ISSN NO. 2320–7418 DOI: 10.22270/jmpas.V10I4.1222

International open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com

Research article

Ema-and useful immunocytochemical marker at differentiation of glandular malignant cells from atypical mesothelial cells on cell block preparation of body effusions

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ABSTRACT

The detection of the malignant cells by cytologic method has long been practised. The detected malignant cells are difficult to be typed specially in the situations of overlapping cytomorphology. The conventional cytologic preparation may harbour lower number of malignant cells but the method of cell block offers high detection rates of such malignant cells. Still the problem remains when the evaluation is short by morphological features and requires immunoexpression studies. The commonest situation encountered of overlap cytomorphology is the distinction between well differentiated adenocarcinoma from reactive mesothelial cells with atypia in effusion. Such a distinction is not only important for the purposes of diagnosis but also for prognostification by stage and management of the patient .The review presents the experiences of the past studies over utility of EMA in detection and segregation of the cells of well differentiated adenocarcinoma infiltrating the effusions from reactive atypical mesothelial cells. The present review is compiled from the publications from various institute across the globe and shares the authors experience for the utility of epithelial membrane antigen over the cell block of effusions at distinction of overlap cytomorphology. Compilation of the study by inclusion of articles by 1,2,3,3,4,5,6,7,8,9,10.The articles were searched through google engine and Pubmed search. The 10 articles dealing onto the role of EMA as a immunocytochemical marker have affirmed the high sensitivity and specificity of EMA at seggregating glandular malignant cells from atypical mesothelial cells on cell block preparation of body effusions.The range of sensitivity and specificity for EMA was found to be 81% -100% and 98.86 -100% respectively.The positive predictive value range from 92-97% and negative predictive value ranging from 88.64 – 100%. All the studies reviewed confirmed the high value of significance (P value for EMA). The systematic review carried out re-affirms the role of EMA in the situations of of indistinguishable cytomorphology of adenocarcinoma from that of benign , reactive , atypical mesothelial cells. The overall diagnostic utility of EMA was conducted to br over 90% in such situations.

Keywords: EMA, Immunocytochemistry, Adenocarcinoma, Mesothelial cells, Cellblock, Effusions

Received - 05-06-2021, Reviewed - 30/06/2021, Revised/ Accepted- 08/08/2021

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INTRODUCTION

Metastasis of the malignant cells to the potential spaces of body cavities happens as a natural course of malignant diseases. This kind of metastasis often results in effusions. Adenocarcinoma is the most common type of glandular cell malignancy that has the propensity to metastasize to these potential spaces and often results to pleural effusions, peritoneal effusions, pericardial effusions and few others. The samples of haemorrhagic, exudative or a serous effusion are often sent to cytopathology laboratories with the purpose of detection of malignant cells. The finding of malignant cells within the effusions is not helping clinicians for the diagnosis but is useful for staging of the disease and appropriate therapeutic intervention.

However it is not so easy to detect, diagnose, type and differentiate the malignant cells even when the primary malignancy is known leave apart the unknown or occult malignant loci. The most common difficulty encountered by the cytopathologist worldwide is:

1. The representative cellularity in conventional cytopathologic smears preparation.
2. The cytomorphological overlaps.

Both these situations results in inappropriate diagnosis that matters to adequate and appropriate treatment of the sufferers of malignant epithelial neoplasia. The difficulty of the cell concentration with conventional cytology has been taken care by cell block preparations of body fluids since past two decades. It's now been an established method of cytologic evaluation. However, the disputed and overlap cytomorphology still remains. The most common overlap cytomorphology that remains indistinguishable from each other are reactive atypical mesothelial cells from that of dissociated or in sheets of cells of adenocarcinoma.

