

Application of Immunohistochemistry to Soft Tissue Neoplasms

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• **Context.**—Soft tissue tumors are composed of numerous and complex diagnostic entities. Because of this complexity and the recognition of an intermediate malignancy category including some tumors with a deceptively bland histologic appearance, soft tissue tumors may represent a major diagnostic challenge to the general practicing pathologist.

Objective.—To correctly diagnose soft tissue tumors with the ancillary use of immunohistochemistry.

Data Sources.—Review of the current literature with emphasis on those tumors for which immunohistochemistry has proven to be particularly useful.

Conclusions.—Immunohistochemistry plays an important role in the diagnosis of soft tissue tumors. One of its

major utilities is to correctly identify a tumor as being of mesenchymal or nonmesenchymal origin. Once mesenchymal origin has been established, histologic subtyping according to specific cell lineage may be achieved with the use of lineage-specific markers. Tumors of uncertain cell lineage and tumors with primitive small round cell morphology are often characterized by a unique immunohistochemical phenotype. In this group of tumors, immunohistochemistry is most widely applied and is of greatest value. Despite the rapid development of molecular genetic techniques, immunohistochemistry still remains the most important diagnostic tool in the diagnosis of soft tissue tumors aside from recognition of morphologic features and clinical correlation.

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Soft tissue tumors (STTs) are composed of a complex group of diagnostic entities. Most STTs are of connective tissue (mesenchymal) origin. Nonmesenchymal STTs, that is, those of hematolymphoid origin, are not covered in this article.

Given the overall rarity of many of these lesions and an ever-growing list of new diagnostic entities, the general pathologist may be faced with a major diagnostic challenge when dealing with these lesions. The usual approach to soft tumor classification is by presumed cell lineage. The World Health Organization classification, for example, divides tumors into adipocytic, fibroblastic/myofibroblastic, so-called fibrohistiocytic, smooth muscle, pericytic, skeletal muscle, vascular, chondro-osseous, and lastly “of uncertain differentiation” category.¹

Immunohistochemistry (IHC) plays an important role in STT diagnosis. The first approach consists in ruling out a nonmesenchymal tumor, followed by trying to define mesenchymal cell lineage. This approach, achieved with a panel of commonly used antibodies, helps narrow down the differential to a more manageable level. In addition, there are specific tumor types requiring a more refined set of immunohistochemical antibodies. Unfortunately, there is also a substantial number of diagnostic entities in which

IHC is of limited or no use. It is important to mention that molecular genetics of STTs has developed at a rapid pace in recent years and has helped in gaining insight into pathogenesis and histogenesis. The more current textbooks including the current World Health Organization edition on tumors of soft tissue and bone reserve specific paragraphs to include the most recent cytogenetic and molecular data. However, many of these tumors have been detected in a small number of cases and confirmatory tests are not yet commercially available. Their diagnostic utility is therefore limited with the exception of several fluorescence in situ hybridization probes, which are being used with increasing frequency, in particular for the diagnosis of synovial sarcoma, Ewing/primitive neuroectodermal tumor (PNET), and alveolar rhabdomyosarcoma (ARMS). It is hoped that detection of tumor-specific alterations and validation through genetic analysis on larger samples will lead to development of new IHC antibodies. These new markers can detect tumor-specific fusion proteins that are either overexpressed or aberrantly expressed as a result of a translocation. Examples of such antibodies are ALK-1, WT-1, and FLI-1.

Given the bewildering number of STTs and likewise continuously growing list of IHC antibodies used in STT diagnosis, this article concentrates on pathologic entities as broad categories and discusses the applicability of IHC (or lack thereof) instead of providing a detailed discussion of individual antibodies. Emphasis is placed on specific tumor entities for which IHC has proven to be particularly useful.

