Original Article

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Pathology Section

Cell Block- A Useful Adjunct in Cytopathology of Serous Effusions

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ABSTRACT

Introduction: Use of the Cell Block (CB) technique in cytology increases the diagnostic accuracy. This technique enables cells to be retrieved in a fluid specimen to form a paraffin block, which concentrates the cells in a limited field without loss of cellular material and preserves tissue architecture. Additional sections can be obtained from CB to perform ancillary studies like Immunohistochemistry (IHC) or molecular studies. The main advantages of the technique are preservation of morphology, feasibility of performing ancillary studies and familiarity of the Haematoxylin and eosin (H&E) stain. The aim of the study was to assess the utility of CB preparation in making sound cytological decisions in samples from Serous Effusions (SF).

Aim: We studied the various advantages of CB preparation along with the feasibility of the use of IHC on CB preparation in arriving at the diagnosis of Carcinomas of Unknown Primary origin (CUP).

Materials and Methods: In this retrospective cross-sectional study we reviewed 30 cases of SF received over a period of one year at the Department of Pathology, of a tertiary care center. Provisional diagnoses made on Conventional Smears (CS) stained by May-Grumwald Giemsa (MGG) and Papanicolaou (Pap) stains were compared with the diagnoses revised after examining H&E stained slides and IHC done on the CB preparation. The advantages of the CB preparation and the utility of this technique in diagnosing cases of CUP were assessed.

Results: A total of 30 cases of effusion samples were reviewed. Of the samples, 21 were peritoneal fluid, 08 were pleural and 01 was from a hydrocele sac. Mean age group was 55 years. Female patients formed 2/3rd of all cases. Cellularity was enhanced following CB preparation due to cell concentration. Architectural patterns were also easier to recognize in CB method in comparison to CS. The cases were grouped into 4 diagnostic categories: malignant, suspicious of malignancy, benign/no-malignant cells seen or inadequate for reporting. By the CB method, an additional 4 cases (7%) were detected as malignant. The malignant effusions were more common in females than males (ratio: 2.5:1). The most common primary site identified was from the ovary. Out of 13 cases of malignant effusions, the primary was known in 8 cases, which included six cases of carcinoma ovary and two cases each of carcinoma of lung from male patients. In the remaining nine cases, the primary was established with the help of CB and IHC.

Conclusion: The CB technique enables sediments from body fluid specimens to be retrieved and to form a paraffin block, which concentrates the cells in a limited field without loss of cellular material and preserves tissue architecture. H&E stained slides can be examined and additional sections can be used to perform ancillary studies like IHC. This study demonstrated a clear advantage of CB technique in diagnosing CUP origin over the conventional techniques of cytology. Therefore, it is recommended that CB technique be routinely employed in all cases of SF to increase the diagnostic accuracy.

Keywords: Cytology, Immunohistochemistry, Malignant effusions

INTRODUCTION

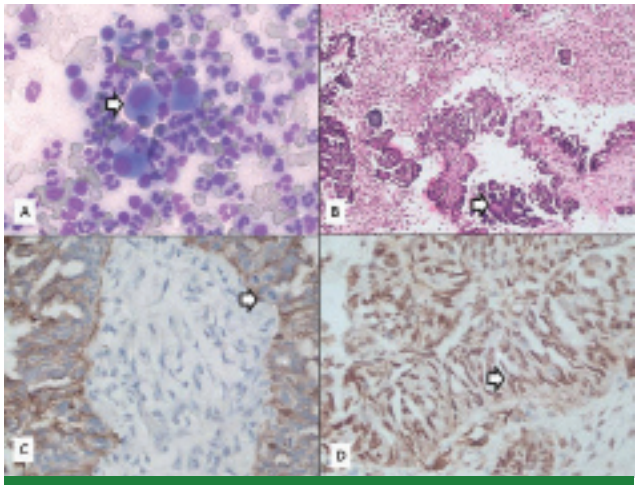
Presence of malignant cells in SF is an important diagnosis given by the cytopathologist, which has serious implications for the patient as it defines incurability of tumors in most situations [1]. Malignant serous effusions can also be the manifestation of a metastatic disease and many-a-time the only clinical clue of an unknown primary [2]. It is imperative for the treating clinician to know whether the malignancy is present and if present which lineage these malignant cells belong too, as it is the deciding factor in planning subsequent management protocols for the patient. CB coupled with IHC is an excellent tool to help arrive at a near definitive diagnosis in cases of SFs [3]. We analysed SF samples received at our center from January 2016 to assess the utility of CB in conjunction with IHC in arriving at a definitive diagnosis in comparison with CS.

