Malignant Mesothelioma

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Introduction

While malignant mesothelioma is a relatively rare neoplasm, it provokes a lot of anxiety due to its poor prognosis, the litigation that follows such a diagnosis, and the difficulty in establishing the diagnosis itself. Mesothelioma presents with a large serosal effusion in over 90% of patients [1], a fact that situates cytology as the primary mode of evaluation and diagnosis. Previous guidelines by the International Mesothelioma Interest Group (IMIG) for the diagnosis and treatment of mesothelioma shed doubt on the role of cytology in the definitive diagnosis of mesothelioma and hence the need for tissue biopsy despite ample literature to the contrary [2]. However, recent guidelines published by several groups acknowledged that cytology can have a role in this diagnosis, and consequently supplemental guidelines for the cytopathologic diagnosis in effusions were published [3–6].

Epidemiologic and Mineralogical Aspects

Malignant mesothelioma is one of very few malignancies directly associated with exposure to a natural substance. The reported rate of asbestos exposure in patients with mesothelioma has ranged between 15% and 80%. This wide range is primarily attributed to the methodology of taking history and asking the right questions as well as inaccurate histories sometimes provided by family members. It is now well established that asbestos exposure can be documented in over 80% of cases [1]. Not only direct exposure is implicated in mesothelioma; secondary exposure of family members has been documented as well to cause mesothelioma in the spouses and children of asbestos workers. It is believed that asbestos fibers are carried on their clothes, etc. Despite the well-documented association of asbestos and mesothelioma, the threshold of exposure is not known yet, in part because of the long latency period between exposure and the development of symptoms (at least 20 years), decades from the exposure, and the far higher prevalence of lung carcinoma with asbestos exposure [7]. In a study by Roggli et al., the authors compared the number of asbestos bodies from patients who died of mesothelioma versus those who died of other diseases and found no correlation with development of the disease [8].

Other causes for mesothelioma have also been reported, albeit very rarely. These include history of radiation and exposure to beryllium, nickel and silica dust, and fiberglass. Few patients may genuinely have no history of exposure [7].

Historically, the use of asbestos has been reported as early as 3000 BC. Its fire-resistant quality made its use very popular for many applications, including incorporation into pottery since antiquity, designing of funeral clothes by the Greeks that would survive cremation of nobility, coating of the feet of victims undergoing trial by fire in the Middle Ages, and manufacturing of purses that safeguard money against fire. While asbestos-related lung disease has also been reported as early as 100 AD, and while rare reports of mesothelioma have been published throughout the last two centuries, malignant mesothelioma as a pleura-based distinct malignancy has only been recognized around the second half of the twentieth century [9].

Asbestos is a general term applied to a group of crystalline hydrated silicates with fibrous geometry defined as having a length three times greater than their width. There are three commonly occurring asbestos varieties: chrysotile, crocidolite, and amosite. Anthophyllite, tremolite, and actinolite occur less commonly and mainly as contaminants. Pleural plaques are strongly dose-dependent, yet the threshold for mesothelioma is unknown. While crocidolite has a more well-established association with mesothelioma than chrysotile, cases with pure exposure to the latter have also been documented [7].

In addition to asbestos, erionite was identified and documented in the villages of Cappadocia, Turkey, as another

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highly potent mineral that induces mesothelioma. Erionite is a member of the zeolite family, a complex group of silicates found in volcanic ash [10].

Clinical Presentation

Despite the banning of asbestos products for several decades, the incidence of mesothelioma is reported as 2500 cases in the USA and 5000 cases in Western Europe annually. In fact, it is projected that the incidence worldwide will peak by 2020 [1].

Mesothelioma can occur in any of the body cavities lined with serosal cells. However, it most commonly arises in the pleural, followed by the peritoneal and pericardial cavities. The ratio of pleura to peritoneum ranges from 3:1 to 11:1 according to the literature. Pleural mesothelioma presents mainly as a unilateral disease, although it might be bilateral in rare cases.

Because of the long latency period that may last up to four decades, the age of presentation is generally around 60 years. Patients with history of exposure in their childhood may present earlier. The presenting symptoms tend to be insidious, and it consequently takes between 3 and 6 months before a definitive diagnosis is established. Most patients initially present with shortness of breath that develops due to the large pleural effusion or nonpleuritic chest pain resulting from significant chest wall and diaphragmatic invasion. Other symptoms may include fever, fatigue, dry cough, and weight loss. Pleural effusion tends to be unilateral in about 95% of cases and bilateral in the remaining 5%. The right pleura is affected in 60% of these patients. Pleural mesothelioma is a disease that predominates in males.

Initial workup by chest X-ray detects large pleural effusions in 80–95% of patients, while the remaining patients may have no detectable fluids. Pleural plaques are also detected in patients with asbestos-related lung disease, and focal or diffuse pleural thickening is also detected, although it may initially be obscured by the large effusion. As the disease progresses, the pleural fluid decreases, becomes loculated, and eventually disappears due to fusion of the visceral and parietal surfaces, forming a rind that encases the lung and extends into the fissures. Computed chest tomography with contrast has recently been proven to be more sensitive, especially in the detection of pleural effusions, assessment of the size of hilar and mediastinal lymph nodes, and evaluation of the presence of pleural masses or rind. Magnetic resonance imaging of the chest with contrast is more useful in detecting chest wall invasion and diaphragmatic spread. Positron emission tomography is used to detect contralateral chest involvement and extrathoracic metastatic sites. The latter information is essential for tumor staging and for treatment planning, particularly surgery [1, 11].

Diagnosis and Treatment

Pleural mesothelioma presents early as small rounded yellow to gray nodules studding the parietal pleura that coalesce as the disease advances to eventually form the characteristic thick pleura otherwise known as “pleural rind.” With disease progression, the parietal and visceral pleura fuse and the effusion disappears. Peritoneal mesotheliomas were reported to have a more variable growth pattern and could present as disseminated carcinomatosis-like pattern or as large omental masses and mimic carcinoma [12].

Since most mesotheliomas are associated with large effusions, cytological examination is logically the first line of workup. However, the effectiveness of cytology is a subject of great controversy. Several factors may contribute to this controversy, including the subtle cytological features that are not easily recognized by pathologists, a general lack of pathologists experienced in mesothelioma diagnosis due to the rare occurrence of the disease, and finally, the fact that some effusions are mostly bloody or lack diagnostic cells. It is recommended that a minimum of 100 mL of fluid and preferably the entire volume of aspirated fluid is submitted for cytological examination. Such volume would allow the preparation of a cell block with optimum cellularity for additional ancillary testing [6].

When a definitive diagnosis by cytological examination is not achieved, a pleural biopsy is the next step. CT-guided needle biopsy of a pleural mass can be up to 87% sensitive, while video-assisted thoracoscopy (VATS) allows direct visualization of the chest with aspiration of the pleural fluid, direct biopsies of the pleural mass, and direct injection of talc. This results in up to 95% accuracy and the highest rate of successful pleurodesis. It is important, however, to recognize that seeding of the tumor along the chest tube and the surgical incision tracts, eventually resulting in chest wall invasion, is a possible complication of VATS in up to 20% of patients.

While a diagnosis of malignant mesothelioma can be made by cytology, VATS with extensive pleural biopsies is still recommended to exclude the presence of a sarcomatoid component. Mediastinoscopy to examine the mediastinal lymph nodes is also essential prior to considering the patient for extrapleural pneumonectomy (EPP). This is because, as previously mentioned, patients with either metastatic lymph nodes or sarcomatoid component do not respond to EPP [1, 6, 11, 13].

Untreated, the mean survival rate is about 6 months. With recent treatment regimens including surgery and chemotherapy, survival rate of up to 5 years has been reported, a fact that underscores the significance of early detection and diagnosis of mesothelioma. For pleural mesothelioma, surgical procedures used for either treatment or palliation include VATS with talc pleurodesis, pleurectomy with decortications

(P/D), and EPP. The latter provides the most complete reduction of tumor and is the only method in which long-term survival is documented. Unfortunately, EPP does not control the nonepithelial variant of mesothelioma, and only 10–15% of patients with the epithelial variant, particularly those with negative mediastinal lymph nodes, seem to benefit from this procedure. Chemotherapy with combination of cisplatin and pemetrexed demonstrated significant survival advantage (12 months) and is currently used as first-line treatment, while radiation therapy is only used to control local chest wall invasion such as implants in chest tube or surgical wound tracts [1, 6, 11].

Considering the localized nature of peritoneal mesothelioma, locoregional therapies have been explored [14]. The most accepted therapy at this time is cytoreduction and hyperthermic intraoperative intraperitoneal perfusion with chemotherapy (HIPEC). First, cytoreduction is performed so as to remove all grossly visible tumor. This is followed by HIPEC which distributes the high-dose IP chemotherapy uniformly to all the peritoneal surfaces. Infusing a clinically relevant hyperthermic IP is known to enhance the cytotoxic effect of multiple chemotherapeutic agents. While experience is limited with peritoneal mesotheliomas due to the rarity of the disease and the variability in its biological behavior, it has been noted that patients with smaller tumor burden and female gender had prolonged survival [15–17]. In a review of 83 patients, epithelioid histology, low mitotic count, complete gross cytoreduction, and pathologically negative lymph nodes were identified as independent factors associated with improved survival [18].

Morphology

Histological Features

Histologically, malignant mesothelioma is divided into epithelioid (50%), sarcomatoid (16%), or mixed variants (34%) [12]. The epithelioid variant may present with a variety of patterns, most commonly tubulopapillary, acinar, and confluent sheets. Well-differentiated tumors present mainly with papillary and tubular architecture (Fig. 5.1). The papillary structures project into large tubular structures and usually contain fibrous cores. The tubular structures form elongated and complex clefts lined by the malignant cells. Henderson et al. noted that they frequently observed an eruptive organizing granulation tissue layer covering the mesothelioma and eventually entrapping the mesothelial proliferation within a fibrous tissue layer at the interface with the adjacent adipose tissue [19]. Metaplastic changes such as squamous differentiation have been described [20]. Less common patterns include signet ring, small cell [21], clear [22], lipid-rich [23], and microcystic. The sarcomatoid variant may be homologous, consisting predominantly of a fibrosarcoma-like proliferation (Fig. 5.2), or contain heterologous stroma, such as osteoid, chondroid, rhabdomyoblastic, etc. [24]. The biphasic variant is a mixture of the epithelioid and sarcomatoid patterns (Fig. 5.3). Rare variants include an undifferentiated (Fig. 5.4), desmoplastic [25, 26], lymphohistiocytoid [27, 28], and deciduoid type [29–31]. Desmoplastic mesothelioma is defined as a mesothelioma in which collagenous tissue constitutes more than 50% of the tumor (Fig. 5.5). The majority of these mesotheliomas are of the sarcomatoid variant.

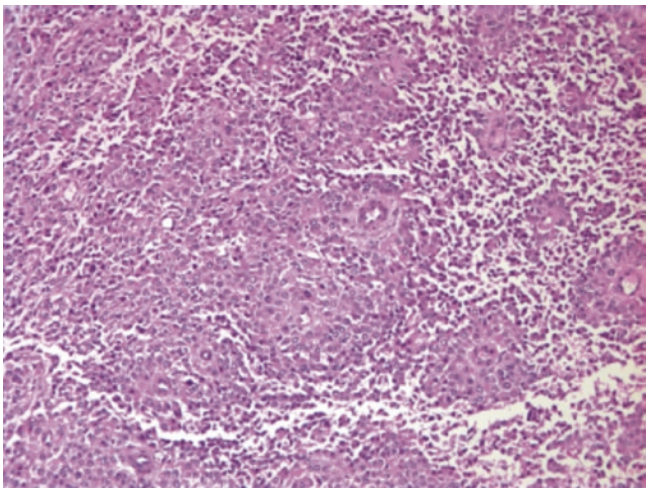

Fig. 5.1 Well-differentiated epithelioid mesothelioma showing papillary and tubular structures admixed with cellular sheets. Notice the overall monotony of the cells; H&E

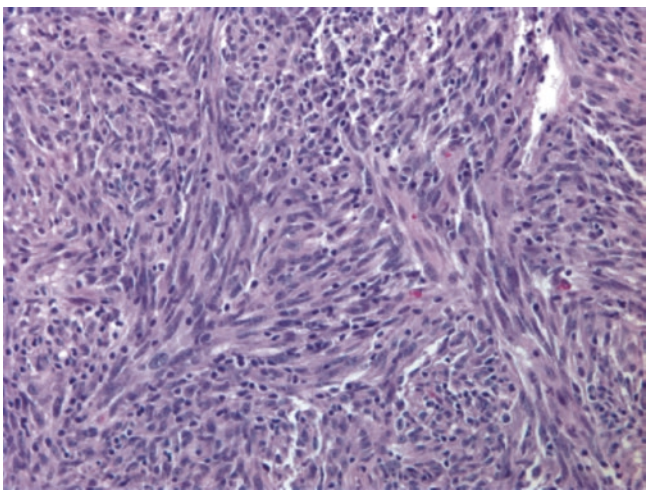

Fig. 5.2 Sarcomatoid variant of mesothelioma consisting predominantly of fibrosarcoma-like proliferations of spindled cells; H&E

While the rare variants are very infrequently encountered, their features are worth noting because of the differential diagnosis they present.

