



Utility Of Cell Block Study As An Addendum In Fluid Cytology – A Prospective Observational Study In A Tertiary Care Hospital

¹Dharani V C, ²Dr. Manjunath H K, ³Dr. Akshatha Basavaraju, ⁴Dr. Bhargavi Mohan, ⁵Dr. Priyadarsini Sinha, ⁶Dr. Priyanka K M, ⁷Dr. Mythri B M, ⁸Dr. Gudrun Koul
^{1,4}Associate Professor, ²Professor, ^{3,7,8}Assistant Professor, ^{5,6}Post Graduate
BGS Global Institute Of Medical Sciences, Kengeri, Bangalore

***Corresponding Author:**
Dr. Manjunath H K

Professor, BGS Global Institute Of Medical Sciences, Kengeri, Bangalore

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Background: Conventional smear examination of body fluids, although a simple and easy technique to perform, has low sensitivity in detecting malignancy compared with cell block examination. Cell block examination as an adjunct to conventional smear has high specificity in the diagnosis of malignancy due to higher cell yield, better evaluation of architectural patterns, and better cell morphology and also source for Immunohistochemical study and FISH.

Materials And Methods: This was a prospective, observational, and analytical study conducted over a **one** year period in a tertiary care hospital. All fluids (peritoneal , pleural, cerebrospinal fluid, BAL, organ cystic fluid, urine and intraoperative collection) remaining after preparation of a conventional smear were processed for a cell block study.

Results:

A total of 260 body fluids were analysed in the present study. The most common age group was between 41 and 50 years, with a male predominance. The most commonly analysed fluid was pleural fluid, followed by peritoneal fluid. Most cases were negative for malignancy (94.5%) and 5.4% were positive for malignancy on conventional smear. On cell block examination, 93% of cases were negative and 6.9% were positive for malignancy. Cell block examination detected 3 additional cases of malignancy that were missed on conventional smears. 1 case of CSF and 2 cases of urine for cytology proved positive for malignancy.

Conclusion:

In the present study, **we observed that** cell block as an adjunct to conventional smear is more beneficial than the conventional smear alone in definitive diagnosis of malignancy. The additional information provided by cell block as an adjunct will help make important clinical decision in evaluation of the patients. Therefore, cell block examination must be used in all cases of body fluids for high diagnostic accuracy and source for IHC , FISH and molecular biology.

Keywords: cell block, body fluids, positive for malignancy, negative for malignancy

Introduction

The serous cavities are generally referred to as body cavities. Under normal conditions, these cavities are collapsed sacs enclosed by the heart, lungs, and intestines. Each cavity is completely enclosed (with the exception of the peritoneal cavity at the points

where it receives the fimbriated ends of the fallopian tubes) and each contains a small amount of fluid. The outer layer of each serous cavity is the parietal layer; the layer in direct contact with the sheathed organ is the visceral layer. Apart from a thin film of fluid,

these layers are in contact with each other, so that under normal conditions each cavity is only a potential cavity. When a cavity contains excess fluid, it becomes an actual cavity. Each cavity is lined by a monolayer of mesothelial cells, beneath which is a layer of connective tissue traversed by blood vessels, lymphatics, and nerves. The close proximity of blood and lymphatic vessels to the mesothelium may be partly responsible for the rapid spread of neoplastic cells in a serous cavity. The accumulation of fluid in a serous cavity in excess of the normal small amount is called effusion. The effusion may be a transudate or an exudate. In exudates, the cellular content is higher, probably due to many inflammatory cells in inflammatory conditions and numerous neoplastic cells in neoplasm. Serous effusions caused by a neoplasm may sometimes be a transudate as well, resulting from lack of reabsorption of serous fluid due to mechanical interference by the neoplasm.¹ Cytologic examination of the serous effusion is of paramount importance because the presence of cancer cells indicates not only that the patient has advanced stage cancer, but also that the cancer is almost always incurable. In addition to detecting malignancy, cytologic examination of various fluids such as pleural, peritoneal, pericardial, cerebrospinal fluid, and cystic fluid also provides information about inflammatory conditions of the serous membranes, parasitic infestations, and infections with bacteria, fungi, and viruses.² Cytologic examination of various body fluids can be performed by conventional stained smears, liquid-based preparations, and cell block techniques. Preparation of conventional stained smears is extremely simple, rapid, and can be examined immediately and is the cytologic equivalent of frozen sections in histopathology.²

The use of the cell block in serous effusions is becoming standard routine practice in many laboratories. The cell block is useful in some specimens that are not anticoagulated and contain a fibrin clot that may be voluminous, and such a clot may contain virtually all of the neoplastic cells in the specimen. Consequently, smears prepared in the remaining fluid after removal of the clot may be free of neoplastic and other cells. In such a situation, sections of the spontaneously formed clot often show numerous obvious neoplastic cells. Cell block examinations may also reveal certain histologic

aspects of a neoplasm, such as papillary, acinar, or ductal formations and also psammoma bodies, which are difficult or impossible to detect in a conventional smear preparation.

