Malignant Mesothelioma Diagnosis

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• ***Context.***—**Malignant mesothelioma is a relatively rare pleural tumor that may mimic benign mesothelial lesions and various other tumors including carcinomas and sarcomas. This makes the diagnosis challenging for the pathologist.**

Objective.—**To provide a brief but useful update on the immunohistochemical, cytogenetic, and molecular markers that are currently available for the diagnosis of malignant mesothelioma.**

Data Sources.—**Reference materials including peer-reviewed publications, text books, and consensus opinion reports among pathologists.**

Conclusions.—**It is important to correlate histologic findings on adequate biopsy samples with clinical and radiologic features. Useful diagnostic mesothelial markers include calretinin, WT-1, cytokeratin 5/6, and D2-40 (podoplanin). It is recommended that at least 2 mesothelial and 2 carcinoma markers with greater than 80% sensitivity and specificity be used for the diagnosis of mesothelioma when all clinical, radiologic, and histologic features are concordant. p16 deletion is reported in up to 70% of primary epithelioid and 90% to 100% of sarcomatoid pleural mesotheliomas. Presence of this homozygous gene deletion is so far the best indicator of mesothelioma. To date, this deletion has not been reported in any benign mesothelial lesion. The impact of various histologic patterns on the clinical and prognostic aspects of mesothelioma is addressed. The pleomorphic pattern, when present in more than 10% of tumor, translates into a highly aggressive behavior and is associated with poor survival. Recent studies have shown that the high-grade subgroup of deciduoid mesothelioma with pleomorphic histologic pattern also has a more aggressive clinical course. Nuclear grade (combination of nuclear atypia and mitotic count) may also prove to be an independent prognostic factor.**

(*Arch Pathol Lab Med.* 2015;139:978–980; doi: 10.5858/arpa.2013-0381-RA)

The diagnosis of malignant mesothelioma continues to be challenging. There are several aspects that have recently been addressed by consensus by pathologists with expertise in the subject.[1] Several recent articles[2–5] have provided much needed data on histologic variants and light microscopic features that are likely to be useful in providing prognostic reliable information. These are reviewed and summarized here.

DIFFERENTIATING BENIGN MESOTHELIAL PROLIFERATIONS FROM MALIGNANT MESOTHELIOMA

In a biopsy, which is first determined to be adequate, the histologic findings must be first correlated with clinical and radiologic features. The key indicator of malignancy remains invasion of preexisting tissue, particularly adipose tissue.[6–9] One must be careful not to mistake "fake fat," which is an artifact and is S100 negative (Figure 1, A and B), for real invasion into preexisting adipose tissue.[10] Two antibodies, glucose transporter 1 (GLUT-1) and insulin-like growth factor II messenger RNA–binding protein 3 (IMP3), have recently been shown to have utility in differentiating benign from malignant mesothelioma, independently from tissue invasion. GLUT-1 shows focal positivity in 67% of mesotheliomas and in 3% of reactive lesions, while IMP3 shows more diffuse positivity in 73% of mesotheliomas and negativity in reactive lesions.[1,11,12]

The most common genetic alteration in malignant mesothelioma is homozygous deletion of the 9p21 locus within a cluster of genes that includes *CDKN2A*, *CDKN2B*, and *MTAP*. p16/*CDKN2A* deletions are reported in up to 70% of primary epithelioid and 90% to 100% of sarcomatoid pleural mesotheliomas. The presence of this homozygous deletion is the best marker of malignancy in a mesothelial lesion, since it has not been reported so far in any of the benign lesions.[13] The fluorescence in situ hybridization (FISH) assay using a commercially available dual-color FISH probe (Abbott Molecular, Des Plains, Illinois) can be reliably performed on archival paraffin-embedded tissue and it is relatively less expensive than other molecular assays.

Immunohistochemistry for loss of p16 protein expression does correlate with p16 deletion but it can be positive in FISH-proven homozygous deletion and it can be negative in the absence of deletion. The staining pattern depends on the type of antibody, assay conditions, and interpretation criteria. Therefore, immunohistochemistry is not recommended as a surrogate method for detection of p16 deletion.[14]

HISTOLOGIC PATTERNS OF EPITHELIOID MALIGNANT MESOTHELIOMA

The microscopic features of various histologic patterns have been well described in the past. Recently, the clinical and

Accepted for publication January 24, 2014.

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The authors have no relevant financial interest in the products or companies described in this article.

