

## CYTOLOGICAL DIAGNOSIS OF SEROUS EFFUSION BY COMPARATIVE APPROACH OF ROUTINE STAINING AND MODIFIED CELL BLOCK TECHNIQUE

Ranjana S. Ranade<sup>1</sup>, Purushotham Reddy<sup>2</sup>, Sujata S. Giriyan<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Pathology, SDM Medical College, Dharwad.

<sup>2</sup>Associate Professor, Department of Pathology, KIMS, Hubli.

<sup>3</sup>Professor and HOD, Department of Pathology, KIMS, Hubli.

### ABSTRACT

#### BACKGROUND

Cytological serous effusions many times poses a diagnostic dilemma between benign and malignant effusions. Study of Conventional Smears (CS) is a routine practise in diagnosis of body fluids. Hence, the present study is undertaken to assess the diagnostic efficacy of Cell Block (CB) preparation by combined approach of conventional smears and cell block preparation.

#### MATERIALS AND METHODS

This study was conducted in cytology section of Department of Pathology for a period of 18 months. Two conventional smears were prepared and stained for Haematoxylin and Eosin and Wright's stain. Cell blocks using 10% alcohol-formalin as fixative agent were prepared and paraffin sections were stained with Haematoxylin and Eosin stain. Both CS and CB were interpreted separately noting cellularity, cellular architecture and cytomorphological details. Statistical analysis for sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy was calculated for combined CS and CB preparation.

#### RESULTS

A total of 300 body fluids were studied of which 41.6% were pleural fluid and 58.4% were peritoneal fluid. Only, 7.7% of case of malignant effusions from both pleural and peritoneal fluid cytology was encountered. An additional 0.67% of cases were diagnosed as malignant effusions by cell block preparation, which otherwise would have been missed with only CS preparation.

#### CONCLUSION

The cell block preparation is simple, rapid and inexpensive technique for serous effusions. The cell block preparation provides better architectural and morphological details when compared to conventional smear preparation, thereby increases sensitivity of cytodiagnosis. Hence, cell block preparation is an useful adjuvant to conventional smear.

#### KEYWORDS

Cell Block, Serous Effusions, Conventional Smear.

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#### BACKGROUND

Cytological examination of serous effusions is of great importance in diagnosis of malignancy. It has helped for staging and prognosis of malignant tumours and also gives information regarding various inflammatory lesions of serous membrane.<sup>1</sup> The major challenging aspect of pathologist is to reliably distinguish between benign and malignant lesions in serous effusions. The accurate identification between malignant and reactive mesothelial cells is a diagnostic problem in Conventional Smear (CS) cytology. The cytodiagnosis of CS has got lower sensitivity due to

overcrowding of cells, cell loss and different laboratory processing methods.<sup>2</sup> The routine use of Cell Block (CB) by agar or plasma thrombin are not cost effective as it needs additional material.<sup>2</sup> However, a new method of modified cell block preparation by using 10% alcohol-formalin as fixative increases cellularity and gives better morphological details.<sup>1</sup> Cell block preparation is simple, reproducible and safe.<sup>3</sup> Further the effectiveness of cell block lies in the availability of diagnostic material for further histologic examination, histochemistry and Immunohistochemistry (IHC) studies for better classification of tumours and identification of infectious causes with microbiological stains.<sup>3,4</sup> Hence, the present study was undertaken to assess the diagnostic efficacy of cell block preparation by combined approach of CS and CB preparation in serous effusions.

#### MATERIALS AND METHODS

A prospective study was conducted in Department of Pathology, KIMS, Hubli, for one and a half years. Fresh samples of pleural and peritoneal fluids received in cytology

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*Corresponding Author:*

*Dr. Purushotham Reddy,*

*Associate Professor, Department of Pathology,*  
*KIMS, Hubli-580021.*

*E-mail: reddy\_puru24@rediffmail.com*

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sections were evaluated for the study. Procedure - From the receiver of the fresh sample, 10 mL of fluid was taken and divided into two equal parts. The first volume was used for conventional smears. The fluid was centrifuged at 3000 rpm for 15 minutes and a minimum of two thin smears were prepared from the sediment and stained with Wrights and Haematoxylin and Eosin stains.