The small cell variant is characterized by sheets of uniform small cells with open nuclei and prominent nucleoli. In

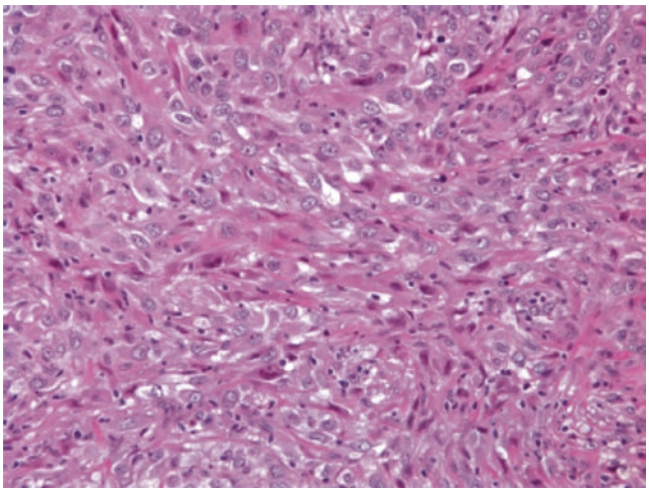

Fig. 5.3 Biphasic variant of mesothelioma exhibiting both the spindle cell proliferation and the epithelioid-type cells; H&E

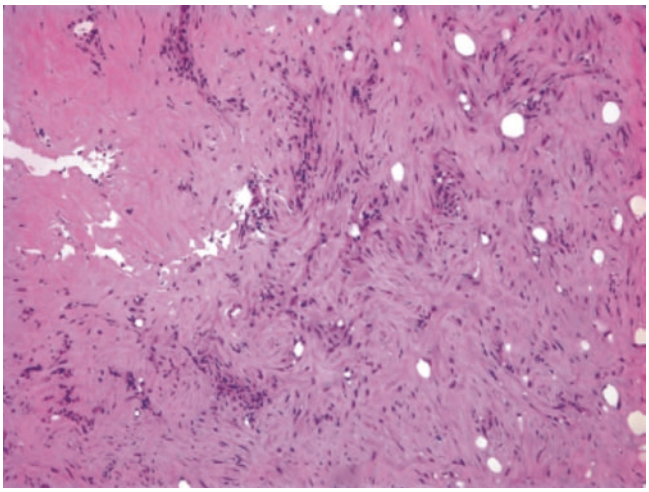

Fig. 5.5 Desmoplastic mesothelioma showing few abnormal spindled cells infiltrating in a very heavily collagenous connective tissue stroma; H&E

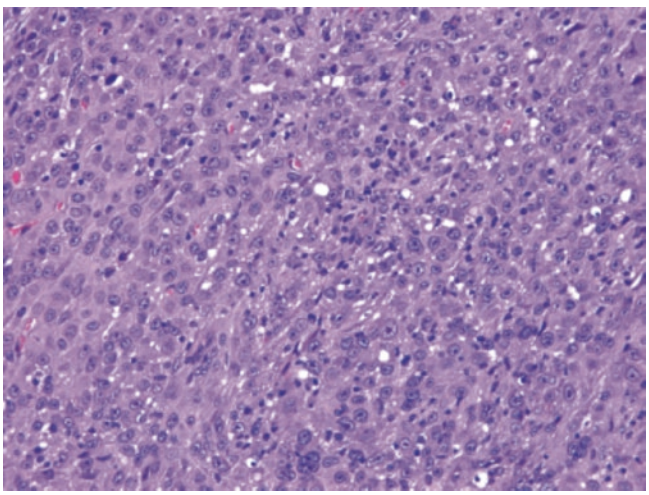

Fig. 5.4 Poorly differentiated mesothelioma appearing predominantly as solid sheets of malignant cells with no papillary, tubular, nesting, or other previously described features. The cells are not readily identified as mesothelial; H&E

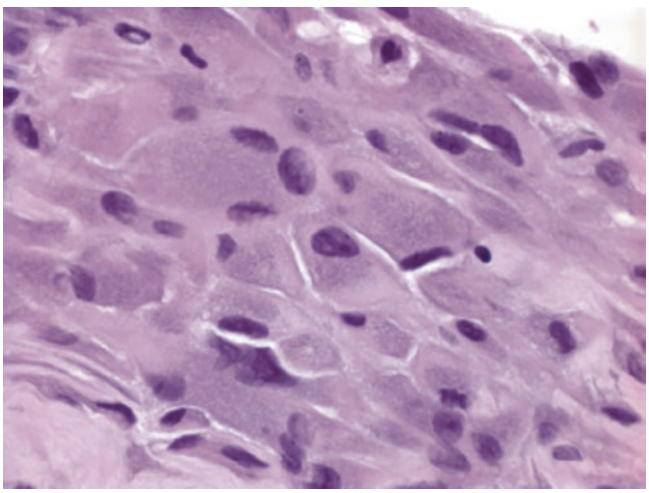

Fig. 5.6 Deciduoid mesothelioma exhibiting large cells with abundant pink glassy cytoplasm and enlarged obviously abnormal nuclei. The cellular features are closely akin to those of decidual cells; H&E

all reported cases, adequate sectioning of the tumor revealed a component of typical epithelioid or sarcomatoid mesotheliomatous patterns. According to Mayall et al. [21], who reported 13 cases, all cases contained frequent areas of necrosis and lymphatic invasion. In some cases, intralymphatic tumors exhibited a typical mesotheliomatous pattern. Mitotic activity was low in all cases (less than 5 per 10 high-power fields). The classic features of neuroendocrine tumors described by Azzopardi [32], such as pseudo-rosettes, streams, ribbons, or tubular growth patterns, salt-and-pepper hyperchromatic nuclei, nuclear molding, and hematoxyphilia in blood vessels, were all consistently lacking in their cases.

The deciduoid variant is a very rare morphologic phenotype first described in 1985 as a diffuse epithelioid mesothelioma occurring in the peritoneum of a 13-year-old female with morphologic resemblance to deciduosis [33]. Since then, it has also been described in the pleural surface and in older patients of both genders [29, 31, 34, 35]. Histologically, this variant presents as sheetlike proliferation of large polygonal cells with abundant pink, glassy cytoplasm and well-defined borders (Fig. 5.6). Some cells have a perinuclear cytoplasmic density. The nuclei are round to oval with vesicular chromatin and single prominent nucleoli. Binucleated cells are also present. Mitotic figures are present and may be abnormal but not frequent.

The lymphohistiocytoid variant presents as sheets of histiocyte-like cells with no evidence of differentiation in the form of tubular or papillary architecture (Fig. 5.7). The cells vary from round to spindle in appearance. The

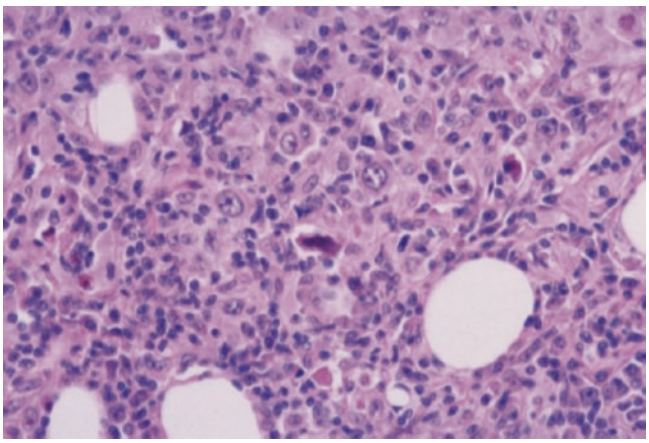

Fig. 5.7 Lymphohistiocytoid mesothelioma presenting as large histiocyte-like cells with abundant clear cytoplasm admixed with a highly lymphocytic background; H&E

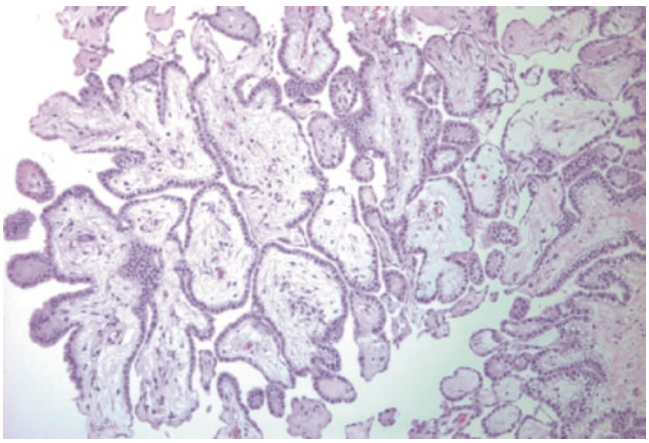

Fig. 5.8 Well-differentiated localized papillary mesothelioma presenting as well-defined papillary proliferation with thick fibrovascular cores covered by a single layer of mesothelial cells; H&E

nuclei are usually round to oval, are vesicular, and contain prominent nucleoli. The cytoplasm is moderate in amount and eosinophilic. A diffuse lymphoid infiltrate predominantly of T cells is noted. The histiocytoid cells have an immunostaining profile that is similar to epithelioid mesothelioma [28].

Localized well-differentiated papillary mesothelioma is another rare variant that arises in the peritoneum and is frequently discovered incidentally during abdominal and pelvic surgeries. It is believed to have an indolent clinical course and may behave either as a benign neoplasm or have a tendency to recur. Histologically it is a localized proliferation of well-developed papillary structures with thick fibrovascular cores covered by a single layer of mesothelial cells (Fig. 5.8). It is important to distinguish this variant from the well-differentiated diffuse papillary mesothelioma that carries a much worse prognosis.

Cytological Features

The Role of Cytology in the Diagnosis of Mesothelioma [3, 36]

The reported sensitivity of cytology for the diagnosis of mesothelioma ranges from 4% to 63%, and many doubt the utility of cytology in establishing this diagnosis [37]. However, the reader should distinguish the probability of establishing the diagnosis by examining serosal effusions from the ability of the pathologist to render the diagnosis from a cellular fluid based on cytological features. In fact, the literature suggests that in experienced hands, mesothelioma diagnosis can be established in up to 50% by cytological evaluation alone and in up to 80% of cases utilizing ancillary techniques [38].

The cytological diagnosis is challenged at two points; the first is that not all malignant mesothelioma effusions contain diagnostic cells. In fact, about 10% of the effusions are bloody and virtually acellular. Sarcomatoid and some of the other rare variants do not exfoliate. In almost all cases, it is the epithelioid component that exfoliates and renders itself to diagnosis. The second challenge was much more significant in the past because of the difficulty in separating mesothelioma from adenocarcinoma. However, with the availability of new immunocytochemical stains, the last decade has witnessed a plethora of literature confirming that mesothelioma can be distinguished from carcinoma with a high degree of accuracy [39, 40]. A more significant morphologic challenge is separating mesothelioma from reactive effusions. Rakha et al. reviewed a total of 154 effusions with histologically proven pleural mesothelioma and were able to either diagnose or suspect mesothelioma in 79 cases, with a sensitivity of 53%. A benign or reactive diagnosis was rendered in 65 cases (42.2%), and 5 cases (3.2%) were considered inadequate for diagnosis. The sarcomatoid variant presented mainly as benign effusion and showed the least sensitivity (20%), with 11/15 cases diagnosed as benign [41]. The lack of exfoliated diagnostic cells in mesothelioma fluids has been attributed to several factors: (1) the tumor could be covered by a thick layer of fibrinous material or fibrosis; (2) the tumor may consist predominantly of fibrous stroma, as in the case of desmoplastic or sarcomatoid tumors [42].

