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Guidelines for Pathologic Diagnosis of Mesothelioma

2023 Update of the Consensus Statement From the International Mesothelioma Interest Group

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• ***Context.***—**Mesothelioma is an uncommon tumor that can be difficult to diagnose.**

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

Data Sources.—**Pathologists involved in the International Mesothelioma Interest Group and others with expertise in mesothelioma contributed to this update. Reference material includes peer-reviewed publications and textbooks.**

Conclusions.—**There was consensus opinion regarding guidelines for (1) histomorphologic diagnosis of mesothelial tumors, including distinction of epithelioid, biphasic, and sarcomatoid mesothelioma; recognition of morphologic variants and patterns; and recognition of common morphologic pitfalls; (2) molecular pathogenesis of mesothelioma; (3) application of immunohistochemical markers to establish mesothelial lineage and distinguish mesothelioma from common morphologic differentials; (4) application of ancillary studies to distinguish benign from malignant mesothelial proliferations, including BAP1 and MTAP immunostains; novel immunomarkers such as Merlin and p53; fluorescence in situ hybridization (FISH) for homozygous deletion of *CDKN2A*; and novel molecular assays; (5) practical recommendations for routine reporting of mesothelioma, including grading epithelioid mesothelioma and other prognostic parameters; (6) diagnosis of mesothelioma in situ; (7) cytologic diagnosis of mesothelioma, including use of immunostains and molecular assays; and (8) features of nonmalignant peritoneal mesothelial lesions.**

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Pathologic diagnosis of mesothelioma continues to evolve. This updated guideline[1] reflects the authors' expert opinion, informed by a thorough review of relevant literature. The contents were discussed at the annual meeting of the International Mesothelioma Panel, with subsequent drafts circulated to obtain consensus among the authors. This guideline is intended to offer a practical reference for the diagnostic pathologist, rather than a mandate.

Challenges in the morphologic classification of pleural and peritoneal mesothelioma are summarized. Morphologic hallmarks and pitfalls are reviewed, acknowledging that many pathologists lack access to some or all of the immunohistochemical and molecular assays discussed here. The role of immunostains to establish mesothelial lineage, separate benign from malignant mesothelial proliferations, and distinguish mesothelioma from other malignant mimics is presented. Given the large number of studies reported from different laboratories, sensitivity and specificity figures quoted in this article for different immunostains should be regarded as average figures obtained by literature review. The evolving role of diagnostic molecular assays is also addressed. Additional topics include recommendations regarding reporting the diagnosis of mesothelioma, the evolving concept of mesothelioma in situ, the role of cytopathology in diagnosis of mesothelioma, and features of nonmalignant peritoneal mesothelial lesions.

Approximately 85% to 90% of mesotheliomas arise in the pleura, with most of the remaining 10% to 15% affecting the peritoneum. Primary pericardial and paratesticular mesothelioma each account for roughly 1%, and considerations specific to these rare locations are not addressed. Location of the tumor (pleural versus peritoneal, or rarely pericardial, paratesticular, or at a metastatic site) and sex of the patient affect the differential diagnosis and thus the diagnostic approach. Regardless of site, a diagnosis of mesothelioma should always be based on compatible morphologic and immunohistochemical results obtained from an adequate tissue sample (typically a biopsy; less often an effusion, exfoliative, or fine-needle aspiration cytology specimen), in the context of appropriate clinical, radiographic, and (when available) surgical findings. A history of asbestos exposure should not be taken into consideration by the pathologist when confirming or excluding mesothelioma. Molecular studies might be necessary in a minority of cases.

When "mesothelioma" is diagnosed without further qualification, it is generally understood to mean diffuse mesothelioma, which represents 99% of pleural and peritoneal mesotheliomas and is characterized by disseminated involvement of the serosal-lined cavity. In contrast, localized mesothelioma (accounting for just 1% of cases) presents as a solitary, circumscribed pleural- or peritoneal-based mass, ranging from 0.5 to 20 cm, with negative effusion cytology. Localized and diffuse mesothelioma are indistinguishable under the microscope, with approximately the same distribution of epithelioid, biphasic, and sarcomatoid tumors and comparable molecular profiles.[2,3] Nonetheless, this distinction is clinically important, as localized mesothelioma has a more favorable prognosis than diffuse mesothelioma. Correlation with clinical and radiographic findings is necessary in all cases of mesothelioma to differentiate localized from diffuse process.

MORPHOLOGIC CLASSIFICATION OF MESOTHELIAL TUMORS

The 2021 World Health Organization (WHO) classification of mesothelioma[4] retains the 3 major histologic subtypes—epithelioid, biphasic, and sarcomatoid—but incorporates several architectural patterns and cytologic and stromal features that are prognostically significant. The diagnostic term *mesothelioma* is recommended, rather than *malignant mesothelioma*. To avoid confusion, the lesion previously termed *well-differentiated papillary mesothelioma* has been renamed *well-differentiated papillary mesothelial tumor*. As before, the term *multicystic mesothelioma* is discouraged, in favor of *(multiloculated) peritoneal inclusion cyst.*[5] In short, the term *mesothelioma* now applies only to malignant tumors.

Epithelioid Mesothelioma

Epithelioid mesothelioma accounts for 60% to 70% of pleural and 80% to 90% of peritoneal mesotheliomas.[6–9] These tumors comprise polygonal, oval, or cuboidal cells that often mimic nonneoplastic, reactive mesothelial cells. Epithelioid mesothelioma exhibits several generally familiar architectural patterns, including tubulopapillary (Figure 1), trabecular (Supplemental Figure 1, see the supplemental digital content file, containing 18 figures and 2 tables), micropapillary (Figure 2), adenomatoid (Figure 3), and solid (Figure 4). The identified architectural patterns should be reported for each tumor in both biopsy and resection specimens, and in definitive resection specimens (ie, extended pleurectomy/decortication or extrapleural pneumonectomy); percentage representation (to the nearest 10%) of each pattern should also be reported.[10]

Tubulopapillary, trabecular, and adenomatoid patterns are associated with a more favorable prognosis. Conversely, any micropapillary component or at least 50% solid pattern is associated with worse prognosis.[11] Micropapillary pattern correlates with a higher incidence of lymphatic invasion. Necrosis is seen in 30% of epithelioid mesotheliomas and is associated with a worse prognosis.[12]

Variant cytologic and stromal features are recognized for epithelioid mesothelioma, and familiarity facilitates proper diagnosis. Those features of known prognostic significance should be reported when present.

Myxoid Stromal Features.—Rare epithelioid mesotheliomas comprise clusters of mildly atypical tumor cells in a matrix of loose myxoid stroma (Supplemental Figure 2). Epithelioid mesotheliomas with at least 50% myxoid morphology and less than 50% solid growth pattern have a favorable prognosis.[13,14]

Rhabdoid Cytologic Features.—Rhabdoid cytologic features are prognostically unfavorable, defined by a variable proportion (15%–75%) of tumor cells morphologically similar to those of rhabdomyoblastic tumors, containing cytoplasmic globules that express cytokeratins but are negative for myogenin (Supplemental Figure 3).[15]

Pleomorphic Cytologic Features.—Epithelioid mesotheliomas with pleomorphic cytomorphology—defined by nuclear enlargement, hyperchromasia, prominent nucleoli, and (often) multinucleation, forming at least 10% of the tumor—behave like sarcomatoid and biphasic mesotheliomas.[16,17] The 2021 WHO classification recommends that tumors with pleomorphic cytomorphology (Supplemental Figure 4) be classified as epithelioid, biphasic, or sarcomatoid, based on the remaining tumor cell morphology, although the

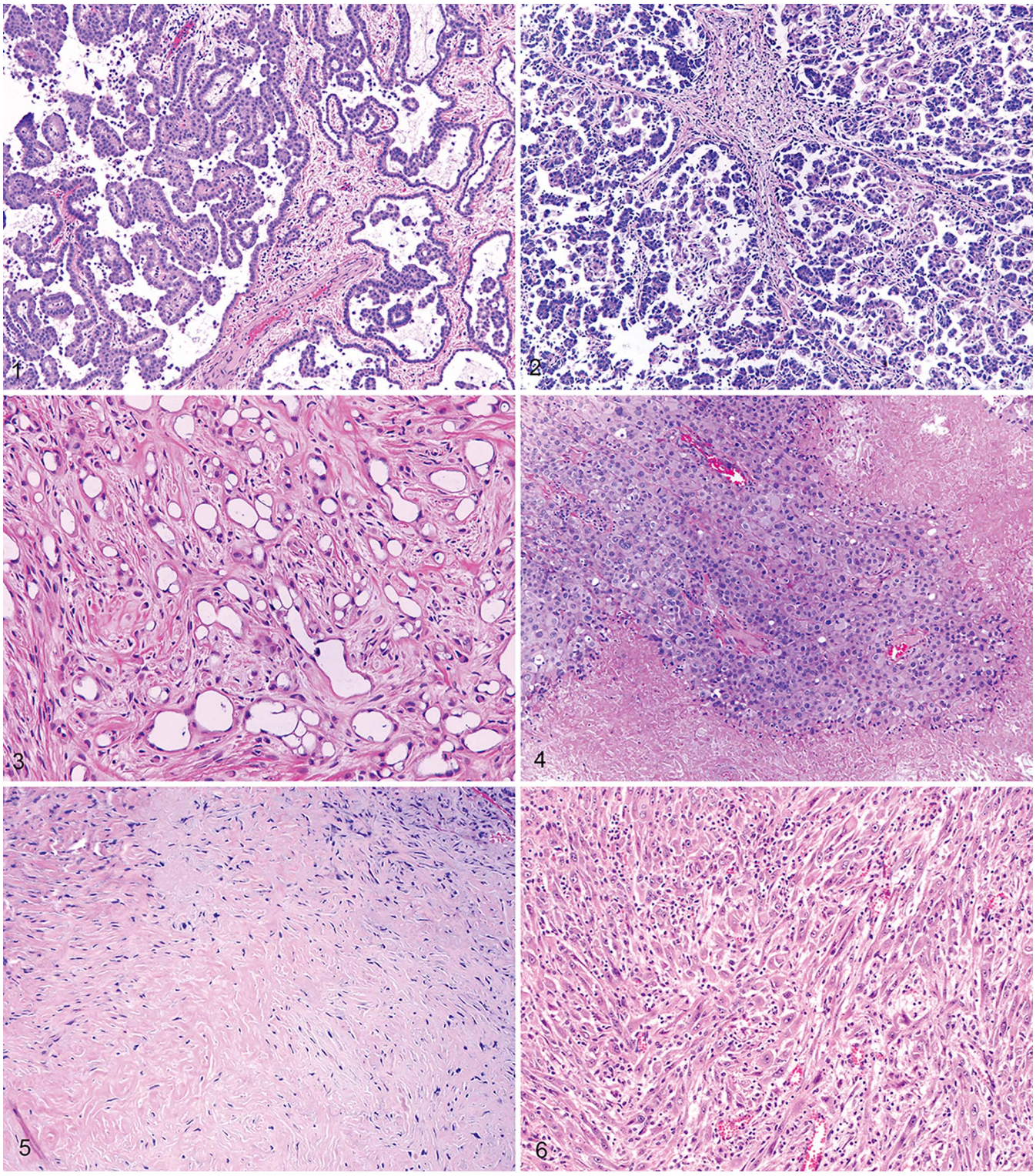

Figure 1. *Epithelioid mesothelioma, tubulopapillary architecture (hematoxylin-eosin, original magnification ×100).*

