The Diagnostic Utility of p16 FISH and GLUT-1 Immunohistochemical Analysis in Mesothelial Proliferations

Sara E. Monaco, MD, Yongli Shuai, MS, Mona Bansal, MD, Alyssa M. Krasinski, MD, and Sanja Dacic, MD, PhD

Key Words: p16; Fluorescence in situ hybridization; GLUT-1; Immunohistochemistry; Mesothelioma

Abstract

Two promising ancillary tests used in the diagnosis of mesothelioma include GLUT-1 immunohistochemical analysis and fluorescence in situ hybridization (FISH) testing for the p16 deletion. This study compared the diagnostic usefulness of p16 FISH and GLUT-1 immunohistochemical analysis in the diagnosis of mesothelial proliferations in 158 cases with a diagnosis of benign (45.4%), atypical (10.4%), or malignant/mesothelioma (44.2%). Of the 70 benign cases, none had a deletion of p16 and 5 cases (7%) were positive for GLUT-1. Of the 68 mesotheliomas, 40 (59%) had a deletion of p16 (sensitivity, 59%; specificity, 100%) and 27 (40%) were positive for GLUT-1 (sensitivity, 40%; specificity, 93%). GLUT-1 showed lower sensitivity in pleural (56% vs 70%) and peritoneal (29% vs 51%) mesotheliomas (P = .004). Our results demonstrate that p16 FISH is a more sensitive and specific test than GLUT-1 immunohistochemical analysis and can be a more reliable ancillary tool to support the diagnosis of mesothelioma.

Malignant mesotheliomas are aggressive malignancies with a poor prognosis and short survival. These tumors primarily occur in the pleural and peritoneal cavities, are more common in men, and have been highly associated with exposure to asbestos. Although the overall incidence of these tumors is low in comparison with the more common lung, breast, and colon cancers, the incidence of mesothelioma is increasing.

Owing to the variety of histopathologic patterns, often bland cytomorphologic features of the tumor cells, and the presence of other entities that can mimic mesothelioma, the diagnosis can be challenging, particularly in small surgical biopsy specimens and cytologic specimens. The diagnosis of mesothelioma can be so challenging that even among experts in the United States-Canadian Mesothelioma Reference Panel, there was a reported disagreement in 22% of the cases during a 5-year period in distinguishing benign from malignant mesothelial proliferations. The mimics include common benign entities, such as reactive mesothelial or histiocytic proliferations, and malignant entities, such as adenocarcinomas. There is also a high false-negative rate in diagnosing mesotheliomas on cytologic specimens owing to diagnostic difficulties and the quality of the specimen (low cellularity, poor cell preservation), which can lead to a delay in diagnosis. In addition, stromal or chest wall fat invasion has been emphasized as being of diagnostic importance in mesotheliomas, and this has resulted in the perception that cytology has a limited role in the primary diagnosis of mesothelioma and created debate between cytopathologists and surgical pathologists. Finally, the issue of litigation in cases of mesotheliomas makes pathologists reluctant to diagnose mesothelioma until they have confirmatory evidence.
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Given the difficulties in making a definitive diagnosis and the poor prognosis, there has been a great interest in investigating new tumor markers, molecular markers, and ancillary studies to help treat patients earlier in the course of disease and to enhance the ability to make a diagnosis of mesothelioma in small tissue samples. Some of the first proposed markers for distinguishing benign/reactive from malignant mesothelial proliferations included immunohistochemical antibodies for epithelial membrane antigen (EMA), p53, bcl-2, desmin, P-glycoprotein, and X-linked inhibitor of apoptosis protein; however, there has been a great deal of inconsistency reported in the reliability of these markers.

More recently, it was suggested that the expression of GLUT-1 is a sensitive and specific marker of malignancies, including mesothelioma. GLUT-1 is a glucose transport protein on the cell surface that is normally expressed in RBCs, the perineurium, the renal tubules, the blood-brain barrier, and the placenta. The expression of GLUT-1 has been relatively undetectable in normal tissues and benign epithelial tumors but expressed in a variety of carcinomas and mesotheliomas. However, the most recent studies questioned the diagnostic usefulness of GLUT-1 immunohistochemical studies in making the distinction between benign/reactive and malignant mesothelial proliferations as well as in other malignancies.

