



TO STUDY THE DIAGNOSTIC UTILITY OF CONVENTIONAL SMEAR, CELL BLOCK AND CEA IMMUNOHISTOCHEMISTRY IN PLEURAL AND ASCITIC EFFUSIONS

Pathology

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ABSTRACT

Background—Cytological diagnosis of serous effusions is simple and non invasive technique and one of the first line of investigations. It is useful especially for diagnosing malignant cases. Besides conventional smear alone, cell block in conjunction with immunohistochemistry can help in providing more accurate diagnosis. **Materials and methods**—A total of 260 cases of ascitic and pleural effusions were studied during the period from April 2021 to April 2022. The fluids were subjected for routine conventional smear and cell block, followed by immunostaining with CEA in suspicious malignant cases, the results were calculated and tabled. **Result**—A total of 81.54% were benign, 6.92% were malignant, 6.92% were suspicious of malignancy on conventional smear. A total 78.8% were benign, 13.1% were malignant, 0.8% were suspicious of malignancy on cell block. The additional yield of malignancy was 6.2%. Cell block sensitivity 57.78 %, specificity 95.35 %, PPV 72.22 %, NPV 91.52 %. IHC CEA was done on 36 cases, 34 of them were malignant and 2 were suspicious of malignancy on cell block. IHC CEA sensitivity 92.31 %, specificity 100 %, PPV 100 %, NPV 100%. **Conclusion**—For the final cytodiagnosis of serous effusions, combination of conventional smear and cell block in addition with IHC helped in increasing the diagnostic yield and more accurate and definite diagnosis.

KEYWORDS

Cell block, Serous effusions, Carcinoembryonic antigen (CEA), Conventional smear

INTRODUCTION:

Serous effusions are accumulations of excess fluid in the serous cavities.¹ Cytological examination of fluids is one of the first line of investigations which is commonly used. It is a quick and inexpensive method and not only does it provides information on various inflammatory and non-inflammatory lesions, in addition it aids in the diagnosis, staging, prognosis, and management of patients with cancer.² The fact that the cell population present in serous effusions is indicative of a considerably broader surface area than obtained by the needle biopsy may be the cause of the diagnostic success of cytological analyses of the fluid.³

However, there are certain limitations with conventional cytological examinations, especially in regards to diagnosing malignant effusions. The accurate identification of cells as either malignant or benign reactive mesothelial cells is a common diagnostic problem.⁴

It has been demonstrated in numerous investigations that cytological analysis of fluids using smears, no matter how thoroughly done, leaves behind a significant residue that is not further examined but may contain important diagnostic information. Sometimes the presence of inflammatory cells and paucity of representative cells in the sample, makes the definitive diagnosis more difficult by light microscopy using routine conventional staining methods.^{5,15} By treating this remaining material as a cell block, embedding it in paraffin, and examining it alongside the standard smears, it may be analysed quickly and easily.^{6,7}

Cell block technique was first introduced by Bahrenburg in 1896. In this method, fluid sediment is fixed, processed and embedded in paraffin. This technique provides a more definitive cytopathological diagnosis.⁸

Cell block provides high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, thereby increasing the sensitivity of cytodiagnosis when compared with conventional smear. The main advantages of the CB technique are preservation of tissue architecture and obtaining multiple sections for special stains and immunohistochemistry.² It bridges the gap between cytology and histopathology.^{7,9,10} Therefore, the cell block technique can be considered as a useful adjuvant in fluid cytology for a final cytodiagnosis, along with the routine conventional smear method.

CB immunocytochemistry is accepted as an indispensable adjunct to cellular morphology in cytology.^{11,12} CEA has been considered as one of the most reliable markers to distinguish adenocarcinoma from malignant mesothelioma, with most of the malignant 4 mesothelioma negative for CEA.¹³ Wang and coworkers were the first to report that mesotheliomas fail to express CEA as determined by the immunoperoxidase method.¹⁴ The percentage of carcinomas positive for CEA is variable, depending on the primary site and the subtype of carcinoma.^{15,16} Nonspecific reactivity for CEA in macrophages and other inflammatory cells was reported previously.^{17,18}

MATERIALS AND METHODS:

The present study was conducted in the Department of Pathology in Govt Medical College, Amritsar over a period of 1 year from April 2021 to April 2022 and included a total 260 samples (ascitic and pleural effusions). All ascitic and pleural fluids were included irrespective of volume and all grossly haemorrhagic fluids were excluded.