The inability to detect invasion of preexisting tissue (not granulation tissue), a key feature in the definitive histologic diagnosis of mesothelioma, has been used for the last several decades as a supportive evidence against the cytological diagnosis of mesothelioma. However, the latest guidelines recognize that the cytological diagnosis relies on different criteria. The updated statement on mesothelioma from British Thoracic Society (BTS) and the guidelines issued by the Asbestos Disease Research Institute (ADRI) accept the cytological diagnosis as sufficient in some patients when correlated with imaging studies, i.e., utilizing imaging studies as an equivalent to the histologic diagnosis of invasion [5, 6].

It is important to recognize that despite the limitation of diagnosing mesothelioma by cytology, it still plays a major role as the initial and least invasive step in the evaluation of the patient. In fact, a definitive diagnosis may be established in a patient with positive radiological and clinical findings, and further workup may not be necessary if the tumor is unresectable. On the other hand, suspicion of mesothelioma or a negative persistent effusion in a patient with positive clinical findings should be followed up aggressively to avoid further progression of the disease and afford a patient at an early stage of the disease the opportunity for surgical treatment or adjuvant therapy [43].

Stepwise Review of Effusions [3, 44]

When evaluating an exudative effusion, the pathologist should answer three questions based on the morphologic features:

1. Are the cells mesothelial or epithelial in origin?
2. If mesothelial, are the cells benign or malignant?
3. If epithelial, what is the primary origin?

Features of mesothelial origin have been described in chap. 1. The following is a summary of these features which tend to be subtle in quiescent effusions, easily detected in reactive effusions, and prominent in mesotheliomas.

1. Cell windows seen in mesothelial cords and within clusters (Fig. 5.9).
2. Cellular clasping and pinching (described as pincerlike) (Fig. 5.10).
3. Cell within cell arrangement (Fig. 5.11).
4. Clusters with scalloped borders (Fig. 5.12).

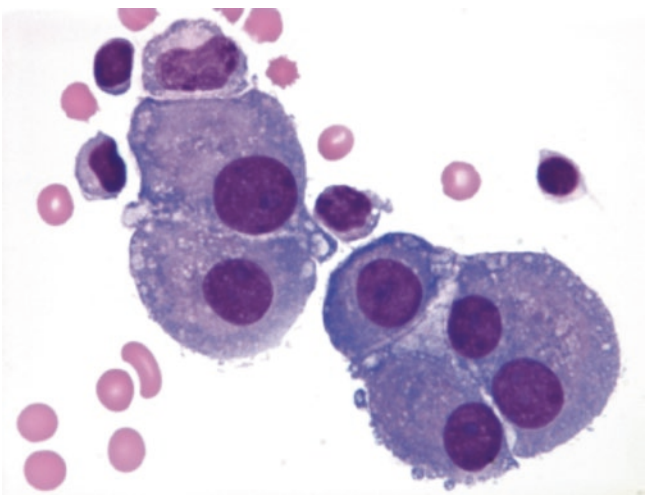

Fig. 5.9 Mesothelial cells in apposition with windows between the adjacent cells. The cells form short cords and small clusters; Diff-Quik

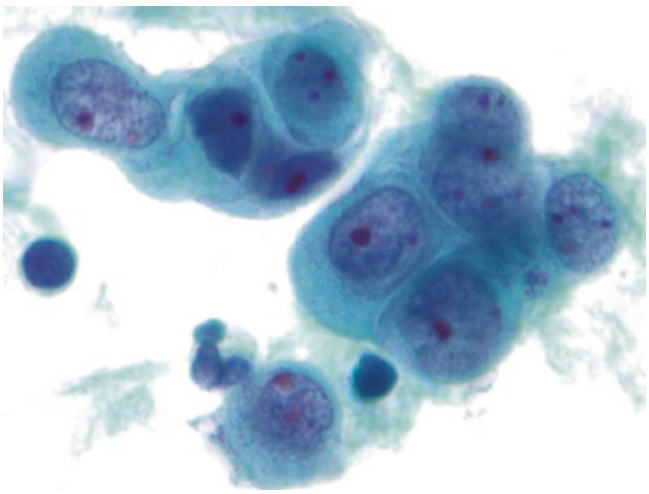

Fig. 5.10 The cytoplasm of one mesothelial cell wraps around the adjacent cell to form the cellular clasping rather than the windows as seen in the short cord in the *top left*; PAP

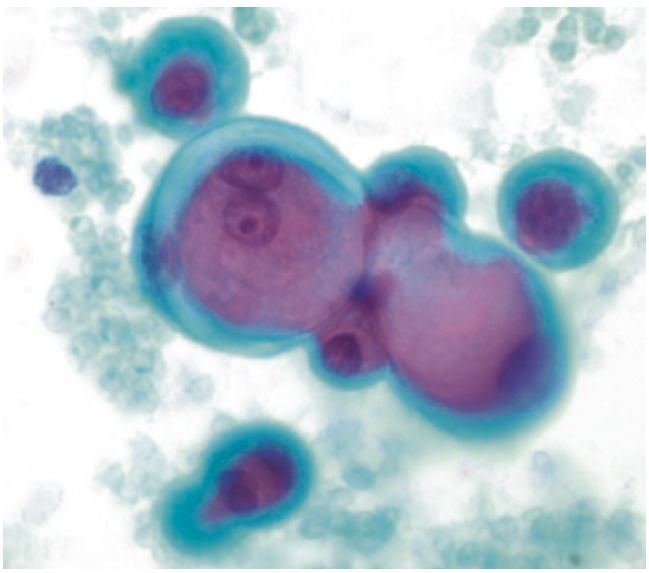

Fig. 5.11 A mesothelial cell might be situated within the cytoplasm of the other cell like a cup sitting on its plate giving the appearance of "cell within cell"; PAP

5. Cells with two-tone cytoplasm, i.e., endo-ectoplasmic demarcation (Fig. 5.12).
6. Vague cell borders or brush border (Fig. 5.13).
7. Sub-membranous glycogen vacuoles. Yellow glycogen might be detected on fixed smears (Fig. 5.14).
8. Perinuclear small fat vacuoles best detected on Romanowsky stain.

Cytological Features of Mesothelioma

The cytological features of mesothelioma appeared in sporadic reports since the nineteenth century. However, the first well-illustrated examples were shown by Dr. Papanicolaou

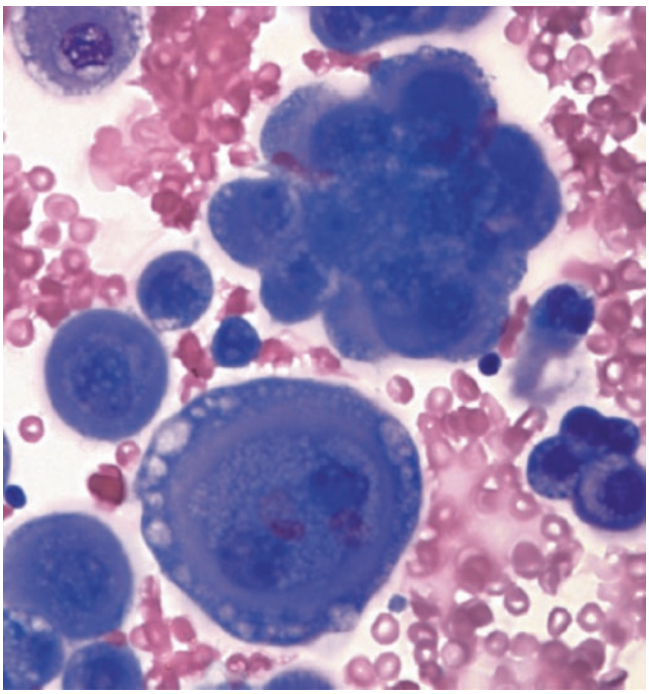

Fig. 5.12 Mesothelial cells from a mesothelioma case forming loose clusters with scalloped borders. The adjacent single cell is markedly enlarged with two-tone cytoplasm and well-defined sub-membranous vacuoles; Diff-Quik

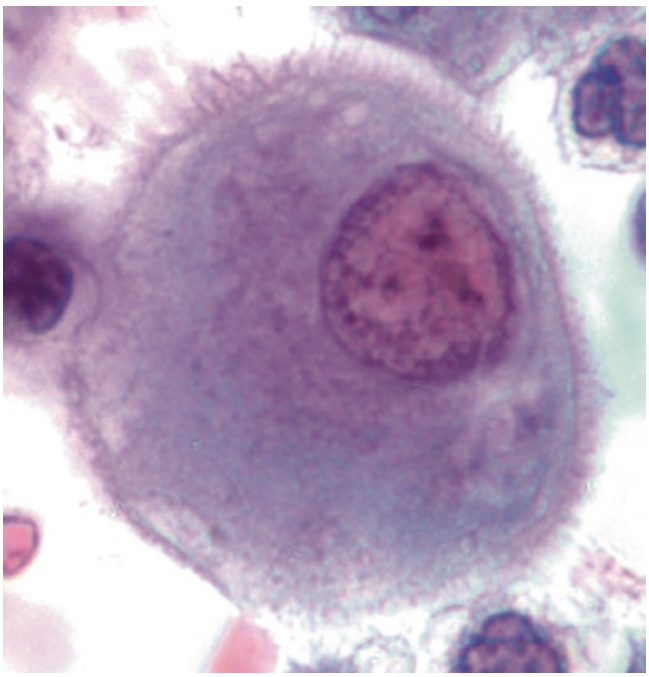

Fig. 5.13 The mesothelial cell has a poorly defined cell circumference with a brushlike border corresponding to the long slender microvilli seen by electron microscopy; PAP

in 1954 [45]. Following that, many reports describing mesothelioma appeared in the literature [38, 42, 46–51].

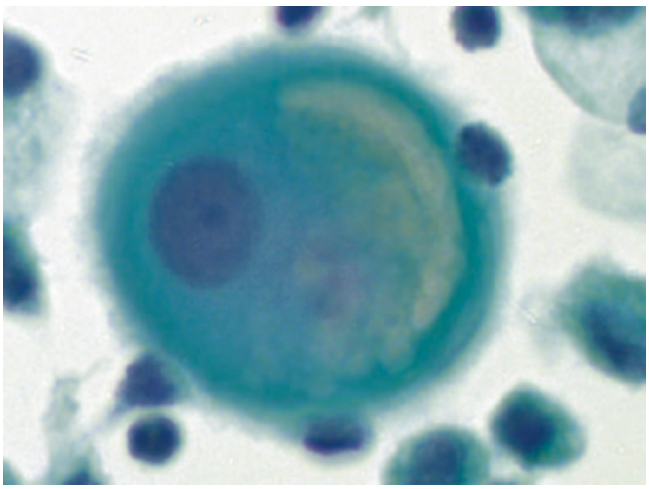

Fig. 5.14 Large cytoplasmic vacuoles are full of glycogen that is sometimes readily recognized as yellow granular material within these vacuoles; PAP

Epithelioid Variant (Table 5.1)

The fluids are usually of large volume, although the entire volume may not be submitted to the laboratory. Grossly, the fluid has been described to have a viscous, tar, or honey-like consistency while processing and smearing. The majority of fluids are moderately to highly cellular and can comprise almost exclusively cellular clusters and spheres (cohesive) (Fig. 5.15), single cells (discohesive) (Fig. 5.16), or a mixture of both (Fig. 5.17), with the latter being the most common. The background is usually very bloody or contains a very viscous material. Chronic inflammatory cells may be seen. However, acute inflammation is not characteristic. Once a drain is permanently installed, the fluid will exhibit a considerable acute inflammatory background.