The homozygous deletion of the 9p21 locus is one of the most common genetic abnormalities reported in mesotheliomas, and the tumor suppressor gene that is most often inactivated is the p16 (CDKN2A) gene. Loss of p16 may be a result of homozygous deletion, promoter hypermethylation, or point mutation, but of these, the homozygous deletion, unique for malignant processes, is the most common abnormality detected. Therefore, fluorescence in situ hybridization (FISH) testing has been used to detect the loss of this gene in mesotheliomas.

Because 2 of the more commonly used tests to help make the distinction of mesothelioma from its benign/reactive mimics include FISH for the detection of the p16 deletion and immunohistochemical analysis for the detection of GLUT-1 expression, the aim of our study was to compare the diagnostic usefulness of these 2 methods in the diagnosis of mesothelioma. By using a large series of surgical and cytopathology specimens, we analyzed a range of mesothelial proliferations, from benign/reactive to malignant.

Materials and Methods

Surgical pathology and cytopathology cases with a diagnosis of benign/reactive mesothelial cells, atypical mesothelial proliferation, and malignant mesothelioma were randomly selected from the archives of the University of Pittsburgh Medical Center, Pittsburgh, PA. The study protocol was reviewed and approved by the institutional review board of the University of Pittsburgh Medical Center (No. 0612074).

The diagnosis of mesothelioma was made based on the morphologic features and the results of ancillary studies. Mesothelial origin was confirmed by using an immunohistochemical panel that included antibodies to calretinin, cytokeratin 5/6, D2-40, WT-1, Ber-EP4, B72.3, thyroid transcription factor-1 (TTF-1) and/or carcinoembryonic antigen. Representative tissue and cell blocks and previously reported high-density tissue microarrays (TMAs) with 26 peritoneal mesotheliomas were selected for p16 FISH and GLUT-1 immunohistochemical studies. All histologic and cytologic slides were reviewed by 2 pathologists (S.E.M. and S.D.), who also reviewed all GLUT-1 immunostains, to provide a semiquantitative score for the staining.

Immunohistochemical Analysis

Immunohistochemical analysis was performed using the anti-GLUT-1 prediluted rabbit monoclonal antibody (clone SPM498, Abcam, Cambridge, MA) according to the standard avidin-biotin-peroxidase complex method without a blocking step. Briefly, sections were submitted to heat-induced antigen retrieval in the presence of CC1 (Cell Conditioner 1, Ventana Medical Systems, Tucson, AZ) using an automated tissue immunostainer (Ventana Medical Systems). The GLUT-1 immunostain for each case was interpreted as previously described. In brief, appropriate positive and negative control samples were used, including RBCs as an internal positive control. The area of GLUT-1 staining was evaluated using a semiquantitative scoring scale from 0 to 3+ (0, <1%; 1+, 1%-25%; 2+, 26%-50%; and 3+, >51%) as a representation of the percentage of cells with positive membranous staining among the population of mesothelial cells (indicated by calretinin or WT-1 immunostain). Immunohistochemical staining was scored independently by 2 observers (S.E.M. and S.D.), and any discrepancies were addressed to reach a consensus score.

The TMAs used were constructed from archival, formalin-fixed, paraffin-embedded samples using 3 cores (each measuring 0.1 cm in diameter) from each case using a manual tissue arrayer (MTA-1, Beecher Instruments, Sun Prairie, WI). The GLUT-1 score was determined as described above for each core in the TMA, and then an average of the 3 cores for each individual case represented on the TMA was calculated for the final score of the case.

