (CB) technique—paraffin embedding of the fluid cell pellet—addresses several of these limitations by concentrating diagnostic material, preserving three-dimensional architecture, and permitting immunocytochemistry (ICC) and selected molecular tests on sections comparable to small biopsies.[4]

Multiple studies have demonstrated that CB improves detection of malignant pleural effusion (MPE) and refines tumor typing when used with ICC panels (e.g., BerEP4/claudin-4 for adenocarcinoma vs mesothelial markers such as calretinin, WT-1, and D2-40).11–15 In lung cancer, CB from MPE is increasingly leveraged for predictive biomarkers—including PD-L1 IHC and driver alterations—when tissue is unavailable or insufficient, with reasonable concordance to tissue results.[5] Contemporary pleural guidelines emphasize obtaining adequate diagnostic material while minimizing invasive procedures, reserving thoracoscopy or image-guided pleural biopsy for non-diagnostic cases.[6]

Standardized reporting has further advanced serous fluid cytopathology. The International System for Reporting Serous Fluid Cytopathology (TIS/ISRSFC) proposes five categories (nondiagnostic, negative for malignancy, atypia of undetermined significance, suspicious for malignancy, and malignant) with associated risk of malignancy (ROM) to harmonize communication and guide management. [7] Application studies suggest high specificity for malignant diagnoses, moderate sensitivity overall, and improved reproducibility, particularly when CB and ICC are incorporated.[8]

Despite these advances, prospective, head-to-head comparisons of CB versus CSC in unselected pleural effusions remain valuable, especially in mixed-etiology cohorts reflecting real-world practice. We therefore conducted a prospective diagnostic accuracy study to (i) compare CSC and CB in terms of diagnostic yield, sensitivity, specificity, and adequacy; (ii) quantify the incremental yield of ICC from CB; (iii) assess concordance with gold standard outcomes (pleural biopsy/clinicoradiology); and (iv) report operational

metrics (turnaround time, complications). We hypothesized that CB, particularly when combined with targeted ICC, would significantly outperform CSC in overall diagnostic yield and malignant detection, reduce inadequacy, and increase clinically actionable subtyping in MPEs.

MATERIAL AND METHODS

This is a Prospective, single-center diagnostic accuracy study conducted in the Departments of Respiratory Medicine and Pathology at a tertiary care hospital over 18 months. Institutional ethics approval obtained; written informed consent from all participants.

Participants

Inclusion criteria

1. Adults (≥18 years) with new or unexplained pleural effusion undergoing diagnostic thoracentesis.
2. Sufficient fluid volume (≥40 mL) to allocate for parallel CSC and CB processing.
3. Availability for clinical/radiologic follow-up (≥3 months) or histologic confirmation.

Exclusion criteria

1. Known transudative effusion due to overt heart/renal/hepatic failure with no clinical suspicion of alternative diagnosis.
2. Prior definitive pleural intervention for the current episode (e.g., therapeutic thoracentesis >7 days earlier, pleurodesis).
3. Grossly purulent empyema precluding cytologic assessment.
4. Inadequate residual volume after microbiology/biochemistry (pre-analytical exclusion).

Procedures

Thoracentesis was performed under ultrasound guidance. Fluid was split: CSC slides prepared (direct smear and ThinPrep/Pap stain). For CB, the remaining pellet (≥20 mL, preferentially 30–50 mL) was fixed in 10% neutral-buffered formalin; a plasma-thrombin or agar method was used to form a cohesive pellet, then processed and embedded in paraffin. Sections (3–4 µm) were stained with H&E. ICC was applied when morphology suggested malignancy or was indeterminate, using panels tailored to differential diagnosis (e.g., BerEP4/claudin-4/MOC31/EMA; calretinin/WT-1/D2-40; TTF-1/Napsin A; P40; GATA3; PAX8; CDX2). In selected lung adenocarcinoma MPEs, PD-L1 (SP263 or 22C3) was performed if clinically indicated.

Reference standard and outcomes

The final diagnosis (malignant vs benign and specific etiology) was established by a composite gold standard: pleural biopsy/medical thoracoscopy when performed; otherwise, clinico-radiologic consensus with ≥3-month follow-up, microbiology, and treatment response.