A few representative published studies have advocated the use of cell block studies in this situation. But the problem still remains as mere cell block study cannot provide the solution at resolution of complexity at above specified cytomorphological overlap. Cell block per se is widely used technique .However, it is possible that 50% of the cases of the metastatic disease will elude its detection in cell block.1

Inability to separate the disputed morphologies of exfoliative, benign, atypical, mesothelial cells from metastatic cells of adenocarcinoma in effusions therefore requires immunomarkers as diagnostic aids. The myriad of architecture and cellular alterations in details has emphasized the cytological characters for distinction of benign reactive mesothelial cells and well-differentiated or borderline malignant cells masquerades each other. To dispense away this equivocality immunochemical studies are indispensable. The best option to resolve this dilemma is to submit the cell blocks of suspected pleural effusions with indistinguishable cytomorphology to the immunohistochemical panel that enables their distinctions.

Studies are available in literature those propose the utility of immunohistochemistry and has bought out the distinction between the peculiar overlap of cytomorphology either by advocating a single immunocytochemical reactive antibodies or the combinations of it 1,2,3,4,5,6,7,8,9,10.. The commonest antibody that has been resourcefully used for distinction between epithelial and mesothelial cells are EMA, E-Cadherins, carcinoembryonic antigen, calretinin and vimentin (1,2,3,4,5,6,7,8,9,10). The studies of Murugan et al1,Nautiyal et al2,Vrinda et al3, Singh et al6, Keith et al7, Ueda etal9 have bought out the distinction between the epithelial malignant glandular cells of adenocarcinoma of well diffentiated type from benign, reactive, atypical mesothelial cells with high sensitivity as close as above 90%. The similar values over 90%have been specified for specificity for immunohistochemical evaluation by above antibodies at diagnosis and distinction carried out over the cell blocks. In the afore specified studies Murugan et al1,Vrinda et al3,Singh et al6,Nautiyal et al2,Ueda et al9,Keith et al7,Subarayan et al4the common antibody that has extensively been used with high sensitivity and specificity at distinction of disputed cytomorphology have been EMA followed by E-cadherin. Antibodies to desmin are next in list that has specificity of 95.12% and positive predictive value of 91.30 % for mesothelial cells (1,3) closely followed by vimentin.

Study of Murugan et al1 and Nautiyal et al2 in their observation enticed that a single or combination of these immunocytochemical markers are competent enough to distinguish between the two cells of different histogenesis. EMA, which is expressed by the glandular epithelial cells of malignant nature constantly expressed even through the grade of adenocarcinoma. EMA is otherwise unassociated with mesothelial cells for its marked expression therefore the detection of EMA can confidently segregate cells of well differentiated adenocarcinoma from that of benign, atypical mesothelial cells on cell blocks studies (1,2,3).

The literature search on this topic for identification of malignant cells, their distinction in the situations of overlap cytomorphology and cytoarchitecture have shown EMA as a common molecular candidate in all the combinations of immunocytochemical panels. Therefore it becomes the molecular expression of EMA remains still a gold standard for the distinction of glandular epithelial cell and mesothelial cells .(1,2,3,4,5,6,7,8,9,10)

The review is constructed over the studies that deliberate on the utility of EMA immunocytochemistry as a potential marker of distinction which has implications not only for diagnosis but also for prognostification and therapeutic implication.

The author of the present review intends to explore the studies that have been dedicated to immunocytochemical assessment of the cell block for EMA as one of and one and one only immunocytochemical marker. The eligibility criteria for the inclusion of studies in this systematic review are made known in the later part of the text.

The single most criteria for the inclusionof this studies within the present reviews objective is the assessment of EMA as a marker in difficult situations of overlap cytomorphology accompanied by its statistics. (Sensitivity, Specificity, Positive Predictive value, Negative Predictive Value and P-value).

