Original Article

The Use of Immunohistochemistry to Distinguish Reactive Mesothelial Cells From Malignant Mesothelioma in Cytologic Effusions

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BACKGROUND: The distinction of benign from malignant mesothelial proliferations in cytologic specimens can be problematic. In this study, the authors investigated the utility of immunohistochemical (IHC) markers in making this distinction. **METHODS:** Archival paraffin-embedded cell blocks of pleural and peritoneal fluids from 52 patients with malignant meothelioma (MM) and 64 patients with reactive mesothelial hyperplasia (MH) were retrieved. IHC stains included desmin, epithelial membrane antigen (EMA), glucose-transport protein 1 (GLUT-1), Ki67, and p53. **RESULTS:** Desmin was positive in 84% (54 of 64) cases of reactive MH and in 6% (3 of 52) of MM cases ($P < .001$). EMA was positive in 9% (6 of 64) of benign and 100% (52 of 52) of malignant cases ($P < .001$). GLUT-1 was positive in 12% (5 of 43) of benign and 47% (7 of 15) of malignant cases. Ki67 showed strong nuclear positivity in >40% of mesothelial cells in 9% (6 of 64) of benign and 16% (8 of 49) of malignant cases ($P = .38$). p53 showed strong nuclear positivity in 2% (1 of 46) of benign and 47% (7 of 15) of malignant cases ($P < .001$). EMA positivity and desmin negativity were found in 2% (1 of 64) of reactive MH cases and 98% (49 of 52) of MM cases ($P < .001$). EMA negativity and desmin positivity were found in 86% (55 of 64) of reactive MH cases and 0% of MM cases. **CONCLUSIONS:** The combination of positive EMA and negative desmin strongly favors MM; conversely, a combination of negative EMA and positive desmin favors a reactive process. Likewise, strong membranous positivity for GLUT-1 and/or strong nuclear staining for p53 favors a mesothelioma. Ki67 proliferative index showed no significant difference between reactive MH and MM cases. ***Cancer (Cancer Cytopathol)* 2010;118:90–6.** © *2010 American Cancer Society.*

KEYWORDS: cytologic effusions, immunohistochemical markers, malignant mesothelioma, reactive mesothelial hyperplasia.

The distinction between reactive mesothelial hyperplasia (MH) and malignant mesothelioma (MM) may be very difficult based only on histologic and morphologic findings; of 217 cases circulated among all members of the US-Canadian Mesothelioma Reference Panel, there was some disagreement about whether the process was benign or malignant in 22% of cases.[1] Frank invasion is regarded as the most important diagnostic feature of malignancy in surgical excision specimens; however, this is not applicable to cytologic examination of effusions.[1] The cytologic features commonly used to identify malignancy, including nuclear pleomorphism, macronucleoli, large cellular aggregates, papillary-like tissue fragments, and cell-in-cell engulfment, are helpful features but have limited use in effusion, because they may also be present in florid reactive MH. It is well recognized that reactive MH can show various degrees of cytological atypia, and that the MM can show very bland cytologic features.[1,2]

Because of the difficulty in distinguishing reactive MH from MM even in tissue specimens, such as small pleural biopsies, several studies have used immunohistochemical markers to distinguish between reactive and neoplastic mesothelial cells[3-8] or between adenocarcinoma, reactive MH, and MM in serous effusions.[9-14] These studies suggest that 2 of the

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Table 1. Antibody Sources, Dilutions, and Fixation Conditions

Antibody	Source	Catalogue No.	Dilution	Fixation
Desmin	Dako	M0760	1:100	10% formalin
EMA	Dako	N1504	1:2	10% formalin
GLUT-1	Thermo Scientific	RB9052	1:200	10% formalin
p53	Vector Laboratories	VPp958	1:30	10% formalin
Ki67	Dako	M7240	1:100	10% formalin

EMA indicates epithelial membrane antigen; GLUT-1, glucose-transport protein 1.

