Comparative Immunohistochemical Analysis of IMP3, GLUT1, EMA, CD146, and Desmin for Distinguishing Malignant Mesothelioma From Reactive Mesothelial Cells

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ABSTRACT

Objectives: *To identify useful biomarkers for differentiating between malignant mesothelioma (MM) and reactive mesothelial cells (RMCs).*

Methods: *Formalin-fixed, paraffin-embedded (FFPE) tissues from 34 MM and 40 RMC samples were analyzed using immunohistochemistry, and the findings were compared.*

Results: *Positive markers for MM included insulin-like growth factor 2 messenger RNA binding protein 3 (IMP3), glucose transporter 1 (GLUT1), epithelial membrane antigen (EMA), and CD146, which showed sensitivities of 94%, 85%, 79%, and 71% and specificities of 78%, 100%, 88%, and 98%, respectively. In sarcomatoid MM, EMA had significantly lower expression than did IMP3, GLUT1, and CD146 ($P < .001$). The areas under receiver operating characteristic curves were the highest for IMP3 (0.95), followed by GLUT1 (0.93). When the optimal cutoff points for IMP3 (30%) and GLUT1 (10%) were used, the sensitivity of IMP3 and GLUT1 for MM was 100%, and the specificity of both for MM was 95%.*

Conclusions: *The combination of IMP3 and GLUT1 is most appropriate for distinguishing MM from RMC using FFPE sections.*

CME/SAM

Upon completion of this activity you will be able to:
- list biomarkers used for the differential diagnosis between malignant mesothelioma (MM) and reactive mesothelial cells (RMCs).
- appropriately select and interpret immunohistochemical markers in the diagnosis of MM among its subtypes and in the differential diagnosis between MM and RMC.
- describe the limitations of using ancillary studies, such as immunohistochemistry and fluorescence in situ hybridization, in the diagnosis of MM.

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Questions appear on p 141. Exam is located at www.ascp.org/ajcpcme.

The global incidence of malignant mesothelioma (MM), an aggressive tumor of the serosal surface, is increasing.[1] Although a multimodality therapeutic approach improves the response to treatment and survival, the prognosis is poor, with a median survival time rarely exceeding 12 months.[1,2] Accurate and early pathologic diagnosis of MM can improve patient outcomes because patients with early stage disease are more likely to be eligible for multimodality therapy, including surgery. However, it is extremely difficult to distinguish between a variety of reactive and neoplastic changes on histologic examination, such as between reactive mesothelial hyperplasia and epithelioid MM and between reactive pleural fibrosis and sarcomatoid MM.[3] MM tumor cells often appear unremarkable, while hyperplastic mesothelial cells exhibit various degrees of nuclear atypia. In addition, it is often

difficult to identify the invasive component of MM on a small biopsy specimen. Superficial entrapment of mesothelial cells by organized effusion is common in benign reactions, and such effusion needs to be distinguished from invasion.[4]

Over the past 20 years, a number of biomarkers have been evaluated in an effort to distinguish between MM and reactive mesothelial cells (RMCs). However, the results of these investigations are contradictory.[3] In their systematic review, King et al[5] showed that epithelial membrane antigen (EMA) and desmin appear to be the most useful biomarkers; however, the diagnostic sensitivities and specificities of these markers are less than 90%, and they play a limited role in daily clinical practice.

Recently, several new biomarkers, including insulin-like growth factor 2 messenger RNA binding protein 3 (IMP3), glucose transporter 1 (GLUT1), and melanoma cell adhesion molecule (CD146 or MUC18) have emerged as good candidates for distinguishing between MM and RMCs.[6-8] However, thus far, no study has reported a single immunohistochemical marker that accurately discriminates between RMCs and MM.

IMP3 is ubiquitously expressed during the early stages of embryogenesis, with only limited expression in postembryonic stages. IMP3 is also expressed by a variety of malignant tumors,[8,9] and strong expression of this protein has been correlated with a poor prognosis, while negative or weak expression is thought to indicate benign or low-grade lesions.[9] In 2008, Hanley et al[10] performed immunohistochemical analyses of IMP3 (anti-L523S) in cell blocks of malignant serous effusions and reported 91% (11/12) reactivity in MM effusions and 0% (0/13) reactivity in benign effusions. In 2011, Ikeda et al[11] reported that IMP3 had a sensitivity of only 36% and a specificity of 96% in effusions of 11 MM and 50 RMC samples. Moreover, with immunohistochemical analyses of histology sections from 45 MM and 64 RMC samples, Shi et al[8] found 73% sensitivity and 100% specificity of IMP3.

