

# Diagnostic Utility of the Immunohistochemical Markers Calretinin (CAL) and Epithelial Membrane Antigen (EMA) in Differential Diagnosis of Reactive Mesothelial Cells (RMC), Malignant Mesothelioma and Metastatic Adenocarcinoma (AC) in Serous Effusions

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**Abstract:** *Objective-*In present study, an attempt was made to compare conventional smear and cell block technique and to differentiate equivocal cytomorphological cases, reactive mesothelial cells and neoplastic cells on the basis of Calretinin and Epithelial Membrane Antigen immunohistochemistry. *Methodology-* In the present study 50 body cavity fluid specimens were evaluated by simultaneous use of smear and cellblock technique in Pathology department. First diagnosis was made on histopathological basis and then paraffin embedded sections were subjected to immunohistochemistry for Calretinin and Epithelial Membrane Antigen. *Results:* The additional yield for malignancy was 18% in cell block as compared to conventional smear. Calretinin proved to be an efficient marker for mesothelial cells with sensitivity 100%, specificity 94.4%, positive predictive value 96.9% and negative predictive value 100% (p-value < 0.05) and Epithelial Membrane Antigen for adenocarcinoma cells with sensitivity, specificity, positive predictive value and negative predictive value of 100%, 93.75%, 90%, 100% respectively (p-value < 0.05). *Conclusions:* Cellularity, morphological details and architectural patterns are better appreciated in conventional smear than cell block, with an additional diagnostic yield of malignancy. If supplemented with immunohistochemistry proved to be an useful adjuvant in evaluating serous effusions for a accurate diagnosis.

**Keywords:** conventional smear, cell block, calretinin, epithelial membrane antigen

## 1. Introduction

Cytological examination of serous effusions have been well accepted and a positive diagnosis is often considered as a definitive diagnosis. It helps for staging and prognosis of the patients in malignancies and also gives information about inflammatory lesions of serous membranes. Diagnostic problems arise in everyday practice to differentiate reactive mesothelial cells and malignant cells by conventional smear (CS) method

Cellblock (CB) method gives better architectural patterns, morphological features and help to differentiate reactive methotelial cells from malignant cells and thereby increases the efficacy of cytodiagnosis.

Cell block technique is one of the oldest method, for the evaluation of body cavity fluids.<sup>[1]</sup> This has not received much attention probably due to lack of standardized cost effective methods. The main advantages of cellblock technique are preservation of tissue architecture and to obtain multiple sections from the same material for special stains and immunohistochemistry.

Immunohistochemistry is an umbrella term that encompasses many methods used to determine tissue

constituents (the antigens) with the employment of specific antibodies that can be visualized through staining.<sup>[3],[4]</sup> In this study we attempt to differentiate mesothelial cells in ascitic/pleural fluid specimens from metastatic carcinomas using a limited panel of two monoclonal antibodies, calretinin and epithelial membrane antigen.

Calretinin is a calcium adhesion protein with molecular weight 29000 and is mainly expressed in the nervous system.<sup>[5]</sup> The "fried eggs" staining pattern has made calretinin Immunohistochemistry more valuable in differentiating mesothelial cells from carcinoma cells.<sup>[6]</sup> EMA is a high molecular weight transmembranous glycosylated protein of the breast mucin complex, which is useful for epithelial differentiation and has been found to be present on both carcinoma and mesothelioma cells.<sup>[7],[8],[9]</sup>

## 2. Methodology

In the present study 50 body cavity fluid specimens were evaluated by simultaneous use of smear and cellblock technique. Half of the fresh samples received were subjected for routine processing and another half of the fluid was subjected for cellblock by using plasma thrombin technique. Paraffin embedded tissue sections were then stained with routine haematoxylin and eosin (H&E) and first diagnosis

was made on the basis of the histopathological findings, then they were subjected for Immunohistochemistry for antibodies calretinin and EMA.

Various parameters such as cellularity, architectural patterns, additional yield of malignancy were compared between conventional smear and cell block. Analysis of discrepancies in both pleural and peritoneal fluid was done by conventional smear and cell block technique. By application of a panel of two antibodies Calretinin and Epithelial Membrane Antigen an attempt was made to differentiate between reactive mesothelial cells and adenocarcinoma cells.