most useful markers are desmin and epithelial membrane antigen (EMA); reactive mesothelial cells have been found to stain positive for desmin and negative for EMA, whereas MM cells have been shown to be negative for desmin and positive for EMA. However, these markers have diagnostic sensitivity and specificity of <90%.[8] In 1 study, glucose-transport protein 1 (GLUT-1), which is a member of the facilitative family of glucose transporters, was found to be positive in 100% of MMs and 0% of reactive mesothelium; however, GLUT-1 was also found to be positive in 96% of lung carcinomas as well.[15] Others have found that for diagnosis of MM in body cavity fluids, GLUT-1 monoclonal and polyclonal antibodies demonstrated a sensitivity of 40% and 67% and specificity of 95% and 74%, respectively, with a cutoff of ≥25% of the cells staining.[14] Some studies have suggested that the proliferation marker Ki67 may be useful in separating reactive mesothelial proliferation from MM.[16,17] The tumor suppressor gene p53 has also been found to be overexpressed more frequently in MM than reactive mesothelial proliferations, with a sensitivity ranging between 41% and 61% and a specificity of 91%.[6,8,18]

These studies of immunohistochemical markers have been mainly performed in tissue specimens, with only rare studies performed on effusion specimens. In the current study, we evaluate the utility of desmin, EMA, GLUT-1, p53, and Ki67 in differentiating reactive from MM cells in cytologic effusions and provide a comparative analysis of their performance.

MATERIALS AND METHODS

Archival paraffin-embedded cell blocks of pleural, peritoneal, and pericardial fluids were retrieved from the pathology files of the University of Michigan Hospital and University of California, San Diego Medical Center to obtain 52 cases of MM and 64 cases of reactive MH. Only cases with cellular cell blocks were selected. The cases of reactive MH were confirmed with review of the previous and/or current medical records.

The MM group of University of Michigan patients (n = 36) consisted of 24 men and 12 women with an age range of 43 to 85 years. All of these patients had confirmatory histologic diagnosis, and were dead of disease, as they were all collected over the years from either consult or legal cases (1992-2006). The MM group of University of California, San Diego patients (n = 16) consisted of 10 men and 6 women with an age range of 46 to 89 years collected from between 2001 and 2009. In this group, 2 cases had a benign diagnosis on the initial cytologic diagnosis, 1 case had a suspicious diagnosis, and 13 had a positive cytologic diagnosis of MM. Diagnosis of MM cases had been previously confirmed by a panel of immunohistochemical stains such as WT-1, calretinin, CK5/6, LeuM1, and B72.3. Confirmatory surgical biopsy was available in 8 cases. All of these patients had clinical and radiological findings consistent with mesothelioma. Clinical follow-up showed 4 of these patients were dead of disease, 6 were lost to follow-up, and 6 were alive with disease.

The corresponding cell blocks were cut 4 μm thick and stained with desmin (Dako, Carpentaria, Calif; 1:100 dilution), EMA (Dako; 1:2 dilution), GLUT-1 (rabbit polyclonal antibody, Thermo Scientific, Waltham, Mass; 1:200 dilution), p53 (Vector Laboratories, Burlingame, Calif; 1:30 dilution), and Ki67 proliferation index (Dako, 1:100 dilution) (Table 1). The incubation and pretreatment time were 30 minutes for all the immunostains. Appropriate positive and negative controls were included.

The results for desmin immunohistochemical stains were recorded as negative when no immunoreactivity was seen, focal/weak if <20% of cells were positive or showed only blush positivity, and positive if strong positivity was seen in ≥20% of cells. The results for EMA and GLUT-1 immunohistochemical staining were recorded as negative (no staining), focal/weak positive if there were a few (<20%) scattered cells that showed a membranous staining pattern or if there was only blush cytoplasmic staining

Table 2. Summary of Results of Immunohistochemical Stains

Stain	Mesothelioma	Reactive	Sensitivity	Specificity	*P* (Fisher Exact Test)
Desmin					
Negative	41	6			
Focal (<20%)	8	4			
Positive (≥20%)	3	54	84%	94%	<.001
Total number	52	64			
EMA					
Negative	0	43			
Focal (<20%) or weak	0	15			
Positive (≥20%)	52	6	100%	91%	<.001
Total number	52	64			
GLUT-1					
Negative to focal (<20%)	8	38			
Positive (≥20%)	7	5			
Total number	15	43	47%	88%	.008
Ki67 proliferative index					
Negative to low (<10%)	21	36			
Moderate (10%-39%)	20	22	57%	56%	.18
High (>40%)	8	6	16%	91%	.38
Total number	49	64			
p53					
Negative to focal (<10%)	8	45			
Positive (≥10%)	7	1			
Total number	15	46	47%	98%	<.001