GLUT1 is a member of the glucose transporter isoform family and facilitates the entry of glucose into cells.[12] It can usually be detected in erythrocytes, the blood-brain barrier, and the placenta but rarely in other organs. It is widely expressed in various human malignancies.[6,11] In 2007, Kato et al[6] reported immunohistochemical expression of GLUT1 in all tested MM samples (40/40, 100%) and no RMC samples (0/40, 0%). However, other immunohistochemical and immunocytochemical studies with GLUT1 reported a sensitivity of 40% to 99% in MM and a specificity of 82% to 100% in RMCs.[11,13,14]

CD146 is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily and serves as a Ca^{2+}-independent adhesion molecule.[15] Shih et al[16] found that CD146/MCAM is immunohistochemically expressed in a variety of malignant tumors, but its expression is limited in normal human adult tissue. Bidlingmaier et al[15] reported the first immunohistochemical analysis of CD146 expression in MM and found that CD146 was expressed in 91% (50/55) of the epithelioid and sarcomatoid MM tissue microarrays, but the specificity was not indicated. In 2010, Sato et al[7] reported a sensitivity of 94% and a specificity of 100% by immunocytochemical analysis of CD146 in pleural effusions for discriminating between MM and RMCs. Thus far, to our knowledge, no other immunohistochemical analysis of CD146 in MM has been reported.

Each of these biomarkers has shown good sensitivity and specificity, but the sensitivities differ, especially among analyses of histologic vs cytologic specimens. In addition, these biomarkers have not yet been comparatively analyzed in formalin-fixed, paraffin-embedded (FFPE) tissues. Therefore, in this study, we conducted a comparative immunohistochemical analysis of MM and RMC cases using antibodies targeting IMP3, GLUT1, CD146, EMA, and desmin to determine which of these antibodies would provide the best combination for distinguishing between MM and RMCs.

Materials and Methods

Case Selection

FFPE tissues from 34 cases of previously confirmed MM and 40 cases of benign lung tissues with RMCs were used. The cases were consecutively extracted from the surgical pathology files from patients seen between 1998 and 2011 at Kanazawa Medical University, Kanazawa University Hospital, and KKR Hokuriku Hospital, in Japan. This study was carried out in accordance with the Helsinki Declaration.

Immunohistochemistry

We cut 4-μm slices from FFPE blocks. Each slice was deparaffinized, treated with 3% hydrogen peroxidase to block endogenous peroxidase activity, and then treated with 0.01 mol/L citric acid (pH 6.0) for 4 minutes at 110°C in a pressure cooker for antigen retrieval. Slides were then incubated for 1 hour at room temperature with antibodies; a summary of the antibodies used is provided in **Table 1**. The slides were immunostained using an UltraTech HRP streptavidin-biotin detection system (Beckman Coulter, Brea, CA), with diaminobenzidine as the chromogen, and then counterstained with hematoxylin. Negative controls were prepared by replacing the primary antibodies with phosphate-buffered saline. Internal controls used were as follows: erythrocytes for GLUT1, endothelium and smooth muscle of vascular walls for CD146,[15] smooth muscle of vascular walls for desmin, and alveolar pneumocytes and plasma cells for EMA. Sections of malignant lymphoma tissue with positive reactions were used as positive controls for IMP3.[4]

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Table 1
Antibodies Used in This Study

Antibody	Clone	Source	Dilution	Pretreatment
GLUT1	Polyclonal	Abcam[a]	1/50	Heat
CD146	N1238	Abcam	1/50	Heat
IMP3	L523S	DAKO[b]	1/100	Heat
EMA	E29	DAKO	1/100	None
Desmin	D33	DAKO	1/15	None

[a] Abcam (Cambridge, England).
[b] DAKO (Carpinteria, CA).
EMA, epithelial membrane antigen; GLUT1, glucose transporter 1; IMP3, insulin-like growth factor 2 messenger RNA binding protein 3.

Immunohistochemical Evaluation

Staining results were scored as the percentage of stained mesothelial or tumor cells in 5% increments. When more than 5% of the mesothelial or tumor cells appeared stained by an antibody, the result was defined as positive.

Statistical Analysis

The χ^2 test or Fisher exact probability test was used to assess the association between categorical variables. *P* values of less than .05 were considered significant with two-sided tests. Receiver operating characteristic (ROC) curves were used to determine the relationship between the sensitivity and specificity of each antibody and to find the best diagnostic cutoff values. The area under the ROC curve (AUC) was calculated and compared among each antibody. The AUC is a measure of how well a parameter can distinguish between RMC and MM; the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test.[17] All statistical analyses were carried out using StatFlex version 6.0 (Artech, Osaka, Japan).

Results

Among the patients with MM, the mean age was 63 years (range, 32-80 years); 31 were men, and 3 were women. Among the patients with RMCs, the mean age was 39 years (range, 13-95 years); 35 were men, and 5 were women. All materials from RMC cases were benign lung tissues surgically removed from patients with pneumothorax who did not have any tumor tissues. Follow-up periods for these patients with RMCs ranged from 8 to 2,362 days (mean, 811 days; median, 466 days). A 64-year-old man died after 1,636 days due to intra-abdominal dissemination of gastric cancer. There were 16 cases with less than 1 year follow-up, for which the mean age was 27 years, excluding 86- and 95-year-old men. No diagnostically challenging cases were included.

A total of 31 MM cases were of pleural origin, while three were of peritoneal origin (two men and one woman). Of the MM cases, 17 (50%) were epithelioid, 11 (32%) were biphasic, and 6 (18%) were sarcomatoid. Of the sarcomatoid cases, two were desmoplastic.

