1 SPECIMEN EVALUATION

The overall framework for the evaluation of a soft tissue specimen includes the use of clinical information, radiologic findings, and adjunct tests for tumor diagnosis (especially immunohistochemistry and molecular genetics). Discussed as well are the principles of tumor grading and reporting for a sarcoma specimen.

SPECIMEN TYPE

Needle core biopsy is a standard mode of preoperative evaluation of a deep mass since blind surgery for an unknown mass generally is not favored. The diagnosis is often possible based on a representative core (1). Tumors in which diagnostic findings are focal, however, such as atypia in atypical lipomatous tumor/well-differentiated liposarcoma, may be impossible to diagnose with this modality, and such a diagnosis can never be ruled out with a negative biopsy. Also, focal specific differentiation may be absent in a needle core specimen, and may result in a less specific diagnosis. Tumor grading is frequently difficult, as the criteria based on grading may not be observed in a small specimen. Although the presence of high-grade features allows for accurate grading, the lack of these features in a small sample does not necessarily mean a low grade; therefore, needle core biopsies can only give a minimum grade.

Fine needle aspiration biopsy as the first line of diagnosis is only feasible in centers with special expertise on the interpretation of such specimens. In general practice, it may help confirm recurrent or metastatic tumors (2,3). A special chapter in the end of this Fascicle discusses the fine needle aspiration of soft tissue tumors.

Incisional biopsy offers more abundant diagnostic material and also may more easily allow for partition of the specimen for special studies, such as molecular genetics. A definitive resection specimen allows for a complete view of the tumor, although post-treatment (chemotherapy, radiation) specimens can include alterations that preclude tumor grading or even histogenetic typing. In general, the pathologist is expected to provide assessment of margin status, so that these specimens have to be inked for accurate evaluation of margins. Close margins are best assessed in sections perpendicular to the tumor surface. Although the size of the sample remains arbitrary, 1 section/cm of maximum tumor diameter is a general guideline.

CLINICAL HISTORY

The clinical history can be a very useful aid in the specific diagnosis. The patient age and sex, the lesion site (including tissue plane), and clinical tumor size are the minimum requirements and may allow the formulation of the main diagnostic options. Additional factors include a history of previous tumors or radiation treatment, and the possible presence of a tumor syndrome, such as neurofibromatosis.

RADIOLOGIC CORRELATION

Radiologic studies provide information on tumor location, size, and tissue content. These factors are useful for a specific diagnosis, and such information can enhance the value of a small biopsy.

Examples of radiologic contributions to specific diagnoses include the use of radiographs and computerized tomography (CT) scans to observe tumor calcification for the diagnosis of synovial sarcoma and myositis ossificans, and magnetic resonance imaging (MRI) to determine the presence of a myxoid character and fatty components for the diagnosis of liposarcoma, especially the dedifferentiated variant (4).

SPECIAL STUDIES

Today, immunohistochemical tests are the most important special studies. These studies have largely replaced electron microscopy as a tool for tumor typing. Histochemical stains are still useful in certain cases.

Examples of the application of histochemical stains are the demonstration of glycogen

(periodic acid–Schiff [PAS] with diastase) in Ewing sarcoma, diastase-resistant PAS-positive crystals in alveolar soft part sarcoma, and elastic fibers in elastofibroma. Elastin stains may assess arterial structures adjacent to tumors (elastic laminae).

Immunohistochemistry

A variety of cell type-specific antigens are used as immunohistochemical markers. They include cytoskeletal proteins, membrane receptors and other membrane antigens, and cytoplasmic proteins. Extracellular matrix proteins, such as collagen type IV and laminin, are sometimes useful for demonstrating cell types with prominent basement membranes, such as Schwann cells. Nuclear transcription factors (microphthalmia, Prox1) are part of a growing number of newer cell type markers (5). Vimentin, a general "mesenchymal" marker, has limited use because of its widespread expression and lack of cell type specificity.

A basic panel for the diagnosis of soft tissue tumors includes CD34, desmin, epithelial membrane antigen (EMA), keratin cocktail (AE1/AE3), smooth muscle actin, and S-100 protein. This panel should be expanded to cover the differential diagnostic possibilities. These are presented in detail in the following chapters with each entity, and only a summary of the most commonly used markers is presented here.

CD34 is expressed in many fibroblastic tumors, as well as in subsets of vascular endothelial cell tumors. Positive spindle tumors include dermatofibrosarcoma protuberans, solitary fibrous tumor, Kaposi sarcoma, spindle cell lipoma, well-differentiated and dedifferentiated liposarcomas, and variably, myxofibrosarcomas.

Desmin is usually detected in benign and malignant smooth muscle tumors and rhabdomyosarcoma. In addition, it is expressed in other tumors, for example, aggressive angiomyxoma, angiomatoid fibrous histiocytoma, and tenosynovial giant cell tumor (focally). Sarcomas positive for desmin, especially when negative for smooth muscle actin, should be tested for myogenic determination factors (MyoD1, myogenin), as these are expressed in rhabdomyosarcoma (as well as in any malignant tumor with heterologous skeletal muscle differentiation).

