Guidelines for Pathologic Diagnosis of Malignant Mesothelioma

2017 Update of the Consensus Statement From the International Mesothelioma Interest Group

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• ***Context.***—**Malignant mesothelioma (MM) is an uncommon tumor that can be difficult to diagnose.**

Objective.—**To provide updated, practical guidelines for the pathologic diagnosis of MM.**

Data Sources.—**Pathologists involved in the International Mesothelioma Interest Group and others with an interest and expertise in the field contributed to this update. Reference material included up-to-date, peer-reviewed publications and textbooks.**

Conclusions.—**There was discussion and consensus opinion regarding guidelines for (1) distinguishing benign from malignant mesothelial proliferations (both epithelioid and spindle cell lesions), (2) cytologic diagnosis of MM, (3) recognition of the key histologic features of pleural and peritoneal MM, (4) use of histochemical and immunohistochemical stains in the diagnosis and differential diagnosis of MM, (5) differentiating epithelioid MM from various carcinomas (lung, breast, ovarian, and colonic adenocarcinomas, and squamous cell and renal cell carcinomas), (6) diagnosis of sarcomatoid MM, (7) use of molecular markers in the diagnosis of MM, (8) electron microscopy in the diagnosis of MM, and (9) some caveats and pitfalls in the diagnosis of MM. Immunohistochemical panels are integral to the diagnosis of MM, but the exact makeup of panels employed is dependent on the differential diagnosis and on the antibodies available in a given laboratory. Depending on the morphology, immunohistochemical panels should contain both positive and negative markers for mesothelial differentiation and for lesions considered in the differential diagnosis. Immunohistochemical markers should have either sensitivity or specificity greater than 80% for the lesions in question. Interpretation of positivity generally should take into account the localization of the stain (eg, nuclear versus cytoplasmic) and the percentage of cells staining (>10% is suggested for cytoplasmic and membranous markers). Selected molecular markers are now being used to distinguish benign from malignant mesothelial proliferations. These guidelines are meant to be a practical diagnostic reference for the pathologist; however, some new pathologic predictors of prognosis and response to therapy are also included.**

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The pathologic diagnosis of malignant mesothelioma (MM) continues to evolve and be refined as more antibodies and molecular tests become available for general use. This is especially applicable to distinguishing benign from malignant mesothelial proliferations, for which im-

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munohistochemistry (IHC) has largely been replaced by tests based on the analysis of molecular alterations in mesothelioma. These methods can be used in both tissue and cytologic specimens. The previous guidelines[1,2] have now been updated with the addition of these new techniques. The basic morphologic description of MM is not repeated here; however, some of the features and subtypes that have recently been shown to have prognostic or clinical significance are highlighted. The IHC panels have been updated to include newer antibodies, such as claudin 4. There is some repetition with the 2013 guidelines, but we thought it was important that the reader not have to go back and forth to prior guidelines to determine what antibodies remain useful. New sections on prognostic factors and staging have been added. As in the past, this review focuses on practical, diagnostic guidelines that are meant to be a reference for the pathologist, rather than a mandate or comprehensive, in-depth review of the literature.

GENERAL RECOMMENDATIONS

The diagnosis of MM should always be based on the results obtained from an adequate biopsy (less commonly, an exfoliative or fine-needle aspiration cytology evaluation) in the context of appropriate clinical, radiologic, and surgical findings. A history of asbestos exposure should not be taken into consideration by the pathologist when confirming or excluding MM. Location of the tumor (pleural versus peritoneal), as well as the sex of the patient will affect the differential diagnosis, as discussed below. The histologic diagnosis of MM should be based on both the appropriate morphology and on appropriate immunohistochemical findings. Specific information on antibody clones and their sources should be obtained from the current literature because that is an evolving area and is outside of the scope of this article. Molecular testing is now more widely available and is diagnostically helpful in selected cases.

BENIGN VERSUS MALIGNANT MESOTHELIAL CELL PROLIFERATIONS

Separating benign from malignant mesothelial proliferations presupposes first that the process has been recognized as mesothelial (which may mean using "mesothelial markers," as discussed below). The diagnostic approach used when distinguishing reactive mesothelial hyperplasia from epithelioid mesothelioma is different from that used when distinguishing fibrous pleuritis from desmoplastic mesothelioma.[3] The major problem areas are discussed below.

Reactive Mesothelial Hyperplasia Versus Epithelioid MM

It is well known that reactive mesothelial proliferations may mimic mesothelioma (or metastatic carcinoma) because reactive mesothelial proliferations may show high cellularity, numerous mitotic figures, cytologic atypia, necrosis, formation of papillary groups, and entrapment of mesothelial cells within fibrosis mimicking invasion (Figure 1). Morphologic features that help in distinguishing reactive, mesothelial hyperplasia from mesothelioma are summarized in Table 1.

The demonstration of tissue invasion (eg, visceral pleural/lung, parietal pleura/chest wall, among others) is a key feature in the diagnosis of MM (Figure 2). Invasion may be highlighted with immunostains, such as pancytokeratin or calretinin. Invasion by mesothelioma is often subtle and may be into only a few layers of collagenous tissue below the mesothelial space and lacking a desmoplastic reaction. When a substantial amount of solid, malignant tumor with histologic features of MM (ie, a tumor mass) is identified, the presence of invasion is not required for diagnosis.

Although certain immunohistochemical stains are more likely to be positive in benign proliferations and others in malignant proliferations, those cannot be solely relied upon in the diagnosis of individual cases. As reviewed recently by Churg et al,[4] staining for p53, desmin, epithelial membrane antigen, glucose transporter 1, and U3 small nucleolar ribonucleoprotein protein (IMP-3) may be useful statistically in separating benign from malignant lesions but are not useful in an individual case. In several recent studies to date, the finding of homozygous deletion of p16 by fluorescent in situ hybridization (FISH) or the loss of BRCA1 associated protein 1 (BAP1) by IHC is found only in mesotheliomas (but not in all mesotheliomas) (Figure 3, A and B).[4–9] We consider these 2 techniques, which can be used together, very useful. These techniques have different efficacies in different locations, and that needs to be considered before selecting a test. Most peritoneal epithelial mesotheliomas do not show a loss of *p16* by FISH, but many show loss of BAP1 by IHC. Conversely, loss of BAP1 is very uncommon in sarcomatous and desmoplastic mesotheliomas at any site.

Fibrous Pleurisy Versus Desmoplastic Variant of Sarcomatoid MM

The identification of features of malignancy in a desmoplastic mesothelioma requires adequate tissue, and large surgical biopsies are generally (but not always) needed. Features to separate fibrous pleurisy from desmoplastic mesothelioma are shown in Table 2. Stromal invasion is often more difficult to recognize in spindle cell proliferations of the pleura than they are in epithelioid proliferations. The invasive malignant cells are often deceptively bland, resembling fibroblasts, and pancytokeratin staining (as opposed to the usual mesothelial markers used in assessing epithelioid proliferations) is invaluable in highlighting the presence of cytokeratin-positive malignant cells in regions in which they would not normally be present: adipose tissue or skeletal muscle deep to the parietal pleura or invading the visceral pleura/lung tissue (or other extrapleural structures present) (Figure 4, A and B).

Reactive fibrous pleurisy tends to show a uniformity of growth, and that can also be highlighted with pancytokeratin staining, which shows regular sheets and sweeping parallel fascicles of bland spindle cells that respect mesothelial boundaries in contrast to the disorganized growth and haphazardly intersecting proliferations seen in desmoplastic/sarcomatoid mesotheliomas. Another helpful clue in desmoplastic MM is the presence of expansile nodules of varying sizes with abrupt demarcation and changes in cellularity between nodules and their surrounding tissue.

Although identification of invasion into adjacent tissues is often straightforward with the aid of pancytokeratin staining, Churg et al[10] have pointed out that fatlike spaces ("fake fat") may be encountered in some cases of organizing pleuritis, probably as a result of artifactual changes in the dense, fibrous connective tissue (Figure 5, A and B). In those regions, horizontally oriented, cytokeratin-positive cells may be encountered around the fatlike spaces (Figure 6). In addition, S100 protein, laminin, and collagen IV are usually positive in true adipose tissue and can help in distinguishing

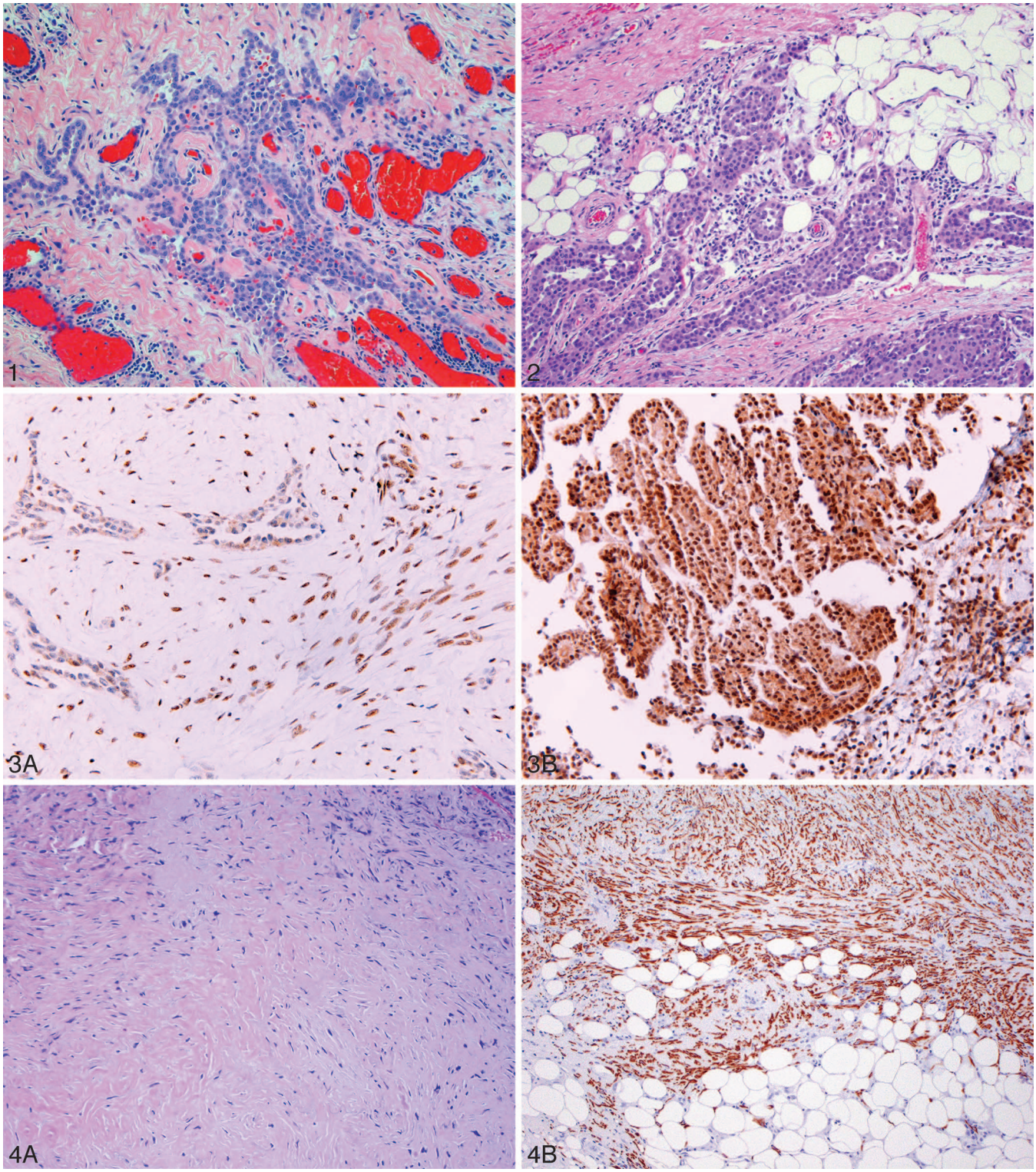


Figure 1. *Reactive mesothelial hyperplasia within fibrous tissue mimicking invasion (hematoxylin-eosin, original magnification ×100).*

