Utility of Glucose Transporter 1 in the Distinction of Benign and Malignant Thoracic and Abdominal Mesothelial Lesions

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Context.—Malignant mesothelioma, of either peritoneum or pleura, is an uncommon cancer. The diagnosis is often difficult to make, in part because of the overlapping morphology of reactive and malignant mesothelial cells. Glucose transporter 1 (GLUT-1) is a glucose transporter typically found on erythrocytes, which is aberrantly expressed in various carcinomas. It has recently been reported as specific and sensitive in discriminating malignant pleural mesothelioma from reactive hyperplasia. The application of GLUT-1 staining in peritoneal mesothelioma has not been fully explored.

Objective.—To determine if GLUT-1 staining is helpful in distinguishing abdominal mesotheliomas from benign, reactive mesothelial lesions and to further study its utility in the thorax.

Design.—Tissue microarrays containing 135 abdominal malignant mesotheliomas and 30 malignant pleural mesotheliomas were stained with an antibody to GLUT-1, as were 56 reactive mesothelial lesions.

Results.—The overall sensitivity and specificity for GLUT-1 in mesothelioma was 53% and 98%, respectively. The sensitivity in epithelioid malignant mesothelioma was 49% and in sarcomatoid/biphasic malignant mesothelioma, 66%. In the thorax, the sensitivity was 50% and in the abdomen it was 54%. The positive predictive value of GLUT-1 immunoreactivity was 98% and the negative predictive value was 40%.

Conclusion.—Glucose transporter 1 staining of thoracic mesotheliomas showed high specificity but lower sensitivity than previously reported. Abdominal malignant mesotheliomas showed similar results. Because of low sensitivity, only positive staining is informative. In both sites, the utility of the stain was limited by nonspecific staining (eg, in necrotic areas) as well as bright labeling of erythrocytes and occasional lymphoid elements. Despite these limitations, GLUT-1 can help differentiate malignant mesothelioma from reactive benign mesothelioma.

carcinoma, and has been linked to prognosis in some sites.\textsuperscript{7–9} Pleural mesothelioma has been studied to some extent, but there is limited data available for abdominal disease. Here we present our findings in a large cohort of thoracic and abdominal mesotheliomas collected at Columbia University Medical Center, New York, New York.

**MATERIALS AND METHODS**

**Patient Selection**

The materials for the study were collected from the files of the Columbia University Medical Center Department of Pathology from 1998–2010 after approval by the Columbia University Medical Center Institutional Review Board. Thirty cases of thoracic mesothelioma were identified. These included 21 epithelioid cases and 9 sarcomatoid or biphasic cases. For controls, 23 whole slide sections demonstrating benign mesothelial hyperplasia of thoracic serosa were stained, as were 15 whole slides demonstrating fibrosing pleuritis. One-hundred and thirty-five cases of malignant mesothelioma of the abdomen were identified. These consisted of 100 samples with epithelioid differentiation and 35 biphasic or sarcomatoid variants. In addition, 3 well-differentiated papillary mesotheliomas were also included. Eighteen cases with reactive mesothelial hyperplasia in the abdomen were stained as controls.

The initial diagnoses were made on the basis of routine and selected immunohistochemical stains at the discretion of the original pathologist, and relevant clinical and radiographic data. Cases were not reclassified for purposes of this study. Tissue blocks from all mesotheliomas were obtained and 4 tissue cores from tumors were used to construct tissue microarrays with an MTA-1 arrayer (Beecher Instruments, Sun Prairie, Wisconsin) as described previously.\textsuperscript{10} For biphasic tumors, cores were obtained from epithelioid and sarcomatoid areas when possible. For rare cases with low tumor density, whole slides were analyzed. When necessary, mesothelial markers were analyzed in conjunction with GLUT-1 to ensure that only the cells of interest were investigated.

**Immunohistochemistry**

The slides were warmed to 95°C and incubated for 8 minutes in Cell Conditioner No. 1. One drop of anti-GLUT-1 (polyclonal, predilute, Dako North America, Carpinteria, California) was then added. The slides and antibody were incubated at 36°C for 32 minutes. Amplifier A and B and peroxidase reactions were performed per the automated Ventana protocol (Ventana Medical Systems, Tucson, Arizona). Erythrocytes served as a positive internal control. Glucose transporter 1 positivity, defined as a minimum of 5% of tumor cells, and estimated percentage of cells showing reactivity were determined by 2 authors (S.M.L. and A.C.B.) who reached consensus on all cases.

