Diagnostic utility of cell blocks and an immunomarker panel in the cytological evaluation of serous effusions

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Abstract

Introduction: Cell blocking (CB) has gained wide acceptance as an integral diagnostic tool in cyto-diagnosis. It allows retrieval and concentration of exfoliated cells in fluid specimens to form paraffin blocks that are interpreted in a biopsy like fashion. The aim of this study was to compare the diagnostic utility of CB to conventional smears (CSs) in the evaluation of serous effusions and to evaluate whether the tested immunomarker panel increases the diagnostic yield of malignancy.

Materials and Methods: Serous effusion specimens were processed using both techniques; CSs and CB. An immunomarker panel (Ber-EP4, EMA E29, D2-40 and desmin) was then applied on CB sections.

Results: CB enhanced the cellularity; it increased the diagnostic yield by 27.4% over CSs. Both nuclear and cytoplasmic details were better demonstrated in CBs. CBs were superior in the preservation of architectural patterns as acini, papillae and sheets. The diagnosis of CBs concurred with that of CSs in 79% of cases, however the diagnostic yield of malignancy increased by 9.6% using CBs. Adding the immunomarker panel totally eliminated the suspicious category and further increased the diagnostic yield for malignancy by 9.7% over CBs and by 19.3% over CSs. Furthermore, categorization of malignant cases into primary malignant mesothelioma and metastatic carcinoma was feasible after thorough interpretation of the combined immunoprofile.

Conclusion: CB represents a cost-effective adjunct to CSs in the evaluation of serous effusions. It increases the cellular yield, provides superior cytomorphology, architectural preservation, and supplies material for ancillary studies.

Keywords: Serous effusions, Cell blocks, Immunohistochemistry, Marker panel.

Introduction

Serous effusions are pathologic regardless of their composition or etiology.[1] Despite the fact that only 10-20% of serous effusions are malignant, yet the presence of malignant cells in effusions indicates poor outcome with a mean survival time of less than 6 months. It is also reported that in 10-20% of cases, no primary tumour is recognized prior to the development of the effusion and thereby effusions may represent the first indication of malignancy.[2,3]

The cytological examination of body fluids is considered an easy, simple and inexpensive procedure, which is used as a definitive test to guide patients' management.[4,5] Yet, the cytologic evaluation of serous effusions is prone to many diagnostic pitfalls; the main problem confronting cytopathologists in every day practice is the challenging distinction between reactive mesothelial cells, adenocarcinoma and malignant mesothelial cells.[2] This can be due to: (a) the overlapping cytological features between malignant mesothelial and metastatic adenocarcinoma cells, (b) the highly atypical features exhibited by reactive mesothelial cells in response to various insults to the serous cavities, (c) the subtle morphologic features of some neoplasms,(d) obscuring of diagnostic cells by inflammatory or mesothelial populations, (e) paucity of representative cells, and absence of the dual cell population in some cases which is the main clue to the diagnosis of metastatic malignancy.[6-9] Furthermore, cellular crowding, overlapping, cell loss, lack of architecture and the artefacts caused by laboratory processing techniques are among the problems of conventional smearing (CS).[5,10]

The cell block (CB) technique, which was first implemented more than a century ago, has now become widely accepted and recommended by most experts as an adjunct to conventional smears in the cytodiagnosis of effusion cytology.[11] It aims at retrieving cellular material and concentrating cells in a small field, with preservation of cytomorphologic details, and better demonstration of architectural patterns -such as acini, cell balls, papillae and rosettes- in a biopsy like fashion. Moreover, it provides archived material that can be used for immunohistochemistry, special stains and molecular studies.[5]

Although, most cases can be diagnosed morphologically based on routine cytological preparations (conventional smears (CSs) and CB preparations), still there is a number of cases in which an unequivocal diagnosis cannot be reliably established. That's why various ancillary techniques have been utilized to supplement the cytomorphological evaluation.

Immunohistochemistry performed on CB preparations is the most widely used technique for that purpose.[9] Most investigators advocated the use of marker panels because no marker yielded unequivocal results. The value of each marker can be improved when utilized as a part of a battery of immunostains, in which each marker supports and controls the others to recognize different cell types. Ideally, two negative and two positive markers are needed for each possible diagnosis. However, up to date consensus has not been reached regarding the optimum antibody panel.[9,12-16]

The current study aimed at evaluating the role of CB preparations along with conventional smears in the

cytodiagnosis of serous effusions. A second aim was to assess the utility of an immunomarker panel, containing human epithelial antigen clone Ber-EP4, epithelial membrane antigen EMA clone E29, D2-40 and desmin clone D33, to differentiate reactive from malignant effusions; whether primary malignant mesothelioma or secondary metastatic malignancies.

Materials and Methods

Sixty two serous effusion samples were collected from the cases submitted to Pathology Department Laboratories, Alexandria Faculty of Medicine, based on inclusion and exclusion criteria. Upon receipt, the volume, colour, clarity of the effusion fluid and the presence or absence of clots or coagulum were assessed.

