https://doi.org/10.46389/rjd-2024-689
www.jrdiabet.ro

Rom J Diabetes Nutr Metab Dis
2024; Volume 31, Issue 1, pages 689-697

A STUDY ON DIAGNOSTIC UTILITY OF CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY

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Abstract

The cytological examination of serous fluids like pleural and peritoneal effusions are well accepted and their positive diagnosis is mostly considered as a definitive diagnosis. It is helps in staging, prognosis, management of patients with malignancies, and it also gives the information about various inflammatory and non-inflammatory lesions. Routine conventional methods have a limitation in differentiating between reactive mesothelial cells and malignant cells Thus Cell Block method overcomes these limitations because of its increased cellularity, preservation of tissue architecture and feasibility of performing immunohistochemistry. This paper aims to compare the sensitivity, specificity and positive predictive value (PPV) of cell blocks and conventional smears of pleural and peritoneal fluid specimens in diagnosing malignancy and to calculate the efficacy of cell blocks in typing malignancy. This study was conducted to compare the diagnostic efficacy parameters of cell block method and routine conventional smears in pleural and peritoneal fluids in the cases of suspected malignancy. A one year study was done in a Government Medical

College on pleural and peritoneal fluids over the suspected cases of malignancy. The total number of cases studied were 140, while specimens taken were a total of 160 pleural and peritoneal fluids. The cytology smears were stained with Papanicolaou stain and cell block preparations of centrifuged deposits were processed, cut at the size of 5 micrometers and the sections were stained by Hematoxylin and Eosin in every case. In the cell blocks slides additional stains and immunohistochemistry were done as required. Diagnosis of malignancy for any tissue based method within a period of 3 months of follow up was taken as the gold standard for analyzing the results. Sensitivity of cell blocks being 0.6521 was closed to double to that of routine cytological smears which was 0.2879. Both the methods were very high in specificity. Cell block method was proved to be superior to conventional smears in terms of pattern recognition and have more advantage whenever there is need for immunohistochemistry. The use of cell blocks in adjunct with conventional cytological smears of body fluids can increase the sensitivity to a considerable extent. It is used further in affirming the diagnosis by pattern recognition or immunohistochemistry.

Keywords: Cell block method, Conventional smear method, Pleural effusion, Peritoneal effusion, Sensitivity

Introduction

The cytological examination of body fluids is plays an important role not only in the diagnosis but also for staging, prognosis and clinical management of the patient. According to Marel M et al cytological study is considered as the best for establishing the diagnosis of pleural fluid malignancies[1]. The preparation of routine cytological smears (CSs) of is simpler process than paraffin sections, it also has low sensitivity for the detection of malignancy. This is attributed to lack of tissue architecture, overcrowding and overlapping of cells, cell loss, artifacts because of suboptimal processing and delaying, huge amount of reactive mesothelial cells, scarcity of representative cells, large amount of inflammatory cells concealing the morphology of atypical cells, mild cyto-morphological features of some of the malignant neoplasms and the useful material is left behind during processing[2,3,4,5]. Even for an experienced observer, the accurate identification of cells as either malignant or mesothilial is always a challenge in conventional cytological smears. The storage of slides in CS study is a practical problem [6,7]. The cell blocks prepared from the residual tissue fluids and fine needle aspirations can be useful when used simultaneously with smears for getting more definitive cyto- pathological diagnosis. They are particularly useful in categorizing tumors that are otherwise not possible alone with smears. It is also useful whenever there is need for special stains or immunohistochemistry. Preparation of Cell blocks is time taking but it has the following advantages to offer, like after completion of cytological preparations the residual material mostly contains a valuable diagnostic evidence which includes tissue fragments. The cell block method additional information which is important in solving diagnostic dilemmas. It also provides additional information like cell enrichment, lesser cellular dispersal, preservation of specific tissue architecture, better morphological details, the

familiarity of Haematoxylin and eosin stain and feasibility to perform ancillary studies like special histochemical stains and immunohistochemistry [2,5,7]. The smears along with cell blocks provide the definitive diagnosis. The storage of slides and blocks of CB preparation for the retrospective studies is comparatively easier than smears.