The present study was conducted to evaluate the importance of cell block examination as an adjunct to conventional smear examination in the detection of cancer cells in liquid cytology. The aim of this study was to compare the role of cell block technique versus conventional smear method in cytological diagnosis of serous fluids.

Materials And Methods

Study design: Descriptive and prospective study

Study period: The present study was conducted from January 2021- December 2022

Study settings: The present study was conducted in cytology section of department of pathology at BGS Global Institute of medical Sciences from January 2021-december 2022. All the body fluid specimens received were processed first for conventional smear cytology and then the remaining fluid was processed for cell block study.

Conventional Smear technique: For conventional smear 5 ml of fluid was centrifuged at 1500 rpm for 15 minutes and a minimum of three thin smears were prepared from the sediment. Two smears were immediately fixed with 95% alcohol and stained with Haematoxylin-Eosin (H&E), PAP stain and other was stained with Giemsa stain.

Cell block study : The fluid samples were centrifuged at 3000 rpm for 10 min using a Remi routine centrifuge to obtain sediment; the sediment was allowed to stand undisturbed for a time period ranging from 2 h to overnight. The supernatant was decanted and the tube was allowed to drain on a filter paper. The residual sediment or fibrin clot was then dislodged using a spatula and wrapped in filter paper, placed in a cassette, and fixed in 10% formalin. This was followed by paraffin embedding and blocks preparation. Then, 4 to 6 μm thick sections were then cut and mounted on albuminized glass slides.

Interpretation of conventional smear versus cellblock: In conjunction with clinical data the smears were categorized as benign, suspicious and malignant lesions based on morphological criteria such as cellularity, arrangement (acini, papillae and cell

balls), cytoplasmic and nuclear details . Comparative evaluation of conventional smear versus cellblock preparations was done and cytomorphological characters were studied to identify the malignancy. Statistical analysis was done using Medcalc statistical software to calculate the sensitivity and specificity of the two methods in picking up malignant cells on body fluid samples.

Ethical clearance: Ethical clearance was taken from institutional ethics committee

Results

In the present study a total of 260 body fluids were studied. Majority of the patients were males (59.3%) followed by females(40.7%).

Graph 1-Sex Distribution

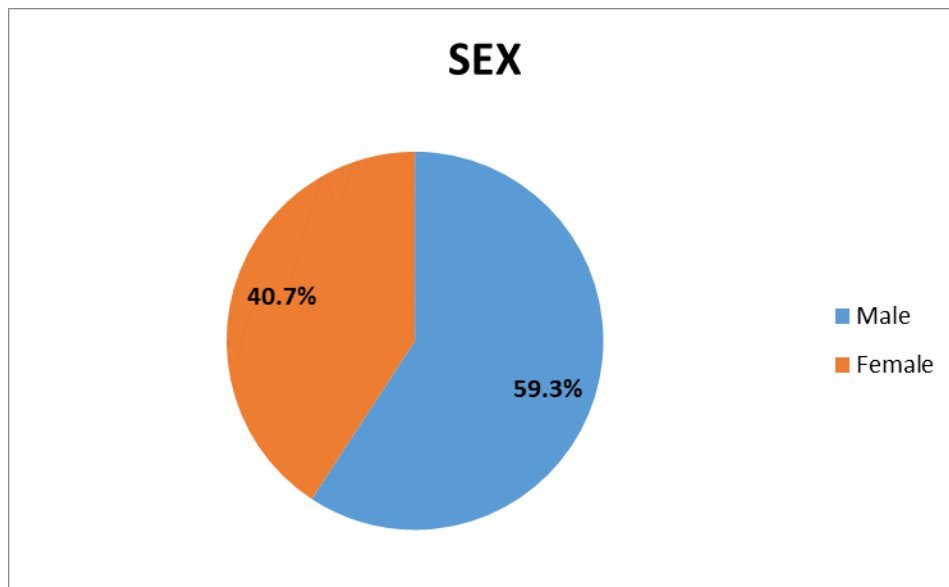
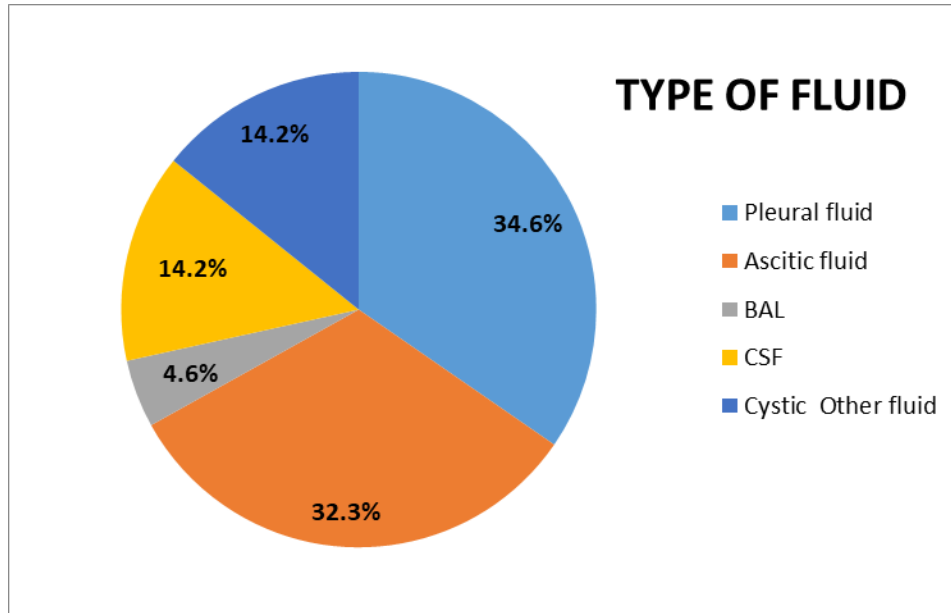


