



CELL BLOCK OVER TRADITIONAL SMEARS IN PLEURAL FLUID CYTOLOGY- A 3 YEARS STUDY IN A TERTIARY CARE CENTRE.

Pathology

Dr. Vidhya. T. C. S Postgraduate, Department Of Pathology, Kmcrl Hubli

Dr. N S Kamakeri Professor, Department Of Pathology, Kmcrl Hubli

Dr. Amaya Bhaskaran Postgraduate, Department Of Pathology, Kmcrl Hubli

ABSTRACT

Introduction: Pleural fluid cytology helps in staging and prognosis of malignant lesions. Recognizing whether cells in serous effusions are malignant or reactive mesothelial cells could pose a diagnostic problem in conventional smears. **Materials And Methods:** A cross-sectional study was conducted out between June 2020 and June 2023. 260 pleural fluid samples were obtained for the purpose of research using both smear and cell-block techniques. Based on the microscopic features all the pleural fluids has been classified as Benign, Suspicious of malignancy or positive for malignancy in both smear and cell block. **Results:** In conventional smear(CS), 237 patients had a benign effusion, 6 had a suspected malignancy, and 17 had a positive result for malignancy. There were 235 cases of benign effusion, 2 cases of suspected malignancy, and 23 cases of malignant effusion in cell block. **Discussion:** In the present study diagnostic yield of malignant pleural effusion in cell block (8.85%) is more than that in conventional smears(6.54%). Hence the diagnostic yield of malignant effusion in cell block method is more when compared to smears. Immunocytochemistry with BerEp4 and calretinin helps if malignant mesothelioma is suspected. **Conclusion:** The study recommends using both smears and cell blocks in parallel to increase diagnostic accuracy. Also cell Block improves the diagnostic potential when paired with IHC in situations of suspected malignant mesothelioma.

KEYWORDS

Pleural fluid cytology, Conventional smears(CS), Cell block(CB)

INTRODUCTION

Thoracentesis is performed for the diagnosis of patients with pleural effusion. With a diagnosis rate of 60% (varying from 40% to 87%), fluid smears are very helpful in cases especially when malignant pleural effusion (MPE) is suspected.¹

It aids in prognosis and staging in addition to being crucial for the diagnosis of malignant lesions. The information regarding other non-infectious and infectious disorders including bacterial, viral fungal and parasite infections of serous membranes can also be examined.²

One typical diagnostic challenge in serous effusions is correctly classifying cells as reactive mesothelial cells or malignant cells. The primary causes of the decreased sensitivity of cytodiagnosis of effusions are alterations brought about by various laboratory processing techniques, cell loss, and overcrowding or overlap of cells.² To differentiate benign from malignant effusions, cytological diagnosis is combined with CB. Also, additional tissue sections can be obtained, immunohistochemistry (IHC), special stains and molecular tests can be performed if CB is available.³

The cell block approach yields better morphological details in addition to increased cellularity because of improved conservation of architectural details such as pattern of arrangement of cells, features of the nucleus and cytoplasm.⁴

Objectives

1. To study the microscopic features of pleural fluid with both conventional cytological smears and cell-block method.
2. To evaluate the role of cell-block in refining the final cytological diagnosis

MATERIALS AND METHODS

A cross-sectional study was carried out between June 2020 and June 2023 in the cytology section of the pathology department of Karnataka Medical College and Research Institute. The study included 260 pleural fluid samples that were collected employing both smear and cell-block techniques. Samples without cell block preparation were not included in the study.

A physical inspection of the appearance, color, and volume was done. Following centrifugation, two smears were prepared for every sample, and Wright's and H&E stains were applied. The residual sediment was formalin-fixed and utilized to prepare CB. Based on the microscopic features all the pleural fluids has been classified as **Benign, Suspicious of malignancy or positive for malignancy** in both smear and cell block.

Cell block preparation: sample was immediately fixed in a 1:1 mixture of 10% alcohol and formalin for an hour. The fixed sample was placed in a centrifuge tube and spun for fifteen minutes at 3000 rpm. After discarding the supernatant 3ml fresh alcohol formalin was added to sediment and left for 24 hours. Next day sediments on filter paper were scooped out, blotted, and sent for standard tissue processing. After cutting a histology slide, H&E staining was done. One case with smears suspicious of malignant mesothelioma was subjected to IHC staining. On CB sections, BerEp4 and Calretinin have been used to rule out malignant mesothelioma.