EXPERIMENTAL

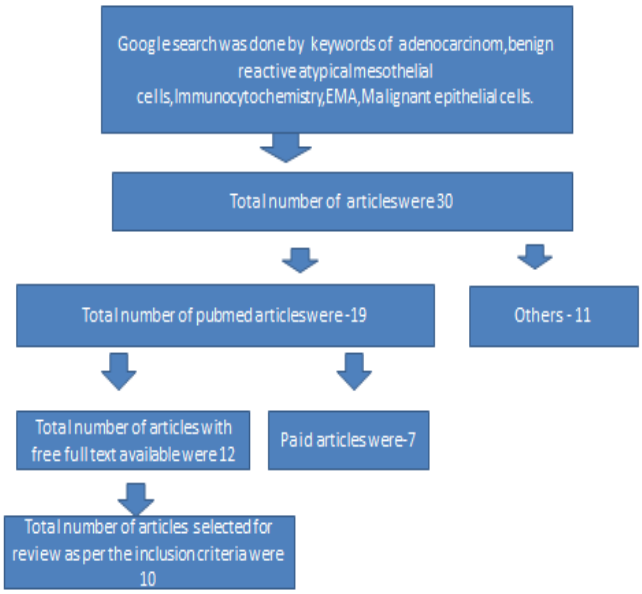
The review on immunocytochemical staining on cell blocks in the effusion meant for confirmation of disputed cytomorphology and overlaps between the cells of well-differentiated adenocarcinoma and benign, reactive mesothelial cells was compiled through the web access of articles freely available on PubMed.

The PubMed search was done with the keywords of cell block plus immunocytochemistry plus EMA plus adenocarcinoma plus benign plus atypical plus mesothelial cells through google drive.

The following was the methodology as depicted in figure 1 that is made with the pre-established criteria for the selection of

original articles.

Figure 1: Literature search strategy for inclusion within review



Total of 10 articles were selected from PubMed which suffice to the inclusion criteria of the present review as described below

1) Minimmal number of Patients in study should be around 20.
2) A comparative statistics for EMA with adenocarcinoma and that of mesothelial cells.
3) The statistical evaluation of the results done with tools of sensitivity, specificity, positive predictive value, negative predictive value and p value.
4) Immunohistochemistry performed by monoclonal antibodies against epithelial membrane antigen by biotin streptavidin method with colour development by 3,3 diaminobenzidine.

STATISTICAL METHOD

The present review intended to collect statistical information regarding the total number of patients of 10 articles, gender distribution, age-ranges, site and location of primary tumour, the type of carcinoma and its grade. Cytological diagnosis on the cell block ,results of immunohistochemistry for EMA ,the sensitivity and specificity immunohistochemistry for EMA on cell block and other auxiliary statistics that pertains to the credibility of EMA immunostain.

RESULTS

The review of ten articles consisted of total of 763 numbers of patients. The distribution of gender was in proportion of male: female of 1:2.1.The age range of patients was from 11 years to 90 years.

The distribution for the site of effusion was as follows: Pleural-279, Peritoneal-261, Pericardial-11, Others-nil. The following are the studies described for the parameters of study population size, the number of fluid, sites, number of preparations, the additional number of cases diagnosed on cell block. (Table 1).

Table1: Number of samples per study for cytology and cell block

Study and Year	Number of patients	No of samples	Additional Number of cases diagnosed on cell block.
Murugan et al, 2009.	43	49	16
Nautiyal et al, 2017.	253	253	Nil
Vrinda et al, 2016	50	50	09
Subarayan et al, 2019.	84	84	-
Knoepp et al, 2012.	66	66	-
Singh et al, 1994.	180	180	-
Keith et al, 1990.	55	55	-
Ueda et al, 2005.	19	19	-
Daste et al, 1991.	109	109	-
Jensen et al, 1995.	94	94	-

The analysis of comparative statistical values from 10 reviewed studies are described in table 2 for EMA in Murugan et al1, Nautiyal et al2, Vrinda et al3, Subarayan et al4, Knoepp et al5,Singh et al6,Keith et al7,Ueda et al9,Daste et al10.statistical values of correlation in studies reviewed for EMA.(Table 2)

