Cytologic Malignancy Versus Benignancy

How Useful Are the “Newer” Markers in Body Fluid Cytology?

Virgane Lyons-Boudreaux, MD; Dina R. Mody, MD; Jim Zhai, MD; Donna Coffey, MD

One of the diagnostic challenges of evaluating body cavity fluids is to distinguish reactive mesothelial cells from malignant mesothelioma and metastatic adenocarcinoma. Various mesothelial and epithelial immunohistochemical markers are currently available to differentiate between metastatic carcinoma and benign or malignant mesothelial cells. Although there are various markers to choose from, it is sometimes difficult to evaluate these stains on cell block preparations because of nonspecific background staining, focal staining, poor staining quality, or paucity of diagnostic cells.

Calretinin is a 29-kd calcium-binding protein expressed in the central and peripheral nervous system. First recognized in 1996 by Doglioni et al as a novel immunohistochemical marker for mesothelial cells, calretinin has proved to be useful for distinguishing reactive mesothelial cells and mesothelioma from adenocarcinoma. However, in effusion cytology, calretinin may not consistently identify mesothelial cells possibly because of different antibody clones used, specimen fixation (alcohol-fixed smears vs formalin-fixed cell blocks), or interlaboratory variation.

Therefore, other mesothelial markers such as HBM-E-1, thrombomodulin, E-cadherin, and WT1 (Wilms tumor gene) have been used to increase the sensitivity of identifying mesothelial cells. Recently, D2-40, a monoclonal antibody directed against oncofetal antigen M2A, has been described as a useful marker of mesothelial cells. Chu et al showed that D2-40 has good sensitivity and specificity for distinguishing epithelioid malignant mesothelioma and reactive mesothelial cells from adenocarcinoma and is at least as valuable as currently available markers in tissue samples. To our knowledge, the utility of D2-40 in cytologic preparations has not been established. However, in a single reported abstract, and subsequent article, Saad et al found that D2-40 has high specificity and good sensitivity for distinguishing epithelioid mesotheliomas from other primary non–small cell lung carcinomas in pleural fluid cytology.

WT1 is a tumor suppressor gene located on chromosome 11p13 that is expressed in urogenital and meso-
derm-derived tissues. WT1 is a useful marker for mesothelial cells in tissue sections and cytologic preparations, however, WT1 also demonstrates nuclear staining in primary peritoneal serous carcinomas, ovarian and endometrial papillary serous carcinomas, high-grade ovarian endometrioid carcinomas, and a small proportion of breast adenocarcinomas. This may present a potential problem when evaluating effusions in female patients.

MOC-31 is a monoclonal antibody that recognizes an epithelial-associated transmembrane glycoprotein of unknown function and is a well-documented epithelial marker that has consistently been shown to detect the presence of metastatic adenocarcinoma cells in effusions with high sensitivity and specificity. Most recently, XIAP (X-linked inhibitor of apoptosis), a monoclonal antibody, has been described by Wu et al as a useful marker that distinguishes malignant from benign and reactive cell populations in body cavity fluids. XIAP is a potent constituent of the inhibitor of the apoptosis family of proteins. Through a specific capsase-mediated pathway, XIAP suppresses apoptotic cell death. Elevated expression of XIAP may explain enhanced survival of cancer cells.

To date, there are various immunohistochemical panels suggested for assessing body cavity fluids. We evaluated the performance of the aforementioned immunostains from cell block specimens of benign and malignant effusions.

MATERIALS AND METHODS

This study was conducted after approval from the institutional review board of The Methodist Hospital. We reviewed a total of 72 formalin-fixed, paraffin-embedded cell blocks from cases accessioned between 1995 and 2005. The cell blocks were previously prepared using a thrombin-based method from pleural and peritoneal fluids of both men and women. The ages ranged from 38 to 88 years with a mean age of 63 years. The cell blocks included 5 mesotheliomas, 19 benign effusions, and 48 adenocarcinomas from the following primary sites: 17 ovarian, 4 endometrial, 12 breast, 2 lung, 7 gastrointestinal, 2 genitourinary, and 4 unknown primary sites. All but 4 cases had clinically or surgically confirmed primary malignancy. Each case was stained with hematoxylin-eosin and immunostained using antibodies against calretinin (1:400; Zymed Lab, San Francisco, Calif), D2-40 (1:75; Signet Lab, Dedham, Mass), WT1 (1:100; Dako, Carpinteria, Calif), MOC-31 (1:10; Dako), and monoclonal anti-XIAP (1:150; DB Biosciences, San Jose, Calif). All cases were stained using Dako immunostainer with polymer-based detection system (EnVision). Each case was reviewed by 2 pathologists (D.C. and V.L.-B.). Cellularity of cell blocks was variable, and in some cases diagnostic tissue was not available for all immunostains. Both calretinin and WT1 were considered positive if nuclear staining was present. D2-40 and MOC-31 were considered positive if membranous staining was present. XIAP was interpreted as positive when granular cytoplasmic staining was present. Each marker was reported as positive (ranging from focal to diffuse and from weak to strong) or negative.

