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Original Research Article

Diagnostic utility of cytospin, cell block and immunocytochemistry in pleural effusion cytology

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ABSTRACT

Background: With the introduction of cytospin, the sensitivity of diagnosing malignancies has increased mainly due to the increase in cellular yield. Cell block also gives the advantage of ancillary testing and allows for retrospective studies. Immunocytochemical markers are used to differentiate and subtype various malignancies in body effusions. Aim of the study was to compare the morphological features of both technique and to assess the diagnostic utility of cell block methods in the cytodiagnosis of pleural effusions.
Methods: This was a Prospective observational comparative study of two cytopreparatory techniques. All samples were examined and processed by cytospin and cell block techniques. Continuous data were expressed as Mean±SD (standard deviation) while categorical data were expressed in number, percentage and compared by chi-square ($\chi 2$) test.
Results: The final diagnosis of both cytospin (147 cases) and cell block (150 cases) techniques was divided into four broad categories: Inadequate, Benign, Suspicious and Malignant. The significant diagnostic cytospin (AUC=0.857, $p<0.001$) in discriminating positive and negative malignant cases with 75.00% sensitivity (95% CI=53.3-90.2) and 100.00% specificity (95% CI=86.7-100.0) and with 100.0% positive predictive value and 81.2% negative predictive value. In contrast, cell block also showed significant diagnostic but with higher accuracy (AUC=1.000, $p<0.001$) and sensitivity 100.00% (95% CI=86.7-100.0) and specificity 100.00% (95% CI=86.7-100.0) and 100.0% positive predictive value and 100.0% negative predictive value than cytospin technique.
Conclusions: Cell block as a technique should be used in routine practice as it not only increases the diagnostic yield but ancillary test can also be done.

Keywords: Cell block, Cytospin, Immunocytochemistry, Pleural fluid

INTRODUCTION

The cytologic study is considered to be the best for establishing a diagnosis of malignancy of pleural fluid.[1] Cytological examination not only helps for the diagnosis of cancer but also for staging and prognosis of disease.[2] It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease. Use of cell-blocks as an adjunct to routine cytology smears of body fluids can increase the sensitivity to a considerable extent.[3] The diagnostic performance of the cytologic study of 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.[4] With the introduction of cytospin the sensitivity of diagnosing

malignancies has increased mainly due to the increase in cellular yield and morphological preservation of cells.

Aim and objective of the study was to know the sensitivity, specificity, positive and negative predictive value of cytospin and cell block in pleural fluid cytology and to correlate with histopathology and immunocytochemistry.

METHODS

This was a Prospective Observational comparative study of two cytopreparatory techniques. This study was conducted in the department of cytopathology laboratory in collaboration with the department of Respiratory Medicine in tertiary care based hospital for one year. Study sample includes 152 pleural fluid samples.

Inclusion criteria

Pleural effusion with or without suspected case of malignancy.

Exclusion criteria

Treated patients and not willing for investigations

All samples were examined and processed to cytospin and alcohol-formalin cell block techniques. Cytospin smears were stained with May-Grunwald-Geimsa, Hematoxylin and Eosin stains. Sections taken from blocks were stained with haematoxylin and eosin stain. Smears and blocks were examined separately by two cytopathologists.In this study TTF-1, CA19-9, calretinin, cytokeratin and other immunocytochemistry markars (ICC) were applied on cell block according to the need.

Statistical analysis

Continuous data were summarised as Mean±SD (standard deviation) while discrete (categorical) in no. and percentage (%). Categorical groups were compared by chi-square ($\chi 2$) test. Sensitivity and specificity of Cytospin and Cell block against IHC was done using ROC (receiver operating characteristic) curve analysis considering IHC the gold standard. Concordance correlation coefficient analysis was done to assess association (concordance, precision and accuracy) between the variables. A two-tailed ($\alpha=2$) $p<0.05$ was considered statistically significant. Analyses were performed on SPSS software (windows version 17.0).