ADIPOCYTIC TUMORS

Hematoxylin-eosin (H&E) morphology and clinical correlation are still the most helpful aids in the diagnosis of

Immunohistochemistry of Soft Tissue Neoplasms—Heim-Hall & Yohe

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Table 1. Immunoprofile of Adipocytic Tumors*

	CD34	MDM2	CDK4
Spindle cell/pleomorphic lipoma	+++	?	?
Well-differentiated liposarcoma	Spindle cells focally +; lipoblasts -	+	+
Dedifferentiated liposarcoma	+/-	+	+

* +++ indicates strongly positive in more than 95% of tumors; ?, unknown (insufficient data); +, positive in more than 70%; -, negative in more than 70%; and +/-, variable.

fatty neoplasms, whereas IHC plays a relatively minor role in their diagnosis. Normal fat cells are generally positive for S100,² but this marker may be lost in neoplastic adipocytes making it unsuitable for determining adipocytic lineage.³

Spindle Cell Lipoma/Pleomorphic Lipoma

Spindle cell and pleomorphic lipoma are 2 closely related benign entities with overlapping histologic features. The spindle cell component in these tumors stains strongly with CD34.⁴ Well-differentiated liposarcoma (WDLS) on the other hand exhibits only focal positivity with CD34 in spindle cells, whereas lipoblasts are negative. When non-adipocytic spindle cell lesions resembling spindle cell lipoma are a consideration, a negative CD34 result is helpful in ruling out spindle cell lipoma.⁵

WDLS and Dedifferentiated Liposarcoma

Some authors have recommended the use of a panel of 2 immunohistochemical stains, MDM2 and CDK4, to be used in the differential diagnosis of WDLS and dedifferentiated liposarcoma.^{6,7} In their studies, most WDLSs expressed both markers, whereas benign lipomatous lesions were generally negative for both. For increased specificity and sensitivity, the authors recommend using these 2 markers in conjunction. They also found that when WDLS dedifferentiates, the high-grade component usually retains MDM2 and CDK4 positivity.⁷ However, the specificity of this finding was limited by the fact that up to 19% of nonlipogenic sarcomas also expressed 1 or both of these 2 markers.⁶

Myxoid Liposarcoma

Myxoid liposarcoma/round cell liposarcoma is a genetically distinct subset of liposarcoma having a characteristic translocation involving the *CHOP* gene on chromosome 12. Variant translocations include t(12;16) and t(12;22) and result in production of a TLS-CHOP or EWS-CHOP fusion protein, respectively. Although the corresponding fusion genes can be detected by molecular techniques, a novel IHC antibody to the TLS/EWS-CHOP chimeric oncoproteins has shown promise in being able to detect most genetic variants of myxoid liposarcoma/round cell liposarcoma. This antibody appears to have good sensitivity and can be used on formalin-fixed paraffin-embedded tissues.⁸ Further studies will be needed to validate the utility of this antibody (Table 1).

FIBROBLASTIC/MYOFIBROBLASTIC TUMORS

Fibrous STTs are a heterogeneous group of spindle cell proliferations composed of a mixture of fibrocytes, fibroblasts, and myofibroblasts. Some of these tumors have been termed fibrohistiocytic because of a morphologic and functional resemblance of cultured tumor cells to histiocytes and positive staining for CD68. However, CD68 is not specific for histiocytes, and immunophenotypic stud-

ies with more specific markers did not confirm monocyte/macrophage derivation. In general, the group of fibrous STTs stains positively with vimentin and variably with muscle markers (smooth muscle actin [SMA], less commonly muscle-specific actin and desmin). Two distinct subsets of fibroblasts can be identified by IHC: myofibroblasts and CD34-positive fibroblasts.⁹

Myofibroblasts are present in reactive lesions and in most fibroblastic neoplasms and display a profile that is intermediate between smooth muscle and fibroblasts. As such, they have some, although less than smooth muscle, actin positivity with variable coexpression of desmin.¹⁰ The subset of CD34-positive fibroblasts is found around blood vessels, in skin adnexa, and in connective tissue septa throughout the body.¹¹

Most fibrous tumors are distinguished by their morphology taken in conjunction with clinical features. Immunohistochemistry is not helpful in differentiating benign from malignant fibrous lesions. The main use of IHC in fibrous tumors is to rule out nonfibrous tumors, although in some entities a characteristic staining profile will help in the distinction from other fibrous lesions.