Fluorescence In Situ Hybridization

Dual-color FISH analysis was performed, as previously described, using a SpectrumGreen-labeled chromosome 9 centromeric (CEP 9) probe and a p16 (CDKN2A) SpectrumOrange-labeled probe (Abbott Molecular, Des Plaines, IL).
Homozygous deletion was defined by loss of both and the average ratio of the gene to CEP 9 copy numbers. Each tumor was assessed by the average and the maximum score. Overlapping cells were excluded from the analysis. At treated slides. Only individual and well-delineated cells were on the H&E-stained sections were identified on the FISH-DAPI (UV 360 nm). The histologic areas previously selected band exciters for SpectrumGreen, SpectrumOrange, and Clara, CA) equipped with filter sets with single- and dual-band excitors for SpectrumGreen, SpectrumOrange, and DAPI (UV 360 nm). The histologic areas previously selected on the H&E-stained sections were identified on the FISH-treated slides. Only individual and well-delineated cells were scored. Overlapping cells were excluded from the analysis. At least 60 cells were scored for each case and control sample. Each tumor was assessed by the average and the maximum numbers of copies of the p16 (CDKN2A) gene per cell and the average ratio of the gene to CEP 9 copy numbers. Homozygous deletion was defined by loss of both p16 gene signals in at least 20% of nuclei that showed at least 1 signal for the CEP 9 probe.

Statistical Analysis

Logistic regression and receiver operating characteristic (ROC) curve analysis were used to assess the accuracy of the 2 biomarkers making the diagnosis of a mesothelioma. The ROC comparisons are performed by using the nonparametric approach of DeLong et al.30 Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). A significance level was set at .05, and all P values reported are 2-sided.

Results

Clinicopathologic Characteristics

Table II summarizes clinicopathologic characteristics of 154 cases included in this study. A total of 70 benign/reactive cases (45.4%), 16 atypical cases (10.4%), and 68 (44.2%) mesotheliomas with sufficient representative material were selected. Of 68 mesotheliomas, there were 27 pleural (40%) and 41 peritoneal (60%) mesotheliomas, which included 61 epitheliod, 3 mixed, and 4 sarcomatoid types. The average age of the patients was 63.2 years (range, 18-89 years), with 104 men (67.5%) and 50 women (32.5%).

The majority of specimens were from surgical pathology biopsies or resections (107 [69.5%]); however, there were also 47 cytology cases (30.5%), including 28 benign/reactive cases, 13 atypical cases, and 6 malignant mesothelioma cases. Of the 16 atypical mesothelial proliferations, 13 (81%) were cytology specimens and 3 (19%) were surgical specimens. Of the 16 atypical cases, 13 (81%) had histologic follow-up, of which 8 (62%) were malignant and 5 (38%) were benign/reactive. All of the atypical cases with histologic follow-up were the cytology specimens. In addition, of the 68 mesotheliomas, only 6 (9%) were cytology specimens and all had follow-up, including 5 cases with histologic confirmation and 1 case in a patient who died shortly after the cytologic diagnosis. The 28 benign/reactive cases diagnosed by cytologic examination also had clinical or pathologic follow-up confirming the diagnosis.

GLUT-1 Immunohistochemical Results

The results of GLUT-1 immunohistochemical staining are summarized in Table II. Of 68 mesotheliomas, 41 cases (60%) showed no staining and 27 (40%) showed positive staining. Positive cases demonstrated mostly 1+ staining (17 cases [25%]), whereas 6 cases (9%) showed 2+ staining and 4 cases (6%) had 3+ staining. GLUT-1 positivity was more common in pleural (15/27 [56%]) than in peritoneal (12/41 [29%]) mesotheliomas. GLUT-1 immunostaining was also observed in 5 (7%) of 70 benign cases (3 cytology fluids and 2 surgical biopsy specimens), but in these cases, staining was weak (1+). Overall, GLUT-1 immunohistochemical studies showed 40% sensitivity and 93% specificity (positive predictive value [PPV], 84%; negative predictive value [NPV], 61%) for making the diagnosis of mesothelioma, and there

