Differential Diagnosis of Reactive Mesothelial Cells and Adenocarcinoma Cells Using Immunocytochemical markers (Ber-EP 4, MOC-31, Calretinin and HBME-1) in Serous Effusions

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Abstract:

The inability to undisputedly distinguish reactive mesothelial cells from metastatic adenocarcinoma cells exfoliated in serous effusions is the most common difficulty encountered by pathologists. Ancillary studies are being useful in improving the accuracy of cytological diagnosis. From all the available methods, Immunochemical stains have excelled in the diagnosis of effusion cytology.A combination of epithelial and mesothelial markers is recommended, as use of a single marker alone can't establish the diagnosis.In present study, we aimed to distinguish adenocarcinoma cells from reactive mesothelial cells with the help of a panel of immunomarkers including; two epithelial cell markers; Ber-EP 4 and MOC 31, and two mesothelial markers; Calretinin and HBME1.

Objective*:* To differentiate reactive mesothelial cells and adenocarcinoma cells by using immunocytochemical markers (Ber-EP 4, MOC-31, Calretinin and HBME-1) in serous effusions.

Subjects and Methods: A total 75 fluid samples containing either reactive mesothelial cells or adenocarcinoma were included in the study.Cytospin smears were made and subjected to immunocytochemistry for Ber-EP 4, MOC-31,Calretinin and HBME-1 staining.

Results: Ber- EP 4, an epithelial marker, showed positive membranous/cytoplasmic expression in 100% of the effusions which were cytologically diagnosed as positive for adenocarcinoma. MOC 31 expressed diffuse membranous staining in 93% of the total cases of adenocarcinoma.HBME-1 was positively stained in 100% of cases of reactive mesothelial effusions. 100% effusions containing reactive mesothelial cells showed both cytoplasmic and nuclear staining with Calretinin.

Conclusion*:* The immunomarkersBer-EP 4 and Calretinin are more effective in distinguishing reactive mesothelial cells and adenocarcinoma, and they should be included in immunocytochemical (ICC) panel.

Key words: Reactive mesothelial cells, Adenocarcinoma, Immunocytochemistry (ICC), Effusion

Introduction

The inability to distinguish reactive mesothelial cells from metastatic adenocarcinoma cells exfoliated in serous effusions and the unavailability of a standard and accurate panel of immunomarkers as a diagnostic aid in solving the problem is the most common difficulty encountered by pathologists in diagnostic cytology worldwide.[1] The main reason is the overlap in cytomorphological features of reactive cells of mesothelial origin and of carcinomatous cells.[2] Ancillary studies are being useful in improving the accuracy of cytological diagnosis.

Reactive changes in mesothelial cells can be present in variety of infectious conditions including chronic inflammation, infarction, liver diseases, systemic diseases, radio-therapy and chemotherapy. Reactive mesothelial cells are also present together with the metastatic neoplastic lesion on the mesothelial surfaces.[3] Adenocarcinomas are the most common tumours that involve the serous membranes of body cavity.[4] The altered cytological features exhibited by

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mesothelial cells due to the reactive and hyperplastic changes can imitate malignancy

As the cancer and infectious diseases have different effect and management therefore, it is important to properly distinguish between malignant and benign effusions.[5] In routine, the patients who are diagnosed with malignancy in the effusions, go through the further testing to confirm the evidence of malignancy because the accumulation of the malignant effusions are suggestive of metastasis and therefore, they are possibly intended to have the aggressive therapies against cancer. Irrespective of the type of cancer present, the existence of the malignant effusions is the indication of poor prognosis.[6] In most of the cases, diagnosis can be made by analyzing the fluid. In case of patients with transudative pleural effusions, there is no further requirement of diagnostic and the built-in diseases for example; liver disease, cardiac failure etc should be treated in a proper manner. The fluid analysis can conclude the effusions as infectious or malignant in the case of exudates.[7]

Although multiple laboratory tests are in use for differential diagnosis of effusion in routine clinical practice, a major percentage is not being diagnosed appropriately.[5]The appearance and color of the fluid provide preliminary information regarding effusions. Whereas the use of indicators including counts of red blood cells and white blood cells, and the chemical tests such as glucose, cholesterol, LDH, protein and amylase provide the additional information relating to the inflammatory characteristics of the fluid.[8] Aninfection indicated by the fluid which is non-purulent and free-flowing can be treated by the use of antibiotics only.[7]