RESULTS

Total 152 sample were received irrespective of sex or age. Cytospin showed 147 cases which was adequate for cytodiagnosis while cell block showed adequate 150 cases for cytodiagnosis. 5 samples were inadequate in cytospin and 2 samples were inadequate in cell block technique. All cases were categories in to acute, chronic, mixed, suspicious, malignant and inadequate (Table 1). All acute, chronic, and mixed inflammatory effusion cases was broadly categories as inadequate:5, benign: 95, malignant:19, suspicious:33 in cytospin technique and in cell block, inadequate:2, benign:98, malignant:26, suspicious:26 (Table 2).

Out of 26 malignant cases in cell block were 10 metastatic adenocarcinoma from unknown origin, 5 from ovarian carcinoma, 3 from Squamous cell carcinoma, 2 of each infiltrating ductal carcinoma breast and malignant mesothelioma,1 of each pleomorphic sarcoma pleura, malignant lymphoma, adenocarcinoma stomach and adenocarcinoma colon where as in cytospin 09 metastatic adenocarcinoma from unknown origin, 5 from ovarian carcinoma, 1 Squamous cell carcinoma, 2 of each infiltrating ductal carcinoma breast and malignant mesothelioma. Immunocytochemistry (ICC) was performed on those cases (n=52) reported as suspicious or positive for malignancy using primary panel of CK(5/6), TTF-1, Calretinin, Ca19.9, and another ICC panel was further decided based on need. The results on ICC were compared with cytospin, cell block and histopathology.

Table 1: Various categories of cases in cytospin and cell block techniques.

Findings	Cytospin (n=152) (%)	Cell block (n=152) (%)
Acute	13 (8.6)	13 (8.6)
Chronic	50 (33.4)	47 (30.9)
Mixed	32 (21.2)	38 (25.0)
Suspicious	33 (21.7)	26(17.1)
Positive for malignancy	19(11.8)	26 (17.1)
Inadequate	5 (3.3)	2(1.32)

Table 2: Final diagnosis of cases in both cytospin and cell block techniques.

Final diagnosis		No. of cases (n) (%)
Cytospin (n=147)	Benign	95(64.6)
	Malignant	19 (12.9)
	Suspicious	33(22.5)
Cell block (n=150)	Benign	98 (65.34)
	Malignant	26 (17.33)
	Suspicious	26(17.33)

The patients were mostly 60-70 years (32.9%) aged followed by 50-60 years (21.7%) and median 55 years. Out of 152 patients, 66 (43.4%) were females and 86 (56.6%) were males. Among all patients, 88.8% have cough, 96.7% dyspnoea, 7.2% haemoptysis and 26.3% weight loss present.

On comparing, the various parameters of pleural fluid, $\chi 2$ test showed significant findings: cellullarity ($\chi 2=96.22$, $p<0.001$), cellular morphology and nuclear preservation

($\chi2$=7.07, p=0.029) and background ($\chi2$=84.31, p<0.001) between two techniques. However, findings of adequacy were found similar (p>0.05) between the two techniques that was did not differ significantly (Table 3).

Table 3: Comparison of cytospin and cell block using various parameters (n=152).

Findings		Cytospin (n=152) (%)	Cell block (n=152) (%)	χ^2 value	p value
Adequacy:	Inadequate	5 (3.2)	2 (1.3)	2.05	0.152
	Adequate	147 (96.8)	150 (98.7)		
Cellullarity	Paucicellular	100 (65.8)	21 (13.8)	96.22	<0.001
	Cellular	47 (30.9)	131 (86.2)		
	Hypercellular	5 (3.3)	0 (0.0)		
Preservation of cellular and nuclear details	Minimal to absent	4 (2.6)	1 (0.7)	7.07	0.029
	Moderate to some preservation	119 (78.3)	104 (68.4)		
	Excellent preservation	29 (19.1)	47 (30.9)		
Background	Large amount/Diagnosis compromised	3 (2.0)	0 (0.0)	84.31	<0.001
	Moderate amount/Diagnosis possible	122 (80.3)	46 (30.3)		
	Minimal/Diagnosis easy	27 (17.8)	106 (69.7)		

Both cytospin and cell block showed good cellular architecture but the overall findings in cell block in terms of cellular architectures like acini, cell ball, and papillary pattern also helped in giving a clue about the location of the primary tumor.