Most mesotheliomas are highly cellular, although some cases are low in cellularity. The individual cells exhibit all the previously described mesothelial features. Mitotic figures may be seen but tend to be inconspicuous, and atypical mitoses are not seen [52]. Examination at scanning magnification reveals a monotonous population of cells that exhibit similar morphologic features yet vary tremendously in size. The cells may vary from the size and shape of benign or reactive mesothelial cells to large or even gigantic cells (Fig. 5.18). Binucleated and trinucleated cells are very frequent and many scattered multinucleated cells can be identified (Fig. 5.19). In fact, the multinucleated cells in mesothelioma have been described to contain between 2 and 50 cells or more nuclei (Fig. 5.20). Despite the obvious nuclear enlargement, the cells retain abundant cytoplasm and consequently have low nuclear-to-cytoplasmic (N/C) ratio.

The nuclei are centrally located and do not exhibit obvious malignant features, contrary to their counterparts in adenocarcinoma and other metastatic epithelial malignancies. Nevertheless, closer examination will reveal nuclear

Table 5.1 Features of malignant mesothelioma

Feature	Description	Comment
Gross appearance	Thick and viscous fluid Bloody in most cases	Tar- or honey-like consistency
Background	Numerous red blood cells Lymphocytosis frequent	Neutrophils are only seen after insertion of drain
Cell population	Monotonous single cell population	No alien population identified
Pattern	1. Predominantly cohesive groups	Morules and clusters
	2. Predominantly discohesive cells	
	3. Mixture of clusters and single cells	Most common pattern
Clusters	1. Cohesive tight clusters	Smooth outline, sphere like
	2. Loose clusters	Knobby borders, berrylike
Cellular features		
Scanning magnification	Mesothelial characteristics	Small to gigantic; cells are large and may attain the size of a small morule
	Wide variation in size	
Cytoplasm	Dense with vague brush border	Blebs may also be seen
	Endo-ectoplasmic demarcation	Two-tone staining
	Sub-membranous vacuoles	Glycogen might be seen
	Small perinuclear vacuoles	Fat droplets
Nucleus	Centrally located	
	Enlarged	
	Frequently 2–3 nuclei	May contain up to 50 nuclei
	Multinucleation common	
Nucleoli	Prominent	Macronucleoli might be seen
	One or more	
Chromatin	Slightly coarse	May be clumped
	Slightly hyperchromatic	
Nuclear membrane	Smooth or slightly irregular	Rarely very irregular
N/C ratio	Low	May be high in few cells
Cytological atypia	Mild to moderate at most	Rare cases are very atypical

atypia in the form of slightly coarse chromatin, irregular nuclear membranes, and most importantly, prominent nucleoli, and sometimes macronucleoli. The cytoplasm in well-visualized cells has two-tone or endo-ectoplasmic demarcation. The cell circumference tends to be hazy due

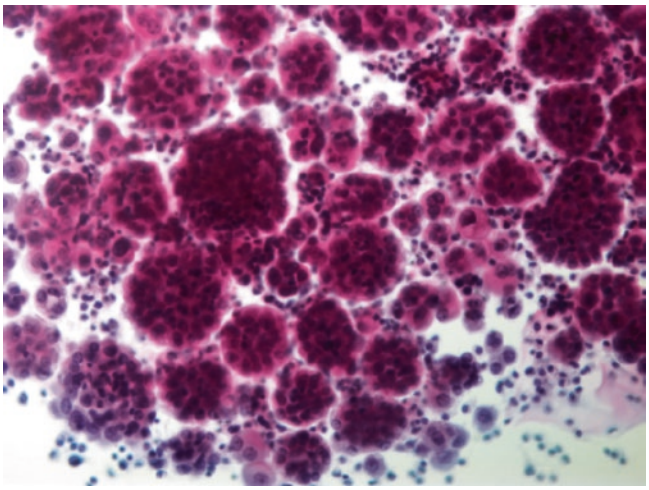

Fig. 5.15 Highly cellular smear of mesothelioma, consisting mainly of cellular spheres and morules; PAP

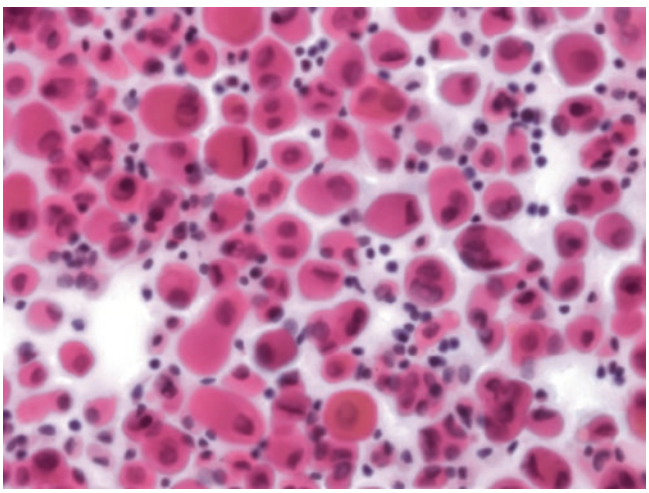

Fig. 5.16 Highly cellular smear consisting of discohesive single cell population of malignant mesothelial cells; PAP

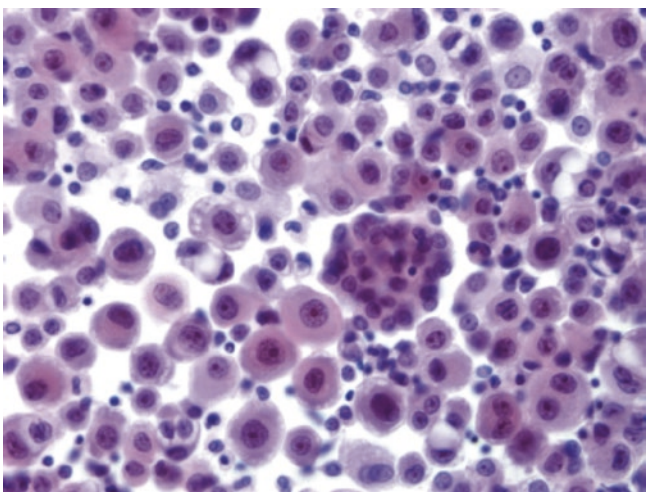

Fig. 5.17 Mesothelioma presenting with a mixture of cellular morules and numerous discohesive single cells; PAP

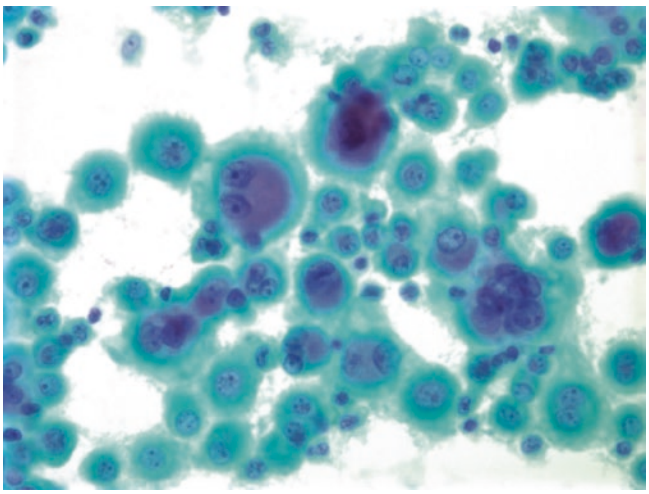

Fig. 5.18 The mesothelioma cells exhibit a wide variation of size ranging from small size similar to those of benign mesothelial cells to very large cells attaining gigantic size. Notice the large cell on the left approaching the same size of the small morule on the right; PAP

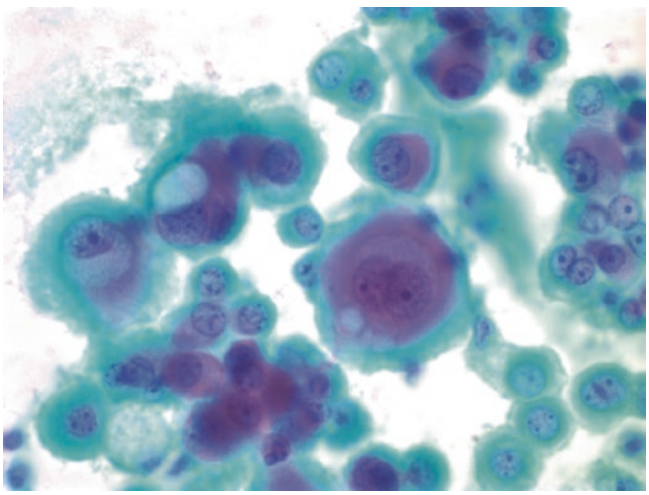

Fig. 5.19 Enlarged mesothelial cells with binucleation and prominent nucleoli; PAP

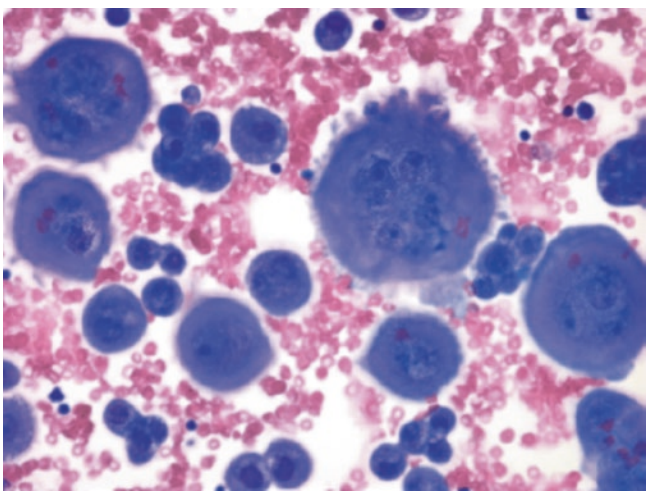

Fig. 5.20 Multinucleation is common in mesothelioma with nuclear number ranging from 2 to 50 or more; Diff-Quik

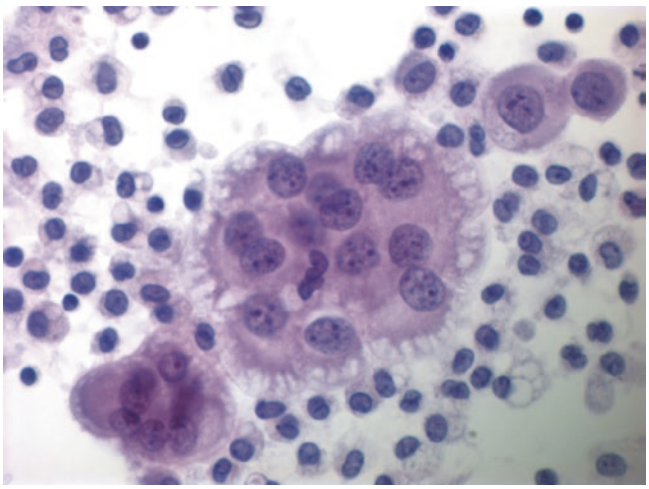

Fig. 5.21 Mesothelioma with high glycogen content appearing as large cytoplasmic vacuoles beneath the cell membrane; PAP

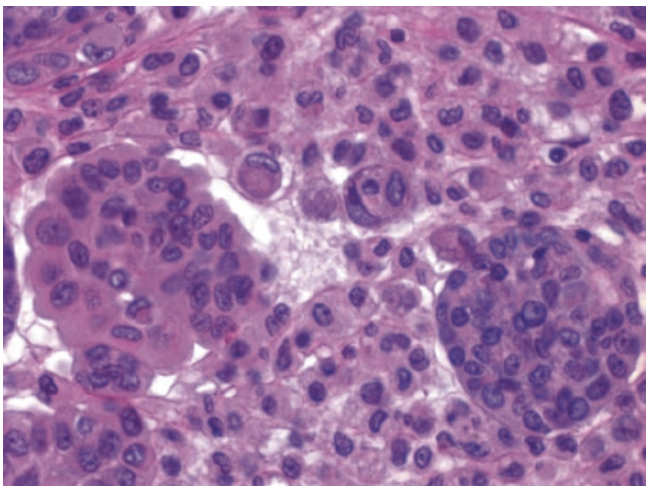

Fig. 5.22 Cell block of a mesothelioma presenting with two types of cellular clusters, the loose cluster having a knobby or scalloped border (berrylike) and the tight spherical group with smooth outline; H&E

to the circumferential brush border formed by the long slender microvilli visualized by electron microscopy. Submembranous vacuoles are frequently noted and sometimes coalesce to form long vacuoles, described as sausage links. It is not unusual, especially in the Papanicolaou-stained smears, to see yellow glycogen clumps within these vacuoles (Fig. 5.21).