Most commonly used method for distinguishing between malignant and non-malignant effusions is an examination of the fluid. In this method, there is a 100 % specificity but sensitivity ranging from 40% to 70 %, because the malignant cells may not be present in the sample, ignored or can't be identified correctly.[6] The cytological screening has low sensitivity due to different reasons, and differentiation between malignant and reactive mesothelial cells is difficult particularly in the long-standing effusions.[9] Additional material can be very useful in challenging cases, especially if the degenerated cells are encountered, because the malignant effusions accumulate again promptly and the cells are usually preserved better in the second tap.[10] Therefore, in many cases there may be a need for multiple samples to establish the diagnosis of malignancy.[11] Cytology of the malignant effusions is significant due to its convenience and noninvasive procedure.[12] However the cytological diagnosis depends on the type of tumor and primary site, and it is highest for ovarian tumors (83%) and lesser for the carcinoma of breast (78%), lung (57%), and mesothelioma (41%).[13] Immunochemical stains have excelled in the diagnosis of effusion cytology. Now they have been widely adopted as complementary tools for correct cytological diagnosis of effusions. Historically these stains have been used on cell block preparations of effusion samples.[14]

At present many mesothelial and epithelial immunochemical markers are available to assort benign and neoplastic mesothelial cells and metastatic carcinoma. Despite the fact that a number of markers are available in market, an optimal panel of antibodies is not feasible yet.[15] In previous studies, immunochemical staining was performed on small surgical biopsies and cell block sections. Very few studies have assessed these markers on cytospin slides.[4] MOC-31, B72.3, Ber-Ep4 and monoclonal carcinoembryonic antigen (CEA) is among most widely used immunomarkers for epithelial cells. Calretinin, WT1 and HBME-1 are commonly used mesothelial markers.[15]Studies advocate to use an antibody panel consisting of both mesothelial and epithelial markers.[16]

The application of these carcinoma markers (Ber-EP 4 and MOC-31) and those for mesothelial cells (HBME-1 and Calretinin) can help in isolating malignant from reactive mesothelial cells, hence will help in making an early and accurate diagnosis.

Objectives

To differentiate reactive mesothelial cells and adenocarcinoma cells using immunocytochemical markers (Ber-EP 4, MOC-31, Calretinin and HBME-1) in serous effusions.

Materials and Methods

It was an observational study,conducted in the Department of Morbid Anatomy and Histopathology at University of Health Sciences Lahore. 75 fluid samples containing either reactive mesothelial cells (RMC) or adenocarcinoma (AC) were included in the study. Detailed examination of fluid including site, total volume, color and presence of blood, mucous, pus or clot and any other necessary details were recorded. After processing the fluids, two alcohol-fixed and one air-dried smear were made from the sediment and subjected to Haematoxylin & Eosin,

Papanicolaou and Giemsa staining. Preliminary diagnosis was made on the cytomorphological features.

Cytospin preparation: Cytospin preparation was made in all cases morphologically classified as reactive mesothelial effusions and adenocarcinomas and were subjected to immunocytochemistry (ICC).

Immunocytochemistry: Cytospin smears and cell block sections were made on poly-L-lysine coated slides and were subjected to ICC for Ber-EP 4, MOC-31, Calretinin and HBME-1 using streptavidin-biotin peroxidase technique. Positive staining is defined as proper cellular expression (cytoplasmic, membranous, or nuclear) in 5% or more of the cells. Less than 5% cellular expression was taken as negative.[3]

Results:

The fluids were cytomorphologically diagnosed and n=31(41%) were diagnosed to have reactive mesothelial effusions, n=36(48%) were malignant and n= 8(11%) were suspicious of malignancy containing reactive mesothelial cells including only a few atypical cells (Table 1).

Table 1.Cytomorphological diagnosis
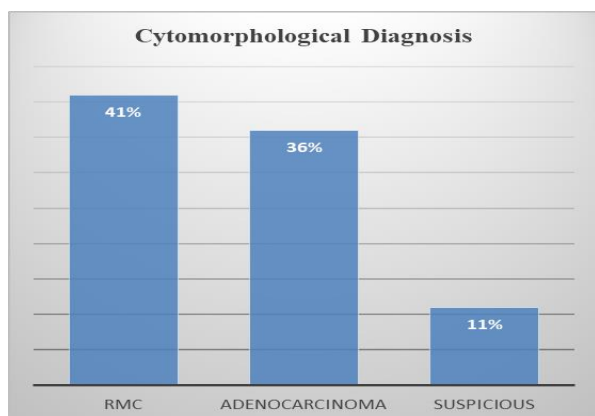
Regarding gender distribution, the number of males presenting with effusions was 23% (n=17), whereas 77% (n=58) cases were females. Male to female ratio was calculated as 1:3.4. In males, n=11 (64.7%) of effusions were of reactive mesothelial effusions and n=6 (35.3%) cases were diagnosed as malignant. Whereas, n=38 (65.5%) of female patients were positive for malignancy and n=20 (34.5%) patients were having benign or reactive effusions.

