Review Article

Morphology and immunohistochemical and molecular markers for diagnosis and guiding therapy in mesothelioma: a narrative review

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Background and Objective: Mesothelioma is a rare tumor that is diagnostically challenging. Morphologic subtyping and nuclear grading have known prognostic value. Historically, the most common use of immunohistochemistry (IHC) was to prove mesothelial or epithelial differentiation. While IHC plays a role in establishing mesothelial lineage, an expanded role for IHC in the differentiation of malignant from benign mesothelial proliferations has emerged. These advances in IHC are a result of better understanding the genetics of mesothelioma. In this background of advanced IHC and an understanding of mesothelioma genetics, immunotherapy has arrived as a potential therapy that may revolutionize treatment. This narrative review aims to summarize the morphologic considerations in diagnosing mesothelioma, the use of IHC in mesothelioma, how IHC relates to mesothelial genetics, and how these topics currently relate to prognosis and potential uses in clinical decision making.

Methods: PubMed database was utilized to search the English literature for topics on mesothelioma diagnosis, morphology, nuclear grade, IHC, and genetics.

Key Content and Findings: One of the most prognostically significant features of mesothelioma is its subtype. Numerous IHC stains exist that aid in establishing mesothelioma lineage. Claudin 4 has recently emerged as an excellent marker of epithelial differentiation. Newer IHC stains have been proposed to separate benign from malignant mesothelioma, with the most notable and widely used being BAP1 and MTAP. Many of these IHC markers have been tied to prognosis. Programmed death ligand-1 (PD-L1) is a nascent marker in mesothelioma that will likely play an expanded role as immunotherapy continues to be investigated in mesothelioma.

Conclusions: The diagnosis and classification of mesothelioma has moved from its earlier forms based purely on histologic subtyping. Numerous IHC markers are now in use which can distinguish benign and malignant mesothelial proliferations. Some of these markers, most notably BAP1 and MTAP, function as disease prognosticators. With the emergence of immunotherapy in mesothelioma, these prognostic and predictive markers take on added significance. As these immunophenotypic and molecular advancements continue to populate the literature, there will be hope that these markers can help guide patients to appropriate and more effective therapies.

Keywords: Mesothelioma; immunohistochemistry (IHC); BAP1; MTAP; claudin 4

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Introduction

Outside of large referral centers, mesothelioma is rarely encountered by the general surgical pathologist, with only around 2,400–3,200 cases per year in the United States, a number which has largely stabilized, but increasing numbers of cases are being reported in other parts of the world, including China (1-3). Mesothelioma is often diagnosed by medical thoracoscopy which has a high diagnostic yield (4-7). In contrast to carcinomas of the lung, which have long been well characterized both immunophenotypically and genetically, use of immunohistochemistry (IHC) in mesothelioma has largely been restricted to determining that a lesion shows mesothelial differentiation, and genetic characterization of mesothelioma was an afterthought. This has changed dramatically over the past decade. While IHC still plays a major role in establishing mesothelial lineage, IHC can also be utilized in the differentiation of malignant from benign mesothelial proliferations. These advances are the direct result of an increased understanding of genetics that underlie mesothelial oncogenesis. This narrative review aims to summarize the most up-to-date data on the morphologic considerations in diagnosing mesothelioma, the use of IHC in mesothelioma, how IHC relates to the current understanding of mesothelial genetics, and how these topics currently relate to prognosis and potential uses in clinical decision making.

Many of these advances have come from international multi-institutional collaborations that have resulted in the highly cited series of pathologic diagnostic guidelines endorsed by the International Mesothelioma Interest Group (IMIG) and authored by IMIG members and mesothelioma experts (8-10). An updated 4th revision of these guidelines is currently in preparation. We present this article in accordance with the Narrative Review reporting checklist (available at https://shc.amegroups.com/article/view/10.21037/shc-23-22/rc).

Methods

English language articles from the PubMed database were searched on multiple occasions between the dates of April 1st, 2023, and May 5th, 2023 (*Table 1*, Table S1). Data search terms included "mesothelioma morphology", "mesothelioma nuclear grade", "mesothelioma transitional pattern", "mesothelioma prognostic features", "immunohistochemistry mesothelioma", "immunohistochemistry mesothelioma prognosis", "BAP1 mesothelioma", "MTAP mesothelioma", "mesothelioma cytology", "mesothelioma PD-L1 expression", and "mesothelioma immunotherapy". The narrative review was created from PubMed search results from the list of search terms, along with the authors' personal knowledge of the literature. The result of this process is summarized in the review below along with selected references.

Morphologic considerations

Assessment of mesothelial morphology starts with subclassification of mesothelioma into three histologic subtypes, epithelioid, biphasic, and sarcomatoid. These histologic subtypes have long been known to correlate to prognosis, with epithelioid morphology associated with the best overall median survival, followed by biphasic, and then sarcomatoid (11-13). Beyond prognostication, this basic histologic subtyping of mesothelioma has proven useful in surgical management, as surgery shows no benefit in patients with sarcomatoid morphology, and many also consider this to be true for patients with biphasic morphology (14,15). While morphologic subtype plays into the decision to proceed to surgical intervention, it should be highlighted that the diagnosis and initial classification of mesothelioma is often made after microscopic examination of only a small sampling of the tumor obtained by percutaneous or video-assisted thoracoscopic surgery (VATS). In this setting, we, along with others, have demonstrated that biphasic morphology is often underrepresented in small biopsies, and that one can expect approximately 20% of epithelioid mesotheliomas to show biphasic morphology if resected or additional tumor sampling is undertaken (16-19). Biphasic or sarcomatoid morphology, if identified in a biopsy, is highly specific for non-epithelioid morphology and should be considered representative of the true histologic subtype of the tumor (16).