Solitary Fibrous Tumor and Hemangiopericytoma

Originally considered a tumor of pericytic origin, hemangiopericytoma (HPC) of soft tissue is now grouped with solitary fibrous tumor (SFT), a tumor of likely fibroblastic origin. The unifying concept has not been universally accepted, especially for tumors in the central nervous system/meninges^{12,13} and the term HPC is still retained in the World Health Organization classification of central nervous system tumors. Arguments in favor of abandoning the term HPC, at least in the soft tissue, are the lack of specificity of HPC-defining histologic features that are shared by many other tumors and the striking overlap of morphologic and immunohistochemical features with SFT.¹⁴ For this reason, the term HPC is now used with decreasing frequency and is often replaced by the term *cellular variant of SFT* or other specific tumor entities with an HPC-like pattern. Although the IHC staining profiles of tumors previously classified as HPC and the more recently recognized SFT differ slightly in the literature, they show considerable overlap and are listed here together for practical purposes. Markers frequently expressed include CD34¹⁵⁻¹⁹ (positive in 44%–95%) and CD99 (positive in 64%–91%).^{20,21} Smooth muscle actin is only rarely positive (<15%),²¹ and desmin is usually negative.^{17,22,23} Endothelial marker CD31 is uniformly negative.²⁴

Dermatofibrosarcoma Protuberans

Much work has been done using immunohistochemical stains to differentiate dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) because these entities have overlapping morphologic features. Dermatofibrosarcoma protuberans is generally factor XIIIa negative and CD34 positive, whereas DF shows the opposite staining.^{25,26}

Table 2. Comparison of Dermatofibroma (DF), Dermatofibrosarcoma Protuberans (DFSP), and Fibrosarcoma Arising in DFSP*

	CD34	Factor XIIIa
DF	-/+	+
DFSP	+	-
Fibrosarcoma in DFSP	+/-	-

* -/+ indicates mostly negative but may show focal staining, especially at the periphery; +, positive in more than 70% of tumors; -, negative in more than 70%; and +/-, variable.

(Table 2; Figure 1, A through F). Occasionally, DF may stain for CD34,^{27,28} especially at the periphery of the lesion.²⁹ CD34 positivity is also helpful in differentiating recurrent DFSP from scar tissue, which is generally CD34 negative.³⁰ Occasionally DFSP can show dedifferentiation with fibrosarcomatous areas. Several studies have shown partial or complete loss of CD34 staining in these areas.^{25,31-33} Other potentially useful markers in the differential diagnosis of DF and DFSP are HMGA1 and HMGA2. Both markers are positive in nearly all cases of DF cases and negative in most DFSPs.²⁹ A smaller study showed that DF stains with CD44 but not with hyaluronate binding protein, whereas DFSP stains in the reverse manner.³⁴

Aggressive Angiomyxoma Versus Angiomyfibroblastoma

Angiomyfibroblastoma and aggressive angiomyxoma are both tumors that occur predominantly in the vulva in women of childbearing age. Differentiating these two is important for prognosis because aggressive angiomyxoma, as the name implies, frequently recurs. Both of these tumors stain for vimentin and estrogen receptor and may stain for progesterone receptor and actin as well.^{35,36} Angiomyfibroblastoma is generally strongly desmin positive,³⁶⁻³⁸ and aggressive angiomyxomas may be positive although the staining is more variable.^{39,40}

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT) has been known in the literature under a number of designations, including inflammatory pseudotumor, plasma cell granuloma, and inflammatory fibrosarcoma. Although the lungs are overall the most common site, this tumor also occurs in the soft tissues. Inflammatory myofibroblastic tumor is composed of fibroblastic/myofibroblastic spindle cells set against a background of a plasma cell-rich inflammatory infiltrate. The neoplastic nature of IMT has been confirmed by the detection of recurrent clonal aberrations involving chromosome 2p23 similar to the ones found in anaplastic large cell lymphoma. The abnormally expressed protein product of the *ALK* gene can be detected with immunohistochemical stain ALK-1 (or p80) in approximately 50% of IMTs (36%–60%).⁴¹⁻⁴³ The staining pattern in IMT is usually cytoplasmic, whereas it can be both nuclear and cytoplasmic in anaplastic large cell lymphoma. ALK staining among mesenchymal tumors is not specific for IMT and has been described in malignant peripheral nerve sheath tumor, rhabdomyosarcoma (RMS), leiomyosarcoma (LMS), and malignant fibrous histiocytoma (MFH). On the other hand, benign fibrous lesions entering the differential diagnosis such as nodular fasciitis, des-