After centrifugation, the effusion fluid samples were evaluated for adequacy of the cytological material for smear and cell block preparation. The received fluid was then divided into two equal portions. The first portion was subjected to conventional smear cytology and the second portion was utilized for the cell block technique, using 5 mL of 10% alcohol formalin (i.e., nine parts of 90% alcohol and one part of 7.5% formalin) as a fixative. After one hour, the fluid was then centrifuged at 2500 rpm for 15 minutes. The supernatant was discarded, and 3 mL fresh 10% alcohol–formalin was added to the sediment and kept at room temperature for one day. The sediment containing the cell button of the fluid sample was scooped out onto a filter paper and processed into formalin fixed paraffin embedded blocks, from which 4–6 µ thick sections were cut and stained with the H&E stain.

Adequacy and evaluation of CSs and CBs

The point scoring system designed by Mair et al[17], which was based on four criteria (background, cellularity, cellular morphology and retention of appropriate architecture), was used for the assessment of the quality of both the CSs and the CBs.

Both the CSs and the CB sections of the 62 chosen cases were then evaluated for: cellularity, architectural pattern, cell arrangement and cytomorphologic features (both nuclear and cytoplasmic). Each case was then reported to be either reactive/ negative for malignancy, suspicious or positive for malignancy by both techniques.

Immunohistochemical staining

Five micrometre-thick sections of formalin-fixed paraffin embedded cell blocks were cut and mounted on positively charged coated slides (Polysine, Bio Optica, Milano, Spain) slides. The sections were deparaffinised in xylene and rehydrated in descending ethanol grades. Sections were incubated for 10 minutes in 3% hydrogen peroxide to block endogenous tissue peroxidase. An immunohistochemical panel was then applied, that included: D2-40, epithelial membrane antigen EMA clone E29, desmin clone D33 and human epithelial antigen clone Ber-EP4. All primary antibodies were mouse monoclonal ready to use antibodies and were provided by Dako (Denmark). For D2-40 and Ber-EP4, heat induced antigen retrieval was done in a microwave oven for seventeen minutes in sodium citrate buffer (0.01M Na-citrate monohydrate, pH 6.0), whereas, for EMA E29 and desmin, heat induced epitope retrieval was done in a microwave oven for twenty minutes in EDTA buffer. The slides were then incubated with biotinylated goat anti-polyvalent secondary antibody followed by streptavidin peroxidase, then sections were counterstained with Harris hematoxylin. Appropriate positive (tissue sections from colon for Ber-EP4 and desmin, lymphangioma for D2-40 and colonic adenocarcinoma for EMA) and negative controls (obtained by excluding the step of the addition of the primary antibody) were included for each batch of slides.

Interpretation of the immunostained slides

All slides were scored semi-quantitatively using an Olympus microscope.

D2-40 positivity was assessed as brown membranous or cytoplasmic staining in the reactive as well as the malignant mesothelial cells. Cases were considered positive when at least 5% of cells were stained, the staining intensity was evaluated using a three-tiered scale: (1+: weak positivity, 2+: moderate positivity, 3+: strong positivity). Regarding the percentage of the cells stained, it was recorded as follows: (0 (<5%), 1+ (5– 25%), 2+ (26–50%), and 3+ (>50%).[18, 19] Ber-EP4 positivity was assessed as brown cytoplasmic and/or membranous staining of malignant epithelial cells. The intensity score for Ber-EP4 staining was evaluated using a 4 tiered scale (score 0: no staining; 1: weak; 2: moderate; 3: strong), the percentage of cells stained too was scored on a 4-tiered scale (score 0: no staining; 1 :< 10%; 2: 10–50%; 3 :> 50%). Finally, both scores were added to evaluate the staining index for each case. The cut off value for Ber-EP4 positivity was at staining index of score 2.[20]

EMA E29 positivity was assessed as thick brown spiky membranous staining in the malignant mesothelial cells and brown cytoplasmic and/ or membranous staining (but not spiky) in malignant epithelial cells. Cases that showed < 10 % positivity for EMA E29 were considered negative.[21] The percentage of cells stained was scored as: (negative: 0%- <10% of cells staining; 1+, 10 %- <25% of cells staining; 2+, 25% to <50 % of cells staining; 3+, 50% to <75 % of cells staining; and 4+, 75% or more of cells staining),[22] while the intensity of staining was evaluated using a 4 tiered scale (score 0: no staining; 1: weak; 2: moderate; 3: strong).[23] Desmin positivity was assessed as brown cytoplasmic staining pattern in reactive mesothelial cells. Immunoreactivity for Desmin was considered positive when at least 10% of cells were stained.[24] The intensity of staining was evaluated using a 4 tiered scale (score 0: no staining; 1: weak; 2: moderate; 3: strong).[25]

Statistical analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Science) program for statistical analysis ((Chicago, IL, USA) version 21) and

MedCalc for Windows (version 12). Using SPSS, qualitative data were described using number and percent. Quantitative data were described using measures of central tendency (mean and median) and dispersion (SD, minimum, and maximum).

Qualitative data were compared using chi-square (X^2) and Monte Carlo Exact test (MCP) when needed. Significance of the results obtained in all applied statistical tests was considered at the 5% level, below which the results were considered to be statistically significant. Using MedCalc program, the strength of agreement between different diagnostic techniques was evaluated using weighted Kappa (K).