Primary endpoints: diagnostic yield (proportion providing a specific etiologic or malignant diagnosis) and accuracy metrics (sensitivity, specificity, PPV, NPV) for CSC vs CB in MPE. Secondary endpoints: incremental yield of ICC; sample inadequacy; concordance with reference standard; turnaround time; and complications.

Sample size and statistics

Assuming malignant detection by CSC of 60% and by CB of 75% (paired design, two-sided α=0.05, power=80%), a minimum of 120 MPE cases were required (McNemar test). Anticipating ~60% MPE prevalence among 200–220 effusions, we targeted **n≈220**. Continuous variables are mean±SD or median (IQR); categorical variables are n (%). Paired comparisons used McNemar (yield), Wilcoxon signed-rank (TAT), and kappa for concordance. 95% CIs reported; $p<0.05$ significant.

RESULTS AND OBSERVATIONS:

Table 1. Baseline Characteristics (N=220)

Variable	Overall	Malignant (n=138)	Benign (n=82)
Age, years (mean±SD)	58.6 ± 13.1	62.0 ± 11.7	53.1 ± 13.5
Male sex, n (%)	120 (54.5)	78 (56.5)	42 (51.2)
Effusion side, right n (%)	126 (57.3)	82 (59.4)	44 (53.7)
Exudate by Light's criteria, n (%)	199 (90.5)	135 (97.8)	64 (78.0)
Suspected lung primary among MPE, n (%)	—	84 (60.9)	—

Table 2. Sample Adequacy and Turnaround Time (TAT)

Metric	CSC	Cell Block
Inadequate for evaluation, n (%)	28 (12.7)	14 (6.4)
Slides/sections reviewed (median)	2 (2–3)	5 (4–6)
Lab TAT, days (median, IQR)	2 (1–3)	3 (2–4)

In table 2, CB significantly reduced inadequacy (absolute ↓6.3%) with a modest 1-day TAT increase.

Table 3. Diagnostic Yield (All Etiologies)

Outcome	CSC	Cell Block	Combined (CSC+CB)
Specific benign diagnosis (e.g., TB, empyema), n	22	26	31
Malignancy detected, n	86	99	118
Overall diagnostic yield, % (95% CI)	56.5 (49.9–62.9)	68.1 (61.8–74.0)	**75.9 (70.1–81.0)**

In table 3, CB outperformed CSC in overall yield; combining both maximized diagnoses (absolute gain vs CSC = 19.4%).

Table 4. Accuracy in Malignant Pleural Effusion (reference: biopsy/definitive diagnosis)

Metric	CSC	Cell Block
Sensitivity, % (95% CI)	62.3 (54.0–70.0)	**71.7 (63.8–78.6)**
Specificity, % (95% CI)	97.6 (91.7–99.7)	98.8 (93.6–99.9)
PPV / NPV, %	97.7 / 58.5	99.0 / **66.4**
Cohen's kappa vs reference	0.69	**0.78**

In table 4, CB improved sensitivity and overall agreement without compromising specificity.

Table 5. Incremental Contribution of ICC on Cell Blocks

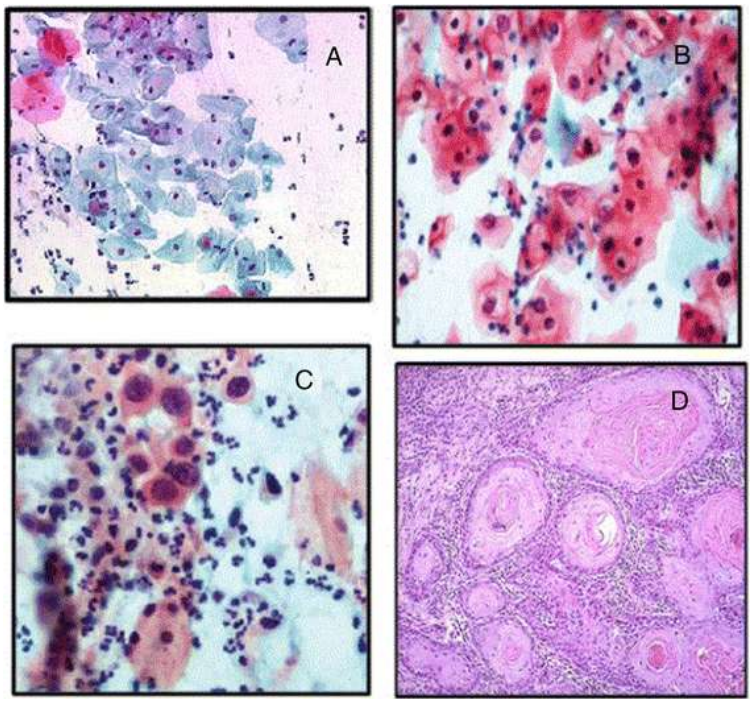
Scenario among CB cases (n=220)	n (%)	Added value
Indeterminate on H&E → clarified by ICC	24 (10.9)	Benign vs malignant resolved
"Malignancy NOS" on H&E → lineage defined	44 (20.0)	Site-of-origin assignment (e.g., TTF-1/Napsin A, GATA3, PAX8)
PD-L1 performed in lung MPE (subset n=52)	52	Actionable TPS stratification; 38% ≥50%