MATERIALS AND METHODS

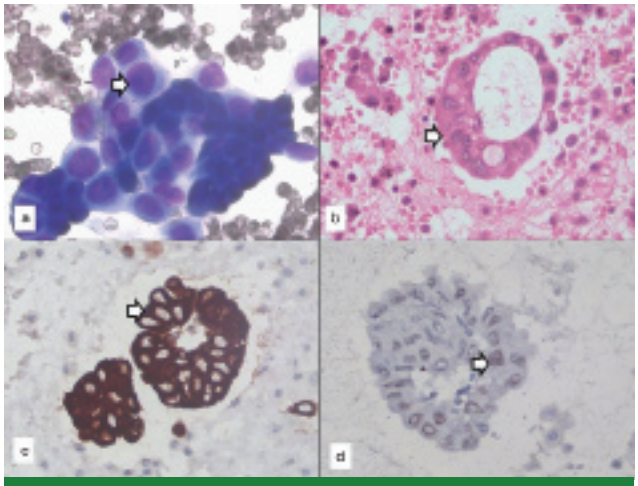
A retrospective cross-sectional study of SF samples received at the Pathology Department of a tertiary care center in

Western India between January 2016 and October 2016 was carried out. All 30 consecutive SF samples received during this period, for evaluation of malignant cells were included in this study. SF samples received for cell count and clinical chemistry analysis without a specific requisition for cytology were excluded from the study. The study was conducted following approval from the institutional ethical committee.

The volume, turbidity and color of the samples were noted. The samples were subsequently homogenized and processed for both CS and CB preparations. The conventional smears included both direct smears and cytospin smears from the fluid sample. Direct smears were made by placing a drop of homogenized sample on a glass slide and making a smear similar to a routine peripheral blood picture. Cytospin smears were made using the Shandon Thermofisher Cytocentrifuge machine. This includes a funnel with the homogenized sample being latched onto a glass slide with a filter paper interface. The assembly is placed in a specialized centrifuge machine which leads to formation of a monolayered sheet of cells within a small circumference. Both the direct and cytospin smears were stained by May-Grumwald Giemsa and Papanicolaou stains. The remaining sample was processed for making a CB by centrifuging at 2500 rpm for 15 minutes to obtain a cell pellet after discarding the supernatant. The cell pellet was mixed with 2 drops of thromboplastin and 2 drops of pooled plasma. The clotted cell pellet was fixed in 10% formalin to complete the process of making a CB. The CB was subsequently processed as a routine surgical tissue. The CB sections were stained with H and E stain and some special stains such as Periodic Acid Schiff and Mucicarmine were done as warranted by the case. The cases were reviewed with respect to whether the initial 4-tiered opinion of inadequate; No malignant cells seen; Suspicious for malignant cells or, positive for malignant cells made on the CSs was revised after review of the CB. The panel of IHC was also analyzed which supplemented the revised diagnosis [Table/Fig-1,2]. The relevant clinical features and cytomorphological findings of CS and CB preparation were entered as an excel data sheet.



[Table/Fig-1]: Illustration of a case of serous papillary ovarian carcinoma with malignant peritoneal effusion.
(a) Romanowsky stained conventional smear (1000X) shows large atypical cells; (b) Hematoxylin and Eosin stained section from cell block (100X); (c) CK 7 Immunohistochemistry (400X) on the cell block; (d) WT1 Immunohistochemistry (400X) on the cell block.



