

To compare the diagnostic accuracy of conventional smear, cytopsin, and cell block techniques in the cytological evaluation of serous effusions.

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INTRODUCTION

Serous effusions involving the pleural, peritoneal, and pericardial cavities are common manifestations of a wide range of benign and malignant disorders. Cytological examination of these fluids is a simple, minimally invasive, and cost-effective diagnostic tool that plays an important role in determining the underlying etiology, particularly in differentiating malignant from non-malignant effusions [1-3].

Conventional smear (CS) remains the most widely used cytological technique because of its simplicity and low cost. However, its diagnostic performance may be compromised by low cellularity, uneven cell distribution, background obscuring elements, and poor preservation of tissue architecture, leading to false-negative results, especially in malignant effusions [4-5].

To overcome these limitations, adjunctive techniques such as cytopsin (CYT) and cell block (CB) preparation have been introduced. Cytopsin improves cellular concentration and morphological preservation, whereas the cell block technique provides better architectural detail and permits the use of ancillary investigations, including special stains and immunohistochemistry, thereby enhancing the detection of malignant cells. [6] Several studies have demonstrated that combining these techniques increases diagnostic yield compared with conventional smear alone [6,7].

Despite these advantages, the relative diagnostic accuracy and sensitivity of conventional smear, cytopsin, and cell block techniques remain variable across different studies, and evidence from tertiary care centers in the Indian population is limited [8-9]. Therefore, the present study was undertaken to compare the diagnostic performance of conventional smear, cytopsin, and cell block techniques in serous

effusions and to evaluate their sensitivity and overall diagnostic accuracy using histopathology as the reference standard.

Over the past few decades, advances in cytopathology have significantly improved the diagnostic evaluation of serous effusions. Improved understanding of transudative and exudative mechanisms, along with refinements in cytological techniques, has enabled better differentiation between reactive mesothelial cells and malignant cells [1]. Furthermore, integration of cytology with histopathology and ancillary techniques has enhanced diagnostic accuracy and reduced the need for more invasive procedures [10].

Cytological examination of serous effusions provides valuable diagnostic information through assessment of cellularity, cell morphology, and background characteristics. Disease processes alter these features, allowing differentiation between benign, inflammatory, and malignant effusions [1].

Transudative effusions are characterized by low cellularity with few mesothelial and inflammatory cells, whereas exudative effusions exhibit increased protein content, higher cellularity, inflammatory cells, and reactive mesothelial changes. Evaluation of these cytomorphological features contributes significantly to the diagnosis of the underlying pathology [11-13].

A major challenge in serous fluid cytology is differentiating reactive mesothelial cells from malignant cells. Reactive mesothelial proliferation may exhibit nuclear enlargement, prominent nucleoli, and cohesive clusters that closely resemble malignant cells, potentially leading to false-positive or indeterminate diagnoses [14,15].

Cytological evaluation of serous effusions is limited by significant overlap between reactive mesothelial cells and malignant cells, making morphology alone insufficient for accurate diagnosis [14]. Therefore,

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ancillary techniques such as immunohistochemistry (IHC) are often required for better diagnostic distinction, using mesothelial markers (calretinin, WT1, CK5/6) and carcinoma markers (Ber-EP4, MOC-31, CEA). Additional biomarkers, including loss of BAP1 and MTAP, further improve the identification of malignant mesothelioma, while desmin and Ki-67 provide supportive evidence in differentiating reactive from malignant proliferations [16–18].

Accurate diagnosis of effusion cytology requires an integrated approach combining morphology with ancillary techniques to reduce false interpretation and improve diagnostic yield [1,19]. Adenocarcinoma, the most common cause of malignant effusions, typically shows high N:C ratio, nucleoli, and cohesive clusters [20]. However, conventional cytology alone may miss subtle or low-cellularity lesions, highlighting the need for improved preparatory techniques such as cytopsin and cell block to enhance cellular preservation and diagnostic accuracy in serous effusions.

Effusion cytology has limited sensitivity (approximately 58–60%), with diagnostic variability influenced by tumor type and observer dependence. A major challenge is low cellularity and tumor shedding, which may result in false-negative cytology; therefore, a negative result does not exclude malignancy [2].