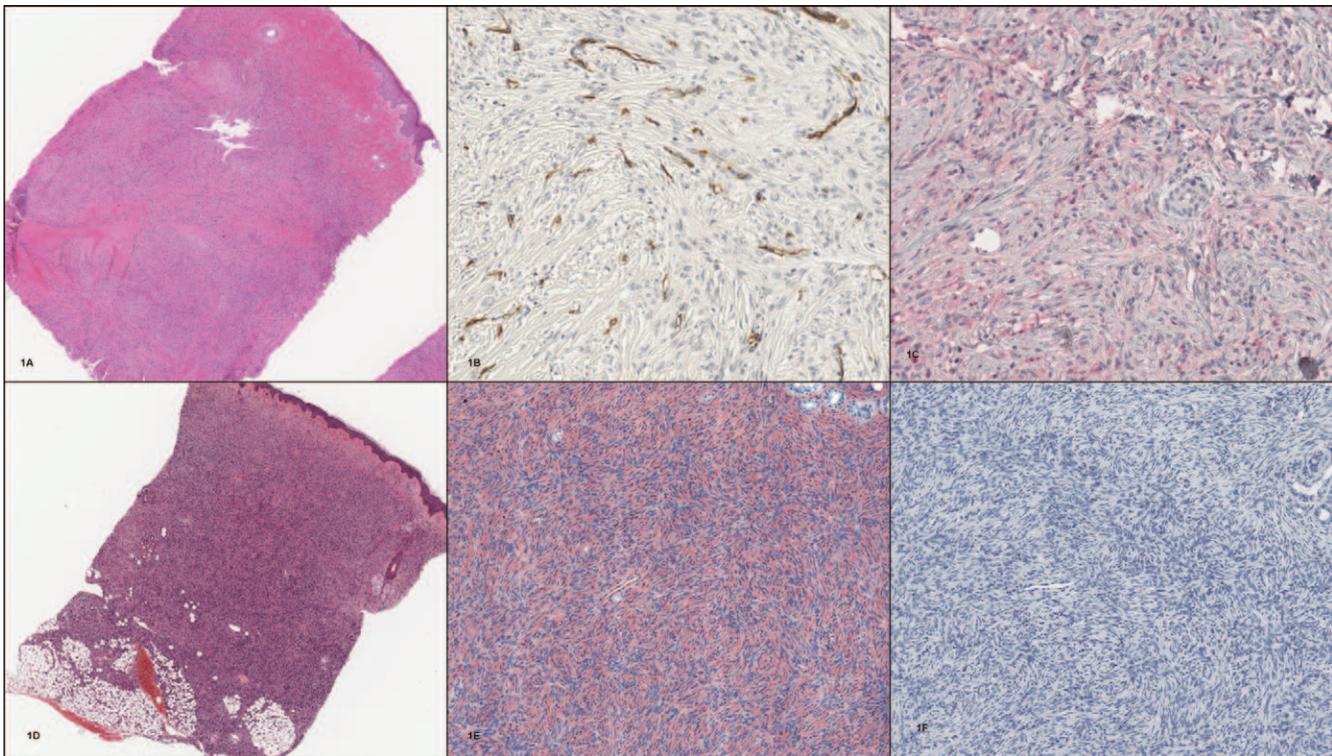


Figure 1. A comparison of dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) by hematoxylin-eosin, CD34, and factor XIIIa. Dermatofibroma stains for factor XIIIa but not for CD34, whereas DFSP has the opposite pattern. A, Dermatofibroma by hematoxylin-eosin (original magnification $\times 2$). B, CD34 stain of DF (original magnification $\times 20$). C, Factor XIIIa stain of DF (original magnification $\times 20$). D, DFSP by hematoxylin-eosin (original magnification $\times 2$). E, CD34 stain of DFSP (original magnification $\times 10$). F, Factor XIIIa stain of DFSP (original magnification $\times 10$). Courtesy of Thomas L. Davis, MD, South Texas Dermatopathology.

moid, myofibroma, and leiomyoma are ALK negative.⁴⁴ The spindle cells in IMT also stain with vimentin, HHHF35, and SMA.^{45–47} Less consistent positivity is seen with desmin (~50%)^{45,46} and epithelial markers AE1/AE3 and CAM 5.2 (approximately one third).^{45,48,49} Skeletal muscle markers MyoD1/myogenin, c-Kit,^{46,47,50} and S100⁵⁰ are negative.