Figure 2. *Epithelioid malignant mesothelioma invading fat (hematoxylin-eosin, original magnification ×100).*

Figure 3. *A and B, BAP1 immunohistochemical staining in malignant mesothelioma (MM). A, Nuclear BAP1 staining is lost in this epithelioid MM (cytoplasmic staining is nonspecific). Note that adjacent stromal cells have normal nuclear staining. B, BAP1 nuclear staining is retained in this MM, which is not helpful in making the diagnosis (original magnification ×200 [A and B]).*

Figure 4. *A and B, Desmoplastic mesothelioma. A, Proliferation of bland-appearing spindle cells with haphazard growth pattern. B, Keratin staining highlights infiltration into fat (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×100 [B]).*

Table 1. Reactive Mesothelial Hyperplasia Versus Mesothelioma	
Mesothelial Hyperplasia	**Mesothelioma**
• Absence of stromal invasion (beware of entrapment and en face cuts)	• Stromal invasion usually apparent (highlight with pancytokeratin staining)
• Cellularity may be prominent but is confined to the mesothelial surface/pleural space and is not in the stroma	• Dense cellularity, including cells surrounded by stroma
• Simple papillae; single cell layers	• Complex papillae; tubules and cellular stratification
• Loose sheets of cells without stroma	• Cells surrounded by stroma ("bulky tumor" may involve the mesothelial space without obvious invasion)
• Necrosis rare	• Necrosis present (occasionally)
• Inflammation common	• Inflammation usually minimal
• Uniform growth (highlighted with cytokeratin staining)	• Expansile nodules; disorganized growth (highlighted on cytokeratin staining)
Usually Not Useful	
• Mitotic activity • Mild to moderate cytologic atypia	

it from fake fat, which is negative for all 3 (Figure 7, A through F).

CYTOLOGICAL DIAGNOSIS OF MM

Mesotheliomas often present with recurrent serous effusions that are submitted for cytologic evaluation. Even though the cytologic features of MM were described more than 50 years ago and have been further refined in numerous subsequent research, establishing a definitive diagnosis of MM by cytologic examination alone remains controversial.[11,12] The published sensitivity of cytology for the diagnosis of mesothelioma ranges from 30% to 75%.[13] That broad range of sensitivity (high false-negative rate) is probably related to sampling, rather than interpretation, but one has to acknowledge that there is a broad overlap in atypical features and in immunoreactivity across benign reactive and malignant mesothelial cell proliferations. Many of the cytologic features (scalloped borders of cell clumps; intercellular windows with lighter, dense cytoplasm edges; and low nuclear to cytoplasmic ratios) are shared between reactive and malignant epithelioid mesothelial cells. Usually the malignant cells in sarcomatoid MM are not shed into the effusion fluid, which may only contain the overlying reactive epithelioid mesothelial cells that may mislead the pathologist. Inability to assess invasion of preexisting tissue (not granulation tissue)—one of the key histologic diagnostic features of MM—in exfoliative cytology specimens further hinders definitive cytologic diagnosis and underscores the importance of close correlation with clinical and imaging findings.

Similar to histologic specimens (as discussed in other sections of this article), application of immunocytochemical and molecular techniques, either on smears or on cell blocks, substantially increases diagnostic accuracy.[14–18] Similar to tissue specimens, FISH that demonstrates homozygous deletion of *p16* is particularly useful in cytologic specimens, as well as in cases in which the differential diagnosis is MM versus reactive mesothelial cells.[19–21] Loss of BAP1 expression by immunocytochemistry is also a useful adjunct to distinguish MM from reactive mesothelial proliferations.[22]

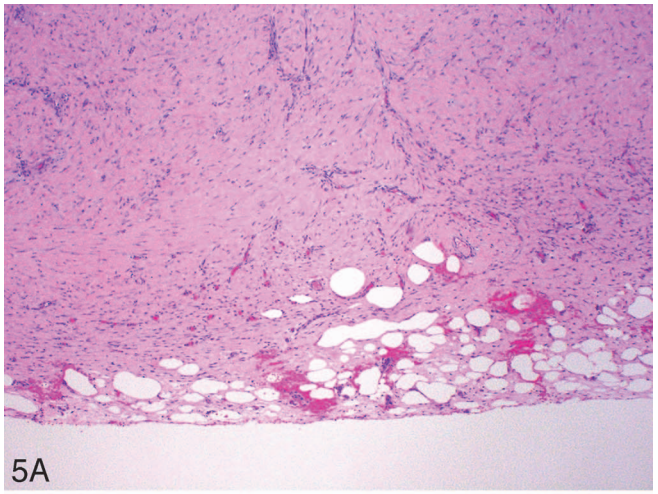
Emerging data that indicate subtyping of epithelioid MM according to morphologic features and nuclear grade[21] are important to predicting survival and suggest that a cytologic diagnosis of *malignant mesothelioma epithelioid type* might not be sufficient in the future.

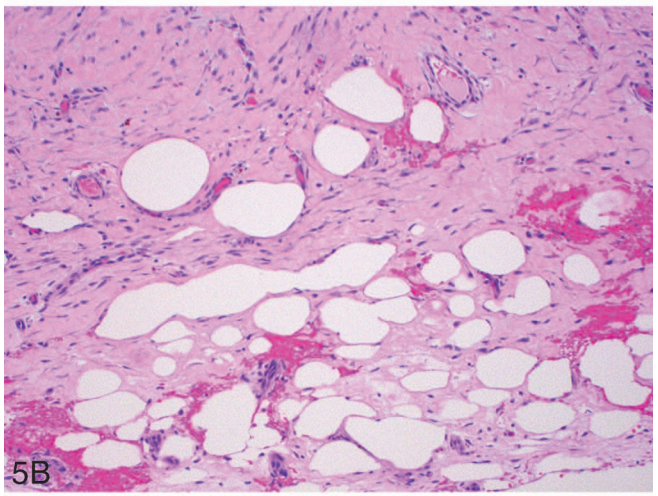
Interestingly, not all mesotheliomas readily exfoliate tumor cells; hence, sarcomatoid mesotheliomas are rarely diagnosed on effusion cytology. In such cases, fine-needle aspiration, combined with core biopsy (or larger tissue samples), are necessary to establish the diagnosis. Diagnostic difficulties and the frequent litigation in cases of MM continue to make pathologists reluctant to diagnose mesothelioma in the absence of histologic confirmation.

The differential diagnosis and use of IHC and molecular markers in cytologic specimens is similar to that in tissue sections (see above and below). Claims continue to be published that positive staining for epithelial membrane antigen, p53, IMP-3, CD146, or glucose transporter 1 can be used to define a cytology specimen as malignant.[23] As is true

Table 2. Fibrous Pleurisy Versus Desmoplastic Mesothelioma[a]	
Fibrous Pleurisy	**Desmoplastic Mesothelioma**
• Storiform pattern not prominent	• Storiform pattern often prominent
• Absence of stromal invasion	• Stromal invasion present (highlight with pancytokeratin staining)
• Necrosis, if present, is at the surface epithelioid mesothelial cells (where there is often associated acute inflammation)	• Bland necrosis of paucicellular, collagenized tissue
• Uniform thickness of the process	• Disorganized growth, with uneven thickness, expansile nodules, and abrupt changes in cellularity
• Hypercellularity at the surface with maturation and decreased cellularity deep (so-called zonation)	• Lack of maturation from the surface to the depths of the process
• Perpendicularly oriented vessels	• Paucity of vessels, without orientation
Usually Not Useful	
• Cellularity • Atypia (unless severe) • Mitotic activity unless numerous atypical mitotic figures	

[a] Data derived from Mangano et al,[135] 1998.





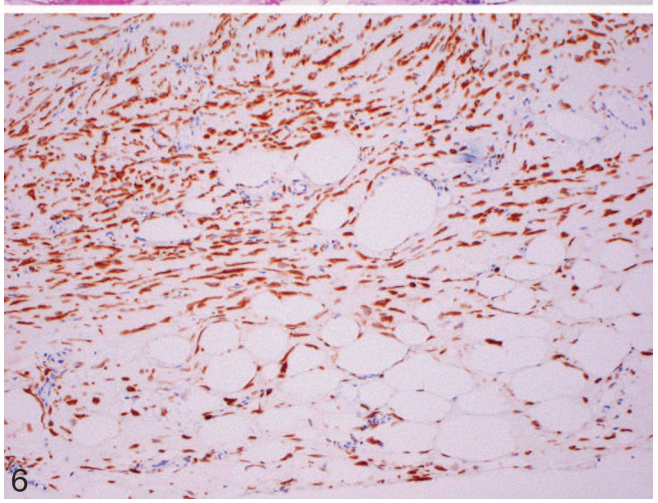


Figure 5. *A and B, Fake fat in a pleural biopsy from a patient with effusion and fibrosis (hematoxylin-eosin, original magnifications ×40 [A] and ×100 [B]).*

Figure 6. *Stain for keratin AE1/AE3 showing horizontal, keratin-positive, reactive spindle cells around fake fat (see Figure 4, B, for comparison with adipose tissue) (original magnification ×100).*

of tissue biopsies, in our view, those markers provide no more than statistical differences between benign and malignant cases and should not be used to diagnose individual patients.

HISTOLOGIC FEATURES OF MM

Most MMs are readily identified or strongly suspected on routine hematoxylin-eosin staining where they exhibit 3 major histologic subtypes, divided into epithelioid, sarcomatoid, or mixed (biphasic) categories in the updated 2015 World Health Organization classification.[24] Good interobserver variation has been reported in distinguishing these subtypes.[25] Multiple patterns have been described within these subtypes, some of which have been shown to correlate with overall survival (see below). The recognition of the various histopathologic patterns is also helpful diagnostically and will guide the differential diagnosis and selection of appropriate markers. However, most mesotheliomas have several patterns, and a biopsy may not be representative of the whole tumor. Thus, the pattern may be included as a comment or in the microscopic description, but the major histologic subtype must be given in the final diagnosis.

Epithelioid MMs are composed of polygonal, oval, or cuboidal cells that often mimic nonneoplastic, reactive mesothelial cells. Sarcomatoid MMs usually consist of spindle cells but can be composed of lymphohistiocytoid cells and/or contain heterologous rhabdomyosarcomatous, osteosarcomatous, or chondrosarcomatous elements.[26,27] Biphasic MMs contain both epithelioid and sarcomatoid areas within the same tumor.[24,28–31] Sarcomatoid areas may sometimes be difficult to distinguish from reactive stroma, in which case concordant BAP-1 loss is helpful in reaching a diagnosis (see Immunohistochemical Staining in MM section below).

