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Does pleural fluid cell block is alternative to Thoracoscopy guided diagnostic techniques in malignant pleural effusion? Study of 500 cases in tertiary care setting in India

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Abstract

Background: Malignant pleural effusion missed routinely because of less diagnostic yield of conventional fluid cytology like fluid cytology. Fluid cell block is underutilsed techniques routinely and less utilized in diagnostic panel due to lack of expertise in filed of cytopathology. **Materials and methods:** Prospective multicentric study conducted during Jan 2014 to June 2020 in Venkatesh chest hospital, and Pulmonary Medicine, MIMSR medical college Latur, to find out diagnostic yield of conventional pleural fluid cytology& pleural fluid 'cell block' in malignant pleural effusion and compare yield of pleural fluid cell block with conventional cytology technique. The study included 500 cases of unexplained, exudative pleural effusion with ADA<30/IU/liter and pleural fluid cytology is either positive for malignant cell with or without cell type differentiation, or cytology suspicious for malignant cell. All cases were subjected to cell block preparation. Statistical analysis was done by using chi-test. **Observation and analysis:** In study of 500 cases, mean age of group was 68±9.5 years and adenocarcinoma were predominant malignancy in 79% cases, mesothelioma in 6% cases, squamous cell carcinoma in 7% cases & 8% cases were having primary tumor outside the thoracic cavity. In study cases pleural fluid cytology was positive in 42% cases (210/500), and pleural fluid cell block was positive in 96% cases (480/200) in detecting malignant pleural effusion (p<0.0001), Remaining six and two cases were diagnosed by using image guided and thoracoscopy guided pleural biopsies respectively. IHC was done in all pleural fluid cell block preparation for calretinin, cytokeratin and EGFR. **Conclusion:** Pleural fluid cell block is sensitive, superior, cost effective and specific diagnostic method over conventional pleural fluid cytology. 'Cell block' specimens are enough for primary diagnosis and IHC analysis necessary for cell typing. It will decrease need for more invasive and costlier diagnostic methods like thoracoscopy and image guided pleural biopsies. We recommend cell block for every exudative pleural fluid sample with ADA<30 IU/liter.

Keywords: Malignant Pleural Effusion, Pleural Fluid Cell Block, Cytology, Lung Cancer.

INTRODUCTION

Lung cancer is a leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for around (80%-85%), of lung cancers [1]. Although pleural effusion is one of the clinical signs of malignant disease, its accurate diagnosis is sometimes difficult. Determining the diagnosis of pleural effusion is important in planning the appropriate management and in the prognostication of the malignant disease [2-4]. Thoracentesis and/or closed pleural biopsy are generally considered as the first step for diagnosis of pleural effusion because these procedures can be easily performed even in outpatients. Some studies have reported that the diagnostic yield of cytology by thoracentesis was (62%), to (90%), and that of closed pleural biopsy was (40%), to (75%) [4].

Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions [5]. Accurate identification of the exact nature of cells (benign/ malignant/reactive) is often a practical problem in conventional cytology smears (CS), due to overcrowding of cells, cell loss and different laboratory processing methods [6].

On the other hand, cell block is also a useful method to evaluate pleural effusion by enabling observation of tissue architecture and providing additional sections that are easily available for special stains and immunochemistry [7,8].

Quincke in 1882, first published detailed description of cancer cells in abdominal and pleural fluids using cell films from sediment [9]. while Bahrenburg first introduced cell block technique or paraffin embedding of sediments in 1896 [10]. Many techniques for CB are described like the plasma thromboplastin method [11], bacterial agar method [5,11], simplified cell block technique [11,12], compact cell block technique [13], histogel technique [14], and Fixed sediment method (FSM) [11].

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In this study we assessed diagnostic yield of pleural fluid cell block in comparison to conventional fluid cytology. We also assessed utilization of cell block specimens for immunohistochemistry analysis.

MATERIALS AND METHODS

Prospective multicentric study conducted in Venkatesh chest hospital, and Pulmonary Medicine, MIMSR medical college Latur during Jan 2014 to June 2020, to find out diagnostic yield of conventional pleural fluid cytology in malignant pleural effusion and its comparison with pleural fluid cell block specimens. We also analyzed immunohistochemistry analysis of cell block specimens. Total 500 cases of unexplained, exudative pleural effusion were enrolled in study after ethical committee approval and written informed consent of patient.