The second volume of fluid was immediately fixed in 10% alcohol and formalin in 1:1 proportion for one hour followed by centrifugation at 3000 rpm for 15 minutes. The sediment pellet was removed after decanting supernatant and was again fixed with 3 mL 10% alcohol-formalin mixture for 24 hours. Subsequently, the sediment was scooped on a filter paper. The filter paper containing sediment was processed like routine histopathological specimens.

Fluids aspirated from cystic lesions, intraoperative fluid, cerebrospinal fluid, synovial fluid and pericardial fluid were excluded from the study. Cytological diagnosis were derived separately by studying cellular details like cellularity, cellular arrangements, cytoplasmic and nuclear details in Conventional Smears (CS) and Cell Block (CB) preparation. The cellular material in CS was considered as mild when there are 5-50 nucleated cells/hpf, medium when 50-200/hpf and marked when >200/hpf. The cellular material in CB was considered mild when 50-200 cells/hpf, moderate when 200-1000/hpf, marked when >1000/hpf and inconclusive when there are no cells observed in CB preparation. The reason for lack of cellularity is possibly due to technical error or degenerated sample. Architectural pattern was said to be excellent when architectural pattern was closely reflecting histology, moderate when preservation of architecture was noted like follicles, acini, papillae, syncytiae and single cell pattern and minimal to absent when nondiagnostic. Statistical analysis for sensitivity, specificity, PPV, NPV and diagnostic accuracy was calculated for combined CS and CB preparation.

**RESULTS**

A total of 300 body fluids were studied, of which, 41.6% were pleural fluid and 58.4% were peritoneal fluid. Fifty percent of cases were seen in 4<sup>th</sup> and 5<sup>th</sup> decades in both males and females. Male-to-female ratio was 1.8:1.

Cellularity	Conventional Smears		Cell Block	
	Cases	Percentage	Cases	Percentage
Nil	0	0%	61	20.3%
Mild	97	32.3%	69	23%
Moderate	158	52.7%	110	36.7%
Marked	45	15%	60	60%

**Table 1. Distribution of Cellularity in CS and CB Preparation**

Diagnosis	Conventional Smears		Cell Block	
	Cases	%	Cases	%
Negative for malignant cells	118	94.4%	93	74.4%
Positive for malignant cells	7	5.6%	8	6.4%

No cellularity	0	0%	24	19.2%
<b>Total</b>	<b>125</b>	<b>100%</b>	<b>125</b>	<b>100%</b>

**Table 2. Comparison of Analysis for Pleural Fluid by CS and CB Preparation**

Cell block preparation yielded one more additional case of positive for malignancy out of 125 cases when compared to CS preparation. However, no cellularity was noted in 19.2% of CB preparation. The reason for lack of cellularity was due to technical errors like inadequate sampling or degenerated samples.

Diagnosis	Conventional Smears		Cell Block	
	Cases	%	Cases	%
Negative for malignancy	161	92%	123	70.3%
Positive for malignancy	14	8%	15	8.3%
No cellularity	0	0%	37	21.1%
<b>Total</b>	<b>175</b>	<b>100%</b>	<b>175</b>	<b>100%</b>

**Table 3. Comparison of Analysis for Ascitic Fluid by CS and CB Preparation**

Similar to pleural fluid, ascitic fluid also yielded one more additional case of positive for malignancy in CB preparation when compared to CS smears. However, no cellularity was seen in 21.1% of cases.