The most frequent histologic type of MM is epithelioid. The common secondary growth patterns of epithelioid MM are readily recognized by most pathologists: tubulopapillary, acinar (glandular), adenomatoid (also termed *microglandular*), and solid. Psammoma bodies may be present in any of the patterns. Some epithelioid MMs have a distinctive feature consisting of clusters of tumor cells floating in pools of hyaluronic acid. Less commonly, tumor cells may be clear, deciduoid, signet ring, small cells, or rhabdoid cells or may have an adenoid cystic pattern.[31] Of note, a micropapillary pattern (without central fibrovascular core) should be classified as something other than *tubulopapillary* because a micropapillary pattern correlates with a higher incidence of lymphatic invasion.[32] In addition, tubulopapillary epithelioid mesotheliomas require distinction from well-differentiated papillary mesotheliomas (WDPMs), which are classified as a separate subtype in the 2015 World Health Organization classification,[24] although WDPMs can (rarely) show invasive foci.[33] Recently, epithelioid mesotheliomas with marked nuclear pleomorphism in more than 10% of the tumor have been shown to behave like sarcomatoid and biphasic variants, with a proposal that a "pleomorphic" MM variant be recognized as an adversely prognostic epithelioid pattern.[34,35] Similarly, deciduoid MM with pleomorphism is associated with more aggressive behavior.[36] The differential diagnosis for lymphohistiocytoid pattern, classified as *epithelioid*, includes nonneoplastic inflammatory process, non–Hodgkin lymphoma, and Hodgkin lymphoma.[37,38]

Secondary patterns of sarcomatoid MM may demonstrate anaplastic and giant cells with a differential diagnosis of high-grade sarcoma, osteosarcomatous areas with a differential diagnosis of osteosarcoma, or chondrosarcomatous areas with a differential diagnosis of chondrosarcoma.[39–41]

A paucicellular distribution of bland, neoplastic spindle cells between bands of dense collagenous stroma that resemble a pleural plaque is the distinguishing feature of desmoplastic MM. This type of MM may not be suspected unless frankly sarcomatoid areas of the tumor are found.

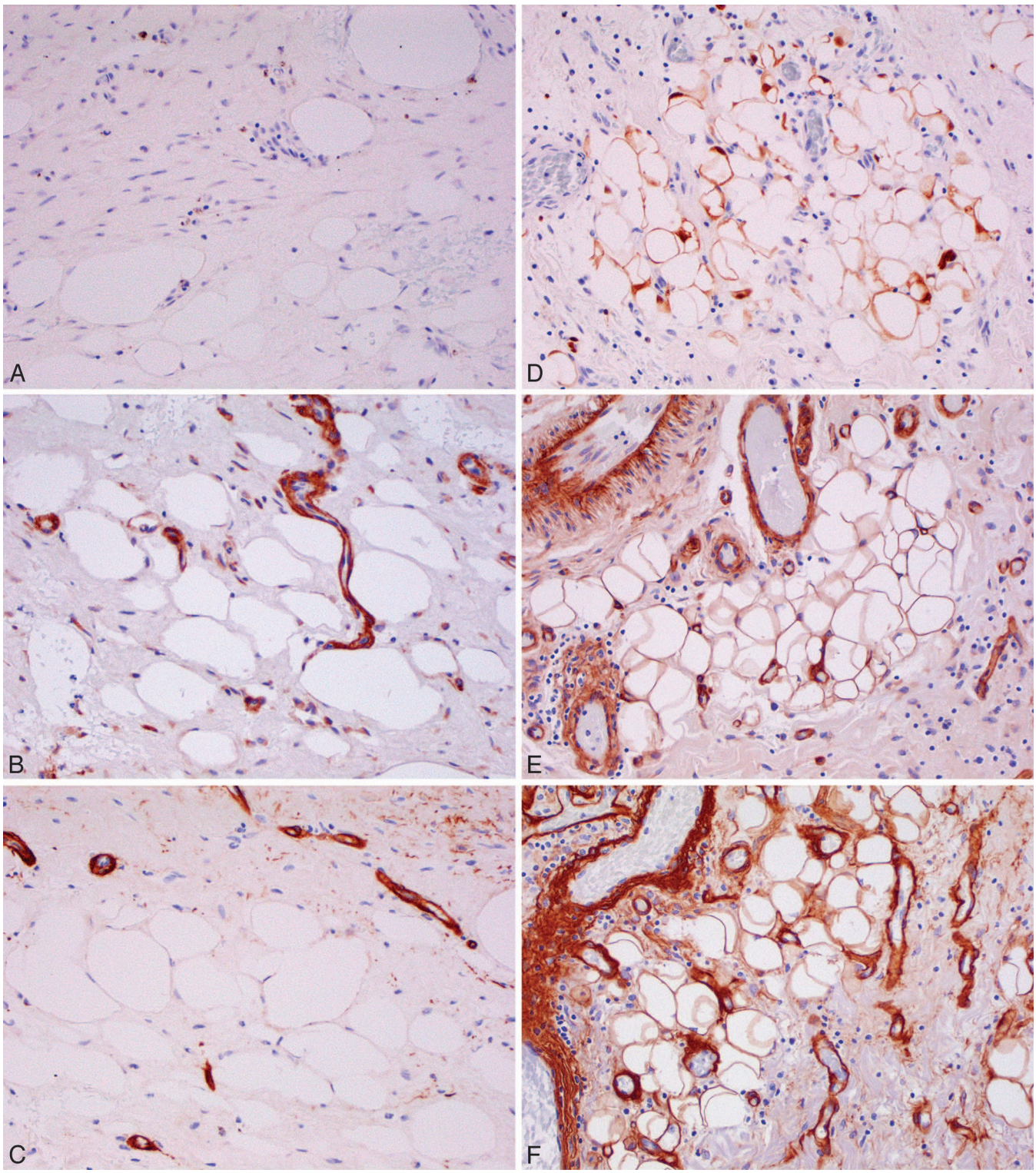


Figure 7. *A through F, S100-, laminin-, collagen IV-negative cells, respectively, in fake fat (A through C), and S100-, laminin-, collagen IV-positive cells, respectively, in true fat (D through F) (S100, original magnification ×200 [A and D]; laminin, original magnification ×200 [B and E]; collagen IV, original magnification ×200 [C and F]).*

Heterologous differentiation within a mesothelioma is a rare, but well-established, feature that occurs more frequently in sarcomatoid variants, although it can also be seen with biphasic and epithelioid morphologies, most commonly taking the form of osteosarcomatous or chondrosarcomatous elements, although rarely, rhabdomyosarcomatous or angiosarcomatous elements may be present.[42,43]

GRADING AND PROGNOSTIC MARKERS IN MALIGNANT MESOTHELIOMAS

Although histologic grading has not traditionally been performed, a recent study of resected epithelioid MM showed that a 3-tiered nuclear grading score based on mitotic activity and nuclear atypia was strongly predictive of

survival.[25,44] In one study,[45] tumoral CD10 expression correlated with aggressive histologic types and higher mitotic activity and was an independent prognostic factor for patients with malignant pleural mesothelioma.

DIFFERENTIAL DIAGNOSIS OF MM ACCORDING TO THE HISTOLOGIC SUBTYPE

In general, the differential diagnosis for MM depends on its basic histologic category. Epithelioid MM needs to be distinguished from carcinomas and other epithelioid cancers, whereas the differential diagnosis for sarcomatoid MM includes sarcomas and other spindle cell neoplasms, and the differential diagnosis of mixed MM includes mixed or biphasic tumors, such as synovial sarcoma and metastatic sarcomatoid/pleomorphic carcinoma of lung. Tubulopapillary epithelioid mesotheliomas require distinction from WDPMs, which are classified as a separate subtype in the 2015 World Health Organization classification,[24] although WDPMs can (rarely) show invasive foci.[33] Solid, well-differentiated MM needs to be distinguished from reactive mesothelial hyperplasia, solid adenocarcinoma, and even squamous cell carcinoma because of the abundant pink cytoplasm. Solid, poorly differentiated MM needs to be distinguished from lymphomas and poorly differentiated carcinomas. Clear cell MM needs to be differentiated from clear cell renal cell carcinomas, clear cell carcinomas of the lung, clear cell melanoma, and other clear cell tumors that can metastasize to the pleura.[46–49] Signet-ring cell mesotheliomas need to be distinguished from signet-ring cell adenocarcinomas of the lung and metastatic carcinomas of the gastrointestinal tract with signet-ring cell features.[50] Small cell mesotheliomas need to be distinguished from small cell carcinomas of the lung, desmoplastic small round cell tumors, lymphomas, and other tumors with small cell morphology.[51] Desmoplastic mesotheliomas may mimic fibrous pleuritis. Because each broad histologic category has its own distinctive differential diagnosis, the immunostains selected for further workup of a patient with MM are dictated by the tumor's histologic category.[52]

MORPHOLOGIC FEATURES RELATED TO PERITONEAL MM

The morphology of peritoneal MM (PMM) is similar to that of pleural MM with epithelioid and sarcomatoid types, with the epithelioid type including the common tubulopapillary/papillary and solid histologies. In the peritoneum, however, several site-specific issues are recognized.

Histologic Subtypes

Although epithelioid and sarcomatoid types can be seen in PMM, the incidence of biphasic tumors is lower than in pleural disease, and pure sarcomatoid tumors are very rare.[53,54] As in pleural MM, the biphasic and sarcomatoid subgroups have a significantly poorer prognosis and are less amenable to treatment overall.[55,56] A minimum of 10% spindled growth has been proposed for a pleural MM to be designated *biphasic*, but the less-common occurrence of biphasic histology and the distinctly poorer prognosis of patients with that subtype of PMM may make a minimum value less practical. It remains unclear whether identification of any component of malignant spindled histology portends a poor prognosis in PMM.[57]

Benign, Multicystic Mesothelioma

Benign multicystic mesothelioma is composed of multiple mesothelial-lined cysts and represents a rare but well-described entity that may enter the differential diagnosis of mesothelial neoplasia. This lesion is nearly always encountered in the peritoneum, although rare cases with pleural involvement have been described.[58] These cystic proliferations are lined by bland mesothelial cells and lack significant stratification, papillation, or atypia. If defined in this fashion, this process does not metastasize, but it can recur.[59]

Well-Differentiated Papillary Mesothelioma

The WDPM type is also an important subgroup that is encountered much more frequently in the peritoneum than it is in the pleura. These generally noninvasive papillary neoplasms are lined by bland mesothelial cells with low-grade nuclei. The nuclei are small, smooth-contoured, and do not contain nucleoli. Mitoses are rarely present. In a recent series[60] of WDPM in women, 1 of 26 patients (4%) had recurrent disease, and none died of disease-related causes. No association with asbestos exposure was identified. The largest tumor in that series was 2.0 cm; however, many cases were multifocal. Setting a size limit to be used in this diagnosis was proposed, but it is clear that bona fide cases can exceed 2.0 cm. A recent article[33] reported 20 cases of WDPM with invasive foci in the papillae, and the authors concluded that those cases appeared to be prone to multifocality and recurrence but that they rarely gave rise to life-threatening disease. It is acknowledged that bulky disease is one feature against WDPM diagnosis. In summary, when narrowly defined by morphologic criteria, WDPM has an excellent prognosis, although recurrent disease can occur. Because the natural history of this subgroup is distinct from PMM, it is an important morphologic distinction from architecturally similar, but more-aggressive, papillary epithelioid MM.[61,62]

HISTOCHEMICAL STAINING IN MM

The cytoplasmic vacuoles in adenocarcinomas frequently contain epithelial mucin, highlighted by periodic acid–Schiff after digestion and mucicarmine stains. Epithelial mucin can also be positive by Alcian blue but it is not digested by hyaluronidase. Although MM vacuoles do not generally show positive results with periodic acid–Schiff after digestion, as seen in adenocarcinomas, there are rare, published examples of epithelioid MM that show positive results with periodic acid–Schiff after digestion.[63] Mesothelial cells may have vacuoles containing hyaluronic acid that stain positive with Alcian blue and are digestible by hyaluronidase. Mucicarmine may also stain hyaluronic acid in MM; thus, mucicarmine stain is not recommended for distinguishing MM from adenocarcinoma. With widespread application of IHC panels, there is only occasional indication for using histochemical stains, for example, in tumors expressing contradictory immunohistochemical markers.