Table 1-Age Distribution

AGE	NUMBER OF CASES	PERCENTAGE OF CASES
0 - 10 yrs	27	10.30%
11 - 20 yrs	10	3.84%
21 - 30 yrs	22	8.46%
31-40 yrs	43	16.50%
41 - 50 yrs	48	18.40%
51 - 60 yrs	40	15.30%
61 - 70 yrs	45	17.30%
71 - 80 yrs	19	7.30%
81 - 90 yrs	6	2.30%
Total cases	260	100%

The most common age was in 5th decade and the least common was in 8th decade

Graph 2: Type of fluids



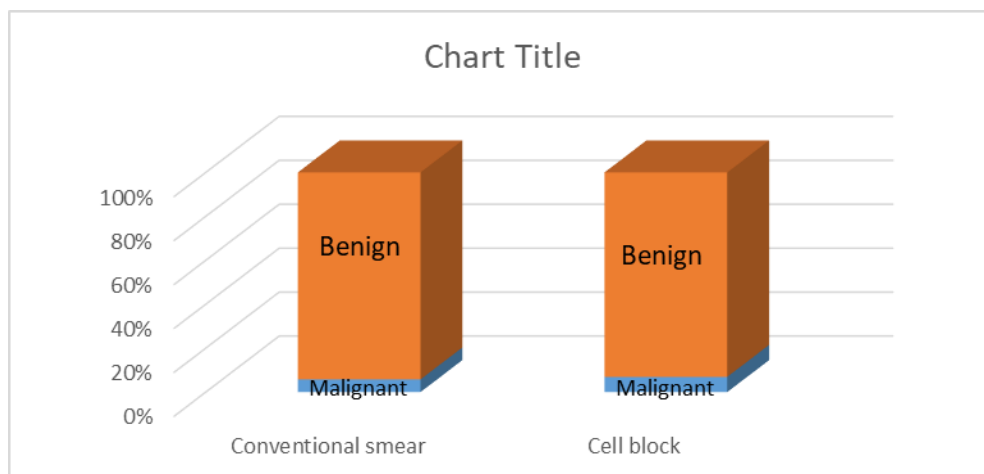
In the present study the most common fluid received was pleural fluid (34.6%) followed by ascitic fluid (32.3%). CSF fluids were also received in good number accounting for 14.2%

Table 2: Cytological Diagnosis

FLUID	NUMBER OF CASES	PERCENTAGE OF CASES
Positive for malignancy	12	4.6
Suspicious of malignancy	3	1.1
Negative for malignancy	245	94.3
Total cases	260	100%

