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ORIGINAL ARTICLE

# Reactivity with the EpCAM-specific antibodies MOC-31 and Ber-Ep4 in plasma cell neoplasms: a potential diagnostic pitfall in cytology samples

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**KEYWORDS**
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**Objective** Epithelial cell adhesion molecule (EpCAM) is a protein expressed on surfaces of healthy epithelia, and is overexpressed in dysplasias and carcinomas. Immunohistochemistry (IHC) utilizing antibodies that react with EpCAM, such as MOC-31 and Ber-EP4, distinguish reactive mesothelial cells from carcinomas in serous effusions. IHC is crucial in effusions with singly dispersed atypical cells, a scenario with a broad differential, including hematopoietic malignancies. Plasma cell neoplasms (PCN) are the second most common hematopoietic malignancy, manifesting as multiple myeloma or plasmacytoma, with 6% of cases developing serous cavity involvement. Most PCNs are readily recognizable; however, variants that deviate from the classic cytomorphology risk erroneous diagnosis. This study demonstrates EpCAM expression in a subset of PCNs, highlighting a potential diagnostic pitfall in serous effusion cytology.

**Methods** A 10-year retrospective search for cytology specimens with a diagnosis of PCN was performed. All cases demonstrating CD138/CD38 and monoclonal immunoglobulin expression, and adequately cellular cell block were included. IHC analysis for MOC-31 and Ber-EP4 was performed using Ventana Benchmark Ultra. Scoring was performed as follows: total IHC score equals the positive proportion (0 = no positive tumor cells; 1 = <1%; 2 = 1–10%; 3 =11–33%; 4 = 34–66%; 5 = 67–100%) plus staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). A score > 4 was considered positive.

**Results** 2 of 28 (7%) PCNs demonstrated positivity for MOC-31 and Ber-Ep4.

**Conclusion** A subset of PCNs in cytology samples show positivity for MOC-31 and Ber-EP4 which could result in misinterpretation as carcinoma.



## Introduction

Epithelial cell adhesion molecule (EpCAM) is an integral transmembrane protein that is important in cell adhesion, proliferation, and differentiation. It is normally expressed on

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the basolateral surfaces of most epithelia in healthy individuals, and is overexpressed in most epithelial dysplasias and carcinomas in a membranous to cytoplasmic pattern. This overexpression is currently being exploited in numerous EpCAM-targeted immunotherapeutic clinical trials for a wide range of carcinomas.[1-3]

EpCAM is reported to be expressed exclusively in epithelial tissues and the immunohistochemical (IHC) surrogates targeting it, MOC-31 and Ber-Ep4, are routinely used in cytopathology practice to help distinguish reactive mesothelial cells from metastatic carcinomas in serous effusion fluids. A common approach in this differential is to utilize 2 carcinoma markers and 2 mesothelial markers, in which combined use of MOC-31 and Ber-Ep4 have been shown to be highly reliable.[4] In effusions with predominantly singly-dispersed atypical cells, the application of IHC is crucial, as the differential diagnosis is broad and includes hematopoietic malignancies.

Plasma cell neoplasms (PCNs) are the second most common hematopoietic malignancy, with an estimated