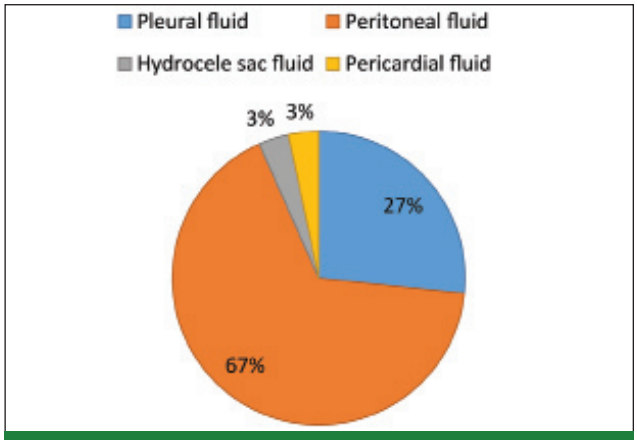
[Table/Fig-2]: Illustration of a case of Adenocarcinoma lung with malignant pleural effusion.
(a) Romanowsky stained conventional smear (1000x) shows large atypical cells; (b) Hematoxylin and Eosin stained section from cell block (100X) shows glandular fragments; (c) CK 7 Immunohistochemistry (400X) on the cell block; (d) TTF1 Immunohistochemistry (400X) on the cell block.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Medcalc statistical software to calculate the sensitivity and specificity of the two methods in picking up malignant cells on SF samples.

RESULTS

Cases of SFs sent for assessment for presence of malignant cells were included in the study. A total of 30 effusion samples which were processed both for CS and CB preparation were reviewed. The distribution of the samples had a predominance of peritoneal effusions, followed by pleural effusions [Table/Fig-3]. The age group was 21 years

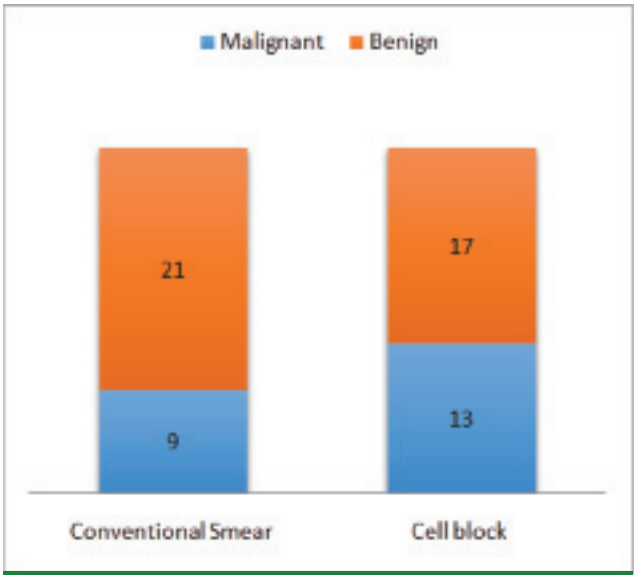


[Table/Fig-3]: Distribution of samples of serous effusions.

to 90 years with a mean of 55 years [Table/Fig-4]. Most of the cases were from female patients [Table/Fig-4]. The most common malignancy was ovarian malignancy. The CSs were sufficient to help arrive at a diagnosis of suspicious malignancies and were positive for malignancy in 9 cases. However, review of the CB preparation slides could help diagnose additional 4 cases as malignant effusions [Table/Fig-5]. The origin of the malignant effusions just based on

Age (in years)	<40	4
	40-59	11
	>60	15
Sex	Male	10
	Female	20

[Table/Fig-4]: Age and sex wise distribution of cases.



[Table/Fig-5]: Distribution of benign and malignant effusions diagnosed by conventional and cell block methods.

cytology could not help arrive at the primary origin of the tumor. However, the architectural pattern of malignant cells in the CB preparation helped decide the probable primary origin of tumor in 7 cases. The panel of antibodies helped in arriving at a definitive diagnosis including the primary origin of the tumor in all 13 cases of malignant effusions [Table/Fig-6]. Of the 13 cases, IHC was instrumental in diagnoses of the primary malignancy in 4 cases of CUPs [Table/Fig-6].

