

COMPARATIVE STUDY FOR THE USE OF DIFFERENT TECHNIQUES IN SEROUS FLUID CYTOLOGY

Mahendra Singh¹, Lubna Khan², Yogendra N. Verma³, Neelima Sachan⁴, Chayanika Pantola⁵, Adrija Pathak⁶, Rachita Gulati⁷, Anuradha Gautam⁸

HOW TO CITE THIS ARTICLE:

Mahendra Singh, Lubna Khan, Yogendra N. Verma, Neelima Sachan, Chayanika Pantola, Adrija Pathak, Rachita Gulati, Anuradha Gautam. "Comparative Study for the use of Different Techniques in Serous Fluid Cytology". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 18, March 02; Page: 3154-3161, DOI:10.14260/jemds/2015/456

ABSTRACT: BACKGROUND: Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions. The diagnosis of cancer in a pleural, pericardial, or peritoneal fluid is of capital importance for the patient and the attending physician or surgeon. It helps in staging, prognosis and management of patients of malignancies, and also give information about various inflammatory and non-inflammatory lesions. Diagnostic problem arises in everyday practice to differentiate reactive atypical mesothelial cells from malignant cells by routine conventional smear method. **AIM:** To compare the morphological and cytological features of the conventional smear method with those of cytocentrifuge and cell block and also to compare the diagnostic yield of cytocentrifuge and cell block methods with conventional smear in the diagnosis serous effusion. **MATERIAL AND METHODS:** Ninety eight samples were subjected to diagnostic evaluation. Along with conventional smear, fluids were subjected to cytocentrifuge and cell block technique. Cell blocks were prepared using 10%formalin as a fixative agent. Smears obtained by each of techniques were scored for different parameters. Statistical analysis with Wilcoxon rank sum test was performed and also Index of Qualitative Variation (IQV) was calculated to compare the results obtained by each of the above three methods. **RESULTS:** Cellularity, diagnostic yield and sensitivity for malignancy was more for Cytocentrifuge and Cell Block technique. **CONCLUSION:** The cytocentrifuge and cell block method provide high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared to conventional smear method.

KEYWORDS: Cytocentrifuge, Cell block, conventional smear, fluid cytology, morphological features.

INTRODUCTION: Accumulation of fluid in a serious cavity in excess of the normal amount is referred to as an effusion. Fluid samples are invaluable specimens and help in diagnosis of particular lesional pathology, identifying the immediate precipitating factors causing the effusion and mostly, results in a specific diagnosis. Cytologic evaluation is the best way to detect the presence of malignancy in body cavity fluids.⁽¹⁾The general cytologic examination can be performed easily, quickly, and inexpensively. Although a positive diagnosis is highly reliable, a negative result does not rule out a malignant cause. Adenocarcinomas, well differentiated squamous carcinomas, small-cell carcinomas, malignant melanomas, large-cell lymphomas, and acute leukaemia's are accurately classified when present in effusions. The diagnostic performance of the cytologic study of the fluid may be attributable to the fact that the cell population present in sediment is representative of a much larger surface area than that obtained by needle biopsy.⁽²⁾The technique still widely used in most cytology laboratory of India is the centrifugation and sedimentation smear preparation technique.

ORIGINAL ARTICLE

With this technique, failure of collecting relatively few cells from any fluid and keeping them on the slide during staining is responsible for large number of unsatisfactory smear.

Thus resulting in effusion being reported as negative or atypical without definitive diagnosis and false negative diagnosis.

This study is to compare the morphological features and diagnostic yield of conventional, cytospin and cell block technique in the study of cytology of serous effusions.

MATERIAL AND METHODS: The present study is prospective study carried out in the department of Pathology over a period of two years.

Sample Size: All randomly taken 98 cellular samples comprising of pleural, peritoneal and pericardial fluids received over a period two years in the department of Pathology were the subject of study. The fresh samples obtained were examined by naked eye for physical characteristic and then divided into three equal parts. One part was subjected to routine centrifuge practiced in our laboratory thin smear preparation after centrifugation at 1200rpm for 5 minutes. Other part was subjected to cytocentrifuge i.e.300microlitre of fluid was placed in cytospin funnel with the filter paper placed between the slide and the funnel, then subjected to centrifugation at 700 rpm for 6 minutes.