Figure 2. *Epithelioid mesothelioma, micropapillary architecture (hematoxylin-eosin, original magnification ×100).*

Figure 3. *Epithelioid mesothelioma, adenomatoid architecture (hematoxylin-eosin, original magnification ×200).*

Figure 4. *Epithelioid mesothelioma, solid architecture, with necrosis (hematoxylin-eosin, original magnification ×100).*

Figure 5. *Desmoplastic mesothelioma (hematoxylin-eosin, original magnification ×100).*

Figure 6. *Transitional mesothelioma (hematoxylin-eosin, original magnification ×100).*

presence of a pleomorphic component should be documented and its poor prognostic significance noted.[4,18]

Lymphohistiocytoid Cytologic Features.—Mesothelioma with lymphohistiocytoid features is defined by polygonal tumor cells morphologically similar to histiocytes, admixed with a marked lymphoid infiltrate (Supplemental Figure 5, A). Immunostains might be necessary to distinguish this entity from a nonneoplastic inflammatory process, lymphoepithelial carcinoma, or lymphoma. "Lymphohistiocytoid features" does not simply refer to markedly inflamed mesothelioma; the tumor cells must show histiocytoid morphology. Mesothelioma with lymphohistiocytoid features can be classified as epithelioid, biphasic, or sarcomatoid from the morphology of the non-lymphohistiocytoid component. Lymphohistiocytoid features in sarcomatoid mesothelioma impart a favorable prognosis, although prognostic significance is less clear for epithelioid tumors.[19]

Clear Cell, Deciduoid, Signet Ring, Small Cell, and Adenoid Cystic Features.—These features are not prognostically significant (Supplemental Figure 5, B; Supplemental Figure 6). However, familiarity with these morphologies facilitates distinction, respectively, from clear cell carcinomas, sarcoma, and melanoma[20]; florid deciduosis or deciduoid carcinomas[21]; signet ring cell adenocarcinomas of the lung and gastrointestinal tract[22]; small cell carcinomas, sarcomas, and lymphomas[23]; and adenoid cystic carcinoma. Note that mesotheliomas with small cell–like morphology do not show true neuroendocrine differentiation, nor do they stain with neuroendocrine markers, and use of the term *small cell mesothelioma* is discouraged, to avoid confusion with small cell carcinoma.

Sarcomatoid Mesothelioma

Sarcomatoid mesotheliomas account for 5% to 15% of pleural and less than 5% of peritoneal mesotheliomas.[8,9,24] They are composed of infiltrating sheets of spindle cells with variable cytologic atypia (Supplemental Figure 7). Tumors can show necrosis, atypical mitoses, and/or heterologous (including rhabdomyosarcomatous, osteosarcomatous, and chondrosarcomatous) elements, which when extensive must be distinguished from true sarcomas.[25] Sarcomatoid mesothelioma has a significantly poorer prognosis than epithelioid mesothelioma.[12,24,26]

Desmoplastic Mesothelioma.—Desmoplastic mesothelioma is a pattern of sarcomatoid mesothelioma characterized by a hypocellular population of bland spindle cells, arranged in a haphazard (patternless) fashion between bands of dense collagenous stroma (Figure 5). While the stroma is similar to that seen in pleural plaque, the tumor cells are arranged in a haphazard fashion as opposed to a linear arrangement usually parallel to the surface in the latter.[27] Desmoplastic mesothelioma might not be suspected unless frankly sarcomatoid areas or areas of invasion into adipose tissue or lung are found. A diagnosis of "desmoplastic mesothelioma" requires desmoplastic features in at least 50% of a tumor in a definitive resection specimen. If desmoplastic morphology is seen in a smaller biopsy specimen, the diagnostic qualification "with desmoplastic features" is recommended.

Transitional Features.—Transitional features in mesothelioma are an uncommon and recently described pattern, defined by sheetlike growth and cytomorphology intermediate between sarcomatoid and epithelioid tumor cells, with elongated but plump and cohesive cells (Figure 6). These were likely variably classified historically, but they are now regarded as a pattern of sarcomatoid morphology, as their prognosis and transcriptomic profile is similar to sarcomatoid mesotheliomas.[28,29] Reticulin staining can be helpful in identifying the transitional pattern, as staining is present around individual cells as in sarcomatoid mesothelioma, in contrast to surrounding clusters of cells in the epithelioid subtype.[29]

Biphasic Mesothelioma

Biphasic mesothelioma accounts for 15% to 30% of pleural and 10% to 20% of peritoneal mesotheliomas.[6–9] These tumors contain malignant epithelioid and sarcomatoid components (Supplemental Figure 8), with transitional morphology regarded as sarcomatoid in this context (ie, if transitional features are seen in an otherwise epithelioid mesothelioma, a diagnosis of biphasic mesothelioma should be rendered). In a definitive resection specimen, at least 10% of each component must be present to render the diagnosis of biphasic mesothelioma. In biopsy specimens, biphasic mesothelioma should be diagnosed if malignant epithelioid and sarcomatoid components are present, regardless of the percentage of each component (ie, <10% epithelioid or sarcomatoid component does not preclude a diagnosis of biphasic mesothelioma in a biopsy—this change in the 2021 WHO classification[4] reflects the typically higher percentage of sarcomatoid morphology seen in resection versus biopsy specimens).[7,11,30] In peritoneal tumors, some evidence suggests that even focal (<10%) sarcomatoid morphology imparts a poorer prognosis than is typical of epithelioid peritoneal mesothelioma,[31] so it is prudent to note even focal (<10%) sarcomatoid growth in resected peritoneal mesotheliomas.

The prognosis of biphasic mesothelioma is intermediate between that of pure epithelioid and pure sarcomatoid mesothelioma.[8,32] While data are limited, there is some suggestion that biphasic mesotheliomas with predominant (>50%) sarcomatoid morphology have a poorer prognosis,[33,34] so the percentage contribution of the epithelioid component and the sarcomatoid component should be reported in biopsies and resections of biphasic mesothelioma.

Historically, expert pathologists showed moderate interobserver agreement in diagnosis of biphasic mesothelioma, though improved agreement can be attained through application of strict diagnostic criteria.[28,34,35] Epithelioid mesothelioma associated with a reactive spindled mesothelial population presents a challenging differential for biphasic mesothelioma, particularly when the spindled component lacks overtly atypical morphologic features. In this setting, immunohistochemical and molecular studies may help define the spindled mesothelial component as malignant (see Ancillary Studies in Diagnosis of Biphasic Mesothelioma below).

MOLECULAR PATHOGENESIS

Although an exhaustive review of the molecular biology of mesothelioma is beyond the scope of this guideline, a brief overview is provided as context for subsequent discussion of diagnostic molecular assays and immunostains. The genes most commonly affected by somatic mutation and copy number alteration in mesothelioma are *BAP1*, *NF2*, *CDKN2A*, *TP53*, *LATS1/2*, and *SETD2*, with mutation rates in particular genes varying by both tumor site and

histotype.[36,37] Gene fusions are rare in mesothelioma, though a subset of peritoneal mesotheliomas in young patients harbor *ALK* rearrangements or *EWSR1*::*ATF1* or *EWSR1/FUS*::*CREB* fusions, while rare cases of *EWSR1*::*YY1* fusion are reported in peritoneal mesothelioma in middle-aged patients.[38–42] *ALK* rearrangements are very rarely detected in pleural mesothelioma.[43,44]

BAP1

BRCA1-associated protein-1 (BAP1) is a tumor suppressor gene encoding a deubiquitylase with roles in cell cycle regulation, cellular differentiation, cell death, and DNA damage response.[45] *BAP1* is inactivated in approximately 60% of pleural and 70% of peritoneal mesotheliomas by missense, truncating, and splice site mutations; truncating fusion events; and copy number loss via chr 3p21.1 deletion. *BAP1* mutations appear to be an early event in mesothelioma pathogenesis, representing the most common molecular alteration in reported cases of mesothelioma in situ (see Mesothelioma In Situ below). *BAP1* alterations are found more often in epithelioid than in sarcomatoid tumors.[46] Germline *BAP1* mutations produce the *BAP1* tumor predisposition syndrome (see Germline Predisposition to Mesothelioma below).

CDKN2A

CDKN2A (*p16*) resides on the chr 9p21 locus alongside *CDKN2B* (*p15INK5*), *p14RF*, and *MTAP*. Homozygous deletion of *CDKN2A* is found in approximately 70% of pleural mesotheliomas (including 90%–100% of sarcomatoid mesotheliomas and 40%–70% of epithelioid and biphasic types) but just 10% to 15% of peritoneal mesotheliomas.[47–49] *CDKN2A* point mutations are exceptionally rare in mesothelioma.[48] Like *BAP1* alterations, *CDKN2A* deletions are an early event in mesothelioma pathogenesis and are occasionally detected in mesothelioma in situ (see Mesothelioma In Situ below). Some evidence suggests that *CDKN2A* deletion plays a role in evolution from epithelioid to biphasic mesothelioma in some cases.[48] Approximately 75% to 90% of mesotheliomas with *CDKN2A* deletion show codeletion of the neighboring *MTAP* gene,[47,49] which can therefore be used as an immunohistochemical surrogate for *CDKN2A* deletion.

NF2

NF2, located in the chromosomal region 22q12, encodes Merlin, a tumor suppressor protein in the Hippo signaling pathway.[50] *NF2* inactivation in mesothelioma is predominantly via truncating mutations or gene deletion, and is somewhat more common in biphasic and sarcomatoid (~70%) than in epithelioid (~40%) tumors.[48] *NF2* inactivation appears to be a fairly late event in mesothelioma pathogenesis, and intratumoral heterogeneity for *NF2* alterations is common.[51]

ESTABLISHING MESOTHELIAL LINEAGE

Reactive and malignant mesothelial proliferations can overlap morphologically with nonmesothelial lesions and malignancies. Establishing mesothelial lineage is a crucial early step in proper diagnosis of a serosal lesion. Although mesothelial cells show certain characteristic morphologic features, panels of "mesothelial" and "epithelial" immunostains are now almost universally applied in routine diagnosis to confirm the morphologic impression of mesothelial differentiation. Markers of specific epithelial lineages also play a role, depending on the clinical and morphologic differential diagnosis. Rarely, the differential for mesothelioma includes nonepithelial (eg, mesenchymal, hematolymphoid) tumors, to which the immunopanel must be tailored.