Some authors have shown that there may be a prognostic significance associated with the percentage of epithelioid morphology in biphasic mesothelioma, ranging from 50–80% epithelioid morphology, but currently there is not enough data in the published literature to draw any firm conclusions (13,20). If a clinician does wish to incorporate the percentage of epithelioid morphology of biphasic mesotheliomas into any future treatment algorithm or, for the purposes of enrollment into a clinical trial, it is important to note that only a fair degree of agreement exists between biopsies and resection specimens in the quantification of percent epithelioid morphology (16).

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Table 1 The search strategy summary

Items	Specification
Date of search	Between 1st April 2023 and 5th May 2023
Databases and other sources searched	PubMed
Search terms used	Free text searches only using the following search terms: "mesothelioma morphology", "mesothelioma nuclear grade", "mesothelioma transitional pattern", "mesothelioma prognostic features", "immunohistochemistry mesothelioma", "immunohistochemistry mesothelioma prognosis", "BAP1 mesothelioma", "MTAP mesothelioma", "mesothelioma cytology", "mesothelioma PD-L1 expression", and "mesothelioma immunotherapy"
Timeframe	No timeframe restrictions on data
Inclusion and exclusion criteria	Inclusion criteria: • English language texts only • All peer-reviewed studies and article types were acceptable • Studies focused on pleural mesothelioma unless discussion pertained to peritoneal mesothelioma Exclusion criteria: • Non-English language text • Full text unavailable via https://pubmed.ncbi.nlm.nih.gov/ links • Non-peer reviewed studies
Selection process	Appropriate articles were selected by one author (Schulte JJ) based on relevance to topic being discussed

Recently, a new morphologic pattern, transitional, has emerged in the literature. Transitional mesothelioma is defined as a pattern in which the mesothelial cells have lost some epithelioid morphology (plump and elongated; not as round as typical epithelioid cells), but are not overtly sarcomatoid (they retain some cellular cohesion in the form of sheet-like growth that is more typical of an epithelioid morphology) (21). In a previous edition of the World Health Organization (WHO), given the cellular cohesion of the transitional cells, transitional was considered a pattern of epithelioid mesothelioma (21,22). Studies now show that transitional pattern is strongly associated with poor prognosis, similar to the prognosis reported for sarcomatoid mesothelioma (13,23,24). The similarities in prognosis between transitional pattern and sarcomatoid mesothelioma are likely a result of underlying genetic similarities (25). While expert consensus originally advocated for transitional mesothelioma to be a pattern that could be observed in both epithelioid and sarcomatoid mesothelioma (21), the most recent WHO classification places transitional mesothelioma into a cytologic feature of sarcomatoid mesothelioma (26,27). Given that transitional is now regarded as a cytologic feature of sarcomatoid mesothelioma, an epithelioid mesothelioma with a transitional component should be regarded as biphasic mesothelioma (26).

Beyond basic histologic subtyping and transitional features, numerous other architectural, cytologic, and stromal features have been found to be prognostically important in mesothelioma. A comprehensive review of all these features is beyond the scope of this text and has been outlined in other recently published works to which the reader if referred (10,21,27). Nevertheless, the reader should be made aware of one very powerful prognostic tool in epithelioid mesothelioma. This prognostic tool is the nuclear grading system. Nuclear grading in mesothelioma was originally developed by researchers at Memorial Sloan-Kettering Cancer Center in New York (28). This three-tier grading system (nuclear grades 1, 2, and 3), based on nuclear atypia and mitotic activity, was shown to stratify patients with epithelioid mesothelioma into three distinct prognostic groups; nuclear grade 1 (*Figure 1*) showed a median survival of 28 months, nuclear grade 2 (*Figure 2*) was 14 months, and nuclear grade 3 (*Figure 3*) was only 5 months. Subsequently, a large multicenter study by Rosen *et al.* reaffirmed these findings, but also showed that the presence of necrosis is an independent marker of adverse outcome (29). Rosen *et al.* also suggested that by combining the three-tier nuclear grade with the presence of necrosis, it is possible

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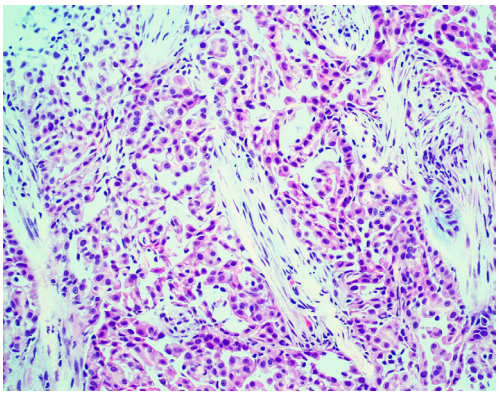

Figure 1 Nuclear atypia grade 1. Notice proliferation of monotonous mesothelial cells without significant nuclear pleomorphism and lack of prominent nucleoli (H&E, 200×). H&E, hematoxylin and eosin.