<table>
<thead>
<tr>
<th>Table II</th>
<th>Characteristics of 154 Study Cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Result</td>
</tr>
<tr>
<td>Mean (range) age (y)</td>
<td>63.2 (18-89)</td>
</tr>
<tr>
<td>Male</td>
<td>104 (67.5)</td>
</tr>
<tr>
<td>Female</td>
<td>50 (32.5)</td>
</tr>
<tr>
<td>Type of specimen</td>
<td></td>
</tr>
<tr>
<td>Cytology</td>
<td>47 (30.5)</td>
</tr>
<tr>
<td>Biopsy/resection</td>
<td>107 (69.5)</td>
</tr>
<tr>
<td>Final diagnosis</td>
<td></td>
</tr>
<tr>
<td>Benign/reactive</td>
<td>70 (45.4)</td>
</tr>
<tr>
<td>Atypical</td>
<td>16 (10.4)</td>
</tr>
<tr>
<td>Malignant</td>
<td>68 (44.2)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage) unless otherwise indicated.
was a difference in diagnostic accuracy between peritoneal and pleural mesotheliomas. Statistical analysis demonstrated GLUT-1 immunohistochemical sensitivity of 29% and specificity of 100% in the diagnosis of peritoneal mesothelioma (PPV, 100%; NPV, 22%). On the other hand, 56% sensitivity and 92% specificity were observed in the diagnosis of pleural mesotheliomas (PPV, 75%; NPV, 83%).

Of the 16 atypical cases, 3 (19%) were positive for GLUT-1, with 2 cases showing 3+ and 1 case showing 1+ staining. Two of these positive cases were lost to follow-up, but one 3+ GLUT-1+ case was proven to be a benign process on follow-up, the benign cases were all negative for the p16 deletion. Of 9 atypical cases that were negative for deletion, 5 were benign and 4 were malignant. Overall, in the 13 atypical cases with histologic follow-up, including 8 mesotheliomas on follow-up and 5 benign/reactive proliferations on follow-up, the benign cases were all negative for the p16 deletion. However, 1 benign case showed 3+ positivity on immunostaining for GLUT-1. In the 8 malignant cases on follow-up, 4 (50%) were positive for the p16 deletion, whereas only 2 (25%) were positive for GLUT-1 (1+ and 3+ positivity). Thus, FISH for p16 correlated better with the histologic follow-up in this atypical group than did the GLUT-1 immunostaining profile.

Table 3 summarizes the results of p16 FISH in comparison with GLUT-1 immunohistochemical analysis. Of 70 benign/reactive mesothelial proliferations, 65 (93%) showed the absence of p16 deletion and negative GLUT-1 immunohistochemical results. There were 5 (7%) false-positive GLUT-1 cases that were all negative for the p16 deletion (Table 3). In the atypical category, 8 cases (50%) were negative for the p16 deletion and GLUT-1. Follow-up failed to demonstrate malignancy in any of these cases. In 2 cases (13%), there was positivity for the p16 deletion and GLUT-1, and 5 (31%) cases were positive for the p16 deletion but negative for GLUT-1. Only 1 case (6%) was positive for GLUT-1 and negative for the p16 deletion (Table 3).

Of 68 mesotheliomas, 16 cases (24%) showed deletion of p16 and positive GLUT-1, 17 cases (25%) were negative for both, 11 cases (16%) were positive for GLUT-1 and negative for p16 deletion, and 24 (35%) were positive for the p16 deletion and negative for GLUT-1 (Table 3). A representative example of a mesothelioma case with GLUT-1 staining and p16 FISH results is shown in Image 2A.

Table 2
Summary of GLUT-1 Immunohistochemical Results and p16 FISH Results in 154 Cases of Reactive, Atypical, and Malignant Mesothelial Proliferations