The immunohistochemical expression of each marker in MM and RMC tissues is summarized in **Figure 1**. To distinguish MM from RMCs, immunohistochemical positivity for IMP3, GLUT1, EMA, and CD146 had sensitivities of 94%, 85%, 79%, and 71% and specificities of 78%, 100%, 88%, and 98%, respectively, and negativity for desmin had 48% sensitivity and 97% specificity. Representative staining patterns for each marker are shown in **Image 1**, **Image 2**, and **Image 3**. Immunostaining for detection of GLUT1, EMA, and CD146 was generally concentrated around the cell membrane, with only occasional cytoplasmic staining. However, cytoplasmic staining of these antibodies was often observed in sarcomatoid MM. IMP3 and desmin showed cytoplasmic staining in both MM and RMC cases. In RMCs, IMP3 and CD146 showed focal and weak cytoplasmic staining, while EMA showed weak cytoplasmic or apical staining. In MM, the proportion of positive tumor cells ranged from 10% to 95% (mean, 51%) for GLUT1, 5% to 95% (33%) for CD146, 5% to 100% (60%) for IMP3, 5% to 100% (59%) for EMA, and 5% for desmin. In RMC cases, the proportion of positive mesothelial cells ranged from 5% to 30% (mean, 12%) for desmin, 10% to 40% (19%) for IMP3, 10% to 35% (19%) for EMA, and 5% for CD146. Positive rates for GLUT1, CD146, IMP3, and EMA in epithelioid, biphasic, and sarcomatoid

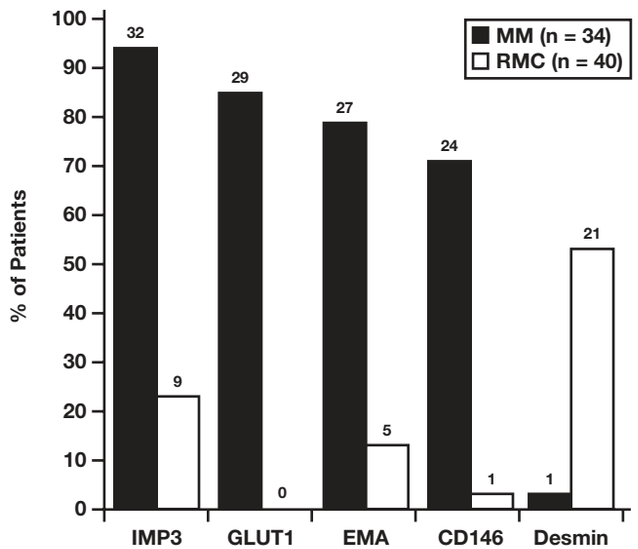

Figure 1 Expression of each marker in malignant mesothelioma (MM) and reactive mesothelial cell (RMC) specimens. EMA, epithelial membrane antigen; GLUT1, glucose transporter 1; IMP3, insulin-like growth factor 2 messenger RNA binding protein 3.

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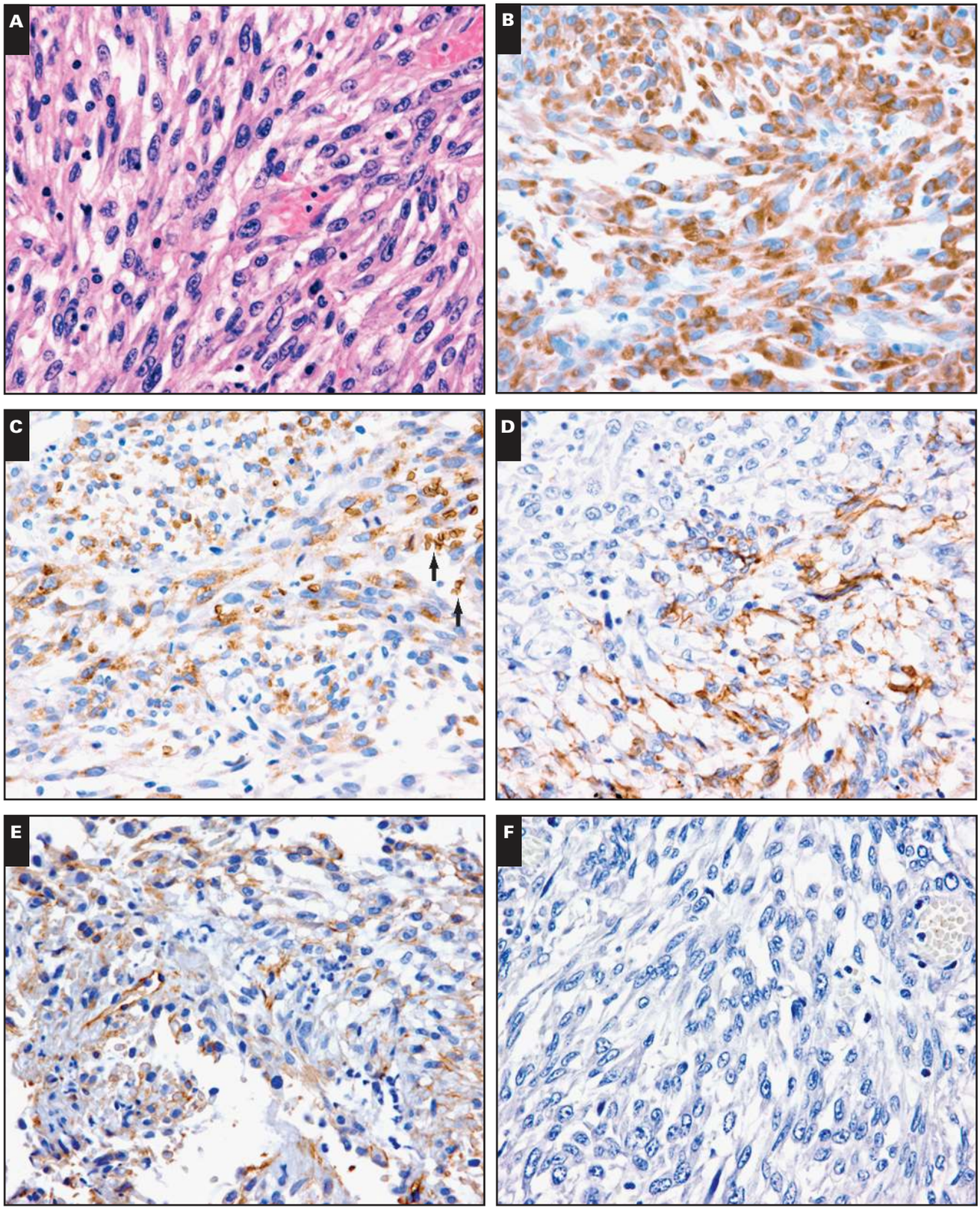