**RESULTS**

The pattern of staining in positive cases ranged from patchy (Figure 1, A and B) to diffuse (Figure 1, C and D). Biphasic mesotheliomas often showed positivity in both epithelioid and spindle regions (Figure 1, E and F). Benign cases were generally negative, with erythrocytes serving as an internal control (Figure 1, G).

**Thorax**

Fifteen of 30 malignant thoracic mesotheliomas showed GLUT-1 immunoreactivity (50% sensitivity). Of 21 epithelioid cases, 13 were negative and 8 were positive. Of the 9 biphasic or sarcomatoid cases, 7 were positive in 1 or both regions. Eight of these had a sarcomatoid population represented on our tissue microarray. Five of the 8 were positive in the sarcomatoid component and 3 were negative. Two cases that were negative in the sarcomatoid area were positive in the epithelioid areas. One that was negative in the epithelioid area was weakly positive in the sarcomatoid component. (Table 1).

**Abdomen**

Seventy-three of 135 peritoneal mesotheliomas showed immunoreactivity (54% sensitivity). Fifty-one of 100 epithelioid mesotheliomas were immunoreactive. Twenty-two of 35 biphasic variants were positive, with 14 showing positive staining in the sarcomatoid areas and 21 showing positivity in the epithelioid areas. Twenty cases had both sarcomatoid and epithelioid areas represented in our study. Twelve were positive in both areas (Figure 1, E and F), while 4 were negative in both. Of the 4 cases with discordant results, 2 were positive in epithelioid regions, but not sarcomatoid, and 2 showed opposite patterns of reactivity (Table 2).

**Overall**

The overall sensitivity of GLUT-1 in malignant mesothelioma of all sites was 53%. Specificity was 98% (2 false positives). Thoracic specificity was 95% and abdominal specificity was 100%. The positive predictive value was 98% and the negative predictive value was 40%. The results are summarized in Tables 3 and 4.

**DISCUSSION**

In our series, positive GLUT-1 immunostaining was helpful in the diagnosis of malignancy in mesothelial proliferations. Sensitivity in thoracic and abdominal disease was 50% and 54%, respectively. The sensitivity was higher in biphasic cases than in purely epithelioid histology (66% and 49%, respectively).

Staining was sometimes quite patchy, with fewer than 10% of cells staining in 16 cases (4 pleural, 12 abdominal). Our tissue microarray was produced to include a high density of tumor cells from each case. Small biopsy specimens may not contain high tumor density. In this setting, patchy staining could be quite detrimental. Because of this limited sensitivity, a negative stain should not be considered informative (negative predictive value = 40%), but because of high specificity, a positive stain does strongly favor malignancy (positive predictive value = 98%).

Although we found the specificity to be quite high, we did note 2 false positives. One was a mesothelial hyperplasia in a patient with a persistent effusion, which was called atypical and suspicious at the time. One year post biopsy, the patient was alive without evidence of malignancy. One case of fibrinous pleuritis showed weak and focal positivity.

In addition to the lack of sensitivity, other factors also limit the value of GLUT-1 immunohistochemistry. For biphasic cases, the results were commonly, but not always, concordant, thus presenting another possible source of confusion. Furthermore, the strong staining of red blood cells could lead to either false-positive or false-negative interpretations. False positives could occur in the setting of red cells intermixed with, and being mistaken for, mesothelial cells. False negatives may have occurred if weak but positive staining in tumor cells was overlooked because the interpreting pathologist expected to see intense positivity similar to that seen in the erythrocytes. Further confusion may be added by nonspecific staining in necrotic areas and staining of lymphoid cells. Based on
A, Epithelioid mesothelioma with papillary morphology. B, This case was focally positive for glucose transporter 1 (GLUT-1). C, Epithelioid mesothelioma with pleomorphic nuclei and necrosis (upper right). D, This case showed diffuse positivity for GLUT-1. E, Biphasic mesothelioma with spindle cells (arrow) and epithelioid clusters (arrowhead). F, Glucose transporter 1 was positive in both the spindle cells (arrow) and epithelioid
these issues, use of GLUT-1 staining is best performed in conjunction with stains for mesothelial differentiation.

