Tumors of serous membranes of difficult diagnosis: Role of immunohistochemistry

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Abstract:
The most common differential diagnosis of tumors involving serosal surfaces is adenocarcinoma versus malignant epithelial mesothelioma. This should no longer be a difficult diagnosis if an appropriate panel of markers indicative of epithelial or mesothelial lineage is properly performed and interpreted. Our currently recommend immunodiagnostic panel will be discussed in some detail.
At this time, the most difficult differential diagnosis is in the distinction between reactive and neoplastic mesothelial proliferations, particularly fibrous pleurisy versus desmoplastic malignant mesothelioma. Unfortunately, immunohistochemistry and other ancillary tests play a minor role in this area of diagnostic difficulty, and incorrect diagnoses are still common.
Examples of cases with this differential diagnosis that were misdiagnosed, with emphasis on the criteria needed to minimize diagnostic error, will be discussed.

Introduction:
The term mesothelium is generally reserved for the monolayer of flattened cells with epithelial features that lines serous cavities. The term derives from a combination of the cells mesodermal origin and their epithelial phenotype. The major function of mesothelium is to provide a smooth, low-friction surface, to facilitate the gliding motion of the lungs in the pleural cavity, the heart in the pericardial sac, and the viscera in the abdominal cavity. This process is assisted by: (a) the presence of myriad surface microvilli, (b) a thick glycocalyx, rich in sialic acid, and (c) the secretion of glycosaminoglycans, especially hyaluronic acid, by the mesothelial cells. The mesothelial cells overlay an ill-defined layer of mesenchymal cells that perform a substantial role in the regeneration of the surface mesothelium.

Although neoplasms of mesothelial origin are rare, they have received a disproportionate amount of attention given their relation to occupational and environmental exposure to asbestos. Such neoplasms are a leading cause of lawsuits in developed countries and often present difficult diagnostic problems to the surgical pathologist. This handout, therefore, focuses primarily on the diagnostic aspects of mesothelioma. However, to better understand some of the various histologic appearances of mesothelium-derived neoplasms, it is helpful to briefly review some features of mesothelial reactions to injury. For a comprehensive review of this subject see the excellent reviews by Whitaker et al. (1;2).

THE REACTIVE MESOTHELIUM

Regeneration

Following a variety of injuries to the mesothelium, including chemical or mechanical exfoliation, the proximity of an inflammatory or neoplastic process, or its exposure to asbestos fibers, there is an initial loss of the surface-cell monolayer followed by the deposition of fibrin, leukocytes, and macrophages(3) Soon, particularly when the irritative stimulus persists, a proliferation of spindle-shaped cells, indistinguishable from fibroblasts but with a characteristic immunophenotype, develops underneath this layer of fibrin. Evidence suggests that these fibroblast-like cells play an important role in the restoration of the mesothelial surface layer.

Raftery (4) provided evidence that perivascular cells resembling fibroblasts proliferate under areas of experimentally denuded peritoneum, and as suggested earlier by Hertzler (5), these cells appeared to be the forerunners for a newly formed mesothelial layer.

Immunohistochemical studies with antibodies to keratins (cytokeratins) supported the observations of Raftery (4). Bolen et al. (6) examined normal and reactive, nonneoplastic serosal tissues by light-microscopic and ultrastructural immunocytochemical methods. Normal surface mesothelium expressed both low- and high-molecular-weight keratins, whereas the resting submesothelial cells expressed vimentin as the only detectable intermediate filament. However, reactive, proliferating, subserosal fibroblast-like cells co-expressed low-molecular-weight keratins and vimentin. Additionally, in common with myofibroblasts, these cells were found to express muscle-specific actin, a contractile protein probably necessary for their migrating properties (6) During the process of mesothelial repair, these specialized fibroblast-like cells gradually acquire more cytoplasm and, as they approach the surface, become rounded and develop epithelial phenotypic and immunophenotypic characteristics, such as (a) expression of high- and low-molecular-weight keratins, and (b) loss or reduction of vimentin expression. Thus, their immunophenotype and phenotype begin to resemble those of the surface

mesothelium. It is important to emphasize that this appears to be an exclusive property of the submesothelial mesenchyme, because proliferating fibroblasts in other regions of the body have not been shown to express keratins by immunohistochemical methods. At any rate, this morphologic and immunophenotypic diversity of reactive mesothelial cells is often recapitulated in neoplasms of mesothelial origin.

Of more practical importance is the fact that these cytokeratin expressing subserosal spindle cells, in response to a variety of irritative stimuli, may be a source of diagnostic error, particularly with the desmoplastic variant of malignant mesothelioma. Characteristically—and this is diagnostically important— the long axes of reactive cells tend to run parallel to the surface, and these cells are uniformly distributed in the vicinity of the irritative stimulus (which may be an underlying neoplasm, for example). They usually have bland cytologic appearance. Pancytokeratin immunostains will reveal an abrupt and well-delimited boundary with the underlying cytokeratin-negative fibroblasts and other stromal cells.

MESOTHELIAL HYPERPLASIA VERSUS MESOTHELIOMA

Distinction between epithelial mesothelioma and adenocarcinoma is now seldom difficult, due to recent immunohistochemical progress. However, the separation between hyperplastic and neoplastic mesothelial proliferations continues to be a difficult diagnostic challenge for which immunohistochemistry is of limited value (7).

Epithelial proliferations

Any chronic irritation may cause hyperplasia of the epithelial-appearing, mesothelial cells of the serous mesothelial surface, as well as the sub-mesothelial cytokeratin-expressing spindle cells. Because it may be very difficult to distinguish the epithelial component of such hyperplasias from incipient, well-differentiated epithelial mesothelioma, it is very important to base the diagnosis in such cases on a careful evaluation of the total clinical and radiological picture (7;8).

True invasion of stromal tissue, particularly adipose tissue and skeletal muscle remains the most reliable criterion of malignancy in epithelial-appearing mesothelial proliferations. However, it is very important not to confuse entrapped mesothelial cells with invasiveness. Entrapped mesothelial cells are usually close to the pleural surface and are well delimited from the underlying adipose tissue, which they do not invade. Reactive fibrosis and inflammatory infiltrates are often present around and near the entrapped mesothelial cells. Clearly, large and non-fragmented biopsy specimens are necessary to avoid error.

The appearance of the pleural surface, particularly at the time of thoracoscopy or thoracotomy plays a central role in this differential diagnosis. The presence of confluent tumor nodules over large areas of the pleural surface favors malignancy. In the absence of tumor nodules, the histological evidence must be very clear before a diagnosis of malignancy is rendered. Often, malignant mesothelioma may be made up of monotonous tumor cells with little or moderate atypia. Furthermore, reactive mesothelial cells may show atypical features. Thus, cytological atypia, unless severe, may not be a reliable criterion for malignancy. The presence of tubular and papillary patterns of growth favors mesothelioma, as they are rarely seen in mesothelial hyperplasia.