The slide was then fixed in 95% ethanol for 15minutes and stained with haematoxylin and eosin; and the third part for cell block technique. The third part of the fluid was fixed in 10% formal alcohol in the ratio of 1:1 and kept for 1 hour. After fixation it was centrifuged at 2000 rpm for 10 minutes. The supernatant was poured off and the cell button formed was obtained on Whatmanfilter paper number 1. The sediment was wrapped in the same filter paper and processed in histokinette and the smears obtained by each of the above technique were evaluated for features such as background, cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale (Table-1) according to the Mair et al scoring system.⁽³⁾

Table 1: Criteria for the assessment of the quality of smear and cell block

Volume of obscuring background blood or clot	Large amount : diagnosis greatly compromised	0
	Moderate amount: diagnosis possible	1
	Minimal: diagnosis easy, specimen of textbook quality	2
Amount of diagnostic cellular material present	Minimal or absent: diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant: diagnosis simple	2
Degree of cellular degeneration and cellular trauma	Marked: diagnosis impossible	0
	Moderate : diagnosis possible	1
	Minimal : good preservation	2
Retention of appropriate architecture and cellular arrangement	Minimal to absent: non diagnostic	0
	Moderate: some preservation e.g., follicles, papillae, acini, syncytia or single cell pattern	1
	Excellent architectural display closely reflecting histology: diagnosis obvious	2

ORIGINAL ARTICLE

RESULTS: The present study was conducted on 98 randomly taken samples of serous effusions and were subjected to biochemical and cytological examination. The cytological examination was performed using conventional smear, cytospin, and cell block preparation. The majority of fluids were of pleural origin (74%) and rest were of peritoneal effusions. There was male preponderance with a sex distribution ratio of 1.55:1. Majority of cases were reported in patients more than 30 years of age (80.5%). Most of the cases were of different reactive effusions (74%) and on biochemical examination of reactive effusion fluids 75% were exudative according to Light's criteria. Cause of reactive effusions were mainly tubercular, chronic inflammatory diseases, bacterial and viral infections. 26% of the cases were malignant effusions and 95.8% of malignant effusions were exudative. Out of 24 malignant effusions only 14 were diagnosed on conventional smear and 23 in cytocentrifuge smear.

Thus using cytocentrifuge smear diagnostic yield increased by 34.2% and using cell block it increased by 41.7%.(table 2&3). On applying statistics it was found that difference of median scores for centrifuge & cytocentrifuge as well as centrifuge & cell block on each parameter were statistically significant ($p < 0.0001$). However, the difference between cytocentrifuge & cell block on each parameter was statistically insignificant ($p > 0.05$) (table-4)

Type of malignancy	Smear	Cell block	Cyto-centrifuge
Adenocarcinoma	05	08	09
Poorly differentiated carcinoma	03	05	05
Mesothelioma	01	01	01
Squamous cell carcinoma	01	03	02
Non Hodgkin lymphoma	01	01	01
Total	11	18	18

Table 2: Distribution of malignant pleural fluid cases on the basis of conventional smear, cell block and cytocentrifuge smear examination

Type of malignancy	Smear	Cell block	Cytocentrifuge
Adenocarcinoma	01	03	03
Mucin secreting adenocarcinoma	00	02	01
Unclassified	02	01	01
Total	03	06	05

TABLE 3: Distribution of malignant peritoneal fluid cases on the basis of smear, cell block and cytocentrifuge preparation

Parameters	Comparison of methods		
	Conventional &cyto centrifuge	Conventional & cell block	Cyto centrifuge & cell block
Background	<0.0001	<0.0001	0.141
Cellularity	<0.0001	<0.0001	0.341
Cell morphology	<0.0001	<0.0001	0.113
Cell distribution	<0.0001	<0.0001	0.061

Table 4: Statistical significance of difference of each parameter

Significance of difference of each parameter for two groups was performed using Wilcoxon rank sum test. It revealed that difference of median scores for Centrifuge & Cyto centrifuge as well as Centrifuge & Cell block on each parameters was statistically significant ($p < 0.0001$). However, the difference between Cyto centrifuge & Cell block on each parameter was statistically insignificant ($p > 0.05$).