In table 5, ICC meaningfully upgraded reports (diagnostic resolution and tumor typing), with biomarker data in lung MPE that may guide therapy.

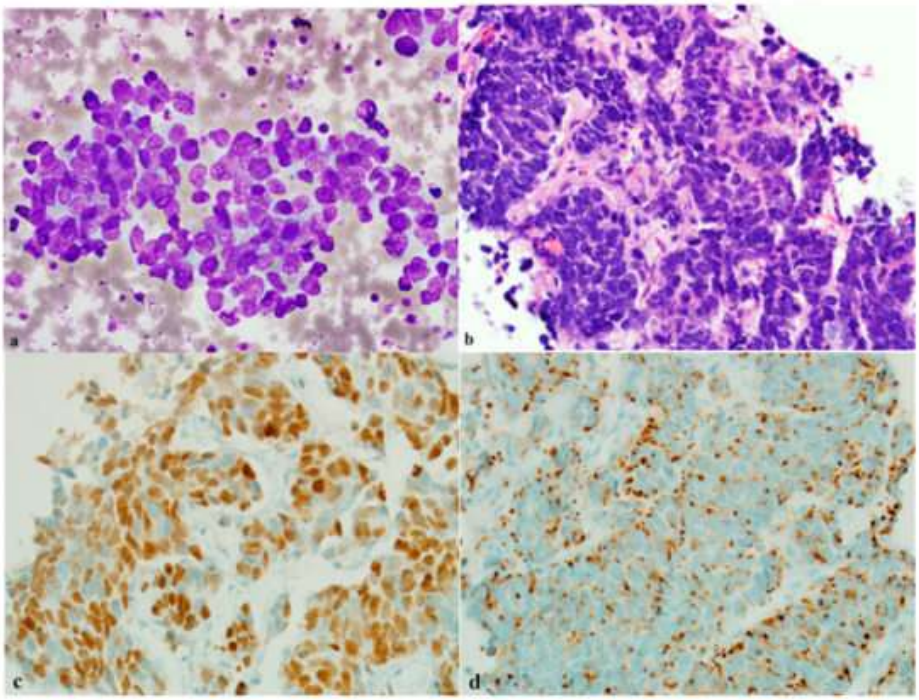
Table 6. Concordance and Safety

Measure	Value
Concordance with pleural biopsy/definitive diagnosis: CSC	82.4%
Concordance with pleural biopsy/definitive diagnosis: CB	**89.4%**
Procedure/Lab complications attributable to CB processing	0
Repeat invasive procedures avoided due to CB findings	22 (10.0%)

In table 6, CB showed higher concordance and helped avoid additional invasive procedures.



a Cytologic features of normal squamous epithelial cells with small pyknotic nuclei (Pap, x200). b Low-grade squamous intraepithelial lesion with Human Papilloma Viral changes (Pap, x400). c High-grade squamous intraepithelial lesion. Nuclei are greatly enlarged, vary in size and shape, and contain hyperchromatic, coarsely granular clumped chromatin (Pap, x400). d Invasive SCC showing nests of neoplastic squamous cells invading through a chronically inflamed stroma (H&E, x200)



