The 2021 WHO Classification of Tumors of the Pleura: Advances Since the 2015 Classification

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Received 29 October 2021; revised 14 December 2021; accepted 31 December 2021
Available online - 10 January 2022

ABSTRACT

Substantial changes in the 2021 WHO Classification of Tumors of the Pleura and Pericardium since the 2015 WHO Classification include the following: (1) pleural and pericardial tumors have been combined in one chapter whereas in the 2015 WHO, pericardial tumors were classified with cardiac tumors; (2) well-differentiated papillary mesothelioma has been renamed well-differentiated papillary mesothelial tumor given growing evidence that these tumors exhibit relatively indolent behavior; (3) localized and diffuse mesothelioma no longer include the term “malignant” as a prefix; (4) mesothelioma in situ has been added to the 2021 classification because these lesions can now be recognized by loss of BAP1 and/or MTAP by immunohistochemistry and/or CDKN2A homozygous deletion by fluorescence in situ hybridization; (5) the three main histologic subtypes (i.e., epithelioid, biphasic, and sarcomatoid) remain the same but architectural patterns and cytologic and stromal features are more formally incorporated into the 2021 classification on the basis of their prognostic significance; (6) nuclear grading for epithelioid diffuse mesothelioma is introduced, and it is recommended to record this and other histologically prognostic features in pathology reports; (7) BAP1, EZH2, and MTAP immunohistochemistry have been found to be useful in separating benign mesothelial proliferations from mesothelioma; (8) biphasic
mesothelial tumors since 2015 (Table 1). For each entity, the pathologist may include BAP1, CDKN2A, NF2, TP53, SETD2, and SETDB1. © 2022 International Association for the Study of Lung Cancer. Published by Elsevier Inc. All rights reserved.

**Keywords:** Mesothelioma; World Health Organization classification; Pleura; Histopathology

**Introduction**

The 2021 (fifth edition) **WHO Classification of Thoracic Tumors** has recently been published.¹ Pleural and pericardial tumors have been combined in one chapter whereas in the 2015 WHO, pericardial tumors were classified with cardiac tumors.² The objective of this review is to summarize changes in the classification of pleural and pericardial tumors that represent advances in our understanding and affect the diagnosis of these tumors.

Although the three main histologic subtypes (i.e., epithelioid, biphasic, and sarcomatoid) remain, there have been significant changes in the classification of mesothelial tumors since 2015 (Table 1). For each entity, the 2021 classification provides essential and desirable diagnostic criteria, which may be useful to the practicing pathologist (Table 2). The prefix “malignant” has been omitted from localized and diffuse mesothelioma because all mesotheliomas are regarded as malignant now that well-differentiated papillary mesothelioma (WDPM) has been renamed WDPM tumor (WDPMT), given its relatively indolent behavior.³,⁴ Therefore, the term malignant is not needed to separate mesotheliomas from WDPMT. Localized mesothelioma remains distinct from diffuse mesothelioma because localized mesotheliomas have been found to be associated with better prognosis when completely resected.⁵,⁶

The 2015 WHO classification⁷ recognized promising advances in the field of mesothelioma pathology, including histologic features with prognostic significance, nuclear grading of epithelioid diffuse pleural mesothelioma, and the use of BAP1 immunohistochemistry (IHC) and homozygous deletion of CDKN2A (9p21; encoding p16) by fluorescence in situ hybridization (FISH) in the separation of mesothelioma from reactive mesothelial proliferations, but these advances were not thoroughly incorporated into the classification until 2021.¹ Recent advances in the understanding of genomics of mesothelioma have led to increased recognition of a new entity, mesothelioma in situ (MIS), which was not formally recognized previously. Criteria for MIS have now been established and are included in the 2021 WHO classification.

Mesenchymal tumors of the pleura have been moved to a new chapter titled “Mesenchymal tumours of the thorax.” An additional section in the “Metastases” chapter titled “Metastasis to the pleura” was added to the 2021 classification. The 2021 **WHO Classification of Thoracic Tumors** also includes a new chapter “Genetic tumour syndromes involving the thorax,” which includes a section titled “BAP1 tumour predisposition syndrome.” A review to address updates in hematolymphoid tumors of the pleura and pericardium will be published separately.