Table 2: Statistical value of various studies for EMA

EMA	Sensitivity	Specificity	Positive predictive value	Negative predictive value	P value
Murugan et al	100%	97.37%	97.5%	100%	<0.0001
Nautiyal et al	91.89%	100%	-	-	-
Vrinda et al	100%	93.75%	90%	100%	<0.05
Subarayan et al	88.1%	92.86%	92.5%	88.64%	<0.0001
Knoepp et al	98%	-	-	-	-
Singh et al	97%	-	-	-	-
Keith et al	96%	-	-	-	-
Ueda et al	100%	100%	-	-	<0.01
Daste et al	81%	-	-	-	-

The following is the distribution of the primary of adenocarcinoma that presented as effusions as collected in total of 10 studies. (Table 3)

Table 3: Distribution of the primary adenocarcinoma in 10 studies.

Serial No.	Site Of Primary	Number of Cases
1.	Unknown	16
2.	Ovary	52
3.	Endometrium	11
4.	Lung	30
5.	Breast	11
6.	Git	21
7.	Pancreas	9

DISCUSSION OF REVIEW

Murugan et al[1] over his observation on cell block have found it to be best single marker of adenocarcinoma with sensitivity of 100% and specificity of 97.3%. Their study advocated calretinin for identification of reactive mesothelial cells with sensitivity of 100% and specificity of 92%. The comparison between calretinin and desmin in their study has shown desmin to be more specific but had poor sensitivity of 56.25%. E-cadherin's, CEA and vimentin were found to have unsatisfactory predictive value that prompts to conclude for preclusion of their use as a single useful diagnostic marker.

The study of Murugan et al[1] suggested the panel instead of a single marker at confirmation of cell of origin, cell typing with the results that EMA and negative calretinin and desmin for adenocarcinoma and negative EMA or CEA for reactive mesothelial

cells. If their combinations are used their sensitivity and specificity reaches to 100% to disclose the identity of adenocarcinoma and reactive mesothelial cellsin overlap cytomorphology.

Nautiyal et al[2] have observed the strong membranous and cytoplasmic positivity of EMA in 34 cases of adenocarcinoma and calretinin positivity in 38 cases categorize as reactive or atypical mesothelial cell hyperplasia .The study advocate the use of calretinin and EMA for the capacity to distinguish adenocarcinoma cell from mesothelial cell. The study of Vrinda[3] et al observed that there is a diagnostic addition of 18%if cell blocks are used as per the conventional smear cytology. Their study has observed calretinin as an efficient marker for mesothelial cell with sensitivity of 100 and specificity 94.4%.Its positive predictive value was found to be 96.9% and negative predictive value-100%.The observations made for EMA for diagnosis of adenocarcinoma cells proved to have sensitivity-100%,specificity -93.75%,positive predictive value-90%,negative predictive value-100%.

The study has advocated use of immunohistochemistry as an adjuvant in evaluating the effusion with low cellularity on conventional cytology and overlap cytomorphological. Study conducted by Subarayan et al[4] showed that EMA had sensitivity of 88.1%and specificity 92.86 for metastatic cells of adenocarcinoma while calretinin demonstrated 100% sensitivity and specificity of 97.62 % for mesothelial cells. The study advocated calretinin immunostaining in difficult situation for appropriate distinction of reactive atypical mesothelial cells from adenocarcinoma cells.

Study of Daste et al[10] made their observation that EMA had strong sensitivity and specificity at diagnosis of adenocarcinoma cells on the cell block. Thirty- two effusions (74%) of effusions showed staining of EMA for adenocarcinoma cells while it was negative for mesothelial cells. Knoepp et al [5]observered sensitivity of 91% of EMA for adenocarcinoma cells and was positive in 1% of reactive effusion. The authors concluded that Epithelial membrane antigen can be useful in detection of metastatic carcinoma cell in malignant effusion more so if the morphology of the cells are confusing.