DISCUSSION: The information provided by body fluid analysis serves several functions; it is a complete diagnostic modality which aims at pointing out the etiology of effusion. Both malignant and non-malignant causes of effusion can be identified by the relatively non-invasive technique of fluid cytology. With this basis the present study on cytology of fluids was taken up. The diagnostic significance of the cytologic study of the fluid may be attributable to the fact that cell population present in the sediment is representative of a much larger surface area than obtained by needle biopsy.⁽⁴⁾ This study is in accordance with Thapar et al (2009) who also used the same criteria by Mair. They noticed that 26% of smears and 12% of the cell block were unsuitable for diagnosis. 20% of smears and 21% of cell blocks were diagnostically adequate. 54% of smears and 67% of cell blocks were considered diagnostically superior.⁽⁵⁾

Lack of morphological details of the representative cells contributes to considerable difficulties in making conclusive diagnosis on conventional smears. In order to overcome these difficulties, in this study an attempt was made to prepare and analyse routine centrifuge, cyto centrifuge and cell block from the same sample. In this study due consideration was given to age, sex, site of effusion, clinical findings and investigations to arrive at final diagnosis and also to identify primary malignant lesion.

In cell blocks, one could very often observe glandular formations and acinar groupings that are less beautifully manifested in smears.^(6,7) Cyto centrifuge preparations can preserve the cellular details and reduce the overlapping of cells, enabling precise interpretation than conventional smears.⁽⁸⁾ The use of cyto centrifuge and cell block not only increases the cellularity as compared to routine centrifuge, but also the cells were evenly distributed. The cellular morphology, nuclear and cytoplasmic details, were better appreciated on cyto centrifuge and cell block technique. Also cell block carries additional advantage of performing Immunohistochemistry which aids in the diagnosis and can be used for retrospective analysis.⁽⁹⁾

So the diagnostic yield increased by 34.2% on cyto centrifuge smear examination and by 41.7% on cell block preparation.

ORIGINAL ARTICLE

Dekker and Bupp reported the samples obtained by mini cell block technique and conventional smear technique of malignant peritoneal effusions and concluded that on cell block examination additional diagnostic yield was 38%.⁽¹⁰⁾ Improved sensitivity of smear in diagnosis of malignant effusion may be due to the use of Cytospin machine that helps to make a monolayer sheet of cells so that morphology of cells can be better studied.^(11,12,13)

In evaluating the cytological details brought out by each technique, cytocentrifuge was superior in demonstrating cellularity, cell retrieval, less cellular crowding, better cytoplasmic and nuclear preservation than routine method. Sections prepared from cell block showed good architectural pattern (Cell balls, acinar pattern, papillary structures). Nuclear and cytoplasmic preservations were as good as compared to cytocentrifuge.

In this study there is a significant difference between the results obtained by conventional smear as compared to cytocentrifuge and cell block. However the difference between cytocentrifuge and cellblock was not significant. Mao et al, studied 99 cases of effusion using cytospin and cell block technique.⁽¹⁴⁾

He found that the difference between the two techniques was statistically insignificant. However when coupled with immunohistochemical findings and clinicopathological findings the difference in the diagnosis was statistically significant. Thus Immunohistochemistry when applied on cell block sections, is useful in detection of the primary origins of the tumour cells in effusion fluids.⁽¹⁵⁾ Combination of morphologic examination, immunohistochemical findings and clinicopathologic correlation can further improve the rate of positive diagnosis.

