

Immunohistochemistry in the Evaluation of Atypical Epithelial Cells in Effusions

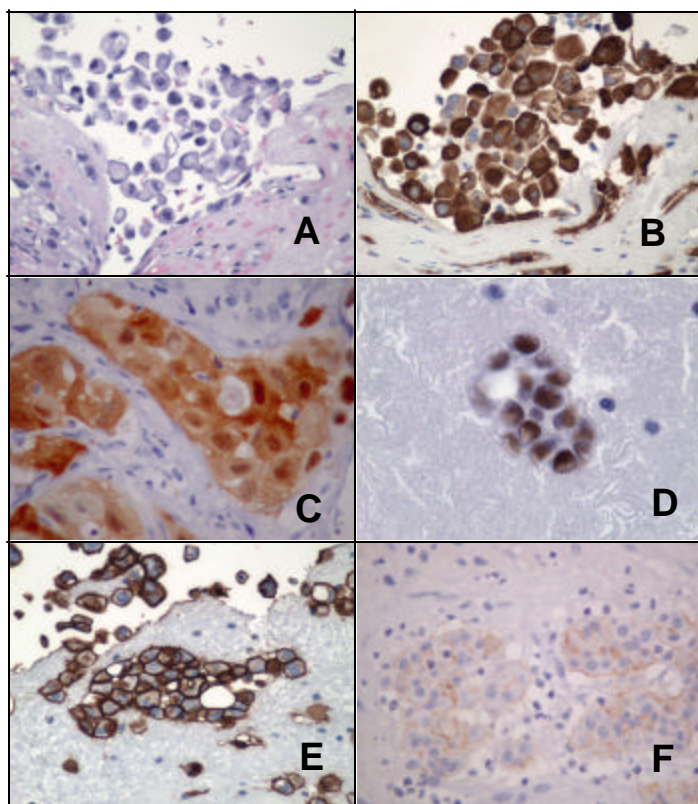
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Evaluation of body fluids for the presence or absence of malignant cells is a common and often challenging task for pathologists. This month (at the suggestion of one of our readers), we will briefly review our approach to assessing body fluids for epithelial malignancy, which involves the use of several classes of markers.

General Epithelial Markers: Cytokeratin AE1/AE3 and EMA: These antibodies are particularly useful when dealing with mixed cell populations (epithelial cells, mesothelial cells, histiocytes, etc). Cytokeratin AE1/AE3 gives you a baseline of the number of epithelial cells present, and will stain benign and malignant mesothelial cells as well as most carcinomas (with the exception of hepatoma and some cases of neuroendocrine carcinoma, renal cell carcinoma, adrenal carcinoma, and prostate carcinoma, in which case low molecular weight cytokeratin is a better choice). Additionally, if the cytokeratin AE1/AE3 stain is negative in the atypical cells of interest, you should consider the possibility of lymphoma, melanoma, or sarcoma. EMA is particularly useful in this study of effusions, **since strong diffuse cytoplasmic staining with EMA nearly always signals the presence of an abnormal neoplastic cell population**, usually carcinoma, although some cases of mesothelioma and lymphoma may show this finding. Most reactive mesothelial cells are negative with EMA, although some may show faint membrane staining and on rare occasions patchy moderate membrane staining. Intense, thick reactivity restricted to the cytoplasmic membrane is a feature of a certain subset of epithelial mesotheliomas, and may be one of the first clues to the presence of that tumor.

General Mesothelial Markers: Calretinin and nuclear WT1: Calretinin is a very good marker of mesothelial cells, which we use on virtually every case of a body fluid



H&E (A) of pleural fluid in 65F with corresponding EMA stain (B), showing strong diffuse cytoplasmic EMA. This usually reflects malignancy, in this case breast carcinoma. Strong Calretinin and WT1 alone are insufficient to diagnose mesothelioma, as pulmonary squamous carcinoma can be strongly calretinin positive (C), and nuclear WT1 is typical in metastatic serous carcinoma involving effusions (D). Strong membrane staining with MOC-31 (E) usually reflects carcinoma, although some cases of reactive mesothelial hyperplasia show patchy weak staining with MOC-31 (F).