Before undertaking immunostains for evaluation of mesothelioma, the responsible laboratory should have performed a rigorous validation to determine ideal conditions for routine use in their hands. Immunostains should be interpreted with caution in minute biopsies, in those with crush artifact (which may induce false-positive or false-negative staining), and around the edges of biopsy specimens (which may show artifactual positive immunostaining). Careful attention should be paid to avoid misinterpretation of immunostaining in entrapped benign mesothelial or epithelial structures.

Broad-Spectrum Cytokeratin

Immunohistochemical stains for broad-spectrum cytokeratin (eg, pancytokeratin, AE1/AE3, CAM 5.2, CK OSCAR) are highly sensitive for mesothelioma, including sarcomatoid mesothelioma. In one large study, 93% of sarcomatoid mesotheliomas exhibited immunoreactivity for at least 1 cytokeratin; that percentage may be even higher if a cytokeratin cocktail is used, there is adequate sampling of the tumor, and the tissue is well fixed.[24] For sarcomatoid neoplasms, cytokeratin positivity is additionally useful in excluding spindle cell sarcoma or melanoma,[52] although rare sarcomas and melanomas can be positive for cytokeratins, and areas of heterologous differentiation in mesothelioma are often cytokeratin negative. Note that reactive mesothelial stroma is also keratin positive; keratin stain does not differentiate benign from malignant mesothelial proliferations.

Broad-spectrum cytokeratins are virtually 100% sensitive for epithelioid mesothelioma. If an epithelioid malignant neoplasm causing diffuse serosal thickening is negative for multiple broad-spectrum cytokeratins, other diagnoses should be considered, such as melanoma, epithelioid hemangioendothelioma or angiosarcoma, and lymphoma.

Occasional tumors do not stain with any marker. This often reflects artifact, such as overfixation in formalin, or alcohol fixation followed by antigen retrieval (commonly used for cytology specimens), so some knowledge about the fixative is important, as is proper laboratory validation for alcohol-fixed tissue.[53–55] Assessment of internal controls is helpful. If needed, vimentin can be used to assess baseline immunoreactivity of the tissue.

Mesothelial and Epithelial Immunomarkers

The most common morphologic differential diagnosis for mesothelioma is carcinoma, and panels of mesothelial and epithelial immunomarkers are routinely used to establish mesothelial lineage and distinguish benign or malignant mesothelial proliferations from epithelial mimics. The specific markers used will depend on the differential diagnosis, and as noted above, nonepithelial (eg, mesenchymal, melanocytic, or hematolymphoid) tumors may occasionally enter the differential, requiring appropriate immunopanel modifications.

The best-characterized and most common mesothelial markers include calretinin (Supplemental Figure 9), CK5 or CK5/6 (Supplemental Figure 10), WT1 (Wilms tumor-1;

Supplemental Figure 11), and podoplanin (D2-40) (Supplemental Figure 12). Each of these markers shows greater than 80% sensitivity for epithelioid mesothelial proliferations, with lower sensitivity for sarcomatoid proliferations.[56,57] HEG1 is a promising mesothelial marker for epithelioid tumors, with similar sensitivity to other mesothelial markers, and possibly greater specificity in the differential with carcinoma of the lung.[58] Importantly, none of these markers is entirely specific for mesothelial origin, and all can be positive (usually focal, though sometimes diffuse) in a subset of carcinomas.[56] Further, different mesothelial immunostains show different patterns of positive staining. For calretinin, combined cytoplasmic and nuclear staining is typically present in mesothelial cells. For WT1, only nuclear staining is considered positive, and cytoplasmic-only staining should be disregarded. CK5/6 is cytoplasmic, and D2-40 and HEG1 show membranous staining. While there is no validated standard for the percentage tumor cell staining required to be called "positive," using 10% seems like a reasonable minimum.

The most common and generally most reliable epithelial markers are claudin-4 (Supplemental Figure 13), MOC-31 (Supplemental Figure 14), and Ber-EP4, all of which show membranous staining. Of note, claudin-4 may occasionally show dotlike cytoplasmic reactivity, which should be interpreted as negative. A variety of older markers (including CEA [carcinoembryonic antigen], CD15 [LeuM1], BG-8, and B72.3) also remain reliable options. When properly validated, each of these markers is greater than 80% sensitive for epithelial lineage (albeit less sensitive for sarcomatoid carcinoma) and greater than 80% specific in distinction from mesothelial tissues.[56,57] Note that diffuse MOC-31 and Ber-EP4 expression are more specific for epithelial lineage than patchy staining, as ~10% to 15% of mesotheliomas show patchy MOC-31 or Ber-EP4 staining.[56,59]

Since none of these markers are perfectly sensitive or specific, it is recommended that, in addition to broad-spectrum cytokeratin, 2 mesothelial and 2 epithelial markers be included in a first-line immunopanel to establish mesothelial lineage. If results are concordant, the diagnosis can be considered established. If discordant, the immunopanel can be expanded for a second round of staining, with additional antibodies selected according to the differential diagnosis. A different tissue block can also be stained, if available. Given the range of reliable options and the likelihood of interlaboratory variation, no specific first-line antibody panel is recommended. Instead, each laboratory should test staining conditions for the antibodies of choice, ideally verifying sensitivity and specificity of at least 80% with appropriate controls.

Emerging evidence suggests that the novel mesothelial marker HEG1 and the epithelial marker claudin-4 may be sufficiently sensitive and specific to be used as a 2-marker panel to distinguish epithelioid mesothelioma from non–small cell lung carcinoma.[58] In the authors' experience, claudin-4 is sufficiently reliable to serve as the sole epithelial marker in most differentials[59] (though claudin-4 is not expressed in proximal renal tubules or hepatocytes and is therefore not highly sensitive for renal cell or hepatocellular carcinoma). Despite its high sensitivity, HEG1 is not yet in widespread clinical use, and its expression in 50% of serous ovarian cancers and 100% of thyroid cancers limits its application. At present, it seems prudent to continue using immunopanels to establish mesothelial lineage.[59–62]

Markers Useful in Specific Differentials

In addition to the broad-spectrum epithelial markers discussed above, immunomarkers specific to particular types of carcinoma are useful in certain differential diagnoses.

Immunohistochemistry in Diagnosis of Epithelioid Mesothelioma.—Tables 1 and 2 list markers that are useful in distinguishing epithelioid pleural mesothelioma from adenocarcinoma and squamous cell carcinoma of the lung, respectively. TTF-1 (thyroid transcription factor-1; 8G73/1 DAKO clone) and Napsin A are highly specific for lung adenocarcinoma in the differential with epithelioid mesothelioma.[63,64] WT1 is expressed in just 2% of squamous cell carcinomas and is therefore highly specific for epithelioid mesothelioma in this differential,[65] while claudin-4 and p40 (and, to a lesser degree, p63) are specific for squamous cell carcinoma in this setting.[65–69] p40 also assists in distinguishing squamous cell carcinoma from adenocarcinoma.[70]

Because most breast carcinomas express estrogen receptor, gross cystic disease fluid protein-15, and/or mammaglobin, these markers are often useful in distinguishing metastatic breast carcinoma and mesothelioma.[71] Calretinin and CK5/6 can be positive in high-grade basal-type breast carcinomas, which may also be negative for estrogen and progesterone receptor.[72,73] SOX10 expression favors breast cancer in this scenario. One-third to one-half of epithelioid mesotheliomas are positive for GATA3, limiting its usefulness in this scenario.[71,74]

Supplemental Table 1 lists markers that are considered useful in distinguishing mesothelioma and metastatic renal cell carcinoma. Because of their sensitivity and specificity, calretinin, D2-40, and CK5/6 are the best mesothelial markers in this context.[75] A panel of epithelial markers may be necessary, as claudin-4, MOC-31, and Ber-EP4 are expressed in 90%, 40%, and 40% of renal cell carcinomas, respectively. Carbonic anhydrase IX is expressed in virtually all epithelioid pleural mesotheliomas and therefore not useful to distinguish it from renal cell carcinomas.[76] PAX8 or PAX2 can be very useful, as these are expressed in most renal cell carcinomas but not in pleural mesotheliomas,[76–78] though PAX8 (using both polyclonal and monoclonal antibodies) is positive in ~15% of peritoneal mesotheliomas.[79] The sensitivity and specificity of renal cell carcinoma (RCC) marker and CD15 for renal cell carcinoma are not high.[76] Most renal cell carcinomas express CD10, but half of epithelioid mesotheliomas are also positive.[80]

Serous ovarian carcinomas are virtually always positive for WT1 and may be positive for HEG1 (50%), CK5/6 (~30%), and D2-40 (~20%).[56,81] Conversely, as noted above, ~15% of peritoneal mesothelioma are PAX8 positive, more often in women (25%) than men (5%).[79,82] Estrogen and progesterone receptor expression is rare in peritoneal mesothelioma (7% and 2%, respectively) (Table 3).[82] Strong diffuse p53 (ie, >80% staining throughout tumor) does not exclude peritoneal mesothelioma, as 15% harbor *TP53* mutation.[83]

Adenocarcinomas of the gastrointestinal tract (Supplemental Table 2) and prostate can be distinguished from epithelioid mesotheliomas by the demonstration of CDX2[84] and prostate-specific antigen (and more recently NKX3.1),[85]

Table 1. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Epithelioid Mesothelioma Versus Lung Adenocarcinoma[a]

Marker	Current Value/Comments
Epithelioid mesothelioma (Positive mesothelioma markers)	
Calretinin	Positive in 95% of epithelioid mesotheliomas; staining is often strong and diffuse and must be both nuclear and cytoplasmic; 5%–10% of lung adenocarcinomas are positive, usually focal
Cytokeratin 5 or 5/6	Positive in 91% of epithelioid mesotheliomas; 5%–20% of lung adenocarcinomas are positive, usually focal
WT1	Positive (nuclear) in 88% of epithelioid mesotheliomas; lung adenocarcinomas virtually always negative
D2-40 (podoplanin)	Positive (membranous) in 93% of epithelioid mesotheliomas; ~3% of lung adenocarcinomas focally positive
HEG1	Positive (membranous) in 94% of epithelioid mesotheliomas; lung adenocarcinomas virtually always negative
Lung adenocarcinoma (Positive carcinoma markers)	
Claudin-4	Positive (punctate or continuous membranous staining) in 99% of lung adenocarcinomas, usually strong and diffuse; mesotheliomas virtually always negative
CEA	Positive in 84% of lung adenocarcinomas; <5% of epithelioid mesotheliomas positive, typically focal
TTF-1	Positive (nuclear) in 82% of lung adenocarcinomas, with virtually all nonmucinous lung adenocarcinomas positive; mesotheliomas are negative (8G7G3/1 DAKO clone most specific)
Napsin A	Positive (granular cytoplasmic staining) in 83% of lung adenocarcinomas; mesotheliomas virtually always negative
B72.3	Positive in 85% of lung adenocarcinomas; 2% of epithelioid mesotheliomas positive
BG8	Positive in 96% of lung adenocarcinomas; 7% of epithelioid mesotheliomas positive, typically focal
MOC-31	Positive in 92% of lung adenocarcinomas; 8% of epithelioid mesotheliomas (or, in one recent study,[59] up to 35%) are positive, usually focal
Ber-EP4	Positive in 96% of lung adenocarcinomas; 15% of epithelioid mesotheliomas (or, in one recent study,[59] up to 35%) are positive, usually focal