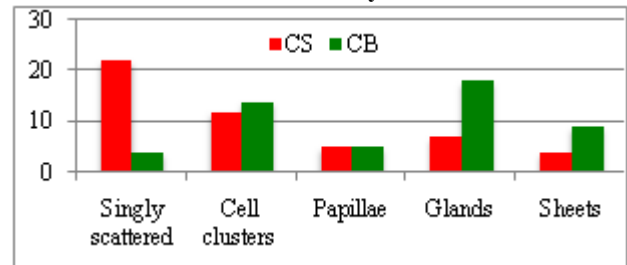
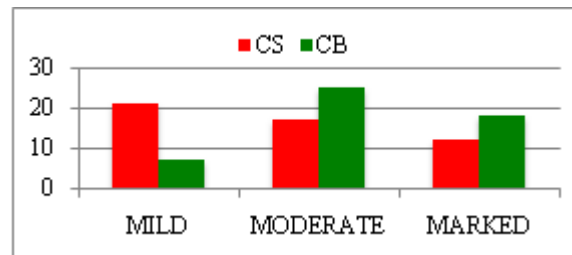
### 3. Results

Total 50 body cavity fluid samples were received, out of which 14 were pleural fluids and 36 were peritoneal fluids. The age varied from 11 to 90 years. The maximum number of samples were in the age group of 51 – 70 years. Male to female ratio being 1.38:1. Both in pleural and peritoneal fluids cellularity was more in cell block method as compared to conventional smear method. Architectural patterns, like cell balls, papillae, glands and three dimensional clusters were better appreciated in cell block than in conventional smears. By conventional smear 33 cases were diagnosed as benign, 8 cases as suspicious and 9 cases as malignant whereas by cell block method 32 cases were diagnosed as benign and 18 cases as malignant. No suspicious case was diagnosed by cell block method. Thus additional diagnostic yield came out to be 18% by cell block method.

In present study, 100%(32/32) of cases showed immunoreactivity with calretinin, with only 5.5% (1/18) of adenocarcinoma cases being positive for calretinin, 94.5% of cases were negative. For Epithelial Membrane Antigen 100% (18/18) of adenocarcinoma cases showed positivity with Epithelial Membrane Antigen, only 6.25% (2/32) of reactive mesothelial cells cases showed positivity with EMA, 93.75% of cases were negative.

By statistical analysis Calretinin proved to be the best single marker for Reactive Mesothelial Cells with a sensitivity of 100% and specificity of 94.4% with a positive predictive value of 96.9% and a negative predictive value of 100%. By using chi square test, p-value<0.05. Epithelial Membrane Antigen was found to be 100% sensitive for adenocarcinoma cells with a specificity of 93.75%, positive predictive value of 90% and a negative predictive value of 100%, p-value<0.05.

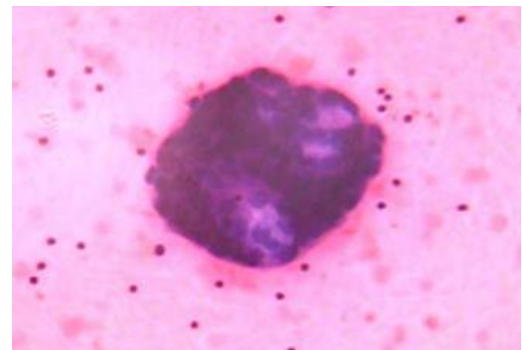
#### Diagrammatic Representation Of Cellularity And Architectural Pattern In Conventional Smear(CS) And Cell Block(CB)



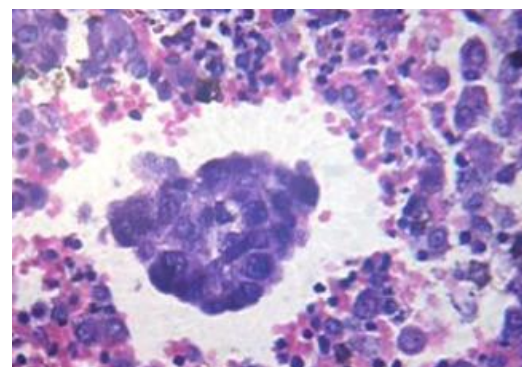
**Table:** Statistical Analysis of Calretinin and Epithelial Membrane Antigen in present study.

	Calretinin	Epithelial Membrane Antigen
RMC(n=32)	32(100%)	2(6.25%)
AC(n=18)	1(5.5%)	18(100%)
Sensitivity	100%	100%
Specificity	94.4%	93.75%
Positive Predictive Value	96.9%	90%
Negative Predictive Value	100%	100%
P-VALUE	<0.05	<0.05

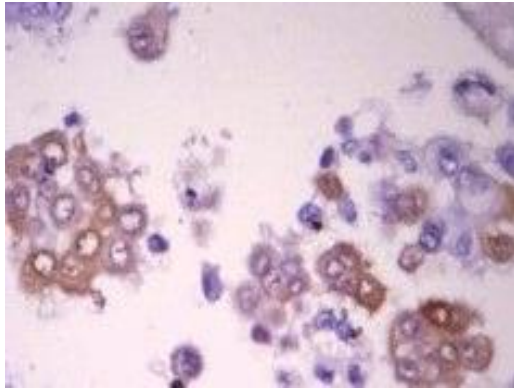
AC-adenocarcinoma, RMC- Reactive Mesothelial Cells