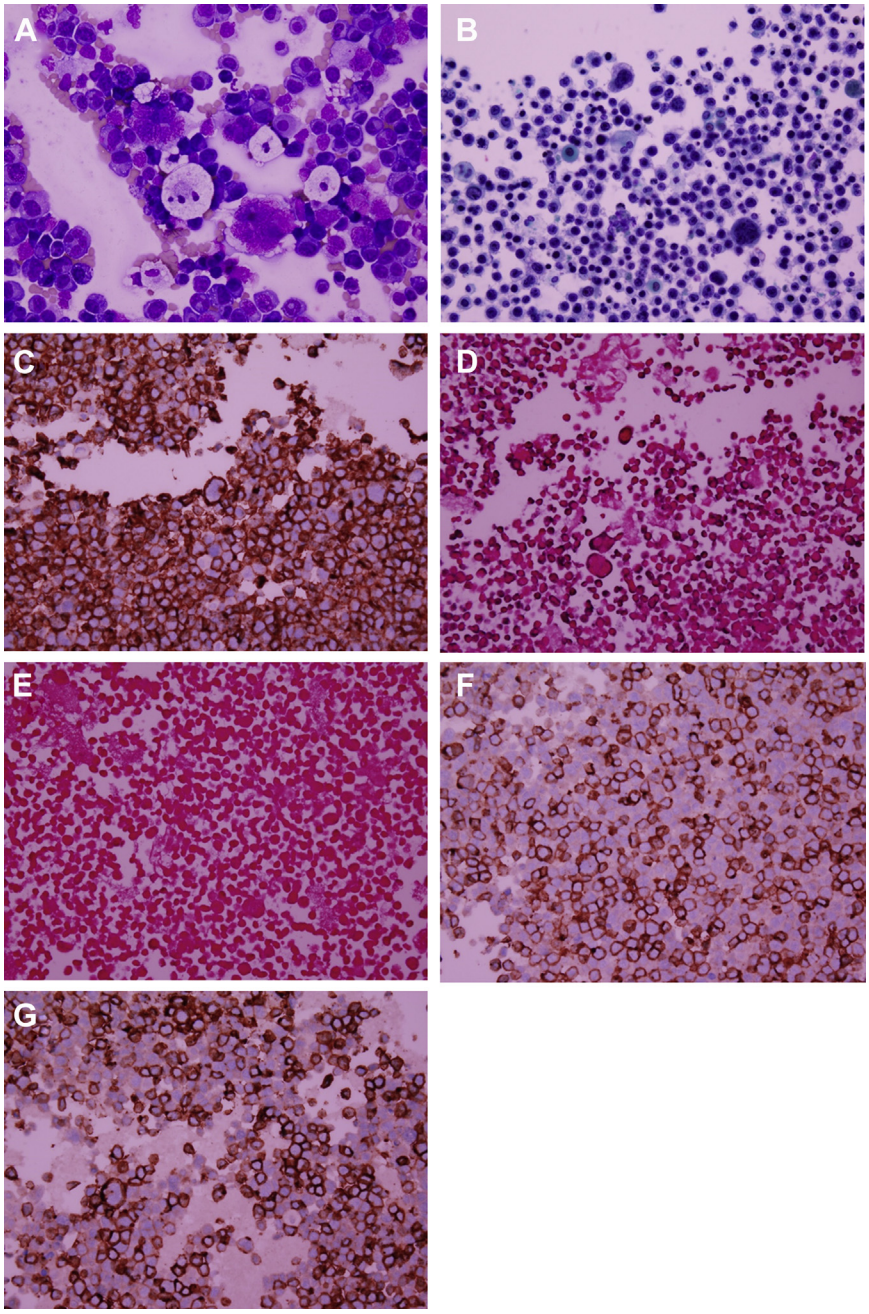


**Figure 1** Case 1, pleural fluid, cytomorphologic features and staining pattern of EpCAM-positive PCN. A, Pleomorphic variant; Diff-Quik stained cytospin, magnification ×600. B, Papanicolaou-stained ThinPrep, ×400. C, Positive CD138. D, Positive kappa in situ hybridization. E, Negative lambda in situ hybridization. F, Positive MOC-31, IHC score = 4 + 3 = 7. G, Positive Ber-EP4, IHC score = 4 + 3 = 7.

annual incidence of approximately 30,000 new cases per year in the United States, and a worldwide 5-year prevalence of approximately 230,000 individuals.[5] The clinical presentation is usually in the form of multiple bony lesions, or as a single osseous or extramedullary lesion (plasmacytoma). Six percent of patients with multiple myeloma develop involvement of the serous cavities, also known as myelomatous serous effusions.[6] Most plasma cell neoplasms show classic cytomorphology and are readily identified without IHC. However, unusual morphologic variants exist that include polymorphous, small cell, histiocytoid, blastic, pleomorphic, spindle cell, and clear cell variants, among others.[7] These less-common variants run a high risk of erroneous diagnosis as they mimic a wide range of malignancies.