Image 1 Representative staining patterns in a sarcomatoid malignant mesothelioma. **A**, H&E, ×400. **B**, Insulin-like growth factor 2 messenger RNA binding protein 3 was diffusely positive in the cytoplasm of tumor cells, ×400. **C**, Glucose transporter 1 was positive in tumor cells as well as erythrocytes (arrows), ×400. **D**, Epithelial membrane antigen showed focal positivity in tumor cells, ×400. **E**, CD146 was positive in cell membranes of tumor cells, ×400. **F**, Desmin was negative, ×400.

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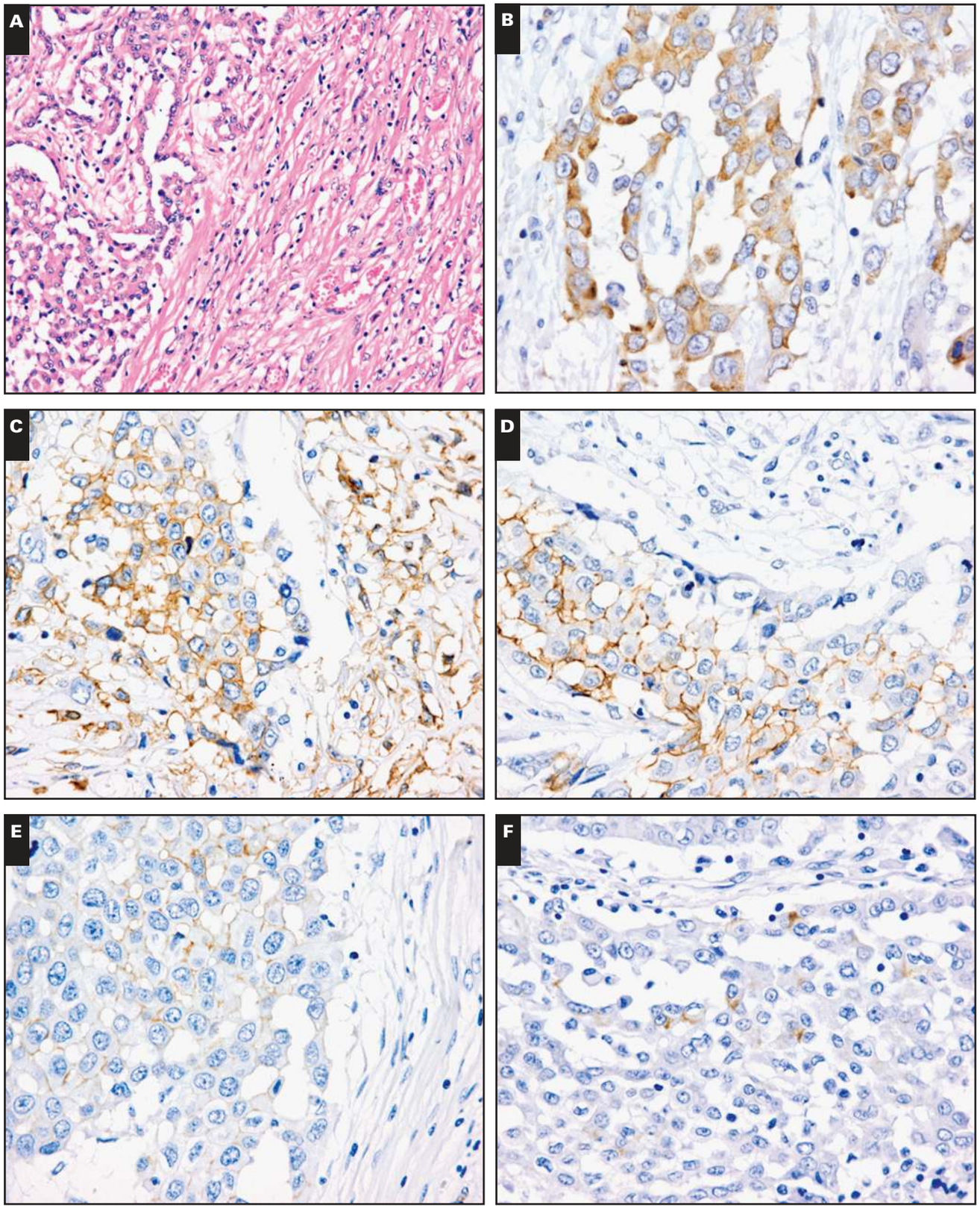