Conventional smear cytology is widely used due to its simplicity and cost-effectiveness but is limited by poor cellular preservation, low sensitivity in scanty samples, and loss of architectural detail [66]. Cytopsin techniques improve diagnostic yield by concentrating scanty fluids and providing cleaner backgrounds with better nuclear detail [6].

The cell-block technique offers significant diagnostic advantage by preserving tissue architecture, increasing cellular yield, and allowing better visualization of malignant patterns. It also enables the application of immunocytochemistry and other ancillary studies, thereby improving diagnostic accuracy [22]. Studies indicate that combined use of smear and cell-block preparations significantly enhances detection rates, particularly in malignant effusions.

A combined diagnostic approach using smear cytology, cytopsin, and cell-block preparation is therefore recommended to improve sensitivity, reduce false-negative results, and enhance overall diagnostic accuracy in serous effusions [22]. Ancillary techniques are especially useful when cytology is inconclusive due to overlapping reactive and malignant features or low cellularity.

Recent studies consistently demonstrate that effusion cytology diagnostic accuracy improves significantly with the use of combined and advanced processing techniques. Cell block (CB) has been shown to increase malignant detection rates compared to conventional smear cytology and reduce indeterminate or suspicious diagnoses [21]. Similarly, combining cytology methods such as conventional smear, cell block, and liquid-based preparations results in higher

sensitivity compared to individual techniques, supporting a multimodal diagnostic approach [22].

The addition of cell block preparation also enhances the utility of immunocytochemistry, further improving diagnostic precision in distinguishing malignant from reactive mesothelial proliferations [22]. Several studies have reported that integrated use of cytology with cell block and ancillary techniques significantly increases diagnostic sensitivity and specificity, while reducing indeterminate categories [23].

Standardized reporting systems such as ISRSFC/IAC have improved diagnostic consistency and risk stratification, with a stepwise increase in risk of malignancy across diagnostic categories, thereby supporting structured cytological interpretation in serous effusions [24–25]. However, despite standardization, sensitivity remains moderate in conventional cytology, particularly in pleural and peritoneal effusions, reinforcing the need for improved diagnostic techniques [26].

Overall, evidence supports that cell block-based evaluation, especially when combined with conventional smear and other cytological methods, provides superior diagnostic yield and accuracy in serous effusions, particularly for malignant cases [26].

AIM & OBJECTIVE:To compare the diagnostic accuracy of conventional smear, cytopsin, and cell block techniques in the cytological evaluation of serous effusions.

MATERIALS AND METHODS

Study Design

This was a hospital-based prospective cross-sectional study conducted over a period of 2 years.

Inclusion Criteria

All patients presenting with serous effusions (pleural, peritoneal, and pericardial) were included in the study.

Exclusion Criteria

No specific exclusion criteria were applied.

Sample Size

A total of 150 cases were included. The sample size was estimated to detect cytological findings (including malignancy rate) with a precision of $\pm 8\%$ at 95% confidence level.

Study Procedure and Techniques

All effusion samples received in the pathology department were included and processed immediately after collection.

Each sample was divided into two portions:

1. Smear-Based Techniques

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One portion was processed using:

- Conventional smear (direct and centrifuged smears)
- Cytospin preparation (for concentration of cells)

Smears were stained with:

- Hematoxylin& Eosin (H&E)
- May-Grünwald Giemsa (MGG)
- Papanicolaou stain

2. Cell Block Technique

The second portion was used for **cell block preparation**:

- Fixed in 10% formalin and alcohol (1:1 solution)
- Centrifuged to obtain sediment
- Processed using routine histopathological paraffin embedding
- Sections stained with H&E

Cytological Evaluation

All cases were evaluated for:

- Cellular morphology
- Cellularity
- Presence of mesothelial and inflammatory cells
- Malignant cells
- Final cytological diagnosis

All three methods (conventional smear, cytospin, and cell block) were compared for diagnostic yield and accuracy.