The clinical data of patients viz..date of admission, age, sex, relevant clinical and radiological findings were noted from the patient records. Routine examination of all samples was done and then processed further by the conventional technique and also by CB technique before microscopic examination.

All ascitic and pleural fluids were divided into two equal parts. One part was for conventional smear preparation (CS), and the other part was used for CB preparation.

For conventional smear, the fluids were centrifuged at 2500 rpm for 10 minutes in plastic test tubes and supernatant decanted. Minimum of two thin smears were prepared from the sediment and H&E staining and Giemsa staining were done on centrifuged smears.

The other portion of fluid specimen were processed by Fixed Sediment Method of Cellblock according to Nathan et al. The fluid specimens were fixed in ethanol formalin fixative (9 parts absolute alcohol & 1 part 10% formalin) in the ratio of 1:1 for one hour. After fixation the specimen were centrifuged at 2500 rpm for 10-15mins. Supernatant was poured off and sediment drained by inverting the tube on filter paper. Supernatant was poured off and sediment drained by inverting the tube on filter paper. Multiple thin sections of 4-5 micron thickness

from paraffin blocks were obtained, and stained with Haematoxylin and Eosin stain and examined microscopically.

The cytological smears and block sections were examined separately for cellularity, architectural patterns and morphology to render a cytological diagnosis for each case and the findings were studied to identify the malignancy and the most probable primary site. Yield of malignancy was identified by both methods.

Also CEA immunostaining was done in suspicious malignant cases.

RESULTS:

In our study, there were 260 fluids. The mean age of patients presenting with effusion was 37.43 ± 16.73 . The most common age group of presentation was between 21-30 years with a total of 80 cases (30.77%). The least common age group of presentation was above 70 years with a total of 9 cases (3.46%). The percentage of males presenting with effusion was higher (59.62%) as compared to that of female (40.38%).

On conventional smear, 212 (81.54%) cases were benign, 18 (6.92%) cases were malignant, 12 (4.62%) cases were inadequate and 18 (6.92%) cases were diagnosed as suspicious of malignancy.

On cell block, the number of cases diagnosed as benign were 205 (78.8%), malignant cases were 34 (13.1%), suspicious of malignancy were 2 (0.8%), and there were 19 (7.3%) inadequate cases. Cell block sensitivity 57.78 %, specificity 95.35 %, PPV 72.22 %, NPV 91.52 %.

Smear method gave a 6.9% yield of malignancy, which was increased to 13.1% on cell block method. The additional yield of malignancy was found to be 6.2% by cell block method.

IHC CEA Was done on 36 cases, 34 of them were malignant and 2 were suspicious of malignancy on cell block. 23 malignant cases came positive on CEA and 11 were negative. One suspicious of malignancy came positive on CEA and 1 was negative. IHC CEA sensitivity 92.31 %, specificity 100 %, PPV 100 %, NPV 100%.

In ascitic fluid, there were 6 cases of ovarian carcinoma, 6 cases of GIT carcinoma, 2 cases of endometrial carcinoma and others constituting 4 cases. Out of these, 13 cases positive for CEA marker and 7 cases were negative.

Out of 15 malignant pleural fluid, 5 cases were of breast carcinoma, 5 cases were lung carcinoma, 1 case of GIT carcinoma and others constituting 4 cases. 11 cases were positive for CEA marker and 4 were negative.

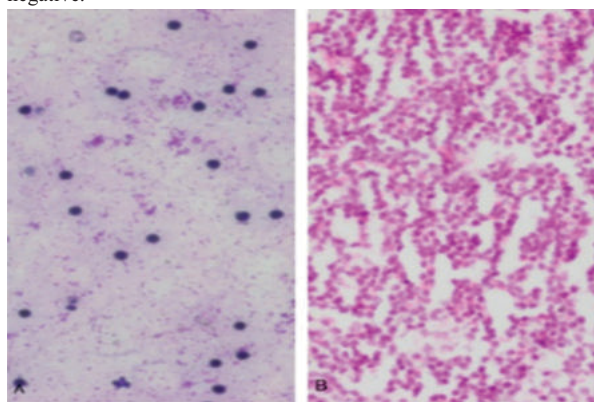


Figure 1: A - Benign features on cytology (Giemsa stain x 400)
B - Benign features on cell block (H & E x 400)