Image 2 A staining pattern for each antibody in a biphasic malignant mesothelioma. **A**, H&E, ×400. **B**, Insulin-like growth factor 2 messenger RNA binding protein 3 was positive in the cytoplasm of tumor cells, ×400. **C**, Glucose transporter 1 showed a membranous staining pattern in epithelioid and sarcomatoid tumor cells, ×400. **D**, Epithelial membrane antigen showed focal membranous staining in epithelioid tumor cells, ×400. **E**, CD146 was weakly positive in epithelioid tumor cells, ×400. **F**, Only this biphasic malignant mesothelioma case showed focal desmin positivity. Staining for desmin, ×400.

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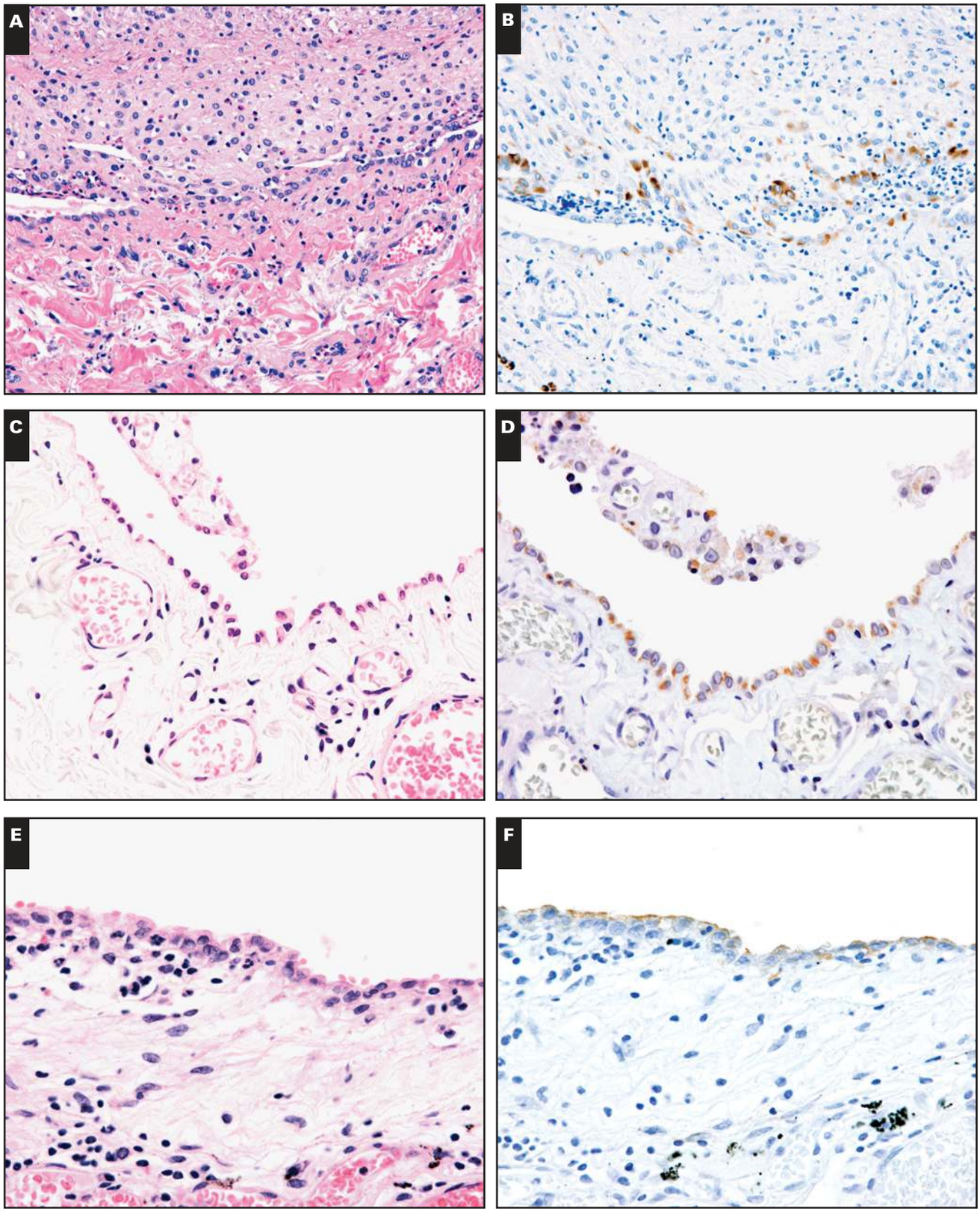