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

[a] Data derived from Chapel et al.[56,57]

respectively. SATB2 (a marker of colorectal adenocarcinoma and osteosarcoma) is typically negative in mesothelioma.[86]

Immunohistochemistry in Diagnosis of Sarcomatoid Mesothelioma.—The role of broad-spectrum cytokeratin immunostains in diagnosis of sarcomatoid mesothelioma is discussed above. In a cytokeratin-positive sarcomatoid malignancy, distinguishing sarcomatoid mesothelioma from sarcomatoid carcinoma requires a panel of immunomarkers, as sarcomatoid mesotheliomas are often negative for 1 or more mesothelial markers, and none of these markers is entirely specific. D2-40 and calretinin are each expressed in 50% to 60% of sarcomatoid mesotheliomas,[24,87–89] though staining may be focal, and specificity is limited, as D2-40 and calretinin each stain ~20% to 25% of sarcomatoid lung carcinomas. D2-40 reactivity in entrapped lymphatics or reactive mesothelial elements is a potential pitfall. Claudin-4, MOC31, and BerEP4 also show low sensitivity for sarcomatoid areas of carcinomas—approximately 33%, 38%, and 23%, respectively, across studies.[59] Positive staining for TTF-1, Napsin A, or p40/p63 supports a diagnosis of sarcomatoid lung carcinoma. Diffuse GATA3 expression is seen in 70% of sarcomatoid mesotheliomas, but focal GATA3 expression can also be seen in sarcomatoid lung carcinoma,[90–92] while 29% of sarcomatoid urothelial carcinomas and 50% of sarcomatoid (metaplastic) breast carcinomas express GATA3. PAX8 is positive in 44% to 69% of sarcomatoid renal cell carcinomas but generally negative in sarcomatoid mesothelioma.[93] Conversely, sarcomatoid renal cell carcinoma is reportedly negative for CK5/6 and calretinin, though CK5/6 is particularly limited by its low sensitivity for sarcomatoid mesothelioma.[75]

Synovial sarcoma is characteristically cytokeratin positive, and angiosarcoma and melanoma may also express cytokeratins. The diagnosis of synovial sarcoma can be confirmed with mutation-specific SS18-SSX and SSX-C-terminus immunostains, or with molecular confirmation of the distinctive X;18 translocation. Immunohistochemistry for TLE-1 can be used but is less specific.[94]

After extensive workup and with appropriate clinical and radiologic features, 5% to 10% of sarcomatoid mesotheliomas are cytokeratin negative.[25,89,95,96] In the absence of convincing cytokeratin positivity, positive calretinin and/or D2-40 staining should not be interpreted as evidence of mesothelial differentiation, as these markers are variably positive in some sarcomas (including synovial sarcoma, malignant peripheral nerve sheath tumor, and angiosarcoma),[87,97] for which additional immunohistochemical markers would be warranted. The expanded differential might include epithelioid hemangioendothelioma, angiosarcoma, liposarcoma, myogenic or neurogenic sarcoma, undifferentiated pleomorphic sarcoma, malignant solitary fibrous tumor, melanoma, and histiocytic sarcomas. Morphology and clinical context should guide the

Table 2. Immunohistochemical Markers Used in the Differential Diagnosis of Pleural Epithelioid Mesothelioma Versus Squamous Cell Carcinoma of the Lung[a]

Marker	Current Value/Comments
Epithelioid mesothelioma (Positive mesothelioma markers)	
Calretinin	Positive in 95% of epithelioid mesotheliomas; staining is often strong and diffuse and must be both nuclear and cytoplasmic; 40% of lung squamous cell carcinomas positive, usually focal
CK5/6	Positive in 91% of epithelioid mesotheliomas; 98% of lung squamous cell carcinomas positive
WT1	Positive (nuclear) in 88% of epithelioid mesotheliomas; 2% of lung squamous cell carcinomas are positive
D2-40 (podoplanin)	Positive (membranous) in 93% of epithelioid mesotheliomas; 60% of lung squamous cell carcinomas positive
HEG1	Positive (membranous) in 94% of epithelioid mesotheliomas; negative in lung squamous cell carcinomas
Lung squamous cell carcinoma (Positive carcinoma markers)	
Claudin-4	Positive (punctate or continuous membranous staining) in 95% of lung squamous cell carcinomas; mesotheliomas virtually always negative
CEA	Positive in 92% of lung squamous cell carcinomas; <5% of epithelioid mesotheliomas positive, typically focal
p40 or p63	Positive (nuclear) in >95% of lung squamous carcinomas, typically strong and diffuse; 5% and 15% of epithelioid mesotheliomas are positive for p40 and p63, respectively
BG8	Positive in 80% of lung squamous cell carcinomas; 7% of epithelioid mesotheliomas positive, typically focal
MOC-31	Positive in 91% of lung squamous carcinomas; 8% of epithelioid mesotheliomas (or, in one recent study,[59] up to 35%) are positive, usually focal
Ber-EP4	Positive in 87% of lung squamous carcinomas; 8% of epithelioid mesotheliomas (or, in one recent study,[59] up to 35%) are positive, usually focal

Abbreviations: BG8, blood group 8; CEA, carcinoembryonic antigen; CK, cytokeratin; WT1, Wilms tumor-1.

[a] Data derived from Chapel et al.[56,57]

differential and selection of appropriate immunohistochemical stains. Note that muscle-specific actin (HHF-35) and α-smooth muscle actin are often positive (occasionally diffusely) in sarcomatoid mesothelioma,[98] though desmin expression is quite rare in sarcomatoid mesothelioma.[98,99] Rare tumors cause diffuse pleural thickening, show extensive heterologous differentiation (eg, osteosarcomatous), and are negative for cytokeratin and mesothelial markers. After exclusion of a separate primary, these can be regarded as consistent with mesothelioma.

Table 3. Peritoneal Mesothelioma Versus Serous Ovarian Carcinoma[a]

	Mesothelioma Markers
Calretinin	Positive in 85%–100% of peritoneal mesotheliomas; 5% of SOCs positive
Podoplanin (D2-40)	Positive in 93%–96% of peritoneal mesotheliomas but also 20% of SOCs
CK5/6	Positive in 53%–100% of peritoneal mesotheliomas but also 30% of SOCs
WT1	Positive in 95% of peritoneal mesotheliomas and virtually 100% of SOCs
HEG1	Positive in 50% of SOCs
	Epithelial Markers in Serous Ovarian Carcinoma
Claudin-4	Positive in 98% of SOCs. Negative in peritoneal mesotheliomas
MOC-31	Positive in 98% of SOCs and just 5% of peritoneal mesotheliomas
BG8	Positive in 73% of SOCs and 3%–9% of peritoneal mesotheliomas
Estrogen receptor	Positive in 60%–93% of SOCs; positive in 7% of peritoneal mesotheliomas
Progesterone receptor	Positive in most SOCs but only 2% of peritoneal mesotheliomas
PAX8	Positive in virtually all SOCs; 15% of peritoneal mesotheliomas positive (including 25% of peritoneal mesotheliomas in women)
Ber-EP4	Positive in 98% of SOCs and 9%–13% of peritoneal mesotheliomas
B72.3	Positive in 80% of SOCs, though often only focal; 0%–3% of peritoneal mesotheliomas positive
CEA	Positive in 10% of SOCs and 0% peritoneal mesothelioma, but sensitivity in SOCs is too low compared with other choices

Abbreviations: BG8, blood group 8; CEA, carcinoembryonic antigen; CK, cytokeratin; SOC, serous ovarian carcinoma; WT1, Wilms tumor-1.

[a] Data derived from Chapel et al.[56,57]

Histochemical Stains for Cytoplasmic Mucin

In current practice, histochemical staining is rarely used by experienced practitioners to distinguish mesothelioma from carcinoma (eg, in tumors expressing contradictory immunohistochemical markers). The cytoplasmic epithelial mucin in adenocarcinomas is positive by periodic acid–Schiff after diastase digestion (PAS-D) or by Alcian blue after hyaluronidase treatment. In contrast, cytoplasmic vacuoles in mesothelioma are generally negative by PAS-D, and the cytoplasmic hyaluronic acid in mesotheliomas stains positively with Alcian blue but is digestible by hyaluronidase.[100] PAS-D can be positive in hyaluronidase crystals. Mucicarmine can be positive in mesothelioma or adenocarcinoma and is not recommended for this distinction.

Electron Microscopy

The ultrastructural features of mesothelioma are well described.[101] However, the advent of novel immunomarkers has reduced the role of electron microscopy in routine diagnosis. Electron microscopy occasionally helps establish a diagnosis of mesothelioma when immunohistochemistry is equivocal, though tumors without diagnostic morphologic and immunophenotypic features of mesothelioma frequently lack specific ultrastructural findings, as well.[102,103] Formalin-fixed material retrieved from a paraffin block may be satisfactory, as microvilli and tonofilament bundles tend to be preserved. As routine diagnostic experience with electron microscopy wanes, such cases are likely best referred to subspecialists with expertise in this domain.

DISTINGUISHING BENIGN VERSUS MALIGNANT MESOTHELIAL PROLIFERATIONS

Separating benign from malignant mesothelial proliferations requires certainty that the process is mesothelial (see Establishing Mesothelial Lineage above). The diagnostic approach used when distinguishing reactive mesothelial hyperplasia from epithelioid mesothelioma differs from that used when distinguishing fibrous pleuritis from desmoplastic mesothelioma.[104] While morphology is paramount, a supportive immunophenotype is necessary for a definitive diagnosis of mesothelioma. The role of molecular studies in routine diagnosis is also evolving.

Reactive Mesothelial Hyperplasia Versus Epithelioid Mesothelioma

Reactive mesothelial proliferations may mimic mesothelioma, as they can show high cellularity, numerous mitoses, cytologic atypia, necrosis, papillary formations, and mesothelial entrapment within fibrosis, mimicking invasion (Figure 7).[104] Morphologic features that help distinguish reactive mesothelial hyperplasia from mesothelioma are summarized in Table 4.