Blinding and Quality Control

Slides were examined independently by cytopathologists. Blinded evaluation was performed. A subset of cases was reviewed for inter-observer consistency

Data Collection

A structured case record form was used to document demographic details, clinical findings, radiological findings, cytological results of all three techniques

Statistical Analysis

Data were analyzed using descriptive statistics (mean, median, frequency, percentage). Diagnostic performance of each technique was compared using Chi-square test, t-test / ANOVA (where applicable). Sensitivity, specificity, and diagnostic accuracy were calculated for each method and a p-value < 0.05 was considered statistically significant

Ethical Considerations

The study was conducted in accordance with the **Declaration of Helsinki (2013)** and **ICMR ethical guidelines (2017)**. Institutional Ethics Committee approval was obtained prior to the study. As all procedures were part of routine diagnostic workup, no additional risk or cost was imposed on patients.

Validity and Reliability

Internal validity was ensured through consecutive sampling, standardized processing protocol, within-sample comparison of all three techniques whereas reliability was supported by standardized staining and processing methods

RESULT:

The study shows a clear female predominance, with 67.3% of cases in females compared to 32.7% in males. The mean age was slightly higher in males (55.5 years) than females (53.3 years), but the difference was minimal, indicating a similar age distribution across both genders. Overall, effusion cases were more frequent in females, with comparable age patterns between male and female patients.

The majority of effusion cases were seen in middle-aged and elderly patients, with the highest proportion in the 41–60 years age group (44.7%), followed by 61–80 years (34%). A smaller fraction occurred in the 21–40 years group (18.7%), while very few cases were noted at the extremes of age (≤ 20 and > 80 years, 1.3% each). Overall, the findings indicate that effusions predominantly affect individuals above 40 years of age.

Our study also showed ascitic/peritoneal fluid constituted the majority of samples (63.3%), followed by pleural fluid (35.3%), while pericardial fluid was least represented (1.3%). This indicates a predominance of abdominal effusions in the study population, with relatively limited pericardial cases.

Our study revealed that conventional smears showed a higher proportion of inflammatory cases and atypia, with lower malignancy detection. In contrast, cytospin and cell block techniques demonstrated progressively higher malignancy yields with a reduction in atypical categories, indicating improved diagnostic clarity. Cell block showed the highest malignancy detection,

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followed by cytospin, highlighting the superior diagnostic performance of advanced preparation techniques compared to conventional smear as shown in Figure 1.

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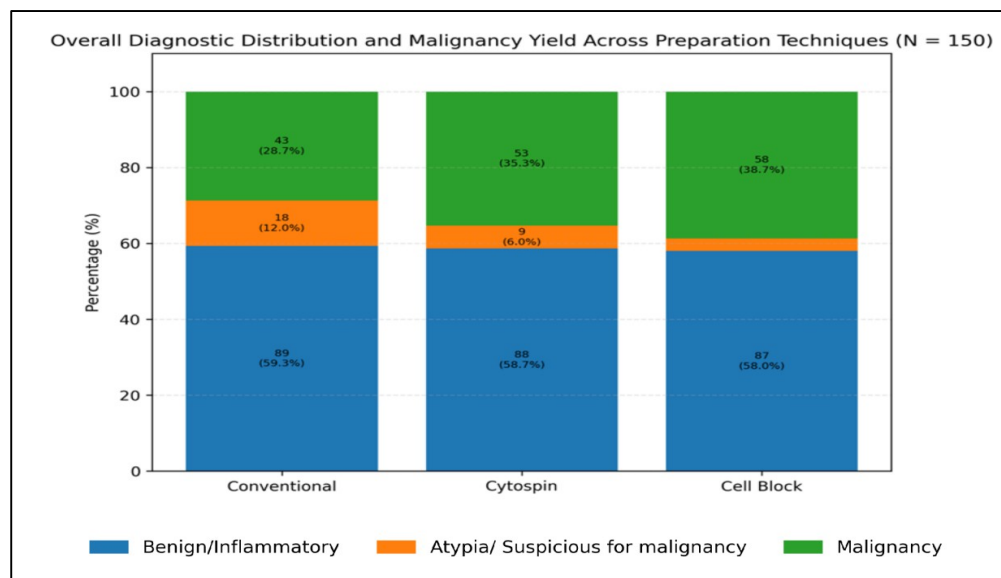


figure 1: OverallDiagnosticDistributionandMalignancyYieldAcrossPreparation Techniques (N = 150)