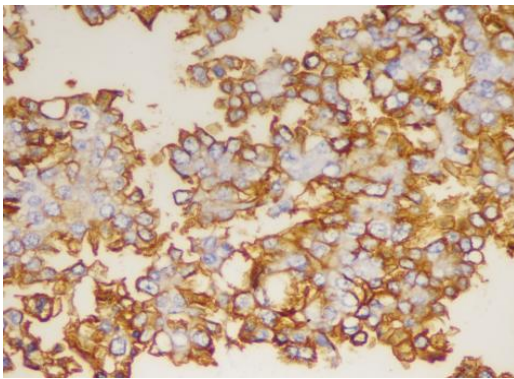
Photomicrograph showing cell ball in conventional smear (400X, Giemsa)



Photomicrograph showing cell ball with pericellular lacunae in cell block (400X, H&E)



Photomicrograph showing calretinin immunoreactivity in reactive mesothelial cells



Photomicrograph showing epithelial membrane antigen membranous and cytoplasmic immunoreactivity

#### 4. Discussion

Evaluation of body fluids for the presence or absence of malignant cells is a challenging task for pathologists, but due to the increasing prognostic implications and therapeutic challenges involved when a patient is diagnosed with the presence of malignant cells in serous effusions justify the continuous need for refinement of the existing diagnostic procedures and protocol.

The present study included 14 pleural fluids and 36 ascitic fluids with maximum samples belonging to the age group of 51-70 years. Male to Female ratio was 1.38:1 and Cirrhosis (37%) being the most common clinical diagnosis in ascitic fluid and Pulmonary Tuberculosis (12.5%) in pleural fluid. We got increased cellularity and better morphological details that is preservation of architectural patterns like three dimensional clusters, better nuclear and cytoplasmic preservation and intact cell membrane in cell block as compared to conventional smear. Similar findings were noted in various studies.<sup>[10],[11],[12]</sup> Diagnostic yield for malignancy was significantly increased, the documented study identified additional 18% (9 cases) malignant lesions by cellblock method when compared to conventional smear study. According to various studies additional diagnostic yield for malignancy was noted if conventional smear technique is supplemented by cellblock method.<sup>[10],[12],[13]</sup>

In our study, among the malignant lesions diagnosed by cellblock method in cohort of 50 samples of both pleural and peritoneal fluids primary was more common in ovary (28%), followed by carcinoma of breast (17%), carcinoma of GIT

and lung carcinoma (5.5%).

In our study we observed that staining for Calretinin was positive in 32/50 (64%) effusion smears, these 32 were all cases of reactive effusions. Adenocarcinoma cells show reaction with Calretinin in only 1 out of the 18 malignant cases. The sensitivity of Calretinin for detection of mesothelial cells was 100% with 94.4% specificity, positive predictive value 96.9% and negative predictive value of 100% (p-value < 0.05). The current work revealed positive staining for Epithelial Membrane Antigen in 18/50 (36%) effusion smears, with all the 18 cases being malignant, mesothelial cells revealed negative staining for Epithelial Membrane Antigen except in 2 Reactive Mesothelial Cells cases where they were focally positive for Epithelial Membrane Antigen. Overall, Epithelial Membrane Antigen exhibited 100% sensitivity, and specificity of 93.75%, positive predictive value of 90% and negative predictive value of 100% (p-value 0.05).

As inferred by other authors,<sup>14,15</sup> our study showed that no single marker on its own is capable of predicting the presence or absence of consistency, although a combination of Calretinin positivity and Epithelial Membrane Antigen negativity showed best sensitivity and specificity for mesothelial cells. Epithelial Membrane Antigen positivity and Calretinin negativity proved to be highly efficient for adenocarcinoma cells. Accordingly, it has been suggested that a minimum of two markers should be selected, as the expression of antigens in metastatic malignancies is often heterogeneous.

#### 5. Conclusion

To conclude Cellblock technique is simple, inexpensive and does not require any special training or instrument. Cellularity, morphological features were better identified and sensitivity for malignant lesion by cellblock method was significantly increased as compared to conventional smear. Calretinin proved to be best marker for mesothelial cells and Epithelial Membrane Antigen for adenocarcinoma cells.

#### 6. Future Scope

The cell block technique when used as an adjuvant to routine smear examination increases the diagnostic yield of malignancy because of better preservation of the architectural pattern which helps in timely and effective management of patients. Immunohistochemistry also gives better results on the tissue in the cell block than cytological smears particularly in cases where there is a diagnostic dilemma between the malignancy and reactive changes.

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