Fibrous proliferations

In the distinction between fibrous pleuritis and desmoplastic malignant mesothelioma, particular attention should be placed to the presence of zonation as it strongly favors a reactive process (7) By zonation is meant a higher cellularity (and at times atypia) of spindle-shaped, cytokeratin positive, mesothelial cells towards the pleural surface, with less cellular and more collagenous layers beneath (see illustrative case #4). Often fibrinous deposits and capillaries growing perpendicular to the surface are noted in reactive processes. However, it is important to emphasize that a reactive and fibrous pleuritis may be caused by the presence of an underlying malignancy, including mesothelioma. Ample sampling, as partial decortication of the pleural lesions, is therefore, very helpful. Thorough and deep sampling, to include underlying fatty and muscle tissue of lesions over the thoracic pleura is essential to avoid diagnostic error. It cannot be overemphasized that a firm diagnosis of malignant mesothelioma, particularly of the desmoplastic type, rests on the clear demonstration of invasiveness.

Role of immunohistochemistry in the distinction between hyperplasia and neoplasia

Immunohistochemistry, as will be discussed later in detail, helps to identify the mesothelial lineage of the proliferating cells, but is of limited value to distinguish between mesothelial hyperplasia and mesothelioma. However, pancytokeratin stains may be useful to identify invasiveness.

Although some have reported that p53 protein is frequently over-expressed by mesothelioma and not by reactive mesothelial proliferations (9-11), others, however, have found otherwise (12-14) My own unpublished observations are more in keeping with the latter studies, as only seven of 38 epithelial mesotheliomas showed immunohistochemical over-expression of p53. Additionally, studies using molecular biologic methods have confirmed a low rate of p53 mutations in mesothelioma (14) Nonetheless, in an isolated case, strong immunostaining for p53 by the majority of the mesothelial cells supports mesothelioma over hyperplasia.

Recently Kumaki et al. reported immunohistochemical detection of Telomerase Reverse Transcriptase (TERT) on 67 of 68 malignant mesotheliomas and in none of 19 benign mesothelial lesions. The immunohistochemical study was validated by in situ hybridization with TERT antisense probes (15) It thus appears that immunohistochemical staining for TERT may assist in distinguishing mesothelioma from reactive processes. Unfortunately, however, TERT immunostaining is difficult to reproduce with currently existing antibodies.

PLEURAL FIBROSIS AND PLEURAL PLAQUE

Chronic injury, to the pleural surface may result in the formation of dense layers of fibrous connective tissue involving visceral as well as parietal pleura. Among causes of pleural fibrosis are, asbestosis and other pneumoconiosis, inflammatory pleurisy, including rheumatoid pleuritis, and, most commonly, bacterial pneumonias. Pleural fibrosis also may result from the introduction of irritants into the pleural cavity to promote therapeutic pleural fusion (pleurodesis).

A frequent area of diagnostic difficulty is in the distinction between fibrous pleuritis and desmoplastic malignant mesothelioma and is now one of the most common requests to the US-Canadian Mesothelioma Reference Panel (7) This difficult differential diagnosis will be discussed in some detail later.

These forms of pleural fibrosis should not be confused with pleural plaques that are, in the vast majority of cases, caused by asbestos exposure (16;17).

Pleural plaques arise over the parietal pleura particularly at the lower chest and the diaphragmatic pleura. Usually they develop from 2 to 3 decades following asbestos exposure. Grossly they consist of well-delimited, irregularly shaped, raised grayish-white to ivory plaques ranging in size from tiny specks to several centimeters. They have a cartilaginous consistency and often calcify. Microscopically, they consist of dense strands of virtually acellular, intensely hyalinized, collagen fibers that feature a reticulated, mesh-like appearance, a pattern that has been referred to as “basket-weave” Pleural plaques are clinically indolent but serve as a reliable marker of asbestos exposure. It has been shown that pleural and peritoneal plaques contain asbestos fibers, particularly chrysotile (18-20).

BENIGN TUMORS OF THE PLEURA AND SUBPLEURAL TISSUES

Benign Localized Epithelial Mesothelioma (adenomatoid tumor)

Benign localized epithelial mesotheliomas of the pleura are exceedingly rare. The few that have been reported were found incidentally at lung resection for other conditions. Histologically they were similar to their more common pelvic peritoneal counterparts, more commonly referred to as adenomatoid tumor (21-23).

Localized Fibrous Tumor

These rare neoplasms, also called solitary fibrous tumors and formerly localized fibrous mesothelioma, usually are discovered as asymptomatic lesions on routine chest radiographs, in patients of any age, with no sex predilection and with no evident relation to asbestos exposure. Most of these tumors arise at the level of the visceral pleura. Although they may grossly appear to infiltrate the pulmonary parenchyma, they usually have a sharply delimited pushing border and are often pedunculated. They tend to measure several centimeters in diameter and are usually rounded, firm, white, and scar-like in their macroscopic appearance. Histologically, they are composed of a mixture of spindle-shaped fibroblast-like cells lying within a variable amount of collagenous stroma. Although a storiform pattern of growth may be focally present; more commonly, the cells are distributed haphazardly. Cell atypia and mitoses are uncommon, but foci of degeneration and cystic change may be present, especially in the large ones (24) Nuclear pleomorphism and mitoses may be seen in the larger tumors but do not necessarily correlate with poor prognosis if the tumor is circumscribed (25;26)

Although most follow a benign course, local recurrence may develop in as many as 16% of the cases, but can be successfully managed by repeated resection (27;28).

Localized fibrous tumors have a characteristic immunophenotype (CD34 positive, cytokeratin negative) useful to distinguish them from fibrous and desmoplastic mesothelioma, that invariably express cytokeratins and generally are CD34 negative (29;30).

Although initially thought to be of mesothelial origin, solitary fibrous tumor is now believed to originate in the subpleural non-mesothelial mesenchyme. Indeed, tumors with similar morphological features and immunophenotype occur at several extrapleural locations, further supporting its mesenchymal origin (31-34).

MALIGNANT MESOTHELIOMA

Etiology, Incidence, and Pathogenesis

Since 1960, after pioneering studies on the incidence of mesothelioma in South African asbestos miners by Wagner et al. (35), the mesothelial carcinogenesis of inhaled asbestos fibers has become firmly established (36-42) Additionally, it is generally accepted that all types of asbestos fibers are capable of causing malignant mesothelioma, usually decades after their inhalation. Moreover, a correlation between the intensity and duration of exposure to asbestos fibers and the risk of developing mesothelioma is well documented (43;44) It also is evident, however, that some mesotheliomas, especially many of those occurring in young people, may not be related to asbestos exposure (45). The incidence of asbestos-related mesothelioma cases has ranged in different series from 10% to 99% (46). Although non–asbestos-related malignant mesotheliomas undoubtedly exist, it is difficult to determine their true incidence for two main reasons: (a) there is no defined threshold of exposure to asbestos fibers in relation to the development of mesothelioma [additionally, the issue of threshold is complicated by the possible effect of individual susceptibility (47)]; and (b) there is a decades-long period of latency between the exposure to asbestos and development of the neoplasm (43;48). It was suggested (49) that even a minimal exposure, such as that which may occur in the household, may be sufficient to induce mesothelioma. In a review of 668 cases of malignant mesothelioma, McDonald and McDonald found that only 50% of the cases in men and 5% of the cases in women were associated with occupational exposure to asbestos (50) However, Vianna et al. (49) proposed that the wide variation in estimates of asbestos exposure in patients with malignant mesothelioma was due to inadequate occupational histories.