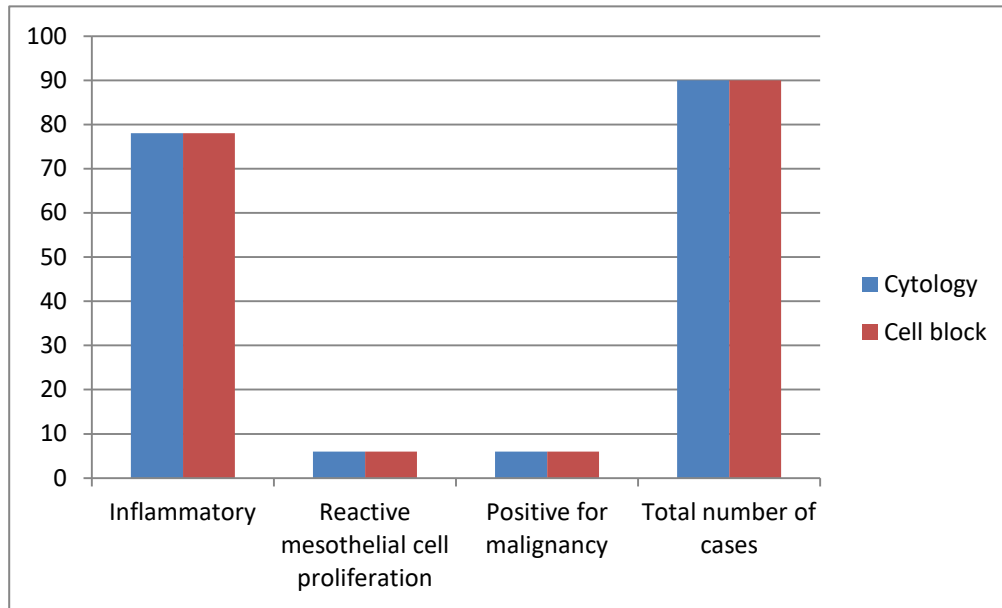
In the present study out of 260 cases of various body fluids majority were negative for malignancy accounting for 94.5% and the rest were positive for malignancy with 5.4% cases.

Graph 3: Distribution Of Benign And Malignant Fluids In Conventional Smear And Cell Block Study



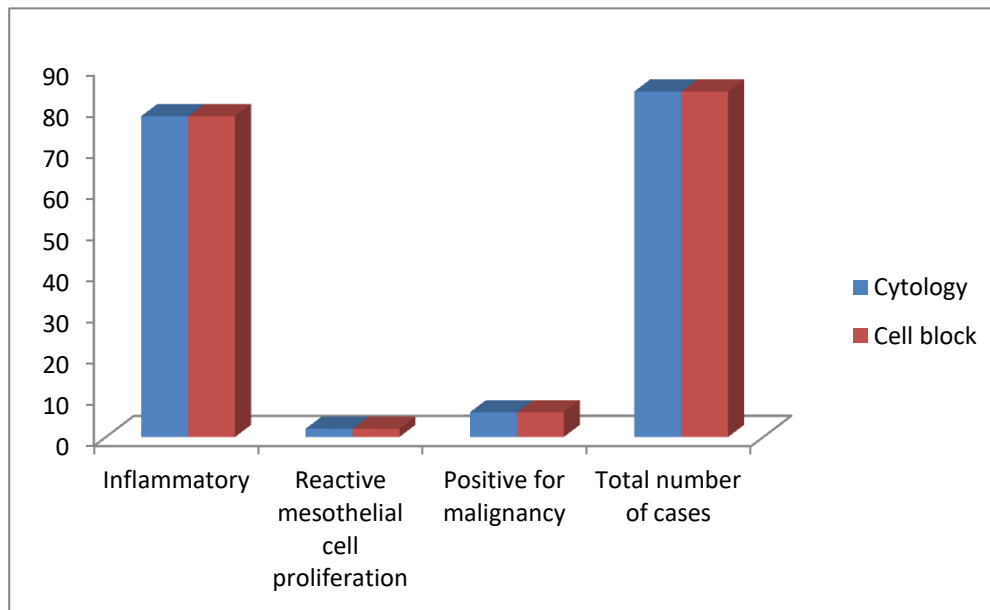
In this study conventional smears were able to detect 15 cases of malignancy while cell block study helped to detect 3 more cases of malignancy missed on conventional smear

Graph 4: Pleural Fluid Lesions



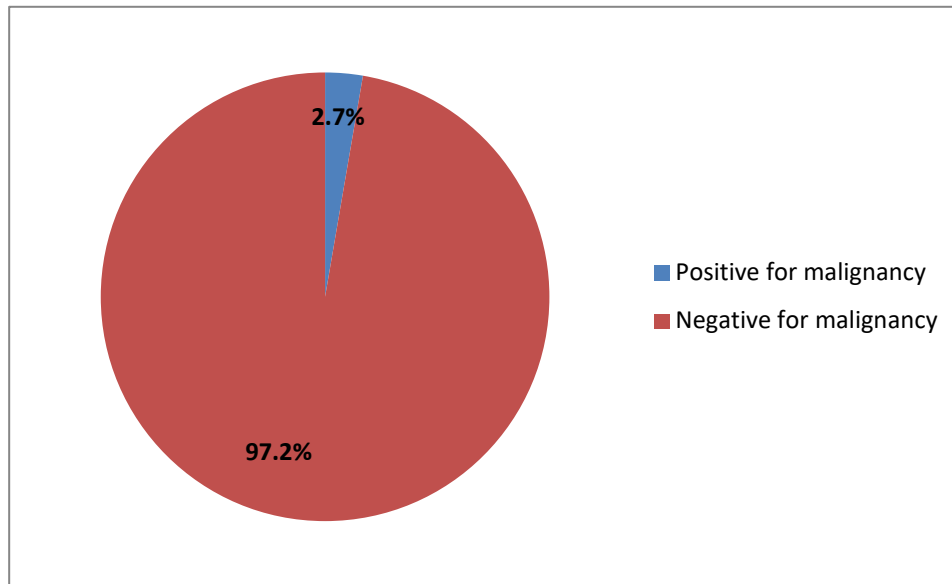
In our study out of 90 pleural fluid cases both conventional cytology and cell block study showed 78 cases of inflammatory lesions and 6 cases of reactive mesothelial hyperplasia and 6 cases were positive for malignant cells.