Image 3 Pneumothorax cases with reactive mesothelial cells (RMCs). The specimen from a 13-year-old girl showed focal desmin positivity in RMCs. **A**, H&E, ×200. **B**, Staining for desmin, ×200. The specimen from an 18-year-old man showed focal insulin-like growth factor 2 messenger RNA binding protein 3 (IMP3) positivity in the cytoplasm of RMCs. **C**, H&E, ×400. **D**, Staining for IMP3, ×400. The specimen from a 22-year-old man showed focal apical staining of epithelial membrane antigen (EMA) in RMCs. **E**, H&E, ×400. **F**, Staining for EMA, ×400.

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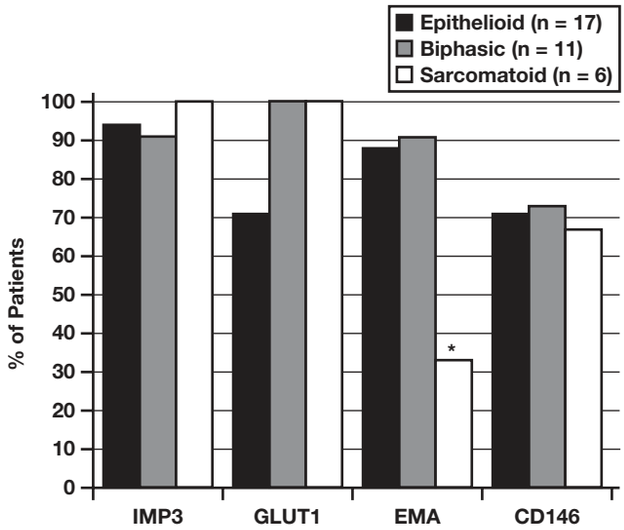
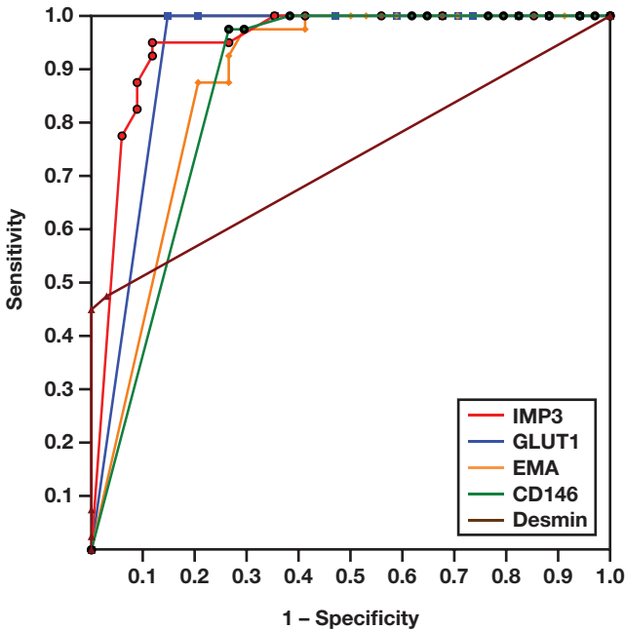


Figure 2 Expression of each marker according to subtypes of malignant mesothelioma. *The expression of EMA was significantly lower in the sarcomatoid than in the epithelioid or biphasic types compared with that of IMP3, GLUT1, and CD146 ($P < .001$). EMA, epithelial membrane antigen; GLUT1, glucose transporter 1; IMP3, insulin-like growth factor 2 messenger RNA binding protein 3.



	IMP3	GLUT1	EMA	CD146	Desmin
ROC (AUC)	0.95	0.93	0.87	0.86	0.73
±SE	0.029	0.037	0.046	0.048	0.059
Optimal cutoff	30%	10%	30%	5%	0%

Figure 3 Receiver operating characteristic (ROC) curves for each antibody for discriminating between malignant mesothelioma and reactive mesothelial cells. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the ROC curve (AUC) is a measure of how well a parameter can distinguish between two diagnostic groups. EMA, epithelial membrane antigen; GLUT1, glucose transporter 1; IMP3, insulin-like growth factor 2 messenger RNA binding protein 3.

MM are presented in **Figure 2**. The expression of EMA was significantly lower in sarcomatoid than in epithelioid and biphasic MM types compared with that of IMP3, GLUT1, and CD146 ($P < .001$). IMP3 and GLUT1 tended to have a higher expression in sarcomatoid MM but without statistical significance. All the MM cases showed positive staining for at least two of the positive markers, except for one case of a relatively small biopsy specimen, which showed positive staining for IMP3 only (pleural; epithelioid type). All five MM cases that were negative for GLUT1 (four pleural and one peritoneal; all epithelioid) showed positive staining for IMP3; two MM cases that were negative for IMP3 (both pleural; epithelioid and biphasic) were positive for GLUT1.