Results

The current study included 62 serous effusion cytology samples. The mean age of the patients was 57.19 years (range 20 -82 years). The maximum number of samples (n=18, 29%) were from patients in the age group of 51-60 years. Female patient samples (59.7%) outnumbered male patient samples (40.3%). Out of the 62 studied cases, 44 cases (71%) were pleural effusions, 16 cases (25.8%) peritoneal effusions and 2 cases (3.2%) pleuropericardial effusions.

Patients with pleural effusion presented most commonly complaining of dyspnea (43 cases (97.7%)), chest pain (8 cases (18.2%)) and cough (7 cases (15.9%)). Thirty four cases (77.3%) had a positive history of malignancy. The commonest primary tumour site was the lung followed by the breast and ovary. All 16 patients with peritoneal effusion presented with abdominal distension (100%), and 6 cases (37.5%) presented with associated abdominal pain. Thirteen cases (81.25%) had a positive history of malignancy. The commonest primary tumour site was the ovary followed by the pancreas. As for the two pleuropericardial effusion cases, one case presented by dyspnea (50%) while the other complained of cough (50%). The case that presented by cough had a history of lung cancer, and the other case that presented by dyspnea had no history of malignancy.

The gross examination of the submitted fluid revealed that the majority of the cases had a turbid aspect (n=56, 90.3%), and were yellowish in colour in 27 cases (43.5%). As regards the volume of the effusion samples submitted, it ranged from 4 cc up to 1700 cc. Biochemically, 21% of cases were transudates, while 79% were exudates.

Cytopathologic evaluation of the H&E stained smears and cell blocks

A comparative assessment was performed between the CSs and CBs as regards the cellular yield. The CB technique revealed significantly higher cellular yield; (MCP <0.001). Overall, cell blocks provided an even distribution of cells, with reduction of cellular overlapping. Both nuclear and cytoplasmic details were better demonstrated and preserved in CB sections compared to CSs, as they provided intact cell membranes, distinct chromatin details, and all individual cellular features were more sharp and crisp.

Cell blocks were superior to CSs in the identification of signet ring cells. Despite this difference, statistical significance was not reached; (P=0.07). Conversely, the cell block technique revealed a statistically significant difference compared to CS as regards detecting cytoplasmic vacuolization (P=0.003). Nuclear membrane irregularities were better identified in cell block sections. Nuclear chromatin and nucleolar pattern were better preserved as well. The CB technique revealed a statistically significant difference compared to conventional smearing as regards the detection of nuclear membrane irregularities (P=0.004), and the identification of nucleoli (P<0.0001). While the difference between both techniques regarding the preservation of nuclear chromatin pattern did not reach statistical significance. (P=0.08).

Cell blocks concentrated the cellularity in smaller fields with better appreciation of the architectural patterns. Compared to CS, CBs allowed identification of patterns that were not detected by CS for example; acini and sheets in benign reactive smears. In addition, it allowed better preservation of organoid patterns as cell clusters, papillae, acini and sheets in malignant smears. Both CSs and CBs were able to identify cell clusters as well as single cell pattern. These two patterns were observed in both reactive and malignant effusion cases. When a single cell pattern was detected, the determination of the nature of cells depended on the evaluation of the cytomorphologic features, and when cell clusters were observed, they were either loose mesothelial groupings that were formed of < 20-25 cells/ cluster -with retention of the mesothelial windows and the characteristic knobby scalloped borders formed by the cell cytoplasm- or they were cohesive epithelial clusters formed of >25 cells, with the characteristic community border formed by the nuclei. No statistically significant difference was noted between the CSs and the CBs regarding the detection of different architectural patterns (P > 0.05).

Diagnostic ability of conventional smearing and cell block technique in the evaluation of the type of serous effusions.

The distribution of the studied cases as regards the cytodiagnostic category is illustrated in (Table 1).

Table 1: Diagnostic ability of conventional smears and cell blocks in the evaluation of the type of serous effusions (reactive, suspicious or positive for malignancy)

	Conventional smear cytology Total (N=62)		Cell block technique Total (N=62)	
	No.	%	No.	%
Diagnosis				
Reactive	18	29	11	17.75
Suspicious	8	12.9	9	14.5
Positive for malignancy	36	58.1	42	67.7

A significant good agreement was observed between CS and CB preparation in the evaluation of the type of serous effusions, (K (95% CI)=0.711(0.566 to 0.855), P<0.001)). Conventional smear and cell block diagnoses concurred in 49 out of 62 cases (79%); both techniques diagnosed 11 cases (17.75%) as reactive, 3 cases (4.8%) as suspicious, and 35 cases (56.4%) as positive for malignancy. However, a discrepancy was observed between the two techniques in 13 cases (21%). Moreover, using CBs increased the diagnostic yield of malignant cells by 9.6% compared to the conventional smears, as CSs identified only 36 cases (58.1%) as positive for malignancy, while the CB technique diagnosed 42 cases (67.7%) as positive for malignancy, as illustrated in (Table 2).

Table 2: Agreement between CSs and the CB technique

Conventional Smear	Cell block			
	Reactive	**Suspicious**	**Positive for malignancy**	
Reactive	11	5	2	18 (29.0%)
Suspicious	0	3	5	8 (12.9%)
Positive for malignancy	0	1	35	36 (58.1%)
	11(17.75%)	9 (14.5%)	42 (67.7%)	62

Evaluation of the immunostained cell block sections and analysis of the results of the combined immunoprofile of the utilized marker panel.

