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Modified alcohol-formalin cell block: a valuable adjunct for cytodiagnosis in suspected malignant pleural effusions

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Abstract

Introduction: Pleural fluid cytology is essential for diagnosing malignant pleural effusions and other pleural pathologies. Conventional smears (CS) are commonly used to study pleural effusions, but the modified cell block (MCB) method can enhance diagnostic accuracy. This study compares the diagnostic yield of CS alone versus combined with MCB in pleural fluid cytology. The aim of this study was to determine the comparative diagnostic value of CS techniques and a combined approach using the MCB method in the cytological analysis of pleural fluid.

Method: This cross-sectional study included samples of pleural fluid over 2 years from Nov 2016 to Oct 2018. Each fluid sample was divided into two parts for evaluation using (CS+MCB techniques. Ethical approval was obtained from the institution. Clinical parameters, along with microscopic findings such as cellularity and morphological preservation on CS and MCB, were compared.

Result: In 102 samples, the combined method (conventional smear+MCB) showed a significantly higher diagnostic yield for malignancy with an additional 9 cases (180%), compared to CS alone. MCB demonstrated superior cellularity and morphological preservation.

Conclusion: Combining CS with MCB in our study resulted in enhanced cellularity and morphological details, leading to more accurate diagnoses. This study highlights the role of MCB in significantly improving the diagnostic accuracy of pleural fluid cytology, as compared to CS alone, especially in malignancy.

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Introduction

Pleural effusions are commonly encountered in clinical practice worldwide, with diverse aetiologies ranging from benign inflammatory conditions to malignancies. Cytological examination of pleural fluid is a minimally invasive and essential diagnostic tool for differentiating benign from malignant effusions. [1,2] Globally, malignant pleural effusions are frequently associated with metastatic carcinomas and primary pleural tumours such as mesothelioma, with increasing incidence reported in many regions, including South Asia and India.[3,4] Conventional smear (CS) cytology remains widely used due to its simplicity and rapid turnaround, but it suffers from limitations such as low cellular yield and poor architectural preservation, which can reduce diagnostic accuracy. [5-7]

Several studies have demonstrated that the modified cell block (MCB) technique is relatively cheap and easy to perform, enhances cellularity, preserves tissue architecture, and allows for ancillary studies like immunohistochemistry and molecular testing, thereby improving diagnostic yield. [8-10] Despite this, the routine use of MCB in pleural fluid cytology remains limited in many clinical settings, including regional and local laboratories, due to factors such as cost, resource availability, and lack of awareness. [11,12] Moreover, differentiating reactive atypical mesothelial cells from malignant cells remains a diagnostic challenge with CS alone, contributing to diagnostic uncertainty.[13,14]

This study aims to compare the diagnostic efficacy of CS alone versus a combined approach using MCB in pleural fluid cytology. The objectives are: (1) to establish diagnoses using CS; (2) to establish diagnoses using MCB; and (3) to assess and compare the diagnostic accuracy of CS alone versus combined CS and MCB techniques.

Method

This study was done on 102 samples of pleural fluid received routinely in the Department of Pathology, Sri Siddhartha Medical College, Tumkur, India, over 2 years from Nov 2016 to Oct 2018. The consent and fluid collection were done by the respective clinical departments where the patients were admitted. This study received ethical clearance from the institutional review board.

All samples received in the department were included in the study. Fluids were immediately processed to avoid cellular loss due to delay. In cases where immediate processing of pleural fluid samples was not feasible, the specimens were preserved at 4°C with processing completed within 12 hours to minimise cellular degradation. Each pleural fluid sample was divided into two and subjected to examination by both CS and MCB methods. Physical examination of the fluid was done to note colour, appearance, presence of clot and mucin. The fluid was divided into two parts.

In CS, one half of the sample was centrifuged at 2500 rpm for 15 minutes, supernatant discarded, sediment smears prepared and stained with Papanicolau stain.

In MCB, the remaining half of the sample was fixed for 1 hour by adding an equal volume of a modified fixative composed of 10% alcohol-formalin and centrifuged at 2500 rpm for 15 minutes. Supernatant was discarded, and 3 ml of 10% alcohol – formalin was added to the cell button obtained and left overnight. The cell button was transferred onto a filter paper, processed like routine biopsy specimens, embedded in paraffin, sections cut and stained with routine H and E stain.