RESULTS

Calretinin

All mesothelioma cases were positive for calretinin, whereas 58% of benign effusions stained with calretinin (Figure 1). Staining was nuclear and usually focal in mesothelioma cases and benign effusions. Calretinin was only focally, weakly positive in 1 case of adenocarcinoma (unknown primary). This most likely represented background staining (Table 1). The sensitivity and specificity of calretinin for identifying mesothelial cells from our data are 67% and 98%, respectively (Table 2).

D2-40

All cases of mesothelioma and all benign effusions showed clean membranous staining with D2-40 (Figure 2). No background staining was noted. In most cases, D2-40 stained more mesothelial cells per case than calretinin did. No tumor cells from any of the adenocarcinoma cases stained with D2-40. However, background reactive mesothelial cells were strongly positive for D2-40 and nicely contrasted with the negative tumor cells (Figure 3). Sensitivity, specificity, positive predictive value, and negative predictive value all reached 100% in our study (Table 2).

WT1

Sixty percent of mesothelioma cases (Figure 4), 27% of adenocarcinoma cases, and 50% of benign effusions showed nuclear staining with WT1. The primary sites of the WT1-positive adenocarcinoma cases included 47% (8/17) of ovarian cases (3 ovarian papillary serous carcinomas, 3 ovarian serous borderline tumors, 2 ovarian tumors of unknown type) (Figure 5), 25% (1/4) of endometrial cases (1 papillary serous carcinoma), 17% (2/12) of breast cases, and 50% (2/4) of unknown primary cases. Sensitivity and specificity of WT1 as a mesothelial marker were 52% and 73%, respectively (Table 2).

MOC-31

All 48 cases of adenocarcinoma showed positive membranous staining with MOC-31 (Figures 6 and 7). Even cases with few tumor cells demonstrated clean membranous positivity with no background staining. All mesothelioma cases and benign effusions were negative for MOC-31. Sensitivity, specificity, positive predictive value, and negative predictive value all reached 100% (Table 2).

XIAP

XIAP showed only focal positive staining in 4 (80%) of 5 mesothelioma cases (Figure 8) and 51% of adenocarcinoma cases. Sensitivity for XIAP in identifying malignant cases was 54% in our study (Table 2). Overall, XIAP stained 54% of malignant cases. Staining pattern was granular cytoplasmic and was focal even in cases with abundant, morphologically obvious tumor. Primary sites of the XIAP-positive cases of adenocarcinoma included 2 (50%) of 4 endometrial (Figure 9), 6 (40%) of 15 ovarian, 6 (60%) of 10 breast, 2 (100%) of 2 lung, 3 (50%) of 6 gastrointestinal, 2 (100%) of 2 genitourinary (renal and prostate), and 1 unknown primary. XIAP also demonstrated weak and focal staining of benign mesothelial cells in 2 (11%) cases of benign effusions (Figure 10).

COMMENT

There are several immunohistochemical markers for distinguishing mesothelial cells from metastatic adenocarcinoma in body cavity fluids. Some of the most commonly used epithelial markers include MOC-31, Ber-Ep4, B72.3, and monoclonal carcinoembryonic antigen. Some mesothelial markers commonly used are calretinin, WT1, and HBME-1. More recently, D2-40 was recognized as a sensitive and specific marker of mesothelial cells on formalin-fixed tissue specimens.

In our study, we found D2-40 to be a more sensitive and specific marker of mesothelial cells than calretinin. The
Figure 1. Calretinin-positive mesothelioma. Malignant mesothelial cell nuclei staining with calretinin (original magnification ×440).