We used the ROC curves for IMP3, GLUT1, EMA, CD146, and desmin to assess the ability of each marker to distinguish between MM and RMC **Figure 3**. The AUC was the greatest for IMP3 (0.95), followed by GLUT1 (0.93), EMA (0.87), CD146 (0.86), and desmin (0.73). Considering the optimal cutoff points on the ROC curve, if the cutoff points were set at 30% for IMP3 and 10% for GLUT1, then the sensitivity and specificity between MM and RMC would be 100% and 95%, respectively, when using the combination of only these two markers. If the cutoff points were set at 40% for IMP3 and 0% for GLUT1, then the sensitivity and specificity between MM and RMC using this marker combination would be both 100%.

Discussion

The current immunohistochemical study aimed to investigate several biomarkers in an effort to identify the best-possible combination among these biomarkers for the differentiation between MM and RMC. Every biomarker showed good sensitivity and specificity, especially IMP3, GLUT1, and EMA, which are most likely to be used in combination in a clinical setting. The sensitivity of CD146 in FFPE sections was lower than expected, and the sensitivity of desmin was too low to be considered practical for routine testing. Previous studies aimed at differentiating between MM and RMC also reported variations in the sensitivity and specificity of different antibodies.[2,3,5-8,10,11,14] Those differences probably relate to differences in (1) study populations and the histology of the cases; (2) specimen types, such as exfoliated cells, cell blocks,

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tissue microarrays, and whole sections; (3) fixation methods; (4) the types of antibodies used; (5) the method of antigen retrieval; and (6) the interpretation of immunostains.

With each antibody, the proportion of positive cells also varied widely from case to case. In their study, Kato et al[6] described similar findings: GLUT1-positive cells varied in number from only a few cells to almost all cells in MM cases. They also indicated that GLUT1 negativity in small samples does not exclude malignancy. Shi et al[8] reported that of the MM cases, 82% showed IMP3 expression in more than 50% of malignant cells, 6% showed IMP3 expression in 25% to 50% of malignant cells, and 12% showed IMP3 expression in 5% to 25% of malignant cells. Considering the data of previous reports and the findings of this study, it is evident that no single biomarker has yet been shown to accurately distinguish between MM and RMCs. This is similar to the differentiation between MM and adenocarcinoma, in which a combination of at least two antibodies is recommended for accurate distinction. In this study, IMP3, GLUT1, and EMA were considered the best candidates among the tested biomarkers for differentiating between MM and RMCs. IMP3 and GLUT1 showed higher AUC values than other biomarkers and were mutually complementary. Use of these two antibodies will produce perfect sensitivity and specificity, especially if the cutoff value is set at 40% for IMP3. A widely positive reaction for either IMP3 or GLUT1 would strongly suggest that the lesion is MM, even when differences in immunostaining reactions in different laboratories are taken into account. On the other hand, a negative reaction with both these antibodies strongly favors the diagnosis of RMCs. When epithelioid MM is suspected, a strong, circumferential, membranous staining pattern of EMA is also a useful indicator of a diagnosis of MM.

We reported, for the first time, the results of an immunohistochemical study of CD146 in MM and RMCs. The immunohistochemical sensitivity of MM was lower (71%) than that of immunocytochemistry (94%).[7] Sato et al[7] used the clones OJ79 and EPR3208 in their study. Because OJ79 is indicated for use only for frozen sections, we at first tried to use EPR3208 and N1238 clones. In our investigation, both of these clones showed high specificities, but EPR3208 had a much lower sensitivity (data not shown). Thus, we used clone N1238 for the present study. The optimal methods and best clones to use for CD146 immunostaining of FFPE tissues remain to be fully elucidated.

CD147 is another novel biomarker that was recently described by Pinheiro et al[18]; we did not include CD147 in our study, but it may be worth investigating this biomarker in the future. CD147 is a chaperon of monocarboxylate transporters, which are responsible for transmembrane cotransport of lactate, also known as an extracellular matrix metalloproteinase inducer. In immunohistochemical analyses of effusions, CD147 was positive in eight of nine MM cases and one of 11 RMC cases and showed equal sensitivity and specificity with GLUT1.[18]

Fluorescence in situ hybridization (FISH) for analysis of the 9p21 deletion, the locus harboring the *p16/CDKN2A* gene, is an alternative diagnostic method to immunohistochemistry for the differentiation between MM and RMCs.[19] Studies using FISH have shown that homozygous deletion of 9p21 can be detected in 56% to 88% (mean 68%) of effusions, tissue microarrays, and whole sections of MM.[13,19-24] Although 9p21 deletion analysis by FISH is a specific method for detecting malignancy, the mean sensitivity of this method is not very high (68%). Takeda et al[25] reported that FISH analysis using multiple probes, including 9p21, 1p36, 14q32, 22q12, 5p15, 7p12, and 8q24, revealed at least one genomic abnormality in all cases (42/42) of MM but no abnormalities in 15 cases of benign mesothelial lesions. Although the results make the FISH with multiple probes an attractive diagnostic method, it is laborious and expensive to perform on a routine basis.