IMMUNOHISTOCHEMICAL STAINING IN MM

A definitive diagnosis of MM requires a workup, including IHC and, in some cases, histochemical stains for mucin. The role of IHC varies depending on the histologic type of mesothelioma (epithelioid versus sarcomatoid), the location of the tumor (pleural versus peritoneal), and the type of tumor being considered in the differential diagnosis (adenocarcinoma, squamous cell carcinoma, malignant melanoma, epithelioid

Table 3. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Malignant Mesothelioma Versus Lung Adenocarcinoma Involving the Pleura

Marker	Current Value/Comments
Epithelioid mesothelioma (positive mesothelioma markers)	
Calretinin	*Very useful*. Is demonstrated in nearly all epithelioid mesotheliomas when antibodies to human recombinant calretinin are used. The staining is often strong and diffuse and is both nuclear and cytoplasmic; 5%–10% of lung adenocarcinomas are positive, but the staining is usually focal.
Cytokeratin 5 or 5/6	*Very useful*. Expressed in 75%–100% of mesotheliomas. About 2%–20% of lung adenocarcinomas can be focally positive.
WT1	*Very useful*. Approximately 70%–95% of mesotheliomas show nuclear positivity. Lung adenocarcinomas are negative.
Podoplanin (D2-40)	*Very useful*. About 90%–100% of mesotheliomas show positivity along the cell membranes; ≤15% of lung adenocarcinomas are focally positive.
Lung adenocarcinoma (positive carcinoma markers)	
Claudin 4	*Very useful*. Essentially all lung adenocarcinomas are positive. Immunoreaction is often strong and diffuse and occurs along the cell membrane in a continuous or punctate pattern. Mesotheliomas are negative.
MOC31	*Very useful*. About 95%–100% of lung adenocarcinomas are positive; 2%–10% of mesotheliomas show focal staining.
CEA	*Very useful*. About 80%–100% of lung adenocarcinomas are positive; <5% of mesotheliomas are focally positive.
B72.3	*Very useful*. About 75%–85% of lung adenocarcinomas are positive. Very few mesotheliomas are positive.
BER-EP4	*Very useful*. About 95%–100% of lung adenocarcinomas are strongly positive; ≤20% of mesotheliomas are focally positive.
BG8 (Lewis^{Y})	*Very useful*. Approximately 90%–100% of lung adenocarcinomas are positive; 3%–7% of mesotheliomas show focal reactivity.
TTF-1	*Very useful*. About 75%–85% of lung adenocarcinomas show nuclear positivity (usually all nonmucinous lung adenocarcinomas are positive). It is not expressed in mesotheliomas.
Napsin A	*Very useful*. About 80%–90% of lung adenocarcinomas show cytoplasmic staining. It is not expressed in mesotheliomas.

Abbreviations: WT1, Wilms tumor-1; BG8, blood group 8; CEA, carcinoembryonic antigen; TTF-1, thyroid transcription factor-1.

hemangioendothelioma, among others). The immunohistochemical approach is also different depending on whether the tumor is sarcomatoid or epithelioid. Because biphasic mesotheliomas have an epithelioid component, the differential diagnosis is similar to that of epithelioid mesotheliomas.

Immunohistochemical staining for pancytokeratin is useful in the diagnosis of mesothelioma because virtually all epithelioid MM and most sarcomatoid MM will produce positive results. In a large study, 93% of sarcomatoid mesotheliomas exhibited immunoreactivity for cytokeratin (CK); that percentage may be even higher if a cocktail of keratins is used, there is adequate sampling of the tumor, and the tissue is well fixed.[64] Sarcomatoid MM with osteosarcomatous or chondrosarcomatous differentiation may be negative for keratin staining. If an epithelioid malignant neoplasm causing diffuse pleural thickening is keratin negative with pancytokeratin immunostaining (using multiple keratins, including AE1/AE3, CAM 5.2, and CK5/6), other possible differential diagnoses should be considered, such as malignant melanoma, epithelioid hemangioendothelioma or angiosarcoma (although some of those can have positive keratin results), and malignant lymphoma. In those circumstances, it is recommended that a screening panel be performed to address those possibilities. Such a panel might include CD45, CD20, CD3, or CD30 for large cell lymphomas; S100 and HMB-45 for melanoma; and CD31, CD34, and ERG (or FLI-1) for angiosarcoma and epithelioid hemangioendothelioma. Because antibodies to podoplanin (D2-40) will stain epithelioid vascular tumors, it is not a good marker for this differential diagnosis. Further confirmatory staining may be useful if one or more of those screening markers are positive. Ultrastructural studies may be of benefit in particularly difficult cases.

On occasion, a tumor may not stain with any marker. That lack of staining can be caused by a variety of reasons, including overfixation in formalin. Negative immunoreactivity may also occur in alcohol-fixed tissues if antigen retrieval is used, so some knowledge about the fixative is important. If needed, vimentin may be used to assess immunoreactivity.

As the role of IHC has evolved, it has become a standard to use panels of positive and negative antibodies that vary depending on the differential diagnosis (see Tables 3 through 6). Because there is variability in staining among different antibody clones and among separate laboratories, no specific panel of antibodies is recommended. It is best for each laboratory to test staining conditions for the antibodies of choice with appropriate controls. If possible, antibodies should be chosen with a sensitivity or specificity of at least 80%.

There is no absolute number of antibodies that can be recommended for the diagnosis of MM. Workup can be done in stages. An initial workup could use 2 mesothelial markers and 2 markers for the other tumor under consideration based on morphology (adenocarcinoma, squamous cell carcinoma). If the results are concordant, the diagnosis may be considered established. If they are discordant, a second stage, expanding the panel of antibodies, may be needed. Additional antibodies should be selected according to the differential diagnosis. In addition, a different block, if available, can be stained. The pattern of immunohistochemical staining is important with certain antibodies, such as calretinin, where both cytoplasmic and nuclear staining is required to support a diagnosis of mesothelioma, and Wilms tumor-1 (WT1), which should have only nuclear staining. There is no standard for the percentage of tumor cells that should be positive, but some have used a 10% cutoff for membranous and cytoplasmic staining.

Pleural Epithelioid Mesothelioma Versus Carcinoma

The differential diagnosis of epithelioid pleural mesothelioma can be greatly facilitated by the use of IHC. Many markers are now available that can assist in distinguishing

Table 4. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Malignant Mesothelioma Versus Squamous Carcinoma of the Lung Involving the Pleura

Marker	Current Value/Comments
Epithelioid mesothelioma (positive mesothelioma markers)	
WT1	*Very useful*. Up to 95% of mesotheliomas show nuclear positivity. Lung squamous carcinomas are negative.
Calretinin	*Somewhat useful*. Essentially all mesotheliomas are positive, often strongly and diffusely, with nuclear and cytoplasmic staining. About 40% of lung squamous carcinomas are positive, but the staining is often focal.
Podoplanin (D2-40)	*Not useful*. About 80%–100% of mesotheliomas are positive; 50% of lung squamous carcinomas also stain.
Cytokeratin 5 or 5/6	*Not useful*. Expressed in 75%–100% of mesotheliomas and 100% of lung squamous carcinomas.
Lung squamous carcinoma (positive carcinoma markers)	
p40 or p63	*Very useful*. 100% of lung squamous carcinomas show strong and diffuse nuclear positivity for either marker. About 2.5% and 7% of mesotheliomas are focally positive for p40 and p63, respectively.
Claudin 4	*Very useful*. About 95% of squamous cell carcinomas are positive. Mesotheliomas are negative.
MOC31	*Very useful*. About 97%–100% of lung squamous carcinomas are positive; 2%–10% of mesotheliomas show focal staining.
BG8 (Lewis^{Y})	*Very useful*. About 80% of lung squamous carcinomas are positive; 3%–7% of mesotheliomas show focal staining.
BER-EP4	*Useful*. Approximately 85%–100% of lung squamous carcinomas are positive; ≤20% of mesotheliomas are focally positive.
Cytokeratin 5 or 5/6	*Not useful*. All lung squamous carcinomas (100%) and most mesotheliomas (75%–100%) are positive.

Abbreviations: WT1, Wilms tumor-1; BG8, blood group 8.

this tumor from metastatic carcinoma originating either in the lung or in distant organs, such as the kidney, breast, or ovary. Tables 3 and 4, respectively, list the markers that are, at present, considered to be useful in distinguishing epithelioid pleural mesotheliomas from lung adenocarcinomas and those that can help in discriminating between epithelioid pleural mesotheliomas and squamous cell carcinomas of the lung. Because none of these markers are 100% specific for these various types of tumors, the International Mesothelioma Interest Group recommends that at least 2 mesothelial and 2 carcinoma markers, in addition to cytokeratin (using a broad spectrum anti-cytokeratin antibody), be included in any immunohistochemical panel.[2] Based on sensitivity and specificity, calretinin (Figure 8, A and B), cytokeratin 5 or 5/6 (Figure 9, A and B), WT1 (Figure 10, A through C), and podoplanin (D2-40) (Figure 11, A and B) are the best positive mesothelioma markers, whereas claudin 4 (Figure 12, A and B), MOC31 (Figure 13, A through C), and BER-EP4 are the best overall carcinoma markers.[65–67] Because of their high specificity for lung adenocarcinomas, TTF-1 and napsin A are more useful than other markers because they can be used to confirm the lung origin of an adenocarcinoma.[68] Antibodies to p40 (or p63, which is less useful because it cross-reacts with adenocarcinoma), claudin 4, MOC31, BER-EP4, and carcinoembryonic antigen (CEA) are regarded as the best positive carcinoma markers for assisting in the differential diagnosis between epithelioid mesotheliomas and squamous cell carcinomas because those markers are commonly expressed in squamous cell tumors, but they are usually absent in epithelioid mesotheliomas.[69–71] p40 is more useful than the other 3 markers because, in addition to being strongly and invariably expressed in squamous cell carcinomas but absent in mesotheliomas, it may assist in distinguishing squamous cell carcinomas from pulmonary adenocarcinomas. Because WT1 is expressed in most epithelioid mesotheliomas but absent in squamous cell carcinomas, it is the best positive mesothelioma marker for discriminating between those malignancies.[69]

Other carcinomas that metastasize to the pleura and that can potentially be confused with mesothelioma are those that originate in the breast, kidney, gastrointestinal tract, and ovary; the latter 2 are addressed primarily in the section Immunohistochemical Issues in Peritoneal Mesothelioma. Because most breast carcinomas express estrogen receptor, gross cystic disease fluid protein-15, or mammaglobin, immunostaining for those markers can be useful in distinguishing a mesothelioma from a metastatic breast carcinoma.[72] GATA3 is a marker that is frequently positive in breast carcinomas; however, one-third to one-half of epithelioid mesotheliomas also express GATA3.[72,73] Table 5 lists markers that are considered useful in distinguishing between mesothelioma and metastatic renal cell carcinoma. Because of their sensitivity and specificity, calretinin, podoplanin (D2-40), and keratin 5/6 are the best positive mesothelioma markers.[74] Among the carcinoma markers, PAX8 or PAX2 is most useful because they are both expressed in most renal cell carcinomas[75,76] but not in mesotheliomas[67,77]; however, PAX8 will sometimes stain peritoneal mesotheliomas and benign mesothelial cells (Figure 14, A and B). Renal cell carcinoma marker and CD15 can also be useful, but the sensitivity and specificity of these markers for renal cell carcinomas are significantly less than that of PAX8 or PAX2. Adenocarcinomas of the gastrointestinal tract and prostate can be distinguished from epithelioid mesotheliomas by the demonstration of CDX2 and prostate-specific antigen, respectively.

Immunohistochemical Issues in Peritoneal Mesothelioma

Diffuse malignancies of the peritoneum include PMM and secondary peritoneal carcinomatosis in the clinical, imaging, and gross pathologic differential diagnosis in many cases. In pleural disease, pseudomesotheliomatous carcinoma (defined as a carcinoma that grows along the pleura encasing the lung) is most often from an adenocarcinoma of pulmonary origin, whereas peritoneal carcinomatosis can have an ovary, fallopian tube (previously considered as primary peritoneal carcinomas), gastric, pancreatic, colonic, and more rarely, breast origin.[54,78] Therefore, IHC panels have to be adjusted accordingly.