Experimental and epidemiologic evidence (39;50-54) suggest that other etiologic agents may be associated with mesothelioma as well. Such agents include radiation, minerals such as silica and beryllium, and synthetic fibers, although evidence for the latter is far from conclusive (43) The finding of Simian virus 40-like DNA sequences in cases of human malignant mesothelioma (55;56) suggested that SV40 virus which was a contaminant of some polio vaccines, may be implicated as a co-carcinogen or directly causing mesothelioma. However, this remains controversial at the present time (57-59).

Malignant mesothelioma is a rare tumor, but its true incidence is difficult to ascertain because it is underreported. McDonald and McDonald (50) estimated the combined U.S.–Canadian incidence to be 2.8 per million male and 0.7 per million female persons, but noted a steady increase in cases in men, which they attributed to occupational exposure to asbestos. Significant increase in the incidence of pleural mesothelioma among white men older than 55 during the years 1973–1980 was reported by Spirtas et al. (60) after a study of incidence rates based on data from population-based cancer registries in New York State (exclusive of New York City), Los Angeles County, (California), and the SEER Program of the National Cancer Institute. In this study, even a fter histopathologic review, a notable upward trend remained.

The pathogenesis of malignant mesothelioma remains unclear. The presence of asbestos fibers in the vicinity of the serosal surfaces appears to be a crucial pathogenic factor. In both epidemiologic and experimental studies, differences in the tumorigenicity of asbestos fibers were found, depending of the composition and physical characteristics of the fibers. Contributions of other factors (including heredity and exposure to other carcinogens such as tobacco smoke) to the pathogenesis of mesothelioma are not supported by epidemiological data (61).

Clinical Features

Malignant mesothelioma of the pleura is about 3 times more common in men than in women (50;62) Most cases occur in patients between ages 50 and 70 years. Chest pain and shortness of breath are the most frequent initial symptoms; these are followed by weakness, fatigue, and weight loss. Clinical signs of pleural effusion are by far the most common finding at the initial physical examination, and in some cases, may precede the development of clinically detectable mesothelioma by several years (63) Chest roentgenograms or computerized tomography will reveal irregular pleural thickening that is most apparent after evacuation of the pleural fluid. Irregular thickening of the interlobar fissures is another characteristic radiographic feature of pleural mesothelioma. In patients who have had a long or intense exposure to asbestos fibers, the presence of a pleural plaque will frequently be discovered by these examinations. The effusions, which often are bloody, tend to recur rapidly after evacuation, but, with progressive obliteration of the chest cavity by the growing tumor, they may subside. There may be involvement of pericardium and mediastinum, as well as invasion of the soft tissues of the chest wall, particularly at biopsy sites or at the location of chest tubes after surgery. Metastases are uncommon in the early stages of the disease, but they may

be seen in later stages and are a frequent finding at autopsy. Nonetheless, cases of metastatic mesothelioma with initial presentation as lymph node metastases have been reported. However, most of these have been peritoneal mesotheliomas (64).

The average survival time from the onset of symptoms is approximately 15 months, but much longer survivals have been reported (65;66). Patients with pure epithelial mesotheliomas tend to survive longer than those with a sarcomatoid mesothelioma (62;67).

The treatment of mesothelioma is generally ineffective. Surgery is of limited benefit for pleural mesothelioma. Radiation therapy or intracavitary instillation of radioactive substances have not been shown to be effective (38;68;69). However, combinations of surgical resection, radiation and chemotherapy have been found to prolong life in selected cases (70).

Pathologic Findings

Gross Findings

The gross appearance of mesothelioma depends on its stage at diagnosis. When diagnosed early, numerous small nodules or plaques extending over the visceral and parietal pleural surfaces characterize it. Later, confluence of the nodules results in a rind-like mass that encases and compresses the lungs. Typically, the tumor is thickest in the dependent portions of the lung and the diaphragmatic surface. At autopsy, invasion of the chest wall, mediastinum and lung, and distant metastases are common. The tumor tissue may be firm, yellowish, and leathery, particularly in the desmoplastic variant, or it may be soft and gelatinous, especially in the poorly differentiated epithelial types. In the latter, abundant foci of degeneration and necrosis are commonly seen at autopsy. The gross appearance of mesothelioma may be complicated by its intermingling with asbestos-related fibrous plaque.

Classification

Three major histologic types of diffuse malignant mesothelioma are recognized: epithelial, fibrous (sarcomatous), and mixed (biphasic), in each of these, cellular differentiation varies over a wide range. Diagnostic difficulties are presented by cases at all levels of differentiation. For example, well-differentiated epithelial mesothelioma must be distinguished from reactive mesothelial hyperplasia, poorly differentiated epithelial mesothelioma from metastatic undifferentiated carcinoma and other poorly differentiated neoplasms, and epithelial mesothelioma of intermediate degree of differentiation must be distinguished from pleural involvement by an adenocarcinoma of lung origin or from a distant metastasis from an adenocarcinoma arising elsewhere in the body. The distribution of the various histologic types varies from series to series, but usually epithelial mesothelioma predominates. Since virtually all series are composed of consultation cases, they tend to underestimate the percentage of biphasic cases, because these are easier to diagnose. I reviewed my consultation files for 100 consecutive malignant pleural mesotheliomas, all confirmed by immunohistochemistry, over the years 2000-2001. Of these 82 were of the epithelial type, seven were biphasic, and 11 were sarcomatoid, including two cases of the desmoplastic type.

Unusual variants of malignant mesothelioma include clear cell mesothelioma, a form of epithelial mesothelioma that may resemble metastatic renal cell carcinoma (71;72); lymphohistiocytoid malignant mesothelioma, a variant of sarcomatous mesothelioma that mimics malignant lymphoma or other lymphoproliferative disorders (73;74), and deciduoid malignant mesothelioma a large cell type of mesothelioma resembling exuberant ectopic decidual reaction. Although deciduoid mesothelioma is more commonly seen in the peritoneal cavity, cases have also been reported involving the pleura (75;76).

Infrequently, epithelioid hemangioendothelioma and epithelioid angiosarcoma involving the pleura may also enter the differential diagnosis (see illustrative case #6)(77-80).

Thymomas arising from ectopic thymic tissues may rarely present as pleural-based tumors, occasionally with total encasement of the lung and clinically and macroscopically mimicking malignant mesothelioma. Histologically they are indistinguishable from classical mediastinal thymomas (81;82). However, distinction from mesothelioma, particularly its lymphohistiocytoid variant, may be difficult on morphologic grounds alone, particularly with scant or fragmented biopsies.

The diagnosis of these unusual forms of mesothelioma is greatly aided by the use of immunohistochemistry.

