

# A Comparative Study of Serous Effusions by using Routine Staining Methods and Modified Cell Block Technique

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## Abstract

**Background** Cytological examination of serous fluids is an important diagnostic modality. However, accurately distinguishing reactive or malignant changes is diagnostically challenging. Cell block (CB) technique is dependable procedure as it improves the diagnostic accuracy. Advantage of cell block is the potential to make many sections for special stains and other ancillary techniques. **Objective** To compare the utility of CB technique compared to conventional smear in fluid cytology. **Material & Methods** The study comprised of analysis of serous effusion samples of pleural, peritoneal and pericardial cavities. All the samples were studied by both conventional smear method and modified cell block technique. **Results** In the present study, a total of 100 serous effusions were studied by using comparative approach of routine staining methods and modified cell block technique. By using conventional smear method, 84 cases were reported as inflammatory, 16 were diagnosed as malignant. Out of 100 cases of cell block preparation, 76 were inflammatory in nature and 20 cases were found to be malignant in nature. Four cases which were reported as negative for malignancy by conventional smear method were proven malignant on cell block thus increasing the diagnostic yield. **Conclusion** Cell block method of the study of body fluids is simple, rapid and cost effective and increases the diagnostic yield compared to the routine methods. It increases the diagnostic accuracy when used as an adjunct to conventional smears.

## Key Words

Serous Effusions, Conventional Smear, Cell Block

## Introduction

Cytological study of serous fluids is a commonly used investigation and is a comprehensive diagnostic modality that serves several functions. It helps in establishing the cause of effusion as well as plays therapeutic and prognostic role. [1,2] Conventional cytological smears requires thorough screening for distinguishing benign from malignant because conventional smear method has limitations due to suboptimal processing, delaying artefacts and cellular overlapping. Cell blocks prepared from these

centrifuged deposits are useful and dependable adjuncts to conventional smears for establishing a more definite cytopathological diagnosis. [3]

Cell block study refers to the study of sections of the centrifuged deposits of exfoliated cells after fixation and processing as is done in routine histopathology. The CB technique due to natural cohesion of the cells helps in interpreting microscopically tissue fragments rather than individual cells. Cell block preparation takes time

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comparable to that of routine paraffin sections and the final cell block sections show clusters of cells that look more like a histological section than a cytological smear demonstrating better architectural patterns helping in correct diagnosis.<sup>[4,5]</sup> Also, immunohistochemical markers can be applied to cell block. The aim of this study was to compare conventional smear cytology of serous effusions with cell block method and utility of combined approach to increase the cytodiagnosis of these effusions.

### Material and Methods

The present study comprised of a prospective analysis of serous effusion samples of pleural, peritoneal and pericardial cavities obtained from both male and female patients of all age groups received in the Department Of Pathology, Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu (J&K), over a period of one year, after obtaining clearance from Institutional Ethics Committee(ASCOMS/IEC/RP&T/2018/297). During this study a total of 100 cases were examined. Patient's relevant clinical history, clinical examination findings, various pathological, biochemical and radiological findings were recorded after obtaining valid consent. The specimen was subjected to gross examination. This was followed by both smear and cell block preparation from the fluid specimen and subsequent microscopic examination of the same. Immunohistochemistry was done wherever applicable. The fluid samples received were centrifuged at 2000 revolutions per minute for 10 minutes. The supernatant was discarded. To prepare wet film one drop of sediment was transferred to a clean glass slide and mixed with a drop of 1% toluidine blue. After mounting with cover slip, wet film was observed under microscope for immediate identification of cell morphology. Both air dried and wet fixed smears were made from the remaining sediment and stained by Giemsa and Papanicolau (PAP) stains respectively, using standard methods.<sup>[6,7]</sup> For haemorrhagic fluids, glacial acetic acid was used as a hemolysing agent and then these were processed. Improved Neubauer counting chamber was used for cell counts. All cases were analyzed for biochemical parameters and cytology.

Fixed Sediment Method was used to prepare the cell blocks. Sediment or tissue fragments were mixed in 10% buffered formalin and was allowed to stay for at least 6 hours.<sup>[8]</sup> It was followed by processing of the sediment like a routine tissue biopsy followed by hematoxylin and eosin staining of sections. If sediment was bloody, the blood was hemolysed prior to the addition of fixative, by

adding 1 ml glacial acetic acid. Sections prepared from cell blocks were assessed for cellularity, architectural details and nuclear and cytoplasmic features.

### Results

During our study, 100 fluid specimens were examined. Out of 100 samples, 71 were pleural fluid, 26 were ascitic fluid, 03 were pericardial fluid. It was observed that the maximum number of patients were males accounting to 24.6% and in the age group of 31-40 years, followed by 12 patients (19.7%) in the age group of 51-60 years. The least number of patients were in the age group of 0-10 years accounting to 1.6%. Among females the maximum number of patients were in the age group of 41- 50 years accounting for (23.1%) followed by 8 (20.5%) patients in the age group of 51-60 years. The least number of patients were observed in the age groups of 0-10 years, 71-80 years and 81-90 years accounting to 2 patients each (5.1%). The Male to Female ratio was 1.6:1. Among both the sexes the maximum number of patients were in the age group of 41-60 years, accounting for 40% followed by 18 (18%) in the age group of 31-40 years. Least number of patients were in the age group of 71-80 years accounting for only 2%.

It was observed that out of 71 cases of pleural fluid, number of inflammatory cases were more i.e. 67 (94.37%) compared to malignancy being 4 (5.63%). In 26 cases of ascitic fluid, number of inflammatory cases were more 14 cases (53.85%) compared to malignant which were 12 cases (46.15%). Three cases of pericardial fluid were included in our study, all of which were inflammatory (*Table 1*).