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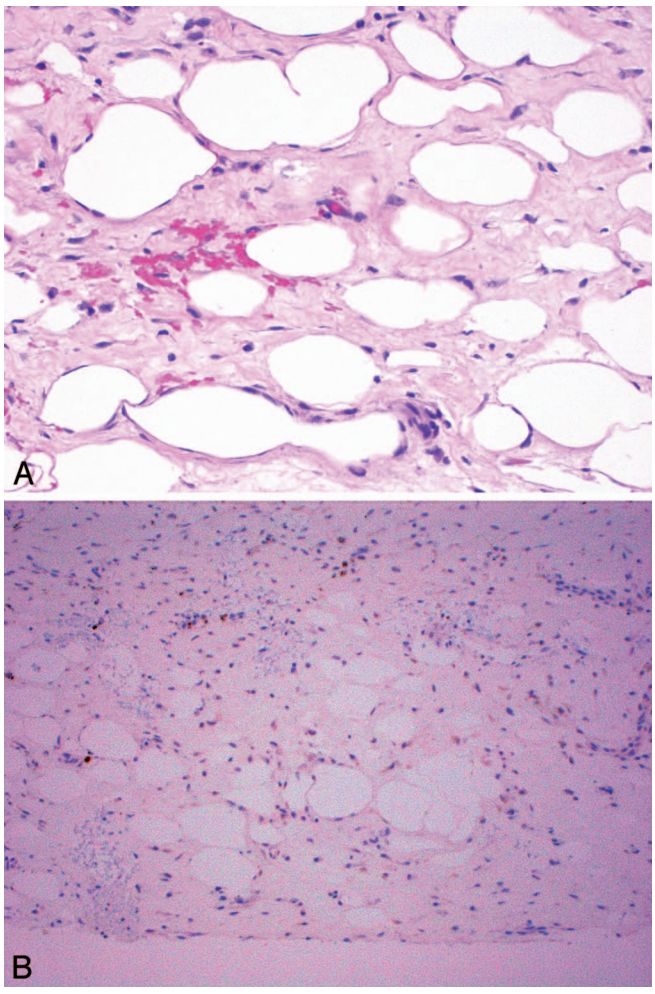

Figure 1. *"Fake fat" phenomenon: irregular airspaces due to pulling artifact in a case of pleural fibrosis in a 3-year-old child with fistula that is negative for S100. A, Hematoxylin-eosin. B, Immunohistochemistry for S100 (original magnification ×200 [A]; original magnification ×100 [B]).*

prognostic significance of many of these patterns have been demonstrated. Two studies have shown that the pleomorphic pattern (Figure 2), when present in more than 10% of tumor, portends a highly aggressive behavior and a poor survival similar to that of sarcomatoid mesotheliomas.[2,3] In one study of 232 patients,[2] the pleomorphic subtype was associated with the worst survival, followed by solid, micropapillary, tubulopapillary, and trabecular patterns. The micropapillary pattern was significantly associated with lymphatic invasion, which has also been demonstrated in other tumors such as lung carcinoma. A study of 21 deciduoid mesotheliomas[4] has shown that the high-grade subgroup (showing wide variation in size and shape of cells, frequent loss of cell cohesion, marked nuclear atypia, and mitotic activity >5 per 10 high-power fields) had a much more aggressive clinical course, when compared to those deciduoid tumors that were more cohesive, less pleomorphic, and had low mitotic activity (Figure 3, A and B).

PROPOSED NUCLEAR GRADING SYSTEM FOR EPITHELIOID MESOTHELIOMA

A study of 232 patients from a single institution has proposed a nuclear grading system for epithelioid meso-