The cellular clusters are of two types. The first are loose clusters with knobby or scalloped borders, also described as berrylike. The second are tight clusters or spheres with smooth borders also known as morules (Fig. 5.22). In the former type, the crowded cells forming the clusters can be easily visualized and intercellular windows can be identified. The cytoplasm with its characteristic mesothelial features can be visualized, particularly in those cells located at the knobby borders. The cells in the morules are very tightly cohesive and therefore frequently

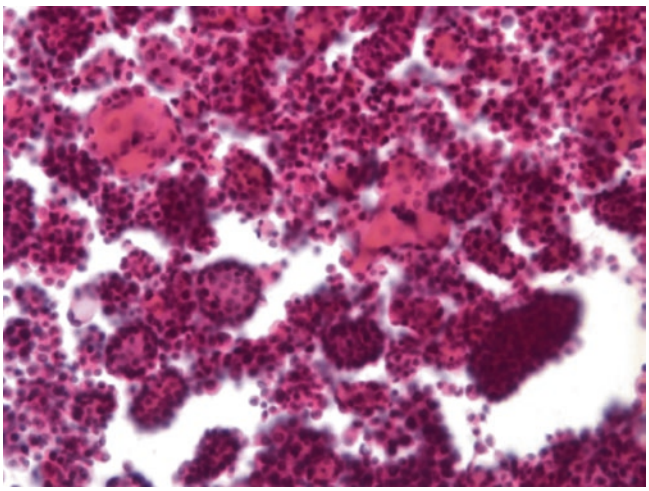

Fig. 5.23 A well-differentiated mesothelioma with papillary features presenting as highly cellular smear consisting predominantly of papillary groups with complex branching and obvious collagen cores; PAP

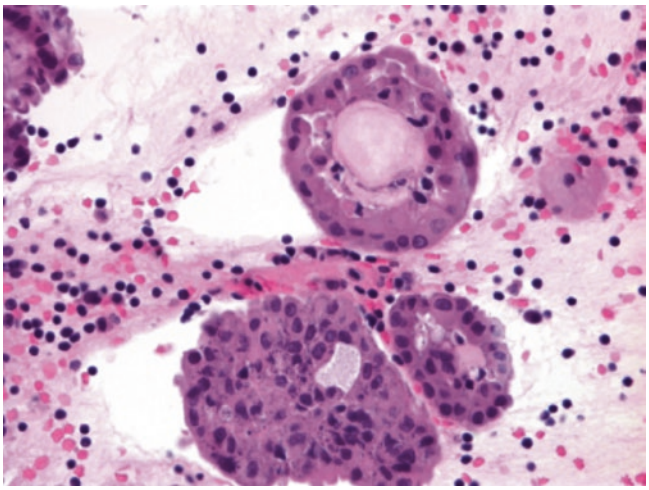

Fig. 5.24 Cell block of the corresponding papillary mesothelioma showing the papillary groups with central collagenous cores; H&E

difficult to discern. Large branching and papillary clusters may be seen and rarely predominate (Fig. 5.23). The clusters may contain an amorphous eosinophilic core that stains negative with PAS, positive with Van Gieson stains, and bright blue with the Martius Scarlet Blue technique, indicating that it comprises collagen (Fig. 5.24). On electron microscopy, these cores were found to consist of whorls with periodicity of 640 Å, confirming their collagenous origin [53].

Whitaker et al. identified five features to be of particular value in the diagnosis of mesothelioma, namely, the presence of cell aggregates, multinucleation, brushlike borders, close opposition of cell borders, and the characteristic two-tone cytoplasm [39].

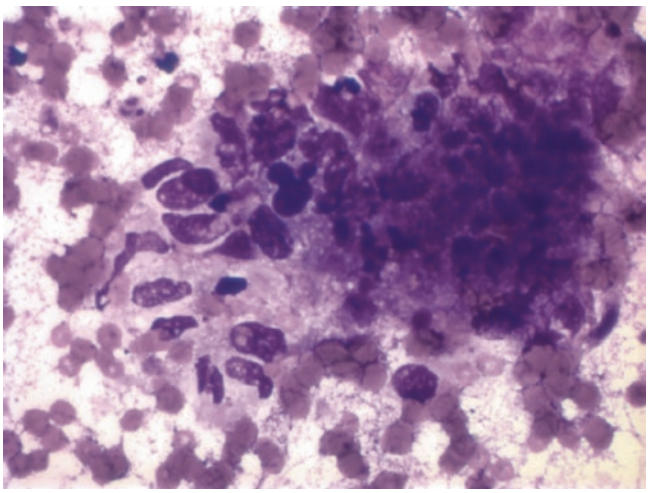

Fig. 5.25 Sarcomatoid mesothelioma presenting as bloody and sparsely cellular smear with rare clusters of spindled cells; Diff-Quik

Sarcomatoid and Biphasic Variants

While most of these mesotheliomas present with persistent effusions, they seldom exfoliate the sarcomatoid malignant cells in the fluids. Consequently, the fluids tend to be bloody and virtually acellular. Rarely, very few malignant spindled or highly atypical large cells are seen with diligent search (Fig. 5.25). The biphasic type may exfoliate, but only the epithelioid component is found in the effusion.

Other Rare Variants

Because of their rarity, very few cases have been reported in the cytology literature and the following features are mainly based on the author's experience. Effusions with the small cell variant have low cellularity. The exfoliated cells are small in size and show the immunophenotypic profile of mesothelioma rather than that of small cell carcinoma (Fig. 5.26a, b). The lymphohistiocytoid variant may present with cellular effusion consisting predominantly of lymphocytes and histiocyte-like cells that stain as mesothelial cells. The deciduoid variant tends to have large and cellular effusions with highly atypical cells that have definitive malignant features, but may not be initially recognized as mesothelial in origin [54–57] (Fig. 5.27a, b). The majority of the reported cases had the immunostaining pattern of mesothelioma.

Localized well-differentiated papillary mesothelioma has mainly been described in peritoneal washes or fine needle aspirates. However, two cases with ascitic fluids were described [58, 59]. Cytological evaluation revealed papillary clusters formed mainly of a collagenous core surrounded by one layer of mesothelial cells.

While most of the cytology literature has focused on pleural effusions, Patel et al. reviewed 49 cases of peritoneal mesothelioma, including 6 peritoneal washes obtained after

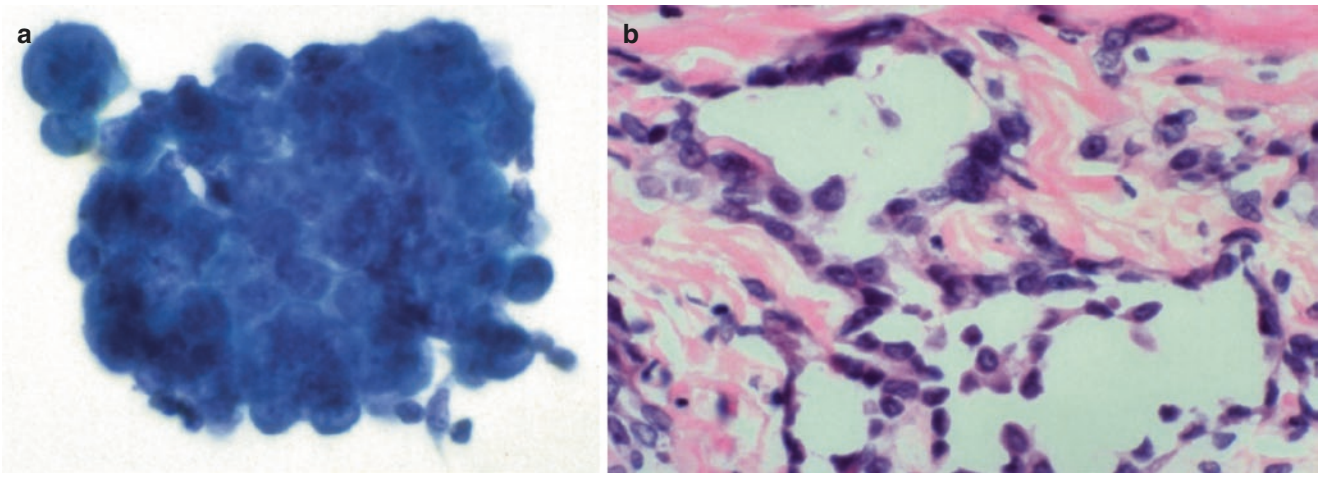

Fig. 5.26 (**a**) Mesothelioma of the small cell variant presenting as a sparsely cellular smear with few clusters as the one shown. The cells are tightly cohesive and exhibit molding simulating small cell carcinoma; PAP. (**b**) Mesothelioma of small cell variant, corresponding biopsy showing small mesothelial cells invading the fibrous stroma; H&E

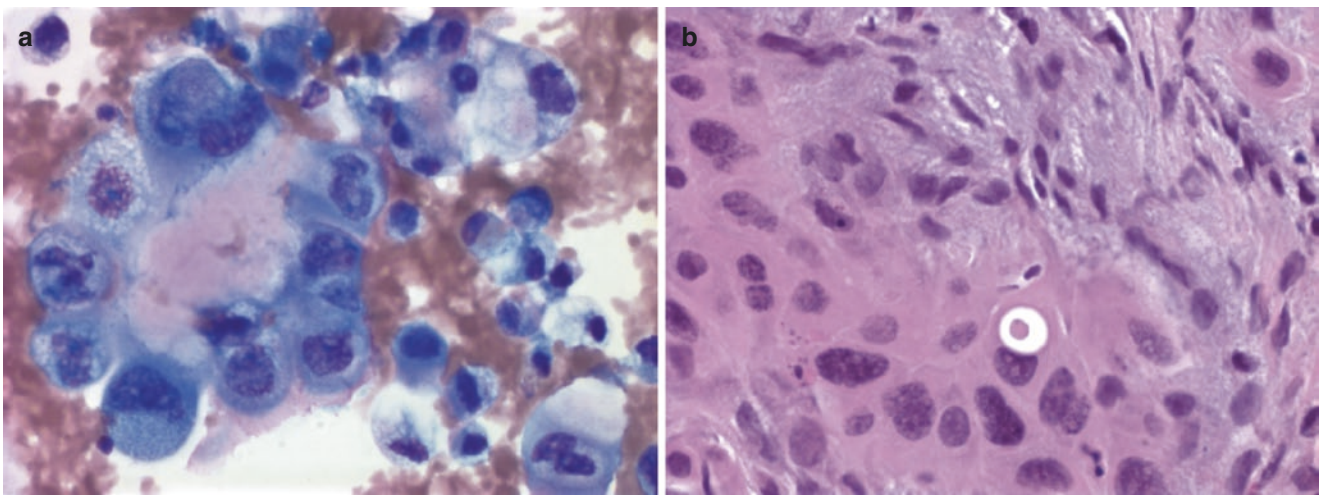

Fig. 5.27 (**a**) Deciduoid mesothelioma presenting as enlarged highly atypical cells with abundant cytoplasm. While occasional cells show two-tone cytoplasm and sub-membranous vacuoles, the mesothelial origin is difficult to ascertain without the confirmatory immunostains; Diff-Quik. (**b**) Deciduoid mesothelioma, corresponding peritoneal biopsy showing a highly atypical infiltrative mesothelial proliferation; H&E

cytoreduction and HIPEC and reported as negative for residual mesothelioma [52]. The peritoneal fluids ranged from 15 to 1000 mL in volume and were predominantly moderate to highly cellular. The smears were typically bloody or had proteinaceous background. The discohesive single cell presentation was uncommon in these cases, but otherwise cytological features similar to those of pleural mesothelioma were described. Mitotic activity was noted in one-third of the cases, but no atypical mitotic figures were identified. Peritoneal washes presented with some different features. When compared to effusions, peritoneal washes were more likely to contain broad, irregular branching sheets, frequently containing several hundreds of malignant cells. Mitotic figures were more readily observed in washes, particularly within the cellular sheets. Peritoneal washes post-HIPEC were uniformly bloody and generally low in cellularity. Residual malignant cells manifested as few small scattered clusters and small sheets admixed with clusters of reactive mesothelial cells. To address this challenge, the authors recommended comparing these samples with diagnostic material evaluated prior to therapy.

