

## A COMPARATIVE STUDY ON DIAGNOSTIC YIELD BETWEEN CENTRIFUGED METHOD OF CYTOLOGY AND CELL BLOCK TECHNIQUE

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### Abstract

Cytological examination of serous fluids is of paramount importance in disease diagnosis. Cell blocks are particularly useful when the cytological abnormalities are misleading, such as in reactive mesothelial cells or obscure as in occasional well differentiated adenocarcinoma. In the present study, these methods are applied to pleural and peritoneal fluids to conclude the diagnosis as inflammatory, benign or malignant lesion. A sample of 110 fluids obtained during August 2011 to August 2012 in the Department of Pathology, Narayana Medical College, Nellore. Out of 110, 76 (69%) are pleural and 34 (31%) were peritoneal samples. Men were 56.4% and women were 43.6% and the mean age of the study was 50.19 with a standard deviation of 14.81. Among 110 fluids 15 were clinically diagnosed as malignant effusions. Out of which 11 were reported as positive for malignant cells and 4 cases were negative. 3 cases were reported as positive for malignant cells in which they were clinically not diagnosed. Out of 110 cases, 12 were reported as positive for malignant cells by smear whereas 14 cases were reported as positive for malignant cells by cell block. The diagnostic yield is increased by cell block technique. The cell block technique not only increased the positive results, but also helped to demonstrate better architectural patterns which could be of great help in approaching the correct diagnosis of the primary site.

**Keywords:** Cell Block Technique, Cytospin smear, Diagnostic Yield, Adeno carcinoma

### Introduction:

The accurate identification of cells as either malignant or benign reactive mesothelial cells in serous effusions is an everyday diagnostic problem. Cytological examination of serous fluids is of paramount importance. Apart from the finding of cancer cells, cytological examination of pleural, peritoneal and pericardial fluids may also reveal information regarding various inflammatory conditions of serous membranes, various bacterial, viral and fungal infections and parasitic infestations.<sup>1</sup> The cytological diagnosis of effusions has a lower sensitivity which is attributed to bland morphological details of cells, overcrowding or overlapping of cells, cell-loss and changes due to different laboratory processing methods.<sup>1</sup>

Cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to

smears for establishing a more definitive cytopathologic diagnosis.<sup>2</sup> Apart from increased cellularity, better morphological details are obtained by cell block method which include preservation of the architectural pattern like cell balls and papillae and three dimensional clusters, better nuclear and cytoplasmic preservation, intact cell membrane, crisp chromatin details.<sup>1,3</sup> The cell block technique now takes an intermediate position between histological and cytological technique. The main advantage of cell block technique is that the cells resemble those seen in histology. Cell blocks are particularly useful when the cytological abnormalities are misleading, such as in reactive mesothelial cells, or obscure as in occasional well differentiated adenocarcinoma.<sup>4</sup>

### Objectives:

1. To provide comparative study of centrifuged method of cytology and cell block.

2. To study body fluids i.e. pleural & peritoneal for the presence or absence of local or systemic pathology of neoplastic & inflammatory lesions.

3. To assess the diagnostic yield of cell block preparation method in increasing the sensitivity of cytodiagnosis of serous effusions.

**MATERIALS AND METHODS**

The present study was done during the year August 2011 to August 2012 in the Department of Pathology, Narayana Medical College, Nellore. During the period, a total of 210 samples of pleural and peritoneal fluids were collected, of which 110 samples were included in the study after following the exclusion criteria. Subjects who did not give consent, who failed to undergo clinical and radiological investigations, whose sample was inadequate for cell block, were excluded.

After clinical examination and radiological investigation, sample obtained was first examined by naked eye for physical characteristics and divided into two halves. Half of the specimen was centrifuged at 1500 rpm for 15 min and the smear prepared was stained with haematoxylin and eosin. For the cell block preparation, other half of the fluid specimen was fixed in a 1:1 solution of alcohol and formalin for one hour. After fixation, the specimen was centrifuged at 2500 rpm for 10-15 min. The supernatant fluid is discarded and the sediment is wrapped in filter paper and processed in histokinette as part of routine paraffin section histopathology. Special staining including PAS was done when needed. The slides were evaluated for cellularity, arrangement, cytoplasmic and nuclear details. Measures were taken to avoid subjective variation by blindfolding the smears.

The results obtained were subjected to analysis using Microsoft Excel spreadsheet and SPSS for statistical significance.