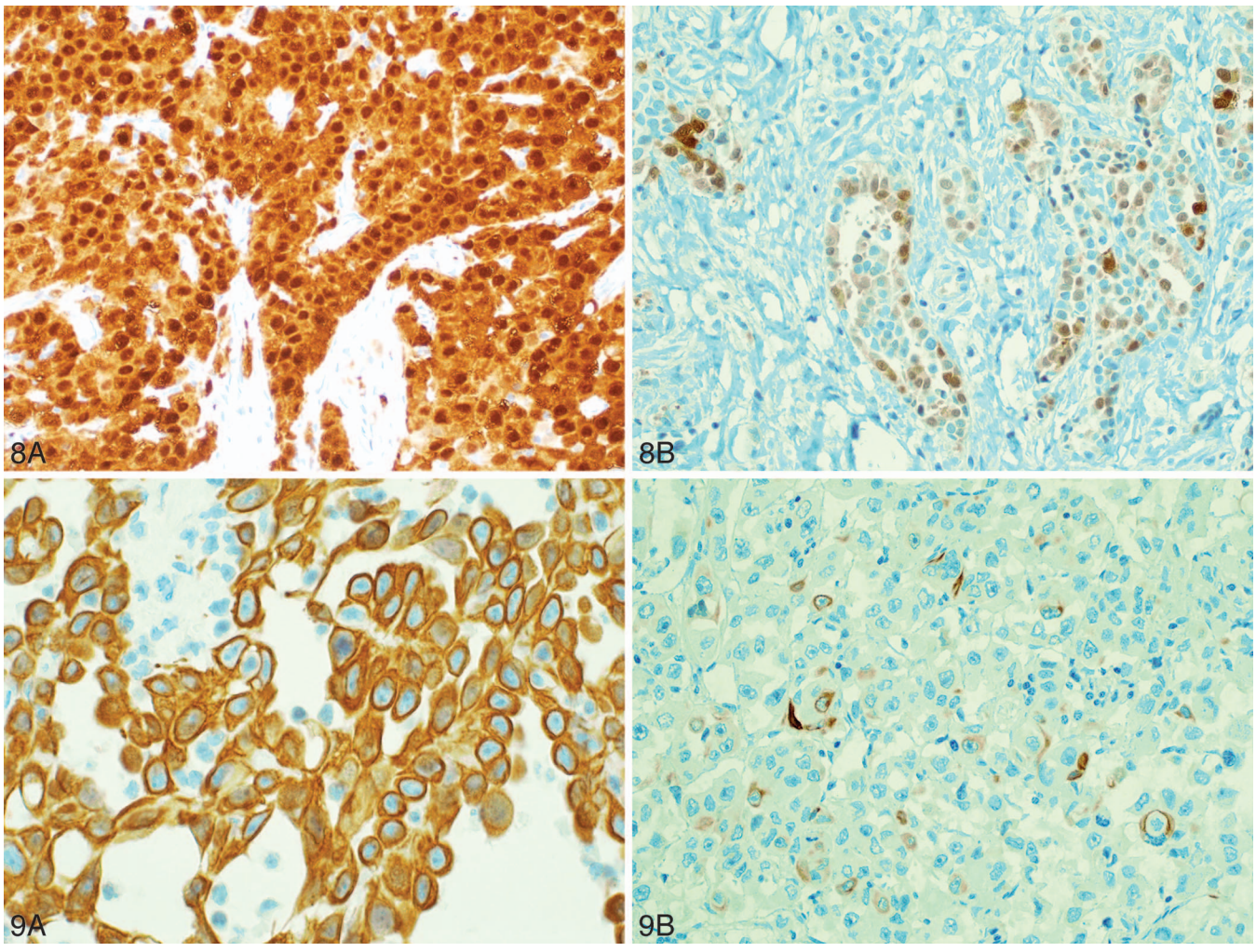


Figure 8. *A and B, Calretinin staining. A, Malignant mesothelioma has diffuse, strong nuclear and cytoplasmic positivity. B, Adenocarcinoma is usually negative but may show focal positivity as shown here (original magnifications ×200 [A] and ×400 [B]).*

Figure 9. *A and B, Keratin 5/6 staining. A, Malignant mesothelioma with strong reactivity. B, Large cell carcinoma with only focal reactivity (original magnification ×400 [A and B]).*

Most studies have focused on differentiating PMM from papillary serous carcinoma (PSC); the findings are summarized in Table 6.[67,78–81] There have been fewer data directly comparing the profile of PMM to pancreatic, gastric, and colon carcinoma.[82,83] The markers useful in women include calretinin and possibly, podoplanin (D2-40) (which can also be positive in some cases of PSC) for positive markers in PMM, and claudin 4, MOC31, BG8, and, with less specificity, BER-EP4 for positive adenocarcinoma markers. Although specific, B72.3 staining may be too focal in many PSC cases, although a positive result is useful. The high frequency of reactivity for the mesothelioma markers CK5/6 and WT1 in PSC and the less-frequent staining for CEA in PSC limits the ability of those markers to discriminate among these entities. However, CEA may also be useful in the setting when PSC is not in the differential diagnosis. H-caldesmon has been reported to be highly useful as a mesothelial marker[77]; however, other studies have not shown this.[84] Strongly positive estrogen receptor staining may be helpful in difficult cases, as would a positive result for progesterone receptor. A very useful marker to address the problem of tumors of Müllerian origin in women and tumors of renal origin in all patients is PAX8.[84,85] PAX8 is a transcription factor involved in the development of the thyroid, kidney, and Müllerian systems. Although focal or weak nuclear staining can be seen in a few cases of MM, a high percentage of ovarian, tubal, endometrial, and renal tumors show immunoreactivity that is frequently diffuse and intense (Figure 14, A and B). This marker is very promising when added to a panel to differentiate abdominal MM from carcinoma.

In male patients, WT1 (nuclear staining) and podoplanin (D2-40) are useful markers, in addition to calretinin, for MM, and for nonserous adenocarcinoma, claudin 4, B72.3, MOC31, BG8, and BER-EP4 all have high sensitivity and specificity.[86]

Sarcomatoid Mesothelioma

Sarcomatoid mesotheliomas are diffuse neoplasms composed of infiltrating, solid sheets of spindle cells with variable cytologic atypia. The presence of necrosis, atypical mitoses, and/or heterologous elements is helpful for diagnosis. A frequently useful initial IHC panel includes

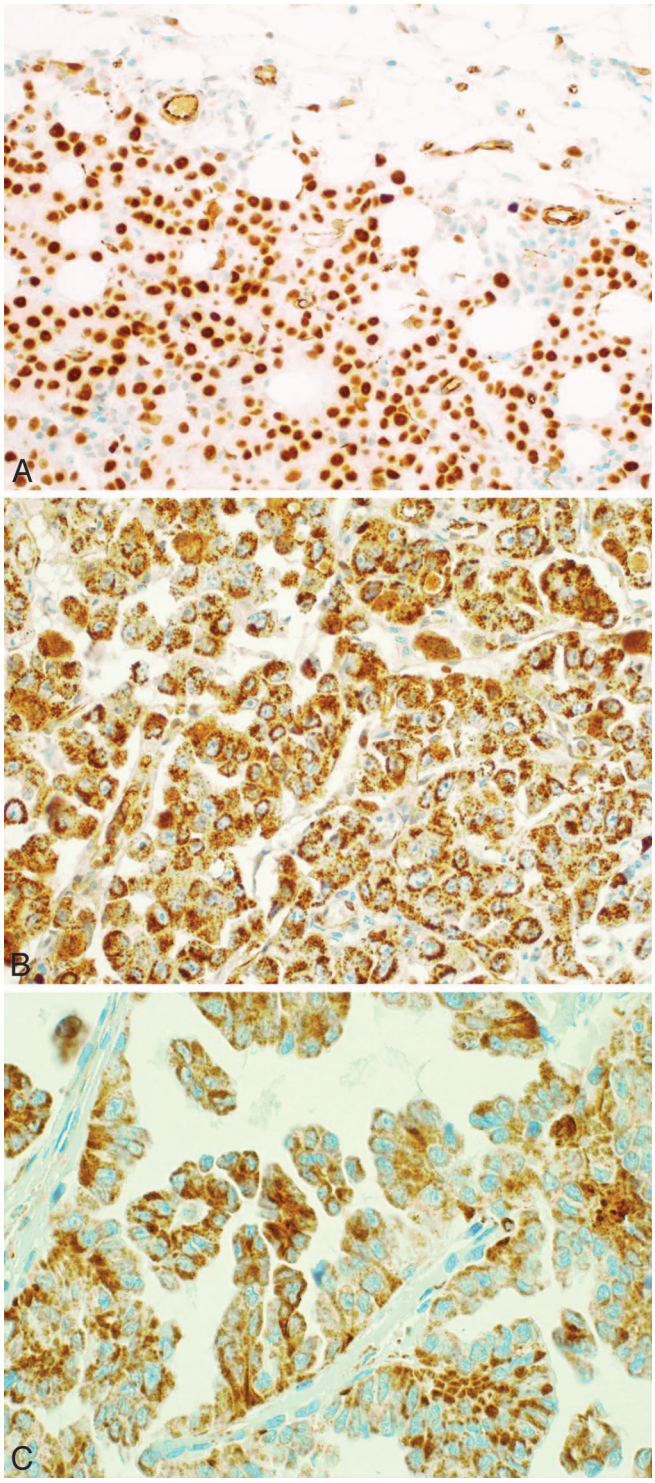


Figure 10. *A through C, Wilms tumor-1 (WT1) staining. A, Strong nuclear staining in malignant mesothelioma invading fat. Note that endothelial cells show cytoplasmic staining only. B, Strong granular cytoplasmic staining in large cell carcinoma. C, Cytoplasmic staining in adenocarcinoma of lung (original magnifications ×200 [A] and ×400 [B and C]).*

AE1/3, OSCAR, KL1, CK18, or CAM 5.2 antibody to exclude a spindle cell sarcoma.[85,87,88] Affirmative markers that are used in the evaluation of epithelioid mesothelioma, such as WT1 and CK5/6, as well as adenocarcinoma markers, such as claudin 4, MOC31, BER-EP4, and CEA, do not provide much added utility in sarcomatoid tumors and should be avoided, particularly when there is limited tissue. Podoplanin (D2-40) and calretinin can be expressed in sarcomatoid mesotheliomas in a variable percentage of cases, with calretinin being the more frequently positive marker.[64,89–92] About 30% of sarcomatoid mesotheliomas express calretinin, which may be extremely focal.[92,93] When positive, podoplanin (D2-40) shows a higher sensitivity and specificity within the differential diagnosis of pleural sarcomatoid mesothelioma and pulmonary sarcomatoid carcinoma. However, false-positivity is a major pitfall and can occur by the misinterpretation of positive podoplanin (D2-40) reactivity within benign entrapped lymphatics or reactive mesothelial and fibrous elements.[89]

A histologically malignant sarcomatoid tumor that stains strongly and diffusely positive for cytokeratin usually limits the differential diagnosis to sarcomatoid mesothelioma, sarcomatoid carcinoma of the lung, and on occasion, synovial sarcoma, angiosarcoma, or other metastatic extrapulmonary sarcomatoid tumors, such as renal cell carcinoma. The diagnosis of synovial sarcoma can be confirmed by molecular testing for its distinctive X;18 translocation. Positivity for TTF-1, napsin A, and p40/p63 support a diagnosis of a sarcomatoid lung carcinoma involving the pleura. Sarcomatoid renal cell carcinoma can metastasize to the pleura and grow like an MM producing a pseudomesotheliomatous sarcomatoid-type pattern. Differential cytokeratin-positivity profiles, other than CK5/6, have not been reported to date in the differential diagnosis of these 2 tumors. CK5/6 has been reported to be negative in sarcomatoid renal cell carcinomas, but the low sensitivity of CK5/6 as a marker in sarcomatoid MM greatly limits its utility.[74] One series[74] reported calretinin negativity in all 4 sarcomatoid renal cell carcinomas tested, but it would be prudent to incorporate additional gross and clinical correlations. The sensitivity of renal cell carcinoma marker in sarcomatoid renal cell carcinoma is low and its utility limited.[94] PAX8 stains less than 45% of sarcomatoid renal cell carcinomas, and its specificity in relationship to sarcomatoid MM is not known.[95] Published data on PAX2 staining in sarcomatoid renal cell carcinoma is sparse. Focal cytokeratin positivity has been reported in many different types of sarcomas; however, it is also possible that this positivity represents entrapment of benign pleural elements.