<table>
<thead>
<tr>
<th>Table 1. 2021 WHO Classification of Tumors of the Pleura and Pericardium: ICD-O Coding and Terminology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors</td>
</tr>
<tr>
<td>Mesothelial tumors</td>
</tr>
<tr>
<td>Benign and preinvasive mesothelial tumors</td>
</tr>
<tr>
<td>Adenomatoid tumor</td>
</tr>
<tr>
<td>Well-differentiated papillary mesothelial tumor</td>
</tr>
<tr>
<td>Mesothelioma in situ</td>
</tr>
<tr>
<td>Localized mesothelioma</td>
</tr>
<tr>
<td>Diffuse mesothelioma, NOS</td>
</tr>
<tr>
<td>Sarcomatoid mesothelioma</td>
</tr>
<tr>
<td>Epithelioid mesothelioma</td>
</tr>
<tr>
<td>Mesothelioma, biphasic</td>
</tr>
</tbody>
</table>

¹Morphology codes are taken from the ICD-O, third edition, second revision (ICD-O-3.2) (REF 1256). Behavior is coded /0 for benign tumors; /1 for unspecified, borderine, or uncertain behavior; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumors.
²Change in terminology of a previous code.
³This new code was approved by the International Agency for Research on Cancer/WHO Committee for the ICD-O at its meeting in October 2020.

**Significant Advances in Pleural Mesothelioma**

**WDPM Has Been Renamed WDPMT**

WDPM of the pleura is very rare.³ Given their relatively indolent behavior, WDPM has been redesignated as WDPMT in the 2021 WHO classification to distinguish them from diffuse mesothelioma.¹,⁴ The definition of WDPMT is now restricted to tumors consisting of papillary formations covered by a single layer of bland mesothelial cells that lack stromal invasion (Table 2; Fig. 1A and B). Accurate diagnosis of this rare tumor requires histologic examination of the entire lesion to exclude the possibility of superficial sampling from a component of an invasive diffuse mesothelioma. WDPMT typically appears as an arborescent mass or nodularity on the visceral or parietal pleura. Patients often present with dyspnea owing to recurrent pleural effusions. Asbestos exposure has been documented in some cases of
WDPMT, but there are insufficient data to implicate causation owing to the rarity of this tumor.3,4

The papillary cores in WDPMT are paucicellular, ranging from myxoid to fibrovascular or hyalinized, and they lack inflammation (Fig. 1C). The thin mesothelial surface lining consists of flat to cuboidal cells with inconspicuous nucleoli, negligible mitotic activity, and immunoreactivity for conventional mesothelial markers.

Low-grade epithelioid diffuse mesothelioma can have florid papillary growth that appears deceptively similar to WDPMT if biopsies are superficial and do not sample deeper areas of invasive growth. Papillae can also occasionally form in reactive pleuritis, but they tend to be more broad based and have reactive cytologic changes and conspicuous accompanying inflammation, in contrast to WDPMT.

Pleural WDPMT can have variable behavior, including a protracted clinical course.3 Data on tumors with architectural features of WDPMT that have focal invasion are too sparse to permit definitive classification.4 CDKN2A homozygous deletion has not been reported in WDPMT, and BAP1 is usually retained. BAP1 loss has only been reported in exceptional cases with synchronous or subsequent diffuse mesothelioma,7 raising the

