International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2019; 2(1): 187-190
Received: 12-11-2018
Accepted: 16-12-2018

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Analyzing diagnostic utility of cytospin and cell block techniques in serous effusion cytology

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DOI: https://doi.org/10.33545/pathol.2019.v2.i1c.28

Abstract
Introduction: Fluid cytology plays a pivotal role delineating benign from malignant effusions, tumor staging and diagnosing recurrences.
Objectives: To assess the diagnostic utility of cytospin and cell block methods in cytodiagnosis of serous effusions.
Material and methods: A total of 240 cases inclusive of 77 pleural and 163 peritoneal effusions were analyzed by cytospin and cell block preparation following conventional smear cytology.
Results: Out of 240 cases, 13% were malignant pleural effusion and 14.7% were malignant peritoneal effusion. Cell block diagnosed additional 4% and 2.4% cases of malignant origin in pleural and peritoneal fluid respectively and also aided in ascertain the primary site. Few rare cases such as effusion in synovial sarcoma and squamous cell carcinoma were also diagnosed.
Conclusion: Cell block technique aids in cytodiagnosis of malignant effusions but has demerits also, hence both cytospin and cell block should be used as complement.

Keywords: Cytospin, cell block, cytology

1. Introduction
Serous effusion cytology plays a crucial role in guiding clinical decisions. Serous effusions are the mirrors reflective of a much larger surface area than what could be obtained on needle biopsy [1]. The relative access of performing pleural and peritoneal taps makes it an ideal sample of diagnostic utility in ascertaining the etiology of effusions. Its role in determining tumor stage and tumor recurrence adds further merit. Even in the era of personalized medicine and targeted therapies serous effusion cytology remains indispensable in aiding diagnosis and also archives representative material for future molecular studies [2].
In the present one year prospective study we analyzed samples of pleural and peritoneal effusion received at a tertiary care hospital using simultaneous cytospin (CS) and cell block (CB) techniques supplemented by immunocytochemistry (ICC) where needed. The aim of this study was to assess the utility of two different laboratory techniques in delineating malignant effusion from the non-malignant effusion. We also attempted to identify the tumor type and its origin.

2. Material and methods
In a one year prospective study from Jan 2018 to Dec 2018, 240 samples of peritoneal and pleural fluid were analyzed by both cytospin and cell block preparation. Relevant clinical details including age, sex, symptoms, radiological investigations and clinical diagnosis was obtained. Conventional smear cytology and biochemical analysis was performed for all samples. Both air dried direct and centrifuged smears that were stainedby Leishman-Giemsa (LG) stain. The Shandon Thermo fisher Cytocentrifuge was used to prepare cytospin smears. CS smears were prepared by placing 10 mL fluid in the cytospin funnel with filter paper being placed between the funnel and the slide, followed by centrifugation at 750 rpm for 5 min resulting in formation of a monolayered sheet of cells within a small circumference. Two such smears were prepared. One smear was air dried and stained with LG stain while the other smear was fixed in 95% ethanol and was Papanicolaou (PAP) stained.
CB preparation involved centrifugation of 10 mL of fluidfor 15 minutes at 2500 rpm. After removal of supernatant fluid, the sediment was mixed with two drops of pooled plasma, followed by addition of two drops of thromboplastin and was allowed to stand for 5

min so as to enable clot formation. The formed clotted cell pellet was fixed in 10% buffered formalin and processed as routine histopathology to obtain CB sections obtained which were stained with Hematoxylin and Eosin (H&E). The cases were reported under three categories; Benign, Suspicious for malignancy or Positive for malignant cells. Further attempt were made to characterize the malignancy based on cytoarchitecture and ICC result. Statistical analysis was performed using medcalc software to estimate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

3. Results

Our study cohort included 240 samples of serous fluid effusion comprising of 163 samples of peritoneal fluid and 77 samples of pleural fluid. The age range was comparable for both the effusions being 26 - 80 years (mean 54 year) and 20 - 84 years (mean 53 year) for peritoneal and pleural fluid respectively.

Amongst the 163 cases of peritoneal fluid, 72 were female and 91 were male. On CB a total of 24 (14.7%) malignant effusions [Table 1] were noted with diagnosis of additional 4 cases in comparison to CS.

Table 1: Comparison of results between CS and CB in peritoneal fluid

Result category	CS	CB
Benign	137 (84.0%)	139 (85.3%)
Suspicious	6 (3.7%)	0 (0%)
Malignant	20 (12.3%)	24 (14.7%)

While in the pleural fluid category males (53 cases) outnumbered the females (24 cases). CB confirmed the diagnosis of malignant effusion [Table 2] in 10 (13%) cases with diagnosis of additional 3 malignant cases.