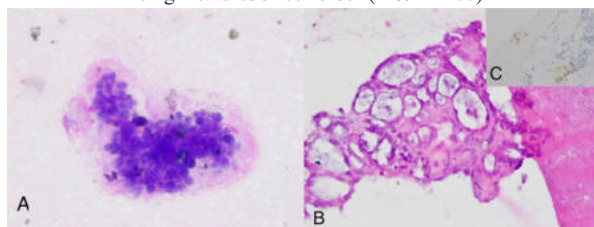


Figure 2: A - Features suspicious of malignancy on cytology (Giemsa

stain x 400).

B - Features of malignancy on cell block (H & E stain x 400).

C - Positive CEA IHC on malignant cell block (x100)

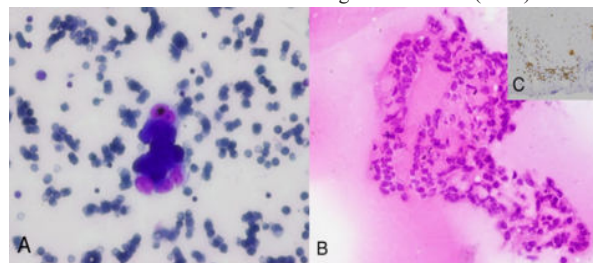


Figure 3: A - Features of malignancy on cytology (Giemsa x400).

B - Features of malignancy on cell block (H & E x400)

C - positive CEA IHC on malignant cell block (x100)

DISCUSSION :

The cytological examination of serous effusions is a non invasive technique and is available in most hospital and is well accepted as an initial investigation. It is an important tool in clinical medicine and a positive diagnosis on fluid examination is often considered the definitive diagnosis.^{19,20} Cytological examination of serous effusions is of paramount importance in the diagnostic algorithm and has therapeutic as well as prognostic implications. Reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional centrifuged smears.^{21,20,22} Cytological examination of body fluids commonly involves the use of direct or sediment smears (conventional smear technique).²³ In conventional smear, there can be overlapping or overcrowding of the cells, cell loss, artifacts and poor background staining, all of which can be due to improper smear, fixation and staining techniques.^{24,25,26,27} To enhance the diagnosis from conventional smear, the sediment from centrifuged fluids can be processed as cell block for histology. Although CB technique has been described for nearly a century and is well-known among cytopathologists, it is still underprescribed by clinicians.

Cellularity is higher by CB compared with CS and is concentrated in one small area that can be evaluated at a glance, with all cells lying in the same focal plane of the microscope.^{7,2} This is useful in screening the material in lesser time. Similar findings were noted in studies by Yang GC et al,²⁸ Dekker A and Bupp PA,⁷ and Thapar M et al.⁴

In addition, CB provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin; there is also less difficulty in microscopic observation, in spite of the presence of excess blood in the background.² The architectural patterns such as glands, sheets, three-dimensional cell clusters, and cell balls are commonly demonstrated in CB, resulting in increased sensitivity of diagnosis of malignancy by CB method.²

However, CB entails a risk of losing material during preparation, especially in the case of fixation technique that might explain negative CB results but positive CS results in some cases.

We received 260 samples of pleural and ascitic fluids. Out of this pleural fluid samples contributed more i.e. 80.3 % and ascitic fluids contributed 19.6 %. Our results correlated with the studies done by Sears & Hajdu²⁹ and Van de Molengraft et al.³⁰

Maximum number of samples were in the age group of 21 to 30 years, and this is in accordance with the study done by Kumar SH et al.³¹ In this study, the mean age of patients presenting with serous effusion was 37.43 ± 16.73 .

The percentage of males presenting with serous effusions was higher (59.62%) as compared to females (40.38%) with a M:F ratio of 1.4:1 and this is in accordance with study done by Padmavathi A et al.³² Bansode S et al³³ have reported M: F ratio of 2.1:1.