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**Table 2. 2021 WHO Classification of Tumors of the Pleura and Pericardium: Essential and Desirable Diagnostic Criteria**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Essential Criteria</th>
<th>Desirable Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign and preinvasive</td>
<td>• Focal proliferation of tubular spaces or vacuoles lined by flattened or cuboidal</td>
<td>• Immunohistochemistry for mesothelial markers, if needed</td>
</tr>
<tr>
<td>mesothelial tumors</td>
<td>mesothelial cells in a fibrous stroma</td>
<td>• Immunohistochemical staining for L1CAM, a marker of TRAF7 mutation, may be useful</td>
</tr>
<tr>
<td>Adenomatoid tumor</td>
<td>• Lack of diffuse or multifocal spread along pleura and absence of malignant</td>
<td>• BAP1 expression retained and absence of homozygous deletion of CDKN2A</td>
</tr>
<tr>
<td></td>
<td>histologic features, such as invasive growth into underlying stroma, cytologic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>atypia, necrosis, or sarcomatoid patterns</td>
<td></td>
</tr>
<tr>
<td>Well-differentiated papillary</td>
<td>• Papillary stromal formations covered by bland mesothelium</td>
<td>• Papillary stromal formations covered by bland mesothelium</td>
</tr>
<tr>
<td>mesothelial tumor</td>
<td>• No stromal invasion</td>
<td>• No stromal invasion</td>
</tr>
<tr>
<td>Mesothelioma in situ</td>
<td>• Pleural effusions (nonresolving)</td>
<td></td>
</tr>
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<td></td>
<td>• No thorascopic or imaging evidence of tumor</td>
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<tr>
<td></td>
<td>• Single layer of mesothelial cells (with or without atypia) on pleural surface</td>
<td></td>
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<tr>
<td></td>
<td>• No histologic features of invasive growth</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>• Loss of BAP1 and/or MTAP by immunohistochemistry and/or CDKN2A homozygous deletion by FISH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Multidisciplinary discussion of diagnosis</td>
<td></td>
</tr>
<tr>
<td>Localized pleural mesothelioma</td>
<td>• Presentation as a solitary localized mass by imaging, surgical findings, and</td>
<td>• Multidisciplinary discussion to confirm the diagnosis</td>
</tr>
<tr>
<td></td>
<td>histology</td>
<td></td>
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<tr>
<td></td>
<td>• Examination of a surgical resection specimen revealing lack of invasion beyond the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>circumscribed borders of the tumor</td>
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</tr>
<tr>
<td></td>
<td>• Histologic features of diffuse mesothelioma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Immunohistochemical evidence of mesothelial origin</td>
<td></td>
</tr>
<tr>
<td>Diffuse pleural mesothelioma</td>
<td>• Diffuse pleural thickening by a malignant neoplasm with epithelioid, sarcomatoid,</td>
<td>• Loss of BAP1 and/or MTAP by immunohistochemistry, and/or CDKN2A loss by FISH</td>
</tr>
<tr>
<td></td>
<td>or biphasic histology</td>
<td>Or mutations in BAP1 or CDKN2A demonstrated by next-generation sequencing</td>
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<td></td>
<td>• Invasion of adjacent structures (i.e., adipose tissue, skeletal muscle, and/or</td>
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<td></td>
<td>lung parenchyma), tumor necrosis, or formation of unequivocal malignant tumor nodules</td>
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<td></td>
<td>• Desmoplastic mesothelioma is characterized by dense collagenized tissue</td>
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<tr>
<td></td>
<td>separated by malignant mesothelial cells arranged in a storiform or so-called</td>
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<td></td>
<td>patternless pattern, which must be present</td>
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<td></td>
<td>in ≥50% of the tumor in definitive resection specimens</td>
<td></td>
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<td></td>
<td>• Biphasic mesothelioma is mesothelioma revealing ≥10% each of epithelioid and</td>
<td></td>
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<td></td>
<td>sarcomatoid patterns in definitive resection specimens or any percentage of each</td>
<td></td>
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<td></td>
<td>component in smaller biopsy and cytology specimens</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Immunohistochemistry confirming mesothelial origin</td>
<td></td>
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</tbody>
</table>

FISH, fluorescence in situ hybridization.
possibility of diffuse papillary mesothelioma mimicking WDPMT.

MIS

MIS is now recognized as a distinct entity and is regarded as a precursor to invasive mesothelioma. The diagnosis requires multidisciplinary correlation among histologic, immunohistochemical and/or molecular, clinical, and radiologic findings (Table 2). MIS is defined as a single layer of relatively bland mesothelial cells growing along the pleural surface (Fig. 2A) that have lost BAP1 (Fig. 2B) or MTAP by IHC or homozygous deletion of CDKN2A by FISH (Fig. 2B).8–10 Most often, the cells have a cuboidal shape and inconspicuous nucleoli. In occasional cases, the cells form simple papillary structures, but still with a single layer of covering cells.

Invasive mesothelioma must be absent to make a diagnosis of MIS. A layer of markedly atypical large mesothelial cells or mesothelial proliferations consisting of piled up cells growing along the pleural surface or forming exophytic papillary structures protruding from the pleural surface is more likely to be surface spread from a concurrent invasive mesothelioma than MIS. Pleural fluid cytology specimens in isolation of other data cannot separate MIS from invasive mesothelioma. The latter determination requires information on the presence or absence of invasive tumor, which may be obtained from direct observation of the pleura or imaging in conjunction with a pleural biopsy.

Most patients with MIS present with recurrent pleural effusions of unknown cause. By definition, there must be no evidence of tumor on imaging or by direct visual inspection of the pleura. The diagnosis requires multiple samples (ideally 100–200 mm²) from different areas of the pleura in addition to clinical and radiologic information.