All four markers were evaluated semi-quantitatively to assess both proportion and intensity scores. All studied cases were analysed for the combined immunoprofile of the four markers; EMA E29, Desmin, D2-40 and Ber-EP4 and 5 patterns were observed, (Table 3).

Out of the 62 studied effusion cases, immunostaining performed on cell blocks categorized 48 cases (77.4%) as positive for malignancy and 14 cases (22.6%) as reactive /negative for malignancy. Among the 48 malignant cases, the immunomarker panel categorized a single case (1.6%) as primary malignant mesothelioma, and 47 cases (75.8%) as metastatic malignancy. Representative photomicrographs of different effusion cases are demonstrated in (Fig. 1-5).

Table 3: Analysis of results of combined immunoprofile of (EMA E29, Desmin, and D2-40 and Ber-EP4)

Immunomarkers				Diagnosis			
EMA-E29	**Desmin**	**D2-40**	**Ber-EP4**	**Positive for malignancy**		**Reactive**	
				No.	%	No.	%
Positive	Negative	Negative	Positive	46	74.2		
Positive	Negative	Positive	Negative	1	1.6		
Positive	Positive	Negative	Positive	1	1.6		
Negative	Positive	Positive	Negative			13	20.9
Positive	Positive	Positive	Negative			1	1.6

Using weighted Kappa, a significant good agreement was observed between the immunomarker panel and CB cytomorphology in the categorization of the type of serous effusions (K (95% CI)=0.756(0.613 to 0.898), P<0.001)). The immunomarker panel and CBs concurred in the diagnosis of 52 cases (83.8%); 42 malignant and 10 reactive. The immunomarker panel increased the diagnostic yield of malignancy by 9.7% (6 cases) over the CB technique.

On the other hand, the immunomarker panel and conventional smearing agreed in the diagnosis of only 49 cases (79%); 13 reactive and 36 malignant cases. Using weighted Kappa, there was a significant moderate agreement between both techniques in the diagnosis of the type of serous effusions; (K (95% CI) =0.655(0.479 to 0.830), P<0.001)). Following examination of the immunostained CB sections, 12 additional cases (19.3%) were identified as positive for malignancy, (Table 4).

Table 4: Agreement between the immunomarker panel and Conventional Smearing

Immunohistochemistry	Conventional Smear			
	Reactive	**Suspicious**	**Positive for malignancy**	
Reactive	13	1	0	14 (22.6%)
Suspicious	0	0	0	0 (0%)
Positive for malignancy	5	7	36	48 (77.4%)
	18(29%)	8 (12.9%)	36 (58.1%)	62

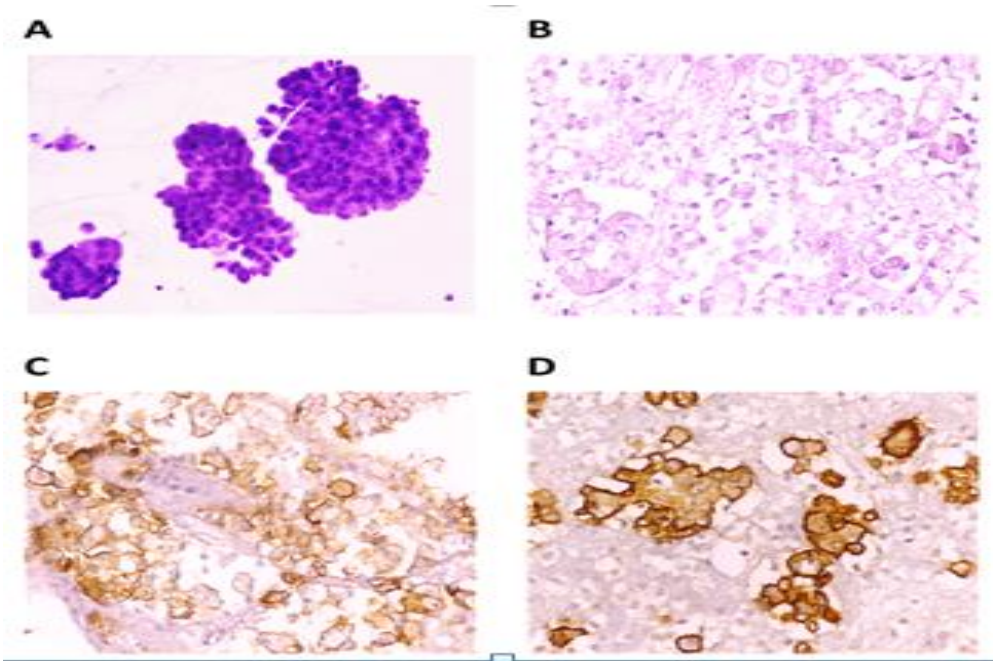

Fig. 1: A=Mesothelial cell clusters in CS, B=CB showed single cells and gland-like patterns, C=cells showed strong membranous staining for D2-40, D=strong spiky membranous staining for EMA, and were negative for desmin and Ber-EP4 (not shown), (All x400).