Despite the limitations listed above, GLUT-1 immunostaining does appear to be potentially valuable, particularly given the lack of reliable markers of malignant mesothelial cells. Selected other studies of ancillary tests that may be helpful in the distinction of mesothelioma from reactive mesothelium are discussed below, and several representative immunohistochemical studies are summarized in Table 5.

Other Studies of GLUT-1

The original report by Kato et al describing GLUT-1 immunoreactivity in pleural mesothelioma demonstrated 100% sensitivity and 100% specificity (when the differential was mesothelioma versus reactive mesothelioma) in samples from 48 pleural mesotheliomas and 40 reactive mesotheliomal proliferations obtained at a Japanese cancer referral center. Our sensitivity was much lower in both pleural and peritoneal disease. Our findings are more consistent with what has been found in other organ systems and by subsequent studies that looked at surgical resections and biopsies of mesothelial lesions.1,2 Thus, the difference in sensitivities reported by our group and the Kato group2 may represent preanalytic variables, though the nature of the specific variable(s) involved is unclear. We did not collect data on the ethnic background of our patients; therefore, we cannot draw any conclusions related to the possibility that there may be a true biological difference between Japanese and American patients with mesothelioma.

Monaco et al11 recently looked at pleural and peritoneal mesotheliomas and compared GLUT-1 immunohistochemistry to p16 deletion by fluorescence in situ hybridization (FISH). They investigated 68 cases of mesothelioma and 70 benign cases and reported sensitivity of 40% and specificity of 93% for GLUT-1 immunohistochemistry. Both the sensitivity and specificity reported by these authors were similar to ours, albeit lower. Their lower sensitivity seems to stem from the fact that, for their peritoneal cases (41), staining occurred at a much lower rate (29%) than for their pleural cases (56%).11 We performed staining for 135 peritoneal cases and did not identify a difference in rates of positivity between pleura and peritoneum (50% and 54%, respectively).

The lower specificity may be related to the source of the material. Of 5 false positives, 3 were cytologic specimens, which seem to show lower specificity. Several other recent studies have looked at GLUT-1 in cytology specimens and all have reported lower specificity than for surgical pathology material. One group looked at alcohol-fixed cytology specimens and found that monoclonal GLUT-1 was 100% sensitive and 80% specific for the diagnosis of mesothelioma over reactive mesothelioma in a study that included 11 epithelioid mesotheliomas (10 from the thorax) and 50 reactive effusions. Ikeda et al12 found that polyclonal GLUT-1 had 91% sensitivity and 82% specificity in another study of cytology material. Hasteh et al13 studied cell blocks made from effusion specimens and found GLUT-1 positivity in 47% of 15 mesothelioma cases, after setting a 20% cutoff for positivity. Their specificity, however, was only 88% in 43 benign cases. Shen et al14 investigated both monoclonal and polyclonal GLUT-1 staining in cell blocks prepared from effusions from both the thorax and the abdomen. Their study included 35 mesotheliomas (32 thoracic, 3 abdominal) and 38 benign effusions (32 thoracic and 6 abdominal). If any degree of staining was accepted as positive, then the sensitivity of monoclonal GLUT-1 was 63% and the specificity was 82%. Under the same conditions, the sensitivity of polyclonal GLUT-1 was 83% and the specificity was 63%.14 These data are similar to the aforementioned Hasteh series,13 which was also cell-block based.

Overall, it seems GLUT-1 immunohistochemistry is much less specific in cytology specimens.