Figure 2. D2-40–positive mesothelioma with a membranous staining pattern (original magnification ×440).

Figure 3. D2-40–positive background benign mesothelial cells in metastatic adenocarcinoma case, membranous staining pattern (original magnification ×440).

Figure 4. WT1-positive mesothelioma, nuclear staining pattern (original magnification ×1000).

Table 1. Percentage of Mesothelioma and Adenocarcinoma Cases and Benign Effusions Positive for Selected Immunostains

<table>
<thead>
<tr>
<th>Immunostain</th>
<th>Mesothelioma (n = 5)</th>
<th>Adenocarcinoma (n = 48)</th>
<th>Benign Effusion (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calretinin, % (No./total No.)</td>
<td>100 (5/5)</td>
<td>2 (1/48)*</td>
<td>58 (11/19)</td>
</tr>
<tr>
<td>D2-40, % (No./total No.)</td>
<td>100 (5/5)</td>
<td>0 (0/48)</td>
<td>100 (18/18)†</td>
</tr>
<tr>
<td>MOC-31, % (No./total No.)</td>
<td>0 (0/5)</td>
<td>100 (48/48)</td>
<td>0 (0/18)</td>
</tr>
<tr>
<td>WT1, % (No./total No.)</td>
<td>60 (3/5)</td>
<td>27 (13/48)</td>
<td>50 (9/18)†</td>
</tr>
<tr>
<td>XIAP, % (No./total No.)</td>
<td>80 (4/5)</td>
<td>51 (22/43)†</td>
<td>11 (2/18)†</td>
</tr>
</tbody>
</table>

* Most likely represents background staining.
† Cellularity of cell blocks was variable, and in some cases diagnostic tissue was not available for all immunostains.

Table 2. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Selected Immunohistochemical Markers

<table>
<thead>
<tr>
<th>Calretinin</th>
<th>D2-40</th>
<th>WT1</th>
<th>MOC-31</th>
<th>XIAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>67</td>
<td>100</td>
<td>52</td>
<td>100</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>98</td>
<td>100</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td>PPV, %</td>
<td>94</td>
<td>100</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td>NPV, %</td>
<td>85</td>
<td>100</td>
<td>76</td>
<td>100</td>
</tr>
</tbody>
</table>

Sensitivity and specificity of D2-40 reached 100%, whereas that of calretinin was 67% and 98%, respectively (Table 2). The positive predictive value and negative predictive value for calretinin and D2-40 are 94% and 85% and 100% and 100%, respectively (Table 2). Noticeably more mesothelial cells displayed clean membranous staining with D2-40, even in cases with scant mesothelial cells present. Although calretinin stained all cases of mesothelioma, nuclear staining was focal and sometimes weak. Calretinin...
Figure 5.  WT1-positive ovarian adenocarcinoma, nuclear stain (original magnification ×1000).

Figure 6.  MOC-31–positive adenocarcinoma, membranous staining pattern (original magnification ×440).

Figure 7.  MOC-31–positive adenocarcinoma, membranous staining pattern (original magnification ×440).

Figure 8.  XIAP-positive mesothelioma with granular cytoplasmic staining pattern (original magnification ×440).

Figure 9.  XIAP-positive endometrial adenocarcinoma with granular cytoplasmic staining pattern (original magnification ×1000).

Figure 10.  XIAP-positive benign mesothelial cells. Benign mesothelial cells showing granular cytoplasmic staining with XIAP (original magnification ×1000).
failed to stain morphologically obvious mesothelial cells in 42% of benign effusions. In the past, some studies have found calretinin to be an efficient marker of mesothelial cells in cytology preparations as well as in paraffin-embedded tissue sections, whereas other studies have concluded that calretinin is not the best marker for mesothelial cells in cytologic preparations. Recently, Chu et al. concluded that D2-40 is as sensitive as calretinin for detecting cells of mesothelial origin. Although D2-40 is found to be at least comparable to calretinin in formalin-fixed tissue sections, its usefulness in body cavity effusions, to our knowledge, has not been established. Our study clearly indicates that D2-40 is more sensitive than calretinin in highlighting mesothelial cells as D2-40 unequivocally stained the few mesothelial cells present in scant cell block material. We support the use of D2-40 as a valuable mesothelial marker in effusion cytology.

