Histopathology

- **Chapter 9:** Approach to the Serosal Biopsy
- **Chapter 10:** Morphologic Variants and Their Mimics
- **Chapter 11:** Mucin Histochemistry and Electron Microscopy
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- **Chapter 13:** Atypical Mesothelial Proliferations
A serosal biopsy may be deemed adequate when a good sample of mesothelium is present. This is more likely to be the case when open, laparoscopic, thoracoscopic, or radiologically guided biopsies are performed. The resting mesothelium is usually an attenuated layer of cuboidal cells (Fig. 9-1). A significant proportion of blind needle biopsies of the pleura contain muscle and fat without any mesothelium, and these should be reported as unsatisfactory. Generally, as with all branches of histopathology, the larger and more targeted the biopsy, the more likely one is to establish a correct and definitive diagnosis. In one comparative analysis of varied antemortem biopsy techniques in 45 subsequent autopsy-proven cases of diffuse malignant mesothelioma, needle biopsies were positive in only 16%, whereas open biopsies were positive in 95% to 100%.

In another series of 188 patients, needle biopsies provided a positive diagnostic yield in only 21%, whereas larger, thoracoscopic specimens gave a rate of 98%. Biopsy size governs diagnostic yield and is itself dependent on underlying biopsy method and user technique and experience.

The most common artifacts observed in attempted pleural biopsies relate to crush effect or misappropriated tissue. The observation of a compressed eosinophilic membrane, derived from the skin epidermis, juxtaposed with underlying spindle cells may be misinterpreted as pleura rather than chest wall. The crushing of inflammatory cells may raise concerns of either metastatic small cell carcinoma or occult lymphoproliferative disease and prompt immunohistochemical evaluation.

After evaluating specimen adequacy, the next important consideration is to determine whether the biopsy shows definite malignancy or a reactive epithelioid, spindle cell proliferation or has mixed biphasic elements present (Fig. 9-2; Tables 9-1 to 9-3).

**EPITHELIOID CELL PROLIFERATIONS**

If a cellular epithelioid proliferation exists, it is important to establish its underlying nature: whether it is mesothelial, epithelial, or of another alternate histogenesis. Cytomorphologic features are useful in experienced hands to separate reactive epithelioid mesothelial cells from histiocytes, metastatic carcinoma, and other epithelioid malignancy; however, there are significant limitations on relying on morphologic characteristics alone because reactive mesothelial cells may demonstrate significant atypia and diffuse malignant mesotheliomas may exfoliate deceptively bland tumor cells. Considerable overlap exists (Figs. 9-3 and 9-4). Extreme caution should be exercised in diagnosing diffuse malignant mesothelioma on cytologic grounds alone, as exfoliative cytologic preparations do not allow for the evaluation of frank invasion, the only absolute criterion of malignancy. Indeed, diagnosing diffuse malignant
FIGURE 9-1: Resting mesothelium showing thin attenuated surface mesothelium on omental fat connective tissue.

FIGURE 9-2: Approach to serosal biopsy interpretation.
mesothelioma on a cytologic specimen is essentially impossible and is not recommended. Additionally, sarcomatoid diffuse malignant mesotheliomas infrequently exfoliate diagnostic material, so in those cases a negative result holds no diagnostic weight. Immunohistochemistry has an important role in separating reactive from neoplastic mesothelial proliferations and determining underlying tumor histogenesis. Immunohistochemistry is reviewed in detail in Chapter 12.

The biopsy should ultimately be interpreted with knowledge of the clinical, radiologic, and direct observational findings of the sampling procedure (the thoracic and/or abdominal surgical opinion). This is most easily done through multidisciplinary meetings where the clinician, oncologist, surgeon, radiologist, and surgical pathologist are present. Diagnostic decision making will in part be impacted by knowledge of relevant concomitant medical conditions. In the pleura, for example, a patient may have had a pneumothorax,