RESULTS

The age distribution was 6–87 years, with a mean of 54.03 ± 15.22 years at presentation. The age group of 51 to 60 was the most prevalent. The majority of those affected were men, including 149 men and 111 women. Architectural patterns that were frequently seen in the CB approach included glands, acini, papillae, three-dimensional cell clusters, and cell balls; in contrast, singly distributed cells were more frequently found with the CS method.

In conventional smear(CS), 237 patients had a benign effusion, 6 had a suspected malignancy, and 17 had a positive result for malignancy. There were 235 cases of benign effusion, 2 cases of suspected malignancy, and 23 cases of malignant effusion in cell block. Compared to men, women suffered malignant effusions with greater frequency (Figure 2).

Of the 237 benign effusions that were observed in the smear, 8 turned out to be positive for malignant cells in the cell block procedure, and 229 of them remained benign. Six smears were suspect for malignancy; of these, one proved to be malignant, three changed into benign pleural effusions with hyperplasia of mesothelial cells, and the remaining two remained suspect because of reduced cellularity. Furthermore, of the 17 malignant pleural effusions noted in the smear, 14 were shown to be malignant, while the remaining three showed no malignancy in the cell block report. Additionally 9 cases had been verified as positive for malignancy in cell block.

All 23 cases of malignant pleural effusion were adenocarcinoma deposits. Most common primary was lung adenocarcinoma (8 cases), 7 breast carcinomas, 4 gastrointestinal malignancies and 4 were of unknown origin.

One case that was highly suspicious for malignant mesothelioma in the smear turned out to have deposits of adenocarcinoma in the cell block. For this particular case IHC revealed strong positivity for BerEp4 and negative results for calretinin. Consequently, secondary

adenocarcinoma deposits were confirmed by IHC.(Shown in Figure 5) When correlated with the cell block report, the cytological smear had a specificity of 98.71%, sensitivity of 63.64%, positive predictive value of 82.35%, and negative predictive value of 96.62%.

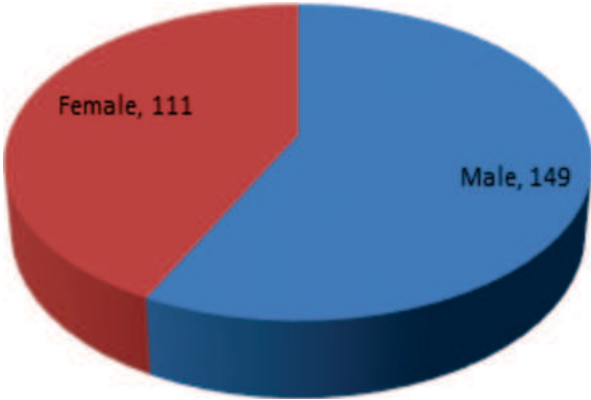


Figure 1:Gender distribution

Table 1: Distribution of age group in cases of pleural effusion

AGE GROUP	MALE	FEMALE
<11 years	1	-
11- 20 years	-	3
21-30 years	12	6
31-40 years	17	13
41-50 years	32	17
51-60years	36	31
61-70 years	33	24
71-80 years	15	14
>80 years	3	2

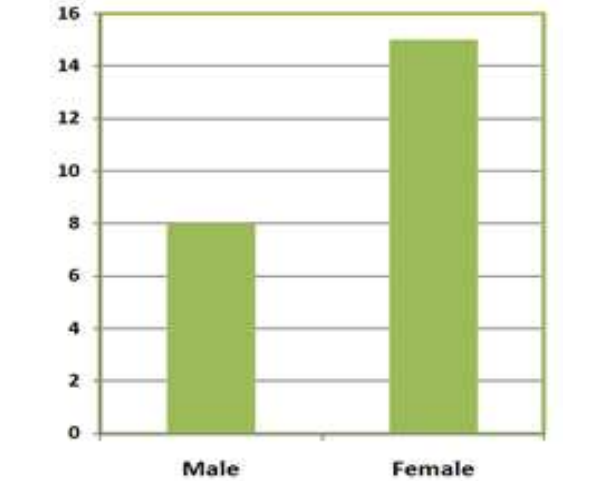


Figure 2: Gender distribution in Malignant pleural effusions

Table 2: comparison of smear vs cell block in pleural fluid cytology.