Demonstration of tissue invasion is a key feature in diagnosis of mesothelioma (Supplemental Figure 15). Invasion by mesothelioma is often subtle, involving only a few layers of collagenous tissue subjacent to the mesothelial space and eliciting no obvious desmoplastic reaction. Invasion may be highlighted with immunostains, such as pancytokeratin or calretinin. However, inflammatory pleural processes can entrap mesothelial cells in granulation tissue deep to the pleura, typically arranged parallel to the pleural surface. Tubular collections of reactive mesothelial cells may also be seen, again parallel to the pleural surface. These patterns do not connote malignancy. Diagnosis is best performed in a well-oriented specimen (ie, cut perpendicularly to the pleural surface, including the full thickness of the pleura with adjacent adipose tissue, skeletal muscle, and/or lung parenchyma), as a tangential section (ie, taken parallel to the pleural surface) can give a false impression of a full-thickness mesothelial proliferation.

When a substantial amount of solid, malignant tumor (ie, tumor nodule[s]) with histologic features of mesothelioma is identified, the presence of invasion is not required for diagnosis. Tumor necrosis is also a feature of malignancy.

Fibrous Pleuritis Versus Sarcomatoid or Desmoplastic Mesothelioma

Identification of malignant features in desmoplastic mesothelioma requires adequate tissue, and large surgical biopsy samples are generally (but not always) needed. Features separating fibrous pleuritis from desmoplastic mesothelioma are shown in Table 5.

Fibrous pleuritis tends to show a uniformity of growth, with regular sheets and sweeping parallel fascicles of bland spindle cells that respect mesothelial boundaries. In contrast, sarcomatoid or desmoplastic mesothelioma shows disorganized growth, haphazardly intersecting fascicles, and expansile nodules of varying sizes with abrupt demarcation and changes in cellularity between nodules and their surrounding tissue (Figure 8, A). These different patterns can be highlighted by a broad-spectrum cytokeratin immunostain. Note that reactive mesothelial stroma is also keratin positive; keratin stain does not differentiate benign from malignant mesothelial proliferations; however, it can show the pattern of growth.

Stromal invasion is often more difficult to recognize in spindle cell than in epithelioid proliferations, as the invasive malignant cells in the former are often deceptively bland, resembling fibroblasts. Broad-spectrum cytokeratin staining is invaluable in highlighting bland, cytokeratin-positive malignant cells in areas where they would not normally be present (eg, adipose tissue, skeletal muscle, or lung or other visceral tissue in the pleura or peritoneum) (Figure 8, B). Although identification of invasion is often straightforward with the aid of broad-spectrum cytokeratin staining, fatlike spaces (termed *fake fat*) can be encountered in organizing pleuritis, probably reflecting artifactual changes in dense, fibrous connective tissue (Figure 9, A).[105] In such cases, horizontally oriented, cytokeratin-positive cells may be seen around the fatlike spaces (Figure 9, B). In addition, S100 protein, laminin, and collagen IV are usually positive in true adipose tissue and can help distinguish it from "fake fat," which is negative for all 3 markers (Figure 9, C).

Immunohistochemical and Molecular Studies

In small biopsy specimens, morphology alone may be inadequate for a definitive diagnosis of malignancy. Many immunostains previously purported to distinguish benign from malignant mesothelial proliferations—including GLUT1, IMP3, desmin, and epithelial membrane antigen (EMA)—are of little diagnostic value in individual cases. However, loss of nuclear BAP1 by immunohistochemistry, loss of cytoplasmic methylthioadenosine phosphorylase (MTAP) by immunohistochemistry, and homozygous deletion of *CDKN2A* by FISH,

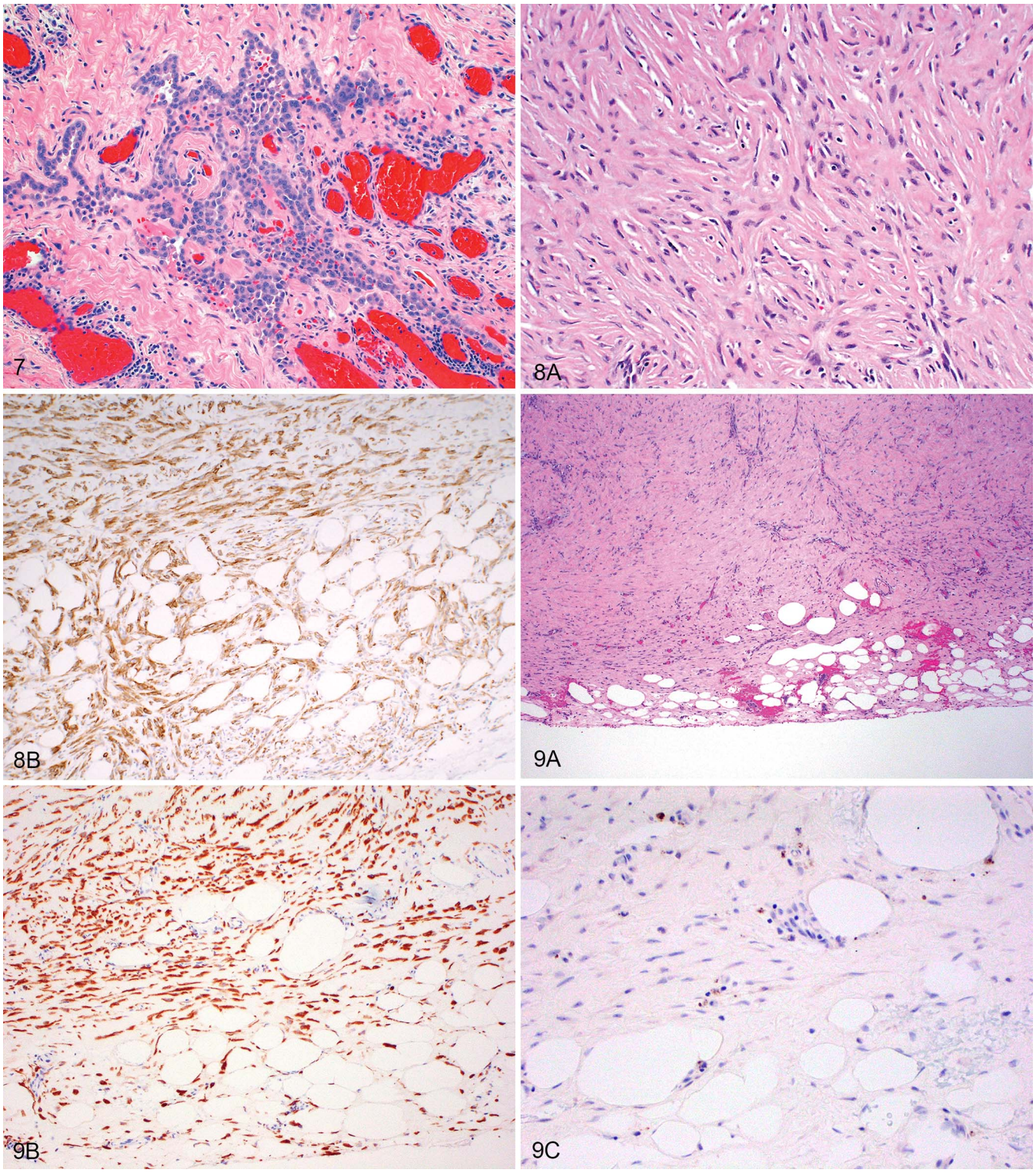

Figure 7. *Reactive mesothelial hyperplasia within fibrous tissue mimicking invasion (hematoxylin-eosin, original magnification ×100).*

Figure 8. *Desmoplastic mesothelioma. A, Bland-appearing spindle cells with haphazard growth. B, In a different focus, keratin highlights infiltration into fat (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×100 [B]).*

Figure 9. *"Fake fat." A, Fake fat in a pleural biopsy specimen from a patient with effusion and fibrosis. B, Keratin AE1/AE3 highlights horizontal, keratin-positive, reactive spindle cells around fake fat. C, S100 is negative in fake fat (hematoxylin-eosin, original magnification ×40 [A]; original magnifications ×100 [B] and ×200 [C]).*

Table 4. Reactive Versus Malignant Mesothelial Proliferations	
Mesothelial Hyperplasia	**Epithelioid Mesothelioma**
Morphologic Features	
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	Tumor necrosis present (occasionally)
Inflammation common	Inflammation usually minimal
Uniform growth (highlighted with cytokeratin staining)	Expansile nodules; disorganized growth (highlighted on cytokeratin staining)
Ancillary Studies[46,48,56,82,130]	
BAP1 loss	
100% specific for malignancy in differential with reactive mesothelium	
50%–60% sensitive for pleural mesothelioma	
60%–70% sensitive for peritoneal mesothelioma	
Sensitivity greater for epithelioid than biphasic/sarcomatoid	
MTAP loss	
100% specific for malignancy in differential with reactive mesothelium	
50% sensitive for pleural mesothelioma	
Sensitivity greater for biphasic/sarcomatoid than epithelioid	
5%–10% sensitive for peritoneal mesothelioma	
CDKN2A homozygous deletion	
100% specific for malignancy in differential with reactive mesothelium	
70% sensitive for pleural mesothelioma	
Sensitivity greater for biphasic/sarcomatoid than epithelioid	
10%–15% sensitive for peritoneal mesothelioma	

Abbreviation: MTAP, methylthioadenosine phosphorylase.

though not present in all cases of mesothelioma, are by definition never found in benign mesothelium (Supplemental Figures 16 and 17).[48,106–113] Both BAP1 and MTAP immunostains must be interpreted in the presence of a positive internal control, typically intratumoral inflammatory or stromal cells. These 3 techniques are very useful and can be applied in an algorithmic fashion in both tissue sections and cell block preparations (Figure 10).