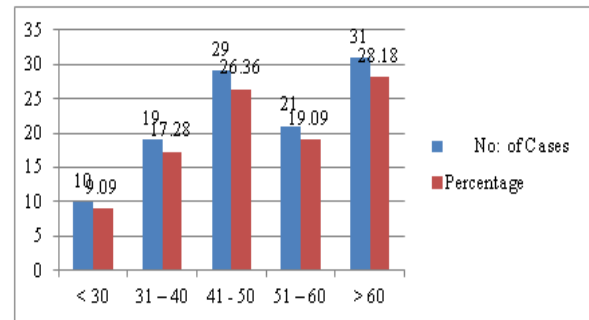
**RESULTS:**

The present study was done during the year August 2011 to August 2012 in the Department of Pathology, Narayana Medical College, Nellore. During the period, a total of 210 samples of pleural and peritoneal fluids were collected, of which 110 samples were included in the study after following the exclusion criteria.

**Table1: Representing the sample source**

Type of Fluid	Number	Percentage (%)
Pleural	76	69%
Peritoneal	34	31%
<b>Total</b>	<b>110</b>	<b>100%</b>

Among the 110 samples collected, 76 (69%) were pleural and 34 (31%) were peritoneal.



**Figure 1: Representing the age distribution of cases under study**

In the present study, males were 62 (56.4%) and females were (43.6%) with the mean age of 50.19 years with a standard deviation of 14.81 years and ranging from 18 to 85 years.

**Table 2: Comparison of smear versus cell block of all fluids**

Cellularity	Number of cytospin smears	Number of Cell blocks	$\chi^2$ Value	P Value
Lymphocytes	10(9.09%)	10(9.09%)	0.00	1.0
Neutrophils	18(16.36%)	18(16.36%)	0.00	1.0
Mixed inflammatory cells	47(42.73%)	44(40%)	0.17	0.68
Lymphocytes + Reactive mesothelial	20(18.18%)	24(21.82%)	0.455	0.5
Malignant cells	10(9.09%)	14(12.73%)	0.75	0.38
Suspicious for malignancy	2(1.82%)	-	0.497	NS
Unsatisfactory smear	3(2.73%)	-	0.247	NS
<b>Total</b>	<b>110(100%)</b>	<b>110(100%)</b>	<b>0.497</b>	<b>0.9</b>

NS – Not significant.

Among 110 fluids, 3 cases of unsatisfactory smear on smear examination, 3 cases showed lymphocytes with reactive mesothelial cells on cell block. 2 cases were suspicious for malignancy on smear examination which were confirmed as malignancy by cell block and 2 cases showing mixed inflammatory cells showed positivity for

malignancy 10 cases were positive for malignant cells on smear examination. 14 cases were positive for malignancy on cell block.

**Table 3: Efficacy of Centrifuged smear with cell block in detecting malignant cells**

Centrifuged smear (N=110)	Cell block Positive for malignant cells	Cell block Negative for malignant cells	Total
Positive for malignant cells (12)	12	0	12
Negative for malignant cells (98)	2	96	98
Total	14	96	110

Sensitivity= 85.7%

PPV=100%

Diagnostic accuracy=98.2%

Specificity=100%

NPV=97.5%

Kappa Statistic=0.84

Out of 110 cases, 12 were reported as positive for malignant cells by smear. 14 cases were reported as positive for malignant cells by cell block. The diagnostic yield is increased by cell block technique.

**Table 4: Efficacy of clinical diagnosis with pathological diagnosis (Cell Block) in detecting malignancy**

Clinical diagnosis (N=110)	CELL BLOCK		Total
	Positive for malignant cells	Negative for malignant cells	
Malignant effusions (15)	11	4	15
Others (95)	3	92	95
Total (110)	14	96	110

Sensitivity=78.57%

PPV=73.33%

Diagnostic accuracy=93.6%

Specificity=95.83%

NPV=96.84%

Kappa Statistic=0.85

Among 110 fluids, 15 were clinically diagnosed as malignant effusions. Out of which 11 were reported as positive for malignant cells and 4 cases were negative. 3 cases were reported as positive for malignant cells in which they were clinically not diagnosed.

**DISCUSSION**

In this study, we attempted to prepare and analyse both centrifuged smear and cell block preparation from the specimens to compare the diagnostic utility of each of two of the techniques. The cell block method is the oldest method of processing cytological material as described by Mandlbaum in 1900 for studying exudates.

The most common effusion was pleural followed by peritoneal correlating with the study of Meenu Thapar et al. This is in correlation with other studies

which may be due to prevalence of tuberculosis in the region of our study.<sup>5</sup>

In a study conducted by Ringenberg QS et al, 69 cytologically malignant peritoneal fluids were diagnosed. In 2 cases, no review or follow up data could be obtained. In another two cases the cytological diagnosis was wrong. The most common age group was (30-95 years) with a median age of 62 years.<sup>6</sup> In our study, 4 cases of malignant peritoneal fluid were diagnosed. The most common age group was about 60years with a median age of 65 years and correlates with Ringenberg QS study.