Out of the total 260 serous effusions, non-malignant lesions were more as compared to the malignant lesions. Our findings correlate with studies conducted by Sujathan K et al,²⁰ Bodele AK et al,²² Nilay Shah et al³⁴ and Goswami FJ et al³⁵ in which non-malignant lesions were more than malignant lesions.

On conventional smear, out of 260 fluids, 212 (81.5%) cases were reported as benign, 18 (6.9%) as suspicious of malignancy, another 18 (6.9%) as malignant and 12 (4.6%) were reported as inadequate for opinion.

On cell block, 205 (78.8%) cases were reported as benign, 2 (0.8%) cases as suspicious of malignancy, 34(13.1%) as malignant and 19(7.3%) as inadequate for opinion.

Out of 212 cases diagnosed as benign on conventional smear, 202 remained benign on cell block, 1 diagnosed as suspicious of malignancy, 2 reported as malignant and 7 cases were inadequate on cell block. Out of the 18 cases diagnosed as suspicious of malignancy on conventional smear, 2 cases were diagnosed as benign on cell block, 1 case remained suspicious of malignancy and 15 cases were reported as malignant on cell block. Similar findings were seen in the study by Sujathan K et al.²⁰ where the number of cases suspicious for malignancy on Conventional Smear were 5 (6%) and 1 (1%) on Cell Block. Similarly Shivakumaraswamy U et al.,² Dey S et al.,³⁶ Bodele AK et al.²² and Ponnatt TS et al.³⁷ also observed the number of cases.

The number of malignant cases in ascitic fluid were increased from 9 cases in conventional smear to 20 cases in cell block. The number of malignant cases in pleural fluid were increased from 9 in conventional smear to 14 in cell block.

Thus, conventional smear method gave a 6.9% yield of malignancy, which was increased to 13.1% on cell block method. The additional yield of malignancy was found to be 6.2% by cell block method. This is in accordance with a study by a study done by Bodele AK et al.²².

In our study, the sensitivity of cell block is 57.78%, specificity is 95.35%, PPV is 72.22%, NPV is 91.52%.

The common causes of benign pleural effusion were pulmonary tuberculosis pneumonia and non specific chronic inflammation. Similar findings were also seen in studies done by Sujathan K et al.²⁰ and Shah N et al.³⁴ in which most of the cases of benign pleural effusions were due to pulmonary tuberculosis.

The most common cause of benign peritoneal effusions was cirrhosis of liver. Similar findings were seen in study conducted by Shah N et al.³⁴

In our study, to supplement the CB, we have incorporated IHC CEA in 36 cases – 34 of which were malignant on cell block and 2 cases were suspicious of malignancy on cell block. IHC CEA came out positive on 24(67%) cases. This addition of marker enabled us to clearly ascertain the primary malignancy in these cases.

There were 12 (33%) which were negative for IHC CEA. In these cases, a definite primary malignancy could not be identified mostly because of low cellularity where the representative cells could not be identified in deeper sections for further IHC. Also in some cases, panel of markers need to be applied for definite diagnosis.

The sensitivity of IHC CEA is 92.31%, specificity is 100.00%, PPV is 100%, NPV is 99.15%.

The most common cause for malignant ascites in female was ovarian carcinoma (30%). This is because most women with ovarian carcinoma present with advanced stage of the tumour with ascites being the most common symptom at the time of detection. 108 Similar findings were found in studies done by Monte SA et al.,³⁸ Karoo ROS et al.,³⁹ Badal VK et al.⁴⁰, Khan N et al.,³⁵ and Mahmmod G et al.⁴¹ Other causes of malignant ascites were carcinoma of GIT (10%) and carcinoma of breast (10%) and others (15%).

In ovarian carcinomas, CEA is most frequently positive in mucinous carcinomas (70– 80%) and, to a lesser extent (20%), in serous carcinomas.⁴²

Most adenocarcinomas stain positively with antisera to CEA. CEA is negative in mesothelial cells, endometrial cells, and all normal ovarian tissue.⁴³

CONCLUSION:

Cell block technique is a simple, safe and reproducible procedure that does not require any special training or instrument. It provides a higher

yield of malignancy than conventional smear. Morphological features are better identified by cell block method, when compared with conventional smear method. Multiple sections can be obtained if required for special stain or IHC study, which will help identifying the primary location of tumour.

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