If the initial round of cytokeratins proves to be negative or if there is only focal cytokeratin positivity, additional blocks should be selected and stained, and cytokeratin antigen retrieval techniques, as well as antibody source and dilutions, should be reviewed. A vimentin stain is useful in assessing the general antigenic integrity of the tissue. Particularly in the absence of convincing cytokeratin positivity, calretinin and/or podoplanin (D2-40) positivity alone should not be interpreted as evidence of mesothelial differentiation. These markers are variably positive in many different types of sarcomas for which immunohistochemical markers should be added at this point. The expanded differential diagnosis might include other sarcomas (epithelioid hemangioendothelioma/angiosarcoma, synovial sarcoma, liposarcoma, myogenic, or neurogenic tumors), malignant solitary fibrous tumor, melanoma, and lymphoma. The marker panel should be expanded accordingly to include antibodies such as CD31, ERG, FLI1, CD34, STAT6, desmin, myoglobin, S100, SOX10, and CD45. Muscle-

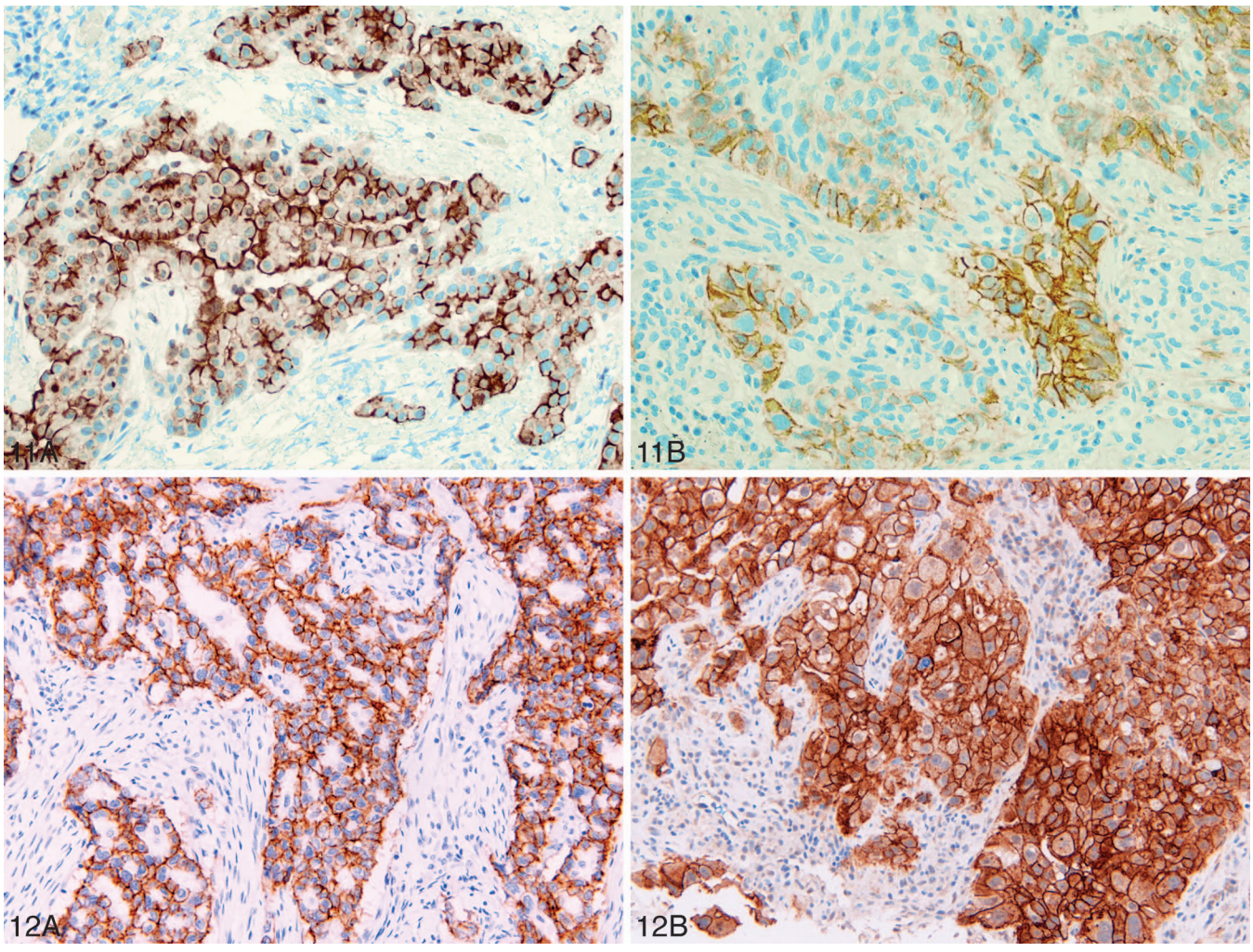


Figure 11. *A and B, Podoplanin (D2-40) staining. A, Strong membranous staining in malignant mesothelioma. B, Focal staining in squamous cell carcinoma (original magnifications ×200 [A] and ×400 [B]).*

Figure 12. *A and B, Claudin 4 shows strong membranous staining in well-differentiated (A) and poorly differentiated (B) carcinomas (original magnification ×200 [A and B]).*

specific actin (HHF-35) and α-smooth muscle actin are often positive, at least focally, and on occasion, more diffusely, in sarcomatoid mesotheliomas.[96] In contrast to reactive mesothelial cells, desmin positivity in pure sarcomatoid mesotheliomas is quite rare.[96,97] After extensive workup and with appropriate clinical and radiologic features, cytokeratin-negative sarcomatoid mesotheliomas are recognized in the literature with a frequency of about 5% and in 10% of tumors with heterologous elements.[42,85,91,98]

The use of molecular markers in the diagnosis of MM is covered in detail in the following section, but it should be noted that homozygous deletion in the region of 9p21 (p16) is seen in most sarcomatoid pleural mesotheliomas,[5] whereas only a few show loss of BAP1 expression as assessed by IHC.[4,99]

MOLECULAR MARKERS IN MM

Key molecular alterations in the pathogenesis of MM have been known for decades, but their potential diagnostic and prognostic implications have only recently been more extensively investigated.[100] One of the most common genetic alterations in MM is the homozygous deletion of the 9p21 locus within a cluster of genes that includes cyclin-dependent kinase inhibitor (CDKN)-2A, CDKN2B, and methylthioadenosine phosphorylase.[101–105] Several cytogenetic and molecular studies have reported *p16/CDKN2A* deletions in up to 80% of primary pleural MM, depending on the histologic subtype (90%–100% of sarcomatoid mesothelioma; 70% of epithelioid and mixed types). In contrast, that deletion occurs in approximately 25% of peritoneal MM.[19,105,106] Besides homozygous deletion, point mutations and DNA methylation occur less frequently at the same genetic locus.[103] *p16/CDKN2A* is present in all healthy cells and is essential for normal cell-cycle control, and therefore, its loss may be a helpful marker of malignancy. Deletions of *p16/CDKN2A* occur only in MMs, whereas point mutations and DNA methylation may occur in benign mesothelial cells as well.[107] Therefore, the detection of this deletion can be a useful approach for distinguishing benign from malignant mesothelial proliferations. It should be emphasized that

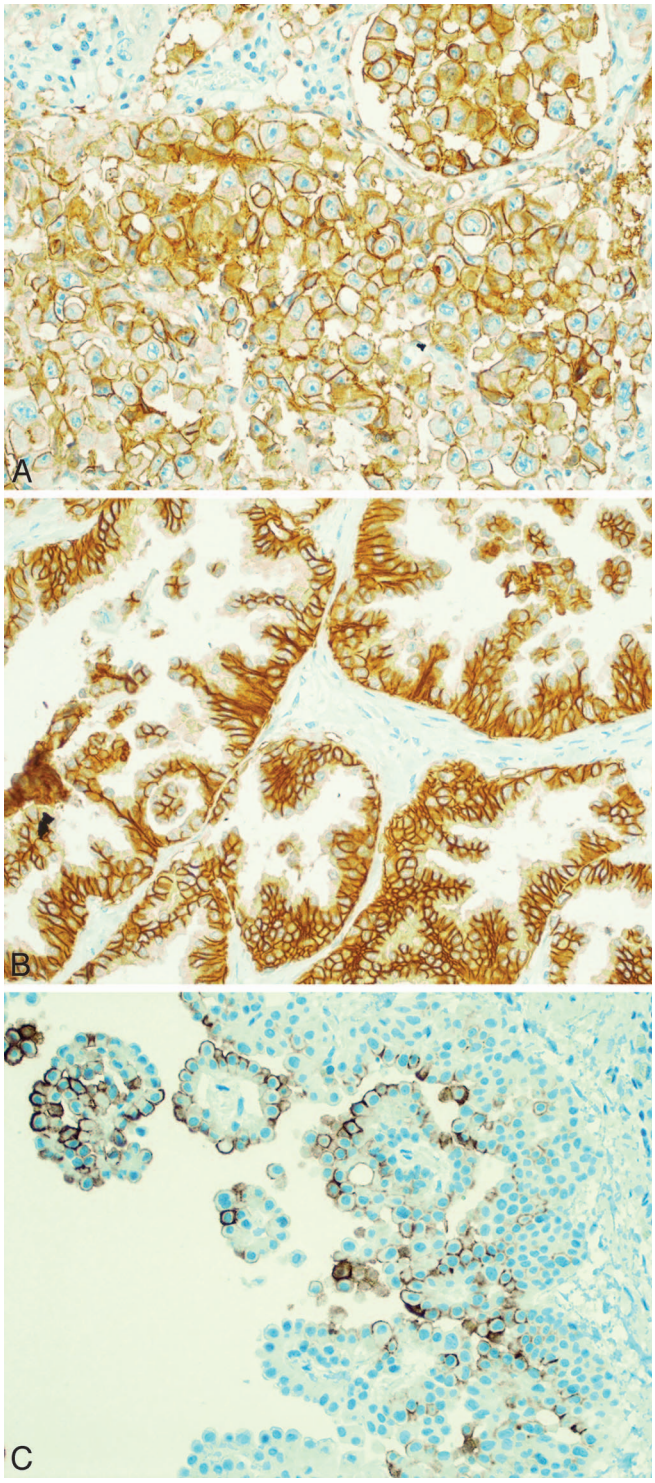


Figure 13. *A through C, MOC31 staining. A, Large cell carcinoma with membranous staining. B, Papillary adenocarcinoma of the lung with strong staining. C, Focal staining in malignant mesothelioma (original magnifications ×400 [A and B] and ×200 [C]).*

this technique is not useful for distinguishing MM from adenocarcinoma (as discussed below).