CONCLUSION: Routine centrifuge is not satisfactory in reporting fluids with scant cellularity. Hence for fluids with scant cellularity cytocentrifuge and cell block are useful methods. Also the morphology of the cells were well appreciated by cytocentrifuge and cell block as compared to routine centrifuge, thus aids in accurate diagnosis.

In our study the diagnoses which were missed or incompletely diagnosed on routine centrifuge were diagnosed accurately by the other two techniques. Also there was statistical difference between the results obtained by the three techniques. Thus cytocentrifuge and cell block proved to be superior method for the study of effusion as compared to routine centrifuge.

REFERENCES:

1. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology-Improved preparation and its efficacy in diagnostic cytology. *AmJ Clin Pathol* 2000;114: 599-606
2. Stevens MW, Fazzalari NL, Crisp DJ. Lymph node cellular morphology: Comparative study of imprints and cytocentrifugesmeared. *J Clin Pathol* 1987;40:751-55.
3. Mair, Dunbar F, Becker PJ, DuPlessis W. Fine Needle Cytology: Is aspiration suction necessary? A study of 100 masses in varioussites. *ActaCytol* 1989;33:809-13.
4. Gaur DS, Chauhan N, Kusum A, Harsh M, Talekar M. Pleural Fluid Analysis-Role in Diagnosing Pleural Malignancy. *J Cytol*2007;24:183-88.
5. Thapar M, Mishra RK, Sharma A, Goyal V. Critical analysis of cell block versus smear examination in effusion. *J Cytol*2009;26:60-4.
6. Udasimath S, Arakeri SU, Karigowdar MH. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. *J Cytol* 2012; 29:11-15

ORIGINAL ARTICLE

7. Bodele AK, Parate SN, Wadadekar AA, Bobhate SK, Munshi MM. Diagnostic utility of cell block preparation in reporting of fluid cytology. *J Cytol* 2003;20:133-35.
8. Shah P, Deshmukh R. Exfoliative Cytology and Cyto centrifuge Preparation of Oral Premalignant and Malignant Lesions. *Acta Cytol* 2012;56:68-73
9. Sherwani R, Akhtar K, Abrari A, Hajra S. Pleural effusion cytology as an aid in the diagnosis of pulmonary tuberculosis. *J Cytol* 2006;23:123-27.
10. Dekker A, Bupp PA. Cytology of serous effusions. An investigation into the usefulness of cell blocks versus smears. *Am J Clin Pathol* 1978;70:855-60.
11. Naylor B. Pleural, Peritoneal fluids. In: Bibbo M. *Comprehensive Cytopathology*. 1st Ed. Philadelphia: WB Saunders, 1991; 551-621.
12. Gieisinger KR, Stanely MW, Raab SS, Silverman JF, Abati A. In: Natasha A. *Effusions. Modern Cytopathology*. 2nd Ed. Philadelphia: Elsevier, 2004;258-59.
13. Kinni SR. Serous Effusions. In: Mtichell W. *Colour atlas of Differential Diagnosis in Exfoliative and Aspiration Cytopathology*. 1st Ed. USA: Williams & Wilkins, 1999;119-142.
14. Mao YY, Yang M, Lui DG, Lin MH, Zhang LQ, Chen ZQ. Evaluation of immunohistochemistry staining and cytologic diagnosis by using cell block sections prepared with effusion fluid cytology specimen. *Zhonghua Bing Li Xue Za Zhi* 2009; 38:547-50.
15. Robertson KC, Dahlberg SE, Edberg SC. Clinical and Laboratory Analyses of Cytospin Prepared Gram Stains for Recovery and Diagnosis of Bacteria from Sterile Body Fluids. *J Clin Microbiol* 1992;30:377-80.

Fig. 1: From same case showing atypical cells with better appreciated morphology on cyto centrifuge smear.