	CYTOLOGY SMEAR	PERCENT AGE (%)	CELL BLOCK	PERCENT AGE (%)
BENIGN	237	91.15	235	90.38
SUSPICIOUS OF MALIGNANCY	6	2.31	2	0.77
POSITIVE FOR MALIGNANCY	17	6.54	23	8.85

Table 3: Discrepancies in diagnosis in smear when compared to cell block

	Benign			Suspicious of malignancy			Positive for malignancy		
Cytological smear	237			6			17		
Cell block	B	S	M	B	S	M	B	S	M
	229	-	8	3	2	1	3	-	14

(B-Benign, S-Suspicious of malignancy, M- positive for malignancy)

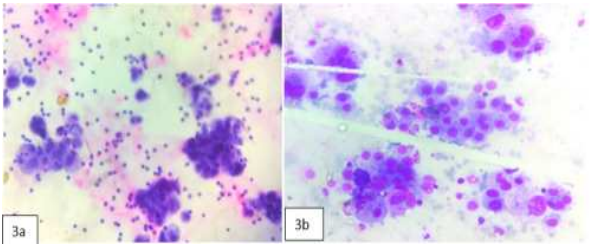


Figure 3: Smears showing malignant cells arranged in clusters and singles 3a.H&E staining(400x) 3b.Wright's stain(400x)Figure 3:

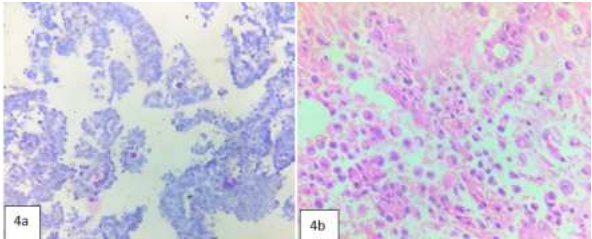


Figure 4a: Photomicrograph of CB showing atypical cells in papillae and psammoma bodies in (H&E stain, 200x)

Figure 4b: Photomicrograph of CB showing atypical cells in glandular pattern and singles in CB (H&E, 200x)

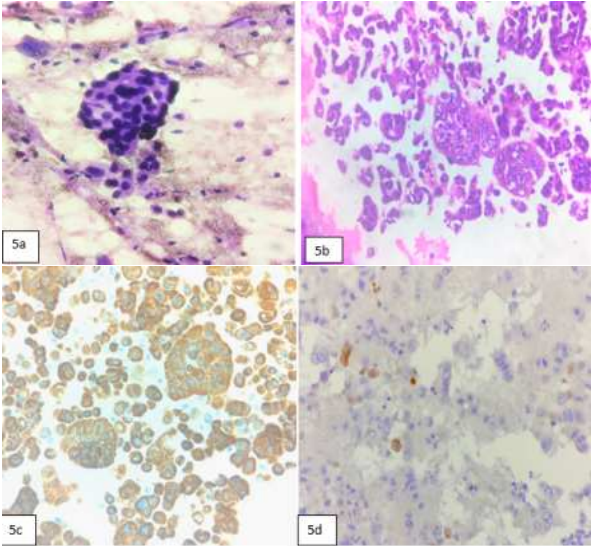


Fig-5: a) Smear-atypical mesothelial cell-suspicious of malignant mesothelioma,400x .b)Cell block showing atypical cells-adenocarcinoma deposits,400x .c)BerEp4:strong membranous and cytoplasmic positivity 400x; d) Calretinin: Suspected cells displaying negative stain 200x

DISCUSSION

Cytological analysis of serous effusions is crucial for prognostic, therapeutic, and diagnostic purposes. The current study's male to female ratio of 1.34:1 is consistent with Grandhi B. et al.'s⁴ , where they also found a male preponderance with a male to female ratio of 1.06:1. According to the current study, the age group of 51–60 years old has the highest sample percentage (25.76%), which is consistent with the findings of Shivakumaraswamy et al⁷. Compared to men, women experienced malignant effusions more frequently, which is consistent with a research by Shivakumaraswamy et al⁷.

Table 4: Comparision of CS and CB findings with other similar studies

Categories	Aswini Gude MD et al ²		Shivakumaraswamy et al, ⁹		Grandhi B et al., ⁴		PRESEN T STUDY	
Sample size	71		60		139		260	
	CS	CB	CS	CB	CS	CB	CS	CB
BENIGN	58	56	54	50	127	127	237	235
SUSPICIOUS OF MALIGNANCY	11	0	5	0	5	0	6	2

POSITIVE FOR MALIGNANT CELLS	2	15	1	10	7	12	17	26
------------------------------------	---	----	---	----	---	----	----	----

As compared to cytological smears (2.31%), the percentage of suspicious malignant effusion in cell blocks (0.77%) is lower, which is consistent with findings from earlier research by Aswini Gude MD et al², Grandhi B et al.,⁴ and Shivakumaraswamy et al.,⁹ (see Table 4).