In a total of n= 75 cases of effusions, number of pleural fluids were n= 35 (46.7%) and n= 40 (53.3%) were the cases of peritoneal effusions. Regarding the gross appearance of these effusions, n= 47 (62.7%) of the fluids were yellow in color whereas n= 28 (37.3%)

fluids were blood-stained. Among the reactive mesothelial effusions, n=19 (61.3%) cases were yellowish in appearance and n=12 (38.7%) were blood-stained fluids, while n=28 (63.6%) of the malignant effusions were non-hemorrhagic and only n=16 (36.3%) of malignant effusions were blood-stained. in the patients with pleural effusions, most common malignancies included breast carcinoma (77.3%), followed by ovarian carcinoma (9%), GIT cancers (9%), and lung carcinomas (4.5%). In peritoneal effusions, 27.5% effusions were had no history of malignancy. Whereas in peritoneal effusions due to malignancies, ovarian carcinoma was most common comprising 62% of malignant peritoneal effusions, followed by 13.7% of breast carcinomas, 10.3% of lung cancers, 6.9% of GIT carcinomas, 3.4% endometrial carcinoma and 3.4% of lymphomas.

Ber-EP 4, an epithelial marker, showed positive membranous/cytoplasmic staining in 100% of the effusions which were cytologically diagnosed as positive for adenocarcinoma and were negative in all the cases of reactive mesothelial effusions (Fig 1).
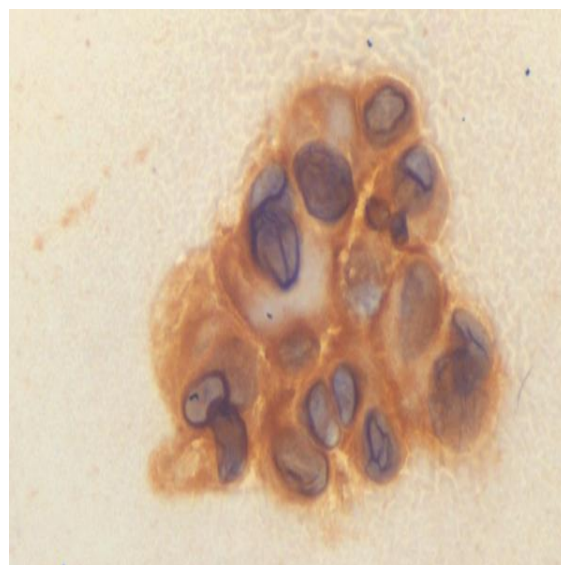

Figure 1. Cells of adenocarcinoma showing membranous and cytoplasmic staining with Ber-EP 4 (400x).

MOC 31 expressed diffuse membranous staining in 93% of the total cases of adenocarcinoma. While all cases of reactive mesothelial effusions were negative (Fig 2). All the effusions containing reactive mesothelial cells showed both cytoplasmic and nuclear staining with Calretinin, a mesothelial marker. Cells of adenocarcinoma also expressed weak cytoplasmic staining but were taken as negative because of the

recommended staining pattern of Calretinin. Calretinin expressed no nuclear staining in any of the adenocarcinoma cells in current study (Fig 4, 5).
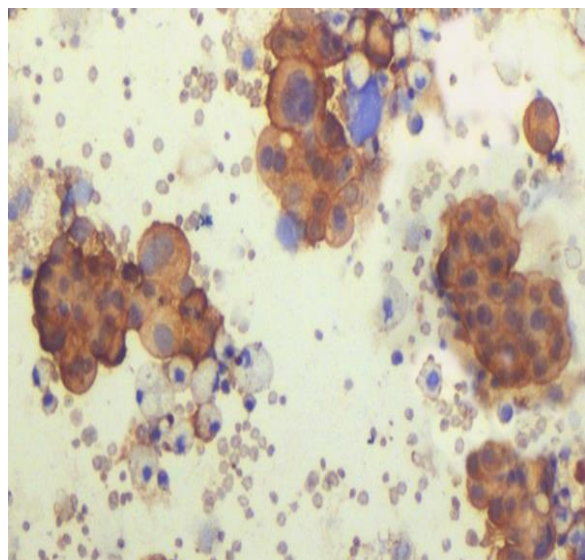

Figure 2. MOC-31 labeling the cell of adenocarcinoma in diffuse membranous and cytoplasmic pattern (400x).