Inclusion criteria

1. Recurrent and unexplained, exudative pleural effusion.
2. Exudative pleural effusion with pleural fluid ADA <30/IU/liter
3. Hemorrhagic or reddish colored pleural effusion with ADA <30/IU/liter.
4. Pleural fluid cytology is either positive for malignant cell with or without cell type differentiation, or cytology suspicious for malignant cell.
5. Clinical and radiological feature suggestive of malignant pleural effusion.

Exclusion criteria

1. Transudate pleural effusion.
2. Exudative pleural effusion with ADA>44 IU/Liter and high index of suspicion for tuberculosis.
3. Bilateral pleural effusion with co-morbidity like heart failure, kidney disease, or hypoproteinemia, anemia.
4. Cases not willing to participate in study or not willing for pleural fluid aspiration.

Methodology

Cases attending outdoor unit after scrutinizing inclusion and exclusion criteria with high index of suspicion of malignancy on clinical and radiological criteria were enrolled in study. All 200 study cases undergone pleural fluid aspiration and at least 100 ml pleural fluid is aspirated as per standard guidelines for thoracentesis. Pleural fluid aspiration was done under ultrasound guidance and aspirated fluid was divided in to two aliquots, one sent for cytology and second for cell block preparation. All study samples were evaluated by two different cytopathologists and oncopathologists having expertise in field of thoracic oncology. Those cases not diagnosed by fluid cytology or cell block were undergone image guided pleural biopsy and thoracoscopy guided pleural samplings to confirm the diagnosis.

Procedure of pleural fluid cytology

We usually take 20 ml pleural fluid and centrifuged @ 2500 RPM for 10 minutes and supernatant discarded and three to five smears prepared from sediment and sent for cytology analysis. Then one to two smears was prepared after air drying and it was stained with the May-Grunewald-Giemsa stain. The other two smears were immediately fixed in (95%), alcohol, and were stained with Hematoxylin-Eosin stain.

Cytology results were categorized as-

1. Cytology suspicious for malignant cells or malignant cells with undifferentiated morphological type.
2. Cytology showing clear morphological malignant cells differentiation.
3. Cytology negative for malignant cell or showing benign cellularity.

Procedure of 'pleural fluid cell block

Cell block processing for serous effusion- Modified Thromboplastin method is used. After centrifugation at 2500 rpm for 10 minutes, drain the supernatant or pipette out the supernatant cell and residual sediment was formed. Excess supernatant was blotted out, 2 drops of plasma added to the tube and then 4 drops of thromboplastin added and allowed to clot for 20 minutes. Then inert the tube and collect the cell block on filter paper. We followed standard protocol for cell block preparation and all specimens were embedded in paraffin and sectioned at 4 µm thickness (process clot as any tiny biopsy specimen), and sent for IHC analysis after histology confirmation.

Technical considerations for cell block preparation as we specifically recommend are:

1. Pooled plasma remains well in a freezer up to one month.
2. Thromboplastin is to be kept in the refrigerator.
3. Reagents should be brought to room temperature before processing.

Cell block preparation results were categorized as-

1. Histology showing malignant cell undifferentiated type.
2. Histology showing malignant cells with exact differentiation.
3. Histology negative for malignant cell or showing benign cellularity.

Cell block specimens after primary evaluation and confirmation as malignancy was send for IHC analysis for reanalysis of primary diagnosis by cell block method and mutation analysis in tumor cells to avail exact treatment to have excellent treatment outcome. In IHC analysis, we specifically recommend for EGFR, ROS, Calretinin, Carcinoembryonic antigen and ALK analysis.

Cell block immunohistochemistry specimen's results were categorized as-

1. Confirmatory & sample sufficient
2. Confirmatory & sample insufficient

The statistical analysis was done using chi-squared test. *P* value was considered significant if it was below 0.05 and highly significant in case if it was less than 0.001.