Histologic Features and Differential Diagnosis

The epithelial type, if sufficiently differentiated, is characterized by mixed papillary and tubular patterns of growth. A variable proportion of solid growth, sometimes with a marked discohesivess of the tumor cells, may be increasingly present in the less differentiated types and is the prevailing histologic architecture in poorly differentiated epithelial mesothelioma. The tumor cells are usually cuboidal, with a bulging, dome-like apical portion, but may be flattened and rarely columnar. This is a helpful feature in its distinction from adenocarcinoma, in which the cells are often predominantly columnar. A common finding in well-differentiated epithelial mesothelioma is the presence of single or multiple, sharply delimited, clear, round cytoplasmic vacuoles. These resemble those commonly seen in localized benign epithelial mesothelioma (adenomatoid tumor) and under

electron microscopy are identifiable as intracytoplasmic lumina. Because adenocarcinomas, as well as epithelioid hemangioendotheliomas, and other neoplasms may contain intracytoplasmic lumina, this finding is of limited diagnostic value. In adequately fixed material, especially in very thin sections, from well-differentiated epithelial mesotheliomas, a brush-border–like rim corresponding to the abundant long microvilli noted by ultrastructural examination may be seen on the free portions of the cell surfaces as well as on the surface of the cytoplasmic lumina. This is a useful characteristic in distinguishing epithelial mesothelioma from adenocarcinoma, because the latter seldom has brush borders. The presence of these brush borders may be enhanced by immunostains to several marker molecules located at the cell membrane. Microcalcifications (psammoma bodies) may be seen, particularly in the tubulo-papillary forms of epithelial mesothelioma. They are of no diagnostic value.

Diffuse fibrous mesothelioma (sarcomatoid or sarcomatous mesothelioma) is made up predominantly of malignant-appearing spindle cells growing in short fascicles within a variable amount of fibrous stroma. A storiform pattern of growth and focal hemangiopericytoma-like patterns are frequent. Although not frequent, multinucleated atypical cells may be present and may give the neoplasm an appearance not unlike malignant fibrous histiocytoma. However, sarcomatous mesothelioma may also be well differentiated, the neoplastic cells having a bland cytologic appearance, resembling reactive fibroblasts. Considerable amounts of collagenous stroma may separate the neoplastic cells. Such well-differentiated sarcomatous mesotheliomas closely resemble the fibromatoses and are often designated as the desmoplastic variant of malignant fibrous mesothelioma (83;84) They differ from the fibromatoses in that their neoplastic cells consistently express cytokeratins. However, their distinction from reactive pleural fibrosis is often difficult, as will be discussed later (see illustrative cases 4 and 5). Sarcomatoid mesothelioma also may imitate hemangiopericytoma, schwannoma, and other sarcomas (85).

The mixed type, also named biphasic malignant mesothelioma, is the most easily diagnosed. As the name implies, it consists of a mixture of epithelial and sarcomatous cellular components. For this reason, it has often been compared with synovial sarcoma, with which, however, it has only a superficial resemblance. Nonetheless, synovial sarcoma may present as a pleural based lesion and mimic mesothelioma clinically and morphologically (86;87). The epithelial component of synovial sarcoma is often mucicarmine positive. Moreover, several epithelial markers are expressed by synovial sarcoma (88) Carcinosarcoma of the lung with pleural involvement, a rare occurrence, may also simulate biphasic mesothelioma. Such cases can be identified by appropriate use of immunohistochemical procedures (89).

Clearly, malignant mesothelioma exhibits a wide scope of differential diagnosis, which ranges from metastatic carcinoma of various origins to several types of sarcomas and includes benign reactive processes. Although some cases of mesothelioma are sufficiently typical to permit a relatively firm diagnosis based on routine morphologic examination, in a large proportion of cases, its diagnosis is often imprecise and prone to be controversial. It has been correctly stated that interobserver variability is greater for the diagnosis of malignant mesothelioma than for most other human neoplasms (90) Thus, numerous ancillary procedures, in particular histochemistry, electron microscopy and immunohistochemistry have been successfully employed to improve the reliability of mesothelioma diagnosis.

Adequacy of biopsy material

Decades ago, many believed that autopsy was the only way to ascertain a diagnosis of mesothelioma. Currently, with adequate biopsy, a firm diagnosis is nearly always possible. In many instances of differential diagnosis between malignant epithelial mesothelioma vs. adenocarcinoma, a needle biopsy, if representative of tumor tissue, may suffice. Indeed, with the help of immunohistochemistry, it is now possible, in many instances to diagnose malignant epithelial mesothelioma based solely on cytological preparations of pleural fluid, as will be discussed in further detail later.

However, distinguishing between reactive hyperplasia and epithelial mesothelioma, or fibrous pleuritis and desmoplastic malignant mesothelioma, requires more extensive sampling, preferably through thoracoscopy or thoracotomy. This is so, because demonstration of invasiveness is of foremost importance in this differential diagnosis.

Ancillary Diagnostic Procedures

Because no single test is currently capable of providing an unambiguous confirmation of the diagnosis of mesothelioma, such diagnosis must be based on the accumulation of evidence. Nonetheless, recent advances in immunohistochemistry have markedly improved the accuracy of mesothelioma diagnosis; to the point of rendering obsolete, many previously used tests.

Histochemistry

Mucins

Histochemical stains for mucins, once essential for the distinction between epithelial mesothelioma and adenocarcinoma are now less relevant because of their low sensitivity. Many adenocarcinomas and poorly differentiated carcinomas do not produce detectable mucins. Moreover, bonafide examples of mesothelioma have been shown to exhibit focal mucicarmine

staining, even after hyaluronidase digestion (91). I no longer rely on this test for the differential diagnosis between epithelial mesothelioma and adenocarcinoma.

Glycogen

Abundant glycogen is characteristic of mesothelioma, in which it has been described as exhibiting a fine, granular distribution versus a more coarse distribution in adenocarcinoma (90;92) However, this subtle feature is inconstant, and in my experience, of little diagnostic usefulness. Thus, along with the mucicarmine stain, the Periodic Acid Schiff stain is of little value for the distinction between mesothelioma and adenocarcinoma.

Histochemistry of Glycosaminoglycans

The histochemical demonstration of glycosaminoglycans (GAGs), and in particular, hyaluronic acid (such as Alcian Blue stains), has long been considered to be an important adjuvant in the diagnosis of mesothelioma, because mesothelial cells and their neoplasms produce large quantities of hyaluronate. However, hyaluronate leaches out of the biopsy specimen when placed in water-based fixatives causing false negative results (93) For these, and other reasons, these histochemical stains are no longer needed for the diagnosis of mesothelioma.

Quantitative Analysis of Glycosaminoglycans and Carcinoembryonic Antigen in Serous Effusions

Harington et al. introduced quantitative hyaluronate assays in serous effusions as useful for the diagnosis of mesothelioma (94) Later studies reported levels exceeding 200 mg. of hyaluronic acid per liter of fluid in mesothelioma (95-97) However, Hillerdal et al., using radioimmunoassays, reported elevated hyaluronic acid in serous effusions not due to mesothelioma (98).

Several studies have shown that levels of carcinoembryonic antigen (CEA) in effusion fluid or serum might help to distinguish adenocarcinoma from mesothelioma. Increased CEA levels were found in effusions from patients with adenocarcinoma but not with mesothelioma (99;100).

Nonetheless, these quantitative assays have failed to gain acceptance in actual clinical practice and are unlikely to do so in the future.

Electron Microscopy (EM)

Prior to recent progress in immunohistochemistry, EM was helpful to distinguish between epithelial mesothelioma and adenocarcinoma, particularly when the tumors were histologically well differentiated. The ultrastructural hallmark of epithelial mesothelioma consists in the presence of long, thin, branching “bushy” microvilli, devoid of a glycocalyx coating, over much of their free cell surface.