<table>
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<th>Table 9-1 Diagnostic algorithm for epithelioid cell morphology</th>
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<td><strong>Mesothelioma</strong></td>
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| Glandular | Adenocarcinoma  
Lung  
Colon  
Kidney  
Ovary  
Prostate  
*Mesothelial hyperplasia* |
| Solid epithelioid | Carcinoma  
Melanoma  
Germ cell neoplasia  
*Nodular mesothelial hyperplasia* |
| Small cell | Carcinoma  
Lymphoma  
Askin tumor  
Desmoplastic round cell tumor |
| Clear cell | Carcinoma  
Kidney  
Lung  
Melanoma (clear cell sarcoma)  
Germ cell neoplasia  
"Pecoma" |
| Lymphohistiocytoid | Lymphoepithelial carcinoma  
Thymic epithelial tumor  
Germ cell neoplasia  
Lymphoma  
*Inflammatory process* |
| Pleomorphic | Carcinoma  
Melanoma  
Sarcoma  
Lymphoma  
Germ cell neoplasia |
which could have resulted in a florid mesothelial proliferation in association with the features of a reactive eosinophilic pleuritis (Fig. 9-5). Distinction between reactive and malignant mesothelial proliferations is frequently problematic particularly in small biopsies. It should be borne in mind that both reactive and malignant mesothelial proliferations can be present simultaneously, for example, malignant mesothelioma may occasionally present with pneumothorax. Eosinophil-rich infiltrates (seen commonly postpneumothorax) may be seen in certain drug reactions, in persons with autoimmune conditions, parasitic infestation, and certain rare hematolymphoid disorders (in particular myeloid leukemia, T-cell lymphoma, and Hodgkin lymphoma). Lymphoid-rich infiltrates may suggest tuberculosis and/or underlying lymphoproliferative disease. Formal hematopathologic referral should be initiated for accurate diagnosis, and this will be facilitated by multi-color immunofluorescence flow cytometric analysis, cytogenetic testing, as well as routine immunohistochemistry.
Patients with collagen vascular disease may develop pleural effusion and exuberant mesothelial proliferations, with histiocyte and neutrophil-rich infiltrates and necrotic debris. The observation of necrosis, in particular, is worrisome of suspected underlying malignancy, but in subjects with rheumatoid nodules, necrosis may be seen and also in those with tuberculous pleurisy.

Large areas of necrosis may preclude a diagnosis of malignancy since there may be too few viable cells for the assessment of morphology or immunophenotype. However, necrosis within substantial areas of otherwise viable mesothelial cells raises the possibility of malignancy (albeit the stated caveats with tuberculosis, collagen vascular disease, etc.). Sometimes following prolonged surgery, acute inflammatory changes are seen within the pleura; these should not be interpreted as evidence of preexisting inflammation.

In the pleura and peritoneum, reactive mesothelial cell entrapment may be seen in persons with recurrent effusions of any causation on account of the successive cycles of inflammatory stimulation, mesothelial proliferation, and reparative fibrosis. In the peritoneum, marked reactive mesothelial cell entrapment may be seen in association with liver cirrhosis.
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and ascites, endometriosis, and around omental–mesenteric decidual reactions. Florid mesothelial proliferations can occur in hernia sacs (as so-called nodular histiocytic mesothelial hyperplasia) and in the pericardium. On occasions these reactive mesothelial proliferations may exhibit marked atypia simulating malignancy. It is important to know whether clinically a diagnosis of diffuse malignant mesothelioma is seriously considered because a pathologist should be reluctant to make such a diagnosis if the clinician thinks the condition is more likely to be benign. Occasionally a pleural biopsy will be sectioned parallel to the pleural surface, which may give a mistaken impression of the mesothelial cells forming a solid tumor.

Diffuse malignant mesothelioma may involve lung and with intra-alveolar growth mimicking organizing pneumonia and desquamative interstitial pneumonia. The morphologic features, which should be taken into account with respect to assessment of malignancy, include (1) extent and complexity of cellular proliferation, (2) cytologic atypia, (3) zonation, (4) presence and severity of inflammation, and (5) invasion.

Reactive mesothelial proliferations usually occur in parallel layers and sheets, whereas the presence of complex papillary structures in pleural biopsies points toward malignancy. Cytologic pleomorphism, mitoses, and inflammation are unreliable determinants of benign versus neoplastic mesothelial proliferation. Indeed, if there is severe inflammation evident, then associated mesothelial proliferations need to be interpreted with great caution. Diffuse malignant mesothelioma may be complicated by empyema, and the overlying inflammation may mask the presence of diffuse malignant mesothelioma. Unequivocal invasion of structures such as fat, chest wall muscle, or lung parenchyma allows for a confident diagnosis of malignancy to be made.