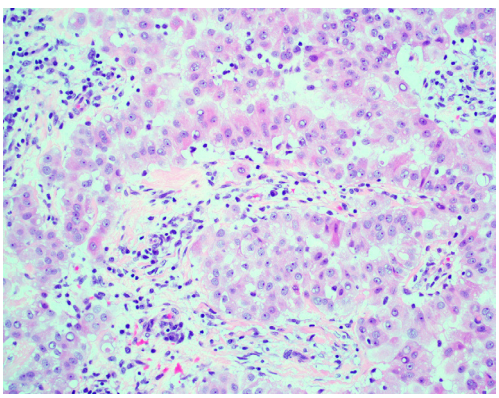

Figure 2 Nuclear atypia grade 2. Mesothelioma showing some nuclear pleomorphism with variably conspicuous nucleoli (H&E, 200×). H&E, hematoxylin and eosin.

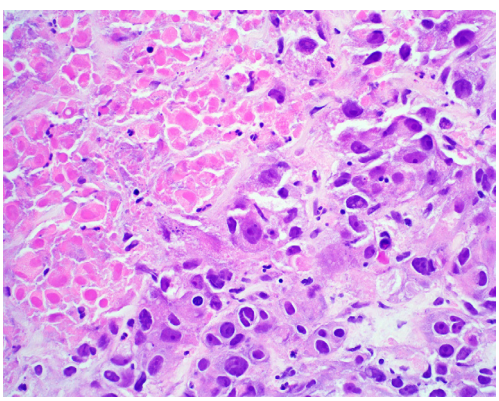

Figure 3 Nuclear atypia grade 3. Mesothelioma with obvious and marked nuclear pleomorphism. Notice necrosis in the upper left corner (H&E, 400×). H&E, hematoxylin and eosin.

to further stratify these patients into distinct prognostic groupings (29). While never formally studied prior to adoption, expert consensus recommended the creation of a two-tier (high and low) nuclear grading system in which all nuclear grade 1 epithelioid mesotheliomas and nuclear grade 2 epithelioid mesotheliomas without necrosis are classified as low grade, while nuclear grade 2 epithelioid mesotheliomas with necrosis and all nuclear grade 3 epithelioid mesotheliomas are high grade (21,26). A later study showed that the two-tier grading system can be applied to biopsy specimens (30). The two-tier grading system is now recommended by the WHO to be reported on any specimen type and is a required synoptic reporting element in the College of American Pathologists' (CAPs') synoptic reporting for mesothelioma (27,31). Additional grading systems for mesothelioma have been proposed that incorporate other histopathologic parameters and can be applied to non-epithelioid subtypes of mesothelioma (32-34). Given the poor outcome typical of non-epithelioid mesotheliomas, it is the opinion of the authors and other experts that the clinical utility of such grading schemes is currently unclear and of limited value. There is only incomplete data that exists to suggest that histologic grading of epithelioid mesothelioma plays a role in treatment selection (35), and it is not yet routinely used in clinical decision-making guidelines. Nonetheless, it is obvious that nuclear grading remains one of the most powerful and robust prognostic tools generated by simple microscopic examination of hematoxylin and eosin (H&E) sections. With the widespread endorsement of nuclear grading by expert mesothelioma pathologists, the WHO, and CAP, it is anticipated that nuclear grading will someday be incorporated into clinical diagnostic and therapeutic algorithms.

IHC in the diagnosis and classification of mesothelioma

IHC is an indispensable tool that is utilized by the pathologist in essentially every case of mesothelioma that is diagnosed. The principal use of IHC in mesothelial pathology is establishing mesothelial lineage. Published diagnostic guidelines support use of an IHC panel in the work-up of mesothelial lesions (10). The panel approach encourages using at least two mesothelial markers and two epithelial markers in the panel at a minimum (10). For example, an epithelioid pleural based lesion that is positive for two mesothelial markers and negative for two

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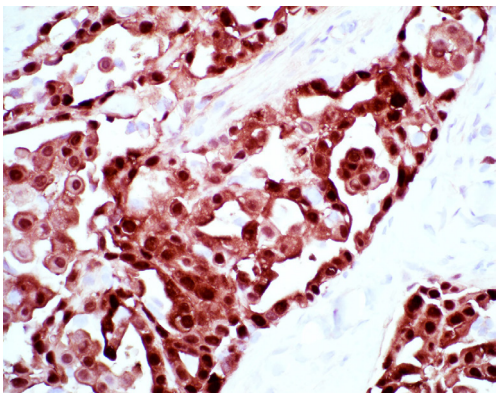

Figure 4 Calretinin IHC in mesothelioma. Calretinin is a highly sensitive but nonspecific marker of mesothelial differentiation characterized by both cytoplasmic and nuclear reactivity (IHC with DAB chromogen, 400×). IHC, immunohistochemistry; DAB, diaminobenzidine.