How long MIS can persist without development of an invasive mesothelioma is unclear, although progression 12 to 92 (median: 60) months after a biopsy diagnosis of MIS has been reported.8

Histologic Features With Prognostic Significance in Pleural Diffuse Epithelioid Mesothelioma

Historically, the major histologic types of diffuse mesothelioma have been the main histologic indicators of prognosis. It is well known that patients with sarcomatoid and biphasic tumors have significantly worse
overall survival compared with patients with epithelioid tumors. In recent years, there has been increased recognition of histologic factors with prognostic significance that could improve risk stratification of patients with epithelioid diffuse pleural mesothelioma and inform clinical management decisions. Although architectural patterns were discussed in the 2015 classification, architectural patterns, cytologic features, and stromal features are more formally incorporated in the 2021 classification (Table 3 and Fig. 3). In addition, a nuclear grading system for epithelioid diffuse pleural mesothelioma (Table 4) has been developed, and its prognostic significance has been validated in multiple studies.

The 2021 WHO classification also discusses cytologic features that can be found in epithelioid diffuse pleural mesothelioma that do not carry prognostic significance but should be recognized to avoid misdiagnosis with other entities in the differential diagnosis, including deciduoid, small cell, clear cell, and signet ring features. Nevertheless, sarcomatoid tumors with lymphohistiocytoid cytologic features have been found to have better prognosis.

Histologic features seen in epithelioid diffuse mesothelioma that are associated with better prognosis include tubulopapillary, trabecular, or adenomatoid architectural patterns, lymphohistiocytoid cytologic features, or the presence of myxoid stroma (when predominant, defined as present in more than or equal to 50% of a tumor with less than 50% solid pattern). Myxoid stroma is seen in rare cases of mesothelioma and is characterized by epithelioid tumor cells that typically have no more than mild atypia floating in a matrix of loose mucoid stroma (Fig. 3F). Unfavorable histologic features in epithelioid diffuse mesothelioma include micropapillary pattern (Fig. 3C), solid pattern (Fig. 3A) when present in more than or equal to 50% of a tumor, rhabdoid or pleomorphic cytologic features, or the presence of necrosis. Rhabdoid features are characterized by tumor cells with cytoplasmic globules, resembling rhabdomyoblastic tumors, but express cytokeratins. Mesotheliomas with rhabdoid features should be myogenin negative and do not have the molecular abnormalities observed in rhabdomyosarcoma.

Pleomorphic cytologic features in mesothelioma are characterized by tumor cells with prominent anaplastic, bizarre nuclei, and/or multinucleated tumor giant cells (Fig. 3B). In the 2015 WHO classification, mesotheliomas with pleomorphic features were classified as epithelioid tumors, but in the 2021 classification, these tumors can be classified as epithelioid, biphasic, or sarcomatoid on the basis of coexistent tumor cell morphology.

Lymphohistiocytoid cytologic features can reveal morphologic features that mimic lymphoma or lymphoepithelial carcinoma. Lymphohistiocytoid features are characterized by marked lymphoid infiltrates composed of CD8-positive lymphocytes obscuring polygonal malignant mesothelial cells that have histiocytoid morphology (Fig. 3E). Lymphohistiocytoid features do not simply represent prominent lymphoid infiltration in an epithelioid mesothelioma. Although mesotheliomas with lymphohistiocytoid cytologic features were previously classified under epithelioid mesothelioma, the 2021 WHO classification allows these tumors to be classified as epithelioid, biphasic, or sarcomatoid on the basis of tumor cell morphology.

Mesotheliomas with transitional cytologic features are characterized by elongated yet plump and cohesive tumor cells that seem intermediate between epithelioid and sarcomatoid in morphology and have a sheet-like growth pattern (Fig. 3D). In the 2021 WHO classification, tumors with the presence of transitional pattern are now classified as sarcomatoid owing to recent studies revealing the presence of transitional features to be associated with worse prognosis. Therefore, if transitional features are seen in an otherwise epithelioid tumor, the tumor could be classified as biphasic mesothelioma depending on specimen type and percentage of transitional features seen (see terminology and criteria for reporting in the subsequent texts).

Because most patients with sarcomatoid mesothelioma have a very poor prognosis, there are fewer histologic features that are prognostically significant in these tumors. Nevertheless, sarcomatoid tumors with lymphohistiocytoid cytologic features have been found to have better prognosis.

**Grading of Pleural Diffuse Epithelioid Mesothelioma**

Although preliminary data regarding prognostic significance of nuclear grading in epithelioid diffuse mesothelioma were mentioned in the 2015 WHO classification, a formal grading system was not included. Nevertheless, a two-tier nuclear grading system that incorporates nuclear atypia (Fig. 4A-C), mitoses, and the presence or absence of necrosis is now included in the 2021 classification for epithelioid diffuse mesothelioma.