Graph 5: Ascitic Fluid Lesions



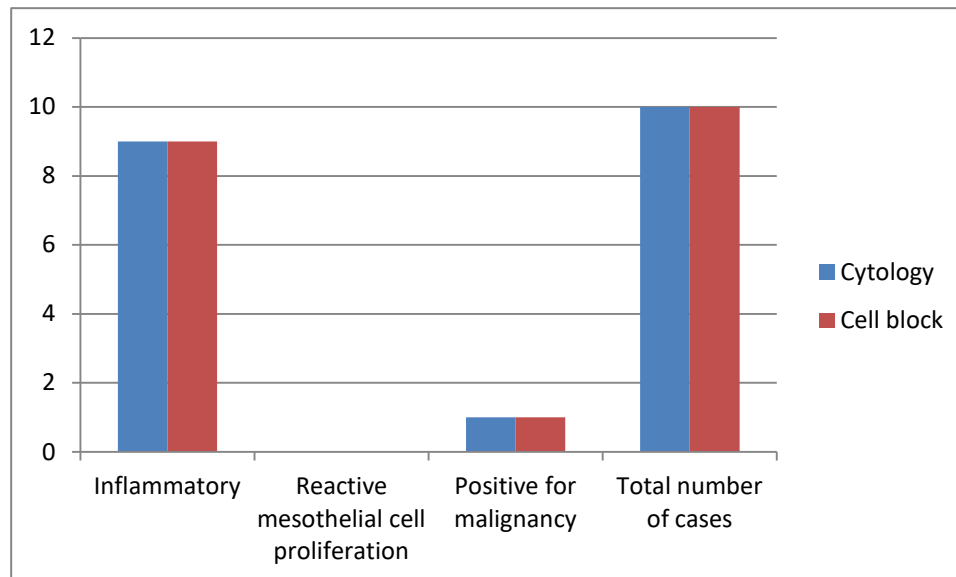
In our study out of 84 ascitic fluid cases both conventional cytology and cell block study showed 78 cases of inflammatory lesions and 2 cases of reactive mesothelial hyperplasia and 6 cases were positive for malignant cells.

Graph 6: Cerebrospinal fluid



Out of 37 cases of CSF analysis one case was positive for malignancy proved both on conventional and cell block study.

Graph 7: Broncho alveolar lavage



In our study out of 12 bronchoalveolar fluid cases both conventional cytology and cell block study showed 9 cases of inflammatory lesions and 2 cases of reactive mesothelial hyperplasia and 1 case was positive for malignant cells.

Other fluids

37 cases of ovarian cyst fluid were studied and all were negative for malignancies. 3 cases of urine samples for cytology were studied of which 2 cases were positive for atypical epithelial cells.

Discussion

Cytological examination of serous effusions has become increasingly common in clinical medicine, so that a positive diagnosis is often considered a definitive test and obviates the need for exploratory

surgery. It is not only important for the diagnosis of malignant lesions, but also helps in staging and prognosis.

The major limitation of this method is that it is poor at distinguishing reactive mesothelial cells from neoplastic mesothelial and epithelial cells. These limitations are due to artifacts associated with the preparation and staining technique, as well as the lack of three-dimensionality, which leads to cell compaction and overlap with poor resolution and difficulty in interpretation. The technique CB can overcome many of these limitations. Cytology of body fluids is more sensitive than blind biopsy for detecting serum malignancy because the fluid provides a more representative sample. The sensitivity for the diagnosis of serosa malignancy ranges from 58 to 71%. The specificity of cytologic diagnosis is very high - false positive diagnosis occur in less than 1%. False positive and false suspicious diagnoses are caused by atypia of repair and regenerative mesothelial cells in pulmonary infarction, tuberculosis, chemotherapy, acute pancreatitis, ovarian fibromas and cirrhosis.¹

Cell block examination increases the percentage of positive results and helps to detect entities such as granulation tissue reactions, cholesterol clefts, squamous cells, skeletal muscle, cartilage, colonies of microorganisms, skin appendages in subcutaneous tissue, fragments of hyperplastic mesothelium with collagenous stroma that are not visible in conventional smear.