Calcifying Fibrous Tumor Versus IMT

Calcifying fibrous tumor is a rare tumor of children and young adults composed of fibroblasts, psammomatous dystrophic calcifications, and lymphoplasmacytic infiltrates that can be confused with IMT. Both of these tumors will stain with vimentin; however, in contrast to IMT, calcifying fibrous tumor is both SMA and ALK negative.^{51,52}

Mammary-Type Fibroblastoma

This tumor, similar to its counterpart in the breast, has a fairly unique coexpression of CD34 and desmin.^{53,54} These stains are therefore useful in differentiating this tumor from SFT (desmin negative) and desmoid fibromatosis (CD34 negative).

Reactive Fibrous Lesions

(Nodular Fasciitis, Proliferative Fasciitis/Myositis, Myositis Ossificans, Ischemic Fasciitis)

Immunohistochemistry is of limited value in the diagnosis of these reactive fibroblastic/myofibroblastic proliferations. In addition to uniform vimentin expression, they are frequently and sometimes strongly positive for SMA and HHHF35, whereas desmin is usually negative.^{55–57} When desmoid tumor is in the differential diagnosis, desmin positivity would favor desmoid over a reactive process. The IHC profile of these reactive lesions is not helpful in ruling out low-grade sarcomas of fibroblastic/myofibroblastic origin, in particular low-grade fibromyxoid sarcoma, fibrosarcoma, and myofibroblastic sarcoma, all of which have a similar staining pattern.

Fibrous Tumors of Infancy

(Fibrous Hamartoma, Myofibroma, Inclusion Body Fibromatosis, Infantile Fibrosarcoma)

Fibrous hamartoma of infancy is a benign pediatric tumor with a characteristic triphasic composition including intersecting trabeculae of fibrocollagenous tissue, areas of primitive-appearing mesenchymal cells, and mature fat. All components of the tumor stain with vimentin. Actin and desmin are negative except for focal staining in spindle cells in the fibrous trabeculae.^{58–60} S100 is negative except in the adipocytic areas.⁶¹

Myofibroma/myofibromatosis is a solitary or multicentric benign tumor that occurs predominantly but not exclusively in childhood. It contains spindled myofibroblastic cells arranged around blood vessels and more immature-appearing round cells in a zoned distribution. Both the myofibroblastic and more primitive component of myofibroma stain for vimentin and SMA, whereas only the myofibroblastic component will stain with HHHF35. Both components are negative for S100, epithelial membrane antigen (EMA), and keratins.^{62,63}

Inclusion body fibromatosis is a rare type of fibromatosis occurring on the digits of infants. It is composed of fascicles of spindle cells containing characteristic intracytoplasmic eosinophilic inclusions. The spindle cells are positive for vimentin and muscle actins. The inclusions

stain positive with actins, although results may vary with tissue preparation.^{64–67}

Infantile fibrosarcoma is histologically similar to adult fibrosarcoma but carries a much better prognosis. The IHC profile is rather nonspecific with vimentin positivity and variable expression of a number of markers including SMA, HHHF35, neuron-specific enolase, desmin, S100, CD34, and cytokeratin (CK). However, staining with MyoD1 and myogenin has not been reported and can be used in the exclusion of RMS.^{68–72}

Other Fibromas

(Nuchal Fibroma, Gardner Fibroma, Collagenous Fibroma, Calcifying Aponeurotic Fibroma, Fibroma of Tendon Sheath)

Nuchal fibroma, also called nuchal-type fibroma, is a paucicellular lesion composed of thick collagen fibers and typically occurs in the posterior neck. Gardner fibroma is histologically identical to nuchal fibroma and can occur in various superficial or deep soft tissue sites. It occurs in association with Gardner syndrome and may be the first manifestation of the syndrome. Both nuchal and Gardner fibroma stain with CD34, which may be helpful in the differential diagnosis with fibromatoses and reactive fibrous lesions.^{73–77} Other specific types of fibromas are collagenous fibroma (desmoplastic fibroblastoma), calcifying aponeurotic fibroma, and fibroma of tendon sheath. They have a nonspecific staining pattern, and IHC is not useful in the differential diagnosis of these entities.^{78–80}

Fibromatoses

(Desmoid Tumor and Superficial Fibromatosis)

Desmoid tumor frequently stains with SMA and sometimes, but usually only focally, with desmin.^{81,82} It is negative for CD34, S100, c-Kit, and epithelial markers. In the differential diagnosis with SFT, CD34 positivity favors SFT over desmoid. Nuclear staining of both superficial and deep fibromatoses with β -catenin has been demonstrated in a limited number of studies and appears useful to differentiate fibromatoses from most sarcomas and other benign fibrous lesions.^{83,84} However, it should not be used in the differential diagnosis of fibromatosis with synovial sarcoma or SFTs, as a significant number of these will also stain with β -catenin.^{84,85} Superficial fibromatoses (plantar and palmar) have a staining profile similar to desmoid tumor. Immunohistochemistry is usually not needed in their diagnosis.