<table>
<thead>
<tr>
<th>GLUT-1 Immunohistochemical Analysis</th>
<th>Benign/Reactive (n = 70)</th>
<th>Atypical (n = 16)</th>
<th>Total (n = 86)</th>
<th>Pleura (n = 27)</th>
<th>Peritoneum (n = 41)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>65 (93)</td>
<td>13 (81)</td>
<td>41 (60)</td>
<td>12 (44)</td>
<td>29 (71)</td>
</tr>
<tr>
<td>1+</td>
<td>5 (7)</td>
<td>1 (6)</td>
<td>17 (25)</td>
<td>11 (41)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>2+</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (9)</td>
<td>3 (11)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>3+</td>
<td>0 (0)</td>
<td>2 (13)</td>
<td>4 (6)</td>
<td>1 (4)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>p16 FISH</td>
<td></td>
<td></td>
<td>28 (41)</td>
<td>8 (30)</td>
<td>20 (49)</td>
</tr>
<tr>
<td>Negative for deletion</td>
<td>70 (100)</td>
<td>9 (56)</td>
<td>40 (59)</td>
<td>19 (70)</td>
<td>21 (51)</td>
</tr>
<tr>
<td>Positive for deletion</td>
<td>0 (0)</td>
<td>7 (44)</td>
<td>21 (31)</td>
<td>9 (33)</td>
<td>12 (29)</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.

"p16 FISH"

Results of FISH testing for the p16 deletion are summarized in Table 2. Of 68 mesotheliomas, 40 (59%) showed deletion of the 9p21 locus. The overall sensitivity was 59% and specificity was 100% (PPV, 100%; NPV, 71%). Deletion of the 9p21 locus was more frequent in pleural (19/27 [70%]) than in peritoneal (21/41 [51%]) mesotheliomas. All of the true benign/reactive cases were negative for deletion. FISH for p16 showed 100% specificity in making the diagnosis of mesothelioma, regardless of anatomic location. However, sensitivity was much better in pleural (70%; PPV, 100%; NPV, 89%) than in peritoneal (51%; PPV, 100%; NPV, 29%) mesotheliomas. Cases positive for deletion showed homozygous deletion of 9p21 in 45% to 100% of analyzed cells.

Of 16 atypical cases, 7 (44%) were positive for deletion. Three positive cases were lost to clinical follow-up, but the remaining 4 cases that were positive for deletion were malignant. Of 9 atypical cases that were negative for deletion, 5 were benign and 4 were malignant. Overall, in the 13 atypical cases with histologic follow-up, including 8 mesotheliomas on follow-up and 5 benign/reactive proliferations on follow-up, the benign cases were all negative for the p16 deletion. However, 1 benign case showed 3+ positivity on immunostaining for GLUT-1. In the 8 malignant cases on follow-up, 4 (50%) were positive for the p16 deletion, whereas only 2 (25%) were positive for GLUT-1 (1+ and 3+ positivity). Thus, FISH for p16 correlated better with the histologic follow-up in this atypical group than did the GLUT-1 immunostaining profile.
Diagnostic difficulties in the interpretation of GLUT-1 immunostaining. Although RBCs serve as a helpful internal control, the positive RBCs within a cell block and small biopsy specimens can be intermixed with benign mesothelial cells and result in falsely interpreting the mesothelial cells as positive. A and B, Benign/reactive mesothelial proliferations. A, Cell block (GLUT-1, ×200). B, Surgical biopsy specimen (GLUT-1, ×400). C and D, Reactive mesothelial proliferations showing weak, patchy, and incomplete membranous staining (0-1+). C, Cell block (GLUT-1, ×400). D, Cell block (GLUT-1, ×200).

Table 3
Comparison of GLUT-1 Immunohistochemical Analysis and p16 FISH

<table>
<thead>
<tr>
<th>Immunohistochemical Result</th>
<th>p16 FISH– for Deletion</th>
<th>p16 FISH+ for Deletion</th>
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<tbody>
<tr>
<td>Benign/reactive mesothelial proliferations (n = 70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT-1–</td>
<td>65 (93)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>GLUT-1+</td>
<td>5 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Atypical mesothelial proliferations (n = 16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT-1–</td>
<td>8 (50)</td>
<td>5 (31)</td>
</tr>
<tr>
<td>GLUT-1+</td>
<td>1 (6)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Malignant mesothelioma (n = 68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT-1–</td>
<td>17 (25)</td>
<td>24 (35)</td>
</tr>
<tr>
<td>GLUT-1+</td>
<td>11 (16)</td>
<td>16 (24)</td>
</tr>
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</table>

FISH, fluorescence in situ hybridization.
* Data are given as number (percentage).