EMA indicates epithelial membrane antigen; GLUT-1, glucose-transport protein 1.

but no membranous staining, and positive if there were ≥20% of mesothelial cells that showed strong membranous accentuation and cytoplasmic staining. For Ki67, the percentage of positive mesothelial cells was estimated in both benign and malignant specimens and categorized as negative (0% of nuclei staining), low (<10%), moderate (10%-40%), and high (>40%). For p53, results were recorded as negative if there was no staining or only blush nuclear staining, focal if there was strong nuclear staining in <10% of cells, and positive if there was strong nuclear staining in ≥10% of cells. For all immunohistochemical stains, the results were independently scored by at least 2 of the authors (F.H., G.Y.L., or C.W.M.), and any discrepant cases were reviewed at a double-headed microscope to achieve consensus.

Fisher exact test of statistical independence was used for statistical analysis of comparison of reactive MH and MM with immunohistochemical staining individually for desmin, EMA, GLUT-1, Ki67, or p53 as well as a combination of the stains. Fisher exact test was calculated using STATA IC10 software (StataCorp, College Station, Tex). All tests were 2-tailed, and a *P* value of <.05 was considered significant.

RESULTS

The summary of results of immunohistochemical stains used in this study is shown in Table 2. An example of a cell block from a case of reactive MH is shown in Figure 1A and of MM in Figure 1B.

For cases of reactive MH, desmin immunohistochemical staining was found to be positive (Fig. 1C) in 54 of 64 (84%) cases, focally positive in 4 of 64 cases (6%), and negative in 6 of 64 cases (9%). For MM cases, desmin was found to be positive in 3 of 52 (6%) cases, focally positive in 8 of 52 cases (15%), and negative (Fig. 1D) in 41 of 52 cases (79%). When using ≥20% of cells staining as the cutoff, desmin had a 84% sensitivity and 94.2% specificity for separating reactive from malignant mesothelium ($P < .001$).

EMA immunohistochemical staining performed on MM cases was positive in all 52 of 52 (100%) cases. Most cases showed strong membranous accentuation, with some cytoplasmic staining (Fig. 1F). Only 6 of 64 (9.4%) cases of reactive MH demonstrated strong membranous staining for EMA. An additional 15 of 64 reactive cases demonstrated weak or focal staining for EMA, frequently with a blush positive cytoplasmic pattern (Fig. 1E). When

using membranous staining in ≥20% of cells as a cutoff, it had a 100% sensitivity and 90.6% specificity ($P < .001$).

GLUT-1 was interpreted as positive when it showed a membranous staining pattern with or without cytoplasmic staining; however, red blood cells are normally positive for GLUT-1, rendering the interpretation of GLUT-1 in effusion cytology difficult, particularly when the specimen is contaminated by large amounts of blood. Seven of 15 (47%) MM cases were positive for GLUT-1 (Fig. 1H), whereas 5 of 43 (12%) reactive cases were positive. For GLUT-1 staining for MM, the sensitivity is 47%, and the specificity is 88% ($P = .008$).

The proliferation marker Ki67 immunohistochemical staining was used to determine the proliferative index. For MM cases, the proliferative index was high in 8 of 49 (16%) cases (Fig. 1J), moderate in 20 of 4 (41%) 9 cases, and negative to low in 21 of 49 (43%) cases. Cellular material was not sufficient to stain for Ki67 in 5 malignant cases. For reactive cases, the proliferative index was high in 6 of 64 (9%) cases, moderate in 22 of 64 (34%) cases, and negative to low in 36 of 64 (56%) cases (Fig. 1I). One difficulty in estimating the Ki67 labeling index in effusion specimens is that lymphocytes in the effusion were also frequently positive. When using moderate or greater (≥10%) proliferative index as the cutoff to be considered positive, Ki67 had 57% sensitivity and 56% specificity ($P = .18$). When only using high Ki67 proliferative index as a cutoff for a positive test, Ki67 had 16% sensitivity and 91% specificity ($P = .38$).

For MM, p53 immunohistochemical staining was strongly positive in 7 of 15 (47%) cases (Fig. 1L) and focal positive in 1 of 15 (7%) cases, whereas the remainder were negative. For reactive mesothelial cells, p53 immunohistochemical staining was positive in 1 of 46 (2%) cases and negative in 45 of 46 (98%) cases (Fig. 1K). When using strong positive p53 staining (≥10%), p53 had 47% sensitivity and 98% specificity ($P < .001$).