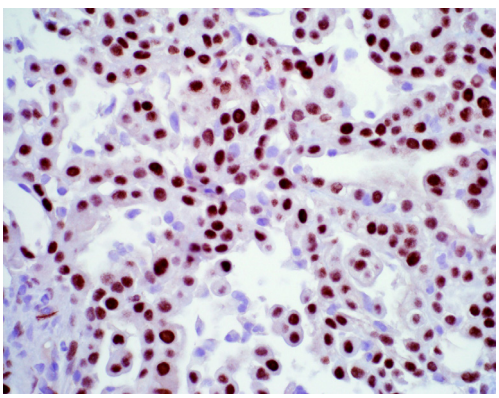

Figure 5 WT-1 IHC in mesothelioma. WT-1 is another highly sensitive but nonspecific marker of mesothelial differentiation characterized by nuclear reactivity (IHC with DAB chromogen, 400×). IHC, immunohistochemistry; DAB, diaminobenzidine.

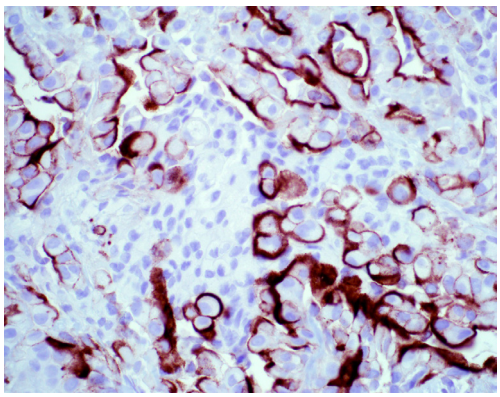

Figure 6 D2-40 IHC in mesothelioma. In contrast to calretinin and WT-1, D2-40 positivity is characterized by membranous reactivity (IHC with DAB chromogen, 400×). IHC, immunohistochemistry; DAB, diaminobenzidine.

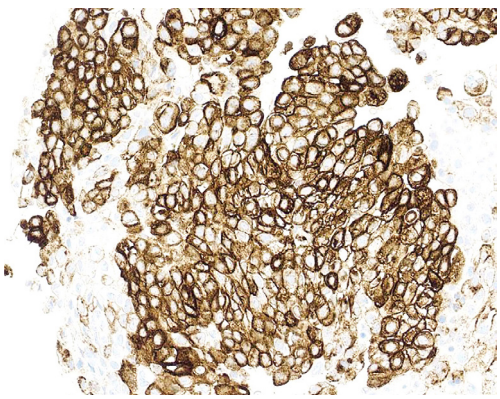

Figure 7 HEG1 IHC in mesothelioma. HEG1 is a new sensitive marker for mesothelial differentiation characterized by membranous expression. The photo shows HEG1 in an epithelioid mesothelioma (IHC with DAB chromogen, 400×). Reactivity is often granular or absent in sarcomatoid mesothelioma. IHC, immunohistochemistry; DAB, diaminobenzidine.

epithelial markers, would be supportive of a diagnosis of mesothelioma. The most commonly used antibodies that support mesothelial lineage include calretinin (*Figure 4*), WT-1 (*Figure 5*), and D2-40 (*Figure 6*), while common epithelial markers include Ber-EP4, MOC31, and carcinoembryonic antigen (CEA) (10,36-38). It is often useful, and necessary, to tailor the specific panel of stains to the differential being considered. For example, CK5/6 stains the majority of mesotheliomas, but also stains nearly all squamous cell carcinomas, so this marker is not useful if the two top diagnostic consideration are mesothelioma and pulmonary squamous cell carcinoma. Similarly, if one wishes to exclude metastatic ovarian carcinoma to the pleura prior to making a diagnosis of mesothelioma, WT-1 is a useless marker given the high degree of expression observed in both these tumors. The individual sensitivities and specificities of these markers and which stains perform best in each diagnostic scenario has been reviewed elsewhere and the reader is referred to these articles for further information (10,36).

While many of the antibodies used in the workup of mesothelial lesions have been in use for years, more recently, a new mesothelial marker, HEG1 (*Figure 7*), and

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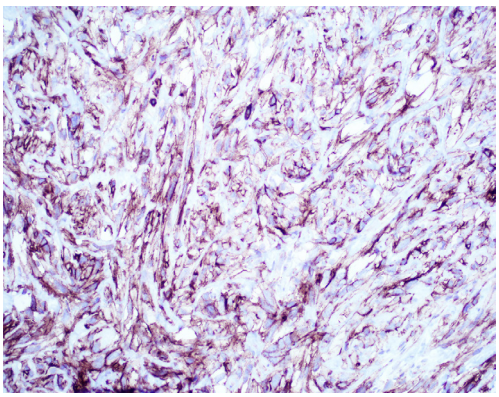

Figure 8 Claudin 4 IHC in sarcomatoid carcinoma. Claudin 4 is a new sensitive marker for epithelial differentiation characterized by membranous expression. The photo shows claudin 4 expression in a pleural-based cytokeratin positive sarcomatoid neoplasm, excluding mesothelioma and allowing for this malignancy to be easily classified as carcinoma (IHC with DAB chromogen, 200×). IHC, immunohistochemistry; DAB, diaminobenzidine.