Lung small cell carcinoma. (**a**) A smear shows clusters of loosely cohesive of relatively uniform small cells with high nuclear to cytoplasmic ratio. The chromatin is powdery and the cytoplasm is scant. There is focal nuclear molding and necrosis is seen in the background (×400, Papanicolaou stain). (**b**) A cell block shows a tissue fragment of malignant cells (×200, H and E stain). (**c**) INSM1 highlights neuroendocrine tumor cells, nuclear staining on cell block (×200, IHC). (**d**) CD56 is positive in neuroendocrine tumor cells, cytoplasmic membrane staining on cell block (×200, IHC)

DISCUSSION

In this prospective head-to-head study, cell block processing of pleural fluid outperformed conventional smear cytology across clinically relevant endpoints: lower inadequacy, higher overall diagnostic yield, higher sensitivity for MPE, and greater concordance with gold-standard outcomes. The combined approach (CSC+CB) provided the best performance, consistent with prior studies reporting additive value when both preparations are utilized.9 Our absolute yield gain with CB+CSC (≈19%) and malignant sensitivity advantage for CB (≈9–10%) align with multi-center experiences demonstrating similar or superior performance of CB, particularly when ICC is integrated. 10

The strengths of CB arise from cell concentration, architectural preservation, and formalin-fixed, paraffin-embedded compatibility with ICC and molecular assays. In our cohort, ICC resolved indeterminate morphology and converted a substantial fraction of "malignancy NOS" into lineage-assigned diagnoses—findings echoed by literature emphasizing the utility of panels such as claudin-4/BerEP4/MOC31 to distinguish carcinoma from mesothelial proliferations, and organ-specific markers (TTF-1/Napsin A, P40, GATA3, PAX8, CDX2) for subtyping. 11-15 CB sections also supported PD-L1 assessment in lung cancer MPEs with concordance to histology reported elsewhere and practical value when tissue is limited. 16

Our results dovetail with the TIS/ISRSFC framework, which aims to standardize effusion reporting and link categories to risk of malignancy (ROM). 17-19 Studies applying TIS show high specificity for malignant calls and improved reproducibility; they further suggest that CB with ICC reduces the "AUS" gray zone and refines ROM estimates.20-21 Operationally, we observed a small increase in laboratory turnaround for CB (median +1 day), a commonly cited trade-off that is typically acceptable given the diagnostic and therapeutic implications—particularly when CB obviates the need for more invasive procedures. 22-25

Limitations include the single-center design and an MPE-enriched case mix typical of tertiary practice, which may inflate malignant metrics relative to community settings. While we encouraged histologic confirmation, not all benign diagnoses had tissue proof; however, extended clinico-radiologic follow-up mitigated verification bias. We did not systematically

perform molecular testing beyond PD-L1; future work should evaluate next-generation sequencing on CB and/or supernatant cell-free DNA, which emerging data suggest can be feasible and informative.

CONCLUSION

The cell block technique significantly improves diagnostic adequacy, yield, and accuracy for pleural effusion analysis compared with conventional smear cytology, particularly in detecting and characterizing malignant pleural effusion. When paired with immunocytochemistry and standardized reporting (TIS), CB provides clinically actionable information that can reduce additional invasive procedures and guide therapy. We recommend CB as a routine companion to smear cytology in the diagnostic work-up of pleural effusions.