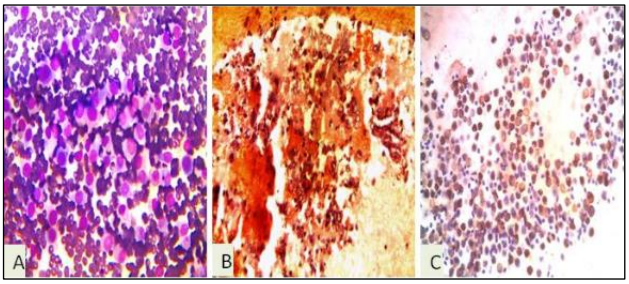

Figure 1: Non Hodgkins lymphoma: Monomorphic, round to oval atypical lymphoid cells diffusely arranged in hemorrhagic background. A and B:Cytospin (Giemsa stain, X40 &H&E Stain, X10), C:Cell Block (LCA Immunocytochemical stain, X10).

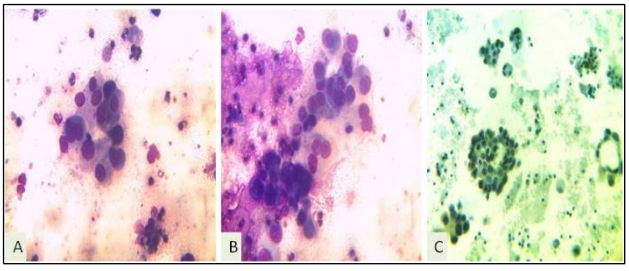

Figure 2: Mesothelioma: Atypical cells arranged in acinar and glandular pattern. Cytospin A and B (Giemsa stain, X40&H&E Stain, X40), C:Cell Block (Calretinin, ICC stain,X10).

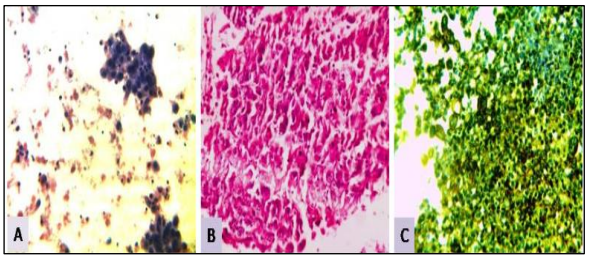

Figure 3: Squamous cell carcinoma: Malignant tumor cells arranged in sheets, clusters as well as lying singly in dirty background. A and B:Cytospin (Giemsa stain, X40 &H&E Stain, X40),C:Cell Block (CK5/6 ICC stain, X10).

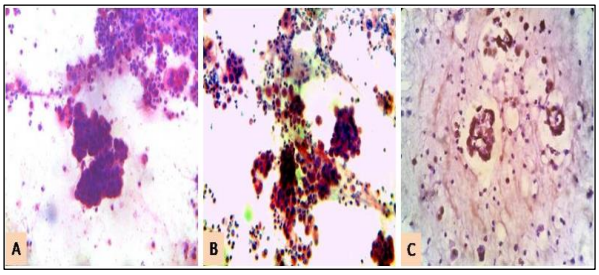

Figure 4: Lung primary adenocarcinoma: Malignant tumor cells arranged in glandular, in sheets as well as singly lying. A and B:Cytospin (Giemsa stain, X40 &H&E Stain, X40), C: Cell Block (TTF-1 ICC stain, X20).

Immunocytochemistry was done in 52 cases of suspicious and malignant pleural effusion. 26 cases were positive and 26 cases were negative in cell block where as in cytospin technique, 19 malignant cases were positive, 5 suspicious cases and rest 26 cases were negative and 2

cases were inconclusive. 26 cases were negative for the three primary ICC panels including Calretinin, CK (5/6) and TTF-1.

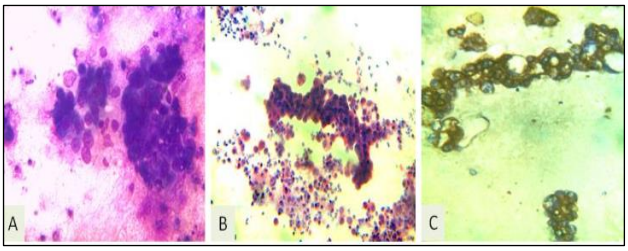

Figure 5: Metastatic adenocarcinoma: Malignant tumor cells arranged in glandular, in papillary pattern as well as singly lying. A and B Cytospin(Giemsa stain, X40) & H&E Stain, X20), C:Cell Block (CDX2 ICC stain, X40).