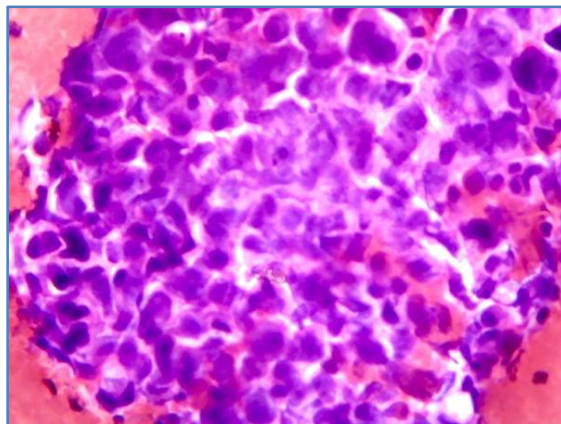


Fig. 1

ORIGINAL ARTICLE

Fig. 2: From same case showing signet ring cell further classifying the above case as mucin secreting adenocarcinoma.

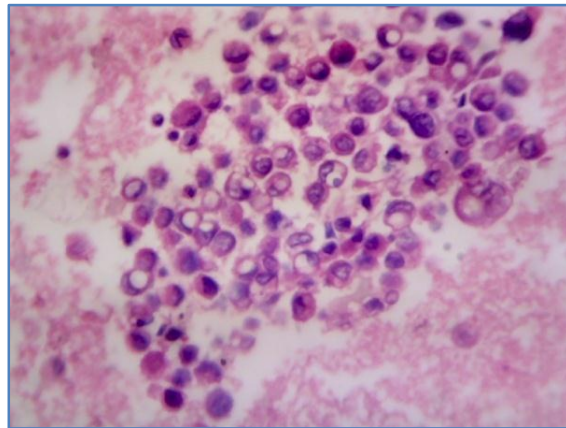


Fig. 2

Fig. 3: Showing clusters of atypical cells diagnosed as poorly differentiated carcinoma on conventional smear.

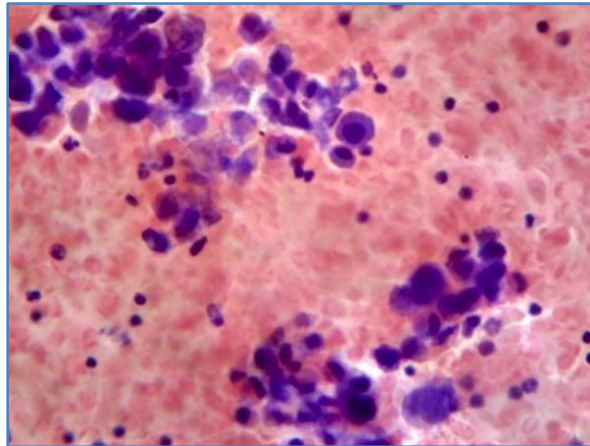


Fig. 3

AUTHORS:

1. Mahendra Singh
2. Lubna Khan
3. Yogendra N. Verma
4. Neelima Sachan
5. Chayanika Pantola
6. Adrija Pathak
7. Rachita Gulati
8. Anuradha Gautam

PARTICULARS OF CONTRIBUTORS:

1. HOD, Department of Pathology, GSVM Medical College, Kanpur.
2. Associate Professor, Department of Pathology, GSVM Medical College, Kanpur.
3. Lecturer, Department of Pathology, GSVM Medical College, Kanpur.
4. Junior Resident, Department of Pathology, GSVM Medical College, Kanpur.

FINANCIAL OR OTHER

COMPETING INTERESTS: None

5. Assistant Professor, Department of Pathology, GSVM Medical College, Kanpur.
6. Junior Resident, Department of Pathology, GSVM Medical College, Kanpur.
7. Junior Resident, Department of Pathology, GSVM Medical College, Kanpur.
8. Junior Resident, Department of Pathology, GSVM Medical College, Kanpur.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Yogendra N. Verma,
L-23, Medical College Campus,
GSVM Medical College,
Kanpur-208002.
E-mail: dryogendra82@gmail.com

Date of Submission: 24/01/2015.
Date of Peer Review: 30/01/2015.
Date of Acceptance: 20/02/2015.
Date of Publishing: 28/02/2015.