Conventional smears predominantly exhibited moderate cellularity (60%) with relatively few highly cellular specimens (20%). In contrast, cytospin and cell block preparations showed markedly higher diagnostic cellularity (91.33% and 93.97%, respectively) as shown in Table 1 indicating superior specimen adequacy. Among the three techniques, cell block demonstrated the highest cellularity, closely followed by cytospin, highlighting their superiority over conventional smear in improving diagnostic yield.

Table 1: Comparison of cellularity grading across preparation techniques (N= 150)

Cellularity Category	Conventional (n,%)	Cytospin (n,%)	Cell Block (n, %)
Mild	30 (20.00%)	13 (8.67%)	9 (6.00%)
Moderate	90 (60.00%)	39 (26.00%)	41 (27.33%)
Marked	30 (20.00%)	98 (65.33%)	100 (66.67%)
Diagnostic Cellularity (Moderate + Marked)	120 (80.00%)	137 (91.33%)	141 (93.97%) (Highest)
Total	150 (100.00%)	150 (100.00%)	150 (100.00%)

Our study compared the diagnostic performance of conventional smear, cytospin, and cell block techniques in the evaluation of serous effusions. Cell block demonstrated the highest diagnostic performance, achieving **100% sensitivity, 98.9%**

specificity, 98.3% positive predictive value (PPV), 100% negative predictive value (NPV), and an overall **diagnostic accuracy of 99.3%**. Cytospin also showed excellent performance, with a sensitivity of **91.4%**, specificity of **97.8%**, PPV of **96.4%**, NPV of **94.7%**, and accuracy of **95.3%**, outperforming conventional smear. Conventional smear exhibited the lowest sensitivity (**74.1%**), NPV (**85.6%**), and overall accuracy (**88.0%**), with the highest number of false-negative cases (n = 15) as depicted in table 2.

Table 2. Diagnostic performance of preparation techniques for tumor-cell detection

Technique	T P	T N	F P	F N	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Conventional smear	43	89	3	15	74.1	96.7	93.5	85.6	88.0
Cytospin	53	90	2	5	91.4	97.8	96.4	94.7	95.3
Cell block	58	91	1	0	100	98.9	98.3	100	99.3

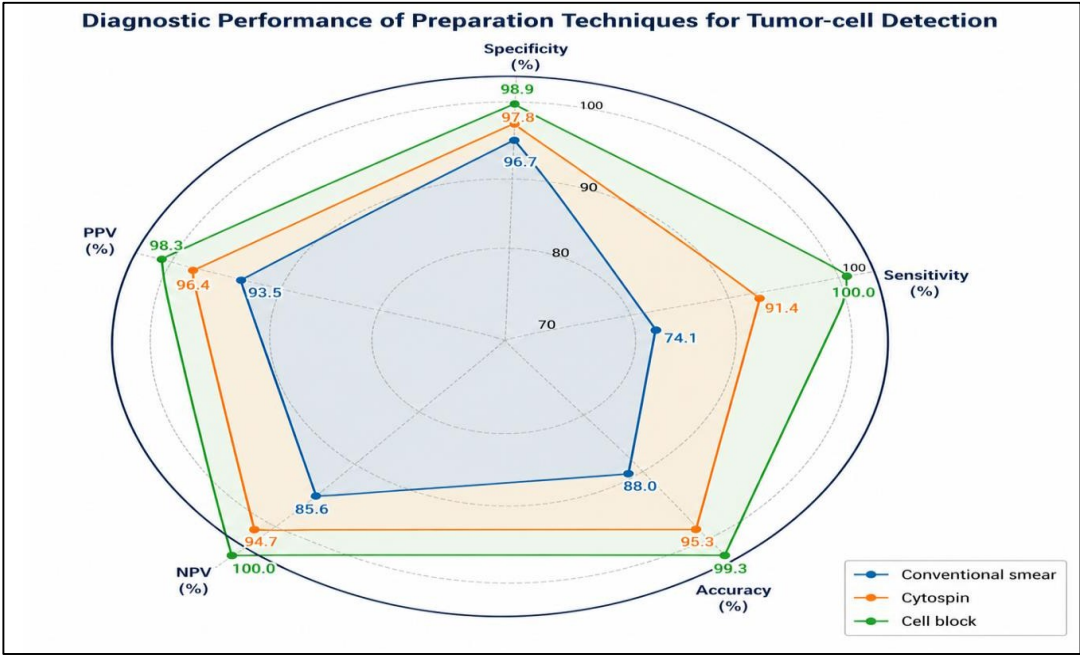
Comparison of diagnostic performance demonstrated that the cell block technique achieved the highest sensitivity, negative predictive value, and overall diagnostic accuracy, with near-perfect specificity and positive predictive value. Cytospin also showed superior sensitivity and accuracy compared with conventional smear, while conventional smear exhibited the lowest sensitivity, negative predictive