Microscopic examination of CS and MCB was done. Data collected was entered on an MS Excel spreadsheet (version 2010) and analysed and interpreted using IBM SPSS version 25. Cytological characteristics such as cellularity, morphological preservation, and the presence of malignant cells were assessed using both methods. Diagnostic accuracy was determined by correlating the cytological findings with clinical and histopathological follow-up data. The diagnostic yield of the two methods was

compared using chi-square tests, with a p-value of less than 0.05 indicating statistical significance.

Result

The study comprised 102 pleural fluid samples, the highest number of 41(40.2%) from the age group 41-50 years, Table 1. Males were 69(67.6%). Out of the 102 samples, 74(72.5%) were exudative and 28(27.5%) were transudative effusions, Figure 1.

Out of 102 samples of pleural fluid, MCB was obtained in only 77 cases due to low cellularity, low fluid volume or other factors obscuring diagnostic material. Amongst the 77 cases, cellularity obtained was more in MCB compared to CS, 53(68.8%) vs. 16(15.7%.) The 5(5%) cases of malignancy on CS were also positive for malignancy on MCB. In addition, nine more cases were detected to be malignant on MCB, resulting in a total of 14(18.2%) cases being reported as malignant on histopathology. These 14 cases were correlated and confirmed on biopsy of the malignant tumour along with serological, radiological and immuno-histochemistry correlation as and when applicable. The 2(2%) cases suspicious for malignancy on CS proved to be benign on MCB, Table 2.

We observed 3D cell clusters, papillary and glandular patterns in our evaluation of MCB slides. The MCB showed better cellular preservation and architectural preservation compared to CS in our study, thereby increasing the diagnostic yield of malignant cases. The majority of the malignant effusions (60%) were seen in males, and the most common underlying cause was lung carcinoma, Figure 2.

Table 1. Age wise distribution of pleural fluid samples, n=102

Age (Years)	n(%)
0-10	0
11-20	0
21-30	1(0.98)
31-40	5(4.90)
41-50	41(40.20)
51-60	33(32.35)
61-70	9(8.82)
71-80	8(7.84)
81-90	5(4.90)
>91	0