Warhol et al. (101) attempted to establish objective criteria for the ultrastructural diagnosis of mesothelioma and concluded that only the length/diameter ratio of the microvilli and the abundance of tonofilaments, were of diagnostic value. Adenocarcinomas have fewer, and shorter microvilli than do mesotheliomas. Furthermore, microvilli in adenocarcinomas are usually present only on the apical portion of the cell surface, whereas they tend to be present in all free cell surfaces in mesothelioma. Burns et al. (102) found a median length/diameter ratio of 11.9 in mesothelioma and 5.28 in adenocarcinoma. However, there are cases in which overlap exists, and these are responsible for a relatively wide “gray zone.” Dardick et al. (103) emphasized that poorly differentiated epithelial mesothelioma and sarcomatous mesothelioma may show no evidence at all of such long microvilli. Moreover, in my experience, practically all mesotheliomas exhibiting long, sinuous microvilli also have a characteristic immunophenotype, with expression of most of the mesothelial markers as well as thick brush-border staining with membrane-based markers.

Distinction of sarcomatous mesothelioma from true sarcomas is also simpler and more accurate by immunohistochemical means than by electron microscopy. Thus, EM is now seldom needed for the diagnosis of mesothelioma, although it remains an essential tool for the identification and quantification of asbestos fibers in lung and pleural tissue (19;104).

Immunohistochemistry

Immunohistochemistry has evolved into the most important ancillary procedure for the histopathologic diagnosis of mesothelioma. Many monoclonal antibodies (Moabs) and antisera to antigens preferentially expressed by mesothelioma or carcinoma are now available. While the value of immunohistochemistry for mesothelioma diagnosis is by now undisputed, there is still no consensus about which is the most appropriate panel of antibodies to use, and there are some lingering discrepancies on how best to interpret some of these immunostains.

Until recently, we chiefly relied on the use of antibodies binding to antigens expressed predominantly by epithelial-derived tumors and seldom by mesothelioma. The downside of this approach has been that a diagnosis of mesothelioma rested

primarily on negative results. Several antibodies of variably restricted specificity for mesothelial cells have recently become available. These new markers have significantly improved the accuracy of mesothelioma diagnosis.

It is important to emphasize that diagnostic specificity is relative, as it depends in great part on the differential diagnosis in any given case. For example, a partially mesothelial-restricted marker, such as Wilms Tumor gene product (WT-1) has a very high specificity when the differential diagnosis is between mesothelioma and adenocarcinoma of the lung in a male patient. When ovarian carcinoma enters the differential diagnosis, this marker's specificity drops dramatically. Thus, awareness of the limitations of most of the immunohistochemical markers that are discussed below is essential to properly utilize them in the most appropriate panel, given the clinical circumstances.

Cytokeratins (Keratins)

Keratins are members of a complex family of at least 20 individually gene-coded filamentous proteins that are the dominant intermediate filament of epithelial cells. Which member(s) of the keratin family is expressed depends on the type of epithelial cell. Neoplasms derived from different epithelia tend to express the same combination of cytokeratins as the parent cell. Thus, the cytokeratin immunophenotype of tumors is often used as a determinant of cell lineage.

The intermediate-filament phenotype of mesotheliomas closely resembles that of reactive and resting mesothelium. When broad-spectrum antibodies (pancytokeratins)— recognizing epitopes shared by multiple members of the keratin family—are used correctly, all mesotheliomas as well as all adenocarcinomas immunostain strongly and diffusely. Therefore, the use of broad-spectrum pancytokeratin antibodies (or cocktails) is not helpful in the context of this differential diagnosis. However, as will be discussed later in more detail, such pancytokeratin antibodies are valuable in the diagnosis of sarcomatous mesothelioma and, to identify invasiveness, particularly in desmoplastic malignant mesothelioma (see illustrative cases 3, 4 and 5).

Many monospecific Moabs to epitopes present in a restricted number of members of the keratin family are currently available. Among these, one binding an epitope shared by cytokeratins 5 and 6 is of diagnostic value for epithelial mesothelioma in paraffin-embedded tissues. Although Moabs to cytokeratins 7 and 20 may help to pinpoint the site of origin of metastatic adenocarcinoma, they are of limited value in the differential diagnosis between adenocarcinoma and epithelial mesothelioma.

Vimentin

Vimentin, the principal intermediate filament of mesenchymal cells, is now known to be expressed by a large proportion of epithelium-derived neoplasms (105) Thus, earlier publications claiming usefulness of the demonstration of vimentin for the differential diagnosis between adenocarcinoma and mesothelioma are now less valid. In fact, we found that a majority of epithelial mesotheliomas do not express vimentin, or do so minimally (106) However, vimentin, using clone V9 without antigen retrieval is a useful universal control to detect overfixation and other types of antigen damage (107).

Mesothelial Markers (positive markers)

This category comprises antibodies recognizing epitopes of molecules expressed mainly by mesothelial cells of epithelial phenotype. These antibodies should thus be regarded as markers of mesothelial lineage, rather than as mesothelioma markers. Since none are fully specific or sensitive enough, there is still need to use them within a diagnostic panel, including epithelial (carcinoma) markers. Based on the literature and our own experimental observations, the first five of the ones discussed below are recommended as the best mesothelial markers available to date.

Calretinin

Calretinin, a calcium-binding protein in the family of S-100 protein, was initially found to be present in central and peripheral neural tissues. Later it was also found in adipocytes, renal tubular cells, Leydig and Sertoli cells, eccrine glands, and mesothelial cells (108;109) As is the case of S-100 protein, it is localized in both cytoplasm and nuclei. Doglioni et al. reported calretinin to be expressed by normal mesothelial cells and mesothelioma, and rarely by adenocarcinoma (108) Recent studies have confirmed a high sensitivity of this marker for epithelial mesothelioma, while uncovering its expression by a small fraction of adenocarcinomas, (110) (111;112) Nonetheless, mesotheliomas usually immunostain for calretinin strongly and diffusely, whereas, in the rare positive adenocarcinomas staining tends to be weak and focal.

Currently there are several commercially available antisera and Moabs to calretinin. It is very important to use the correct one. In an early study we found this marker to be of insufficient sensitivity. We had used the only commercially available antibody at that time, a polyclonal antiserum raised against guinea pig calretinin (Chemicon, Temecula, CA) (106) More sensitive antisera and Moabs have since become available. Using Moab 5A5 (Novocastra, Newcastle upon Tyne, UK) in our

most recent study, comparing a large series of epithelial mesotheliomas with adenocarcinomas of various origins, we found a sensitivity of 96% and a specificity of 83% for this marker (113). Similar results have been reported using polyclonal antisera to human recombinant calretinin (Zymed, South San Francisco, CA) (114).

Cytokeratin 5

Cytokeratin 5, a member of the cytokeratin family, has a restricted distribution in normal and neoplastic tissues. Normal mesothelial cells, myoepithelium and squamous and transitional epithelium express cytokeratin 5 (115), (116), (117;118) Most epithelial mesotheliomas and nearly all squamous cell carcinomas also express this cytokeratin (119), (115), (117) Thus, in the appropriate context of differential diagnosis, it can be a helpful marker for each. On the other hand, expression of cytokeratin 5 by adenocarcinoma is infrequent. Thus, for the common differential diagnosis, mesothelioma versus adenocarcinoma, this is a very useful marker.

As expected, the pattern of immunostaining for cytokeratin 5 is cytoplasmic. If sections are thin and a high-resolution chromogen is employed, a fibrillary pattern of immunoreactivity should be visible. In most cases of mesothelioma and squamous cell carcinoma, the staining affects the majority of the tumor cells.