Clinical Diagnosis	Cases	Percentage
Systemic and circulatory disorders		
Anaemia with hypoproteinemia	10	8.6%
Congestive cardiac failure	8	6.8%
Inflammatory causes		
Tuberculosis	58	49.6%
Pneumonia with pleurisy	28	23.9%
Nonspecific conditions	13	11.1%
<b>Total</b>	<b>117</b>	<b>100%</b>

**Table 4. Clinical Diagnosis of Pleural Effusions in Cases Negative for Malignancy**

Clinical Diagnosis	Cases	Percentage
Systemic and circulatory disorders		
Anaemia with hypoproteinemia	20	12.5%
Congestive cardiac failure	15	9.4%
Renal causes	2	1.2%
Cirrhosis	80	50%
Inflammatory causes		
Tuberculosis	23	14.4%
Nonspecific conditions	20	12.5%
<b>Total</b>	<b>160</b>	<b>100%</b>

**Table 5. Clinical Diagnosis of Peritoneal Effusions Negative for Malignancy**

However, we did not undertake further tests to confirm the clinical diagnosis for other than malignant effusions in both pleural and ascitic effusions. The group malignancy included all those patients who had positive for CS or CB preparations. There were 23 (7.7%) cases of malignant effusions from both pleural and peritoneal fluid cytology. Two cases of mesothelioma was suspected by CS and CB preparations. However, both the cases could not be confirmed by IHC or other ancillary tests. The remaining 21 cases were all of adenocarcinoma deposits.

Architectural Pattern	Conventional Smears		Cell Block	
	Cases	Percentage	Cases	Percentage
Single scattered cells	165	55%	90	30%
Cell clusters	54	18%	78	26%
Papillae	36	12%	15	5%
Glands	0	0%	15	5%
Sheets	45	15%	102	34%

**Table 6. Distribution of Architectural Pattern in CS and CB Preparation for Pleural and Ascitic Fluids**

The morphology of architectural pattern like three dimensional cell clusters, papillae and formation of glands along with crisp chromatin in CB preparation was good when compared to CS preparation.

Primary Site	Male	Female	Total	Percentage
Ovary	-	7	7	33.3%
Breast	-	3	3	14.3%
Lung	1	1	2	9.5%
Rectum	1	-	1	4.8%
Pancreas	1	1	2	9.5%
Stomach	1	-	1	4.8%
Unknown	1	4	5	23.7%
<b>Total</b>	<b>5</b>	<b>16</b>	<b>21</b>	<b>100%</b>

**Table 7. Primary Site of Metastatic Effusions in Male and Female Patients for Pleural and Ascitic Effusions**

Cell Block Preparation	Conventional Smears		Total
	Positive for Malignancy	Negative for Malignancy	
Positive for malignancy	21	2	23
Negative for malignancy	0	277	277
<b>Total</b>	<b>21</b>	<b>279</b>	<b>300</b>

**Table 8. Comparison of Diagnosis between CS and CB Preparation**

Additional 2 (0.67%) cases of malignant effusions were diagnosed by cell block preparations when compared to conventional smear study.

Statistical analysis for cell block preparation showed sensitivity- 100%, specificity- 99%, positive predictive value- 91.3%, negative predictive value- 100% and accuracy- 99%.

**DISCUSSION**

Cytological examination of serous effusions is of importance in diagnostic, therapeutic and prognostic implications. Reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears.<sup>1,2</sup> In this study, an attempt was made to prepare and analyse both smears and cell block from the same specimen. Out of 300 samples, peritoneal fluid contributed 58.4% and pleural fluid samples were 41.7%. Maximum number of samples were noted in the age group of 41-50 years in both males and females. In a study by Sherwani R et al,<sup>5</sup> majority were peritoneal fluid samples predominantly

in the age group of 51-60 years. In our study, males outnumbered females in serous effusions with a ratio of 1.8:1 as maximum number of effusions were due to cirrhosis (45.7%) seen more commonly in males. However, malignant cause for effusions was common in females constituting 76.2%. The reason for this being ovary and breast as primary sites for metastasis constituting 47.6%.