Low-Grade Fibroblastic/Myofibroblastic Sarcomas (Low-Grade Fibromyxoid, Myxoinflammatory Fibroblastic, and Low-Grade Myofibroblastic Sarcoma)

This group of sarcomas may pose a significant diagnostic challenge because of their relatively bland cytologic features. Unfortunately, their IHC staining profiles are not distinctive and overlap with those benign fibrous lesions.^{86,87} The only potential role of IHC is to exclude tumor of distinct nonfibrous cell lineage, that is, a peripheral nerve sheath tumor.

Sclerosing Epithelioid Fibrosarcoma

Sclerosing epithelioid fibrosarcoma is an unusual variant of fibrosarcoma with a distinctive arrangement of epithelioid tumor cells in nests and cords. Despite their epithelioid appearance, tumor cells are only rarely positive for keratins and EMA and are diffusely positive with vi-

Table 3. Initial Immunohistochemical Panel for the Evaluation of Pleomorphic Spindle Cell Neoplasm

Immunohistochemistry Stain	Pleomorphic Neoplasm
Pankeratin positive	Pleomorphic carcinoma
S100 positive	Melanoma
Lymphoid marker positive*	Lymphoma/follicular dendritic tumor
Vimentin positive, keratin negative,† S100 negative,‡ lymphoid negative	Sarcoma

* Leukocyte common antigen, CD20, CD30, CD43, CD21/23.

† Spotty keratin expression may be seen in some sarcomas as aberrant expression.

‡ Focal S100 positivity is seen in some sarcomas including synovial sarcoma and malignant peripheral nerve sheath tumor.

mentin.⁸⁸ S100 expression is likewise rare, and CD34, desmin, CD68, and glial fibrillary acidic protein are negative.⁸⁹

Tenosynovial Giant Cell Tumor and Other Giant Cell Tumor of Soft Tissue

Tenosynovial giant cell tumors may be localized (giant cell tumor of tendon sheath and localized intra-articular tumors) or diffuse lesions (intra-articular type, the so-called pigmented villonodular synovitis, and the diffuse extra-articular type). The osteoclast-like giant cells in these lesions coexpress CD68 and CD45, whereas the mononuclear cells are CD68 positive and CD45 negative.^{90,91} Desmin-positive dendritic cells may be found in the background in up to 50%.⁹²

Giant cell tumor of soft tissue is the soft tissue counterpart of giant cell tumor of bone and is not related to tenosynovial giant cell tumor. The mononuclear cells stain are positive for vimentin and CD68 and focally also for SMA. More rarely, focal S100 and keratin positivity may be found. The giant cells are CD68 positive.⁹³⁻⁹⁵

Plexiform Fibrohistiocytic Tumor

Plexiform fibrohistiocytic tumor is a rare subcutaneous tumor occurring on the extremities of children or young adults. It is composed of interconnected nodules containing mononuclear spindled fibroblast-like or plump histiocyte-like cells and interspersed multinucleated cells. The cells are positive for vimentin, CD68 (in the multinucleated giant cells and mononuclear histiocyte-like cells),^{96,97} and SMA (in the fibroblast-like cells).⁹⁷⁻¹⁰⁰

Pleomorphic Sarcoma Versus MFH

Malignant fibrous histiocytoma as a diagnostic entity has become increasingly disputed. Although MFH was reported to be the most common soft tissue sarcoma in adults older than 40 years and is still listed as such in the current World Health Organization edition of *Pathology and Genetics of Tumours of Soft Tissue and Bone*, it is also said to be, in the same textbook, a "term which may disappear completely." Critics consider MFH a heterogeneous group of pleomorphic tumors for which a better, more specific