In this study, we aim to highlight EpCAM reactivity in a subset of PCNs as a potential diagnostic pitfall in serous effusion cytology. To this end we investigate the frequency of EpCAM reactivity with the antibodies MOC-31 and Ber-Ep4 within a series of PCNs in cytology specimens using data derived from a retrospective single institution survey. We also report the clinicopathologic features of tumors with EpCAM reactivity and explore the possible therapeutic implications of EpCAM-positive PCNs.

## Design

A retrospective search for consecutive patients diagnosed with PCNs on cytology specimens between 2007 and 2017 was performed based on electronic files of the Pathology Department of Washington University at Barnes Jewish Hospital. The study was approved by the Washington University institutional review board (No. 201708140). All cytology specimens from any extraosseous site for which a cell block was created at the time of collection were included in the study. An additional inclusion criterion was adequate cellularity on cell block material, with adequate cellularity being defined as greater than 15 well-visualized tumor cells.

Cytology material was collected in CytoRich Red (TriPath Imaging, Inc, Burlington, NC) and blocks were prepared by suspending the tissue sediment in clot using fresh O plasma and thromboplastin processing. Tissue was then placed in CellSafe mesh capsules (ThermoFisher Scientific, Waltham, MA) for fixation and sectioning. All cases were confirmed as plasma cell neoplasms by combined demonstration of CD138/CD38 and monoclonal immunoglobulin expression by IHC, in situ hybridization, and/or flow cytometry.

IHC analysis for the expression of the EpCAM protein using both MOC-31 and Ber-Ep4 was performed on formalin-fixed, paraffin-embedded cell block sections. MOC-31 clone was obtained from Roche/Ventana Medical Systems (06374409001; Tucson, AZ). Ber-Ep4 clone was obtained from Cell Marque (05435676001; Rocklin, CA). Automated staining instrument utilized was Ventana Benchmark Ultra (Ventana Medical Systems) following the manufacturer's recommended dilutions and staining protocols.

Scoring of EpCAM reactivity was adapted from the Allred model of breast cancer ER/PR staining, whereby an IHC score is calculated as the sum of the positive proportion (0 = no positive tumor cells; 1 = <1%; 2 = 1%-10%; 3