Cases were analyzed for combined immunoprofile of desmin and EMA (Table 3). For cases that were desmin positive and EMA negative, 100% (n = 55) were reactive

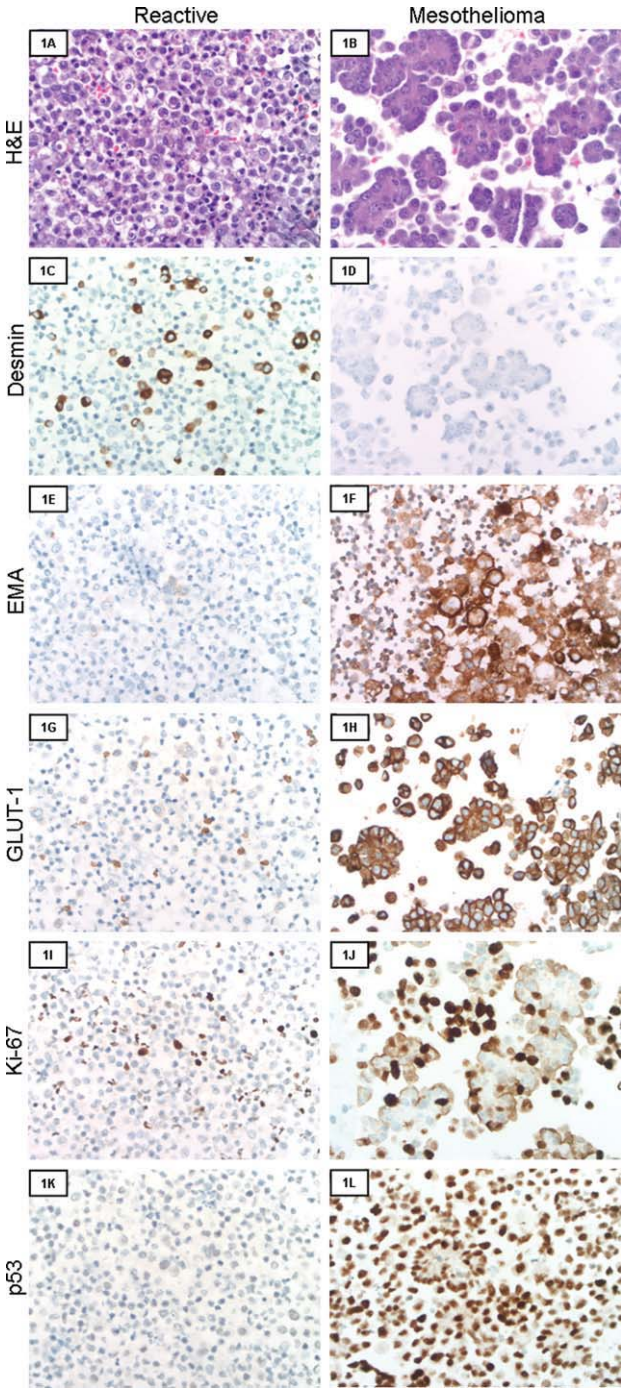

Figure 1. (A) An example of a cell block from a case of reactive mesothelial hyperplasia is shown (H & E). (B) An example of a cell block from a case of malignant mesothelioma is shown (H & E). (C) Desmin: reactive mesothelial cells show strong membranous positivity and cytoplasmic staining. (D) Desmin: malignant mesothelial cells show no to weak and focal staining. (E) Epithelial membrane antigen (EMA): reactive mesothelial cells show no immunoreactivity. (F) EMA: malignant mesothelial cells show strong membranous positivity and cytoplasmic staining. (G) Glucose-transport protein 1 (GLUT-1): reactive mesothelial cells show no immunoreactivity; the background positive cells are red blood cells. (H) GLUT-1: malignant mesothelial cells show strong membranous positivity with some cytoplasmic staining. (I) Ki67: reactive mesothelial cells show low to scattered positive cells. (J) Ki67: malignant mesothelial cells show strong and diffuse positivity. (K) p53: reactive mesothelial cells show negative to weak positivity. (L) p53: malignant mesothelial cells show strong nuclear positivity.