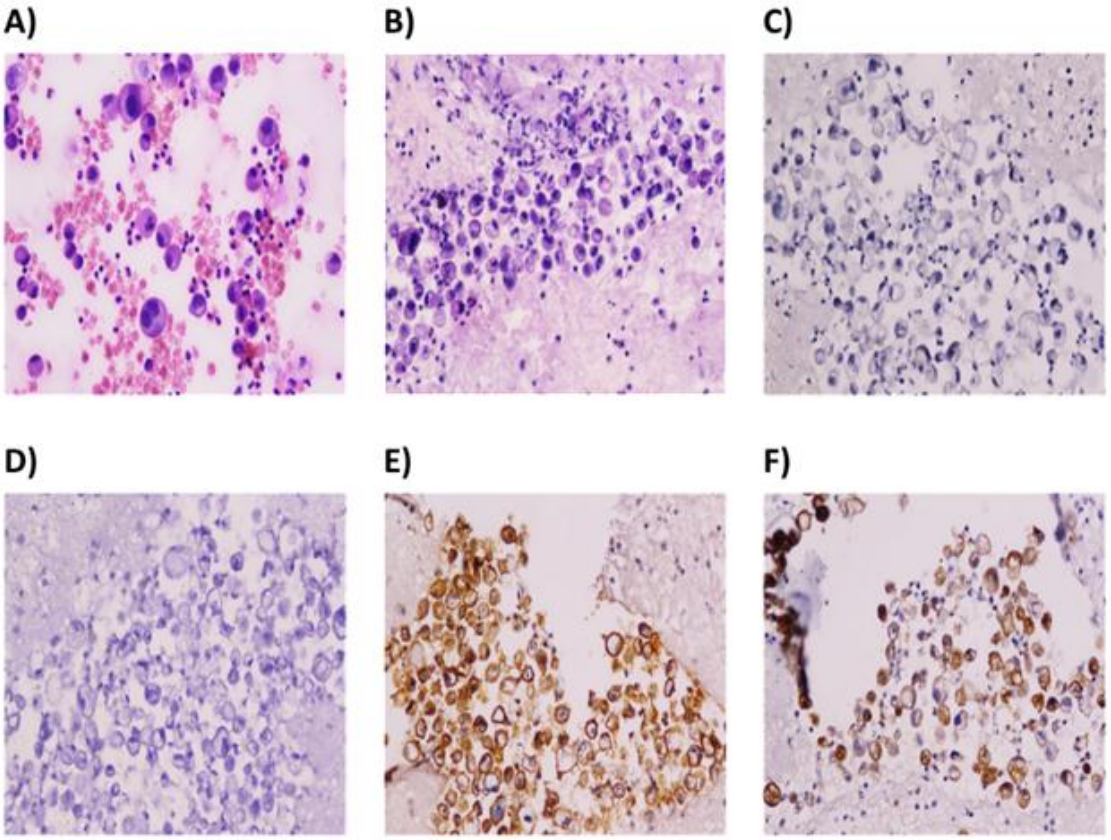

Fig. 2: A=H&E stained smears, B=its CB, show a dishesive suspicious cell population, with scattered signet ring forms. C=cells were negative for desmin, D=negative for D2-40, E=strongly positive for Ber-EP4 and F=for EMA, (All x400).

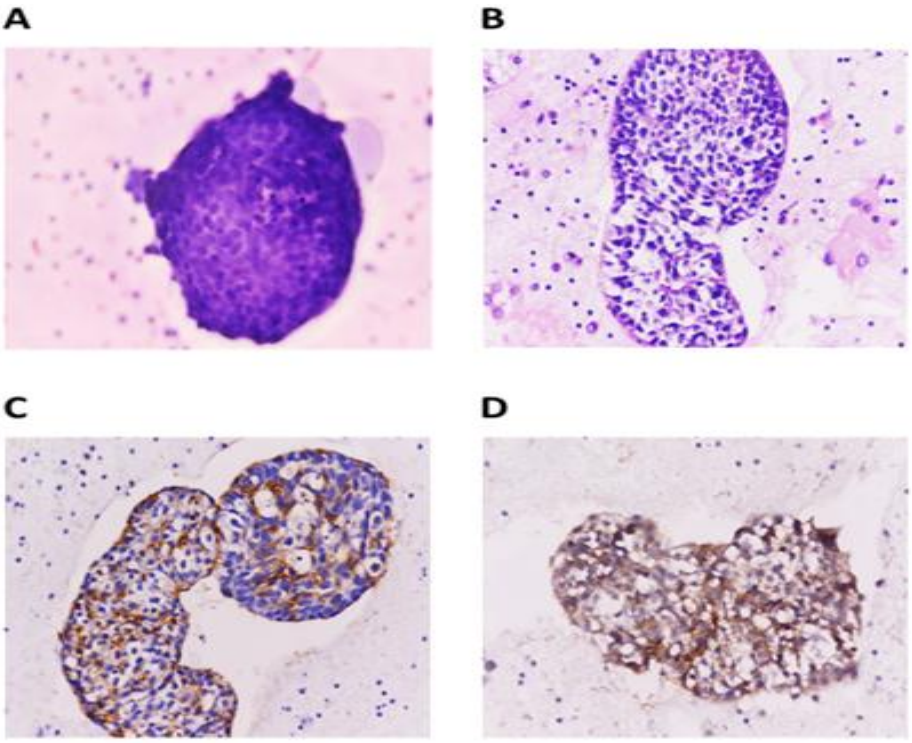

Fig. 3: A=H&E stained smears, and B=CB, display three dimensional cannon balls. The neoplastic cells showed: C=strong cytoplasmic staining for EMA, D=strong membranous staining for Ber-EP4, and were negative for D2-40 and desmin (not shown), (All x400).