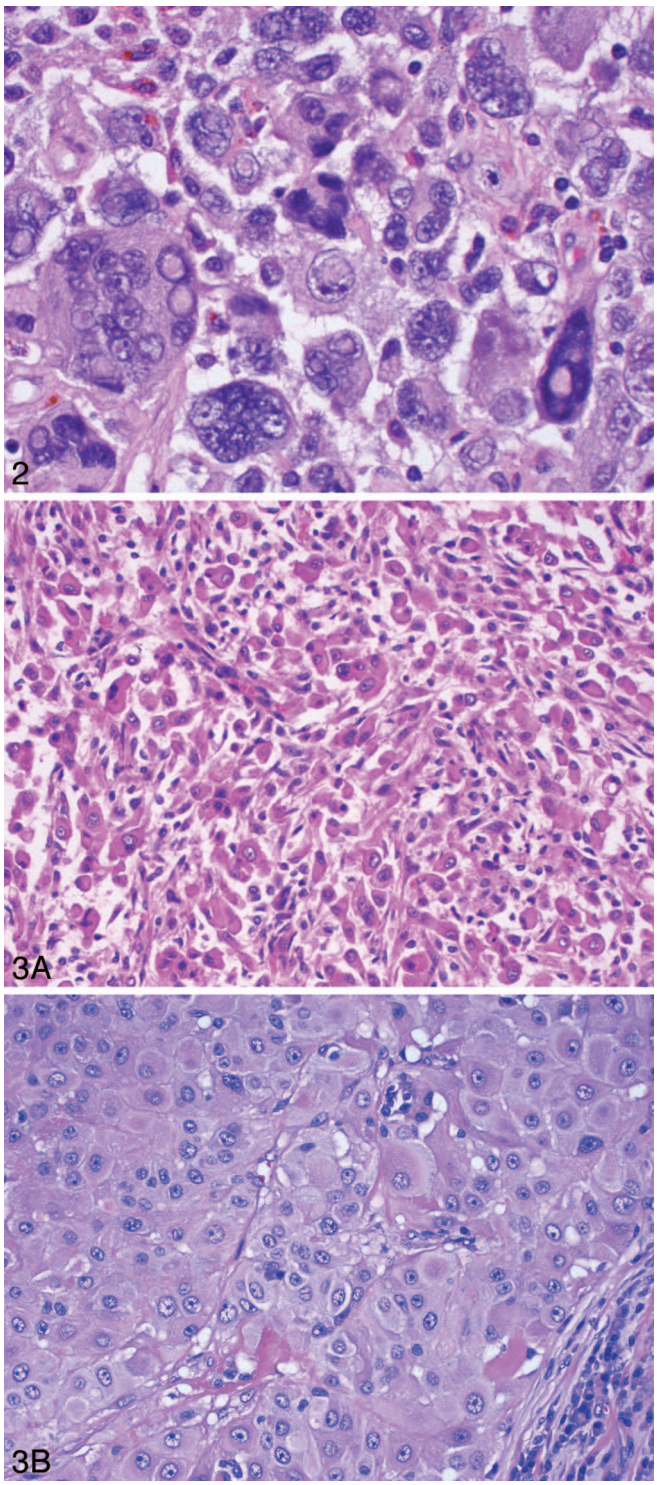

Figure 2. *Pleomorphic variant of epithelioid malignant mesothelioma. Note highly pleomorphic cells, many of which are multinucleated (hematoxylin-eosin, original magnification ×400).*

Figure 3. *Deciduoid malignant mesothelioma. A, High-grade subgroup with variation in cell size, loss of cell cohesion, and nuclear atypia. B, Low-grade subgroup with large cohesive cells and mild pleomorphism (hematoxylin-eosin, original magnification ×200 [A and B]).*

thelioma irrespective of histologic pattern.[5] Nuclear atypia and mitotic count proved to be independent prognostic factors and were used to create a 3-tier nuclear grade, which correlated with MIB-1 labeling index and was an independent predictor of overall survival. Further studies are needed to validate this grading system.

IMMUNOHISTOCHEMICAL PANELS TO DIAGNOSE MALIGNANT MESOTHELIOMA

All variants of epithelioid mesothelioma react with multiple antibodies used to diagnose mesothelioma. This has been verified recently in series of mesothelioma cases with rare histologic patterns, including small cell, signet ring, adenomatoid, clear cell, and with crystalloid structures.[15–19] There is still a wide variation among laboratories as to which antibodies are selected for testing. It is recommended that, when all clinical, radiologic, and histologic features are concordant, at least 2 mesothelial and 2 carcinoma markers with greater than 80% sensitivity and specificity be used. Of course, additional markers would be indicated when any of the above features are discordant. The most useful mesothelial markers are calretinin, WT-1, cytokeratin 5/6 (CK5/6), and D2-40 (podoplanin). The sarcomatoid component of biphasic tumors and pure sarcomatoid mesotheliomas may lose immunoreactivity for most markers in a majority of the cells; however, calretinin and D2-40 are more likely to remain immunoreactive. The most useful general carcinoma markers are MOC31, BG8, carcinoembryonic antigen, and BerEp4. The reader is referred to the consensus statement from the International Mesothelioma Interest Group[1] as well as another recent review[20] for further details.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis between malignant mesothelioma and metastatic carcinoma to the pleura varies with the histologic pattern of mesothelioma, which guides the selection of immunohistochemistry panel. For differentiating lung adenocarcinoma, thyroid transcription factor-1 (TTF-1) and napsinA are most useful; squamous cell carcinoma is p40 positive while WT-1 stains positively in malignant mesothelioma (note: squamous cell carcinoma is positive for calretinin and CK5/6). For renal cell carcinoma, PAX8 or PAX2 nuclear positivity, and for ovarian carcinoma, PAX8 nuclear positivity, differentiates them from malignant mesothelioma. CDX2 is a good nuclear marker for gastrointestinal carcinoma. Estrogen and progesterone receptors are helpful, if positive markers, in breast carcinoma. Genetic alterations such as deletions of 9p are some of the most frequent events in other nonmesothelioma tumor types including non–small cell carcinomas and sarcomas of the lung. Therefore, 9p deletions alone cannot be used to differentiate these neoplasms from mesothelioma.

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