DISCUSSION

Assessment of SFs has been routinely used by clinicians in suspected cases of malignancies [4]. The diagnosis of malignant effusions has important connotations with respect to upstaging the malignancy and conferring a poor prognosis to the patient [5,6]. Presence of malignant cells also restricts use of surgery as a modality of management in some settings [7]. The approach to identification of malignant cells on CSs is restricted to identification of two-cell populations along with cellular patterns and cytological atypia [8]. However CB, though cumbersome in processing and time consuming, has a distinct advantage of offering architectural patterns which help in identifying the lineage of the malignant cells in many instances. CBs being a cell enriched medium, provides an opportunity for the pathologist to analyze many cells in a smaller surface area [9]. The CB also takes precedence over CS in carrying out a panel of IHC markers in establishing the lineage of the malignant cells, especially in a scenario of CUP [2].

Though, the technique of CB is well documented, it has not found its way into routine practice for evaluation of cytological specimens and SFs in particular. The reasons for non-acceptance could be because of lack of standardization in methodology of making a CB and a fear of loss of material during processing [10].The methodology of CB preparation involves making a cell pellet by high speed centrifugation, following which the cell pellet is embedded in a medium such as agar or plasma-thromboplastin to convert it into a solid mass which can be easily manipulated for processing as a routine tissue block, following fixation in 10% neutral buffered formalin [11].

We have attempted to analyze the utility of CB in comparison with CSs in arriving at a definitive diagnosis in conjunction with IHC, especially in cases of unknown primary. Of the total 30 effusion samples, peritoneal effusions were the most common with a female predominance. This is probably due to the higher incidence of peritoneal involvement in ovarian malignancies which was the commonest malignancy in our series [12]. The age at presentation was elderly keeping with our spectrum being carcinomas, which have a proven higher incidence in elderly [13]. CSs were able to detect malignant cells in 9 cases. However, CB was instrumental in definitively diagnosis these 9 cases and in addition 4 other cases as malignancy. This was possible due to cell enrichment and presence of specific architectural patterns seen on the CB preparation [14]. Supplementing the CB was the use of case-based panel of antibodies for IHC [15].This added expression of markers by IHC was instrumental in objectively defining the cell of origin of the malignant cell, which enabled us to clearly ascertain the primary malignancy in 4 cases of CUP [2]. All cases diagnosed as malignant cells with the primary origin were confirmed by imaging and/or histopathological examination of the biopsied or resected tumor mass.

Statistical analysis revealed CB to have a sensitivity of 100% in picking up malignant cells. This is an improvement from earlier studies which showed a sensitivity rate of 90-94% [16-19] [Table/Fig-7]. CB was able to pick up 30% more cases of malignancies in comparison to CS. This is better malignancy pick up percentage in comparison with previous studies except for Dekkar et al., who showed an additional malignant cell detection of 38% [9,10,14-19] [Table/Fig-7]. In cases of CUP, CB with the panel of immunohistocemical antibodies was instrumental in identifying the primary