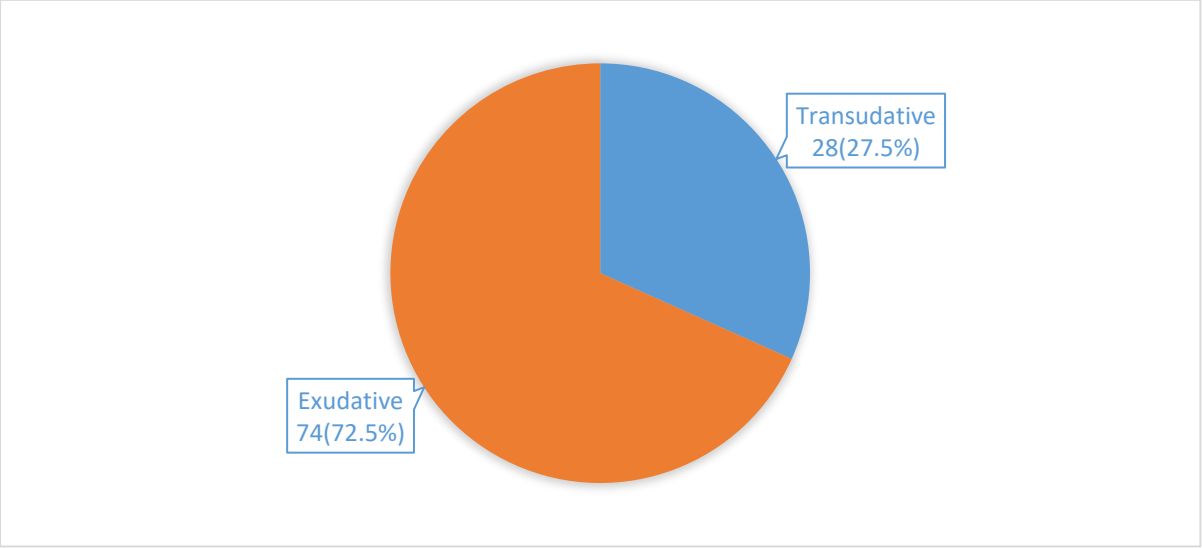

Figure 1. Distribution of type of pleural fluid, n=102

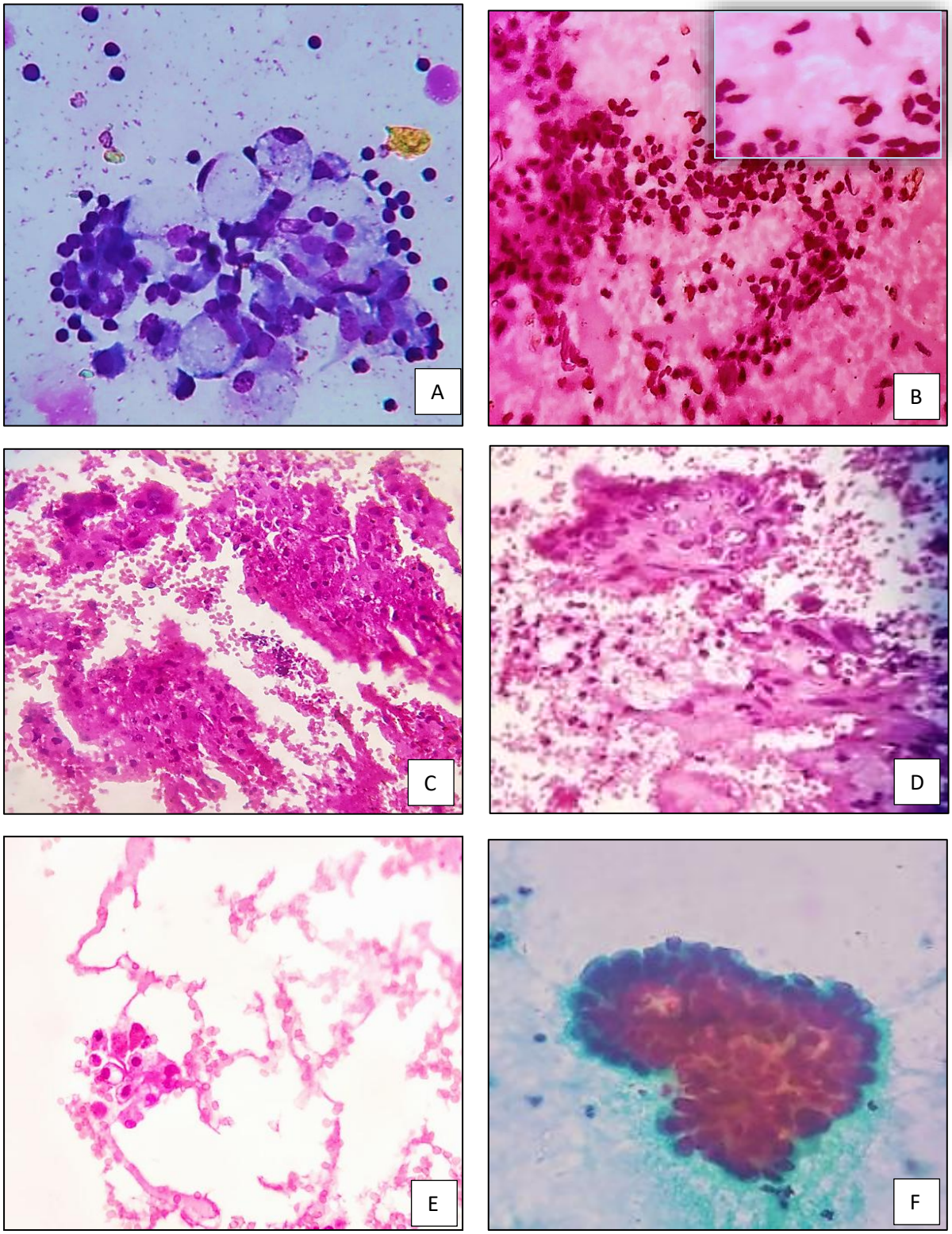

Figure 2. Microscopy of CS and MCB methods for evaluation of pleural fluid samples (A) Cluster of reactive mesothelial cells and lymphocytes in the CS of a pleural fluid. (B) Tuberculosis. MCB of the same fluid revealed granuloma with inset showing epithelioid cells. (C) Squamous cell carcinoma, lung. MCB from a pleural fluid showing malignant squamous cells in sheets. (D) Adenocarcinoma, lung. MCB from a pleural fluid showing malignant cells arranged in glandular pattern. (E) Malignant mesothelioma. CS showing clusters of atypical mesothelial cells. (F) MCB of the same fluid showing malignant mesothelial cells in papillary pattern.

Table 2. Comparison of cellularity and categorisation of nature of fluid on CS and MCB

Variables	Conventional smear, 102 n(%)	Modified cell blocks, 77 n(%)	$\chi 2$ test	p-value
Cellularity			52.34	<0.001
Mild	41(40.2)	12(15.6)		
Moderate	45(44.1)	12(15.6)		
Marked	16(15.7)	53(68.8)		
Nature of fluid			9.44	0.009
Benign	95(93.1)	63(81.8)		
Suspicious	2(2)	0		
Malignant	5(5)	14(18.2)		

Discussion

In the present study, cellularity, categorisation of the nature of pleural fluid and diagnostic yield, including malignancy, were significantly higher by MCB compared to CS, P<0.05.