On the basis of an investigation of a large series of epithelioid diffuse malignant pleural mesothelioma, Kadota et al. first proposed a nuclear grading system combining the two independent prognostic factors on multivariate analysis—nuclear atypia and mitotic count. After this study, Rosen et al. revealed the value of nuclear grade and necrosis in predicting overall survival in epithelioid diffuse pleural mesothelioma in a multi-institutional study that included many experts in mesothelioma pathology. They also revealed that the addition of necrosis to nuclear grade further stratified overall survival.
<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Features/Patterns</th>
<th>Favorable</th>
<th>Unfavorable</th>
<th>Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelioid mesothelioma</td>
<td>Composed of round, epithelioid cells, usually with cohesive architecture, but single cells within a fibrous stroma may also be seen</td>
<td>Architectural patterns: Tubulopapillary, Trabecular, Adenomatoid, Solid, Micropapillary</td>
<td>Architectural patterns: Tubulopapillary, Trabecular, Adenomatoid</td>
<td>Architectural patterns: Solid (≥50%), Micropapillary</td>
<td>Grade (high or low), architectural patterns present (and in definitive resection specimens, such as EPD and EPP, percentages of each pattern; for all other specimens, indicate “with … patterns/features”)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytologic features: Rhabdoid, Deciduoid, Small cell, Clear cell, Signet ring, Lymphohistiocytoid, Pleomorphic</td>
<td>Cytologic features: Lymphohistiocytoid, Low nuclear grade</td>
<td>Cytologic features: Rhabdoid, Pleomorphic, High nuclear grade</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stromal features: Myxoid</td>
<td>Stromal features: Myxoid (if predominant, i.e., when &gt;50% of tumor with &lt;50% solid pattern contains myxoid stroma)</td>
<td>Necrosis (included in grading)</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid mesothelioma, including desmoplastic pattern</td>
<td>Composed of elongated/spindle cells (&gt;2 times longer than wide) arranged in solid sheets or within a fibrous stroma</td>
<td>Cytologic features: Lymphohistiocytoid, Transitional, Pleomorphic</td>
<td>Cytologic features: Lymphohistiocytoid</td>
<td>Cytologic features: Transitional</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stromal features: Desmoplastic, With heterologous differentiation</td>
<td></td>
<td></td>
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<tr>
<td>Biphasic mesothelioma</td>
<td>Composed of both epithelioid and sarcomatoid components (in definitive resection specimens, namely EPD and EPP, ≥10% of each component is required for diagnosis); for smaller samples, including biopsy and cytology specimens, the diagnosis of biphasic mesothelioma can be rendered regardless of percentages of each component present</td>
<td></td>
<td>Percentage of sarcomatoid component should be reported regardless of specimen type</td>
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</tbody>
</table>
survival, allowing classification of epithelioid diffuse pleural mesothelioma into the following four distinct prognostic groups: nuclear grade I tumors without necrosis (29 mo), nuclear grade I tumors with necrosis and grade II tumors without necrosis (16 mo), nuclear grade II tumors with necrosis (10 mo), and nuclear grade III tumors (8 mo). On the basis of these results, a multi-disciplinary group supported by European Reference Network for rare solid adult cancers (EURACAN)/International Association for the Study of Lung Cancer (IASLC) proposed a two-tier system of low and high grade for epithelioid diffuse pleural mesothelioma, based on a combination of nuclear features, mitotic rate, and the presence or absence of necrosis (Fig. 4). According to the grading system described in the EURACAN/IASLC proposal and adopted by the 2021 WHO classification, areas revealing the highest-grade features should be used to assign tumors to low grade or high grade. All nuclear grade 1 tumors (with or without necrosis) and nuclear grade 2 tumors without necrosis are classified as low grade, and nuclear grade 2 tumors with necrosis and any nuclear grade 3 tumors are classified as high grade. The 2021 WHO classification recommends routine reporting of the EURACAN/IASLC nuclear grade in both biopsy and resection specimens of epithelioid diffuse pleural mesothelioma to help identify tumors that may behave more aggressively. Zhang et al. validated this two-tiered grading system in a large study of more than 500 biopsy specimens.

**IHC in Diagnosis of Mesothelioma**

Claudin 4 with membranous and cytoplasmic staining has emerged as a reliable carcinoma marker in the differential diagnosis of mesothelioma and metastatic carcinoma, and it has been found to perform with a higher sensitivity (77%–100%) and specificity (99%–100%) than conventional carcinoma markers. BAP1 (Fig. 5A) and EZH2 IHC and CDKN2A homozygous deletion by FISH (Fig. 5C and D) have emerged as reliable markers for the separation of benign mesothelial proliferation versus mesothelioma, but they are not appropriate for distinguishing mesothelioma from other malignant tumors. In addition, cytoplasmic loss of MTAP expression by IHC (Fig. 5B) occurs in approximately 90% of tumors with homozygous deletion of CDKN2a because these two genes reside within close proximity on the 9p21 region. Therefore, MTAP
IHC is increasingly used in clinical practice as a surrogate marker for CDKN2A homozygous deletion.\textsuperscript{51} It is important to also note that these markers have variable sensitivity for diffuse pleural mesothelioma; therefore, the analysis of retained expression of BAP1, EZH2, or MTAP by IHC or the absence of CDKN2a homozygous deletion by FISH does not exclude the diagnosis of diffuse pleural mesothelioma.