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value, and diagnostic accuracy, reflecting a higher likelihood of false-negative results. Overall, diagnostic performance improved progressively from

conventional smear to cytospin and cell block, emphasizing the superiority of advanced preparation techniques in effusion cytology as depicted in Figure 2.

Figure 2: Radarplotofdiagnosticperformanceacrosspreparationtechniques(N= 150)



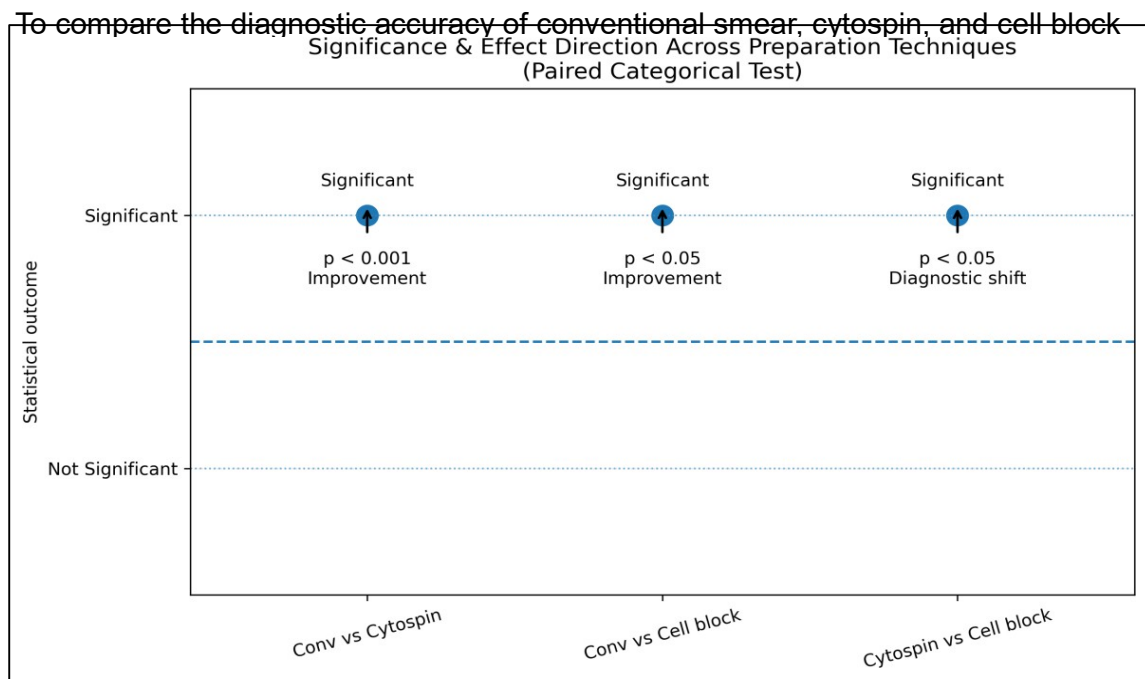
Pairwise comparisons revealed statistically significant differences among the techniques. Both cytospin and cell block demonstrated significantly improved cellularity compared with conventional smear ($p < 0.001$ and $p < 0.05$, respectively) as show in **table 3** and **figure 3**. Although both advanced techniques enhanced cellular yield, cell block provided superior diagnostic categorization.