In serosal biopsies full-thickness mesothelial proliferations or stromal nodules are nearly always malignant, whereas mesothelial proliferations in the superficial cavity–associated regions may be benign or malignant (Fig. 9-6). Stromal invasion can be mimicked by entrapment of mesothelial cells within fibrous tissue in inflammatory processes, but these often have a linear arrangement, which parallels the surface. One needs to be aware that artifactual displacement of mesothelium in closed needle biopsies can occur.

Mesothelial cell inclusions in lymph nodes may mimic metastatic diffuse malignant mesothelioma but must be interpreted with caution. They are usually limited to the subcapsular sinus or show sinusoidal distribution without lymph node effacement. Immunohistochemistry is important in defining their benign nature.
ALGORITHMIC APPROACH FOR EPITHELIOID TUMORS OF THE SEROSA

Distinguishing epithelioid diffuse malignant mesothelioma from various carcinomas and other epithelioid malignancies is facilitated by necessary ancillary testing. Epithelioid malignancies of the serosal tissues are generally either metastatic carcinomas or diffuse malignant mesothelioma. Both are cytokeratin positive. If a diffuse malignant mesothelioma has epithelioid morphology, it should be positive for both broad-spectrum cytokeratins and at least one of the commonly positive mesothelial markers (calretinin, cytokeratin 5/6, WT-1, D2-40). Accordingly, if a serosal-based epithelioid malignancy is negative particularly for varied tested cytokeratins, then diagnoses other than diffuse malignant mesothelioma or carcinoma should be seriously considered. These include malignant melanoma, malignant vascular sarcomas (low-grade epithelioid hemangioendothelioma or high-grade epithelioid angiosarcoma), primitive neuroectodermal tumor (Askin tumor), or some non-Hodgkin lymphomas (notably anaplastic large cell lymphoma) (Table 9-1).

Diffuse malignant mesothelioma may be subject to different morphologic patterns and subtypes in an individual tumor. For this reason there is merit in sampling the tumor widely, particularly when sarcomatoid elements of the tumor predominate (which do not readily allow for definitive diagnosis) and resection or necropsy material is available. It is recognized that the epithelioid component of diffuse malignant mesothelioma is more readily identifiable as such by immunohistochemical markers and other ancillary studies. For this reason, a careful search for epithelioid components is worthwhile, even if they represent a minority component of the tumor.

Searching for epithelioid elements in diffuse malignant mesothelioma may be problematic, but there should be focus on seeking out lymphatic tumor emboli as these often exhibit epithelioid morphology. Metastatic tumor in involved lymph nodes may provide suitable diagnostic tissue to run an immunohistochemical panel, alongside the main tumor. Extreme caution should be exercised in interpreting immunomarkers in areas of necrosis and where pleural diffuse malignant mesothelioma infiltrates lung parenchyma as there will inevitably be cell entrapment.
The morphologic variant forms of diffuse malignant mesothelioma do not have significant prognostic importance (except for the adverse prognosis of desmoplastic diffuse malignant mesothelioma). Instead, they provide the pathologist with an appreciation that diffuse malignant mesothelioma may generate a vast differential of histogenetically diverse tumors, most unrelated to asbestos.

The utility of these immunohistochemical panels is governed by tumor morphology, patient gender, and anatomic site of the neoplasm. In particular, in the female peritoneum, diffuse malignant mesothelioma should be distinguished from primary and secondary Mullerian neoplasms (predominantly serous carcinoma). This is not straightforward because of the common coelomic origin of the tumors and resultant similar morphologic and immunophenotypic characteristics, and it necessitates using a series of immunohistochemical markers to facilitate any suspected diagnosis of diffuse malignant mesothelioma. Incorporating a broad panel of immunomarkers is more reliable for accurate diagnosis than reliance on the morphologic tumor appearances alone. It is recommended by the International Mesothelioma Interest Group to incorporate at least two positive mesothelial markers and two positive epithelial markers together with a broad-spectrum cytokeratin.7

Because of the commercial availability of antibodies, the individual choice of antibody will depend on personal preference and laboratory experience. Some markers have lower specificity and sensitivity than others, and the pathologist should always be mindful of potential false-positive and false-negative rates. The role of immunohistochemistry and molecular cytogenetic testing in the diagnosis of diffuse malignant mesothelioma and its mimics is detailed in other chapters.