Ancillary Tests

Histochemical Stains

Prior to the recent introduction of the currently available wide array of immunostains, particularly mesothelial markers, histochemical stains used to play a major role in the

diagnosis of mesothelioma. At present, these stains do not play such an essential role, with the exception of the rare undifferentiated case that may not express the expected immunostaining profile.

Because of the high glycogen content of mesothelial cells, they stain strongly positive with PAS and convert to negative or weakly positive upon treatment with diastase (PAS-D). Mesothelial cells secrete hyaluronic acid (HA) and acid muco-substances. Consequently, 40–50% of mesotheliomas stain positive with Alcian blue, which converts to negative upon treatment with hyaluronidase. Mayer's Mucicarmine is generally negative in mesothelioma, although rare cases may focally stain positive [60] (Fig. 5.28a). This positive staining will convert to negative with hyaluronidase treatment, confirming a focal nonspecific staining [61].

Measurement of HA in the fluid is believed to be of value. Whitaker et al. measured HA in fluids of reactive mesothelium, mesotheliomas, and metastatic adenocarcinomas. They found that mesothelioma specimens tend to have levels higher than 200 mg/L, and in some cases, levels were as high as 3130 mg/L. The authors noted, however, that some mesotheliomas had levels of less than 90 mg/L and therefore commented that while high HA levels confirm the diagnosis of mesothelioma, low levels do not necessarily exclude it [62]. In a study by Welker et al., the authors reported the cutoff value of 30 mg/L as having maximum diagnostic reliability, with 87% sensitivity and 86% specificity, while a value of 100 mg/L resulted in sensitivity and specificity of 39% and 98%, respectively. The addition of HA measurement to cytology increased the sensitivity from 48% to 71–91%, while only slightly decreasing the specificity to 94–96% [63].

A useful, fast and very affordable yet underutilized stain is Oil Red O to identify the perinuclear fat droplets characteristic of mesothelial cells (Fig. 5.28b) [3].

Electron Microscopy (EM)

Before the introduction of immunoperoxidase stains, EM was the most conclusive method to document mesothelioma. The following are features described as characteristic of mesothelial origin [50]:

1. Cytoplasm rich in intermediate filaments concentrically arranged and particularly concentrated in a ringlike pattern around the nuclear envelope and in the subplasmalemmal position beneath the cell surface. This phenomenon contributes to the endo-ectoplasmic demarcation noticed on light microscopy.
2. Paucity of organelles and mainly glycogen vacuoles seen near the periphery of the cytoplasm.
3. Cell surface rich with microvilli that are distributed throughout the periphery of the cell. Characteristically, these microvilli are bushy, complex, and frequently branching and very long. The microvilli lack glycocalyceal bodies and filamentous core rootlet at their base and usually contain actin-like filaments along their length.

The role of EM in diagnosing mesothelioma is discussed in more detail in Chap. 11.

Immunostains

To date, there is no specific marker that can alone separate adenocarcinoma from mesothelioma, and it is important to use a panel of stains including a minimum of two mesothelial markers and two carcinoma markers. Additional markers can follow if the results of the initial panel are not conclusive [3].

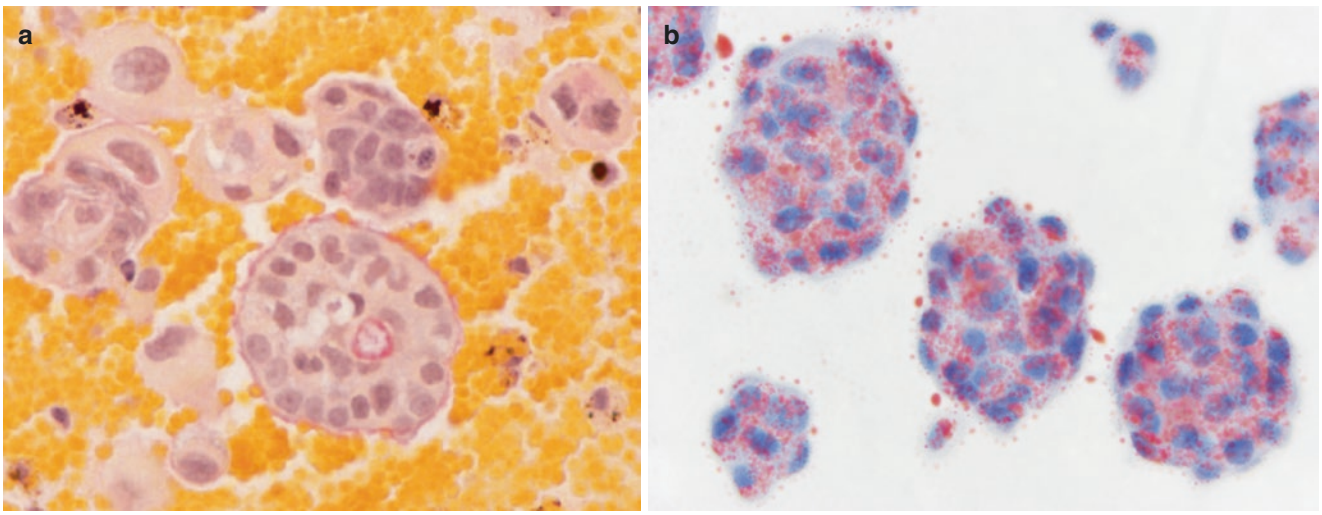

Fig. 5.28 (**a**) Mesothelioma showing rare focal positive staining with Mucicarmine stain. (**b**) Mesothelioma showing distinct perinuclear fat droplets with Oil Red O stain

Positive Mesothelial Markers [64–66]

Mesothelin (Fig. 5.29) was reported to show a diffuse strong staining in up to 100% of mesotheliomas, and some consider a negative stain as strong evidence against mesothelioma. However, it has also been reported to stain a high percentage of adenocarcinomas.

Calretinin (Fig. 5.30) is considered as one of the most sensitive stains for mesothelioma. It strongly and diffusely stains both nuclei and cytoplasm, resulting in a "fried egg" appearance. It was reported to stain from 55% to 100% of epithelioid mesotheliomas cases and 30–60% of sarcomatoid mesotheliomas. The wide range of positivity is likely related to the type of antibody used, and the best results were reported with polyclonal antibodies against recombinant human calretinin. It is worth noting that calretinin was reported to stain carcinomas from various sites of origin including 6–23% of lung ADC, 31–38% of serous carcinomas, 15–74% of breast ADC, 0–10% of renal cell carcinomas, 23–40% of squamous cell carcinomas of the lung, and 41–49% of small cell carcinomas.

Wilms tumor 1 protein (WT-1) (Fig. 5.31) is strongly expressed in the nuclei and has been reported to stain 43–100% of epithelioid mesotheliomas. It was also reported to react with 83–100% of serous carcinoma of the ovary and peritoneum. However, it is negative or very weakly positive in adenocarcinoma of the lung and squamous cell carcinoma and therefore useful in this differential diagnosis [67].

Podoplanin A and D2–40 (Fig. 5.32) are expressed in the cytoplasm of over 90% of epithelioid and 57% of sarcomatoid mesotheliomas. Up to 15% of adenocarcinomas may also show positive staining, though the expression is usually

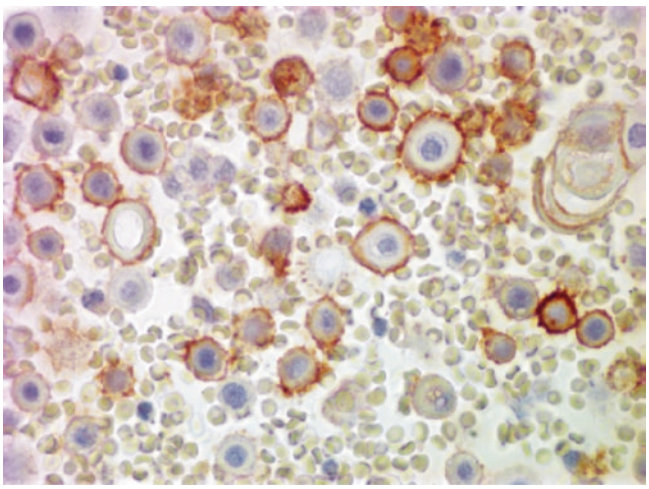

Fig. 5.29 Mesothelioma with positive membranous reaction to mesothelin

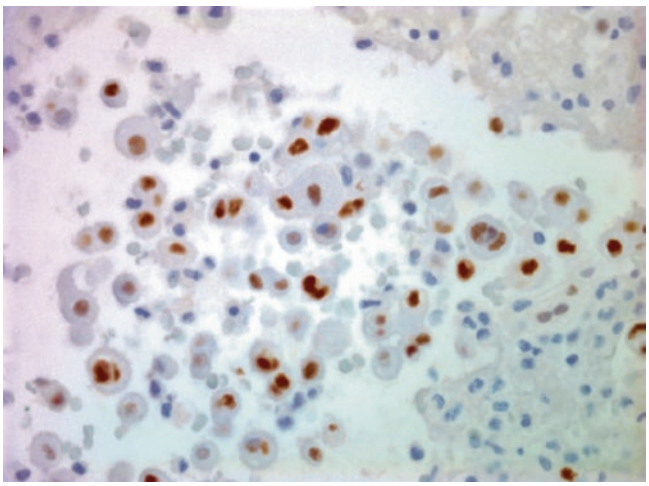

Fig. 5.31 Mesothelioma with positive nuclear reaction with WT-1 antibody

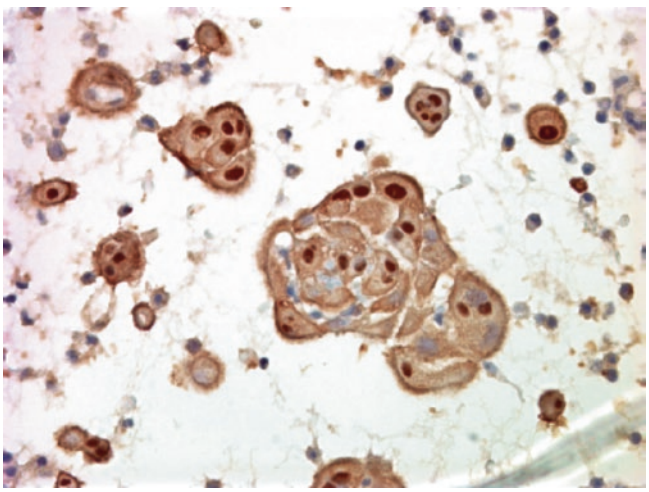

Fig. 5.30 Mesothelioma with positive nuclear and cytoplasmic reaction (so-called fried egg appearance) to calretinin