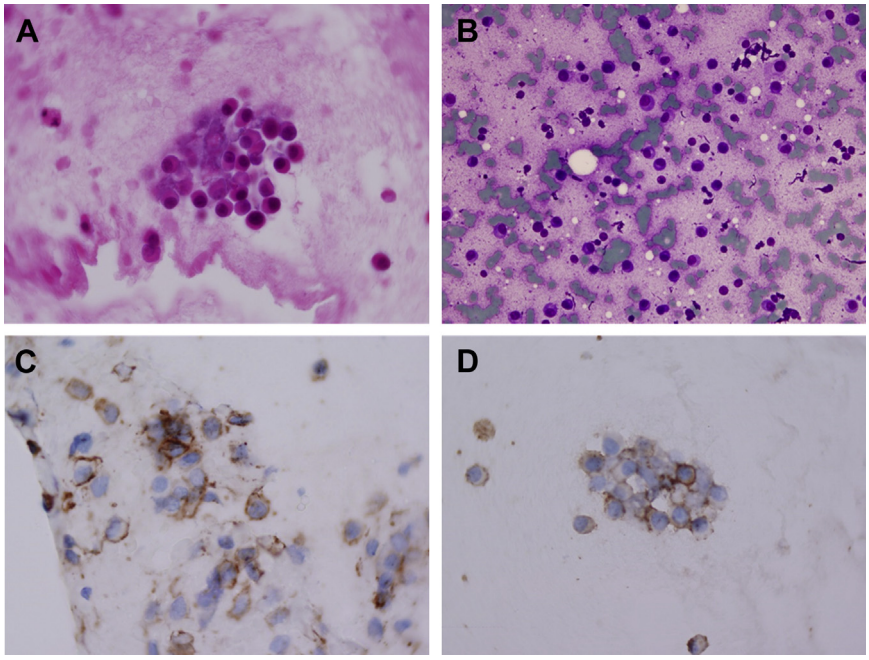


**Figure 2** Case 2, chest wall soft tissue fine-needle aspiration, cytomorphologic features and staining pattern of EpCAM-positive PCN (expressing CD56, CD38, and lambda by flow cytometry). A, Classical variant; hematoxylin and eosin stained cell block, ×1000. B, Diff-Quik stained smear, ×400. C, Positive MOC-31, IHC score = 5 + 2 = 7. D, Positive Ber-EP4, IHC score = 5 + 2 = 7.

**Table 1** Clinicopathological features of EpCAM-positive cases.

| Clinicopathologic feature | Case 1 | Case 2 |
|---|---|---|
| Age | 71 | 66 |
| Sex | F | M |
| Relevant history prior to presentation | 10 year history of unmonitored MGUS | None |
| Followup duration | 2 months | 5 years |
| Outcomes | Dead of disease | Remission |
| PET/CT scan | Peritoneal carcinomatosis; pleura; pericardium; retroperitoneal soft tissue; widespread lymphadenopathy; uterus; no bony lesions | Right ribs and right chest soft tissues; sternal, thoracic and skull bony lesions |
| Specimen site | Pleural fluid | Chest wall |
| PCN morphology | Pleomorphic | Classical |
| EpCAM score | 4 + 3 = 7/8 | 5 + 2 = 7/8 |
| Bone marrow biopsy % clonal plasma cells | 1% | 0% (not involved) |
| Bone marrow biopsy FISH | Monosomy 13; del17p | Negative (marrow not involved) |
| Immunofixation and serum protein electrophoresis (g/dL) | IgG kappa, 3.2 | IgA lambda, 3.0 |
| LDH (U/L) | 524 | 229 |
| Beta 2 macroglobulin (mg/L) | 31.3 | 4.3 |
| Hemoglobin (g/dL) | 9.9 | 12.1 |
| Creatinine (mg/dL) | 12.36 | 1.1 |
| Calcium (mg/dL) | 8.4 | 11.7 |
| Albumin (g/dL) | 2.9 | 3.9 |

Abbreviations: FISH, fluorescence in situ hybridization; LDH, Lactate dehydrogenase; MGUS, Monoclonal gammopathy of undetermined significance; PCN, plasma cell neoplasm; PET/CT, positron emission tomography—computed tomography.

= 11%-33%; 4 = 34%-66%; 5 = 67%-100%) and the staining intensity (0 = no staining; 1 = weak; 2 = moderate; 3 = strong) for a possible total score of 8. EpCAM was considered positive if the IHC score was greater than 4 in the presence of positive and negative control immunoreactivity. Scoring was performed by 2 blinded cytopathologists (CB and RCW). Clinicopathologic features were tabulated and given simply as raw data.

## Results

A total of 28 cases were identified for the study. Specimens included 12 serous effusion fluids (11 pleural, 1 peritoneal) and 16 extramedullary fine-needle aspiration specimens (12 soft tissues, 1 liver, 1 pancreas, 2 lymph nodes). Two of 28 cases (7%) showed reactivity for both MOC-31 and BerEp4 (Figs. 1 and 2). Case 1 displayed a highly pleomorphic variant of PCN presenting as a pleural effusion and carcinomatosis. The specimen was abundantly cellular and composed exclusively of poorly differentiated plasma cells characterized by marked variation in size, bi- and multinucleation, and prominent macronucleoli. Morphologically recognizable, mature-appearing plasma cells were scarce. The tumor cells reacted with a strong staining intensity in approximately 50% of cells (IHC score of 4 + 3 = 7). Case 2 was a chest wall fine-needle aspiration in a patient presenting with chest pain. Smears were highly cellular and displayed a classical, well-differentiated PCN with approximately 75% of tumor cells reacting with a moderate intensity (IHC score of 5 + 2 = 7). Cells were predominantly mature in appearance with little variation in size, clumped peripheral chromatin, and variably present small nucleoli. Full clinicopathologic features of the EpCAM-positive cases are listed in Table 1. The other 26 cases showed complete nonreactivity (IHC scores of 0).