WT1 displays nuclear staining in a variety of tumors including malignant mesothelioma, ovarian and endometrial papillary serous carcinoma, fallopian tube serous carcinoma, and breast adenocarcinoma, to name a few. The specificity of WT1, when used as a mesothelial marker, reached 73% in our study. Although WT1 is relatively nonspecific, it may prove to be helpful in certain settings. In our study, WT1 stained most mesotheliomas, half of benign effusions, and 27% of adenocarcinomas (ovary, endometrial, breast, and unknown primaries). In the male patient, WT1 positivity in a malignant effusion would include malignant mesothelioma as a major diagnostic possibility. It is also positive in desmoplastic small round cell tumor, which is more common in males. However, in the female patient, the possibility of other primaries, especially ovary, should be considered in the differential diagnosis. It would be helpful to add another mesothelial marker, such as D2-40, to reach an accurate diagnosis. Also, if there is an abundance of reactive mesothelial cells and few adenocarcinoma cells, D2-40 may assist in distinguishing mesothelial cells from tumor cells.

Previously, MOC-31 was shown to have diagnostic utility in distinguishing between mesothelioma and metastatic adenocarcinoma in body fluids. In 1999, Morgan et al. reported the sensitivity and specificity of MOC-31 to be 95% and 100%, respectively. We demonstrate the sensitivity and specificity of MOC-31 to be 100% and 100%, respectively. Likewise, other studies have shown similar results. MOC-31 continues to be a highly sensitive and specific epithelial marker in effusion cytology, and our study confirms that MOC-31 is highly effective. Even in cases with limited diagnostic material, MOC-31 showed unequivocal strong membranous staining of rare adenocarcinoma cells with no background staining.

XIAP, a potent constituent of the inhibitor of apoptosis family of proteins, suppresses apoptotic cell death through a specific caspase-mediated pathway. Elevated expression of XIAP may explain enhanced survival of cancer cells. Wu et al. recently described XIAP as a useful marker that distinguishes malignant from benign and reactive cell populations in body cavity fluids. In their study, 67% of malignant effusions displayed positive staining for XIAP with ovarian, endometrial, and lung carcinomas, as well as mesotheliomas showing the highest prevalence of positive staining. Staining pattern varied from focal to diffuse, and intensity ranged from weak to strong. Benign mesothelial cells were reported to be negative for XIAP, except for 2 cases that showed moderate intense staining in histiocytes. In our experience, XIAP stained 54% of malignant cases, showing only focal, weak to moderate staining at best. Even cases with abundant tumor volume showed only focal staining. Most mesotheliomas (80%), most breast adenocarcinomas (60%), half of endometrial cases, and 40% of ovarian cases displayed focal XIAP-positive staining. Both lung carcinoma cases were also positive for XIAP. Two of our benign effusions showed focal, weak staining for XIAP. The specificity of XIAP for the identification of malignant cells is 89% in our study (Table 2). We did not find XIAP to be a reliable immunostain for malignancy as it was not highly sensitive in our study. Staining was very focal and in a relatively low percentage of cells. This could present a potential limitation in scant cell block specimens with few suspicious cells present. At this time, Sun et al have unpublished data (abstract form) that shows that XIAP stains only malignant mesothelial cells, suggesting that XIAP can help distinguish benign from malignant mesothelial cells in effusion cytology. However, we found focal weak staining in both benign and malignant mesothelial cells. Thus, we did not find XIAP to be useful for reliably identifying malignant cell populations as XIAP failed to stain many (almost 50%) of our malignant cases. More studies with a larger number of cases are needed to evaluate the potential usefulness of XIAP in cytologic specimens.

In conclusion, we found that MOC-31 and D2-40 are very sensitive and specific markers of epithelial and mesothelial cells, respectively. Both displayed diffuse membranous staining with no background staining, which is of critical importance in cytology. Compared with calretinin, D2-40 proved to be a more sensitive marker. WT1 proved to be nonspecific, as it was noted to be positive in benign effusions, mesotheliomas, and carcinomas. XIAP is not a sensitive marker for malignancy and has limited value in cytology. We recommend using a panel of immunohistochemical markers, including MOC-31 and D2-40, to improve diagnostic accuracy in body cavity effusions. One limitation of this study is that the number of mesothelioma cases and benign mesothelial effusion cases is low. Future studies with a larger number of cases are needed to confirm our current findings.

References

10. Oates J, Edwards C. HBME-1, MOC-31, WT1, and calretinin: an assess-