Table 3: Pairedcomparison ofcellularityacrosstechniques

Comparison	StatisticalTest	p-value	Interpretation
Conventional smearvsCytospin	Stuart–Maxwell test	<0.001*	Cytospinshowsasignificant upward shift in cellularity grades compared to conventional smear
ConventionalsmearvsCellblock	Stuart–Maxwell test	<0.05*	Cell block demonstrates a significantimprovementin cellularity grading distributioncomparedto

			conventional smear
CytospinvsCell block	Stuart–Maxwell testPairedcomparison ofcellularity acrosstechniques	<0.05*	Cell block shows a significant shift toward diagnostically usefulcellular patterns

Figure 3: Significanceandeffectdirectionofpreparationtechniquecomparisons(N= 150)



DISCUSSION:

Effusion cytology is a rapid, minimally invasive, and cost-effective technique for evaluating serous cavity fluids and often provides the first evidence of underlying malignancy or disease recurrence [27-28]. However, accurate cytological diagnosis remains challenging because reactive mesothelial cells may closely mimic malignant cells, while poorly differentiated malignant cells can be scant or exhibit subtle morphological features, resulting in false-negative or indeterminate diagnoses [1].

Conventional smear cytology remains the cornerstone of effusion evaluation but is limited by low cellularity, poor cellular preservation, obscuring blood or proteinaceous material, and loss of architectural details, which may reduce diagnostic sensitivity [28]. In the present study, conventional smear demonstrated the lowest sensitivity and diagnostic accuracy, with the highest false-negative rate compared with cytospin and cell block techniques. In contrast, cytospin improved cellular concentration and diagnostic yield, while the cell block technique achieved the highest sensitivity, specificity, and overall diagnostic accuracy, highlighting its superiority in the evaluation of serous effusions.

The improved performance of the cell block technique can be attributed to better preservation of tissue architecture, enhanced visualization of cellular clusters, and its suitability for ancillary investigations, particularly immunocytochemistry [29-30]. These advantages contribute to increased detection of malignant cells, reduction of indeterminate diagnoses, and improved diagnostic confidence, especially in low-cellularity specimens.

Standardized reporting systems such as the International System for Reporting Serous Fluid Cytopathology (ISRSFC) further strengthen diagnostic interpretation by reducing reporting ambiguity and improving communication between pathologists and

clinicians [31]. Collectively, the present findings support the routine use of advanced preparation techniques, particularly cell block in combination with cytospin, to improve the sensitivity and overall diagnostic accuracy of effusion cytology.

The present study demonstrated that the **cell block technique achieved the highest malignant detection rate (38.7%)**, followed by **cytospin (35.3%)** and **conventional smear (28.7%)**, confirming the superior diagnostic performance of cell block in the evaluation of serous effusions. Cell block also reduced atypical diagnoses and improved definitive categorization, thereby enhancing overall diagnostic accuracy. These findings are consistent with **Mashru et al. (2023)**, who reported higher malignant detection by cell block (29%) compared with cytospin (17%), with a reduction in suspicious cases and improved sensitivity (100% vs. 81.82%) [29]. Similarly, **Mathew et al. (2024)** observed that cell block identified a greater proportion of malignant pleural effusions than conventional cytology (19% vs. 7%), supporting its superiority in malignant effusion diagnosis [30].

The improved diagnostic performance of the cell block technique can be attributed to enhanced cellular concentration, superior preservation of tissue architecture, better visualization of cellular clusters, and the availability of material for ancillary investigations such as immunohistochemistry. Although cytospin also improved malignant detection compared with conventional smear, **cell block consistently demonstrated the highest diagnostic yield**, supporting its routine use as an adjunct to conventional cytology for improving the sensitivity and diagnostic accuracy of effusion cytology [32].