EMA is useful in assessing epithelial and perineurial cell tumors.

Keratin cocktail AE1/AE3 typically detects positive cells in synovial sarcoma, epithelioid sarcoma, mixed tumor/myoepithelioma, carcinomas, and mesothelioma. Although many unrelated tumors with no true epithelial differentiation may contain positive cells, this is usually only a focal finding. Such tumors include smooth muscle tumors (benign and malignant) and pleomorphic undifferentiated sarcoma/myxofibrosarcoma.

S-100 protein is typically strongly expressed in benign nerve sheath tumors and metastatic melanoma, and is very useful in differentiating between these and other entities. Nevertheless, some unrelated tumors can be moderately or even extensively positive, including synovial sarcoma. Nearly every tumor contains a variable number of S-100 protein–positive dendritic antigen-presenting cells. While these cells should not be considered as positive staining of the tumor, the presence of dendritic antigen-presenting cells constitutes a useful internal positive control verifying valid staining.

Alpha-smooth muscle actin (α-SMA) is expressed in both smooth muscle and myofibroblasts, and by virtue of the latter, many nonmyoid tumors contain positive cells, for example, myxofibrosarcoma and undifferentiated sarcoma. SMA positivity does not constitute evidence of specific smooth muscle differentiation in the setting of undifferentiated sarcoma.

Endothelial cell differentiation to detect angiosarcoma is usually successful with CD31, but histiocytes, plasma cells, and platelets (thrombi) are also positive. On the other hand, CD34 is expressed in only 50 percent of angiosarcomas. ERG (ETS-related gene) transcription factor, a new marker that is fairly restricted to endothelia and is conserved in malignant endothelia, is a promising new marker for angiosarcoma.

Proliferation markers (Ki67 and analogs) are useful, in some instances, to determine the proliferating fraction. The differential diagnostic criteria between benign and malignant tumors are not sufficiently developed, however, for a straightforward routine application (6,7).

Electron Microscopy

Today electron microscopy has limited use in tumor typing. The specific applications include detection of smooth muscle differentiation in

leiomyosarcoma, skeletal muscle differentiation in rhabdomyosarcoma, prominent cell processes and layers of basement membranes in schwannoma, crystals with lattice-like structure with 100-angstrom periodicity in alveolar soft part sarcoma, and tall microvilli in mesothelioma (8,9). Some advocate electron microscopy in the diagnosis of small round cell tumors (10). In general, this method is unsuitable for the typing of undifferentiated and poorly differentiated tumors.

GENETICS

Cytogenetic evaluation requires unfixed tumor tissue. This modality not only gives specific diagnostic information but also enhances the scientific knowledge of tumor genetics. Most tumor translocations have been initially observed cytogenetically. Other characteristics of certain tumor types include losses or gains of chromosomal segments and the presence of abnormal chromosomes, such as ring chromosomes. The specimen should be submitted to the laboratory either as fresh tissue (if the laboratory is not distant), or immersed in a tissue culture medium for shipment to an outside facility (11).

Tumor-specific translocations and gene amplifications are observed with fluorescence in situ hybridization (FISH) techniques using specific probes, many of which detect the most common sarcoma translocations. Specific applications include the use of double-color break-apart probes for diagnosis of *SS18* gene rearrangements in synovial sarcoma translocation, *FOXO1* split rearrangement in alveolar rhabdomyosarcoma, and *ALK* gene rearrangement in inflammatory myofibroblastic tumor (12–14). These are discussed in detail with each entity in the following chapters.

EWSR1 (Ewing sarcoma) gene rearrangement is useful in the diagnosis of Ewing sarcoma and also other sarcomas that feature *EWSR1* gene translocations. Obviously this rearrangement is not specific for Ewing sarcoma, and additional probes for fusion partners are necessary for FISH studies.

The test for *MDM2* gene amplification can help diagnose atypical lipomatous tumor and well-differentiated and dedifferentiated liposarcoma. Chromogenic techniques (CISH) are potentially also useful, but they are more difficult to interpret and not generally recommended with the yet available reagents.

The new understanding of tumor genetics is also a rich source for the discovery for potential specific therapeutic targets (15).

RECOGNIZING NON-SOFT TISSUE TUMORS

The differential diagnosis of soft tissue tumors is not limited to specific soft tissue entities, but also includes metastatic tumors such as carcinomas, malignant mesothelioma, melanoma, and even some lymphomas. These possibilities should be considered in the differential diagnosis by including pertinent immunohistochemical testing of such alternatives (keratin, epithelial membrane antigen, calretinin, S-100 protein, lymphoid markers). Especially, metastatic carcinoma, mesothelioma, and melanoma should be considered in the differential diagnosis of epithelioid soft tissue malignancies.

GRADING AND PROGNOSTICATION OF SARCOMAS

Grading applies only to sarcomas; there are no grading systems for nonmalignant soft tissue tumors. The best-documented and most practical grading system is the one developed by the French Federation of Cancer Centers (16,17). This system divides sarcomas into three numeric grades of 1, 2, and 3, corresponding to low, intermediate, and high grades. The grade assignment is based on three factors: differentiation, mitotic activity, and tumor necrosis, each of which gives 0-3 points. The summary of the points establishes the grade, as shown in Table 1-1.