HBME-1 was positively stained in 100% of cases of reactive mesothelial effusions but also showed positive expression in 7 out of44 cases of adenocarcinoma, while remained negative in 84% of other malignant effusions (Fig 3).
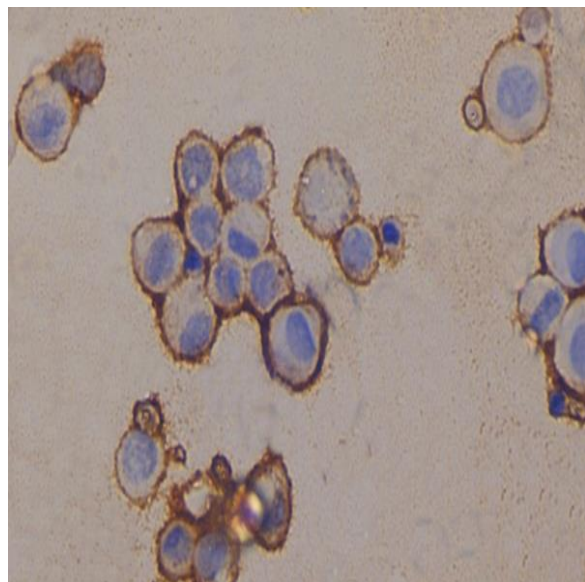

Figure 3. HBME-1 staining the reactive mesothelial cells (membranous pattern) at high power magnification.

A good correlation was observed in positivity of Ber-EP 4, MOC-31, Calretinin and HMBE-1 with the diagnosis (p=0.000; Pearson's correlation).

All the effusions containing reactive mesothelial cells showed both cytoplasmic and nuclear staining with Calretinin, a mesothelial marker. (Figure 4 &5)
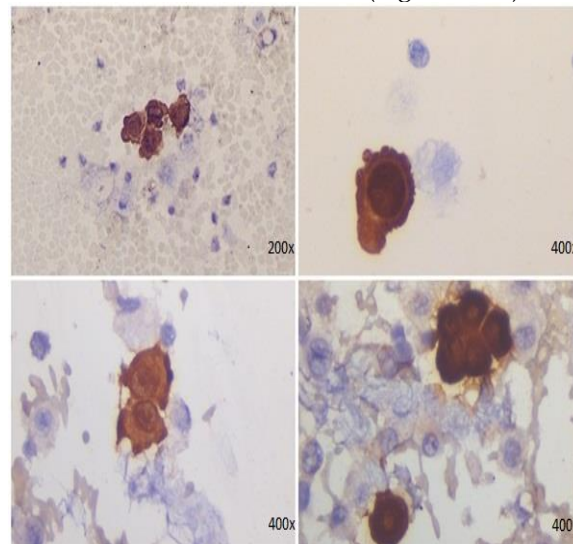

Figure 4. Nuclear and cytoplasmic staining of mesothelial cells with Calretinin (200x)
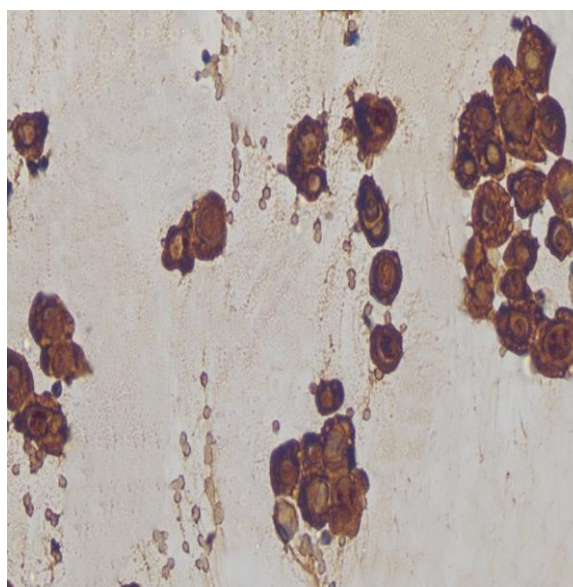

Figure 5. Calretinin labeling the cells of reactive mesothelial cells (200x)

Discussion

On processing large number of pleural and peritoneal effusions, we selected 75 cases of reactive mesothelial effusions and malignant effusions on routine cytology;

containing the representative population on which the immunocytochemical markers can be applied.