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Mesothelioma with GLUT-1 immunohistochemical and p16 fluorescence in situ hybridization (FISH) testing. A and B, Cytomorphologic features of a mesothelioma diagnosed on a pleural fluid specimen with high cellularity and numerous clusters of cells with cytologic atypia. A, Cytocentrifuged specimen (Diff-Quik, ×200). B, Cell block (H&E, ×400). C, The follow-up surgical biopsy confirmed the diagnosis (H&E, ×400). D-F, Ancillary studies on the cell block confirmed that the malignant cells were positive for calretinin (D, ×400), weakly positive (score 1+) for GLUT-1 (E, ×400), and showed homozygous deletion for the p16 gene by FISH (F).
Our results demonstrated that FISH testing for \( p16 \) deletion is a more specific and sensitive test for making the diagnosis of a malignant mesothelioma than is immunohistochemical testing for GLUT-1. The area under the GLUT-1 ROC curve is 0.6628, and the 95% confidence interval is 0.5968 to 0.7288. The area under the \( p16 \) ROC curve is 0.7941, and the 95% confidence interval is 0.7352 to 0.8530. The \( p16 \) ROC curve is significantly different from the GLUT-1 ROC curve \( (P = .004) \). Thus, the homozygous deletion of \( p16 \) detected by FISH was a more reliable way to distinguish the benign/reactive and malignant mesothelial proliferations than immunohistochemical testing for GLUT-1.

### Discussion

During the past decade, there has been a great interest in identifying reliable tumor markers and molecular markers that can be used to enhance the ability to make a definitive diagnosis of mesothelioma, to help in determining the prognosis, and to identify markers to target therapeutically.\(^{11-15,17,26}\) This study looked at 2 of the recently proposed markers in the literature, the deletion of \( p16 \) and the expression of GLUT-1, in a large series of mesothelial proliferations. Our study demonstrates that FISH detection of the \( p16 \) gene deletion has a higher sensitivity and specificity for the diagnosis of mesotheliomas compared with GLUT-1 immunohistochemical studies.

GLUT-1 has been proposed as an immunohistochemical marker of malignancy in aspiration cytology,\(^{20,21}\) and body fluid cytology,\(^{16}\) as well as in surgical pathology of almost any organ site.\(^{16,17,31,32}\) In our study, 60% of mesotheliomas were negative for GLUT-1, and only 4 cases (6%) showed diffuse positivity (3+). In addition, there were a few false-positives detected in our study, including 5 (7%) of the benign/reactive mesothelial proliferations that were GLUT-1+. Overall, the sensitivity was quite low (40%; 30% for peritoneal and 56% for pleural). On the interpretation side, GLUT-1 was difficult, given the numerous background RBCs that are positive, which can make the interpretation of bloody samples difficult, particularly cytology cell block samples. In addition, in cases with weak or partial positivity (score, 1-2+) or heterogeneous staining, it is uncertain if there is enough staining to truly warrant a reliable diagnosis of malignancy. This heterogeneity of GLUT-1 staining has been reported previously as a limiting factor of this test.\(^{17,33-35}\) Prior studies have also shown that negative staining for GLUT-1 does not exclude the possibility of malignancy, which is similar to our results.\(^{20,21}\) In fact, in adenocarcinomas, it seems that more well-differentiated components of tumors stain less intensely, and this finding may explain some of the false-negative results in well-differentiated mesotheliomas.\(^{33,34}\) In addition, the false-positive staining for GLUT-1 in reactive conditions has been reported, and it is hypothesized that expression may be a sign of cellular stress, hypoxia, ischemia, or cellular adaptation to altered metabolism in a nutrient-deprived environment, particularly in exfoliated mesothelial cells in fluid cytology.\(^{14,36}\)