In the present study diagnostic yield of malignant pleural effusion in cell block (8.85%) is more than that in cytological smears (6.54%) and in cytological smears 3 cases of benign were reported as malignant effusion. Hence the diagnostic yield of malignant effusion in cell block method is more when compared to smears which is also correlating with other similar studies conducted by Aswini Gude MD et al², Grandhi B et al.,⁴ and Shivakumaraswamy et al.,⁹

CB has certain advantages over CS in the diagnosis of malignant pleural effusion. Cell loss, artifacts, and poor background staining are less common in CB than they are in CS.^{10,11,12} The current study finds that while the sensitivity of cytological smears is only 63.64%, their specificity is 98.71%. Therefore, the study recommends using both smears and cell blocks in parallel to increase diagnostic accuracy.

CB specimens can be kept in storage, and as in our study, additional IHC staining can be performed if required. The study we conducted has certain limitations. We did not carry out more invasive diagnostic tests to establish a final diagnosis, particularly in known advanced malignant diseases.

CONCLUSION

It is advised to utilize cell blocks and smears in conjunction to increase the accuracy of diagnosis even more. If a cell block is available, IHCs can also be performed. When combined with IHC, Cell Block enhances the diagnostic potential in cases of suspected cancer, provided that sufficient tissue is preserved for CB. As a result, when smears are not definitive, CB combined with IHC may be a helpful adjuvant in assessing fluid cytology for a final cytodiagnosis.

REFERENCES

1. Assawasaksakul T, Boonsamsuk V, Incharoen P. A comparative study of conventional cytology and cell block method in the diagnosis of pleural effusion. *Journal of thoracic disease*. 2017 Sep;9(9):3161.
2. Aswini Gude MD. Cell Block as a Tool of Diagnostic Refinement in Cytological Examination of Pleural and Peritoneal Effusions.
3. Batool S, Sadaf S, Chughtai AS, Qasim A, Zafar A, Jamil A. Diagnostic Accuracy of Cell Block and Immunohistochemistry in Effusion Cytology. *Cureus*. 2023 Feb;15(2).
4. Grandhi B, Shanthi V, Rao NM, Reddy VC, Mohan K. The diagnostic utility of cell block as an adjunct to cytological smears. *International Journal of Medical Research & Health Sciences*. 2014;3(2):278-84.
5. Koyi H, Wilander E. Refined diagnosis of pleural effusions by immunocytochemistry of cell blocks. *Anticancer Research*. 2023 Feb 1;43(2):669-73.
6. Nair GG, Manjula AA. Comparative study of cell-blocks & routine cytological smears of pleural & peritoneal fluids in suspected cases of malignancy. *Indian J Pathol Oncol*. 2015 Apr;2(2):61-8.
7. Nautiyal N, Bhardwaj A, Acharya S, Kishore S, Kudesia S. Diagnostic utility of epithelial membrane antigen (EMA) and calretinin (CAL) in effusion cytology. *Journal of clinical and diagnostic research: JCDR*. 2017 May;11(5):EC36.
8. Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method versus cell blocks method in body fluid cytology: Study of 150 cases. *Ethiopian journal of health sciences*. 2014 Apr 14;24(2):125-30.
9. Shivakumaraswamy U, Arakeri SU, Karigowdar MH, Yelikar BR. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. *Journal of cytology*. 2012 Jan 1;29(1):11-5.
10. Jing X, Li QK, Bedrossian U, et al. Morphologic and immunocytochemical performance of effusion cell blocks prepared using 3 different methods. *Am J Clin Pathol* 2013;139:177-82.
11. Ugurluoglu C, Kurtipek E, Unlu Y, et al. Importance of the cell block technique in diagnosing patients with non-small cell carcinoma accompanied by pleural effusion. *Asian Pac J Cancer Prev* 2015;16:3057-60.
12. Bhanvadia VM, Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method versus cell block method in body fluid cytology: study of 150 cases. *Ethiop J Health Sci* 2014;24:125-31.