RESULT

Total 500 patients between age group 31 to 90 years, with mean age (68±9.5), years, male population constitutes (66%), and females (34%), of total. In study cases only (9%), cases were smoker. Commoner symptoms were shortness of breath in (91.33%), cough in (54.00%), and chest pain (46.66%), cases, and massive pleural effusion (42%), mass with effusion (28%), effusion with fixed mediastinum in (21%),

and bilateral pleural effusion (9%), were commoner radiological abnormalities.

In study of 500 cases with malignant pleural effusion, 210 cases were diagnosed by conventional cell cytology; out of which only 120 cases were diagnosed with clear histological type. Sensitivity of conventional cell cytology in detecting malignant pleural effusion is (42%), (Table 1).

In study of 500 cases with malignant pleural effusion, 480 cases were diagnosed by cell block histology technique; out of which 395 cases were diagnosed with exact histological type. Sensitivity of 'cell block' in detecting malignant pleural effusion is (96%), (Table 2).

In study of 500 cases with malignant pleural effusion, 480 cases were diagnosed by pleural fluid cell block; while only 210 cases were diagnosed by pleural fluid cytology. Pleural fluid cell block has very significant yield as compared to conventional pleural fluid cytology ($p<0.00001$), (Table3).

Pleural fluid cell block has (96%), (480/500), diagnostic yield as compared to conventional cytology having (42%), (210/500), diagnostic yield. Pleural fluid cell block has 2.28 times more detection rate than cytology ($p<0.00001$), (Table 4).

Immunohistochemistry analysis in pleural fluid cell block specimens were confirmatory and sample was sufficient for diagnosis in 90.83% cases, (Table 5).

DISCUSSION

Yield of pleural fluid cytology in study cases

In present study of 500 cases with malignant pleural effusion, 210 cases were diagnosed by conventional cell cytology; out of which only 120 cases were diagnosed with clear histological type. Sensitivity of conventional cell cytology in detecting malignant pleural effusion is (42%), Studies by Rivera *et al* [15]. McGrath et zl [16]. Gupta *et al* [17]. and Hooper *et al* [18]. observed average yield in 60 percent cases, ranging from 40 to 87 percent.

Various studies by Köksal D *et al*. [19], Jing X *et al*. [20], Ugurluoglu C *et al*. [21], and Bhanvadia VM *et al* [22]. documented problem of cell loss, poor background, overlapping and overcrowding whenever hemorrhagic pleural effusion is present and all these issues can be negotiated with cell block sampling.

Studies by Köksal D *et al* [19]. Ugurluoglu C *et al* [21]. Bhanvadia VM *et al* [22]. Dekker A *et al* [23]. and Shivakumar swamy U *et al* [24]. also documented difficulty in differentiating reactive mesothelial cells from malignant process in mesothelial cells, which can be easily made in cell block samples.

Table 1: Yield of pleural fluid cytology in study cases (n=500)

	Yield positive (n=500)	Percentage
Cytology suspicious for malignant cells or malignant cells with undifferentiated type	90	18
Cytology malignant cells differentiation	120	24
	210/500	42

Table 2: Yield of pleural fluid 'cell block' in study cases

	Yield positive (n=500)	Percentage
Histology showing malignant cell undifferentiated type	85	17
Histology showing malignant cells with exact differentiation	395	79
	480/500	96

Table 3: Comparison of pleural fluid cytology and 'cell block' in confirmed cases by these techniques in study cohort (n=480/500)

	Pleural fluid cytology Positive yield (n=210/500)	Pleural fluid 'cell block' Positive yield (n=480/500)
Histology showing malignant cell undifferentiated type	90	85
Histology showing malignant cells with exact differentiation	120	395
Total	210	480

$\chi 2 = 19.52$, $df = 1$, $P < 0.00001$

Table 4: Comparison of overall yield of pleural fluid cytology and 'cell block' in study cohort

	Positive yield	Negative yield
Pleural fluid Cytology (n=500)	210	290
Pleural fluid 'Cell block' (n=500)	480	20

$\chi 2 = 136.32$, $df = 1$, $P < 0.00001$

Table 5: IHC analysis on pleural fluid 'cell block' specimens

	Cell block (n=480)	Percentage
Confirmatory & sample sufficient	436	90.83
Confirmatory & sample insufficient	44	9.17

Yield of pleural fluid cell block in study cases

In study of 500 cases with malignant pleural effusion, 480 cases were diagnosed by cell block histology technique; out of which 395 cases were diagnosed with exact histological type. Sensitivity of 'cell block' in detecting malignant pleural effusion is (96%).