The present study demonstrated that **cell block achieved the highest diagnostic cellularity (93.97%)**, closely followed by **cytospin (91.33%)**, whereas **conventional smear showed lower diagnostic cellularity (80.0%)**. Correspondingly, malignancy detection increased progressively from **28.7% with conventional smear to 35.3% with cytospin** and

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38.7% with cell block, indicating the superior diagnostic yield of advanced preparation techniques. Similar findings were reported by **Shivakumarswamy et al. (2012)**, who observed a 15% higher cellularity and improved malignant detection with the cell block technique compared with conventional smear, attributing this advantage to better preservation of cellular architecture and enhanced sensitivity.[33]

Our study also demonstrated that **cell block reduced atypical diagnoses while increasing definitive malignant categorization**, reflecting its ability to preserve tissue architecture and facilitate recognition of tumor-cell clusters. These findings are consistent with Mashru et al. [29]

The present study demonstrated that **higher cellularity and tumor-cell morphology were significantly associated with malignant diagnosis**, with **cytospin and particularly cell block showing greater diagnostic relevance than conventional smear**. Cell block not only improved cellular yield but also enhanced diagnostic categorization by reducing indeterminate cases and increasing definitive malignant diagnoses. These findings are in agreement with **Salih et al. (2025)**, who reported increasing risk of malignancy across TIS categories using the International System for Reporting Serous Fluid Cytopathology, supporting the value of standardized reporting in effusion cytology [26]. Similarly, **Amita et al. (2024)** demonstrated a progressive rise in the risk of malignancy from benign to malignant TIS categories, reinforcing the diagnostic utility of TIS classification [34]. Collectively, these findings support the combined use of **advanced preparation techniques, cytomorphological assessment, and standardized reporting systems** to improve the sensitivity and diagnostic accuracy of effusion cytology.

CONCLUSION:

The present study demonstrated that both **cytospin and cell block techniques significantly improved the diagnostic performance of effusion cytology compared with conventional smear**. Cell block provided the highest cellularity, malignant detection rate, sensitivity (100%), specificity (98.9%), negative predictive value (100%), and overall diagnostic accuracy (99.3%), while cytospin also showed superior sensitivity and diagnostic yield compared with conventional smear. Both advanced preparation techniques reduced indeterminate diagnoses and enhanced specimen adequacy, with cell block offering the greatest diagnostic clarity through improved preservation of cellular architecture and increased cellular concentration.

The findings support the routine incorporation of **cell block, preferably in combination with cytospin**, as an adjunct to conventional smear for the cytological evaluation of serous effusions. This combined approach improves sensitivity, minimizes false-negative diagnoses, enhances malignant cell detection, and facilitates ancillary investigations such as immunocytochemistry, thereby enabling more

accurate diagnosis and appropriate clinical management.

The present study demonstrated that both cytospin and cell block techniques significantly improved the diagnostic performance of effusion cytology compared with conventional smear. Among the three methods, the cell block technique showed the highest sensitivity (100%), specificity (98.9%), negative predictive value (100%), and overall diagnostic accuracy (99.3%), while eliminating false-negative results and reducing indeterminate diagnoses. Cytospin also showed superior sensitivity (91.4%) and diagnostic accuracy (95.3%) compared with conventional smear, confirming its value as an effective adjunctive preparation technique.

The progressive improvement in cellularity, malignant detection, and diagnostic categorization from conventional smear to cytospin and cell block highlights the importance of advanced specimen preparation in serous effusion cytology. Routine incorporation of cell block, preferably in combination with cytospin, can improve diagnostic sensitivity, reduce false-negative diagnoses, and facilitate ancillary investigations such as immunocytochemistry, thereby enhancing diagnostic confidence and patient management.

LIMITATIONS OF THE STUDY:

The study was conducted at a single tertiary-care center, which may limit the generalizability of the findings. The number of pericardial and hemorrhagic peritoneal effusions was small, limiting subgroup analysis for these less common specimen types. Histopathological confirmation was not available for all cases, and a composite reference standard was used where necessary.

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