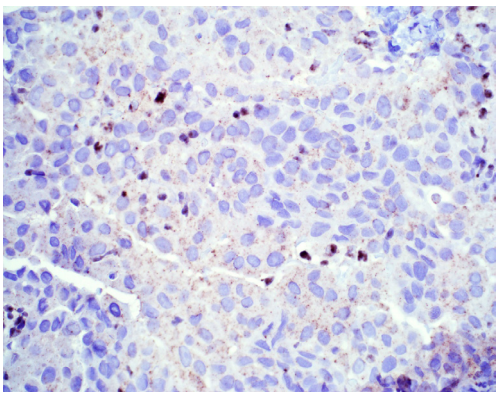

Figure 9 BAP1 in diffuse mesothelioma. This mesothelioma showed loss of BAP1 nuclear expression in the mesothelial cells, consistent with underlying *BAP1* mutation (IHC with DAB chromogen, 400×). Notice retained expression in background inflammatory cells. IHC, immunohistochemistry; DAB, diaminobenzidine.

a new epithelial marker, claudin 4 (*Figure 8*), have come into the spotlight. HEG1 is a highly sensitive marker for epithelioid mesothelioma, with more limited use in biphasic and sarcomatoid mesothelioma (39-42). While HEG1 has great utility in the differential of lung adenocarcinoma versus mesothelioma, its use is diminished if ovarian serous carcinoma is being considered as there is considerable expression of HEG1 in ovarian epithelial tumors. Claudin 4 is the newest epithelial marker in use and it shows superior performance compared to more traditional epithelial markers (Ber-EP4 and MOC31), with some authors advocating that claudin 4 may be sufficient as a stand-alone IHC marker to exclude epithelial differentiation (43-45). The superiority of claudin 4 as a marker of epithelial lineage cannot be overstated. The literature has shown that older epithelial markers can often be expressed in mesothelial cells (up to 35% of cases) (45,46). While claudin 4 is an excellent marker for epithelial lineage, decreased to absent expression has been shown in poorly differentiated and sarcomatoid carcinomas (45,47-49). Given the strong performance of claudin 4 and with the excitement surrounding HEG1 as a new mesothelial marker, some authors have proposed a more limited panel of IHC stains (claudin 4 and HEG1 alone), but until HEG1 is more broadly studied and put into clinical practice, it is difficult at present to recommend deviation from the standard two mesothelial and two epithelial marker panel approach to mesothelioma diagnosis.

The presence of cytologic atypia and mitotic activity alone cannot be used to determine if a mesothelial proliferation is benign or malignant, and the gold standard for determining malignancy has historically relied on invasion of mesothelial cells into underlying tissue (chest wall, fibroadipose tissue, lung, etc.) (10). After establishing mesothelial lineage in a cellular proliferation, it may be difficult to diagnosis the lesion as malignant, especially in small biopsy specimens where underlying tissue may not be sampled. Advancements in the understanding of the genetic underpinnings of mesothelioma have been exploited to aid in the immunohistochemical distinction of benign and malignant mesothelial proliferations. Mutation in BAP1 is a frequent event in the pathogenesis of both sporadic and germline mutated mesothelioma (50-52). Nuclear loss of BAP1 expression by IHC (*Figure 9*) has emerged as a highly specific marker of malignancy in mesothelial proliferations. Loss of nuclear expression of BAP1 indicates a malignant mesothelial proliferation (53-58). While it may be tempting to include BAP1 nuclear loss as evidence of mesothelial differentiation, it should be noted that BAP1 loss can be seen in tumors other than mesothelioma (59). It is still recommended that other mesothelial markers are checked to ensure mesothelial differentiation. Nuclear loss of BAP1, while specific for malignancy, does not have high sensitivity (50–65% across all mesothelioma subtypes) (36). Loss of expression of BAP1 is most sensitive in epithelioid mesothelioma, reaching nearly 80% of cases, with

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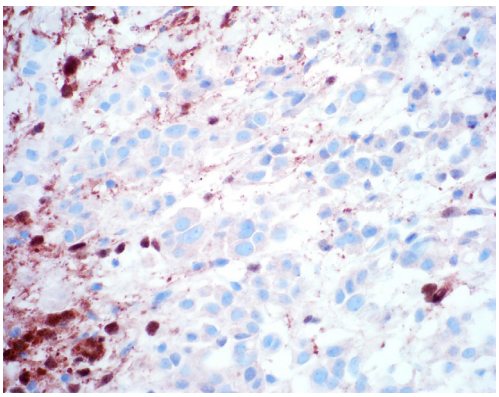

Figure 10 MTAP in diffuse mesothelioma. This mesothelioma showed loss of MTAP expression in the mesothelial cells, consistent with underlying deletion of *MTAP*, a surrogate marker of *CDKN2A* homozygous deletion (IHC with DAB chromogen, 400×). Notice retained expression in background inflammatory cells. IHC, immunohistochemistry; DAB, diaminobenzidine.