Table 2: Comparison of results between CS and CB in pleural fluid

Result category	CS	CB
Benign	66 (85.7%)	67 (87%)
Suspicious	4 (5.2%)	0 (0%)
Malignant	7 (9.1%)	10 (13%)

A comprehensive distribution of malignant diagnosis based on microscopy and ICC is reflected in Table 3.

Table 3: Distribution of malignant serous effusions with histologic findings

Histology	Peritoneal fluid		Pleural fluid	
	CS	CB	CS	CB
Papillary serous carcinoma ovary	19	21	4	4
Signet-ring cell adenocarcinoma stomach	0	1	0	0
Adenocarcinoma gall bladder	1	1	0	0
Adenocarcinoma lung	0	0	2	3
Adenocarcinoma breast	0	0	0	1
SCC alveolus	0	0	1	1
Burkitt lymphoma	0	1	0	0
Synovial sarcoma	0	0	0	1
Total	20	24	7	10

The descriptive statistics pertaining to peritoneal and pleural fluid assessment via CB is provided as per Table 4.

Table 4: Diagnostic efficacy of cell block (95% confidence interval)

Parameter	Peritoneal fluid	Pleural fluid
Sensitivity	87.5% (67.6% - 97.3%)	70.0% (34.7% – 93.3%)
Specificity	99.3% (96.1% - 99.9%)	98.5% (91.7% - 99.9%)
Positive predictive value (PPV)	95.4% (74.8% - 99.3%)	87.5% (48.9% - 98.1%)
Negative predictive value (NPV)	97.9% (94.1% - 99.2%)	95.6% (89.5% - 98.3%)

4. Discussion

Serous effusion cytology forms the first line of diagnostic workup in ascertaining the etiology. Cytological examination methods do vary in different laboratory set ups with most laboratories relying on the conventional smear cytology. Our results reveal the diagnostic efficacy of cytological evaluation using both cytospin and cell block techniques. CB diagnosed additional 4 cases (2.4%) and 3 cases (4%) of malignant etiology in peritoneal and pleural fluids respectively which were either completely negative or suspicious on CS smear. Bhanvadia *et al.* in her study reported additional 10% malignancy by CB preparation in comparison to conventional smear cytology [3].

Our results are in concordance with the work of Shivakumarswamy *et al.*, who in a study of 60 pleural effusions found malignancy in 10 cases (16.67%) [4]. While our study is in discordance with another Indian study by Kushwaha *et al.* who reported malignant pleural effusion in 30.49% cases [5]. We report malignant pleural effusions secondary to carcinoma of ovarian and lung, while Shivakumarswamy *et al.* in his work reported 30% cases due to carcinoma breast followed by carcinoma lung [4].

Rossi *et al.* analyzed 10348 peritoneal effusions and reported only 7% as malignant [6]. In the current study, 14.7% cases were of malignant etiology with ovarian origin in 12.8% cases similar to what has been reported by Creasman *et al*. [7] and Davidson *et al.* [8].

Cell block technique is neither new nor novel but somehow has not received the desired attention and utilization. But, it seems to be gaining wider acceptance in recent times with its scope being realized in effusion cytology and fine needle aspiration cytology [4, 9]. Conventional cytology yields suboptimal results as assessed by Oygluso *et al.* who reported sensitivity of 44.5%, specificity of 95.7%, PPV of 98.7% and NPV of 20% [10]. Thereby entailing the use of adjuvant methods like CS and CB for more reliable cytology results.

Majority serous effusions are secondary to adenocarcinoma, but we encountered a rare cases of pleural effusion due to metastasis of squamous cell carcinoma (SCC) of alveolus [Figure 1]. Tumor cells were evident on both CS and CB slides. Gupta *et al.* in a ten year audit has reported <1% incidence of serous effusions due to metastatic SCC [11].

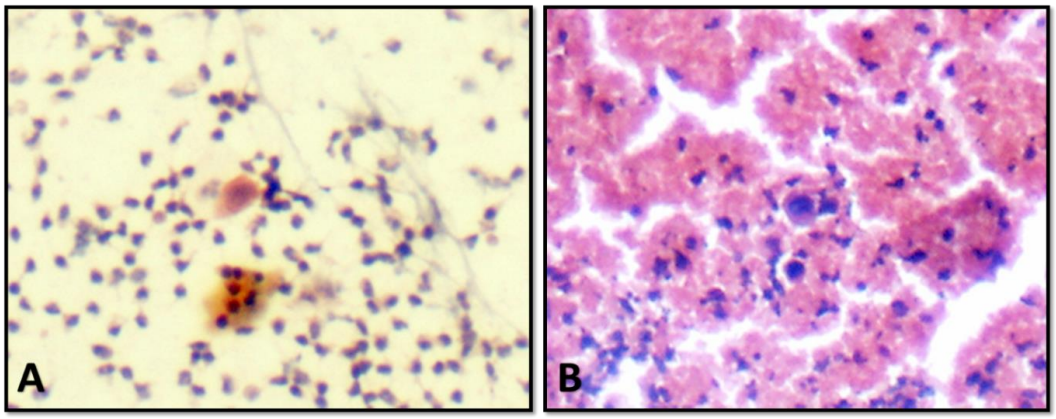

Fig 1: Photomicrograph of cytospin smear showing (A) dysplastic squamous cell with orangeophilic cytoplasm and hyperchromatic nucleus in a neutrophil-rich background, PAP x 100. (B) Cell block section showing two tumor cells in a necroinflammatory background, H&E x 100.