Methodology

160 samples of both pleural and peritoneal fluid from 140 cases were sent to cytopathology laboratory and it constituted the material for the study. Patients with clinical and radiological evidence of malignancy were included in the study. The duration of the study was one year. Consent taken at the time of fluid tap was considered for the study and hence no separate consent was taken. All the samples were initially examined for color and appearance of the fluid. The samples were centrifuged at 2000 rpm for 5 minutes, the supernatant was discarded and the routine smears were prepared from cell button. The smears were stained with Papanicolaou stain. The cell button which was remaining was centrifuged with 203 drops of supernatant at 2000 rpm for 10 minutes. The fixative AAF i.e. Absolute achohol, Glacial acetic acid and 40 % Formaldehyde was added thrice the volume of the material and it was centrifuged for 10 minutes at 2000 rpm.

The test tube was kept in slanting position for about 4-6 hours. The cell button was scrapped and wrapped in filter paper, the gauze piece and paraffin were embedded in the same way as that of the routine biopsy specimens. The sections were taken from these blocks and then the slides were stained with Hematozylin and Eosin stain. Both the smears and the blocks were examined separately. Amongst the following anyone was considered as the gold standard for the confirmation of the diagnosis.

a) Direct FNAC or biopsy of lesion
b) FNAC or biopsy of lymph nodes
c) Sputum cytology
d) Bronchial washing
e) Peritoneal washing

If there were sufficient clinical and radiological evidence for malignancy and the fluid samples also revealed malignant cells they were considered as true positives. In suspicious cases, the immunohistochemistry was done for the confirmation. In case of a negative result the cases were followed for three months period.

Results and Analysis

The results of both the conventional smears and cell blocks were analyzed to calculate the value for sensitivity, specificity, positive predictive value and negative predictive value in relation to their gold standard.

The total number of cases studied were 140 out of which there 85 males and 55 females. From these 140 cases, 160 samples were collected. In 160 samples, pleural fluids were in majority i.e

120 (75%) samples and rest 40 samples (25%) were of peritoneal fluids. Ascitic fluids were 29 samples i.e. 24%. Out of the total samples. Out of the total samples 46% (74 samples) were malignant effusions while 49% (78 samples) were reactive effusions. Out of 74 malignant effusions there were 20 ascitic fluid samples i.e. 27% while the rest 54 were pleural fluids (73%). Out of the total 78 samples of reactive effusions 73% i.e. 57 samples were of pleural effusion. Age wise distribution of the sample is shown in the **Figure 1**.

The group of "malignancy" includes all those cases who had positive tissue diagnosis. The malignant effusions being 74 in number i.e. 46.2% cases. The reactive effusion were 78 in number i.e. 48.7% were categorized as "no malignancy". In the remaining 5.1% of the cases either there wasn't any follow-up or the patient had expired. Expired were the cases who died before any confirmative tissue diagnosis could be made. These cases were removed from the further analysis. The actual diagnosis in conventional smears and cell blocks in relation to the gold standard or the eventual diagnosis is shown in the **Table 1 and 2**. The sensitivity, specificity, positive and negative predictive values for the diagnosing malignancy which is calculated from the Tables 1 and 2 is shown in the **Table 3 and 4**. To calculate these values only two of the above parameters were taken into consideration i.e. malignant cells and no malignant cells. The diagnosis atypical cells, suspicious cells and no material was omitted from the study. The diagnostic efficacy parameters for malignancy in smears were compared in specific group also with ascitic fluid, pleural fluid, and epithelial malignancy **(Table 3 and 4).**

Comparing the results of whole samples, cell blocks had a higher sensitivity of 0.6521 while smear showed nearly half of it i.e. 0.2879. Specificity of both are almost equal.

Table 1: Diagnosis in Conventional cytological smears in relation to final diagnosis

Smear diagnosis	**Final diagnosis**		**Total**
	Malignant	**Non Malignant**	
Malignant cells	19	0	19
Atypical cells	5	2	7
Suspicious for malignancy	3	0	3
No malignant cells	47	76	123
Total	74	78	152

Table 2: Diagnosis in Cell blocks smears in relation to final diagnosis

Smear diagnosis	**Final diagnosis**		**Total**
	Malignant	**Non Malignant**	
Malignant cells	45	2	47
Atypical cells	1	2	3
Suspicious for malignancy	0	0	0

No malignant cells	24	70	94
No material	4	4	8
Total	74	78	152

Table 3:- Diagnostic efficacy parameters in Conventional cytological smears (95% CI)

Parameter	Whole cases	Ascitic fluid	Pleural fluid	Epithelial malignancy
Sensitivity	0.2879	0.2964	0.29	0.2542
Specificity	1	1	1	Nan
Positive predictive value	0.1352	0.1363	0.1323	0.2542
Negative predictive value	0.8647	0.8636	0.8676	0.7457