Historically, a variety of immunohistochemical stains before GLUT-1 have been proposed to help distinguish benign or reactive mesothelial cells from malignant mesothelioma. For example, strong membranous staining for EMA and positive nuclear staining for \( p53 \) have been used to exclude a reactive mesothelial proliferation; however, the sensitivity and specificity have been inconsistent and not entirely reliable.\(^{11-14}\) Similarly, the positivity for desmin has been shown to support a benign/reactive mesothelial proliferation over a malignant one; however, the results have also been inconsistent.\(^{11,15}\) Owing to the variability in the results reported in the literature, the United States–Canadian Mesothelioma Reference Panel discouraged the use of \( p53 \) and EMA immunohistochemical studies.\(^7\) Our findings illustrate that GLUT-1 immunohistochemical analysis also has low sensitivity for detecting mesotheliomas and can be difficult to interpret.

The homozygous deletion of \( p16 \) detected by FISH was a reliable way to distinguish the benign/reactive and malignant mesothelial proliferations in our series of cases and was particularly helpful in pleural cases. Our data show that the majority of mesothelioma cases were positive for the \( p16 \) gene deletion by FISH (41 [59%]), whereas none of the benign/reactive cases were positive for the deletion (100% specificity). In a prior study from our institution looking at a smaller number of cases, there were also no benign/reactive cases with a \( p16 \) deletion by FISH (ie, no false-positives), and the sensitivity for pleural mesotheliomas was about 67%\(^{28}\) which is similar to our findings in this larger series (59% sensitivity; 70% for pleural and 51% for peritoneal). The absence of false-positives was also found in studies at other institutions, including a study performing FISH for the \( p16 \) deletion on ThinPrep slides from cytologic specimens.\(^{29}\) However, it is important to recognize that \( p16 \) FISH is not a perfect test in that it will not be positive in all malignant mesothelioma cases.

Although the group of atypical mesothelial proliferations was the smallest group of cases in our study, this is one of the most important groups in that there is some atypia but insufficient evidence for a definitive diagnosis. In this scenario, a reliable ancillary study would be most helpful for treating clinicians in order to decrease the need for repeated procedures, prevent more invasive surgeries, and decrease the delay in obtaining a definitive diagnosis. In our study, there were 16 atypical mesothelial proliferations, of which 81% (13/16) were cytology cases and had histologic follow-up. The diagnosis of an atypical mesothelial...
proliferation is more common in cytologic specimens than in surgical specimens because the diagnosis of mesothelioma can be more challenging in cytologic specimens owing to the inability to evaluate for tissue invasion and the numerous cytomorphic mimics of mesothelioma, including reactive mesothelial proliferations. Thus, there is a real need for an ancillary test that can reliably distinguish benign/reactive from malignant mesothelial proliferations in these atypical cases, and, in our experience, FISH detection of the homozous p16 deletion has been the most helpful. In our small group of atypical cases, FISH was positive in 50% (4/8) of the histologically confirmed malignant cases and negative in all 5 cases that were benign/reactive on follow-up. However, GLUT-1 showed 3+ positivity in an atypical case that was benign on follow-up.

Our results demonstrate that p16 FISH has higher sensitivity and specificity and was a more reliable diagnostic test for the distinction of malignant mesothelioma from reactive proliferations compared with GLUT-1 immunohistochemical studies. FISH for p16 was particularly helpful in pleural mesotheliomas, with a sensitivity of 70%. Both tests can be performed on tissue sections or cell block sections and do not require a large number of cells. However, one drawback of both of these diagnostic tests is that they can be positive in almost any malignancy and, thus, an immunohistochemical panel to prove mesothelial origin (eg, positivity for calretinin, WT-1, or D2-40 and negativity for BerEP4, B72.3, TTF-1, or MOC-31) should still be the first step in diagnosis.

From the 1Department of Pathology, University of Pittsburgh Medical Center, and 2The University of Pittsburgh Cancer Institute Biostatistics Facility, University of Pittsburgh, Pittsburgh, PA.

Address reprint requests to Dr Monaco: Dept of Pathology, University of Pittsburgh Medical Center, 5230 Centre Ave, Suite WG02.1, Pittsburgh, PA 15232.

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