Cell block diagnosis is depicted in *Table 2*. In a total of 71 pleural fluid cases, 61 (85.91%) cell blocks were inflammatory, 6 (8.45%) were malignant and 4 showed no cellularity. In a total 26 ascitic fluid cases, 12 (53.84%) cell blocks were inflammatory, 14 (53.84%) were malignant. In a total of 3 pericardial fluid cases, all the 3 (100%) cell blocks were inflammatory. A highly significant association was observed between various fluids and cell blocks. Diagnostic yield of malignancy was found to be increased in cell block technique.

### Discussion

In the present study, cyto-diagnosis of serous effusions by using conventional smear methods and modified cell block technique was compared. In the present study, the maximum number of cases were of pleural effusion (71 cases) followed by ascitic fluid (26 cases) and only 3 cases of pericardial effusion. These observations were in concordance with the study done by Chandan *et al.*,

**Table 1. Cytological Diagnosis of Serous Fluids by Conventional Smear Method**

Site	Inflammatory	Malignant	Total
Pleural	67	04	71
Ascitic	14	12	26
Pericardial	03	00	03
Total	84	16	100

**Table 2. Cell Block Diagnosis of Serous Fluids**

Fluids	Cell Block						Total	
	Inflammation		Malignancy		No cellularity		N	%
Pleural	N	%	N	%	N	%	N	%
Pleural	61	85.91	6	8.45	4	5.63	71	100.0
Ascitic	12	46.15	14	53.84	0	0	26	100.0
Pericardial	3	100.0	-	-	-	-	3	100.0
Total	76		20		4		100	100.0

Thapar *et al.*, Matreja *et al.* <sup>[1,5,9]</sup>

By using conventional smear method, maximum (84 cases) were reported as inflammatory, 16 were diagnosed as malignant. Out of 84 inflammatory cases 54 (64.2%) cases were found cytologically to be consistent with diagnosis of chronic inflammation, 16 cases (19.04%) were showing reactive changes and 14 cases (16.6%) were of acute inflammation. These findings were similar to Sherwani *et al* <sup>[10]</sup>

In the present study, total 16 cases of malignancy were reported out of which 12 cases were peritoneal and 4 cases were of pleural effusion. Ovaries were the primary site with 9 cases (69.2%) followed by GIT malignancy with 3 cases (23.1%). These findings correlate with the study conducted by Khan *et al* <sup>[11]</sup>

Out of 100 cases of cell block preparation, 76 were inflammatory in nature which included both acute and chronic types and 20 cases were found to be malignant in nature. Four cases which were reported as negative for malignancy by conventional smear method were proven malignant on cell block thus increasing the diagnostic yield of malignancy. Four cases on cell block preparation showed no cellularity. These findings correlated with studies done by Bodele *et al* , Sujathan *et al* and Rajitha *et al* <sup>[12,13,14]</sup>

Cytological examination of body fluids is an important diagnostic modality used to differentiate between malignant and non malignant effusions. Conventional smear technique is well established method of cytodiagnosis of effusions. However, this technique has lower sensitivity owing to lack of well defined morphological and architectural details.<sup>[5]</sup>

Cell block technique or the paraffin embedding of the

sediments of fluids is one of the oldest methods of preparing materials for microscopic examination. In 1896, Bahrenburg introduced the cell block technique for processing of fluids. Cell block technique increases the positive results by demonstrating a better architectural pattern. The modified cell block technique has an added advantage that multiple sections of the same sediment can be subjected to special stains and other ancillary techniques like immunohistochemistry, in situ hybridization and polymerase chain reaction. <sup>[15]</sup>

In the present study, cell block was found to be diagnostically superior to conventional smears as use of cell block increased the diagnostic yield of malignancy from 16 to 20 cases. Cases which were reported to be inflammatory in smear cytology were proven to be malignant in cell blocks. Dehariya *et al*, Datta *et al* also assessed both the techniques for adequacy and accuracy of diagnosis based on morphological features and found cell block to be diagnostically superior to conventional smears. <sup>[16,17]</sup> Similarly, Bista *et al* also reported additional cases of malignancies by Cell block method compared to conventional smear method. <sup>[18]</sup> In our study four cases showed no cellularity on cell block as also reported by Grandhi *et al* <sup>[19]</sup> Lack of cellularity in cell blocks can be attributed to technical errors, inadequate sampling and degenerated samples and this limitation can be overcome by proper sampling of fresh fluid samples and processing with technical precision.

Morphological and architectural patterns are well preserved with modified cell block method. Cell ball and papillae, three dimensional clusters, nuclear and cytoplasmic details and individual cell characterization are better demonstrated in cell blocks. Reactive

mesothelial cells are responsible for simulating malignancy in conventional smears, largely due to the formation of rosettes, pseudoacini or acini with or without the presence of prominent nucleoli leading to false positive malignant diagnosis.

The modified cell block, thus, effectively puts both features in their proper perspective; i.e., the nucleoli do not appear as prominent as in the smears, and the pseudoacinar or acinar structure can be better appreciated when present which can increase the diagnostic accuracy. <sup>[5,20,21]</sup>

It is advisable to use the modified cell block method as an adjunct to conventional smears in providing a reliable and definitive diagnosis. Combined use of conventional smears and cell blocks helps to reach a conclusive diagnosis in suspicious and malignant cases

### Conclusion

Cell block technique is a simple, convenient and cost effective technique which can be used as an adjunct to conventional smears, in reaching to a more accurate cytological diagnosis, in resource limited setups.

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### Conflicts of Interest

There are no conflicts of interest.

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