Another exceptional case was of pleural effusion due to synovial sarcoma in a 23 year male. The effusion fluid was hemorrhagic on gross examination and cytospin was negative for malignancy. While the tumor cells were detected on cell block and was tested for representative ICC markers [Figure 2]. Abadi *et al.* studied 24 cases of serous effusions in sarcomas and encountered only one case of peritoneal effusion due to synovial sarcoma. Author also highlighted that such a diagnosis is usually made in the setting of a known primary sarcoma [12].

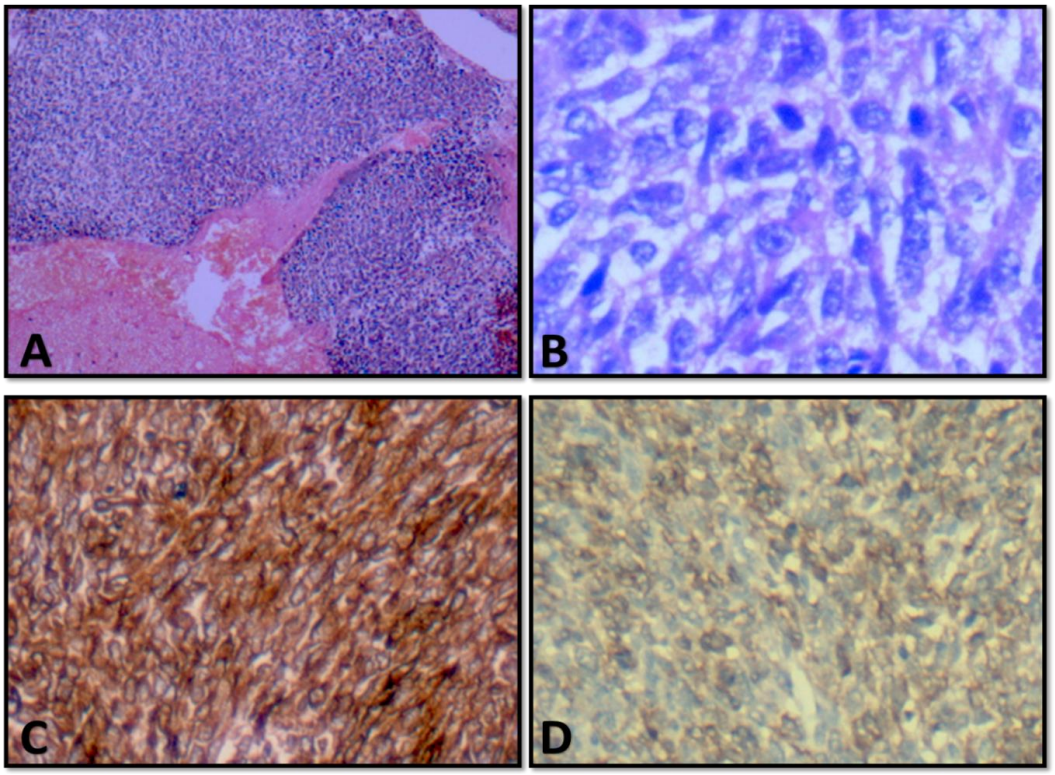

Fig 2: Photomicrograph of cell block section (A) is markedly cellular showing tumor cells in sheets in a hemorrhagic background, H&E x 100.(B) Tumor cells are spindle shaped having round to oval nucleus with coarsely clumped chromatin and marked anisonucleosis, H&E x 400. (C) Positive immunostaining for vimentin and (D) cytokeratin, x 400.

In our study CB preparations undoubtedly aided the diagnosis of additional malignant cases and some with rare diagnosis. No additional equipment was required, it provided superior architectural details and immunocytochemistry could be performed. However, CB technique is marred with disadvantages such as increased turn around time and loss of cellular material during processing [13]. On the other hand CS preparations provide higher cellular yield with better preservation of cell morphology and is less time consuming. Considering the merits and demerits of both the techniques it imperative to use both cytospin and cell block to complement the cytodiagnosis.

5. Conclusion

Cell block technique does offer enhanced diagnostic utility with less investment and we thus recommend the incorporation of cell block technique along with cytospin for augmenting the results of effusion cytology.

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