Differentiation between reactive mesothelial cells and malignant cells in a conventional routine examination is a common problem in cytology.

In the present study, a total of 260 cases of body fluids were examined over a 2-year period. All fluids sent to the cytology laboratory were examined using the conventional smear and cell block technique.

Age And Sex:

The most common age group in our study was the fifth decade and the least common was the eighth decade. Similar results were observed in a study by Ashvini Pandit Mane et al,³ Bhavana et al⁴ and Shubhada et al⁵. In our study, males predominated at 59.3%, similar to the findings of Shivakumar Swamy et al⁶. and Bhavana et al⁴. A study by Bista et al⁷ observed female preponderance.

Distribution Of Fluids:

In the distribution of samples in our study, pleural effusions predominated followed by peritoneal effusions. In a study by Manoj Gopal, Madhakrishna et al.⁸ peritoneal effusions predominated followed by pleural effusions.

Distribution Of Benign And Malignant Fluids:

Of the 260 cases that were studied, 15 (5.7%) were positive for malignancy on both cytology and cell block, of which 3 cases were suspicious for malignancy on conventional smear and confirmed on cell block, while the others were negative for malignancy. Similar findings were observed by Shubhada et al. and Shivakumar Swamy et al. with the majority of cases being negative for malignancy. two cases of ascitic fluid and one case of pleural fluid were found to have florid reactive hyperplasia in the conventional smear but had features of malignancy in the cell block examination.

Benefit Of Cell Block Over Conventional Cytological Smears

In the present study, we aimed to highlight the use of cell block as an adjunct to conventional smears to make a definitive diagnosis when diagnostic problems are encountered with conventional smears.

In 3 cases in which the conventional smears were suspicious for malignancy, the cell block proved to be malignant. In addition, 3 cases designated as florid reactive mesothelial hyperplasia in the conventional smear had atypical cells in the cell block examination. Thus, cell block examination detected 3 additional cases of malignancy that had been missed on the conventional smear. This was possible with cell block examination because of cellular enrichment and details of architectural arrangement such as papillary, acinar, and cell balls, which are difficult to detect in conventional smears. The sensitivity of the cell block in detecting malignant cells was 100% in our study, and the cell block was able to detect 1.2% more malignant cells compared with conventional smears. This percentage is comparable to the studies by Manoj Gopal, Madhakrishna et al, and Dekkar et al. This emphasizes the benefit of cell block study over conventional smear to improve the diagnostic accuracy.^{8,9,10} In our study, 1 CSF tested positive for malignancy in both conventional and cell block examination. The

patient was a 32-year-old man who was diagnosed with carcinoma lung. 2 cases of urine for cytology were suspicious for atypical cells on conventional smear and were positive for atypical urothelial cells on cell block, patient was a 60yr old male diagnosed with urothelial carcinoma.

Conclusion:

Conventional smear in conjunction with cell block examination is highly beneficial in the detection of malignant cells because cell block examination has many advantages, such as higher cell yield, small area for cell spread, and better architectural preservation. Therefore, cell block preparation should be used as an adjunct to conventional cytology in body fluids to improve the diagnostic specificity of malignant effusions. Cell block study are considered to be poor man's fluid specimen for histopathological examination. In positive cases it not only helps in confirming the presence of malignant cells but also helps in morphological typing, and advanced research study .

References

1. Cibas ES, Ducatman BS. Pleural, Pericardia, and Peritoneal Fluids. In: Cytology: Diagnostic principles and clinical correlates. 5th ed. Philadelphia, PA: Elsevier; 2021. p. 141-70.
2. Bibbo M, Wilbur DC. Pleural, Peritoneal, and Pericardial Effusions. In: Comprehensive cytopathology. 4th ed. London: Elsevier/Saunders; 2015. p. 403-48.
3. Ashvini Pandit Mane , Hemant R. Kokandakar , A.R. Joshi. Study of cell block technique in the cytodiagnosis of serous fluids at a tertiary hospital. European Journal of Molecular & Clinical Medicine ISSN 2515-8260 Volume 09, Issue 03, 2022.
4. Grandhi B, Shanthi V, Rao NM, Reddy VC, Mohan KVM. The diagnostic utility of cell block as an adjunct to cytological smears. Int J Med Res Heal Sci. 2014;3(2):278
5. Shubhada B, et al. Evaluation of Cell Block Technique in the Cytodiagnosis of Body Fluids. Int J Sci Res. 2013;14(7): 2319-7064
6. Shivakumarswamy U, Arakeri S, Karigowdar M, Yelikar B. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. J Cytol. 2012;29(1):11-5.
7. Bista P. Comparison of the diagnostic accuracy of cell block with cytology smear in serous effusions. Journal of Pathology of Nepal. 2013; 3: 482- 86.
8. Manoj Gopal Madakshira et al., Utility of Cell Block Preparation in Detection of Malignant Cells in Serous Effusions. National Journal of Laboratory Medicine. 2017 Apr, Vol-6(2): PO26-PO31
9. Joshi DA, Mahajan DN, Karmarkar DPJ, Mahore DSD. Diagnostic utility of various techniques used in body fluid cytology. IOSR J Dent Med Sci. 2014;13(1):13-8.
10. M N G, Puri K, M K S, T R, M B. Diagnostic Utility of Cell Block Method in Pleural Fluid Cytology. J Evid Based Med Healthc. 2014;1(9):1240-5.
11. Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: A review of preparative methods, utility in diagnosis and role in ancillary studies. Cytopathology. 2014;25(6):356-71.