Pleural cell block is easier and it will help in preserving histological characteristics of malignant cells as compared to conventional pleural fluid smears [24]. In previous studies by Nathan NA *et al* [7]. Kern WH *et al* [25]. Axe SR *et al* [26]. Wojcik EM *et al* [27]. Leung SW *et al* [28]. and Norimatsu Y *et al* [29]. in their study observed sensitivity of cell block varied widely from (60%) to (89.4%), may be due heterogenicity in sample volume, aspiration technique, sample type. Thapar M *et al* [6]. & Nathan *et al* [7]. observed (65.7%), & (92.7%), yield respectively. Shion Miyoshi *et al* [30]. observed diagnostic yield of thoracoscopy was (94.2%), and cell block was (71.4%), for malignant pleural disease.

Cellularity is higher by cell block compared with fluid cytology and is concentrated in one small area that can be evaluated at a glance, with all cells lying in the same focal plane of the microscope [23,24]. In addition, cell block provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin; there is also less difficulty in microscopic observation, in spite of the presence of excess blood in the background [24].

Comparison of cytology and cell block in study cases

In study of 500 cases with malignant pleural effusion, 480 cases were diagnosed by pleural fluid cell block; while only 210 cases were diagnosed by pleural fluid cytology. Pleural fluid cell block has very significant yield as compared to conventional pleural fluid cytology ($p<0.00001$), Some studies by Udasimath *et al* [24]. & Thapar M *et al* [6]. have shown additional cases of malignancy on CB by increasing the diagnostic yield by (9%), and (20%), respectively. Various studies by authors Thapar M *et al* [6]. Köksal D *et al* [19]. Ugurluoglu C *et al* [21]. Bhanvadia VM *et al* [22]. Shivakumar swamy U *et al* [22]. showed an additional diagnostic rate of CB to CS around (10–15%), in MPE.

In a study by Dekker *et al* [23]. & Khan *et al* [31]. documented additive yield of cell block in (38%), & (20%), cases respectively with negative cell cytology in malignant pleural effusion. Thaper *et al* [6]. showed a diagnostic yield of (20%), by cellblock preparations. In a study done by Khan *et al* [31]. additional findings were diagnostic in (16%), of malignant cases.

Similar findings were also observed in studies by Dekker *et al* [23]. Takagi *et al* [9]. Chapman *et al* [32]. Vellios *et al* [33]. and Ceyhan *et al* [21]. also documented additional yield of cell block over conventional cell cytology in addition to preservation of cytomorphologic features with minimal shrinkage and aberration.

Contradictory to our observation studies by Shafigh *et al* [34].and Nathan *et al* [7]. documented sensitivity of smears and cell-blocks tended to be similar. Kung *et al* [35]. specially mentioned in their study that cell block entails a risk of losing material during preparation, and fixation and chances of false negative block results in positive conventional fluid cytology scenario.

In this study, pleural fluid cell block has (96%), (480/500), diagnostic yield as compared to conventional cytology having (42%), (210/500), diagnostic yield. Pleural fluid cell block has 2.28 times more detection rate than cytology ($p<0.00001$), In a study by Dekker *et al* [23]. double yield of cell block over fluid cytology smears.

The advantages of the cellblock preparation as we observed in our study are-

1. It will help in identifying exact histological types in many cases whenever cytology is not very sure and having doubtful reports.
2. It will also help in processing the samples as of followed in histopathology samples, special staining's can be possible including immunohistochemistry analysis.
3. Cellularity is well preserved and additional help in identifying histology whenever IHC is not available.
4. In hemorrhagic pleural effusion, in spite of addition of hemolysis after use of chemicals, still lot of hemorrhagic background in smears make it difficult to report as compared to cell block where many such obstacles are not present and ease of reporting.
5. Cost effective to image guided biopsy.
6. Cost effective and less invasive to thoracoscopy guided procedure.
7. It will help to restore slides and slides with block specimen will have additional help in reanalyzing whenever required.