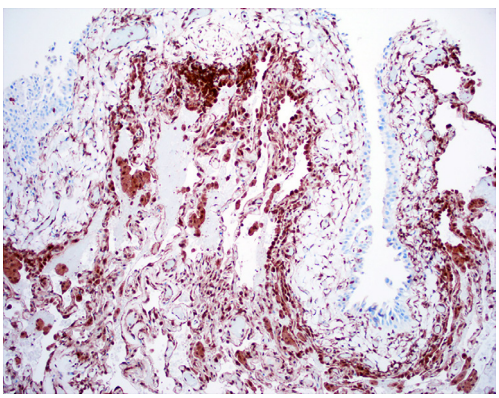

Figure 11 Mesothelioma *in situ*. MTAP IHC shows loss of expression in pleural surface mesothelial cells without invasion of underlying lung parenchyma, diagnostic of mesothelioma *in situ* (IHC with DAB chromogen, 200×). IHC, immunohistochemistry; DAB, diaminobenzidine.

notable drop-offs in sensitivity in non-epithelioid mesothelioma (56). Another common genetic event in mesothelioma is homozygous deletion of *CDKN2A*. Homozygous deletion of *CDKN2A* can be detected by fluorescence in situ hybridization (FISH) and is highly specific for malignancy in mesothelial proliferations (57,60,61). FISH technology is not as accessible or widely available as IHC. Fortunately, methylthioadenosine phosphorylase (*MTAP*), which sits adjacent to *CDKN2A* on chromosome 9 is frequently co-deleted with *CDKN2A* and an IHC stain for MTAP shows a high degree of correlation with *CDKN2A* homozygous deletion (57,60,62,63). In contrast to BAP1 in which nuclear loss of expression indicates malignancy in mesothelial proliferation, cytoplasmic loss of MTAP expression (*Figure 10*) is indicative of malignancy. As with BAP1, it is wise to first ensure that mesothelial lineage has been established with positive mesothelial markers, as MTAP can be lost in a large number of sarcomatoid carcinomas (64). Combining BAP1 and MTAP IHC together can increase the sensitivity for detecting malignancy in mesothelial proliferations (55,57,60,61). BAP1 and MTAP are robust IHC markers, but given imperfect sensitivity in detecting malignancy, some authors have proposed additional combinations of ancillary tests in further attempts to increase sensitivity, including use of p53, merlin, and YAP/TAZ IHC (55,65,66). As these various panels continue to be explored, hopefully a more refined panel will emerge with excellent sensitivity and specificity for malignancy in mesothelial proliferations.

The emergence of BAP1 and MTAP IHC and *CDKN2A* FISH, has allowed for the identification of malignant mesothelial proliferations prior to invasion, termed mesothelioma *in situ* (*Figure 11*) (26,67-69). Mesothelioma *in situ* has long been proposed as a step in oncogenesis, but could not be proven until the development of these ancillary markers. Mesothelioma *in situ* is now an entity in the most recent WHO (27). Very few cases have thus far been collected. What is currently understood is that essentially all patients with mesothelioma *in situ* will go on to have progressive, eventually invasive disease. The time between detection of mesothelioma *in situ* and invasive disease ranges from months to years. An obvious question arises as how to best care for these patients. At present, there is no consensus on treatment recommendations given the novelty and rarity of the diagnosis. Now that pathologists are able to detect mesothermal *in situ* and it is an entity in the WHO, hopefully more cases and series will be reported in the literature and advancements in this space can be made.

Beyond surgical pathology, IHC has also been employed in the classification of serous effusions (70-72). Of note, mesothelial and epithelial markers show excellence performance in pleural fluids, drawing particular attention again to the durability of claudin 4 in identifying epithelial lesions (73). The most interesting application of IHC in serosal fluids has been in the area of BAP1 and MTAP expression (*Figures 12,13*). As in surgical pathology, BAP1 and MTAP IHC have been shown to be able to discriminate benign and malignant mesothelial proliferations with nearly

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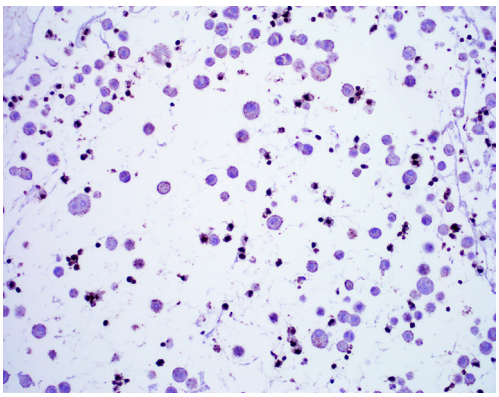

Figure 12 BAP1 in pleural fluid. This patient with recurrent, unexplained pleural effusion showed loss of BAP1 nuclear expression in the mesothelial cells, consistent with a malignant mesothelial proliferation (IHC with DAB chromogen, 200×). Notice retained expression in background inflammatory cells. IHC, immunohistochemistry; DAB, diaminobenzidine.

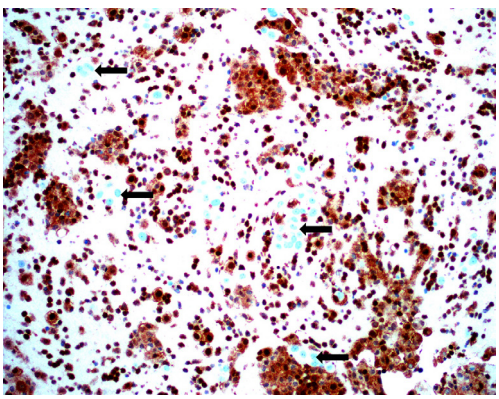

Figure 13 MTAP in pleural fluid. This patient with recurrent, unexplained pleural effusion showed loss of MTAP expression in the mesothelial cells (black arrows), consistent with a malignant mesothelial proliferation (IHC with DAB chromogen, 200×). Notice retained expression in background inflammatory cells. IHC, immunohistochemistry; DAB, diaminobenzidine.