The 2021 WHO classification emphasizes the importance of rigorous validation of ancillary studies, particularly with BAP1 IHC, given the increased acceptance of their utility and increasingly widespread use for the diagnosis of mesothelioma. Reliable validation of nonpredictive marker assays requires 10 positive and 10 negative samples, with results comparable with published data on sensitivities and specificities.\textsuperscript{52} These markers have also been found to be useful in effusion specimens when proper validation of these antibodies on cytology material is performed in individual laboratories.\textsuperscript{52–55} If a laboratory uses fixatives other than buffered formalin, a common practice for cytology specimens, the laboratory is obligated to validate the performance of the test in samples fixed with the alternative fixative against samples fixed in buffered formalin because some antibodies do not perform well after alcohol fixation of specimens.\textsuperscript{53,56}

### Diagnostic Criteria for Reporting Pleural Diffuse Mesothelioma by Specimen Type

The 2021 WHO classification includes specific recommendations for the reporting of pleural diffuse mesothelioma on the basis of specimen type. The classification provides reporting recommendations and templates to assist in reporting diagnoses in definitive resection specimens (i.e., extended pleurectomy/decortication [EPD] and extrapleural pneumonectomy [EPP]) and all other smaller specimens (i.e., smaller biopsy specimens and cytology) (Table 5).

A minimum of 10% of either epithelioid or sarcomatoid component remains the criterion for the diagnosis of biphasic mesothelioma in definitive resection specimens (i.e., EPD/EPP) but is no longer required for the diagnosis of biphasic mesothelioma in smaller specimens, including biopsy and cytology specimens. The percentage of sarcomatoid component should be reported for biphasic mesotheliomas regardless of specimen type. Similarly, desmoplastic mesothelioma can be diagnosed in definitive resection specimens (i.e., EPD/EPP) if more than 50% of the tumor has desmoplastic features, characterized by spindle cells with minimal atypia arranged in a haphazard pattern within a dense/hyalinized stroma. In small biopsy specimens, the designation “with desmoplastic features” is recommended when these morphologic features are present.

For epithelioid diffuse mesothelioma, the grade (high or low) and any architectural patterns and/or cytologic and/or stromal features present in the tumor should be reported. In definitive resection specimens

<table>
<thead>
<tr>
<th>Nuclear grade</th>
<th>1 for mild</th>
<th>2 for moderate</th>
<th>3 for severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitotic count score</td>
<td>1 for low (≤1 mitosis/2 mm²)</td>
<td>2 for intermediate (2-4 mitoses/2 mm²)</td>
<td>3 for high (&gt;5 mitoses/2 mm²)</td>
</tr>
<tr>
<td>Sum</td>
<td>2 or 3 = nuclear grade I</td>
<td>4 or 5 = nuclear grade II</td>
<td>6 = nuclear grade III</td>
</tr>
</tbody>
</table>

Table 4. Nuclear Grading of Pleural Diffuse Epithelioid Mesothelioma

Adapted with permission from Nicholson et al.\textsuperscript{20}
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### Figure 4

The 2021 WHO two-tiered nuclear grading incorporates nuclear atypia. Examples of diffuse pleural mesothelioma with nuclear atypia scores of (A) mild, 1; (B) moderate, 2; and (C) severe, 3, are found.
(i.e., EPD/EPP), the percentages of each pattern/feature should be reported. For all other specimen types, reporting should include “with ... patterns/features.”

Staging should be included in pathology reports for definitive resection specimens (i.e., EPD/EPP) using the TNM staging system.

Differential Diagnoses of Diffuse Pleural Mesothelioma: Thoracic SMARCA4-Deficient Undifferentiated Tumor

Thoracic SMARCA4-deficient undifferentiated tumor (TSDUT) is a recently described entity recognized in the 2021 WHO classification.57–62 TSDUTs are in the differential diagnosis of diffuse pleural mesothelioma because they...