Immunohistochemistry analysis in study cases

In our study we documented that, immunohistochemistry analysis in pleural fluid cell block specimens were confirmatory and sample was sufficient for diagnosis in (90.83%), cases. Analysis was done for EGFR, ROS, calretinin, carcinoembryonic antigen and ALK mutation. Authors Wang W *et al* [35]. Zhou J *et al* [37]. and Liu X *et al* [38]. reported diagnostic yield of cell block in malignant pleural effusion with (81.8%), sensitivity and (80%), specificity in EGFR mutation and (62.5%), to (100%), sensitivity and (100%), specificity for ALK detection. Ensani *et al* [39]. and Ikeda *et al* [40]. also documented similar observation as it will increase diagnostic yield in addition to help in analyzing mutation analysis by IHC.

In our study we documented that, immunohistochemistry analysis in pleural fluid cell block specimens were confirmatory and sample was insufficient for diagnosis in (9.17%), cases. Study by Shion Miyoshi *et al* [30]. documented similar findings in their study because of less cellularity in block and smear as well.

Studies by authors, Esteban JM *et al* [41], Mason MR *et al* [42], Doglioni C *et al* [43], Ellen C *et al* [44], and Cibas ES *et al* [45], documented that distinction of reactive mesothelial cells from malignant cells is always a

diagnostic concern in cyto-diagnosis of serous fluids. In such situations immunohistochemistry may be helpful.

Other important observations in study

A) Does volume of pleural fluid makes difference in yield?

In this study, we observed pleural fluid cell block has (96%), (480/500), diagnostic yield as compared to conventional cytology having (42%), (210/500), diagnostic yield with 100 ml pleural fluid sent for analysis. Baumann MH *et al* [46]. observed that malignant cells are considered to be present heterogeneously within the pleural effusion and can be precipitated by gravity. Position of the patient's body and the site of puncture may affect the diagnostic yield of cytology or cell block from thoracentesis. Shion Miyoshi *et al* [30]. observed added benefit of thoracoscopy guided pleural fluid analysis as heterogenicity of pleural fluid sampling will be less and typing will be easier and also documented added yield in cases with hypocellular smears. A prospective study by Swiderek J *et al* [47]. and Shion Miyoshi *et al* [30]. observed 150 ml pleural fluid will be sufficient to diagnose malignant pleural effusion.

B) Other diagnostic modalities used in study

In this study, 480 cases were diagnosed by pleural fluid cell block technique. Remaining two and eighteen cases were diagnosed by using image guided and thoracoscopy guided pleural biopsies respectively. Reason for required thoracoscopy in these cases were possible hypocellularity in pleural fluid and lesser shedding of malignant cells in pleural fluid. Norimatsu Y e al [29]. Sweeney BJ *et al* [48]. documented that possible hypocellularity or hemorrhagic pleural fluid were reasons for less yield and addition of hemolytic agents will have benefit in increasing yield of pleural fluid smear and block both.

We have used thoracoscopy in eighteen cases and reached diagnosis in them, approximately yield is (100%), Boutin C *et al* [49]. and Tassi GF *et al* [50]. observed yield in more than 90 percent cases and correlating with our study.

CONCLUSION

Pleural fluid cell block is more sensitive, superior, cost effective and specific diagnostic method over conventional pleural fluid cytology in malignant pleural effusion. 'Cell block' specimens are enough for primary diagnosis and IHC analysis necessary for cell typing. Results of cell block are comparable to more invasive and costlier diagnostic methods like thoracoscopy and image guided pleural biopsies. Additionally, it will decrease need for thoracoscopy guided techniques, especially in resource limited setting like India where availability and cost factor make more difference. Although, Thoracoscopy guided techniques are currently considered as 'Gold standard' for undiagnosed exudative pleural effusion, Pleural fluid cell block is good alternative to it.

We recommend 'cell block' as 'most preferred' test for every exudative pleural fluid sample with ADA<30 IU/liter to have early diagnosis. More emphasis should be given to pleural fluid cell block analysis training.

"Compliance with Ethical Standards" –

1. Funding-nil (no funding or any grant utilized)
2. Disclosure of potential conflicts of interest- NIL
3. Informed consent was taken in all cases before procedure as institutional protocol.

Conflicts of interest

None declared.

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