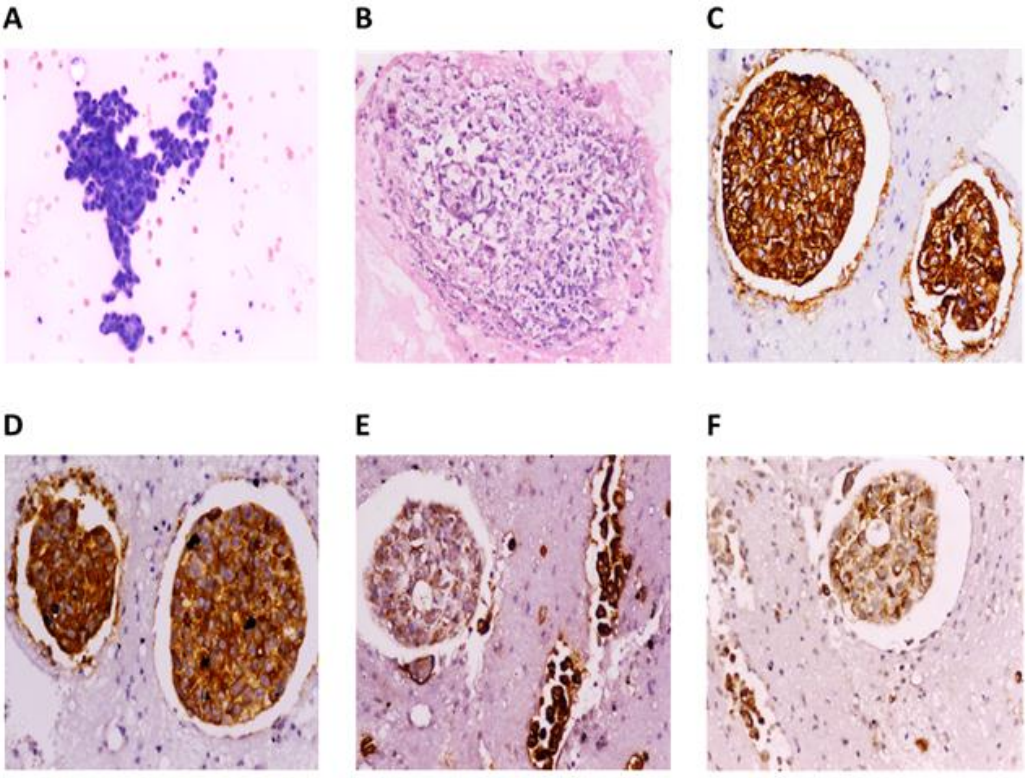

Fig. 4: A=smears feature papillary cell clusters, B=CB displaying a cell ball. C=cells revealed strong membranous and cytoplasmic staining for Ber-EP4, D=strong cytoplasmic EMA staining, E=negative D2-40 and F=focal moderate cytoplasmic staining for desmin, (All x400).

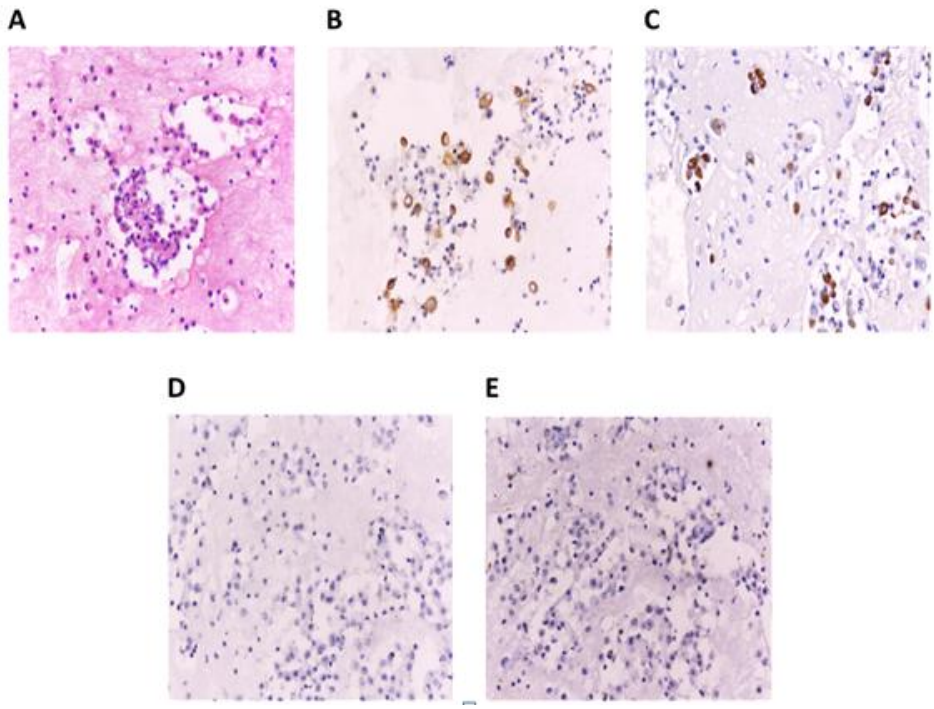

Fig. 5: A=CB shows reactive mesothelial cells singly and in clusters, B=reactive mesothelial cells show strong cytoplasmic positivity for D2-40, C=strong cytoplasmic staining for desmin, D=negative staining for Ber-EP4, and E=negative for EMA, (Allx400).