![Figure 5](image_url)

**Figure 5.** Analysis of loss of (A) BAP1 and (B) MTAP expression by immunohistochemistry (note the presence of internal positive controls) and (D and C) homozygous deletion of CDKN2A detected by FISH are reliable in supporting a diagnosis of mesothelioma when benign mesothelial proliferations are in the differential diagnosis. These ancillary studies can be performed on cytology specimens. BAP1 immunohistochemistry found here was performed on cell block material. FISH, fluorescence in situ hybridization.

**Table 5.** Examples of Pathology Reporting a Diffuse Pleural Mesothelioma in Biopsy and Resection Specimens (i.e., Extended Pleurectomy/Extrapleural Pneumonectomy)

<table>
<thead>
<tr>
<th>Specimens</th>
<th></th>
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<tbody>
<tr>
<td>Small specimens (i.e. biopsy and cytology specimens):</td>
<td></td>
</tr>
<tr>
<td>Tumor site, specimen type:</td>
<td></td>
</tr>
<tr>
<td>Histologic type (epithelioid, biphasic, or sarcomatoid, if desmoplastic features are present, include “with desmoplastic features”)</td>
<td></td>
</tr>
<tr>
<td>High/low grade (use only for epithelioid)</td>
<td></td>
</tr>
<tr>
<td>List all architectural patterns (do not give a percentage) and any cytologic or stromal features present (do not give a percentage)</td>
<td></td>
</tr>
</tbody>
</table>

**Example of a pathology report for a biopsy specimen:**

Pleura (biopsy): epithelioid mesothelioma, high grade. Solid pattern and with rhabdoid cytologic features

**Resection specimens (i.e., extended pleurectomy/extrapleural pneumonectomy):**

| Tumor site, specimen type: |  |
| Histologic type (epithelioid, biphasic, or sarcomatoid/desmoplastic) |  |
| High/low grade (use only for epithelioid) |  |
| List all architectural patterns present (give a predominant pattern and percentages for each pattern listed) and any cytologic and/or stromal features present |  |

**Example of a pathology report for a resection specimen:**

Extended pleurectomy: Epithelioid mesothelioma, high grade. Predominantly tubulopapillary pattern (80%), also with micropapillary pattern (20%) and pleomorphic features (20%).

**AJCC** stage (eighth edition): pT1pN0

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*When a diagnosis of biphasic mesothelioma is made, a comment should be included to indicate the percentage of sarcomatoid component present.

*Using the TNM staging system.

AJCC, American Joint Committee on Cancer.
can involve the pleura. The typical morphologic features of TSDUTs include solid sheets of discohesive epithelioid tumor cells with or without rhabdoid-like features and often with tumor necrosis. The morphologic features of TSDUTs (Fig. 6A) can be similar to some epithelioid mesotheliomas (Fig. 6B). TSDUTs typically are negative for keratins, or have only very focal keratin expression, are often positive for stem cell markers, including CD34, SOX2, and SALL4, and have loss of BRG1 (SMARCA4) by IHC (Fig. 6C–F) or mutations in the SMARCA4 gene by sequencing. The use of mesothelial markers in the workup of pleural tumors should also help differentiate mesothelioma from TSDUTs.

**Genetics**

Several large-scale sequencing studies addressing molecular profiles of diffuse pleural mesothelioma have been published since the 2015 WHO classification...
advancing the understanding of the genomic landscape of diffuse mesothelioma. Most studies focused on improving the histologic classification and prognostic stratification of pleural mesothelioma and revealed intratumoral genomic heterogeneity.\textsuperscript{63-68} Bueno et al.\textsuperscript{63} reported four cluster groups of mesotheliomas on the basis of expression patterns that mostly matched the 2015 WHO histologic classification and correlated with overall survival. Those clusters included sarcomatoid, epithelioid, biphasic-epithelioid, and biphasic-sarcomatoid, recapitulating epithelial-to-mesenchymal transition. Similarly, the TCGA cohort identified four distinct prognostic groups on the basis of genomic, transcriptomic, and epigenomic analysis\textsuperscript{64} (Fig. 7). By combining transcriptome, methylome, and miRNome analysis, Blum et al.\textsuperscript{65} revealed that pleural mesotheliomas have different proportions of epithelioid and sarcomatoid components (E-score and S-score). Alcala et al.\textsuperscript{66} revealed the link between those scores and the mesothelioma microenvironment. Transcriptome analysis of previously recognized epithelioid pattern of transitional mesothelioma provided the rationale for its reclassification as a cytologic feature of sarcomatoid mesothelioma as it revealed genomic characteristics similar to sarcomatoid subtype.\textsuperscript{67} Genomic analysis of pleomorphic mesothelioma revealed molecular characteristics shared with both epithelioid and sarcomatoid subtypes and therefore was reclassified from epithelioid pattern to a cytologic feature that could be associated with either sarcomatoid or epithelioid subtypes.\textsuperscript{69}