100% specificity as shown by a recent meta-analysis (71). These IHC stains along with the concept of mesothelioma *in situ* are likely to bring about significant changes in the practice of serous fluid cytology and the reader is referred elsewhere for a more in-depth discussion (74-76).

While beyond the scope of this review, it is also important to briefly mention the emerging diagnostic revolution underway brought about by digital pathology and artificial intelligence. These emerging technologies have shown various abilities ranging from basic histologic classification of tumors to prediction of underlying genetic alterations in malignancies (77-80). One important application of these technologies is in cytopathology. Cytology specimens may represent the only specimen for several patients with malignant pleural effusions. While the application of digital pathology and whole slide imaging (WSI) lags behind what has been done in surgical pathology, WSI can be employed by cytologists for accurate diagnosis and classification of cytologic preparations (81). Mobile devices may also be of utility in examination of cytology specimens which may improve diagnosis in underserved areas (82). Some work using artificial intelligence has been done specifically in mesothelioma using WSI, and one of these studies demonstrated that use of a deep-learning classification of mesothelioma resulted in improved prediction of patient outcome (24,83). Another recent study highlighted the power of deep learning to identify the transitional pattern in mesothelioma (25). These are exciting developments, yet the technology and its application to mesothelioma diagnosis and classification is still largely in its nascent form. Additional exciting developments are sure to arise in the coming years.

IHC and molecular testing as prognostic markers and in patient management

Some immunohistochemical markers have been shown to be of prognostic importance which in the future may play into treatment decisions. Loss of nuclear BAP1 staining is likely a favorable prognostic marker, especially when viewed in the context of germline mutations (56,58,84,85). Our group recently attempted to discern if the improved survival in patients with BAP1 mutation was a function of favorable histologic features being more common in BAP1 mutated mesotheliomas (86) We found that pleural epithelioid mesotheliomas that harbor BAP1 mutation were more likely to show low nuclear grade than those without BAP1 mutation, or with other mutations; this finding was not statistically significant when looking at peritoneal mesotheliomas alone. One study looking specifically at peritoneal mesothelioma did not show improved survival with loss of BAP1 IHC staining (87). Others have called into question the prognostic ability of BAP1 IHC especially when comparing BAP1 expression across the different histologic subtypes of mesothelioma (88). In contrast to the trend towards a better prognosis seen with BAP1 mutation, *CDKN2A* homozygous deletion and loss of

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Table 2 Mesothelioma versus carcinoma IHC

Carcinoma markers	Mesothelioma markers
Pan-epithelial markers	Calretinin
Claudin 4	WT-1
MOC31	D2-40
Ber-EP4	CK5/6
Other markers	HEG1
TTF-1	
p40	
PAX8	
Other carcinoma lineage specific markers depending on differential diagnosis	

Panel approach to mesothelioma diagnosis: use at least 2 mesothelioma markers and 2 carcinoma markers. IHC, immunohistochemistry.

cytoplasmic MTAP expression by IHC has been shown to be associated with a worse prognosis (87,89-91). While BAP1 and MTAP are the best studied IHC markers relating to prognostication, additional smaller studies have looked at various IHC markers including NF2, p16, and mesothelin, among others (87,89,91-95). If and how these IHC/molecular signatures may be incorporated into treatment approaches is unclear at present. Given what is currently understood of these markers and their likely prognostic ability, it is perfectly feasible to predict that some marker statuses may be used for enrollment into different arms of clinical trials in the future.

One of the more exciting treatment advancements in recent years has been the application of immunotherapy to mesothelioma with notable survival benefits being shown in non-epithelioid mesothelioma (96-98). It should be noted, nonetheless, that immunotherapy in mesothelioma is still in its infancy with only around 5% of patients receiving treatment (2). In the setting of BAP1 mutation, mesothelioma typically takes on an inflammatory immunophenotype, and while this is exciting in the setting of immunotherapy, the more recent data suggests a stronger survival advantage in non-epithelioid mesotheliomas even though BAP1 mutations are more common with epithelioid morphology (97,99). Mesotheliomas have also been shown to have high expression of *VISTA*, a gene associated with immune-checkpoint (100,101). Programmed death ligand-1 (PD-L1) expression has been tested in mesotheliomas and is more commonly associated with non-epithelioid morphology and a worse outcome (102-104). Mesothelioma expression of PD-L1, particularly in the peritoneum, can change over time following prior treatment; what this means for selecting patients for various treatment regimens is unclear at present (105). Neither routine staining nor interpretive guidelines for PD-L1 have been endorsed by any expert panel or clinical guideline group.

Key practical points on mesothelioma morphologic considerations

The above sections highlight the importance of accurate morphologic classification of mesothelioma. Unfortunately, achieving an accurate diagnosis and classification is often challenging. In this section, the authors herein offer some practical points to consider when rendering a diagnosis of mesothelioma.