MH cases. For cases that were desmin negative and EMA positive, 98% were MM and 2% were reactive MH (n = 50). For cases that were both desmin and EMA negative, 100% (n = 3) were reactive MH. For cases that were both desmin and EMA positive, 37.5% were MM and 62.5% were reactive MH (n = 8). Comparison of the test results found a significant difference (Fisher exact chi-square of 104.46, $P < .0001$).

Cases were also analyzed for the combined immunoprofile of desmin, EMA, GLUT-1, and p53 (Table 4). Of cases that had the immunophenotype of desmin positive, EMA negative, GLUT-1 negative, and p53 negative, 100% (n = 34) were reactive MH. Of cases that were desmin positive, EMA negative, GLUT-1 positive, and p53 negative, 100% (n = 3) were reactive MH. Of cases that were desmin negative, EMA positive, GLUT-1 negative, and p53 negative, 100% (n = 4) were MM. Of cases that were desmin negative, EMA positive, GLUT-1 positive, and p53 negative, 80% (4 of 5 cases) were MM and 20% (1 of 5 cases) were reactive MH. Of cases that were desmin negative, EMA positive, GLUT-1 negative, and p53 positive, 100% (n = 4) were MM. Of cases that were desmin negative, EMA positive, GLUT-1 positive, and p53 positive, 100% (n = 3) were MM. Of cases that were desmin positive, EMA positive, GLUT-1 negative, and p53 negative, 100% (n = 4) were reactive MH. Of cases that were desmin positive, EMA positive, GLUT-1 positive, and p53 negative, 100% (n = 1) were reactive MH. No cases with the other possible combinations of stains were observed. The comparison of the test results were significantly different (Fisher exact chi-square of 53.83, $P < .0001$).

We attempted to perform statistical analysis to determine the best immunohistochemical panel to differentiate MM from reactive MH; however; our number of cases was insufficient.

Table 3. Analysis of Results of Combined Immunoprofile of Design and EMA

Desmin	EMA	Mesothelioma	Reactive
Negative	Negative	0	3 (100%)
Positive	Negative	0	55 (100%)
Negative	Positive	49 (98%)	1 (2%)
Positive	Positive	3 (37.5%)	5 (62.5%)
Total number		52	64

EMA indicates epithelial membrane antigen.

DISCUSSION

MM is a diagnostic dilemma for the cytopathologist and surgical pathologist because of the many morphologic similarities between neoplastic cells and their benign counterparts. Mesothelial cells frequently show florid reactive changes in response to many benign conditions such as pulmonary infarction, systemic disease (ie, collagen-vascular diseases), cirrhosis, radiation, underlying neoplasm, chronic inflammation, foreign substance, and infection. The common cytomorphologic features of mesothelial cells in reactive effusion include increase in the cellularity of a monomorphic cell population associated with papillary clusters. The cells are larger than quiescent mesothelium, with some prominence of nucleoli, regular chromatin pattern, and normal nuclear to cytoplasmic ratio.[19] Marked cytologic atypia can also be seen in hyperplastic or reactive mesothelium.[2] The common cytologic features of MM cells are nuclear pleomorphism, macronucleoli, large cellular aggregates, papillary-like tissue fragments, and cell-in-cell engulfment, but MM cells can also be deceptively bland and indistinguishable from benign mesothelial cells.[19] Because cytologic atypia is not a reliable factor, we have investigated a panel of immunohistochemical stains to make this distinction in cytologic effusions.

The intermediate filament protein desmin is a known marker for smooth and skeletal muscle differentiation.[20,21] Several studies have reported positive staining of benign mesothelial cells (reactive MH) in serous fluid and tissue sections for desmin.[3-5,8,9,20] The exact etiology for

Table 4. Analysis of Results of Combined Immunoprofile of Desmin, EMA, GLUT-1, and p53

Desmin	EMA	GLUT-1	p53	Mesothelioma	Reactive
Positive	Negative	Negative	Negative		34 (100%)
Positive	Negative	Positive	Negative		3 (100%)
Negative	Positive	Negative	Negative	4 (100%)	
Negative	Positive	Positive	Negative	4 (80%)	1 (20%)
Negative	Positive	Negative	Positive	4 (100%)	
Negative	Positive	Positive	Positive	3 (100%)	
Positive	Positive	Negative	Negative		4 (100%)
Positive	Positive	Positive	Negative		1 (100%)