ALGORITHMIC APPROACH FOR BIPHASIC AND SARCOMATOID TUMORS OF THE SEROSA

For serosal biphasic and sarcomatoid tumors, the differential diagnoses generally lie between diffuse malignant mesothelioma, sarcomatoid carcinoma, and sarcoma. Reactive conditions may mimic each morphologic type, most notably chronic fibrous pleuritis mimicking desmoplastic diffuse malignant mesothelioma. In certain anatomic sites and genders, specific tumors should be considered at an early stage. For example, in the female peritoneum, malignant mixed mullerian tumor (carcinosarcoma) and malignant teratomatous germ cell neoplasms are far more common than in the counterpart pleural site. Conversely biphasic and sarcomatoid diffuse malignant mesothelioma in the peritoneum is rare. Immunohistochemistry and molecular cytogenetic testing is potentially useful in evaluating these cases and discussed in other chapters (Tables 9-2 and 9-3).

THE UTILITY OF THE ASBESTOS EXPOSURE HISTORY IN EVALUATING SEROSAL BIOPSIES FOR SUSPECTED DIFFUSE MALIGNANT MESOTHELIOMA

Most diffuse malignant mesotheliomas are associated with prior asbestos exposure, and the strength of the association varies with anatomical tumor site, gender, and asbestos fiber type and industry.8,9 Epidemiologic and mineralogic studies show that amphiboles cause the vast majority of diffuse malignant mesothelioma in men. Some authorities believe that
virtually all diffuse malignant mesotheliomas are asbestos related.\textsuperscript{10} Many experts believe that spontaneous or idiopathic mesotheliomas do occur, and highlight non–asbestos-related risk factors for the disease (such as environmental erionite exposure, radiation, and simian virus 40 inoculation).\textsuperscript{11–13}

The background lifetime probability of pleural and peritoneal diffuse malignant mesothelioma combined is estimated to be 3 to 4 per 10,000 individuals.\textsuperscript{9,14} For peritoneal diffuse malignant mesothelioma, the epidemiologic evidence correlating time trends, incidence in both genders, and asbestos exposure suggests that most peritoneal diffuse malignant mesotheliomas are potentially unrelated to asbestos.

Asbestos is undoubtedly associated with a lower percentage of peritoneal diffuse malignant mesotheliomas when compared to pleural counterpart tumors. Historically, peritoneal diffuse malignant mesotheliomas are typically associated with higher cumulative asbestos exposures and exposures to commercial amphiboles. There exists no robust epidemiologic link between peritoneal diffuse malignant mesothelioma and chrysotile exposure.

Because of the association between asbestos and diffuse malignant mesothelioma, it is not uncommon for pathologists to regard the subjects’ asbestos exposure history as a criterion in ascertaining the diagnosis of diffuse malignant mesothelioma. It cannot be overstated that this is incorrect and flawed approach. The pathologic diagnosis of the tumor is based on the combination of the morphologic findings plus ancillary information such as mucin studies, immunohistochemistry, electron microscopy, and molecular analyses. In the presence of adequate pathologic tissue, the asbestos exposure history is irrelevant and should absolutely not be used to overturn an alternate diagnosis.

Only in the absence of diagnostic pathologic tissue can the asbestos exposure history be considered as a factor in the clinical diagnostic decision-making process. If a patient has a history of asbestos exposure and has imaging information, which demonstrates a diffuse pleural based tumor, then because diffuse malignant mesothelioma is the most common tumor to show this imaging feature, it becomes the “default” clinical diagnosis. This may prompt the physician to request a tissue diagnosis. The gold standard for the diagnosis of diffuse malignant mesothelioma is the tissue biopsy result. Cytologic specimens are limited and do not allow for a confident diagnosis. Accordingly, considerable caution should be exercised by the surgical pathologist; a misinterpreted case has far reaching medical and legal consequences.

In the peritoneum and other serosal sites, the approach is different because diffuse malignant mesothelioma is far less common than disseminated carcinomatosis (from the gastrointestinal tract, pancreas, or ovaries) and other tumors. For this reason, diffuse malignant mesothelioma is not the “default” diagnosis, and further investigations, preferably biopsy diagnosis, are necessary.

References