designation should be sought.¹⁰¹⁻¹⁰⁴ In addition to careful search of lineage-typical features by H&E morphology, a panel of IHC stains is recommended to help define cell lineage. The following question arises: How far should one go with ancillary studies in trying to determine specific lineage and what are the clinical/prognostic implications? There is little disagreement over the importance of separating pleomorphic sarcoma from nonmesenchymal malignancies because treatment modalities and prognosis will be entirely different. The main considerations are metastatic sarcomatoid carcinoma, metastatic melanoma, and, in some instances, an unusual hematolymphoid tumor with spindle cell features. The initial battery of IHC markers should always include a broad keratin (pankeratin), S100, and vimentin (Table 3). The use of vimentin is mainly to confirm immunoreactivity of the tissue, and positivity is not specific for mesenchymal differentiation. A positive keratin result should open the door to further clinical workup to detect a primary carcinoma elsewhere. Additional IHC stains, including keratin subtypes, may be used to help narrow this search. A positive S100 stain should be followed by additional melanoma markers and the search for a suspicious skin lesion. Some hematolymphoid neoplasms with predominant spindle cell and pleomorphic features may also be confused with a pleomorphic sarcoma, in particular some Hodgkin lymphomas, some large cell lymphomas including anaplastic large cell lymphoma, and follicular dendritic cell tumor. It is important to remember that some of these tumors may be negative for leukocyte common antigen, and the addition to the screening panel of this marker alone may not be sufficient. When a hematolymphoid neoplasm is suspected, a panel including leukocyte common antigen, CD30, CD2, CD43, and CD21 (or CD23) is appropriate to exclude all of these entities.

Once a nonmesenchymal neoplasm has been excluded, the more difficult step follows, which is subcategorizing a pleomorphic sarcoma (Table 4). Recent publications have stressed a significantly worse prognosis of sarcomas with myogenic differentiation, in particular pleomorphic rhabdomyosarcoma (PRMS) and LMS.¹⁰⁴ Dedifferentiated liposarcoma on the other hand has been shown to behave

Table 4. Immunohistochemical Panel to Subtype Pleomorphic Sarcoma*

	PLS	PLMS	PRMS	PS-NOS
Vimentin	+	+	+	+
MDM2/CDK4	+	-	-	-
SMA	-	++ (diffuse)	-(10% +)	-(may be focally +)
Desmin	-	+(focal)	++	-(rarely focally +)
Myogenin	-	-	+	-

* PLS indicates pleomorphic liposarcoma; PLMS, pleomorphic leiomyosarcoma; PRMS, pleomorphic rhabdomyosarcoma; PS-NOS, pleomorphic sarcoma not otherwise specified; +, positive in more than 70% of tumors; -, negative in more than 70%; SMA, smooth muscle actin; and ++, positive in more than 95%.

less aggressively than most other pleomorphic sarcomas.¹⁰⁵ In addition to a careful search for a component of WDLS by morphology and thorough sampling, IHC for MDM2 and CDK4 may be helpful in separating dedifferentiated liposarcoma from pleomorphic sarcoma not otherwise specified.⁶ The interpretation of positive IHC muscle markers is problematic because diagnostic criteria to define a sarcoma as LMS, RMS, or "myogenic sarcoma" are not uniform. If one relaxes criteria too much, any pleomorphic sarcoma with focal SMA positivity becomes a myogenic sarcoma. Focal SMA positivity has long been recognized and allowed in the diagnosis of MFH and should not be used alone as evidence of specific lineage differentiation (Figure 2, A through D). Recommended criteria for the diagnosis of LMS are diffuse SMA positivity, at least focal desmin positivity, and smooth muscle features on H&E.¹⁰⁶ Criteria required for skeletal muscle differentiation are myogenin (or MyoD1) in addition to desmin positivity, supporting H&E morphology, and, in myogenin-negative cases, ultrastructural evidence of skeletal muscle differentiation.^{105,107,108}

The decision whether to attempt a more precise subcategorization of a pleomorphic sarcoma will ultimately be driven by clinical relevance. At this time, adult sarcoma protocols do not discriminate between histologic subtypes with the possible exception of PRMS, which may be treated according to an RMS protocol, and LMS for which oncologists often use a specific chemotherapeutic agent. Prognostic data reported in the literature are still being based on differing and somewhat subjective criteria, and prospective studies using unified histologic criteria will be needed to correlate prognosis with histologic subtype. It is expected that prognostically significant molecular genetic differences will eventually resolve this controversy and further guide the use of IHC.