In the present study, lymphocyte rich effusion was noticed in 78 cases. Among these, 38 were tuberculosis, 24 were reactive, 16 were nonspecific inflammation. According to Lorenzo M Galindo, lymphocyte rich effusions are relatively common and difficult to give the differential diagnosis.<sup>7</sup> In the

present study, effusions were analysed with the clinical knowledge of age, sex and other clinical details like ESR, peripheral smear examination, ultrasound and radiological findings.

Cytology of pleural effusion is commonly practiced technique and it is particularly important in diagnosis of tuberculous and malignant pleural effusions. The increased incidence of tuberculous pleural effusion was noticed from 32-60 years of age group. This is becoming more common in adult in post primary with reactivation of tuberculosis. ESR was very high in tuberculous pleural effusion when compared to non specific inflammatory conditions. These findings correlated with study made by Sherwani and colleagues.<sup>8</sup> Spieler et al observed cytological features of typical tuberculous effusion with moderate to high cellularity and predominance of lymphocytes.<sup>9</sup> Similar findings observed in the present study.

16(14.5%) cases were diagnosed as nonspecific inflammation by both smear and cell block techniques. It was observed that effusion contained a mixture of inflammatory cells consisting of neutrophils, lymphocytes, occasional macrophages and mesothelial cells in various proportions and a few fibrin strands. Similar findings were observed by Thomas Kransz and colleagues.<sup>10</sup>

To determine the primary source of adenocarcinoma in effusions is very difficult but with knowledge of clinical data taken in to account, the cavity involved and with morphological appearance the diagnosis was made. The cells of adenocarcinoma closely mimic reactive mesothelial cells and the cells of malignant mesothelioma. The typical carcinomatous cells are of variable sizes, exhibit nuclear pleomorphism with

overlapping of nuclei, prominent nucleoli, occasional multinucleated cells and intracytoplasmic vacuoles.

In 2005, Khan et al. in a study of cytodiagnosis of malignant effusion and of determination of primary site found that adenocarcinomas were the commonest type of neoplastic cells found in serous fluids.<sup>11</sup> Similar findings were observed in our study.

Duct cell carcinoma of breast is next common tumor causing pleural effusion in the women.<sup>12</sup> In the present study one case of pleural effusion revealed the primary as duct cell carcinoma of breast. 3 cases of metastatic peritoneal effusions revealed primary in ovary observed as serous adenocarcinoma of ovary. The smears revealed large cells in clusters, acini or papillary clusters, discrete tumour cells, cells with cytoplasmic vacuolation and mitotic figures.

In the study by Sujathan et al, out of 85 samples studied, 21 cell blocks were malignant.<sup>13</sup> 2 samples diagnosed as negative for malignancy by smear technique, were diagnosed as malignancy by cell block method. Thus the use of cell block increased the diagnostic yield of malignancy from 19 to 21.<sup>13</sup> In our present study, out of 110 samples of pleural and ascitic fluids studied, 14 cell blocks were malignant. Two samples diagnosed as negative for malignancy by smear technique, were diagnosed as malignancy by cell block method. Thus the use of cell block increased the diagnostic yield of malignancy from 12 to 14 samples which correlates with the above study.

In a study by Meenu Thapar et al, combined cell block and smear technique was 13% more diagnostic than that of specimens observed by smears only.<sup>5</sup> In 90% of smears, the smears alone were considered diagnostically equal to cell block, while in 10% it was inferior to cell block. These findings were correlated with our study.

**Table 5: Comparison of cell block efficacy on malignant pleural effusion and malignant ascitis with other studies**

	Pleural effusion with lung as primary site as malignancy		Ascitis with GIT and Liver as primary site of malignancy	
	Our study (N=11)	Meenu Thappar et al. (N=16)	Our study (N=3)	Meenu Thappar et al (N=24)
<b>Cytospin smears</b>	63.64%	62.5%	66.66%	75%
<b>Cell block</b>	90.91%	75%	100%	91.67%

The diagnostic yield was more by 27.27% in pleural fluids and 33.34% more in ascitic fluids with Cell block compared to cytospin smears. The same yield was found less with 16.25% and 15.49% respectively in the study done by Meenu Thapar et al.<sup>5</sup>

## CONCLUSIONS

To conclude in the present study, increased diagnostic utility of 10% is noted in cell block method. The cell block technique not only increased the positive results, but also helped to demonstrate better architectural patterns which could be of great help in approaching the correct diagnosis of the primary site.

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