FIGURE 1: a. Cytological smear showing reactive mesothelial hyperplasia in clusters (Pap stain 4X); **b.** Reactive mesothelial cells with binucleate forms (Pap stain 10X); **c.** Mesothelial cell cluster (Pap stain 4X); **d.** Cell block study showing mesothelial cells in sheets and clusters (H&E stain 4X).

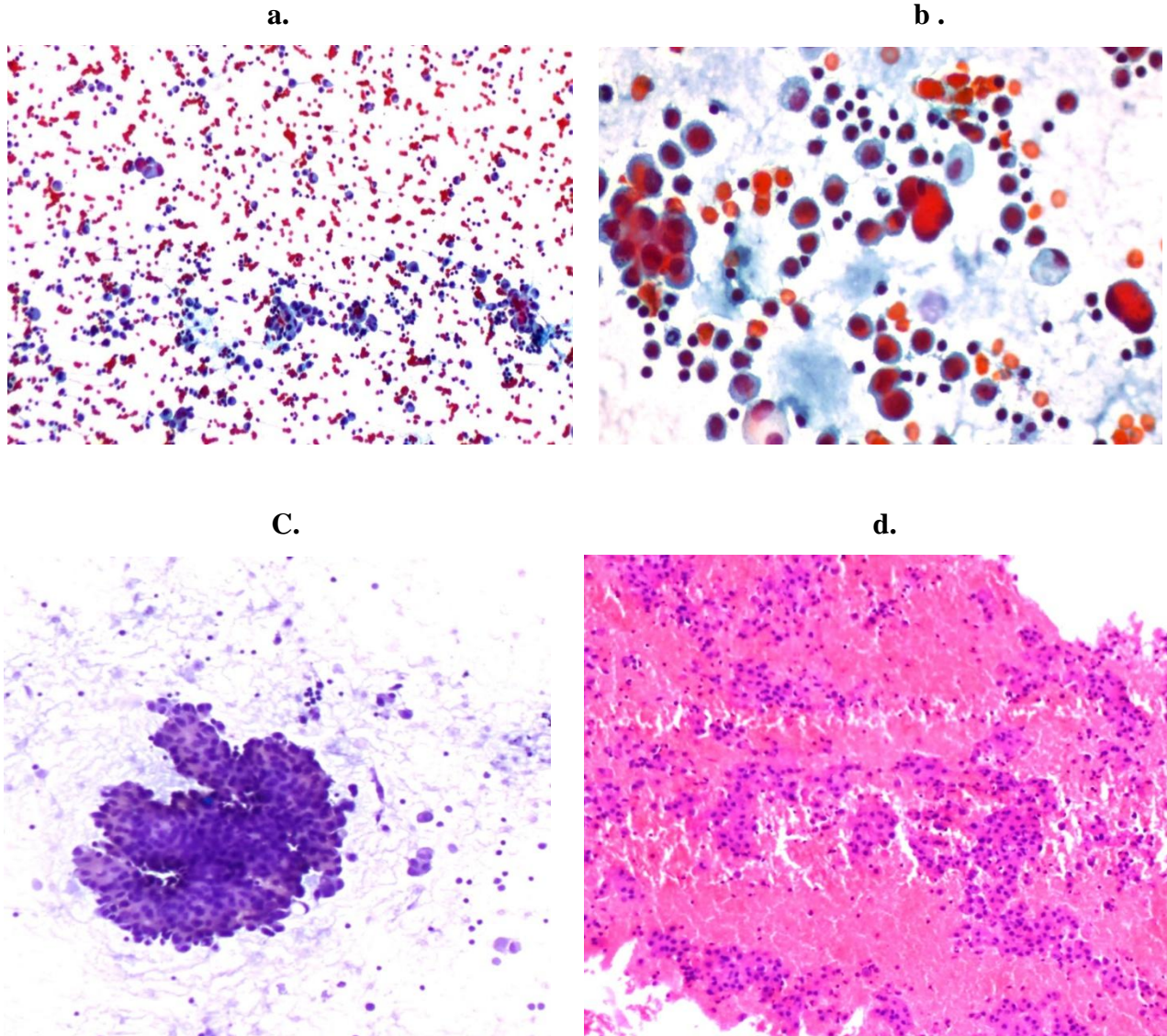


Figure 2: a & b : Cytological smear showing metastatic adenocarcinoma deposits (Pap stain 4x)(H&E stain 4x); c. High power view of cluster of tumor cells (H&E stain 40x); d. Cell block study showing acinar pattern of metastatic adenocarcinoma deposits (H&E stain 10x)