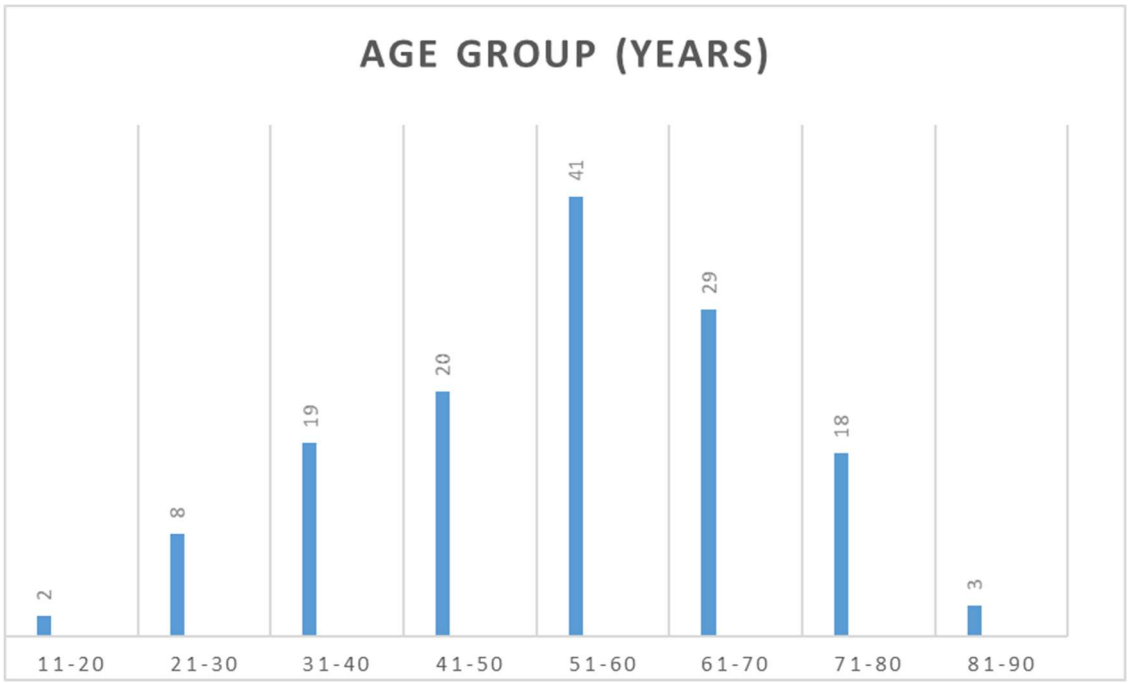
Note - NaN in the above table means that the calculation cannot be performed because the values entered during the calculation had one or more instances of zero and CI means Confidence Interval.

Parameter	Whole cases	Ascitic fluid	Pleural fluid	Epithelial malignancy
Sensitivity	0.6521	0.8743	0.6165	0.6828
Specificity	0.9812	1	0.9824	Nan
Positive predictive value	0.3278	0.3854	0.3219	0.6828
Negative predictive value	0.6721	0.6145	0.6780	0.3171

Table 4:- Diagnostic efficacy parameters in Cell blocks (95% CI)

Note - NaN in the above table means that the calculation cannot be performed because the values entered during the calculation had one or more instances of zero and CI means Confidence Interval.

Figure 1 – Age structure of the sample



Discussion:

Cytological evaluation is the best way to observe and detect the presence of malignant cells in the body cavity fluids. Many studies have the shown the results that fluid examination is superior in diagnosing malignancy [8]. The diagnostic yield is dependent on factors like extent of the disease and the nature of primary malignancy. Most of the laboratories prefer conventional smear over cell blocks. A study did by Oyafuso et al on4297 fluid samples had shown the sensitivity, specificity, efficiency, positive and negative predictive values of smears as 44.55%, 95.7%, 50.1%, 98.7% and 20% respectively [9]. Similar results were obtained by Mother et al also [10]. These studies from the literature show that diagnostic accuracy of effusion cytology by means of conventional smears in not satisfactory and should be improved. Thus the various adjuvant methods were considered. The use of cell block technique has gained considerable acceptance in many years. In this contest it was assessed that whether the diagnostic accuracy can be increased if they are used together. In a study of effusions conducted by Meenu Thaper et al [11]out of 190 cases studied, 120 i.e. 63.15% cases were of different reactive effusions and 70 i.e. 36.85% cases were of malignant effusions. Out of the 120 cases of reactive effusions, 48.3% were of pleural effusions, 45% peritoneal effusions and 6.7% pericardial effusions were seen. The majority of the cases i.e. 22 cases, 18.33% were of tuberculosis. In this study the proportion of malignant and reactive effusions are nearly equal. Tuberculosis is the most common cause of reactive effusion.