Sensitivities of BAP1 immunohistochemistry, MTAP immunohistochemistry, and *CDKN2A* FISH depend on both tumor histologic subtype and primary site. Loss of nuclear BAP1 staining is seen in 60% to 70% of epithelioid pleural mesotheliomas but just 20% of sarcomatoid tumors.[48,107,112,114] Conversely, *CDKN2A* deletion and corresponding loss of cytoplasmic MTAP staining are seen in 60% and 40% of epithelioid pleural mesotheliomas,

Table 5. Fibrous Pleuritis Versus Sarcomatoid or Desmoplastic Mesothelioma	
Fibrous Pleuritis	**Desmoplastic Mesothelioma**
Storiform pattern not prominent	Storiform/haphazard pattern often prominent
Absence of stromal invasion	Stromal invasion present (highlight with pancytokeratin staining)
Necrosis, if present, is at the surface of 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 deeper in the tissue (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	

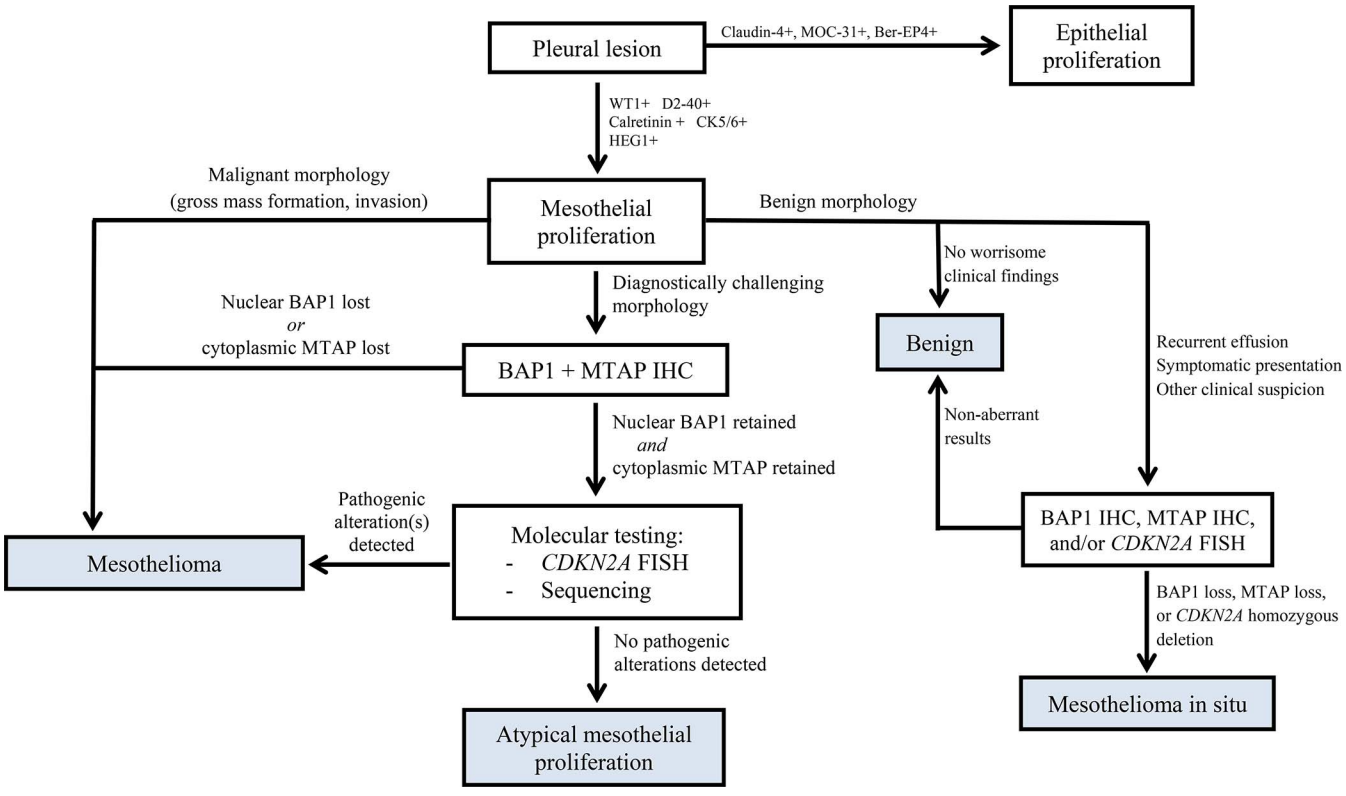

Figure 10. *Algorithm for tissue diagnosis of mesothelial proliferations. An immunopanel of 2 epithelial and 2 mesothelial markers is generally advisable for confirming mesothelial lineage. Abbreviations: CK, cytokeratin; D2-40, podoplanin; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; WT1, Wilms tumor-1.*

respectively, compared to 93% and 75%, respectively, of sarcomatoid pleural mesotheliomas.[107,115,116] Recent data indicate that MTAP immunohistochemistry may be significantly less sensitive than *CDKN2A* FISH in desmoplastic mesothelioma, given its scant cytoplasm, highlighting the continued role of *CDKN2A* FISH in clinical diagnosis.[116]

By primary site, BAP1 loss is seen in 50% to 60% of pleural and 60% to 70% of peritoneal mesotheliomas. Conversely, MTAP loss is seen in 50% of pleural compared to just 5% to 10% of peritoneal mesotheliomas, reflecting the different proportions of histologic subtypes and relative rates of underlying *CDKN2A* deletion at these sites.[48,83]

BAP1 loss can also support a diagnosis of mesothelioma in the differential with certain carcinomas. In particular, BAP1 loss is seen in less than 1% of lung cancers and serous ovarian carcinomas.[117,118] However, BAP1 loss is not specific for mesothelioma in isolation or in all contexts, as it may be seen in ~15% of clear cell renal cell carcinomas and in subsets of intrahepatic cholangiocarcinoma, thymic carcinoma, and melanoma. MTAP loss does not help distinguish mesothelioma from other malignancies in any reported context.

p53 immunostaining has a controversial history in diagnosis of mesothelioma, largely because of prior misunderstanding about wild-type versus aberrant staining, but recent data indicate that strong diffuse p53 immunostaining (ie, in ≥80% of tumor cells) can be seen in mesothelioma but not in reactive mesothelial proliferations. Sensitivity is limited, with strong diffuse p53 staining seen in only ~10% to 15% of pleural mesotheliomas, including rare cases in which this might be the only immunohistochemical evidence for malignancy.[48,119] There is conflicting data on the specificity of null-pattern (ie, complete absence of) p53 staining for underlying *TP53* mutation.

Homozygous or heterozygous *NF2* deletion (detected by FISH or molecular sequencing) is specific for mesothelioma in the differential with reactive mesothelial proliferation.[120] In recent reports, immunohistochemical loss of the *NF2* protein product, Merlin, correlates strongly with underlying *NF2* mutation, and Merlin loss has been detected in 40% to 50% of pleural mesotheliomas.[48,121] Preliminary results are promising, but full endorsement of Merlin immunohistochemistry for this application awaits additional data.

Ancillary Studies in Diagnosis of Biphasic Mesothelioma.—Morphology alone cannot always reliably distinguish biphasic mesothelioma from epithelioid mesothelioma associated with a reactive spindled mesothelial population. Immunohistochemical and molecular studies can attempt to establish a clonal relationship between the epithelioid and spindled populations. Although studies using this approach have shown variable results,[34,122–124] recent data show strong concordance for BAP1 staining pattern (ie, lost versus retained) in the epithelioid and sarcomatoid components of biphasic mesothelioma. In contrast, MTAP staining is more often discordant, with retained expression in the epithelioid component and loss in the sarcomatoid component.[48] At present, it is recommended that BAP1 or MTAP loss (or *CDKN2A* deletion by FISH) in the spindle cell component of a biphasic mesothelial proliferation be regarded as evidence of malignancy (ie, supporting diagnosis of biphasic mesothelioma). Conversely, if BAP1 or MTAP loss is confined to the epithelioid component (ie, retained expression in the spindled component), a diagnosis of biphasic mesothelioma should be made only if the spindled population shows unequivocal morphologic features of malignancy.

Table 6. Grading of Epithelioid Pleural Mesothelioma

MSKCC Grading System for Pleural Epithelioid Mesothelioma (Kadota et al,[128] 2012)

		Score	
Nuclear atypia	Mild (uniform nuclear size and shape)	1	
	Moderate (intermediate-sized nuclei with slight irregularity of shape)	2	
	Severe (bizarre, enlarged, variably sized nuclei; at least 2:1 variation in nuclear size)	3	
Mitotic index (per 10 high-power fields (×40 objective, 0.237-mm^2 field of view)	0–1	1	
	2–4	2	
	≥5	3	
			Composite Nuclear Grade
Combined atypia and mitosis scores		2–3	I
		4–5	II
		6	III

Modified Grading System for Pleural Epithelioid Mesothelioma

Consensus 2-Tier Grading System (Nicholson et al,[11] 2020)	Grade Group (Rosen et al,[12] 2018)		Median Survival, mo (Rosen et al,[12] 2018)
Low grade	1	MSKCC grade I, no tumor necrosis	29
	2	MSKCC grade I, with tumor necrosis, *OR* MSKCC grade II, no tumor necrosis	16
High grade	3	MSKCC grade II, with tumor necrosis	10
	4	MSKCC grade III	8

Abbreviation: MSKCC, Memorial Sloan Kettering Cancer Center.

Molecular Sequencing in Routine Diagnosis and Management.—Tumor molecular profiling with large next-generation sequencing panels has the potential to provide diagnostic, prognostic, and therapeutic information in a single assay. In one study of resection specimens, a 447-gene next-generation sequencing panel showed 95% sensitivity for diagnosis of mesothelioma.[48] At present, routine genomic sequencing of mesotheliomas is performed only in select referral or academic centers, and it is not currently recommended for routine clinical use. Immunohistochemical studies remain the ancillary assay of choice, with targeted molecular studies (eg, *CDKN2A* FISH, *ALK* FISH, sequencing) in select cases.

RECOMMENDATIONS FOR ROUTINE REPORTING OF MESOTHELIOMA

The International Collaboration on Cancer Reporting has recently published a 3rd edition to their guidelines for reporting mesothelioma, which provide a valuable resource for routine practice.[125]

Staging Pleural Mesothelioma

The Union for International Cancer Control and American Joint Committee on Cancer (AJCC) *Cancer Staging Manual*, 8th edition,[126] represents the most widely applied TNM system and should be reported for all pleural mesotheliomas resected via extended pleurectomy/decortication or extrapleural pneumonectomy (now rarely performed). The TNM staging system for pleural mesothelioma evaluates resectability but is generally not a good predictor of prognosis. Importantly, the AJCC 8th edition does not include mesothelioma in situ. There is no consensus TNM staging for peritoneal, pericardial, or paratesticular mesothelioma.