EMA indicates epithelial membrane antigen; GLUT-1, glucose-transport protein 1.

expression of desmin in mesothelial cells is not known; however, the multipotential role of mesothelial cells with possible muscle differentiation and coexpression of desmin have been proposed by some studies.[2,9] Our study also confirmed cytoplasmic expression of desmin in reactive mesothelial cells in 84% of cases; in contrast, only 6% of MM cases showed expression for desmin. Our sensitivity of 84% and specificity of 94% in cytologic effusion specimens are similar to previously reported data collected primarily in tissue specimens. These findings suggest loss of muscle differentiation in MM cells. Our data showed that desmin alone is not completely a reliable marker to differentiate between a reactive and a malignant process, because 9% of our reactive MH cases were negative for desmin, and 6% of our MM cases were positive, with an additional 15% focally positive. However, it is possible that the focal desmin staining in the mesothelioma cases represented a residual population of non-neoplastic mesothelial cells.

EMA is a high molecular weight transmembranous glycosylated protein of the breast mucin complex, which is useful for epithelial differentiation and has been found to be present on both carcinoma and mesothelioma cells.[8,13,19] In mesothelioma cells, EMA staining is mainly seen on the cell surfaces, but in carcinoma cells, EMA stains the cytoplasm of carcinoma cells. Additional immunohistochemical stains should be used to differentiate mesothelial cells from carcinoma. It has been previously shown that EMA stains MM cells but not reactive mesothelial cells with sensitivities ranging from 58% to 100%.[6] All of our MM cases showed a membranous pattern of staining to EMA in >20% of cells. In the 6 cases of reactive MH that showed EMA positivity, the staining was focal and mainly expressed as a weak membranous pattern with blush cytoplasmic staining. Our findings are similar to previous studies, which were primarily performed in tissue specimens.[5,6,7,13]

GLUT-1 is a member of the mammalian facilitative glucose transporters, which passively transport glucose down a concentration gradient. GLUT-1 is expressed in normal tissues, including erythrocytes, renal tubules, and perineurium of peripheral nerves, but has also been found in numerous carcinomas, including carcinomas of the lungs.[15] GLUT-1 has been suggested to be a marker for malignancy, and it has been hypothesized that the increased expression of GLUT-1 helps maintain energy supplies in tumor cells to allow for tumor cell survival. We found that with a rabbit polyclonal antibody, a membrane staining pattern correlated with mesothelioma; however, the sensitivity was only 47%, and the specificity was 88%. These results are similar to those reported in body cavity fluids by Shen et al with both monoclonal and polyclonal antibodies, but have a much lower sensitivity and specificity than reported in tissue specimens by Kato et al.[14,15] The difference between our results, as well those of Shen et al, and those of Kato et al may be related to differences in the cells that have been exfoliated into body cavity fluids versus the cells that are invading into tissue.

Ki67 antibodies recognize a nuclear protein involved in the proliferative portion of the cell cycle.[20] Our data indicate that a high proliferation index (>40%) slightly favors MM, with a specificity of 91%; however, the sensitivity is only 16.7% and is not statistically significant ($P = .38$). There was also no significant difference if a proliferative index of $\geq$10% was used as the cutoff. Other studies performed on pleural biopsies have found that Ki67 labeling index with a cutoff of 9% labeling had a sensitivity of 88% and specificity of 92%.[17] Our results are similar to a prior study by Schonherr et al in effusion specimens, which used a cutoff of 26% and reported a sensitivity of 25% and a specificity of 100%.[16] It is possible that reactive mesothelial cells like any other reactive cells show higher proliferation index. The other possibility is contamination with many lymphocytes in the effusion specimens, which can cause difficulty in estimating the proliferative index by immunostain.