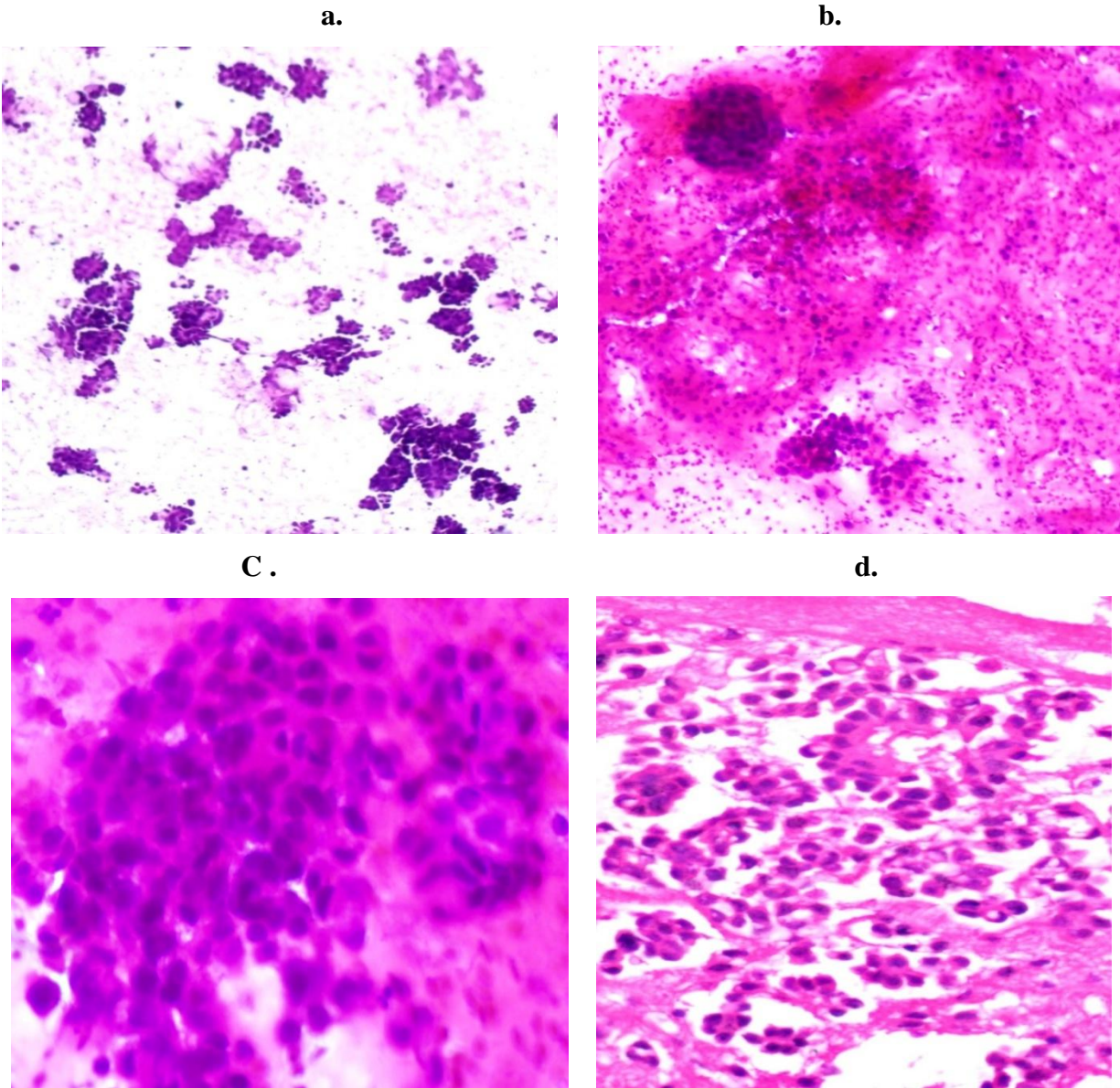


Figure 3 : a & b: Urine cytological smears showing microfilaria (larval stage) parasite (Giemsa 40X) c & d : Cell block sections showing sheathed microfilarial parasite with nuclei till the terminal end. (H&E 40X)