All Positive cases showed immunocytochemical staining according to the tissue of origin.

In case of Non Hodgkin's lymphoma, immunocytochemical markers showed strong cytoplasmic positivity with LCA (Figure 1), pleural effusion with breast Infiltrating ductal carcinoma showed ER and PR negative but Her2Neu positive which correlated with histopathology, malignant mesothelioma showed negative for TTF-1 but nuclear and cytoplasmic positivity for calretinin (Figure 2), ovarian malignancy showed positivity with PLAP and AFP, pleomorphic sarcoma positive for Vimentin, TTF-1, and Calretinin, squamous cell carcinoma positive for cytokeratin (5/6) (Figure 3) and lung adenocarcinoma showed TTF-1 positivity (Figure 4) and metastatic adenocarcinomas showed CDX2 cytoplasmic positivity in tumor cells (Figure 5).

Table 4: Correlation between cytospin and cell block diagnosis with Immunocytochemistry.

Techniques		ICC (n=52)		χ^2 value	p value
		Negative (n=26) (%)	Positive (n=26) (%)		
Cytospin	Negative	26 (100.0)	5 (19.2)	32.50	<0.001
	Positive	0 (0.0)	19 (70.0)		
	Inconclusive	0 (0.0)	2 (0.8)		
Cell block	Negative	26 (100.0)	0 (0.0)	52.00	<0.001
	Positive	0 (0.0)	26 (100.0)		

Table 5: Concordance between cytospin and cell block diagnosis with histopathology (n=22).

Histopathology	Cytospin		Cell block	
	n	Percentage	n	Percentage
Concordance	16	72.7	22	100
No concordance	04	18.2	0	
Could not be commented	02	10.1	0	
Total	22		22	

Table 6: Sensitivity and specificity of final diagnosis of cytospin and cell block techniques against immunocytochemistry (n=52).

Test/Method	AUC	p value	Sensitivity (95% CI)	Specificity (95% CI)	+PV	-PV
Cytospin	0.875	<0.001	75.00 (53.3-90.2)	100.00 (86.7-100.0)	100.0	81.2
Cell block	1.000	<0.001	100.00 (86.7-100.0)	100.00 (86.7-100.0)	100.0	100.0

AUC: Area under curve, CI: confidence interval, +PV: positive predictive value, -PV: negative predictive value.

Comparing the diagnosis of cytospin and cell block with ICC, $\chi 2$ test showed significant association between findings of cytospin ($\chi 2=32.50$, $p<0.001$) and cell block ($\chi 2=52.00$, $p<0.001$) with ICC and suggesting high association and the association was found to be higher with cell block than cytospin (Table 4). Histopathology was available in only 22 cases of pleural effusion. All 22 cases of malignant effusion in cell block showed similar histopathological findings but in case of cytopsin only 16 cases of malignant effusion showed similar histopathological findings and 2 cases were could not be commented and rest 4 cases not showing any concordance with histological findings (Table 5).

To see the diagnostic accuracy (sensitivity and specificity) of cytospin and cell block in diagnosis of malignancy (negative/positive) were compared with diagnosis (negative/positive) of immunocytochemistry.

The significant diagnostic cytospin (AUC=0.857, p<0.001) in discriminating positive and negative malignant cases with 75.00% sensitivity (95% CI=53.3-90.2) and 100.00% specificity (95% CI=86.7-100.0) and with 100.0% positive predictive value and 81.2% negative predictive value.

In contrast, cell block also showed significant diagnostic accuracy (AUC=1.000, p<0.001) and sensitivity 100.00% (95% CI=86.7-100.0) and specificity 100.00% (95% CI=86.7-100.0) and 100.0% positive predictive value and 100.0 negative predictive value than cytospin (Table 6).