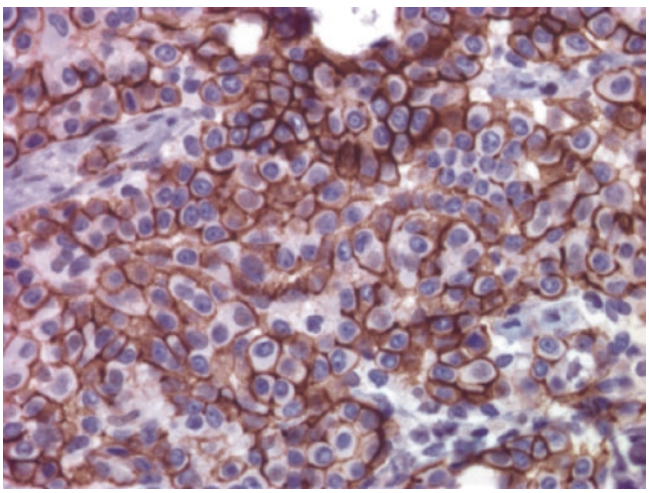

Fig. 5.32 Mesothelioma with distinct membranous staining for D2–40

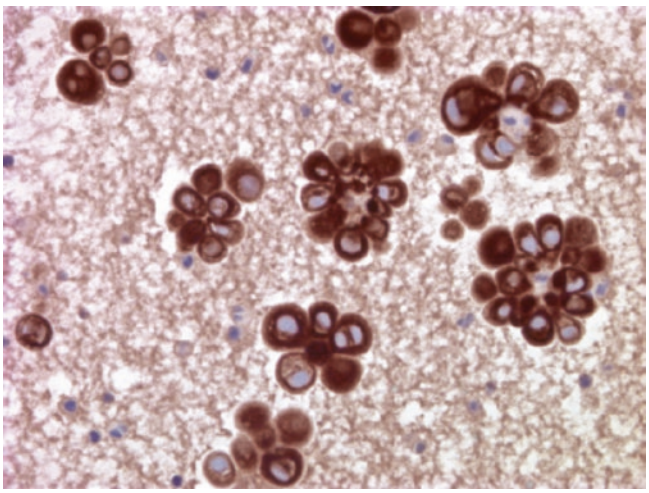

Fig. 5.33 Mesothelioma with strong cytoplasmic reaction with cytokeratin 5/6 antibody

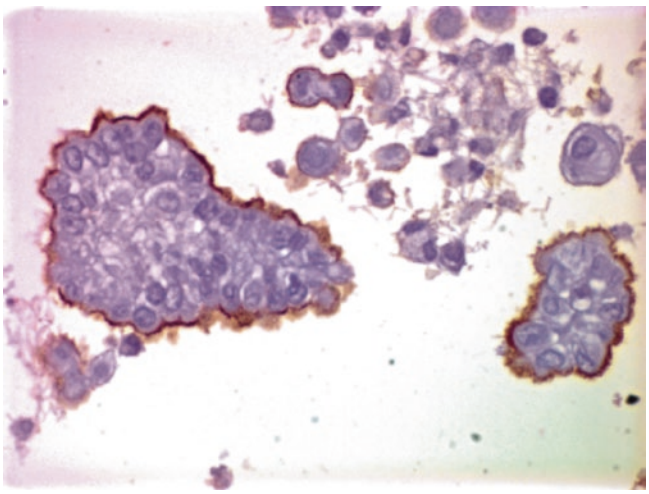

Fig. 5.34 Mesothelioma expressing HBME-1, highlighting the brush border of the cells

weak [68, 69]. Podoplanin is also expressed in squamous cell carcinoma of the lung and serous carcinomas. Bassarova et al. [70] evaluated the diagnostic role of D2–40 in 290 effusions, including 169 ovarian carcinomas and 32 mesotheliomas, and observed frequent staining in the former tumor, concluding that it performed poorly in this differential diagnosis. Ordonez reported similar performance for these two antibodies in surgical specimens [66].

Thrombomodulin stains over 75% of mesotheliomas but was reported to be expressed in up to 25% of adenocarcinomas, although staining is weaker. Staining is also seen in squamous cell carcinoma [71].

Cytokeratin 5/6 (Fig. 5.33) exhibits strong cytoplasmic staining in 65–100% of epithelioid mesotheliomas and a high percentage of squamous cell carcinomas. It has also been reported to stain a significant percentage of breast and gynecologic adenocarcinomas [67]. However, it is predominantly negative in adenocarcinoma of the lung with only 0–19% reported to express CK5/6, attributed to be likely due to squamous differentiation.

HBME-1 (Fig. 5.34) is seldom used now because of the significant staining overlap with adenocarcinomas, particularly of ovarian origin. It is expressed with a distinct membranous or brush border staining in mesothelioma [72],

Negative Mesothelial Markers

Carcinoembryonic antigen (CEA) exhibits cytoplasmic staining in 50–90% of adenocarcinomas, particularly breast (80%), gastrointestinal, and lung origin, as well as in 77–86% of squamous cell carcinoma. While the old literature describes up to 30% staining in mesothelioma, newer clones are more specific and are consistently negative. Of note, most ovarian carcinomas, except for mucinous carcinomas, rarely express CEA, and this marker is therefore not useful by itself in the differential diagnosis between mesothelioma and ovarian carcinoma [73].

B72.3 identifies the Sialyl-Tn sugar group and stains the membrane and/or cytoplasm in over 80% of adenocarcinomas, 75–85% of lung carcinoma, 70–75% serous carcinoma, and 50–70% of breast carcinoma. It is also reportedly expressed in 45–84% of lung squamous cell carcinoma but negative in mesothelioma.

CD15 (Leu-M1) exhibits cytoplasmic staining in a high percentage of adenocarcinomas of various body sites, up to 30% of squamous cell carcinoma and is negative in mesothelioma.

MOC-31 antibody recognizes the membrane protein EpCAM and exhibits strong and diffuse membrane and/or cytoplasmic staining in most adenocarcinomas and squamous cell carcinoma of the lung. It can be focally expressed in 2–15% of mesotheliomas [67, 74].

Ber-EP4 is directed against the same epitope as MOC-31 and exhibits a staining pattern similar to the latter. It may be focally positive in 13–26% of mesotheliomas [74].

BG-8 identifies the Lewis^y sugar group and strongly stains the membrane and/or cytoplasm of over 95% of adenocarcinomas. It can be weakly and focally expressed in 3–9% of mesotheliomas.

Claudin 4 is a transmembrane protein located in the tight junctions. It is expressed in most epithelial cells but not in mesothelioma. It is expressed in most adenocarcinomas of the lung, breast, ovary, and kidney and most squamous and urothelial carcinomas but predominantly negative in mesotheliomas [75].

PAX8 stains as strong nuclear reaction. This stain is essentially negative in mesothelioma and positive in a high percentage of Müllerian tumors, with a sensitivity of 96% and specificity of 100% [76].

MMP-7 is a member of the matrix metalloproteinases, a family of more than 20 zinc- and calcium-dependent enzymes

involved in degrading all components of basement membranes and consequently the physiologic process of tumor progression. Davidson et al. reported that MMP-7 was expressed in 124/307 (40%) of adenocarcinomas and was uniformly negative in all 49 mesotheliomas [77].

Other Immunostains

Pancytokeratin (Fig. 5.35) is usually strongly positive in mesothelioma, squamous cell carcinoma, and adenocarcinoma [78].

Cytokeratin 7 is strongly positive in mesothelioma and in some adenocarcinomas but is expressed in only 30% of squamous carcinomas [79].

Cytokeratin 20 (Fig. 5.36) is variably expressed in mesothelioma and therefore should be cautiously evaluated in the differential diagnosis with adenocarcinomas known to express CK20, such as gastrointestinal tract or urothelial carcinomas that may occasionally mimic mesothelioma [78].

BRCA1-associated protein 1 (BAP1) is a tumor suppression gene encoded by the BAP 1 gene at 3p21.1. Recent studies revealed that BAP1 expression is lost in 57–66% of mesothelioma while expressed in reactive mesothelium with a specificity of 100%. This stain is emerging now as the best immunostain to distinguish mesothelioma from reactive mesothelium [80–82].

Desmin and EMA (Figs. 5.37 and 5.38) play a role in the differential diagnosis between mesothelioma and reactive mesothelium. Mesothelioma has strong membranous EMA staining in the majority of cases which is rarely present in reactive mesothelium. Desmin is preferentially expressed by benign mesothelium and is lost in mesothelioma. Caution should be exercised when evaluating desmin in mesothelioma, since scattered reactive mesothelium in the background may stain positive [69, 83].

E-cadherin and N-cadherin are currently not believed to be of use in the differential diagnosis between mesothelioma and adenocarcinoma. E-cadherin, however, is useful in

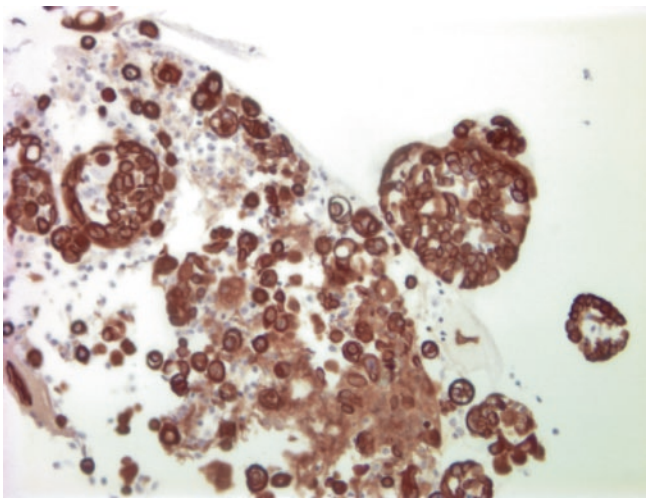

Fig. 5.35 Mesothelioma with strong cytoplasmic cytokeratin 7 staining

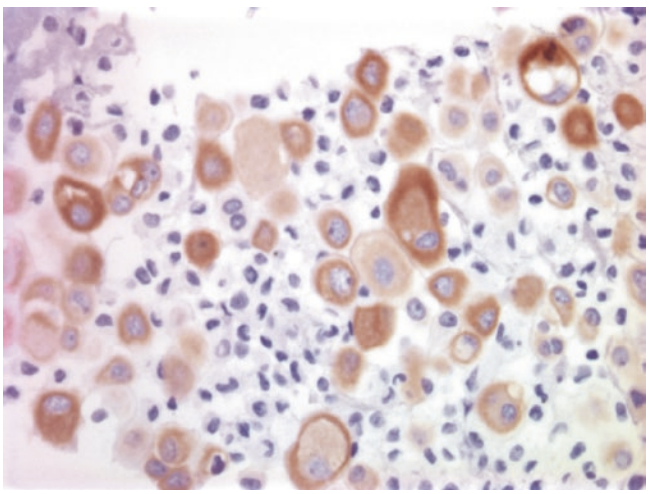

Fig. 5.36 Mesothelioma showing a rare reaction to cytokeratin 20

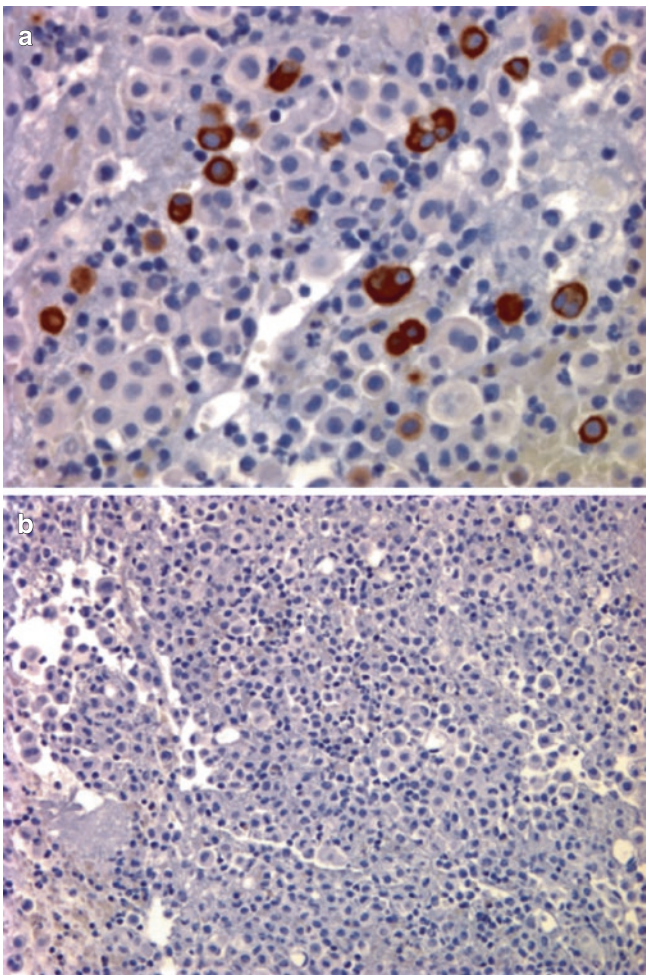

Fig. 5.37 (**a**) Reactive mesothelium showing positive staining for desmin. (**b**) Reactive mesothelium showing negative reaction to EMA