S. No	Sex	Age (in years)	Sample	Primary Lesion	Conventional Smear Opinion	Cell Block IHC Panel	Cell Block Opinion
1	F	37	Peritoneal fluid	Abdominopelvic mass	Benign	Calretinin, CK, CK7, ER, WT1	Benign
2	M	26	Hydrocoele sac fuid	Hydrocoele	Benign	No	Benign
3	F	58	Peritoneal fluid	Carcinoma Endometrium	Benign	No	Benign
4	F	41	Peritoneal fluid	Immunosurvelliance	Benign	No	Benign
5	F	67	Pleural fluid	Carcinoma thyroid	Benign	Calretinin, TTF1	Benign
6	F	34	Peritoneal fluid	Immunosurvelliance	Benign	No	Benign
7	M	60	Pleural fluid	Suspected Lung carcinoma	Benign	No	Benign
8	M	57	Pleural fluid	Cholangiocarcinoma	Benign	No	Benign
9	M	21	Pericardiac fluid	Pericarditis	Benign	No	Benign
10	F	60	Peritoneal fluid	Carcinoma ovary	Suspicious for malignancy	CK7,CK20, Calretinin, WT1, ER	Positive for Malignant cells (Ovarian primary)
11	F	65	Peritoneal fluid	Left adenxal mass	Positive for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
12	F	41	Pleural fluid	Lung carcinoma	Benign	No	Benign
13	F	85	Pleural fluid	Lung mass	Inadequate for reporting	No	Inadequate for reporting
14	F	60	Peritoneal fluid	Abdominopelvic mass	Suspicious for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
15	F	65	Peritoneal fluid	Carcinoma ovary	Benign	CK7, CK20, Calretinin, WT1, ER	Benign
16	M	60	Peritoneal fluid	Ca stomach	Benign	CK7, CK20, Calretinin	Benign
17	F	65	Peritoneal fluid	GI malignancy	Benign	CK7, CK20, Calretinin, CEA	Benign
18	F	54	Peritoneal fluid	Carcinoma ovary	Positive for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for malignancy (Ovarian primary)
19	M	50	Pleural fluid	Pulmonary Koch	Benign	No	Benign
19	F	74	Peritoneal fluid	Primary not known	Benign	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
20	M	65	Peritoneal fluid	Suspected GI malignancy	Benign	WT1, ER, GCDFP, CK7, CK20	Benign
21	M	41	Pleural fluid	Primary not known	Benign	CK7, CK20, TTF1, Calretinin	Positive for Malignancy (Lung primary)
22	M	90	Pleural fluid	Lung mass	Benign	CK7, CK20, TTF1, p63, Calretinin	Positive for malignancy (Lung primary)
23	F	60	Peritoneal fluid	Primary not known	Benign	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
24	F	73	Peritoneal fluid	Abdominopelvic mass	Positive for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
26	F	52	Peritoneal fluid	Ca caecum	Positive for malignancy	CK&, CK20, Calretinin, CDX2	Positive for Malignancy (Colon primary)
27	F	50	Peritoneal fluid	Primary not known	Suspicious for malignancy	CK7, CK20, WT1, CEA, ER	Positive for Malignancy (Ovarian primary)
28	M	49	Peritoneal fluid	Chronic liver disease	Benign	No	Benign
29	F	45	Peritoneal fluid	Abdominopelvic mass	Positive for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)
30	F	62	Peritoneal fluid	Abdominopelvic mass	Positive for malignancy	CK7, CK20, Calretinin, WT1, ER	Positive for Malignancy (Ovarian primary)

[Table/Fig-6]: Distribution of effusion samples received.

Authors	Year	IHC	Findings
Thapar M et al., [9]	2009	Not studied	• CB has a distinct advantage of preserving architectural patterns • An additional 13% of cases were detected to have malignancy by CB method • CB helped in detecting the primary in 83% of cases
Shivkumarswamy U et al., [10]	2012	Not studied	• CB resulted in increased cellularity • CB increased detection of malignancy by 15%
Dekkar A et al., [14]	1978	Not studied	• There was no false negative case with the use of CB • An additional 38% of cases were detected to have malignant cells with the use of CB technique • CB is useful when cytological morphology is misleading
Gong Y et al., [15]	2003	Studied	• CB in comparison with Thin Prep® is better for both non-nuclear and nuclear immunohistocemical markers
Chapman CB et al., [16]	1947	Not studied	• CB is a dependable method to detect malignant cells with cytological anaplasia • CB has an accuracy of 94%
Fetsch PA at al., [17]	2001	Studied	• CB are best suited for IHC on effusions samples • CB have the ease of interpretation, minimal background staining and option of multiple sections • CB can also be archived
Castro-Villabón D et al., [18]	2014	Studied	• CB has advantage of providing multiple sections for immunohistochemistry • Additional 24% cases of malignancy were detected by CB
Bhanvadia VM et al., [19]	2014	Not studied	• CB helped detect an additional 10% malignant cases • CB was able to detect the primary site of malignancy with 90% accuracy

[Table/Fig-7]: Comparison with other studies.

malignancy in all 4 cases, having a specificity of 100%. This is better rate of detection of primary site of malignancy in comparison with earlier studies [9,19] [Table/Fig-7].

The limitation of the study is the limited spectrum of malignancies seen, which is attributed to the patient load at our center.

CONCLUSION

CB is a useful adjunct in evaluating SFs for malignant cells as it has the advantages of smaller area of cell dispersal, increased cell density, preservation of architecture and ability to perform IHC to identify the cell of origin. Hence, CB preparation should be incorporated in routine cytology practice when evaluating SFs to increase diagnostic accuracy.

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