DISCUSSION

Study included all pleural effusion samples irrespective of the clinical or radiological status of the patient we encountered more of benign pleural effusions. In our study benign cytology accounted for 85.7% in cytospin and 82% in cell block. Studies done by J. Archana et al also found similar findings.[5] Most of the samples received were from male patients 86(56.6%). Majority of the benign effusion were chronic effusion 33.4% and 30.9% in cytospin and cell block respectively. More are less similar results were also seen in studied done by J. Archana et al which included 150 effusion samples where they found 43.1% cases of chronic effusions.[5]

The techniques of cell block and cytospin were compared using four parameters: adequacy, cellularity, cytoplasmic and nuclear preservation and background. In this study the findings of cell block in terms of cellularity, cytoplasmic and nuclear preservation and background be more statistically significant as compared to cytospin but in terms of adequacy both techniques showed similar results. Similar conclusions were made by S. Mahendra.[6]

Both cytospin and cell block showed good cellular architecture but the overall findings in cell block in terms of cellular architectures like acini, cell ball, and papillary pattern also helped in giving a clue about the location of the primary tumor. Similar findings were found in studies done by M. Mulkalwar, P. Bista.[7,8]

The 19 cases of positive for malignancy was given by cytospin but cell block yielded 8 more cases (27) of malignancy which was confirmed by IHC. Similar results were found in studies done by Dekker and Bupp et al, Khan et al.[910] The increased yield could be due to the increased diagnostic material with cell block and the more option of ancillary test it gives. Inspite of small sample size and limited duration of study the technique of cell block in pleural effusion cytology was found be both 100% sensitive and specific for diagnosing malignancy which was also shown to have more or less similar results in other studies. S. Bansode,Santoshpawa et al, D.Urvi et al.[11-13]

We compared the results of cell block in those cases where histopathological examination was done. In our study 22 biopsy proven cases of malignancy were available. All 22 cases were found to be positive in cell block technique. Studies done by J. Archana et al, S. Udasimath et al also showed the similar results.[5,14]

One of the problems with the reactive effusion is the way some cell may appear or mimic malignancy which can lead to an equivocal opinion. We have encountered 2 such cases in cytospin where we have given suspicious for malignancy. However on doing cell block and reviewing the slides the morphology were clearly malignant. Similar results were found in studies done by MV. Bhanvadia et al and P. Saswati et al.[15,16]

In this study TTF1 and calretinin were found to be good markers to differentiate between adenocarcinoma and malignant mesothelioma. M.A.Afify et al, A. Khoor et al also came to similar conclusion about TTF-1 and Patricia A. Fetsch and Andrea Abati also mentioned the use of calretinin to be a good marker for differentiating malignant mesothelioma from adenocarcinoma.[17-19] Other IHC markers based on the histopathology also showed good results. By applying IHC to the 27 positive cases of cell block we were able to comment on the primary origin of the malignancy on 19 cases. Similar conclusions were made by S. Mahendra et al.[6] The cell block has been shown to not only increase the diagnostic yield but it provided better architectural preservation like cell ball, papillary pattern, acini along with some excellent nuclear and cytoplasmic details.

Although in our study we did not do subsequent smears or cell block from the sample. The study done by Thapar M et al showed that on subsequent smears and cell blocks from further aspirates enhanced the diagnostic yield of malignancy in both conventional smear and cell block modalities.[20] We should make provision of further follow up in cases of serous effusions and processing of subsequent samples whenever they are aspirated to enhance the diagnostic accuracy. With this study we conclude that cell block should be used as a part of routine study before a sample is been discarded. Similar conclusion was also drawn by D. Köksal et al who conducted studies of effusions by using smears and cell block.[21] They preferred to study paraffin sections before giving the final diagnosis because it was more accurate and as it was easier to demonstrate cellular relationships and pattern with the cell block technique.

Limitations of the present study was that it was a time bound study done on limited number of cases. One more important limitation of our study was the less number of malignant effusion and histological correlation was not available in all cases

CONCLUSION

Cell block proved to be a good technique when combined with routine cytological smear as it increases the diagnostic yield of malignancy. Cell block and cytospin

both had excellent specificity but cell block showed better sensitivity. The cell block technique should be used in routine practice as it not only increases the diagnostic yield but ancillary test and retrospective studies can also be done.

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