Singh et al [6]conducted a study on significance of EMA in the workup of problematic serous effusions and observed that EMA was strongly positive in all cases of malignant effusion because of metastatic adenocarcinoma cells while it was positive in only 3.8% of cases of reactive effusions. The study advocated the use of EMA as a part of ICC panel to differentiate the adenocarcinoma cells from benign reactive mesothelial cells and recommend the use of a panel of antibodies instead of a single marker.

A study was conducted by Keith etal[7] using a panel of antibodies comprising of EMA, B72.3, leu-m1, cytokeratin, LCA, S-100 and vimentin on cell block preparation for the identification of malignant cells in serous effusions. It was found that amongst the antibodies the sensitivity of epithelial membrane antigen in detection of metastatic adenocarcinoma cellsin effusion was highest (96%) followed by CEA (77%), B-72.3 (58%), Leu-M1 (42%).The study favoured the use of ICC panel for confirmation of diagnosis in equivocal cases.

The study conducted by Ueda et al [9]observed that, EMA and MOC-31 was 100% sensitive and showed strong immunoreactivity in all cases of malignant effusions with metastatic adenocarcinoma cells. It was also observed that the sensitivity of smear preparation was more than that of cell block for immunocytochemistry. Jenson et al[8] compared the immunostaning on both the smear as well as cell block for EMA in 94 consecutive serous fluid. The EMA immunostaining was performed differently for smear and cell block with incubation time of 30 min and 16 hrs. respectively. The result of the studies reveals that 11 additional cases of adenocarcinoma could be diagnosed with strong EMA positivity on smear/cellblock.

The results of the studies reviewed by meta-analysis expresses that EMA remains the most sensitive and specific marker that distinguishes adenocarcinoma cells from mesothelial calls as a single marker. The comparative statistics as depicted in table 2 shows that the sensitivity and specificity of the EMA can be enhanced if the combinations of the markers such as calretinin,vimentin,desmin,E-cadherin,MOC-31,MES,P-63,PAX-8,TTF-1, Napsin A, CDX-2, B72.3, LeuM1, Cytokeratin, LCA, S-100, CEA, Ber-EP4, CA-125, HBME-1CA-19-9 are used for discrimination between adenocarcinoma cells and mesothelial cells. The complimentary combination of EMA as reviewed for the present work in the studies of Murugan et al, Vrinda etal, Nautiyaletal, Subarayanetal were Calretinin, Vimentin, E-cadherin in order of their sensitivity and specificity.

The cut off percentage that still remains undiagnosed even on the application of single marker EMA or in combinations with other marker like Calretinin, Vimentin, E-cadherin still remains wide (3.8%)by values of sensitivity and specificity reported in the studies reviewed even when combined immunohistochemical markers are used [1,2,3,4,5,6,7,8,9, 10].

CONCLUSION

The systematic review carried out to evaluate the role of EMA as a single marker on immunohistochemistry of cell block comes close to its diagnostic utility over 90% in the situations of indistinguishable cytomorphology of adenocarcinoma cells from that of benign reactive atypical mesothelial cells.

Though not withstanding to the highest performance but still adds value to the diagnostic work up of the indistinguishable morphologies in resolving or in helping the decision making .This would be an additional exercise for the cytopathologist to be

incorporated in refinement of diagnostic microscopy. The review generalizes the conclusion that EMA alone is a dependable marker to identify adenocarcinoma cells indistinguishable from mesothelial cells on conventional cytology or cell block.

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How to cite this article

Neha Jaiswal*, Arvind Bhake, Anita Sajjanar, 2021. "Ema- and useful immunocytochemical marker at differentiation of glandular malignant cells from atypical mesothelial cells on cell block preparation of body effusions". Jour. of Med. P'ceutical & Allied. Sci. V 10 - I 4, 1222, P- 3459 - 3463. doi: 10.22270/ jmpas.V10I4.1222