Various methods, including polymerase chain reaction–based techniques and FISH, have been used in detection of deletions. The FISH assay can be performed with a commercially available dual-color FISH probe (Abbott Molecular, Des Plaines, Illinois). It can be reliably performed on archival, paraffin-embedded tissue and is relatively less expensive than other molecular assays. Another advantage to this technique over polymerase chain reaction–based assays is the ability to identify homozygous and hemizygous deletions. Furthermore, different tumor areas can be simultaneously analyzed and visualized. In addition, FISH for detection of 9p21 deletions has been shown to be a powerful technique for confirming the diagnosis of MM in effusion and formalin-fixed, paraffin-embedded tissue specimens (Figure 15, A and B).[20,21,106,108,109]

The diagnosis of atypical mesothelial proliferation is more common in cytologic specimens than it is in surgical specimens because the diagnosis of mesothelioma can be more challenging in cytologic specimens because of the inability to evaluate for tissue invasion and the numerous cytomorphologic mimics of mesothelioma, including reactive mesothelial proliferations. Studies showed an overall sensitivity of *p16* FISH in the diagnosis of MM in effusion cytology across all cytologic categories of between 56% and 79%, with a positive predictive value of 100%. In addition, FISH *p16* showed greater sensitivity and specificity than glucose transporter 1 immunohistochemical marker did in cytology specimens.[21] The main challenge in the assessment of *p16* deletion by FISH in cytology specimens when a cell block is available is the presence of admixed, reactive mesothelial cells that could be morphologically indistinguishable from malignant mesothelial cells and could potentially lead to false-negative FISH results.

Although studies showed statistically proven good correlation between *p16* deletion and the lack of p16 protein expression, there is a subset of cases in which p16 protein expression would be maintained despite the presence of *p16* gene deletion and vice versa. This could be explained by the type of antibody, assay conditions, preanalytic variables, and interpretation criteria. Therefore, immunohistochemical assessment for the loss of p16 protein expression would be unreliable and should not be used as a surrogate method for detection of a *p16* deletion.[106]

Homozygous deletion of *p16* can be used as both a diagnostic and a prognostic marker. The presence of a *p16* homozygous deletion correlates with shorter survival in patients with MM.[105,110,111] There is also a correlation between p16 protein loss, as demonstrated by IHC, and a poor prognosis, with increased risk of death in peritoneal mesothelioma, but the association is not as strong.[57,110] There are no molecular markers to help distinguish MM from carcinomas or sarcomas on formalin-fixed, paraffin-embedded tissue. Genetic alterations of 9p are one of the most frequent events in other tumor types, including non–small cell carcinomas of the lung, melanoma, and sarcomas; therefore, deletion cannot be used to differentiate those neoplasms from MM.[112–114] However, detecting t(X;18) is most useful in the differential diagnosis of synovial sarcoma. Begueret et al[115] confirmed the presence of that translocation in 90% of purely sarcomatoid primary synovial sarcoma of the pleura, whereas this translocation has never been detected in MM.[116]

DNA methylation profiles, microRNA dysregulation, and *BAP1* mutations are being studied and are likely to yield important results in understanding pathogenesis and in developing targeted therapy for MM but are not currently used for diagnosis.

Table 5. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Malignant Mesothelioma Versus Metastatic Renal Cell Carcinomas

Marker	Current Value/Comments
Epithelioid mesothelioma (positive mesothelioma markers)	
Calretinin	*Very useful*. Essentially all mesotheliomas are positive, and the staining is often strong and diffuse with nuclear and cytoplasmic staining; 4%–10% of renal cell carcinomas are focally positive.
Cytokeratin 5 or 5/6	*Very useful*. About 75%–100% of mesotheliomas are positive. Renal cell carcinomas are negative.
Podoplanin (D2-40)	*Very useful*. About 80%–100% of mesotheliomas show positivity along the cell membrane. Renal cell carcinomas are negative.
Mesothelin	*Very useful*. All (100%) of mesotheliomas are positive. Renal cell carcinomas are negative.
WT1	*Useful*. Approximately 70%–93% of mesotheliomas show nuclear positivity; 4% of renal cell carcinomas are positive.
Renal cell carcinoma (positive carcinoma markers)	
PAX8	*Very useful*. About 85%–100% of renal cell carcinomas are positive. Mesotheliomas are mostly negative.
PAX2	*Useful*. About 60%–75% of renal cell carcinomas are positive. Mesotheliomas are negative.
Claudin 4	*Useful*. About 90% of renal cell carcinomas are positive. Mesotheliomas are negative.
CD15 (Leu-M1)	*Useful*. About 65% of renal cell carcinomas are positive. Mesotheliomas only rarely show focal positivity. Can stain any necrotic tissue.
RCC Ma	*Somewhat useful*. About 50%–70% of renal cell carcinomas are positive; 28% of mesotheliomas are focally positive.
Napsin A	*Limited utility*. About 30% of renal cell carcinomas are positive. Mesotheliomas are negative.
MOC31	*Limited utility*. About 50% of renal cell carcinomas are positive; 2%–10% of mesotheliomas show focal staining.
BER-EP4	*Not useful*. About 40% of renal cell carcinomas are positive; $\leq$20% of mesotheliomas are focally positive.
CD10	*Not useful*. About 80% of renal cell carcinomas are positive. About 50% of mesotheliomas are positive.
BG8 ($Lewis^Y$)	*Not useful*. About 4% of renal cell carcinomas and 3% –7% of mesotheliomas are positive.

Abbreviations: WT1, Wilms tumor-1; BG8, blood group 8.

ELECTRON MICROSCOPY OF MM

The electron microscopic features of MM are well described.[31,117] The role of electron microscopy is restricted because IHC is faster and often cheaper (and more widely available) in establishing the correct diagnosis. Sarcomatoid mesotheliomas, for the most part, do not show specific ultrastructural features, and tumors that are poorly differentiated by light microscopy and do not demonstrate a typical pattern of immunohistochemical staining usually lack specific features by electron microscopy as well.[118,119] Occasionally electron microscopy is useful in establishing the correct diagnosis when the immunohistochemical results are equivocal or when further support of a diagnosis of either MM or serous carcinoma is needed.[69] Formalin-fixed material retrieved from a paraffin block may be satisfactory because microvilli and tonofilament bundles tend to be preserved.

PITFALLS IN THE DIAGNOSIS OF MM

Morphology and IHC

The first "port of call" for the histologic diagnosis of MM is the morphology. Immunohistochemical stains are important for confirmation of the diagnosis, but they should not be used to force a tumor into the diagnosis of mesothelioma when it does not look like a mesothelioma on hematoxylin-eosin–stained slides; neither should the stains be performed automatically or blindly without considering several factors. As stated previously, the major determinants on which panel to use are (1) the location of the tumor—it will vary as to whether it is pleural, peritoneal, or another serosal surface; (2) the phenotypic problem–benign versus malignant, epithelioid, spindle, biphasic, small cell, or pleomorphic; and (3) the experience of the laboratory. A laboratory employing IHC stains should be performing them frequently, have well established protocols, and have an appreciation of the stains' sensitivities and specificities for various morphologic problems. There is no single, utopian immunohistochemical panel to cover all diagnostic "mesothelial" problems.

One of the problems in comparing the results of particular antibodies from different studies is a lack of standardization in immunohistochemical procedures. This can result in conflicting results for sensitivity and specificity for various antibodies. In their study, King et al[120] tabulated the data for antibody clone, manufacturer, dilution, and antigen-retrieval methods for 5 antibodies employed in separating benign and malignant mesothelial proliferations in 13 studies. The wide variability among the various studies was illustrated. Before use of an antibody for diagnosis, a laboratory should have performed an extensive workup to find the ideal conditions for routine use.[120]

The type of pathologic sample may affect results. For example, tiny needle biopsies may show crush artifact and false-positive immunostaining with various antibodies. In addition, the edges of biopsies may show artifactual positive immunostaining. There may also be variation in interpretation of what is a positive result, illustrated by some laboratories only considering a calretinin result to be positive when there is nuclear staining, whereas a few laboratories consider cytoplasmic staining to be a positive result. That difference can significantly affect the interpretation of the immunohistochemical results.

Another problem associated with IHC may be putting too much emphasis on focal immunopositivity. We would suggest that weak or focal staining of less than 10% of the cells should be considered a negative result when interpreting a panel of stains. Positive immunostaining can also be observed with mesothelial markers in reactive proliferations of submesothelial fibroblasts near nonmesothelial tumors and inflammatory pleural diseases—it is important not to diagnose those cases as mesotheliomas. In contrast,

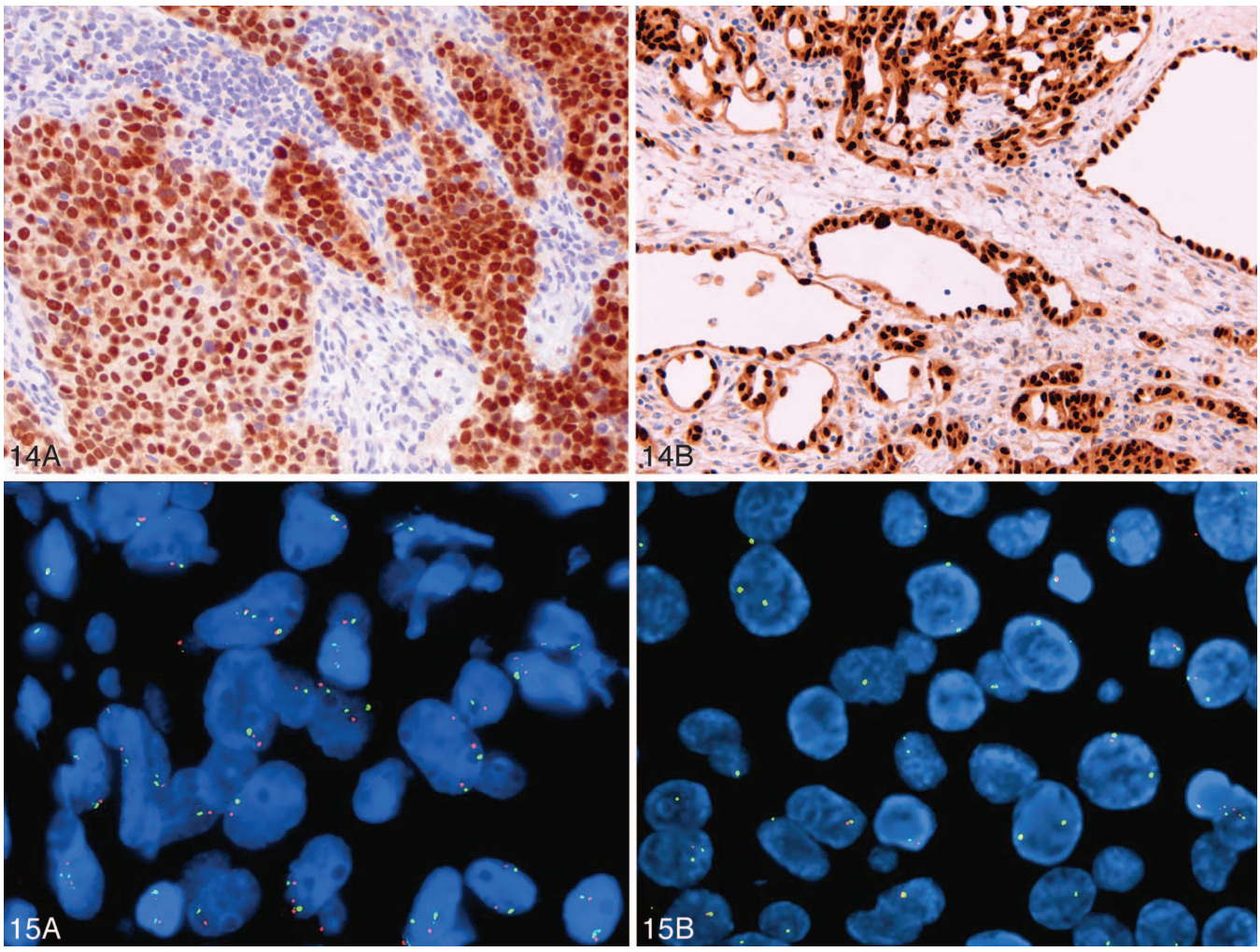


Figure 14. *PAX8 shows strong nuclear staining in metastatic clear cell carcinoma from kidney (A) and in benign mesothelial proliferation from a hernia sac (B) (original magnification ×200 [A and B]).*

Figure 15. *A and B, Fluorescence in situ hybridization (FISH). A, Negative result for* p16 *deletion: 2 green signals (9p centromere) and 2 red signals (p16). B, Positive slide for* p16 *deletion: only 2 green signals (9p centromere) and no red signals (p16) (original magnification ×1000 [A and B]).*

mesotheliomas may invade the underlying lung, and entrapped pulmonary epithelial cells may show positive immunostaining with epithelial markers. Careful correlation with the hematoxylin-eosin sections is necessary to avoid misinterpretation.