Despite well-documented advantages of MCB, its routine use in clinical practice remains limited, with most clinicians relying solely on CS reports. Our findings emphasise the importance of integrating CS with MCB to enhance the accuracy of pleural fluid cytology. Our findings highlight that combining CS with MCB significantly improves diagnostic accuracy, particularly for malignancy, due to MCB's enhanced cellularity, better morphological preservation, and superior visualisation of malignant cells.[15] In this study, 10% alcohol-formalin was used as a fixative for cellblock preparation, which improved cellularity by minimising cell loss through protein cross-linking. Similar findings were reported in a previous study using the same fixative.[16] In our study, out of 102 samples, only 77 samples gave sufficient material for MCB. Limited cell block yield was primarily due to low cellularity in transudative or benign effusions, extremely dilute or haemorrhagic samples that reduced effective cell recovery, and smaller fluid volumes limiting the amount of cellular material obtained.

In our study, the majority of the pleural fluid samples were in the age group 40-50 years (40.2%), which is consistent with other studies.[17,18] In present study, the majority of the samples came from males (68%) compared to females, which is similar to previous studies.[17-20] We found that the majority of samples were exudative (72%), which was similar to one study (91%), but it was contrary to the study, which had more transudative fluids (61%).[18,21] The MCB method yielded a notable increase in cellularity, accompanied by enhanced morphological detail and preservation of architectural patterns, including three-dimensional clusters, cell balls, and papillary and acinar arrangements, when compared to CS, similar to other studies which, in addition to the aforementioned patterns also showed sheets, nests and cords. These observations are consistent with findings reported in prior studies.[21-23]

The information on architectural patterns is an essential clue to the diagnosis of malignancy as well as to identifying the primary site. Our study revealed that five cases initially identified as malignant effusions by CS were confirmed as such by the MCB method. Additionally, nine cases reported as negative for malignancy on CS were subsequently diagnosed as malignant on MCB analysis. All nine cases of malignancy on MCB were confirmed on corresponding histopathological examination along with clinical, serological and radiologic correlation. Two cases suspicious for malignancy on CS proved to be negative for malignancy on MCB. Our evaluation of MCB revealed better preservation of cellular morphology as well as architectural patterns such as 3D cell clusters, papillary and glandular patterns. Thus, in our study, the number of cases detected as positive for malignancy using a combined approach of CS and MCB technique

was increased by 9(180%). This highlights that MCB method demonstrably enhanced the diagnostic yield, particularly in the detection of malignancy, which is similar to the findings from other studies.[21-25] In the present study, among malignant effusions diagnosed majority were those from lung carcinoma, which is consistent with the study by Johnston (36%).[26]

The improved diagnostic utility of the combined method highlights the importance of using MCB as an adjunct to CS. The ability to take multiple deeper sections for extensive analysis, preservation of blocks for future retrieval and analysis, and perform immunohistochemistry and molecular analysis on MCB further enhances diagnostic accuracy, thereby influencing early diagnosis, patient treatment and prognosis.

Our findings align with existing evidence demonstrating MCB's significant advantage in detecting malignant cases.[20-25] Given its cost-effectiveness, ease of use, and superior diagnostic yield, we strongly advocate for the routine use of MCB in conjunction with conventional smear for all pleural fluid cases, to enhance diagnostic accuracy and patient care particularly in cases of malignancy where early diagnosis and management could severely impact prognosis of the patient. Our study comes with certain limitations, where out of 102 samples, only 77 samples had diagnostic material for MCB, and the comparative analysis was restricted to this subset.

Conclusion

This study confirms the advantage of combining conventional smears (CS) and a modified cell block (MCB) technique for accurate pleural fluid cytodiagnosis. Using a simple, cost-effective 10% alcohol-formalin fixative, the MCB method leads to improved cellularity, architectural preservation, and morphological clarity compared to CS. The MCB significantly enhances detection of malignant cases and must be routinely used as a valuable adjunct to CS in pleural fluid cytodiagnosis.

Author contribution

Concept and design: All; Literature search: SMT; Data collection: SMT; Data analysis: All; Draft manuscript: All; Final manuscript and accountability: All

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Conflict of interest

None

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Supplementary material

The data and supplementary material that support the findings of this study are available from the corresponding author upon reasonable request.

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