## Discussion

Studies in the literature have reported that a subset of PCNs react with a variety of epithelial markers, including CAM 5.2, EMA, and AE1/AE3.[8,9] Likewise, a subset of carcinomas have been shown to react with the plasma cell marker CD138.[10] To the best of our knowledge, MOC-31 and BerEp4 reactivity in PCNs has not been previously described. EpCAM is reported to be expressed exclusively in epithelial tissues, and not expressed in hematolymphoid, mesenchymal, neural, or mesothelial tissues. However, review of this body of literature is consistently ambiguous regarding the hematolymphoid tissues and their neoplastic counterparts, with all reports stating negativity in these

tissues and neoplasms as a whole, without giving specific reference to healthy plasma cells or PCNs.[11-15] We found only a single study, from over 25 years ago, reported in the immunology literature by Bergsagel et al, showing positive immunocytochemical staining in 1 of 3 human plasmacytoma cell lines for the EpCAM-specific monoclonal antibody KS1/4.[16] Our findings corroborate those of Bergsagel et al, and further support the observation that a subset of PCNs show reactivity with EpCAM-specific antibodies.

We can only speculate on the reasons why our cases show this aberrant reactivity. Gene expression studies would be the initial step to determine whether these PCNs are truly expressing the EpCAM protein, or whether this is due to some form of cross-reactivity resulting from laboratory processing techniques. There is, in addition, the question of whether or not normal, non-neoplastic plasma cells express EpCAM. At our institution we routinely use MOC-31 and Ber-Ep4 in effusion cytology to differentiate reactive mesothelial cells from carcinoma, with an average utility of 1 to 2 cases per day. Often, the benign cases show abundant chronic inflammation with scattered plasma cells. In our experience, we have not encountered plasma cells within these chronic inflammatory infiltrates to react positively.

That a subset of PCNs may react with antibodies to EpCAM is an important observation and is likely to be helpful in avoiding significant diagnostic errors. For example, in serous effusion cytology cases where PCNs do not exhibit classic cytomorphology, EpCAM-positivity could lead to a misinterpretation of carcinoma.

The clinicopathologic differences between the two positive cases appear quite opposed. Based on these 2 examples, the EpCAM reactivity does not seem to correlate with any particular morphology or disease course. Notably, both cases demonstrated little to no involvement of the bone marrow on iliac crest biopsy. The small sample size of our study, however, limits any generalizations that can be drawn regarding possible unique clinicopathological features of EpCAM-positive PCNs, and also limits the overall confidence in determining the true proportion of PCNs that might react with EpCAM.

It is interesting to note that currently EpCAM is being studied as a target antigen in cancer immunotherapy. Numerous clinical trials involving trifunctional antibodies, EpCAM-targeted chimeric antigen receptor T cells, bispecific T cell engagers, and toxin conjugated EpCAM antibodies, among others, are currently underway.[2,17] In addition, there is approval in Europe and Canada for the intraperitoneal administration of the trifunctional antibody catumaxomab for palliative care in patients with carcinomatous pleural effusions.[18] Should any of these treatments be approved for broader clinical use, it may be worthwhile to consider the possibility of EpCAM-positive PCNs as therapeutic targets of these immunotherapies, especially given the extremely high prevalence and incidence of multiple myeloma.

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