The full range of cell types that an individual marker may stain should be known. For example, WT1 and podoplanin (D2-40) are positive in endothelial cells, which should not be misinterpreted as positive tumor staining in small, crushed biopsies, in particular. Similarly, mesothelial markers may be positive in tumors other than mesothelioma. For that reason, the selection and use of a panel of immunostains and knowledge of the expected results cannot be overemphasized, and reliance on any single "mesothelial" marker in isolation as definitive support for mesothelioma should be avoided or approached with caution. For example, WT1 may be positive in ovarian serous tumors and melanoma, whereas podoplanin (D2-40) may be positive in vascular malignancies and CK5/6 in squamous carcinomas. Calretinin is positive in synovial sarcoma and some germ cell tumors, as well as in a significant percentage of spindle cell thymomas and thymic carcinomas.[121,122] Also of note, calretinin may be positive in breast carcinomas, particularly those tumors with high-grade, basal-type morphology, which may be negative for estrogen and progesterone receptors, which may be particularly problematic given that GATA3 can be positive in mesotheliomas as well as breast cancer.[72,123–125] As such, the significance of positive staining by a single marker should be interpreted within the context of the totality of immunohistochemical, morphologic, and clinical findings.

Entrapment of Benign Mesothelium

Another pitfall leading to misdiagnosis may result from "false" invasion, which can apply to the pleura or chest wall fat. Inflammatory pleural processes may result in mesothelial cells lying quite deeply within the pleura (because of entrapment in granulation tissue), but those cells are usually parallel with the pleural surface. Sometimes tubular collections of reactive mesothelial cells are seen, which are lined up parallel to the pleural surface. That pattern does not connote malignancy. Sometimes a section taken parallel to

Table 6. Peritoneal Malignant Mesothelioma (PMM) Versus Papillary Serous Carcinoma (PSC) and Nongynecologic Adenocarcinoma (AdCa)

Positive Mesothelioma Markers	
Calretinin	*Useful*. Positive in 85%–100% of PMM cases, but reactivity in 0%–38% PSC limits its use as a single marker.
Podoplanin (D2-40)	*Not useful*. Positive in 93%–96% of PMM cases, but wide spectrum of positivity in PSC from 13%–65%.
CK5/6	*Not useful*. Positive in 53%–100% of PMM cases, but positive in 22%–35% of PSC cases.
WT1	*Not useful*. Positive in 43%–93% of PMM, but 89%–93% of PSC are positive.
PSC markers	
Claudin 4	*Very useful*. Positive in 98% of PSC, and negative in all PMM.
MOC31	*Very useful*. Positive in 98% of PSC and 5% of PMM.
PAX8	*Very useful*. Positive in most Müllerian carcinomas; usually negative in PMM.
BG8	*Very useful*. Positive in 73% of PSC and 3%–9% of PMM.
BER-EP4	*Useful*. Positive in 83%–100% of PSC and 9%–13% of PMM.
B72.3	*Limited utility*. Positive in 65%–100% of PSC and 0%–3% of PMM, but many cases show only trace/focal staining.
CEA	*Not useful*. Positive in 0%–45% PSC (average 20%) and 0% PMM, but sensitivity in PSC is too low compared with other choices.
Estrogen receptor	*Useful*. Positive in 60%–93% in PSC, and negative or very low positive rate (0%–8%) in PMM.
Progesterone receptor	*Limited utility*. Lower sensitivity than ER, but uniformly negative in PMM. May be valuable if positive.
PMM versus nongynecologic AdCa (biliary, pancreatic, gastric, colonic)	
Claudin 4	*Very useful*. Positive in 100% of gastric, pancreatic, colonic and biliary adenocarcinomas, and always negative in PMM.
Calretinin	*Very useful*. Positive in 85%–100% of PMM, but also positive in 10% of pancreatic AdCa, so limited as a single marker.
WT1	*Very useful*. Positive in 43%–93% of PMM and 3% of gastric AdCa; negative in pancreatic AdCa.
Podoplanin (D2-40)	*Potentially useful*. Positive in 93%–96% of PMM; negative in pancreatic and gastric AdCa (but limited data).
CK5/6	*Not useful*. Positive in 53%–100% of PMM, but 38% pancreatic AdCa positive.
MOC31	*Very useful*. Positive in 5% of PMM and 87% of AdCa.
BG8 (Lewis^{Y})	*Very useful*. Positive in 3%–9% of PMM and 89% of AdCa.
CEA	*Very useful*. Positive in 81% of AdCa; negative in PMM.
B72.3	*Very useful*. Positive in 84% of pancreas, 89% of bile duct, 98% of colon AdCa; 0%–3% of PMM.
BER-EP4	*Useful*. Positive in >98% of pancreatic and gastric AdCa; 9%–13% of PMM.
CDX2	*Useful*. Positive in 90%–100% of colon, 80% of small intestine, and 70% of gastric carcinomas; negative in PMM.

Abbreviations: BG8, blood group 8; CEA, carcinoembryonic antigen; CK5/6, cytokeratin 5/6; WT1, Wilms tumor-1.

the pleural surface may give a false impression of a full-thickness mesothelial proliferation. Organizing pleuritis may result in a "fake fat" phenomenon, whereby a greatly thickened, fibrotic, paucicellular pleura is associated with circular, fatlike spaces and cytokeratin-positive spindle cells running between those fatlike spaces (Figure 6). However, those cells are parallel to the pleural surface, and vimentin stain will show that there is no cellular lining to the spaces. In contrast, desmoplastic mesothelioma usually shows a downward, rather than horizontal, growth pattern of the keratin-positive spindle cells (Figure 4, B).[10]

Clinical Presentation

Malignant mesothelioma of the pleura typically presents as a unilateral, diffuse tumor in an older patient; however, when the presentation is atypical, it may be misleading. Atypical presentations include a tumor in an uncommon site, such as the pericardium and paratesticular region; presentation as localized (and potentially resectable) mas-s(es), such as lymphadenopathy; and as a pneumothorax.

MESOTHELIOMA REVIEW PANELS

Mesothelioma review panels have been functioning worldwide since the 1960s. These panels continue to serve as a referral source for pathologists facing diagnostic problems and, more recently, to confirm diagnoses for treatment trials. Some of the active panels are summarized in Table 7.

PROGNOSTIC FACTORS IN MM

There have been only modest improvements in the median survival of patients with MM during the past 4 decades, irrespective of treatment. A few persons with the disease do have a significantly improved survival, and that finding has prompted investigation into prognostic factors that may be classified as clinical, hematologic/serum, imaging, pathologic, and molecular. Only the last 2—pathologic and molecular—will be discussed here.

Pathologic factors associated with a poor prognosis include histologic type (nonepithelioid subtypes), especially the desmoplastic-variant sarcomatoid mesothelioma.[126] The pleomorphic-variant phenotype has a poor prognosis.[34,35] Conversely, the myxoid-rich variant epithelioid subtype appears to have a more favorable prognosis.[127] Nuclear grading (degree of nuclear atypia and mitotic count and/or MIB-1 labeling index) has been shown to be a strong predictor of overall survival in diffuse pleural and peritoneal mesothelioma.[44,128] There is emerging data regarding other histologic factors of adverse prognostic importance, including low chronic inflammatory stromal tumor response,[129] high CD10 expression,[45] and loss of p16 expression by IHC,[111] but those factors are not the standard of practice.

Molecular prognostic factors are emerging in MM: chromosomal alterations of the *CDKN2A* locus (9p21.3); homozygous deletion by FISH is a marker of malignancy and poor prognosis (correlation with shorter survival and shorter time to relapse).[130] Homozygous *p16* deletions are present in almost all sarcomatoid mesotheliomas, although

Table 7. International Mesothelioma Panels/Registries	
Panel	**International**
Australian Mesothelioma Panel (Australian Mesothelioma Surveillance Programme)	Sonja Klebe, MD (sonja.klebe@sa.gov.au) Doug Henderson, MD (dhenderson@internode.on.net)
Belgium Mesothelioma Registry	Brigitte Weynand, MD (Weynand@uclouvain.be) Marleen Praet, MD (marleen.praet@ugent.be)
Dutch Mesothelioma Panel	Marc van de Vijver, MD (m.j.vandevijver@amc.uva.nl)
Finland: Reference Center for Pathology of Occupational Diseases, Helsinki University Hospital	Sisko Anttila, MD (Sisko.L.Anttila@hus.fi)
French Mesopanel	Francoise Galateau-Sallé, MD (galateausalle-f@chu-caen.fr; francoise.galateau@lyon.unicancer.fr)
German Mesothelioma Panel	Andrea Tannapfel, MD (Andrea.tannapfel@pathologie-bochum.de)
Italian Registry (Veneto region)	Bruno Murer, MD (Bruno.Murer@ulss12.ve.it)
Japan Mesothelioma Panel	Kenzo Hiroshima, MD (hiroshima.kenzo@twmu.ac.jp)
US-Canadian Mesothelioma Panel	Andrew Churg, MD (achurg@interchange.ubc.ca)

a lower percentage (70%) of epithelioid tumors show such changes. Germline *BAP1* mutations (observed in 1%–2% of mesotheliomas) appear to confer a favorable prognostic effect on overall survival.[131] Somatic mutations are more common in mesothelioma (approximately 60%), although they have no clear prognostic significance.

Gene expression profiling ratios, DNA methylation status of individual genes, and microRNA expression analysis have prognostic utility, although these tests are also not established in routine surgical practice.

STAGING OF PLEURAL MM

The Union for International Cancer Control and American Joint Committee on Cancer, *Cancer Staging Manual*, 7th edition,[132] represents the most widely applied TNM system; however, the 8th edition[133] became available on January 4, 2017. The TNM staging system for malignant pleural mesothelioma evaluates the potential resectability of the disease but is generally not a good predictor of prognosis. There is no consensus TNM staging for any nonpleural mesothelioma cases.

REPORTING OF MM

The International Collaboration on Cancer Reporting[134] has recently described a data set for reporting of MM of the pleura or peritoneum, which includes 8 required and 7 recommended elements that the panel considered essential information.

SUMMARY

This article provides broad guidelines for making a diagnosis of MM, which, although a rare tumor, has a grave prognosis and invariably has medicolegal implications. The salient recommendations are use of histologic features in distinguishing benign from malignant mesothelial proliferations and the use of molecular assays, such as homozygous *p16* deletion, in challenging cases; on biopsy, subtyping should be done, but assigning a further pattern is often not possible. There is limited usefulness from cytology, histochemical stains, and electron microscopy; panels of antibodies were described, which need to be used according to the differential diagnosis in each case. In the typical case in which all features are concordant, 2 mesothelioma markers and 2 carcinoma markers may be adequate for a diagnosis; however, when there are discordant findings, additional markers should be used. The pathologist should always take the clinical, radiologic, and pathologic features into consideration and receive an expert second opinion in difficult cases, as necessary. The best pathologic predictor of prognosis is still the histologic subtype. Nuclear grading of epithelioid MM appears promising. Pathologic staging is useful as a guide to surgical therapy. Other factors affecting prognosis and response to therapy are being studied.

This article has been endorsed by the Board of the International Mesothelioma Interest Group.

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