Large-scale comprehensive testing, such as next-generation sequencing, can be successfully performed on pleural fluid or tissue samples, but identification of druggable targets is very low.\textsuperscript{70,71} Because of the lack of identifiable targets on most of the commercially available next-generation sequencing platforms, testing for predictive biomarkers of response is not recommended as a routine clinical practice at this time.\textsuperscript{72,73}

Somatic mutation burden in mesothelioma is low, usually less than two nonsynonymous mutations per megabase, and with no difference among histologic subtypes.\textsuperscript{63,64} Somatic copy number alterations, primarily deletions, and most frequently \textit{CDKN2A}, are the most common genetic events.\textsuperscript{63,64} \textit{CDKN2A} homozygous deletions most frequently occur in sarcomatoid mesotheliomas, followed by biphasic and epithelioid, and are an established diagnostic marker of malignant mesothelial proliferations.\textsuperscript{44,74} Most frequently mutated genes are \textit{BAP1}, \textit{NF2}, \textit{TP53}, \textit{SETD2}, \textit{DDX3X}, \textit{ULK2}, \textit{RYR2}, \textit{CFAP45}, \textit{SETDB1}, and \textit{DDX51}.\textsuperscript{53,64} In the TCGA cohort, a subset of mesotheliomas with \textit{TP53} and \textit{SETDB1} co-mutations associated with genome-wide loss of heterozygosity which affects more than 80% of the genome ("genomic near-haploidization") was identified mostly in young female patients.\textsuperscript{64} In addition, recurrent \textit{EWSR1/\textit{FUS}-ATF1} fusions have been detected in mesotheliomas in younger adults.\textsuperscript{75}

Recent clinical trials revealed promising results for efficacy of immune checkpoint inhibitors in the treatment of pleural mesotheliomas, but no definitive predictive biomarker has been identified.\textsuperscript{76} The predictive role of programmed death-ligand 1 (PD-L1) expression in pleural mesotheliomas remains controversial, and there is no defined predictive cutoff of response. Nevertheless, PD-L1-positive pleural mesotheliomas respond better to immune checkpoint inhibitors than those that are negative for PD-L1.\textsuperscript{77} Several trials are ongoing to investigate the role and prognostic value of the immune microenvironment of mesotheliomas. Molecular S- and E-scores seem to reflect on mesothelioma microenvironment and may have predictive value.\textsuperscript{65,66} The S-score correlated with the presence of T-cells, monocytes, fibroblasts, and endothelial cells and high expression of PD-L1. The E-score was associated with infiltration of natural killer cells, complement pathway, and VISTA overexpression. These results are consistent with reports of frequent association of PD-L1 protein expression and sarcomatoid mesotheliomas, poor prognosis, and increased lymphocytic inflammation.\textsuperscript{78-83}

\textit{TRAF} mutations have been described in localized pleural mesothelioma\textsuperscript{84} and in adenomatoid tumors of the genital tract\textsuperscript{87} but have not yet been identified in thoracic adenomatoid tumors, although these are exceedingly rare with less than 20 reported in the thoracic region.\textsuperscript{88-90} \textit{BAP1} mutations and genomic near haploidization have also been described in localized pleural mesothelioma.\textsuperscript{86}

**Summary**

In summary, this review provides an update to the changes in the \textit{WHO Classification of Pleural and Pericardial Tumors} on the basis of recent advances since the 2015 classification which have led to improvements in the diagnosis of these tumors.

**CRediT Authorship Contribution Statement**

Jennifer L. Sauter, William D. Travis: Conceptualization, Data curation, Writing - original draft, Writing - review & editing.

Sanja Dacic, Kelly J. Butnor, Andrew Churg, and Kyuichi Kadota: Writing - original draft, Writing - review & editing.

Francoise Galateau-Salle, Richard L. Attanoos, Aliya N. Husain, Andras Khoor, Andrew G. Nicholson,
Victor Roggli, Fernando Schmitt, and Ming-Sound Tsao: Writing - review & editing.

Acknowledgments

The authors wish to thank Professor Ian Cree (Editorial Board Chair), all members of the Editorial Board for the Thoracic Tumor Book, and International Agency for Research on Cancer staff involved in the WHO Classification of Tumours, fifth edition series, for their contribution to the completion of the fifth edition book. The authors also thank Ms. Francis Bodd and Ms. Jessica Lopardo for their assistance with manuscript preparation.

References