Study	No. of Cases	Benign		Suspicious		Malignant	
		CS	CB	CS	CB	CS	CB
Bodele et al <sup>1</sup>	150	118	111	3	0	29	39
Khan et al <sup>6</sup>	75	23	14	10	7	42	54
Present study	300	279	216	0	0	21	23

**Table 9. Comparison of Cytopathology of Serous Effusions in Present Study with Other Studies**

No cellularity was observed in 21% of cases due to technical error such as inadequate sampling or degenerating samples. No cellularity in CB was also observed by Bodele et al.<sup>1</sup> There was better morphological details in CB preparation, which included preservation of architectural patterns such as papillae, acini, three dimensional clusters with better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details. Similar findings were observed by Bodele et al,<sup>1</sup> Sujatha K et al,<sup>2</sup> Velios F and Griffin J.<sup>7</sup>

Reactive mesothelial cells has been a common problem for simulating malignancy in CS due to formation of rosettes, pseudo acini and acini with or without presence of prominent nucleoli. The CB preparations puts both the features in proper prospective, that the nucleoli does not appear as prominent as in CS cytology. The pseudo acinar and acinar structures are better appreciated when present in CB preparations. Similar findings were noted in Dekker and Bupp study.<sup>3</sup> In difficult situations, immunohistochemistry maybe helpful.<sup>8</sup> In the present study, diagnostic yield for malignancy was marginally increased (0.66%) by cell block preparation when compared to CS study. In a study done by Bodele et al,<sup>1</sup> an additional 7% of malignant cases were identified by CB preparations. Dekker and Bupp<sup>3</sup> study reported a 38% of patients had positive for malignancy by CB preparations, which otherwise was negative or atypical in cytological report by CS study. In a study by Khan et al,<sup>6</sup> additional findings in CB were diagnostic in 16% of malignant cases. In a study by Richardson et al,<sup>9</sup> an additional 5% of malignant cases were added by CB preparation. An additional 18 cases for malignant lesions were diagnosed by cell block method in a study by Takagi F.<sup>10</sup> According to various studies, additional diagnostic yield for malignancy were noted if conventional smear technique is supplemented by cell block method. The reason for low yield for malignancy in our study could be probably due to low deception of cancer in both pleural and ascitic effusions constituting 7.7 percent. This being attributed to ours being a general hospital treating mainly noncancerous patients. Secondly, our study never encountered any suspicious for malignancy in conventional smears.

Study	Breast	Lung	Ovary	GIT	Others	Unknown
Sears and Hajdu <sup>11</sup>	24	19	5	4	29	19
Lopez and Cordoz <sup>12</sup>	16	21	4	3	07	49
Johnson <sup>13</sup>	15	36	8	6	16	19
Khan et al <sup>6</sup>	12	69	0	0	0	19
Present study	2	2	0	1	1	2

**Table 10. Comparison of Primary Site for Metastatic Effusion in Pleural Fluid Preparation**

The metastatic effusion in pleural fluid constituted 38.1%, which is less than ascitic fluid. Though the number of malignant effusions are less in our study, breast and lung was encountered equally as most common sites of primary. In a study by Sears and Hajdu,<sup>11</sup> breast was the commonest site. Lopez and Cordoz,<sup>12</sup> Johnson<sup>13</sup> and Khan et al,<sup>6</sup> encountered lungs, the commonest site.

Study	Breast	Lung	Ovary	GIT	Others	Unknown
Sears and Hajdu <sup>11</sup>	32	11	4	15	23	15
Lopez and Cordoz <sup>12</sup>	32	32	0	9	13	14
Khan et al <sup>6</sup>	28	9	0	0	30	33
Present study	0	0	7	3	0	3

**Table 11. Comparison of Primary Site for Metastatic Effusion in Peritoneal Fluid Preparation**

Our study encountered ovary as the common site of primary, whereas all other studies shown above show breast as common site for malignant peritoneal effusions. In a study by Nair and Manjula,<sup>14</sup> GIT was the commonest site followed by ovary for malignant peritoneal effusions.

**CONCLUSION**

The use of cell block preparation as an adjunct to routine cytological smears can increase sensitivity and specificity by obtaining better cellularity, cellular architecture and cellular details, thus increasing diagnostic yield of malignancy in serous effusions.

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