In conclusion, the distinction between MM and RMCs is based on the morphology of the sample, with unequivocal stromal invasion by mesothelial cells. However, a combination of immunohistochemical biomarkers, especially IMP3 and GLUT1, may be very powerful for distinguishing between MM and RMC when stromal invasion is difficult to assess. The use of immunohistochemical findings of CD146 as a biomarker in MM may need further investigation. These immunohistochemical studies to distinguish MM from benign mesothelial lesions may be used to identify potential candidates for molecular targeted therapy in patients with MM. Larger studies that involve multiple institutions will be necessary to confirm the validity of the current results, especially in diagnostically challenging cases.

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References

1. Robinson BW, Lake RA. Advances in malignant mesothelioma. *N Engl J Med*. 2005;353:1591-1603.
2. Ray M, Kindler HL. Malignant pleural mesothelioma: an update on biomarkers and treatment. *Chest*. 2009;136:888-896.

3. Cury PM, Butcher DN, Corrin B, et al. The use of histological and immunohistochemical markers to distinguish pleural malignant mesothelioma and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. *J Pathol.* 1999;189:251-257.
4. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. *Am J Surg Pathol.* 2000;24:1183-1200.
5. King J, Thatcher N, Pickering C, et al. Sensitivity and specificity of immunohistochemical antibodies used to distinguish between benign and malignant pleural disease: a systematic review of published reports. *Histopathology.* 2006;49:561-568.
6. Kato Y, Tsuta K, Seki K, et al. Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma. *Mod Pathol.* 2007;20:215-220.
7. Sato A, Torii I, Okamura Y, et al. Immunocytochemistry of CD146 is useful to discriminate between malignant pleural mesothelioma and reactive mesothelium. *Mod Pathol.* 2010;23:1458-1466.
8. Shi M, Fraire AE, Chu P, et al. Oncofetal protein IMP3, a new diagnostic biomarker to distinguish malignant mesothelioma from reactive mesothelial proliferation. *Am J Surg Pathol.* 2011;35:878-882.
9. Findeis-Hosey JJ, Xu H. The use of insulin like-growth factor II messenger RNA binding protein-3 in diagnostic pathology. *Hum Pathol.* 2011;42:303-314.
10. Hanley KZ, Facik MS, Bourne PA, et al. Utility of anti-L523S antibody in the diagnosis of benign and malignant serous effusions. *Cancer.* 2008;114:49-56.
11. Ikeda K, Tate G, Suzuki T, et al. Diagnostic usefulness of EMA, IMP3, and GLUT-1 for the immunocytochemical distinction of malignant cells from reactive mesothelial cells in effusion cytology using cytospin preparations. *Diagn Cytopathol.* 2011;39:395-401.
12. Olson AL, Pessin JE. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr.* 1996;16:235-256.
13. Monaco SE, Shuai Y, Bansal M, et al. The diagnostic utility of p16 FISH and GLUT-1 immunohistochemical analysis in mesothelial proliferations. *Am J Clin Pathol.* 2011;135:619-627.
14. Shen J, Pinkus GS, Deshpande V, et al. Usefulness of EMA, GLUT-1, and XIAP for the cytologic diagnosis of malignant mesothelioma in body cavity fluids. *Am J Clin Pathol.* 2009;131:516-523.
15. Bidlingmaier S, He J, Wang Y, et al. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. *Cancer Res.* 2009;69:1570-1577.
16. Shih IM, Nesbit M, Herlyn M, et al. A new Mel-CAM (CD146)–specific monoclonal antibody, MN-4, on paraffin-embedded tissue. *Mod Pathol.* 1998;11:1098-1106.
17. Beck JR, Shultz EK. The use of relative operating characteristic (ROC) curves in test performance evaluation. *Arch Pathol Lab Med* 1986;110:13-20.
18. Pinheiro C, Longatto-Filho A, Soares TR, et al. CD147 immunohistochemistry discriminates between reactive mesothelial cells and malignant mesothelioma. *Diagn Cytopathol.* 2012;40:478-483.
19. Onofre FB, Onofre AS, Pomjanski N, et al. 9p21 Deletion in the diagnosis of malignant mesothelioma in serous effusions additional to immunocytochemistry, DNA-ICM, and AgNOR analysis. *Cancer.* 2008;114:204-215.
20. Chiosea S, Krasinskas A, Cagle PT, et al. Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. *Mod Pathol.* 2008;21:742-747.
21. Takeda M, Kasai T, Enomoto Y, et al. 9p21 deletion in the diagnosis of malignant mesothelioma, using fluorescence in situ hybridization analysis. *Pathol Int.* 2010;60:395-399.
22. Chung CT, Santos Gda C, Hwang DM, et al. FISH assay development for the detection of p16/CDKN2A deletion in malignant pleural mesothelioma. *J Clin Pathol.* 2010;63:630-634.
23. Dacic S, Kothmaier H, Land S, et al. Prognostic significance of p16/cdkn2a loss in pleural malignant mesotheliomas. *Virchows Arch.* 2008;453:627-635.
24. Savic S, Franco N, Grilli B, et al. Fluorescence in situ hybridization in the definitive diagnosis of malignant mesothelioma in effusion cytology. *Chest.* 2010;138:137-144.
25. Takeda M, Kasai T, Enomoto Y, et al. Genomic gains and losses in malignant mesothelioma demonstrated by FISH analysis of paraffin-embedded tissues. *J Clin Pathol.* 2012;65:77-82.

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