In our most recent study, comprising only epithelial mesotheliomas and adenocarcinomas of various origins, and excluding squamous cell carcinoma, the sensitivity for epithelial mesothelioma, using a Moab to cytokeratins 5/6 was 76%, with a specificity of 86% (113) Clearly, had we included squamous cell carcinoma, its specificity would have markedly decreased. However, this is not a serious impediment, since the differential diagnosis between mesothelioma and squamous cell carcinoma involving the pleura is rather infrequent, and is seldom difficult, clinically and morphologically. Moreover, with the use of additional immunostains the diagnosis can be readily established in doubtful cases (120;121).

Thrombomodulin (CD141)

Thrombomodulin is a transmembrane glycoprotein with anticoagulant properties, expressed preferentially by mesothelial and endothelial cells. It has also been reported to be expressed by syncytiotrophoblast, dermal keratinocytes and urothelial tumors (122-125) Collins et al. reported all of 31 mesotheliomas and only four (8%) of 48 adenocarcinomas to stain with this antibody (126) The combined literature about this marker reported positive staining in 114 of 141 mesotheliomas (80%), albeit up to 17% of adenocarcinomas also stained (127), (126), (128), (129). Our most recent study with this marker revealed a sensitivity of 64% and a specificity of 92% (113). Thus, although somewhat limited in sensitivity, if positive in a case that is otherwise negative to the epithelial markers of the panel, it strongly favors mesothelioma.

Positive immunostaining with thrombomodulin is predominantly membranous, often exhibiting the thick “brush border” pattern correlating with the presence of long and abundant microvilli. Cytoplasmic staining should not be interpreted as a positive result. Endothelial cell staining is also the rule with this marker providing a suitable internal control for adequacy of the immunostaining.

Wilms Tumor Gene Product

Wilms tumor gene product (WT-1) is a DNA-binding, hence nuclear-based protein, essential in the morphogenesis of the genitourinary tract and mesothelium. In normal adult tissues mesangial cells of the kidney, Sertoli cells of the testis, ovarian stromal cells and ovarian surface epithelium, all mesothelial cells and some stromal cells in the GYN tract express it. It is expressed by epithelial mesothelioma as well as tumors derived from the ovarian surface epithelium, desmoplastic small cell tumor, and expectedly, by Wilm’s tumor (130-132) Adenocarcinoma of the lung rarely expresses WT-1. Thus, within an appropriate context of differential diagnosis, WT-1 can be a useful marker for mesothelioma (88;133;134).

Positive WT-1 immunostains are localized chiefly in the cell nuclei. In most positive mesotheliomas, the stain involves a large proportion of the tumor cells.

In our most recent study, WT-1 had a sensitivity of 81% and a specificity of 61% for mesothelioma. However, if only adenocarcinomas of lung origin are considered, its specificity rises to 95%. On the other hand, as previously stated, this marker is of no value in discerning between epithelioid mesothelioma and serous carcinoma of ovarian or peritoneal origin (114).

Mesothelin

Initially this marker was designated as the CAK1 antigen, identified by the antibody K1 (135) The antigen is a cell surface protein, probably an intercellular adhesion molecule. Now it is identified as Mesothelin by “second generation” Moabs such as the 5B2 clone. Mesothelin is relatively specific for mesothelial cells as well as epithelial cells of the ovarian surface. However, a large subset of pancreatic carcinoma, and squamous cell carcinoma of various sites of origin have been shown to express this marker (136;137).

In our most recent study, we found mesothelin to have a sensitivity of 71% for malignant epithelial mesothelioma and a specificity of 66%. Only immunostains showing discrete membranous distribution should be interpreted as positive. A thick membranous pattern of immunoreactivity is often exhibited by epithelial mesothelioma with this marker.

Miscellaneous Mesothelial Markers

Included in this category are markers of questionable diagnostic value or that how now been made less useful by the availability of more specific mesothelial markers.

HBME-1

This Moab was raised in our laboratory using whole cells from a typical malignant epithelial mesothelioma as immunogen. When carefully interpreted it is helpful in the differential diagnosis between adenocarcinoma and mesothelioma. Only thick membrane pattern of staining, preferably involving most of the cell surfaces, should be interpreted as favoring mesothelioma. Cytoplasmic staining should be disregarded. While some authors have found this marker to be useful for the diagnosis of mesothelioma (138-140), others have not, (111;141;142). Although highly sensitive, HBME-1 is less specific than the preceding mesothelial-restricted markers.

CD44H (a.k.a. CD44S)

Attanoos et al. reported that 75% of 20 mesotheliomas showed strong membrane immunoreactivity for CD44H (the major cell-surface receptor—HCAM—for hyaluronic acid), whereas only three (15%) of 20 pulmonary adenocarcinomas showed focal immunoreactivity for this molecule (143) They advocated the use of CD44H as a positive mesothelial marker to be added to other established immunohistochemical markers to distinguish adenocarcinoma from mesothelioma. We tested, by using the same Moab (Novocastra NCL-CD44-2) under the same conditions as reported by Attanoos et al., a larger series of mesotheliomas and adenocarcinomas (unpublished observations). We found that only 21 (45%) of 46 epithelial mesotheliomas stained for CD44H. Eighteen (15%) of 117 pulmonary adenocarcinomas showed membranous immunostaining, in many cases involving most of the tumor cells. Additionally, in our study, adenocarcinomas of nonpulmonary origin often immunoreacted strongly and diffusely for CD44H. Others have reported similar findings (116;132).

Finally, in a recent, more extensive study, Attanoos et al. concluded that CD44H was of no value for the diagnosis of mesothelioma (144).

N-Cadherin

Based on studies on frozen sections, using a non-commercial Moab to N-Cadherin, Peralta-Soler et al reported strong immunostaining of all epithelial mesotheliomas and only 3 of 16 adenocarcinomas of the lung (145). They later reported similar results with formalin-fixed paraffin-embedded specimens (146) However, attempts to reproduce their results in our laboratory, using the same Moab and methodology were unsuccessful (unpublished data). We found only eight of 37 epithelial mesotheliomas to express this cell-adhesion molecule, by using the same method as reported by Han et al. Only 11 of 129 pulmonary adenocarcinomas expressed N-cadherin in our study, whereas none of 30 colonic and 30 breast cancers did stain.

Other recent studies, using different antibodies have concluded that CD44H is not useful in the differential diagnosis of mesothelioma (116;147).

Epithelial membrane antigen (EMA)

This marker, as identified by Moab E29 is expressed in nearly equal numbers by mesothelioma and adenocarcinoma. However, in the former, a thick membrane pattern of staining is more likely to be observed (148) Nonetheless, overlapping immunoreactivity limits the value of this marker (114).

Human Milk Fat Globule Antigen

This marker, as identified by Moab HMFG-2 was found to be useful in the differential diagnosis of mesothelioma versus adenocarcinoma providing that only membrane staining be interpreted as positive (149). Other studies, have found the results of HMFG-2 immunostains difficult to interpret or of no practical value (106;114;150). I no longer regard this antibody to be a useful marker.