P53 is a 53-kDa protein product of a tumor suppressor gene that regulates cell growth and inhibits cells from entering S-phase. Mutations in p53 are common in malignancies, and mutations in p53 lead to a prolonged half-life and accumulation of high amounts of the protein. We found that p53 staining favors a diagnosis of mesothelioma, but the sensitivity is only 47%. This finding is similar to results from studies primarily performed in tissue specimens.[6,8,18]

Overall, the combination of positive EMA and negative desmin correctly identified 49 of 52 (94%) cases of MM, whereas the combination of negative EMA and positive desmin was seen in 55 of 64 (86%) cases of reactive MH. It is important to recognize that rare cases may strongly coexpress both markers, and that these findings should be interpreted in correlation with the clinical and radiological findings. Strong and diffuse positivity for GLUT-1 or p53 argues in favor of mesothelioma, whereas negative findings cannot exclude mesothelioma. Ki67 proliferative index shows no significant difference between reactive MH and MM.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferation. *Am J Surg Pathol.* 2000;24:1183-1200.
2. Bolen JW, Hammer SP, McNutt MA. Reactive and neoplastic serosal tissue. A light-microscopic, ultrastructural and immunocytochemical study. *Am J Surg Pathol.* 1986;10:34-47.
3. Scoones DJ, Richman PI. Expression of desmin and smooth muscle actin in mesothelial hyperplasia and mesothelioma. *J Pathol.* 1993;169S;188A.
4. Hurlimann J. Desmin and neural marker expression in mesothelial cells and mesotheliomas. *Hum Pathol.* 1994;25: 753-757.
5. Wolanski KD, Whitaker D, Shilkin KB, et al. The use of epithelial membrane antigen and silver-stained nucleolar organizer regions testing in the differential diagnosis of mesothelioma from benign reactive mesotheliosis. *Cancer.* 1998;82:583-590.
6. Attanoos, Griffin A, Gibbs AR. The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and BCL-2. *Histopathology.* 2003;43:231-238.
7. Cury PM, Butcher DN, Corrin B, et al. The use of histological and immunohistochemical markers to distinguish pleural malignant mesothelium and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. *J Pathol.* 1999;189:251-257.
8. King J, Thatcher N, Pickering C, Haselton P. 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.
9. Afify AM, Al-Khafaji BM, Paulino AFG, et al. Diagnostic use of muscle markers in the cytologic evaluation of serous fluids. *Appl Immunohistochem Mol Morphol.* 2002;10:178-182.
10. Ghosh AK, Gatter KC, Dunhills MS, et al. Immunohistochemical staining of reactive mesothelium, mesothelioma and lung adenocarcinoma with a panel of monoclonal antibodies. *J Clin Pathol.* 1987;40:19-25.
11. Duggan MA, Masters CB, Alexander F. Immunohistochemical differentiation of malignant mesothelioma, mesothelial hyperplasia and metastatic adenocarcinoma in serous effusions, utilizing staining for carcinoembryonic antigen, keratin and vimentin. *Acta Cytol.* 1987;31:807-814.
12. Bhalla R, Siddigui MT, Mandich D, et al. Diagnostic utility of D2–40 and podoplanin in effusion cell blocks. *Diagn Cytopathol.* 2007;35:342-347.
13. Walts AE, Said JW, Shintaku IP. Epithelial membrane antigen in the cytodiagnosis of effusions and aspirates: immunocytochemical and ultrastructural localization in benign and malignant cells. *Diagn Cytopathol.* 1987;3:41-49.
14. Shen J, Pinkus GS, Deshpande V, Cibas ES. 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. 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.
16. Schonherr A, Bayer M, Bocking A. Diagnostic and prognostic value of Ki67 proliferation fraction in serous effusions. *Cell Oncol.* 2004;26:57-62.
17. Taheri ZM, Mehrafza M, Mohammadi F, et al. The diagnostic value of Ki-67 and repp86 in distinguishing between benign and malignant mesothelial proliferations. *Arch Pathol Lab Med.* 2008;132:694-697.
18. O'Kane SL, Pound RJ, Campbell A, Chaudhuri N, Lind MJ, Cawkwell L. Expression of bcl-2 family members in malignant pleural mesothelioma. *Acta Oncol.* 2006;45:449-453.
19. Geisinger KR, Stanley MW, Raab S, Silverman J, Abati A. Modern Cytopathology. Philadelphia, PA: Churchill Livingston; 2004:257-280.
20. Dabbs D. Diagnostic immunohistochemistry. Philadelphia, PA: Elsevier; 2006.
21. Van Muijen G, Ruiter DJ, Warnaar SO. Coexpression of intermediate filament polypeptides in human fetal and adult tissues. *Lab Invest.* 1987;57:359-369.