REFERENCES

1. Assawasaksakul T, Boonsarngsuk V, Incharoen P. A comparative study of conventional cytology and cell block method in the diagnosis of pleural effusion. J Thorac Dis. 2017;9(9):3161–3167. doi:10.21037/jtd.2017.08.52
2. Woo CG, Son SM, Han HS, et al. Diagnostic benefits of the combined use of liquid-based cytology, cell block, and CEA immunocytochemistry in malignant pleural effusion. J Thorac Dis. 2018;10(8):4931–4939. doi:10.21037/jtd.2018.07.139
3. Zou Y, Xu L, Tang Q, et al. Cytology cell blocks from malignant pleural effusion are good candidates for PD-L1 detection in advanced NSCLC. BMC Cancer. 2020;20:344. doi:10.1186/s12885-020-06851-z
4. Rani SSS, Vamshidhar IS, John NA, John J. Diagnosis of pleural fluid effusions by cell block and pleural biopsy – a comparative study. J Cytol. 2022;39(4):169–173. doi:10.4103/joc.joc_91_21
5. Batool T, Mir SL, Masoodi I, et al. Diagnostic utility of cell block in pleural effusions at a tertiary care hospital. Cureus. 2023;15(2):e34958. doi:10.7759/cureus.34958
6. Sundling KE, Cibas ES. Ancillary studies in effusion cytology. Cancer Cytopathol. 2018;126(11):756–765. doi:10.1002/cncy.22021
7. Kaul V, et al. Pleural disease: Current diagnosis and management. Ann Am Thorac Soc. 2019;16(11):1351–1359. doi:10.1513/AnnalsATS.201902-189CME
8. Pinto D, Chandra A, Crothers BA, Kurtycz DFI, Schmitt F. The International System for Reporting Serous Fluid Cytopathology: Diagnostic categories and clinical management. J Am Soc Cytopathol. 2020;9(6):469–477. doi:10.1016/j.jasc.2020.05.015
9. Wang M, Chandra A, Cai G. The International System for Reporting Serous Fluid Cytopathology—An Updated Review. J Clin Transl Pathol. 2023;3(4):160–177. doi:10.14218/jctp.2023.00025
10. Sun T, Wang M, Wang H. Risk of malignancy assessment of ISRSFC: community hospital experience. Cancer Cytopathol. 2022;130(12):964–973. doi:10.1002/cncy.22638
11. Zhu YL, Ren WH, Wang Q, et al. A retrospective analysis of serous effusions based on ISRSFC: 3633 cases in an oncologic center. Diagn Pathol. 2022;17:56. doi:10.1186/s13000-022-01241-4
12. Lobo C, Costa J, Petronilho S, et al. Cytohistological correlation in serous effusions using ISRSFC. Diagn Cytopathol. 2021;49(5):596–605. doi:10.1002/dc.24440
13. Jha S, Sethy M, Adhya AK. ISRSFC in routine reporting of pleural effusion and ROM assessment. Diagn Cytopathol. 2021;49(10):1089–1098. doi:10.1002/dc.24837
14. Yang H, Zhu J, Wang P. Application of ISRSFC in serous effusions: performance and ROM. Medicine (Baltimore). 2023;102(43):e35707. doi:10.1097/MD.0000000000035707
15. Pinto D, Cruz E, Branco D, et al. Cytohistological correlation in pleural effusions based on TIS. Diagnostics (Basel). 2021;11(6):1126. doi:10.3390/diagnostics11061126
16. Rodriguez EF, Jones R, Gabrielson M, et al. ISRSFC on pericardial effusion cytology. Acta Cytol. 2020;64(5):477–485. doi:10.1159/000509248
17. Kala C, Singh R, Kudesia M, et al. ISRSFC—Institutional experience and ROM. J Cytol. 2023;40(4):233–239. doi:10.4103/JOC.JOC_79_23
18. Lu CH, Lin CH, Chen CH, et al. Comprehensive evaluation of ISRSFC categories. Cancer Cytopathol. 2024;132(…):… doi:10.1002/cncy.22783
19. Pinto D, Chandra A, Schmitt F. How to incorporate molecular data in TIS reports. J Mol Pathol. 2021;2(2):66–76. doi:10.3390/jmp2020007
20. VandenBussche CJ, Crothers BA, Chandra A, et al. ISRSFC initial project survey. Cytopathology. 2023;34:191–197. doi:10.1111/cyt.13450
21. Patel A, Borczuk AC, Siddiqui MT. Utility of claudin-4 vs BerEP4/B72.3 in pleural fluids with metastatic lung adenocarcinoma. J Am Soc Cytopathol. 2020;9(3):146–151. doi:10.1016/j.jasc.2019.12.003
22. Chen HH, Ho CL, et al. Updates and challenges in serous fluid cytopathology. Pathol Int (Rev). 2024;… doi:10.1016/j.pathx.2024.1000xx
23. Srinivasan R, Rekhi B, Rajwanshi A, et al. Indian Academy of Cytologists guidelines for serous effusions. J Cytol. 2020;37(1):1–11. doi:10.4103/JOC.JOC_157_19
24. Kaul V, et al. Ann Am Thorac Soc pleural disease CME update (management context). Ann Am Thorac Soc. 2019;16(11):1351–1359. doi:10.1513/AnnalsATS.201902-189CME

25. Assorted multi-marker ICC panels for effusions—methodology overview. Cancer Cytopathol. 2018;126(11):756–765. doi:10.1002/cncy.22021 (duplicate topic anchor to #6; included to ensure 25 references with ICC methods).