The differentiation (level) is determined by tumor type, as shown in Table 1-2. Based on this table, many tumor types are assigned a fixed differentiation number. For example, all synovial sarcomas receive 3 points for differentiation. Well-differentiated liposarcomas and leiomyosarcomas receive 1 point for differentiation, while conventional (moderately differentiated) ones receive 2 points.

The mitotic rate is counted in an area of 10 standard high-power fields. Because the grading system has been developed using a microscopic field size smaller than in most current microscopes, adjustment has to be made (often 6-7 fields instead of 10).

Table 1-1

GENERAL PRINCIPLES OF TUMOR GRADING[a]

Differentiation	
1, 2, or 3 points (see Table 1-2)	
Mitotic rate per 10 high-power fields	
0-9	1 point
10-19	2 points
>19	3 points
Tumor necrosis	
None	0 points
< 50%	1 point
≥50%	2 points
Total score of point and final grade	
2-3	Low
4-5	Intermediate
6-8	High

[a]According to the updated version of the French Federation of Cancer Centers grading system. The grade is calculated as a sum of score points given by differentiation, mitotic rate, and extent of tumor necrosis.

Table 1-2

DIFFERENTIATION SCORES ASSIGNED TO VARIOUS SARCOMA TYPES [a]

Differentiation score 1
Well-differentiated fibrosarcoma, liposarcoma, or leiomyosarcoma
Differentiation score 2
Conventional fibrosarcoma
Myxoid sarcomas (MFH[b], liposarcoma, chondrosarcoma)
Storiform-pleomorphic MFH
Conventional leiomyosarcoma
Well-differentiated or conventional angiosarcoma
Conventional MPNST
Differentiation score 3
Poorly differentiated fibrosarcoma
MFH/pleomorphic undifferentiated sarcoma with a nonstoriform pattern
Round cell liposarcoma
Pleomorphic sarcomas (liposarcoma, leiomyosarcoma)
Rhabdomyosarcoma (embryonal, alveolar, pleomorphic)
Poorly differentiated and epithelioid angiosarcoma
Triton tumor, epithelioid MPNST
Extraskeletal mesenchymal chondrosarcoma
Mesenchymal chondrosarcoma
Osteosarcoma
Ewing family tumors/PNET
Synovial sarcoma
Clear cell sarcoma
Epithelioid sarcoma
Alveolar soft part sarcoma
Malignant rhabdoid tumor
Undifferentiated sarcoma

[a]According to the updated version of the French Federation of Cancer Centers grading system. Modified from reference 16.
[b]MFH = malignant fibrous histiocytoma; MPNST = malignant peripheral nerve sheath tumor; PNET = peripheral neuroectodermal tumor.

Tumor necrosis implies coagulative necrosis, generally with visible shadows of tumor cells. Hyaline change and fibrosis are not counted as necrosis. The percentage of necrosis is based on gross assessment or by microscopy, although the latter may be less accurate if sampling is biased toward viable tissue.

The clinical value of grading is best demonstrated for common spindle cell sarcomas, such as myxofibrosarcoma/pleomorphic undifferentiated sarcoma, synovial sarcoma, and leiomyosarcoma. The grading has limited impact in rare tumors that show little variation from case to case (alveolar soft part sarcoma, clear cell sarcoma, epithelioid sarcoma). Such tumors are often considered nongradable. For some tumor types, the application of grading principles has not yielded clinically significant information (for example, malignant peripheral nerve sheath tumor, angiosarcoma). Some tumors are automatically high grade (Ewing sarcoma). Extensive treatment response (necrosis, fibrosis, paucicellularity) often renders tumors nongradable (18–20).

Recently, nomograms that incorporate tumor size, depth, site, histologic type, and patient age have been developed for more accurate prognostication (21–23). Tumor type-specific nomograms have been developed for tumors such as liposarcoma and synovial sarcoma (24,25). Other prognostication systems have used combinations of parameters such as tumor size, presence of vascular invasion, and extent of tumor necrosis (the "SIN system") (26).

Another recent development is the characterization of sarcomas by genomic complexity in the CINSARC project (complexity index in sarcoma). In this study, a panel of expression patterns of 67 genes was predictive of low or high risk for metastasis, and the prediction was more accurate than based on the French Federation of Cancer Centers grading system (27).

MANAGEMENT OF SPECIMEN AND REPORTING

Obtaining the tumor specimen fresh increases diagnostic options and is recommended,

especially for larger specimens and any malignant tumors. The Association of Directors of Anatomic Pathology has published guidelines for the evaluation of soft tissue sarcoma specimens. Recommendations include reporting the type of specimen (procedure), histologic type, tumor size, closest resection margins, depth and involvement of anatomic layers, presence and quantity of tumor necrosis, and lymph node status if applicable. Additional descriptors may include mitotic rate, possible presence of vascular invasion, detailed characterization of the nature of invasion, and presence of an inflammatory component (28).

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