with atypical cells. In effusions, calretinin stains a very high proportion of mesothelial cells, and it is useful to compare the number of cells staining on the cytokeratin AE1/AE3 and calretinin stains. If similar numbers of cells are staining, you may be dealing with a mesothelial proliferation (either reactive or neoplastic), and if there are substantially more positive cells with AE1/AE3 than calretinin, you often find that the fluid contains carcinoma (particularly if you also see strong cytoplasmic EMA). It is important to realize that **a significant proportion of pulmonary squamous carcinomas express strong and diffuse calretinin**, so strong and diffuse calretinin staining alone is not sufficient for a diagnosis of mesothelioma. Nuclear expression of WT1 is also a good marker of mesothelial cells, although in our experience it frequently does not stain as high a percentage of mesothelial cells as does calretinin. Also, it is important to realize that **serous carcinoma nuclei also stain strongly with WT1**, and some endometrioid adenocarcinomas show a lesser percentage of positive cells. As such, strong nuclear reactivity with WT1 significantly outnumbering calretinin-positive cells frequently reflects serous carcinoma involving a body fluid. Some authors advocate the use of cytokeratin 5/6 as a mesothelial marker, and indeed it does stain a significant number of mesotheliomas. However, squamous carcinomas also typically show strong and diffuse staining with cytokeratin 5/6, and many different types of carcinomas show patchy or "low-level" staining with this marker. For that reason, we generally do not employ cytokeratin 5/6 in the workup of effusions unless squamous carcinoma is in the differential diagnosis (a situation that also prompts us to add p63 to the panel of immunostains, since p63 is often strongly positive in squamous carcinomas but is absent in mesothelioma).

Adenocarcinoma-Related Markers: B72.3, Ber-EP4, monoclonal CEA, and MOC-31 (and sometimes TTF-1, villin, estrogen receptor, and GCDFP-15). The four markers B72.3, Ber-EP4, CEA (clone COL1), and MOC-31 are our personal favorites used as a screen for adenocarcinoma. Although occasionally reactive mesothelial cells or mesothelioma may show patchy weak reactivity with MOC-31 or Ber-EP4, strong and diffuse reactivity with 2 or more of these 4 markers strongly favors metastatic adenocarcinoma. If pulmonary adenocarcinoma is in the differential diagnosis, TTF-1 is added to the panel (always negative in mesothelioma), and in female patients estrogen receptor and GCDFP-15 can be useful to screen for breast and female genital tract tumors (as mesotheliomas are negative for these markers). Villin (also negative in mesothelioma) is useful if GI carcinoma is in the differential diagnosis, although a significant number of pulmonary carcinomas express villin. BG-8 can also be used in a similar fashion, although we do not have the years of experience with this

marker that we do with the others listed above. CD15 (Leu-M1) has been advocated by a number of authors, but we have never found this antibody to be of particular use, and no longer employ it in this situation.

SUMMARY

Benign effusion with reactive mesothelial cells: In this situation, one typically finds that the number of positive cells with cytokeratin AE1/AE3 approximates the number of positive cells with calretinin, a subpopulation of cells express nuclear WT1, EMA is negative or only focally weakly positive on the cytoplasmic membrane, and B72.3, Ber-EP4, CEA, and MOC-31 are negative (or you may see focal weak surface reactivity with MOC-31 or Ber-EP4).

Malignant effusion with epithelial mesothelioma: As above, the number of cytokeratin AE1/AE3 positive cells approximates the number of calretinin-positive cells, a subpopulation of cells express nuclear WT1, EMA is strongly positive either in the cytoplasm or on the membrane, and the tumor cells are negative for B72.3, Ber-EP4, CEA, and MOC-31 (or may show focal weak surface reactivity with MOC-31 or Ber-EP4). Hopefully, you will have sufficient cytologic atypia to convince yourself that you are dealing with a neoplastic process.

Malignant effusion with metastatic adenocarcinoma: Cytokeratin AE1/AE3-positive cells outnumber calretinin-positive cells, EMA is strongly positive either in the cytoplasm or on the membrane, and the tumor cells are strongly positive with 2 or more of B72.3, Ber-EP4, CEA, and MOC-31. Alternatively, positivity with TTF-1, villin, or estrogen receptor also establishes metastatic adenocarcinoma, and remember that serous adenocarcinomas typically have strong nuclear WT1 (but they characteristically show MOC-31 and often express estrogen receptor).

Feel free to E-mail me with any suggestions for future topics and I will try to address them if space permits.

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