Pathologic Predictors of Prognosis and Therapy Responsiveness

The 2021 WHO classification[4] also recognizes a variety of important pathologic factors beyond tumor histologic subtype (ie, favorable prognosis for epithelioid, intermediate prognosis for biphasic, and poor prognosis for sarcomatoid tumors).[10,127] As noted above, certain architectural, cytologic, and stromal features are linked to prognosis, and pathologists should routinely report this information.[11] In brief, prognostically favorable morphologic findings include tubulopapillary, trabecular, and adenomatoid architecture; low nuclear grade; high tumor-associated immune microenvironment (eg, lymphohistiocytoid cytologic features); and myxoid-rich stromal matrix.[12,13,128] Conversely, adverse prognosis is associated with any micropapillary or greater than 50% solid architecture; high nuclear grade; rhabdoid, pleomorphic, transitional, or desmoplastic morphology; and necrosis.[15–17,29]

Grading Epithelioid Mesothelioma.—Several studies have validated a 3-tiered grading system for epithelioid mesothelioma of the pleura and peritoneum, based on mitotic activity and nuclear atypia.[8,12,128] After clinician input at a multidisciplinary meeting of mesothelioma experts, necrosis was added to mitotic activity and nuclear atypia to create a 2-tiered grading system (ie, low versus high grade), which better facilitates clinical decision-making (Table 6). The prognostic significance of the 2-tiered system has been

validated in a large series of pleural mesothelioma,[11,26] though its applicability to peritoneal mesothelioma remains unclear.[8] When assigning nuclear grade, tumor foci with the highest-grade features should be used. The current 2021 WHO classification recommends routine reporting according to this 2-tiered grading system for biopsies and resections of epithelioid diffuse pleural mesothelioma.[4]

Molecular Prognostic Factors.—Homozygous deletion of *CDKN2A* and MTAP loss by immunohistochemistry both portend poor prognosis (shorter overall and disease-free interval) among mesothelioma cases.[36,129,130] In contrast, loss of nuclear BAP1 by immunohistochemistry is a favorable prognostic marker, at least partly reflecting the significantly improved prognosis and treatment responsiveness in patients with germline *BAP1* mutation.[131,132] Rates of *CDKN2A* deletion, MTAP loss, and BAP1 correlate with tumor histologic subtype and primary site (see Distinguishing Benign Versus Malignant Mesothelial Proliferations above).

Germline Predisposition to Mesothelioma

Germline testing should now be considered for all patients with mesothelioma, as it affords improved response to platinum-based chemotherapy and relatively favorable prognosis (despite advanced stage at presentation), potential access to novel therapies, and genetic counseling for the patient and family, which is relevant to surveillance for other tumors.[132–134]

Genomic profiling indicates that at least 12% of mesotheliomas arise in carriers of pathogenic germline mutations.[45,132,133,135] *BAP1* germline mutations (which cannot be distinguished from somatic mutations by BAP1 immunohistochemistry) account for approximately half of such cases. The prevalence of germline predisposition is higher in younger patients (ie, germline mutations identified in >50% of patients with mesothelioma and younger than 50 years[134]), in those with peritoneal disease, in tumors with low-grade epithelioid morphology and a high tumor immune response, in patients with longer overall survival (median survival >5 years[135]), and in those with a personal or family history of multiple cancers (especially melanoma, clear cell renal cell carcinoma, and breast cancer).[132]

Targeted Therapies

Pathogenic germline mutations in patients with mesothelioma most commonly affect DNA damage repair pathways, which serve as a potential therapeutic target for poly (ADP-ribose) polymerase (PARP) inhibitors.[136] Use of PARP inhibitors alone or combined with platinum-based regimens in mesotheliomas with germline homologous recombination defects is under evaluation.

US Food and Drug Administration approval for combined nivolumab and ipilimumab in untreated unresectable diffuse pleural mesothelioma followed publication of the CheckMate 743 trial.[137] The benefits are primarily observed among patients with nonepithelioid disease, which is typically most refractory to conventional chemotherapy. By immunohistochemistry, PD-L1 is positive (>1% tumor cell staining) in 10% to 49% of epithelioid, 9% to 67% of biphasic, and 22% to 100% of sarcomatoid mesotheliomas,[138–140] with some variability between PD-L1 antibody clones.[139,140] Routine PD-L1 immunostaining is not currently indicated for mesothelioma.

ALK rearrangements can be identified by immunohistochemistry or molecular testing in a small number of mesotheliomas, with a predilection for children and young adults, women, and peritoneal tumors.[40,141,142] *ALK* rearrangement appears mutually exclusive with other genetic events commonly observed in mesothelioma. *ALK*-fusion–positive peritoneal mesotheliomas afford a small group of patients access to novel targeted treatment with tyrosine kinase inhibitors, with reportedly dramatic treatment response.[143] There is no formal guideline on screening for *ALK* rearrangement in mesothelioma, but given its clinical implications, it appears reasonable to perform ALK immunohistochemistry in young patients and patients with peritoneal tumors, particularly if other molecular alterations (eg, BAP1 loss, MTAP loss, Merlin loss) are not detected.

MESOTHELIOMA IN SITU

Mesothelioma in situ (MIS) as the noninvasive precursor to diffuse mesothelioma was initially proposed in 1992.[144] The morphology of MIS is now recognized as variable and includes flat or cuboidal cells with or without cytologic atypia, small or complex papillary proliferations, or small surface nodules with moderate to severe cytologic atypia. Invasion is absent by definition, and there must be no clinically or radiographically identifiable mass lesion or diffuse process. MIS cannot be diagnosed by morphology alone, and immunohistochemical loss of nuclear BAP1 and/or demonstration of *CDKN2A* homozygous deletion (by FISH or by MTAP immunohistochemistry) must also be demonstrated on a rigorously validated assay with appropriate controls (Figure 11, A through D).[47,111,112,115,145–147]

There are no published criteria on minimum acceptable sample size, but caution is warranted when biopsy samples are very small or crushed. The WHO recommends thoracoscopic evaluation with large biopsy specimens (ideally 100–200 mm^2) from different areas of the pleura in patients with nonresolving effusions.[4] Similarly, no criteria are published for which samples should be tested for BAP1, MTAP, and *CDKN2A* alterations, but a low threshold is suggested for patients with unexplained recurrent effusions, history of occupational exposures, genetic predisposition, history of chest radiation, or atypical histologic features.

The differential diagnosis of MIS includes reactive mesothelial atypia and well-differentiated papillary mesothelial tumor (WDPMT), depending on lesional architecture.[148,149] BAP1 and MTAP immunostains should be performed in WDPMT-like lesions identified on investigation of an effusion or related symptoms, with aberrant results supporting MIS with WDPMT-like morphology, in the correct clinical context. Absence of BAP1, MTAP, and *CDKN2A* alterations does not exclude MIS, and an expert opinion is advisable in difficult cases, given the potential for malignant behavior.

The WHO classification[4] currently only describes MIS in the pleural space, but peritoneal, pericardial, and paratesticular presentations are described, and the same diagnostic criteria can be applied.[150–152] At present, MIS has only been established for epithelioid mesothelioma.

The 2021 WHO classification[4] emphasizes that MIS is a multidisciplinary diagnosis. Communication with the clinical team is especially important because time to progression may range from 1 year[146,149] to 15 years.[153] No treatment guidelines exist currently.

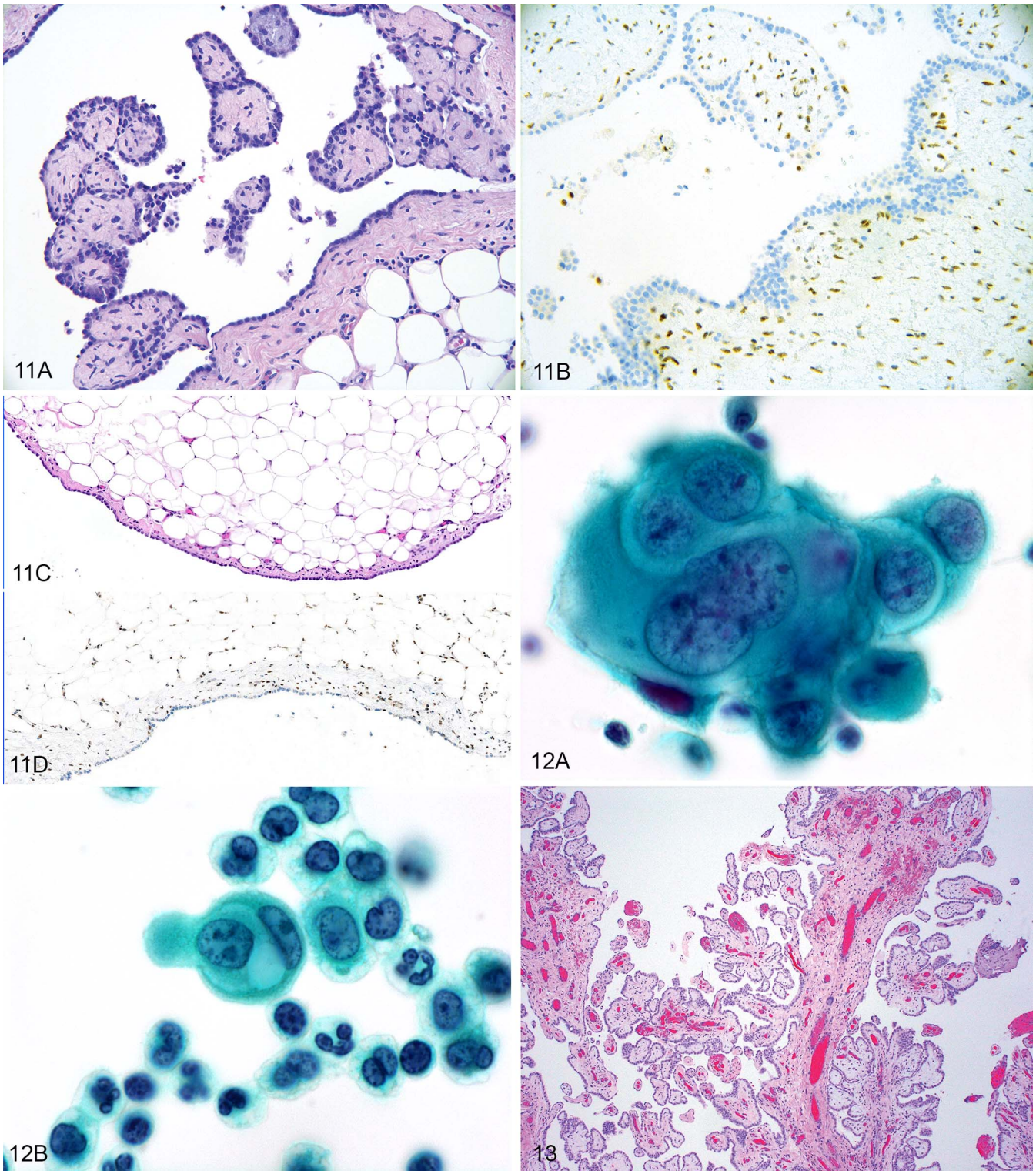

Figure 11. *Mesothelioma in situ. A, Papillary proliferation lined by a flat, cytomorphologically banal mesothelium, with (B) BAP1 loss. C, A separate case shows flat mesothelial lining with (D) BAP1 loss (hematoxylin-eosin, original magnifications ×100 [A and C] and ×200 [B]; original magnification ×100 [D]).*

Figure 12. *Mesothelioma, cytology preparation. Features of mesothelioma include (A) cell-in-cell arrangement and (B) a hump at the cell periphery (Papanicolaou, original magnification ×1000 [A and B]).*

Figure 13. *Well-differentiated papillary mesothelial tumor (hematoxylin-eosin, original magnification ×40).*

CYTOLOGIC DIAGNOSIS OF MESOTHELIOMA

Up to 90% of patients with diffuse pleural mesothelioma present with a pleural effusion. Consequently, cytology fluid specimens are often the first (and in patients who cannot tolerate additional procedures, the only) sample available for diagnosis.