Proving mesothelial lineage

The authors strongly advocate for continuing the practice of an IHC panel approach to establishing mesothelioma lineage (*Table 2*). Pathologists should be aware of the lack of specificity of the so called "mesothelioma markers". Many of the markers of mesothelioma lineage stain carcinomas. Pathologists also need to be cognizant of the fact that mesotheliomas may occasionally show reactivity for classic pan-epithelial markers like Ber-EP4 and MOC31. In their everyday practice, the authors frequently rely on Claudin 4 as the most specific epithelial marker in excluding carcinoma. Pathologists should move to adopt this marker in their own laboratories or order the IHC using an external reference lab when working on a case in which the differential is carcinoma versus mesothelioma.

Benign versus malignant mesothelial proliferations

Separating benign from malignant mesothelial proliferations remains a very challenging task. This is particularly true when dealing with fibrous pleuritis versus mesothelioma. A general rule of thumb is that fibrous pleuritis and other reactive mesothelial proliferations show orderly and predictable arrangement of mesothelial cells. Mesotheliomas often show a full thickness proliferation of mesothelial cells that is often architecturally complex or haphazardly arranged. The morphologic differences between benign and malignant mesothelial proliferation

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Table 3 Stepwise approach to establishing malignancy in mesothelial proliferation

Step		
Step 1	Establish mesothelial lineage	See *Table 2*
Step 2	Identify invasion into underlying tissue	Diagnostic of malignancy if present
Step 3	If no invasion: IHC stains BAP1 or MTAP	Nuclear loss BAP1 = malignant
		Cytoplasmic loss MTAP = malignant
Step 4	BAP1 and MTAP retained—perform CDKN2A FISH	Deletion of CDKN2A = malignant
Step 5	If no deletion of CDKN2A: diagnose "atypical mesothelial proliferation" or go to step 6	Recommend additional tissue sampling
Step 6	Perform molecular sequencing (if available)	Identification of mutations commonly seen in mesothelioma is supportive of malignancy

IHC, immunohistochemistry; FISH, fluorescence in situ hybridization.

have been well described elsewhere. The authors feel that a more recent challenge has not been the separation of a fibrous pleuritis from mesothelioma, but rather the separation of benign hyperplasia and other tumors and proliferations (well differentiated papillary mesothelial tumor, etc.) from mesothelioma. In this case, the authors strongly advocate for the use of the markers of malignancy (BAP1, MTAP, *CDKN2A* FISH; *Table 3*). While these markers are not in widespread use in every laboratory, at least within North America, they are easily orderable through large reference laboratories or academic medical centers. One final note on the morphologic classification of mesothelioma is that the pathologist should not forget about the diagnostic power harbored within a simple cytokeratin IHC. Pankeratins, CK5/6, and CK7 highlight many mesotheliomas. While this is a well-known fact, careful review of cytokeratin staining may reveal very useful information. Cytokeratin may show subtle invasion of individual tumor cells into underlying tissue, proving a mesothelial proliferation is malignant. Cytokeratin may also highlight architectural patterns in a way that may not be obvious on H&E-stained sections. Cytokeratins may show epithelial islands in an otherwise sarcomatoid mesothelioma, allowing for reclassification as biphasic mesothelioma. Also, cytokeratin may highlight subtle spindling of tumor cells in an epithelioid mesothelioma, cluing in the pathologist of the possible presence of transitional or sarcomatoid morphologies.

Diagnosis of mesothelioma in cytology

Recently it has become possible to establish malignancy in mesothelial proliferations on review of serous effusions. The authors have advocated in their own clinical practice, the adoption of BAP1 and MTAP IHC for use in cytology specimens, especially when an adequate cell block is made. Nuclear loss of BAP1 and cytoplasmic loss of MTAP in mesothelial cells is always indicative of a malignant mesothelial proliferation. This is true in both surgical pathology and cytopathology. The one important caveat to the cytologic diagnosis of mesothelioma, is that it is currently unknown how well the cell block is predictive of final subtype. In the limited number of cases in which the authors have helped to establish a diagnosis of mesothelioma on cell block (all of them showing epithelioid morphology), they have advocated for pleural biopsy to subtype the mesothelioma. In a number of these cases, a sarcomatoid component was found in the pleural biopsy, changing the tumor classification from epithelioid to biphasic.

Conclusions

The diagnosis and classification of mesothelioma has moved from its earlier simplistic forms based purely on histologic subtyping. Numerous IHC markers exist which are able to distinguish benign and malignant mesothelial proliferations, even prior to invasion and in effusion cytology specimens. Some of these markers, most notably BAP1 and MTAP, allow for identification of mesothelioma *in situ* and function as disease prognosticators. With the advent of immunotherapy in mesothelioma, there is the potential to significantly alter disease course, and thus far there are some promising results for non-epithelioid mesotheliomas. So long as these immunophenotypic and molecular

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advancements in mesotheliomas continue to populate the literature, there will be an enduring hope that these markers can help guide patients to appropriate and more effective therapies. There will no doubt be much to come on this topic in the next few years.

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Supplementary

Table S1 Search process example

Step	
Step 1	Visit webpage https://pubmed.ncbi.nlm.nih.gov/
Step 2	Enter search term into search bar
	Example: IHC mesothelioma
Step 3	Scan results for original articles pertaining to the search term
Step 4	Select article to review
Step 5	Ensure inclusion criteria are met and there are no exclusionary criteria
Step 6	After sufficient number of articles are reached, conduct next search

IHC, immunohistochemistry.

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