Epithelial Membrane Antigen

The Ikeda group12 found that EMA staining had 100% sensitivity and 74% specificity in alcohol-fixed cytologic specimens in a study that included 11 epithelioid mesotheliomas (10 thoracic) and 50 benign effusions. Hasteh et al13 found EMA staining in 100% of 52 mesotheliomas. They report a specificity of 91% when a cutoff of 20% is used to establish positivity. The Shen group14 considered any staining for EMA as positive and found 86% sensitivity and 87% specificity. Saad et al15 stained cell blocks prepared from effusion specimens from 20 mesotheliomas and 20 benign effusions with 2 different EMA clones. Clone E29 had 75% sensitivity and 100% specificity if 2 specimens with weak staining were

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Table 1. Glucose Transporter 1 Staining in Benign and Malignant Thoracic Mesothelial Lesions

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesothelial hyperplasia, total</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Fibrosing pleuritis, total</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (focal)</td>
</tr>
<tr>
<td>Malignant mesothelioma, epithelioid, total</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
</tr>
<tr>
<td>5%–10% cells</td>
<td>2</td>
</tr>
<tr>
<td>11%–50% cells</td>
<td>6</td>
</tr>
<tr>
<td>&gt;51% cells</td>
<td>0</td>
</tr>
<tr>
<td>Malignant mesothelioma, biphasic/ sarcomatoid, total</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
</tr>
<tr>
<td>5%–10% cells</td>
<td>1</td>
</tr>
<tr>
<td>11%–50% cells</td>
<td>3</td>
</tr>
<tr>
<td>&gt;51% cells</td>
<td>3</td>
</tr>
</tbody>
</table>

clusters (arrowhead). G, This benign case demonstrated mesothelial hyperplasia. Mesothelial cells are GLUT-1 negative, but erythrocytes coating the cluster could cause a diagnostic difficulty (hematoxylin-eosin, original magnifications ×40[A, C, and E]; anti–GLUT-1, original magnifications ×40[B, D, F, and G]).
considered to be negative. Clone Mc5 had 70% sensitivity and 40% specificity. Attanoos et al\textsuperscript{1} looked at a large cohort of benign and malignant mesothelial proliferations in formalin-fixed, paraffin-embedded tissue (40 benign, 60 malignant) and found that EMA had 80% sensitivity and 80% specificity. Cury et al\textsuperscript{16} investigated formalin-fixed, paraffin-embedded pleural biopsies from 31 mesothelioma cases and 34 benign controls. They reported that EMA had 97% sensitivity for mesothelioma; however, they identified staining in 11 of 34 benign cases, such that specificity was 68%. However, if a 10% cutoff for positivity was applied, the specificity would improve to 94%. Roberts et al\textsuperscript{17} also looked at formalin-fixed, paraffin-embedded tissue from 112 mesotheliomas (100 thoracic, 7 abdominal, 5 miscellaneous) and 11 cases of benign, reactive pleura. They reported sensitivity of 68% and specificity of 45% with a 10% cutoff for positivity.

### p53

Hasteh et al\textsuperscript{13} found abnormal nuclear accumulation in 47% of 15 mesothelioma cell blocks. It was rarely seen in 46 benign effusions and had a specificity of 98%. Cagle et al\textsuperscript{18} showed p53 reactivity in 48% of 40 biopsy specimens of pleural mesothelioma. They did not note false positives in 13 benign controls (100% specificity). Consistent with these results were those of Attanoos et al\textsuperscript{1} who reported sensitivity of 45% and specificity of 100% in their series. Conversely, Cury et al\textsuperscript{16} found nuclear reactivity in 97% of their mesothelioma cases, and 9 of 34 reactive cases showed nuclear reactivity in greater than 10% of cells. Therefore, even when we apply a 10% cutoff for positivity (which the authors did not), their specificity was still only 74%. Additionally, Roberts et al\textsuperscript{17} found that p53 reactivity was associated with 70% sensitivity, but only 18% specificity, after applying a 10% cutoff for positivity. The authors suggest that differences in fixation and antigen retrieval may be responsible for the variation in reactivity observed among different groups.

### Desmin

The Hasteh group\textsuperscript{13} found desmin positivity (defined as 25% reactivity) in the benign mesothelial cells, associated with 84% sensitivity and 94% specificity, in the distinction between benign and malignant mesothelium. Attanoos et al\textsuperscript{1} similarly reported sensitivity of 85% and specificity of 90%. In a recent study, Tsukiji et al\textsuperscript{19} looked at 63 epithelioid mesotheliomas (57 thoracic and 6 peritoneal) and 44 cases demonstrating nonneoplastic mesothelial cells. They found that desmin had 86% sensitivity and 90% specificity.