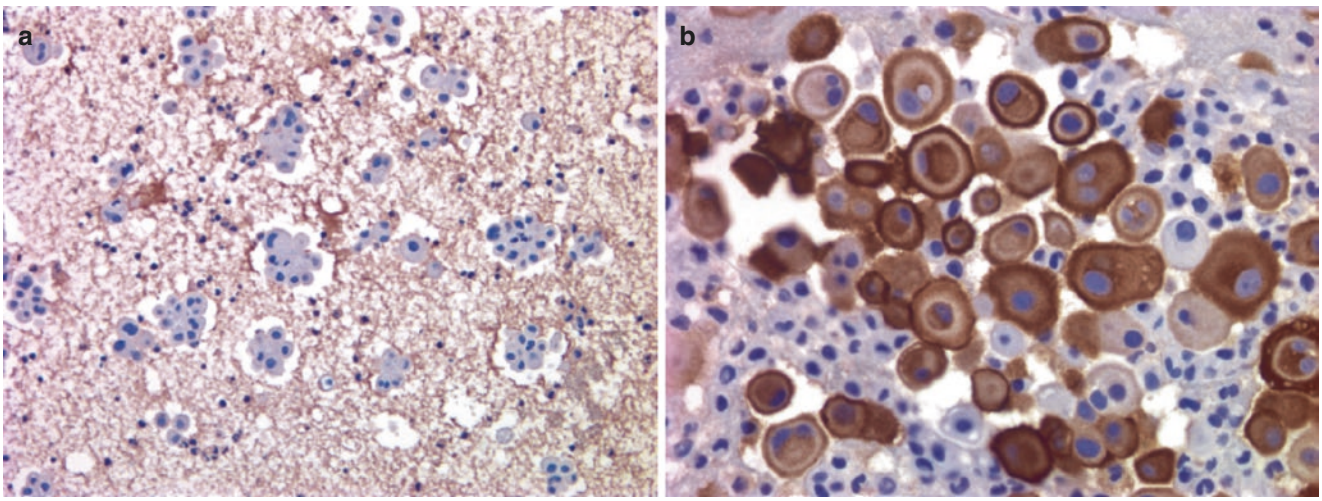

Fig. 5.38 (**a**) Mesothelioma reacting negatively to desmin. (**b**) Mesothelioma reacting strongly to EMA

separating adenocarcinoma, which tends to be positive, from the negatively reacting benign mesothelium [84, 85].

Other Methods

Several other methods, including traditional cytogenetics, fluorescent in situ hybridization (FISH), measurement of secreted biomarkers, and high-throughput technology, are discussed in Chap. 11.

Differential Diagnosis

The differential diagnosis depends on the cytological presentation and degree of atypia recognized. When atypia is subtle, reactive mesothelial hyperplasia is the main differential. When malignancy is identified, it is important to separate mesothelioma from adenocarcinoma of various primary sites.

Mesothelioma Versus Reactive Mesothelium (Table 5.2)

When mesothelium is floridly reactive, it may mimic mesothelioma. The cellularity can be high, with an increase in the number of clusters and multinucleated cells. Mitotic figures may be conspicuous and have been reported to reach their peak in mesothelial surfaces reacting to injury within 48 h [86]. In such cases, examination at low magnification cannot be stressed enough. The cells are monotonous in appearance, i.e., mesothelial in origin, and exhibit a small to moderate size with very subtle difference in size except for a few outliers. Nuclei, while enlarged and somewhat atypical, have vesicular chromatin and smooth chromatin contours even in the multinucleated cells. The nucleoli may be prominent, but no macronucleoli or irregular nucleoli are detected. Clusters tend to be few in most cases and are small with lack of depth, i.e., flat with scalloped borders. Multinucleation is rarely beyond 3–4

Table 5.2 Reactive mesothelium versus malignant mesothelioma

Feature	Reactive mesothelium	Malignant mesothelioma
Cellularity	Moderate	Very high
Cell size	Little variation in size and shape	Wide variation in size from benign to gigantic
Multinucleated cells		
Number	Few scattered cells	Numerous
Nuclei	Rarely exceed 5 nuclei	May contain >50 nuclei
	Benign appearance	Enlarged and atypical
Giant cell	Rare	Characteristic
		May reach the size of adjacent morules
		Normal N/C ratio
Clusters		
Number	More numerous than normal	Innumerable
Morphology	Flat and lack depth	Morules and spheres with depth
Borders	Scalloped	Scalloped or knobby
Cell arrangement	No crowding	Frequently crowded
Nuclei		
Size	Slightly enlarged	Markedly enlarged
Chromatin	Mostly vesicular	Atypia present but vary from subtle to definitive
Nuclear membrane	Smooth or subtle irregularity at most	Subtle to definitive irregularity
Nucleoli	Slight to moderate enlargement	Markedly enlarged or macronucleoli
Cytoplasm	Moderate	Abundant and very dense
Mitotic activity	Can be conspicuous	May not be increased

nuclei and these appear normal. No gigantic cells are seen. In contrast, malignant mesothelioma will present with numerous morules and single cells. The single cells in malignant mesothelioma may appear monotonous, consistent with their mesothelial origin. However, they may vary tremendously in size from that of normal mesothelium to gigantic cells. In the author's experience, such large cells may attain the size of the small adjacent morules, a feature that has not been seen except in mesothelioma. The cellular clusters also vary in size and have a spherical appearance with depth of focus and berrylike borders. The presence of small orangiophilic squamous-like or parakeratotic-like cells were reported to be highly correlated with malignant mesothelioma while rarely noted in reactive mesothelium [87, 88].

In a study by Kimura et al. [89], the authors devised a scoring system of the cytological features in an attempt to separate reactive from malignant mesothelium. They assigned a total score of 10 points, 1 point each for variation in cell size, sheetlike arrangement, cyanophilic cytoplasm with windows/blebs/brush border, mirror ball-like cell clusters, cannibalism, and nuclear atypia. Two features, acidophilic large nucleoli and multinucleated cells with more than eight nuclei, received 2 points each. Mesotheliomas consistently scored more than 5, while reactive mesothelial hyperplasia and metastatic adenocarcinomas scored less than 3 points. A study by Cakir et al. [90] using logistic regression analysis identified the presence of cell ball formation, cell-in-cell engulfment, and monolayer sheets as variables useful in the separation of reactive mesothelium from mesothelioma, with the latter finding favoring a reactive diagnosis.

The role of ancillary testing in separating reactive from malignant mesothelium is somewhat controversial. While proliferation markers such as Ki-67 and MIB-1 may be useful in some cases where mesotheliomas have higher activity, it has been the author's experience that they play a limited role in the floridly reactive effusions, where mitotic activity is very high, with a sensitivity of 17% and specificity of 91% [83]. The differential staining of desmin and EMA seems to be more helpful. It is well established that benign mesothelium expresses muscle markers, particularly desmin, which is progressively lost as the mesothelium becomes malignant, with a sensitivity of 91% and specificity of 94%. On the other hand, EMA is not expressed by benign mesothelium and is expressed in most malignant mesotheliomas, with a sensitivity of 100% and specificity of 94%. p53 may also be helpful in this differential diagnosis and has been shown to be expressed at much higher levels in mesothelioma, with a sensitivity of 57% and specificity of 98%. In the author's experience, GLUT-1 has less utility in this differential diagnosis, with a sensitivity of only 47% and specificity of 88% [83].

Otherwise, reactive mesothelium expresses all the immunomarkers expressed by mesothelioma, such as HBME-1, calretinin, D2–40, and WT-1.

Mesothelioma Versus Adenocarcinoma (Table 5.3)

In over 50% of cases, the distinction between adenocarcinoma and mesothelioma is feasible by routine cytological stains once the characteristic mesothelial features are recognized. Generally, at low magnification, the evaluator should recognize an overall monotony in the type of cells with no alien population in mesothelioma while frequently detecting a two-cell population, namely, carcinoma and benign mesothelium, in adenocarcinoma specimens.

Mesothelioma is characterized by a low degree of atypia. Definitive malignant features are rarely present and mitotic activity is inconspicuous. In contrast, adenocarcinoma generally expresses a noticeable degree of pleomorphism with definitive malignant features and high mitotic activity in most cases.

In the study by Cakir et al. [90], the authors identified the presence of giant atypical mesothelial cells, nuclear pleomorphism, and acinar formation as features useful in distin-

Table 5.3 Mesothelioma versus adenocarcinoma

Feature	Mesothelioma	Adenocarcinoma
Cellularity	Very high	Variable, frequently high
Overall cell features		
Cell type	Monotonous population	Polymorphous population
	One cell type, mesothelial	Two cell types
Pleomorphism	Minimal atypia	Obviously atypical
	Rarely frankly malignant	Rarely subtle atypia
Cell size	Vary from small to gigantic	Generally enlarged and of similar size
N/C ratio	Low	High
Mitotic activity	Inconspicuous	Variable, can be high
Cytoplasm	Abundant	Variable but rarely abundant
	Two-tone	One-tone in most cases
	Sub-membranous vacuoles and brush border	Fine vacuoles throughout or large disfiguring vacuoles
Cell clusters		
Shape	Spheres, morules, and loose clusters	Variable, mainly spheres or clusters
Circumference	Knobby or berrylike	Mostly smooth
	Scalloped borders	
	Cytoplasm forming the border	Nuclei forming the border
Cell-to-cell relation	Cellular windows	No windows
	Cellular clasping	No cell clasping
	Cell within cell	Cell within cell

guishing mesothelioma from adenocarcinoma, with the latter two favoring adenocarcinoma.

In a subgroup of cases, it is truly difficult to distinguish mesothelioma from adenocarcinoma, and immunostains will play a significant role in establishing the diagnosis. As stated above, we recommend using a minimum of two mesothelial markers and two or three carcinoma markers in the initial panel.

While all adenocarcinomas can present a differential with mesothelioma, certain adenocarcinomas should particularly be considered. These include tumors of lung, ovary, and breast origin, since they can present with numerous cell aggregates in a pattern very similar to mesothelioma. Breast carcinoma in particular tends to present as cellular spheres with only a few single cells. However, breast clusters tend to be very large, frequently with irregular contours, and an overall cribriform pattern in contrast to the smaller morules with scalloped borders in mesothelioma. As previously mentioned, in the author's experience, the presence of multinucleated cells with abundant dense cytoplasm and gigantic cells approaching the size of adjacent morules is a feature frequently seen in mesothelioma and rarely encountered in adenocarcinoma. Primary adenocarcinoma of the serosal surface may be difficult to separate from peritoneal mesothelioma. Both present as a primary peritoneal tumor and may overlap with some immunostains. In a study by Ordonez evaluating multiple markers, the best discriminators among the positive markers for mesothelioma were D2–40, podoplanin, and calretinin. The author recommended a panel of Ber-EP4 and MOC-31 in combination with calretinin, and/or D2–40 or podoplanin [91]. However, since Ber-EP4 and MOC-31 are directed against the same epitope, we believe that one of them is sufficient and recommend instead an additional marker such as B72.3, which is highly specific, to be added to the panel [92]. In addition, it is important to remember that mesothelial markers may also be expressed by a subset of ovarian carcinomas. Estrogen (ER) and progesterone (PR) receptor immunostaining is also helpful in the differential diagnosis, with reactivity for ER in up to 88% of ovarian and 86% of primary peritoneal serous carcinomas, and PR staining in up to 60% and 56% of ovarian and peritoneal carcinomas, respectively [93]. As previously mentioned, PAX-8 is very helpful in this differential diagnosis, with negative staining in mesothelioma and frequent reactivity in serous carcinoma [76].

Mesothelioma Versus Poorly Differentiated Squamous Carcinoma (Sqcc)

Fortunately, this differential is very rare. Poorly differentiated Sqcc may present with cellular spheres and large cells with abundant two-tone cytoplasm. It should always be considered when a fluid is suspected to be mesothelioma but staining is inconsistent, e.g., positive staining for both calretinin and carcinoma markers. In these cases, staining with WT-1 and p63 or p40 may be valuable. Sqcc expresses p63 in over 90% of cases and is characteristically WT-1-negative. For further discussion, please refer to Chap. 2 in this book.

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