There are various cytomorphological features that are being used to identify the malignancy. These includes the nuclear pleomorphism, macro nucleoli, arrangement of cells such as the presence of large cellular aggregates, papillary like structures of tissue fragments and the cell-in-cell engulfment.[17] The presence of the intracytoplasmic mucinous vacuoles strongly favors adenocarcinoma over mesothelial cells[18]. In some cases of adenocarcinoma, the cells occur as small groups or isolated, deceptively small and morphologically uniform with inconspicuous vacuolation. These cells may be confused as mesothelial cells. It may not be possible to definitively distinguish reactive mesothelial cells (RMCs) from adenocarcinoma (ACs) based on morphology alone and ancillary techniques are required in such a situation. A plethora of literature encourages commercially available monoclonal antibodies use in the diagnosis of ambiguous effusion cases.[1]

In Pakistan, according to our knowledge, no study has been reported on the utility of antibodies on cytospin slides. In our study, we aimed to distinguish adenocarcinoma cells from reactive mesothelial cells with the help of panel of immunomarkers including two epithelial cells markers; Ber-EP 4 and MOC 31, and two mesothelial markers; Calretinin and HBME 1 on cytospin preparations.

The Ber-EP4 antibody is generated by immunizing mice with cells from the MCF-7 breast carcinoma cell line. The Ber-EP4 antibody reacts with an epitope on two noncovalently bound glycopeptides[20]. It reacts with surface and cytoplasmic glycoproteins of epithelial cells.[3] The Ber-EP4 is very dynamic as a differentiating marker of mesothelioma from adenocarcinoma.[3,4,20-22] In our study, Ber-EP 4 showed positivity in 100% of adenocarcinoma cases and was negative in all cases of reactive mesothelial effusions.

MOC-31 is a monoclonal antibody that identifies an epithelial-associated transmembrane glycoprotein. Over the time it has been shown to positively stain adenocarcinoma cells with great sensitivity and specificity[3]. The previous studies stated the usefulness of MOC-31 as an effective marker in detecting adenocarcinoma cells.[20,22] Various studies reported 100% reactivity of MOC-31 antibody in all the cases of adenocarcinoma in effusions, irrespective of their primary sites. [15, 23] In the present study, MOC-31 was found to be positive in 93% of the total cases of adenocarcinoma. None of the case of reactive effusions was stained positive by this antibody.

Calretinin, 29-kDa calcium binding protein is a member of elongation factor hand proteins. Structurally, it contains a distinct characteristic helix-loop-helix which serves as the calcium-binding site. It is expressed in peripheral and central neural tissues and thought to play an important role in somatosensory transduction and cell cycle [24]. Calretinin has been established as strongly immunoreactive with mesothelial cells [21]. According to other researchers calretinin is a sensitive and specific marker for both benign and neoplastic mesothelial cells. [25] Nevertheless, calretinin has been proved by different studies to be fairly specific for mesothelial cells, and adenocarcinoma cells are weakly stained. [26] Kitazume et al. indicated Calretinin as a good marker for mesothelial cells and no case of adenocarcinoma was stained positive in their study. In the study by Ellen et al., the reactive mesothelial cells of all benign cases demonstrated strong nuclear staining with calretinin, whereas all metastatic adenocarcinomas lacked positive nuclear staining to calretinin. [27] In the present study, Calretinin showed strong nuclear and cytoplasmic staining in 100% cases of reactive mesothelial effusions. No nuclear staining was observed in the cells of adenocarcinoma while Calretinin showed weak cytoplasmic staining in malignant cells. In all cases, Calretinin stained the reactive mesothelial cells present in the background.

HBME-1 is a monoclonal antibody against mesothelial cells that recognizes an unknown microvillus surface antigen. [28] It reacts against normal mesothelial cells. [29] It gives a thick membranous staining pattern due to its reaction with membrane antigen of mesothelial cells [3]. HBME-1 monoclonal antibody is the most challenging mesothelial marker [29]. The sensitivity and specificity of HBME-1 approach 100% and 80% respectively in detecting reactive mesothelial cells. [21] Saleh and co-workers, in their study, stated that Calretinin and HBME-1 are the two most sensitive markers for mesothelial cells[3]. In current study, HBME-1 was positively stained in 100% of cases of reactive mesothelial effusions but also showed positive expression in 7 out of 44 cases of adenocarcinoma.

We concluded the cytospin preparations as an alternative for cell block preparations due to its cost effectiveness and quick and easy procedure. As cytospin preparations are routine procedure in most settings, no special skills and resources are needed for it and therefore, is more feasible and cost effective for small centers and developing countries.

Conclusion

Immunocytochemical panel for distinguishing reactive mesothelial cells from adenocarcinoma cells should include both kinds of immunomarkers; mesothelial markers and epithelial markers. The immunomarkersBer-EP 4 and Calretinin are more effective in distinguishing reactive mesothelial cells and adenocarcinoma.

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