### Novel Markers

Immunocytochemistry for CD146 (cluster of differentiation 146) has recently been reported to be valuable in discriminating pleural mesothelioma from benign mesothelial cells in a study of 21 cytology specimens.\textsuperscript{20} In that study, 19 of the 21 mesothelioma specimens were positive with no false positives in 28 benign cases. Another novel marker of interest is insulin-like growth factor II messenger ribonucleic acid–binding protein 3 (IMP3). In 1 notable study of 45 mesotheliomas and 64 benign cases,\textsuperscript{21} IMP3 showed positivity in 73% of the malignant cases, and none of the benign.

### p16

Loss of p16 has been shown by immunohistochemistry and FISH to be a common oncogenic event in mesothelioma, as well as serving as a marker of poor prognosis (31%–74% prevalence in pleural mesothelioma).\textsuperscript{22} In the peritoneum, lower rates of p16 loss by deletion have been reported.\textsuperscript{11,22–24} Homozygous loss of p16 is not present in benign proliferations, thus its detection strongly favors malignancy. Fluorescence in situ hybridization may show greater sensitivity in cytology specimens, where full nuclei are more easily visualized.\textsuperscript{25}

Monaco et al\textsuperscript{17} directly compared GLUT-1 immunohistochemistry to p16 FISH and found sensitivity of 59% and specificity of 100% for p16 deletion by FISH. This was superior to GLUT-1 immunohistochemistry in their series. There are, however, some limiting factors for this test. Namely, p16 loss is present in only some mesotheliomas; therefore, sensitivity will always be somewhat limited. Additionally, not all laboratories possess the ability to perform FISH, whereas immunohistochemistry is almost universally available.
Table 5. Summary of immunohistochemical markers used to distinguish benign and malignant mesothelial proliferations

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Source, y</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical specimens</td>
<td>63</td>
<td>98</td>
<td>Kato et al., 2007; present study, 2011</td>
</tr>
<tr>
<td>Cytology specimens</td>
<td>74</td>
<td>79</td>
<td>Ikeda et al., 2011; Hasteh et al., 2010; Shen et al., 2009</td>
</tr>
<tr>
<td>EMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical specimens</td>
<td>76</td>
<td>81</td>
<td>Attanoos et al., 2003; Cury et al., 1999; Roberts et al., 2001</td>
</tr>
<tr>
<td>Cytology specimens</td>
<td>88</td>
<td>82</td>
<td>Ikeda et al., 2011; Hasteh et al., 2010; Shen et al., 2009; Saad et al., 2005</td>
</tr>
<tr>
<td>Desmin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical specimens</td>
<td>86</td>
<td>90</td>
<td>Attanoos et al., 2003; Tsukiji et al., 2010</td>
</tr>
<tr>
<td>Cytology specimens</td>
<td>84</td>
<td>94</td>
<td>Hasteh et al., 2010</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical specimens</td>
<td>58</td>
<td>90</td>
<td>Attanoos et al., 2003; Cury et al., 1999; Cagle et al., 1994</td>
</tr>
<tr>
<td>Cytology specimens</td>
<td>47</td>
<td>98</td>
<td>Hasteh et al., 2010</td>
</tr>
</tbody>
</table>

Abbreviations: EMA, epithelial membrane antigen; GLUT-1, glucose transporter 1.

a Values represent pooled results from listed studies.
b The specificity shown represents our interpretation of the original data after applying a 10% cutoff for positivity.

SUMMARY

Ultimately, the distinction of malignant mesothelioma from reactive mesothelial cells must be made primarily on hematoxylin-eosin staining, with proper consideration of the clinical setting. Nonetheless, because of the difficulty in this endeavor, immunohistochemical stains are potentially quite valuable. Use of GLUT-1 has been mentioned as a possible adjunctive test in a recent consensus statement on pathologic assessment of mesothelioma. Our findings support this conclusion, though our sensitivity was much lower than what was initially reported. This much more limited sensitivity is likely to be more representative, based on what has been published by other investigators. Performing GLUT-1 immunohistochemistry on cytology specimens may diminish specificity (see Table 5). This is a significant limitation and certainly suggests a role for newer tests, such as immunohistochemistry for IMP3 and CD146, or FISH for p16 deletion. Ultimately, the optimal workup may include a panel of tests and may need to be tailored to the type of specimen being investigated.

References