Epithelial Markers (negative markers)

Under this designation are included several antisera and Moabs to antigens primarily expressed by carcinoma. Many of these antigens have been characterized as being high-molecular-weight glycoproteins. Until recently these markers, used as a panel, were the main tools available for distinguishing mesothelioma from adenocarcinoma (106). Best among this group are (a) Moabs and antisera to CEA, (b) the Moab designated as Ber-EP4, (c) a Moab designated BG8, (a blood group–related

glycoprotein) and a Moab named MOC-31. Moabs Leu-M1 (CD15) and B72.3 have also been found useful as members of a secondary panel.

Successful application of these antibodies depends, in great part, on their careful titration with a panel of known mesotheliomas and adenocarcinomas to select the titer that stains the lowest number of mesotheliomas. For optimal results, certain rules of interpretation must be observed, as discussed below. It also is important to keep in mind that many early publications using these antibodies antedated the use of heat-induced epitope retrieval (HIER), and are less valid today because the sensitivity of some of these antibodies has been greatly enhanced by HIER, in most instances with no appreciable change in specificity (106).

Carcinoembryonic Antigen (CEA)

This oncofetal antigen has been found by many investigators to be expressed with variable frequency by adenocarcinoma and rarely by mesothelioma (149;151-154) The incidence of CEA immunostaining in adenocarcinoma varies from series to series, but in most reports, about 70% of the carcinomas are CEA positive. The site of origin of the adenocarcinomas is important in their expression of CEA. Gastrointestinal or lung-derived neoplasms tend to be positive more frequently than are adenocarcinomas of other origins. The staining pattern is usually intracytoplasmic. In cases with high expression, some peritumoral stromal staining is often seen.

We found CEA to be positive in 175 (83%) of 211 adenocarcinomas of various origins and in none of 57 epithelial mesotheliomas by using Moab clone CEJ065 and HIER (106) Results with polyclonal antisera are similar although a small number of mesotheliomas did stain (113).

MOC-31

MOC-31 is a Moab binding a glycoprotein of unknown function present in the cell membrane of epithelial cells. Ruitenbeek et al reported in 1994 that 98% of adenocarcinomas and none of five mesotheliomas immunoreacted with MOC-31 (155). Ordóñez, in a more extensive study found 100% of 40 lung adenocarcinomas and 82% of non-pulmonary adenocarcinomas to immunoreact strongly and diffusely with MOC-31. Only 2 of 38 epithelial mesotheliomas stained, but focally and weakly (114;156).

Our most recent re-evaluation of these markers confirms the usefulness of MOC-31 in the differential diagnosis between epithelial mesothelioma and adenocarcinoma (157).

BG-8

This Moab has been found to bind to the blood-group antigen Lewis^{y} Jordan et al. reported strong, diffuse, and homogeneous staining in 18 primary lung adenocarcinomas with this antibody, whereas mesotheliomas did not stain or did so in less than 10% of the cells (158). We expanded these studies by including a larger series of adenocarcinomas and mesotheliomas with HIER pretreatment (106) Our results largely confirmed those of Jordan et al. When a 10% cut-off was used, none of 57 epithelial mesotheliomas was considered positive, whereas 114 of 123 pulmonary adenocarcinomas were unambiguously BG-8 positive.

Several points are important to keep in mind in the interpretation of BG-8 immunostains. As already stated, a semiquantitative approach is necessary because, in a small number of mesotheliomas, fewer than 10% of the tumor cells may (usually weakly) stain. On the other hand, the staining of adenocarcinomas, particularly those of lung origin, usually involves from 60% to 100% of the tumor cells and is commonly intense The sensitivity of the antibody is markedly enhanced by the use of HIER. Staining with this antibody is diffusely and homogeneously cytoplasmic, with membrane accentuation. As warned by Jordan et al., a coarse granular pattern of staining may occasionally be seen in mesothelioma, for unknown reasons, and should not be misinterpreted as true antigen expression (158). In our cases, using HIER, such spurious staining was rarely observed (106).

BG-8 is a useful member of the mesothelioma panel, capable (when used together with CEA and Ber-EP4 or MOC-31) of identifying most adenocarcinomas (113).

Ber-Ep4

This Moab recognizes an epitope present in two glyproteins present in most epithelial cells but not in mesothelial cells. Latza found immunoreactivity with this antibody in 99% of 144 epithelial tumors of various origins whereas none of 14 mesotheliomas did stain (159) Sheibani, a year later, reported Ber-Ep4 immunoreactivity in 87 adenocarcinomas of various sites of origin and in only one of 115 mesotheliomas (160). Later, however, Gaffey et al. reported immunoreactivity for this marker in 10 of 48 mesotheliomas (161). Ordóñez reported up to 26% of 70 mesotheliomas immunostaining for Ber-Ep4, albeit focally and

affecting only a small proportion of cells, whereas in lung adenocarcinoma the staining was invariably diffuse and positive in 100% of the cases (162). Thus, a cut-off level could be reasonably applied to Ber-Ep4, as is the case with BG-8.

Moreover, I believe that differences in the interpretation of the immunostains accounts for much of the discrepancy in the literature about this marker. Latza emphasized the predominant basolateral immunoreactivity when using this Moab. If predominantly apical membrane staining is found, it should not be interpreted as positive. In my experience, most mesotheliomas show entirely negative stains with Ber-Ep4 and those that do stain show a very small percentage of cells staining, usually weakly and without the basolateral accentuation shown by adenocarcinoma. Thus, when a quantitative approach, plus careful observation of the pattern of staining is taken into consideration, Ber-Ep4 is a useful diagnostic marker, particularly to distinguish adenocarcinoma of the lung from mesothelioma.

Thyroid transcription factor-1 (TTF-1)

TTF-1 is a tissue-specific nuclear transcription protein important in the morphogenesis of the lung and thyroid gland. Recent immunohistochemical studies have shown that it is often expressed by adenocarcinoma and neuroendocrine carcinoma of the lung. Conversely, TTF-1 is not expressed by mesothelioma, squamous cell and large cell carcinoma of the lung or adenocarcinoma on non-lung origin, with the exception of those arising in the thyroid gland. (132;163-166).

Consequently, TTF-1 can be helpful in the distinction between primary adenocarcinoma of the lung and pleural mesothelioma. Only nuclear stains should be interpreted as positive. Usually staining involves a majority of nuclei. Because normal pneumocytes express TTF-1, they provide adequate internal control in biopsies including lung tissue.

Leu-M1 (CD15)

Sheibani et al. reported that the antigen detected by the Moab Leu-M1, a myelomonocytic marker was also detectable in a large percentage of non-hematopoietic epithelial neoplasms (167) Of a group of 179 adenocarcinomas of various origins, 119 (58%) were found to immunoreact with Moab Leu-M1, whereas none of 18 mesotheliomas did stain. Later the same investigators expanded the study and compared the expression of CD15 in 50 pulmonary adenocarcinomas and in 28 pleural mesotheliomas (168) Focal cytoplasmic staining was found in 47 (94%) of the adenocarcinomas and in none of the mesotheliomas. Subsequent studies showed that addition of HIER did not significantly affect these results (106).

It must be kept in perspective that the expression of CD15 is often quite focal and that, if the sample size is small, the number of false-negative results is expected to increase. It also is important to emphasize that areas of necrosis should be avoided in the interpretation of this stain because degenerating leukocytes in and around these foci may lead to false-positive readings. If the biopsy is small or necrosis is abundant, it is best to avoid using this marker.