Discussion

The presence of malignant cells in serous effusions has significant prognostic and therapeutic implications.[26] The available techniques for assessment of serous effusions include cytological examination using CS and the CB technique or serous membrane biopsies. Both can be followed by immunohistochemistry, biochemical, bacteriologic analysis or cytogenetic studies.[27]

Despite being a simple procedure, CS has limitations; poor staining, fixation and preparation artefacts, lack of preservation of tissue architecture, cellular overcrowding, and scarcity of the diagnostic cells in some cases.[28,29] One of the most common problems in effusion cytology is the distinction between adenocarcinoma and mesothelial cells, both reactive and neoplastic. Therefore, in order to reach an accurate diagnosis, good cytological preparations and reliable ancillary methods are needed.[30]

The traditional CB technique can be traced back as early as 1896, when it was first introduced using a celloidin embedding medium.[31] The diagnosis of malignancy was found to be easier by the CB method compared to CSs mostly because of the better preservation of the tissue architecture and the easier differentiation between metastatic adenocarcinoma, mesothelioma and reactive mesothelial cells. However, because of the discrepancy between the results of CBs and CSs in some cases, it is recommended that both should be performed, each being complementary to the other, aiming to increase the diagnostic accuracy.[32]

In the current study, we aimed to evaluate the diagnostic utility of the CB technique in the cytologic diagnosis of serous effusions. A second aim was to assess the utility of an immunohistochemical panel in terms of improving the diagnostic accuracy.

In the current study, a comparative assessment of the cellular yield of both CBs and CSs was performed. A statistically significant difference between both techniques was observed (MCP <0.001), as the CB technique revealed a high cellular yield in 27.4% of cases more than smears. This is because cell blocks were more able to concentrate cellular elements in smaller fields compared to the problem of cell dispersal in CSs. Katti et al,[33] Shivakumarswamy et al[6] reported similar results, however, Nathan et al[34] reported that the cellularity of CSs and CBs prepared from body fluids were more or less identical and even slightly better in smears.

In this study, in agreement with others,[4,35,36] CBs, compared to CSs, demonstrated better preserved individual cell characteristics. Both nuclear and cytoplasmic details were better appreciated. The CB technique revealed a statistically significant difference compared to CSs as regards the identification of nucleoli (P<0.0001), detection of nuclear membrane irregularities (P=0.004) and detection of cytoplasmic vacuolization (P=0.003).On the other hand, Qamar et al[37] observed that individual cellular details were much better in cytospin preparations compared to CBs.

In the present work, and in accordance with others,[33,36,37] CBs were superior to conventional smearing regarding their ability to delineate the architectural patterns. Cell blocks were able to highlight the architecture in a biopsy like fashion. In our study, the single cell pattern was the commonest pattern detected in reactive effusion cases,

while more complex patterns such as: cell balls, clusters, papillae and acinar formations were detected in malignant effusions.

As regards the categorization of the effusion cases, a significant good agreement (K=0.711) between CSs & CB technique was observed. This is higher compared to Castro-Villabón et al[38] as the agreement between the two techniques in their study was moderate (K=0.59).

Furthermore, the results of the current study revealed a discrepancy between both techniques in 13 out of 62 cases (21%). Similar findings were reported in previous studies; [6, 39] for example, Köksal et al[39] also reported 4 additional cases diagnosed as malignant by the CB technique, three of which were suspicious on CSs and one was reactive. They also reported 9 cases in which the diagnosis of both techniques were concordant.

In the current study, the diagnostic yield for malignant cells increased by 9.6% following cell blocking. This finding is in agreement with other studies; Köksal et al[39] reported a 10% increase in the diagnostic yield of malignancy, and Thapar et al[10] reported a 13 % increase in the malignancy pick up percentage. Similar findings were also observed in other studies.[6,36,40] These findings could be attributed to the higher cellularity, the better preservation of specific architectural patterns, and the crisp nuclear and cytoplasmic details provided by CBs compared to CSs.

Because of the many diagnostic dilemmas in effusion cytology, a definitive diagnosis is often difficult based on observing the morphology alone, therefore, ancillary techniques are used to increase the diagnostic accuracy.[41] Immunocytochemistry (ICC) is optimally applied on CBs.[16] Because no single marker yielded unequivocal results, many studies have investigated different combinations of markers.[9] It is suggested that the best panels used to differentiate malignant mesothelioma, reactive mesothelial cells and metastatic tumours should include at least two positive mesothelial and two positive epithelial markers.[42]

One of the goals of the present work was to test the extent of improvement of the diagnostic accuracy of a four-immunomarker panel in distinguishing metastatic carcinoma from meosothelial cells, both reactive and malignant. D2-40 was utilized to highlight mesothelial cells, whether reactive or malignant, desmin was used to highlight reactive mesothelial cells, Ber-EP4 to stain epithelial cells and EMA E29 with its different staining patterns to differentiate malignant mesothelial cells and metastatic carcinomatous cells.