Amongst 120 pleural fluid samples, 54 i.e 45% were diagnosed as malignant and 66 samples i.e 55% were reactive effusions. Adenocarcinoma was the most common cyto-pathologic diagnosis seen in the malignant pleural effusions in the study. Out of the diagnosis made during the study, Primary adenocarcinoma of lung had the 50% of the share. In 4cases the diagnosis non-small cell

carcinoma was given as the clear differentiation could not be made. In many cases the primary site could not be identified, although adenocarcinoma was the confirmed. Breast was identified as the primary site in 8 cases. Lymph nodes were also involved in 4 cases. Squamous cell carcinoma was the uncommon of malignant effusion. Small cell carcinoma was also not common, both of them had 2 cases each. Out of the reactive effusions nearly half of the cases were due to tuberculosis.
Amongst Ascitic fluids, 40% were malignant and 56 % were reactive. Adenocarcinoma of GI tract accounted for the most of the cases of malignant ascites followed by carcinoma of ovaries. Among the case of reactive effusions, cirrhosis accounted for 37.6% followed by tuberculosis (5 cases). Out of the 74 samples of malignant effusions, 19 i.e. 26 % samples were reported as positive for malignancy by the conventional cytological smears. While cell block method had shown 45 positive cases of malignancy i.e. 61%. As compared to the study Thaper's study had similar findings, but much lower was for smears. Sensitivity of smears in demonstrating malignancy is 28.79% in this study, while for cell blocks it is 65.21 %. But for both conventional smears and cell blocks the specificity is very high. In the studies of Shafigh at al [12] and Nathan et al [13] the sensitivity of smears and cell blocks is similar whereas the cell blocks have proved to be superior in our study. According to the literature, the reason for lower sensitivity can be because of the limitations in the methodology, invasive nature of the neoplasms and few sampling errors. (). The following reasons can be stated:

a) In majority of the cases only one specimen was examined.
b) Pick up rate can be increased if 4 slides are examined in each case. In our study the slides examined were either 1 or 2.
c) The preparation technique may have some faults that require correction.
d) Cell morphology is difficult to interpret whenever there is hemorrhagic background.

Sensitivity of cell block in ascitic fluid is higher than pleura fluid, it is in accordance with Mother et al[10]Specificity of both the conventional smears and cell blocks is almost equal. There were only 2 cases that that were falsely reported as malignant in cell blocks. Basically both of them were the cases of tuberculous effusion and there was monotonous population of the reactive mesothelial cells showing anisonucleosis. Distinction of reactive mesothelial cells from the malignant cells is always a diagnostic concern in cytodiagnosis of serous fluids like pleural and peritoneal fluids. In such situations immunohistochemistry can be of great help.
Examination of Papanicolaou stained smears have various advantages. It is a quick procedure, it does not need extra time for processing and cutting of the paraffin blocks. All it requires the expertise in identifying the malignant cells in the smear. The cell blocks being a tedious process have many advantages to offer. If the material is copious then it gets very difficult to sample in case of conventional smear. Preparation of cell blocks solves this problem. Cell block is also useful when special stains are required. The block can be easily stored and multiple sections can be taken. Cell blocks play an important role in immunohistochemistry. In case of cytology slides the de-staining of slides for immune stating is a laborious task and also results in loss of material. Also the results from the immune staining smears is poor unless the procedure is frequently practiced.

In this study, most of the malignant effusions were adenocarcinomas, either primary or malignant. Cell blocks had better sensitivity than the smears in detecting epithelial malignancies. There were 4 cases of squamous cell carcinoma but neither conventional smear nor the cell blocks could pick it. As far as lymphomas were concerned the 4 cases of T cell lymphomas, 2 cases of follicular lymphoma and 1 case of diffuse large B cell lymphoma were seen.

Conclusion

The study concludes that for cytological examination of all fluid samples, smears should be used in conjunction with cell blocks methods in order to increase pickup rate speciaaly in case of suspected malignancy. This becomes very important when the repetition of samples is not feasible. So the sensitivity of the can be increased to certain extent when the cell clocks are used as an adjunct to smears. This can be further used in highlighting the diagnosis by pattern recognition or immunohistochemistry.

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