B72.3

Moab B72.3 was generated by using a membrane-enriched fraction of a human breast carcinoma (169) It was found to stain a large proportion of adenocarcinomas of non-breast origin and no mesotheliomas (170) The pattern of staining of adenocarcinomas is membranous and cytoplasmic, often focally distributed. In our post-HIER reexamination of this marker, we found that 80.5% of the adenocarcinomas were positive, whereas only two (3.5%) of 57 mesotheliomas did stain (139) Omission of HIER greatly decreased the number of B72.3-positive adenocarcinomas without altering its specificity. Although, B72.3 may be useful as an epithelial marker in the mesothelioma vs. adenocarcinoma panel CEA, Bg8, MOC-31 and BerEp4 have largely superseded it.

The mesothelioma diagnostic immunohistochemistry panel

Most cases of differential diagnosis between epithelial mesothelioma and adenocarcinoma can be correctly diagnosed with a six-antibody panel (three mesothelial and three epithelial) Calretinin, Cytokeratin 5/6, WT-1 and CEA, MOC-31 and Bg8. It is possible that smaller (4 antibody panels) may be sufficient, particularly when dealing with well, or moderately differentiated tumors (114) In fact, data from logic regression statistical analysis of our most recent evaluation of these markers shows that three antibodies may be sufficient for this purpose in many instances. For example, lack of immunoreactivity with Bg8 and positive calretinin or lack of MOC-31 expression established the diagnosis with a perfect specificity and a 96% sensitivity (157).

Nonetheless, a practical approach would be to use three mesothelial and three epithelial markers for all cases suspected of being an epithelial mesothelioma. Only a few poorly differentiated tumors will be found to need expansion of this six-antibody panel.

Additionally, the panel should be modified if the differential diagnosis includes carcinoma of ovarian origin or squamous cell carcinoma, as previously discussed. Moreover, if other possibilities beyond adenocarcinoma enter the differential diagnosis additional markers may become necessary, usually as a secondary panel. For example, markers of vascular lineage such as CD31 and vonWillebrand factor should be included if epithelioid hemangioendothelioma is suspected (see illustrative case 6). The p53-related gene, p63 should be added if the histological features suggest squamous cell carcinoma (120).

Cytologic Diagnosis of Mesothelioma

It is generally agreed that the cytologic diagnosis of malignant mesothelioma is difficult. Furthermore, the distinction between malignant mesothelial cells, adenocarcinoma cells, and atypical irritated mesothelial cells is a common and particularly challenging occurrence in the practice of cytology. Naylor, who studied seven cases of malignant mesothelioma, concluded that cytologic examination could only suggest the diagnosis of mesothelioma (171) According to Naylor, two important criteria needed to be met for the cytologic diagnosis of malignant mesothelioma. First, the cells have to exhibit the usual cytological characteristics of malignancy, and second, they had to display features typical of mesothelial cells. Cytologic features supporting the diagnosis of mesothelioma are the knobby outline of cell clusters (morula formations), cytoplasmic vacuolation, binucleation, and a fuzzy cell surface due to the presence of abundant long microvilli, best seen with immunostaining for Thrombomodulin, Mesothelin or HBME-1.

The use of immunohistochemical studies in cell blocks of serous effusions has greatly facilitated the differential diagnosis between epithelial mesothelioma and adenocarcinoma, provided that Naylor's first criterion is met since reactive atypical mesothelial cells will be positive to most of the mesothelial-restricted markers. Thus, if the cytological features are unquestionably malignant, and mesothelial markers are positive and epithelial markers are not, a cytological diagnosis of malignant mesothelioma is possible. However, even if there is uncertainty as to whether suspicious cells are malignant or not, immunohistochemistry may still be useful. If the mesothelial markers are negative, and one or more of the epithelial markers are positive in the suspicious cells, the results would strongly favor adenocarcinoma over atypical mesothelial hyperplasia.

These criteria, it should be emphasized, apply only to epithelial and biphasic forms of mesothelioma. Sarcomatous and desmoplastic mesothelioma shed infrequently into serous effusions and seldom can be diagnosed by cytological means.

Flow Cytometry

Determination of DNA aneuploidy on serous effusions may serve as a marker for malignant cells. However, its usefulness to distinguish mesothelioma from carcinoma is controversial. El-Naggar et al. compared the DNA flow-cytometric characteristics of 23 epithelial mesotheliomas and 41 pulmonary adenocarcinomas from paraffin-embedded blocks (172) They reported that 78% of the mesotheliomas were diploid versus a statistically significant 88% aneuploidy rate for the adenocarcinomas. Additionally, these authors found a significantly higher proliferative rate in adenocarcinoma than in mesothelioma. Similar findings were reported by Esteban and Sheibani (173). Thus, it would appear that the finding of a diploid tumor by flow cytometry favors a diagnosis of mesothelioma over adenocarcinoma. Pyrhönen et al., by using fresh-frozen tumor samples, reported 16 (52%) of 31 mesotheliomas as diploid and found that the ploidy status was of no prognostic value in mesothelioma (174). Similar lack of prognostic value was reported by Dazzi et al.(175). Frierson et al. studied effusion fluids and compared them with paraffin-embedded samples (176) They found a 53% rate of aneuploidy in mesothelioma and concluded that aneuploidy in an effusion specimen containing atypical mesothelial cells would strongly support a diagnosis of mesothelioma. It thus would appear that, in some circumstances, flow-cytometric DNA analysis could be diagnostically useful.

Antibodies useful for the differential diagnosis between adenocarcinoma and epithelial mesothelioma

Antibody	Specificity	Clone	Source	Dilution	Pretreatment
Calretinin	Mesothelial	5A5	Novocastra	1:50	8 min. pressure cooker citrate buffer pH 6.0
Cytokeratin 5/6	Mesothelial	D5/16B4	Chemicon	1:2000	Steam 20 min. in citrate + 10 min. in pronase
Mesothelin	Mesothelial	5B2	Novocastra	1:25	8 min. pressure cooker citrate buffer pH 6.0
WT-1	Mesothelial	Polyclonal	Santa Cruz	1:1000	8 min. pressure cooker citrate buffer pH 6.0
Thrombomodulin	Mesothelial	1009	Novocastra	1:250	Steam 20 min. in citrate + 10 min. in pronase

HBME-1	Mesothelial	HBME-1	DAKO	1:250	8 min. pressure cooker citrate buffer pH 6.0
CEA	Epithelial	Polyclonal	DAKO	1:4000	Steam 20 min. in citrate buffer pH 6.0
Bg8	Epithelial	F3	Signet	1:250	Protease XXIV x 5 min.
MOC-31	Epithelial	MOC-31	DAKO	1:50	Protease XXIV x 5 min.
BER-EP4	Epithelial	Ber-Ep4	DAKO	1:250	Protease XXIV x 5 min.
CD15	Epithelial	MMA	B.D.	1:50	Steam 20 min. in citrate buffer pH 6.0

Chemicon, Chemicon International Inc., Temecula, CA; DAKO, DAKO Corporation, Carpenteria, CA; Santa Cruz, Santa Cruz Biotechnology, Santa Cruz, CA; Novocastra, Novocastra Laboratories, Newcastle upon Tyne, UK; Signet, Signet Laboratories, Dedham, MA; B.D., Becton Dickinson, San Jose, CA;

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