Cytomorphology

The typical cytologic features of epithelioid mesothelioma were described more than 50 years ago and have been refined in numerous subsequent publications.[154–156] Mesothelioma can manifest cytologically as a highly cellular effusion with obvious nuclear atypia, numerous large tissue fragments and cell clusters, or as cellular fluid with single and clustered mesothelial cells exhibiting only subtle atypia, overlapping with reactive mesothelium. Cell-in-cell arrangements, a hump at the cell periphery, multinucleated cells, papillary groups with basement membrane cores, and orangeophilic cells are features concerning for mesothelioma (Figure 12, A and B). Sarcomatoid mesotheliomas generally do not shed malignant cells into effusions.

Ancillary Studies

As in tissue specimens, the diagnosis of mesothelioma in cytology specimens is a 2-step process in which both mesothelial lineage and malignancy must be established.[157–159] Immunocytochemistry plays an invaluable role in both steps and can be performed on smears or cell blocks, although use of cell blocks is preferable, as they undergo processing similar to formalin-fixed paraffin-embedded tissue specimens, thereby enabling preparation of serial sections for immunostaining and/or molecular studies. Given that cell blocks can be prepared in various ways, it is important to recognize that differences in fixation can affect immunostaining results and to use appropriate controls.[53,54] When cell blocks are not prepared, the cell-transfer technique (in which a Papanicolaou-stained sample is divided into several pieces and transferred to multiple slides) can facilitate multiple immunocytochemical stains on limited materials.[160]

The same antibodies used to distinguish mesothelial from epithelial lineage in tissue samples (see Establishing Mesothelial Lineage above) can be applied to cytology specimens, provided they have been validated for this application in the responsible laboratory. As in tissue specimens, it is currently recommended to use 2 epithelial and 2 mesothelial markers, though claudin-4 immunocytochemistry can be used as the sole epithelial marker if well validated. When a cytology specimen comprises single cells or loose aggregates, the differential diagnosis may also include melanoma, lymphoma, sarcoma, germ cell, and other tumors, and the immunopanel should be tailored to the differential diagnosis.

BAP1 and MTAP immunocytochemistry, and *CDKN2A* FISH have been validated for use in cell block preparations (Supplemental Figure 18).[108,110,154,158,159,161,162] In distinction of mesothelioma from reactive mesothelial proliferation in pleural effusion cytology specimens, a recent meta-analysis of 65 studies found 100% specificity for *CDKN2A* homozygous deletion by FISH, and 99% specificity for both BAP1 loss and MTAP loss by immunocytochemistry. The same study found a diagnostic sensitivity of 83% for the combination of BAP1 immunocytochemistry and *CDKN2A* FISH.[163] FISH for *NF2* deletion is also reportedly specific for mesothelioma in effusion cytology, though not yet in widespread use.[120,164] Although previously viewed as potentially useful markers, it is now clear that positive staining for EMA, IMP-3, CD146, or GLUT1 alone is insufficient to diagnose a cytology specimen as malignant.

Limitations

The inability to assess stromal invasion and sarcomatous elements, coupled with the grim prognosis and expensive and toxic therapy associated with the diagnosis, has contributed to a general reluctance to render a primary definitive diagnosis of mesothelioma based solely on effusion cytology. The reported sensitivity of cytologic diagnosis for mesothelioma is 30% to 75%, though specificity is 99% to 100%.[157,165] Sensitivity is almost certainly lower for sarcomatoid mesothelioma, which does not typically shed cells in effusions. Although the epithelioid component of biphasic mesothelioma can shed mesothelioma cells into effusions, biphasic mesothelioma cannot be differentiated from epithelioid mesothelioma on cytology effusion. Additionally, cytology alone cannot distinguish invasive mesothelioma from MIS,[166] and correlation with clinical and radiographic findings is recommended in all cases. Architectural subtyping and grading of epithelioid mesothelioma also cannot be performed on cytology specimens.

Non–Effusion Cytology Specimens

The above discussion pertains principally to effusion cytology, which is by far the most common setting for a cytologic diagnosis of mesothelioma. Similar considerations apply to other types of cytology samples. Mesothelioma may rarely be diagnosed in sputum or bronchial washing, lavage, or brushing, and a few studies from the 1980s report use of transthoracic or endoscopic bronchial ultrasound-directed fine-needle aspiration biopsy (FNAB) for diagnosis of mesothelioma. Though rarely reported, definitive primary diagnosis of mesothelioma using these techniques is possible in the appropriate clinical and radiologic context. The cytomorphology in such cases resembles that of mesothelioma in effusions, with high cellularity and papillary clusters, though other features characteristic of mesothelioma in effusions (eg, cell-in-cell arrangements, multinucleated cells) are less commonly observed. Because FNAB directly samples a clinical mass lesion, sarcomatoid mesothelioma is more likely to be diagnosed in an FNAB specimen than in effusion cytology.

MORPHOLOGIC FEATURES OF OTHER PERITONEAL MESOTHELIAL LESIONS

Peritoneal Inclusion Cysts

The WHO Classification of Tumors of the female genital tract (which includes peritoneal mesothelial tumors) encourages the diagnostic term *peritoneal inclusion cyst* and discourages use of *multicystic mesothelioma* (and similar terms) to avoid confusion with (malignant) mesothelioma.[5] Peritoneal inclusion cyst(s) may comprise 1 or multiple cysts lined by bland mesothelial cells without significant stratification, papillary formations, or infiltration of soft tissues. Lesions can be unifocal or multifocal within the pelvis

and abdomen. Local recurrence rates as high as 50% have been reported in studies of florid multifocal lesions,[167] though a recent study including a more representative population of peritoneal inclusion cysts, as currently defined in the WHO, found a local recurrence rate of just 3%.[168] It remains unclear whether peritoneal inclusion cysts represent reactive or neoplastic lesions, though BAP1 and MTAP are universally retained.[169]

Well-Differentiated Papillary Mesothelial Tumor

Well-differentiated papillary mesothelial tumor (WDPMT) occurs principally in the peritoneum and is often an incidental surgical finding. Tumors are generally unifocal and small (<2 cm), though larger and/or multifocal examples are reported with otherwise classic morphology.[170] WDPMT is composed of slender papillae with hyalinized to myxoid cores lined by a single layer of bland mesothelial cells (Figure 13).[171] Mitoses are rare to absent. Infiltration of underlying soft tissue is absent by definition, though occasional WDPMTs show so-called invasive foci, characterized by confluent papillary growth and/or percolating mesothelial nests/cords within papillary stroma (ie, confined to the WDPMT).[172] Recurrent mutations in *TRAF7*, *CDC42*, *EHD1*, *ATM*, *FBXO10*, and *SH2D2A* have been reported.[173,174] *BAP1* and *CDKN2A* alterations are absent, with BAP1 and MTAP retained by immunohistochemistry.[169] Most (60%–95%) are positive for PAX8.[171,175]

"Invasive foci" and multifocality are associated with increased recurrence risk and should be reported when present. Malignant transformation is rare and can occur years or decades after original diagnosis. It is uncertain whether such cases represent true WDPMTs, versus MIS with WDPMT-like morphology and/or early epithelioid mesotheliomas with WDPMT-like foci.[176] BAP1 immunohistochemistry (and, if necessary, MTAP immunohistochemistry or *CDKN2A* FISH) should be performed on WDPMT-like lesions discovered during clinical workup for effusion or associated with multifocal or diffuse serosal involvement. BAP1 or MTAP loss supports a diagnosis of WDPMT-like mesothelioma or MIS, but retained staining does not exclude mesothelioma and requires multidisciplinary correlation.[148]

Adenomatoid Tumor

Adenomatoid tumors are small, circumscribed, nodular lesions, most often involving the uterus and fallopian tube, and rarely other peritoneal sites.[177,178] An association with immunosuppression has been noted.[177] Tumors comprise acini, cords, and nests of plump to flattened, bland mesothelial cells. Single cells may be noted, often with a signet ring appearance. Stringlike bridges characteristically span tumor lumina, which may contain hyaluronic acid–rich myxoid material. BAP1 and MTAP are retained.[179] *TRAF7* mutations have been reported in adenomatoid tumors at various sites.[177]

Unusual and Provisional Entities

Rare peritoneal mesothelial lesions show mixed features of peritoneal inclusion cyst, WDPMT, and adenomatoid tumor. The pathogenesis and prognosis of these hybrid lesions remain unclear.[171] Occasional noninvasive, morphologically bland, and unifocal or oligofocal nodular mesothelial proliferations defy easy classification. A provisional entity of "solid papillary mesothelial tumor" attempts to encompass some of these lesions, which appear to show indolent behavior.[180] In such cases, a descriptive diagnosis with recommendation for clinical and radiographic correlation with close follow-up is generally appropriate.

CONCLUSIONS

This article provides broad guidelines for diagnosis of mesothelioma, which, though uncommon, carries a grave prognosis and frequently has medicolegal implications. We emphasize that morphology remains the cornerstone for classification of mesothelial proliferations in both biopsy and resection specimens. Numerous prognostically and clinically significant morphologic features are discussed, and these should be routinely reported in the diagnostic report, whenever possible. Immunohistochemistry and molecular studies play a growing and evolving role in diagnosis and management of mesothelioma. Immunohistochemical panels are routinely applied to establish mesothelial lineage, distinguish mesothelioma from malignant mimics, and distinguish mesothelioma from reactive mesothelial proliferations. Specific immunopanels should be tailored to the clinical and morphologic differential, and immunostains should be carefully validated for optimal performance. Molecular studies, including FISH and targeted sequencing panels, are useful in challenging cases with nondiagnostic morphologic and immunophenotypic findings. These same principles apply to the cytologic diagnosis of mesothelioma, with recognition that cytology does not permit evaluation of stromal invasion, that tissue fixation protocols may impact immunostain performance in cytology preparations, and that sarcomatoid mesothelioma does not typically shed in effusions. Importantly, the pathologist must always correlate morphology and ancillary study results with clinical, radiographic, and operative findings. Despite (or perhaps in part because of) rapid evolution in the field of mesothelioma diagnosis, this remains a challenging and evolving area of surgical and cytopathology, and expert opinion should be sought in difficult cases, when needed.

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

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