To the best of our knowledge, only few previous studies have addressed the use of a single panel of immunomarkers to differentiate these three diagnoses at the same time,[12,24] yet many studies have utilized panels to differentiate only between two groups, for example, mesotheliomas versus adenocarcinomas,[43,44] adenocarcinomas versus reactive mesothelial cells,[9,13] or mesotheliomas versus reactive mesothelial hyperplasia.[45]

In the present study, Ber-EP4 membranous and/ or cytoplasmic staining was detected in all suspected epithelial cells detected in CB sections. D2-40 stained all reactive as well as malignant mesothelial cells. It was able to highlight reactive mesothelial cells and was completely negative in metastatic carcinoma cells. D2-40 was also positive in the background reactive mesothelial cells in the malignant cases and was utilized as an internal positive control.

The current study has attempted to investigate the utility of the concurrent use of EMA clone E29 and desmin, as a part of an immunomarker panel, to differentiate reactive from malignant mesothelial cells. In addition, the pattern of EMA E29 staining was carefully analysed to differentiate metastatic carcinoma from malignant mesothelioma cells in our study. Based on the results of the current work, EMA E29 exhibited a high sensitivity in differentiating between reactive and malignant mesothelial cells; all reactive mesothelial cells were negative to EMA E29 with the exception of a single case. Desmin stained reactive mesothelial cells in all cases (100%).

Following the analysis of the combined immunoprofile of the studied cases, a single case was diagnosed as malignant mesothelioma (EMA E29 positive with a thick membranous staining pattern, D2-40 positive, desmin negative and Ber-EP4 negative). Among the 47 cases of metastatic malignancy, 46 cases were EMA E29 positive, Ber-EP4 positive, D2-40 and desmin negative. A single case revealed tight epithelial clusters and tri-dimensional balls, against a background of singly and clustered mesothelial cells, which showed focal desmin positivity (in 10% of cells) with a moderate staining intensity in one of the epithelial clusters. These clusters, however, exhibited strong cytoplasmic positivity for EMA E29 as well as strong positive cytoplasmic and membranous staining for Ber-EP4, and were negative for D2-40. Thus, the combined immunoprofile as well as the cytomorphology of the smears and the CB confirmed that case to be positive for metastatic malignancy. Davidson et al[46] also reported desmin positivity in 2 out of 98 (2%) carcinoma cases included in their study, in contrast, Hyun et al[24] reported that desmin was negative in all adenocarcinoma cases included in their study.

Regarding the reactive cases, the majority (13 out of 14) were EMA E29 negative, Ber-EP4 negative, desmin positive and D2-40 positive. A single case revealed focal positivity (in 10% of cells) for EMA E29 in reactive mesothelial cells. In that case, the cells were morphologically benign by both conventional smears and cell blocks, and they exhibited strong positivity to both desmin and D2-40, which was consistent with a mesothelial origin. These cells, however, were negative to Ber-EP4 which excluded an epithelial origin. Following comprehensive analysis and literature review, this case was reported as reactive. Shen et al[22] reported similar findings. On the other hand, Saad et al[21] stated that EMA clone E29 was negative in all reactive mesothelial cells and stained the majority of malignant mesothelioma.

In the present work, a significant good agreement (K=0.756), was observed between the CB cytomorphology and the final diagnosis following application of the immunohistochemical markers on CB sections, where concordance was noted in 52 cases (83.8%). Conversely,

immunohistochemistry changed the diagnosis of 10 cases (16.2%), and increased the diagnostic yield of malignancy by 9.7% when compared to the diagnosis based on CB cytomorphology alone. Dey et al[36] have applied immunohistochemistry on 13 suspicious cases by the CB and the CSs in their study, out of which 3 cases were confirmed as malignant, 3 cases were confirmed as reactive, and in 6 cases immune-staining confirmed the CB findings.

Among our cases, a significant moderate agreement between the diagnosis achieved by CSs and the applied immunomarker panel was observed. Both techniques agreed in the diagnosis of 49 cases (79%); including 13 reactive cases and 36 malignant cases. On the other hand, the immunopanel helped to eliminate the suspicious category, and increased the diagnostic yield of malignancy by 19.3%, as immunohistochemistry labelled 12 additional cases as positive for malignancy over CSs.

Conclusion

CBs as adjuncts to CSs allow a more accurate refined cytologic diagnosis compared to CSs alone. IHC performed on CB sections can further increase the diagnostic accuracy and the diagnostic yield of malignancy; it aids much in the classification of effusions into benign and malignant and in the categorization of malignant cases into primary and metastatic. Therefore, preparation of CBs along with CSs from all serous effusion samples submitted to the cytopathology laboratories is strongly recommended to be routinely performed.

Conflicts of Interest: None.

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How to cite this article: Tayel HY, EL-Gendi S M, Shafeik HA, Hassan SA, Diagnostic utility of cell blocks and an immunomarker panel in the cytological evaluation of serous effusions, *Arch Cytol Histopathol Res* 2019;4(1):47-56