Myxofibrosarcoma

Formerly included under the category of MFH (myxoid MFH), this tumor is now considered a separate entity with more defined histologic and clinical characteristics. It encompasses a range from low- to high-grade tumors. The low-grade variant may be difficult to distinguish from benign myxomatous lesions, whereas high-grade variants show considerable overlap with pleomorphic sarcoma not otherwise specified. The IHC profile is not unique, and focal SMA or muscle-specific actin positivity can be seen just like in pleomorphic sarcoma or so-called MFH. Desmin, S100, keratins, CD68, and factor XIIIa¹⁰⁹ are generally negative.

Gastrointestinal Stromal Tumor

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. Although not strictly speaking a tumor of soft tissue in the anatomical sense, it is discussed here because of its unique place among mesenchymal tumors and the importance of IHC in diagnosis, treatment, and prognosis. Before the discovery of the *KIT* receptor kinase gene mutations and associated expression of Kit protein detected by IHC, many GISTs were classified as smooth muscle tumors.

Kit (CD117) positivity is seen in the great majority of GISTs (>95%). Given the effectiveness of receptor tyrosine kinase inhibitor imatinib mesylate (Gleevec, Novartis, East Hanover, NJ) in the treatment of GIST, Kit positivity has

traditionally been required as a diagnosis-defining feature for enrollment in imatinib-based protocols.

Kit positivity is usually strong, diffuse cytoplasmic with frequent dotlike accentuation of the Golgi zone. Mesenchymal spindle cell tumors in the differential diagnosis with GIST include leiomyoma, LMS, SFT, desmoid tumor, and schwannoma, all of which are Kit negative.¹¹⁰ Additional tumors with rare, usually just focal Kit positivity include angiosarcoma, Ewing sarcoma, metastatic melanoma, and a few others.¹¹¹ CD34 positivity is seen in 60% to 70% of GISTs with relatively higher frequency in GISTs of esophageal and rectal origin and in benign gastric GISTs.¹¹⁰ CD34 positivity is also observed in 10% to 15% of smooth muscle tumors and occasionally in schwannoma making it a less specific marker. Desmin is useful in distinguishing GIST from true smooth muscle tumor with only rare GISTs expressing focal desmin (<5%).^{112,113} S100 is focally positive in 5% to 10% of GISTs, whereas schwannoma is diffusely positive. Smooth muscle actin is positive in 30% of GISTs¹¹³ and is therefore not helpful in distinguishing GISTs from smooth muscle tumors or other tumors with possible SMA expression (desmoid tumor, SFT). β -Catenin has been used by some investigators to differentiate GIST from desmoid tumor with the former being consistently negative and the latter positive in most cases.¹¹⁴

Not all tumors fulfilling the clinical and morphologic criteria of GIST are Kit positive and whether these tumors can be diagnosed as GIST is a matter of debate. This small subset of Kit-negative GISTs makes up about 4%^{115,116} and occurs relatively more frequently in the stomach and omentum. Despite Kit negativity, these tumors may respond to imatinib treatment and should therefore not be denied kinase inhibitor therapy.¹¹⁷ There are several explanations for this apparent discrepancy, which are briefly addressed in the following.

Mutational Analysis of GIST and Response to Treatment

After the first discovery of *KIT*-activating mutations in GIST,¹¹⁸ numerous studies have further explored the genetic profile of these tumors. *KIT*-activating mutations are found in 85% to 90% of GISTs and may involve different sites within the *KIT* gene (most frequently exon 11). An additional subset, making up about 5% of GISTs, harbors an alternate mutation of a related receptor tyrosinase kinase gene, platelet-derived growth factor receptor α (*PDGFRA*). Lastly, there is a subset of GISTs (5%–10%) lacking mutations in either kinase (so-called wild type). It is important to emphasize that the extent and pattern of Kit staining by IHC does not correlate with the type of *KIT* mutation and does not predict response to treatment. In GISTs lacking demonstrable *KIT* mutations, *KIT* may be nevertheless strongly activated, possibly because of *KIT* mutations that are not readily detected by conventional screening methods or, alternately, because *KIT* is activated by nonmutational mechanisms.¹¹⁹ It has been shown, however, that imatinib response correlates with the location of mutations on the *KIT* and *PDGFRA* genes (best response with exon 11 mutations), whereas GISTs lacking either mutation are less likely to respond.^{120,121} Whether to perform mutational analysis as part of the standard diagnostic workup for patients with GIST is a matter of controversy but at this time does not appear practical. Immunohistochemistry for detection of *PDGFRA* in Kit-negative tumors has been successfully used in several research

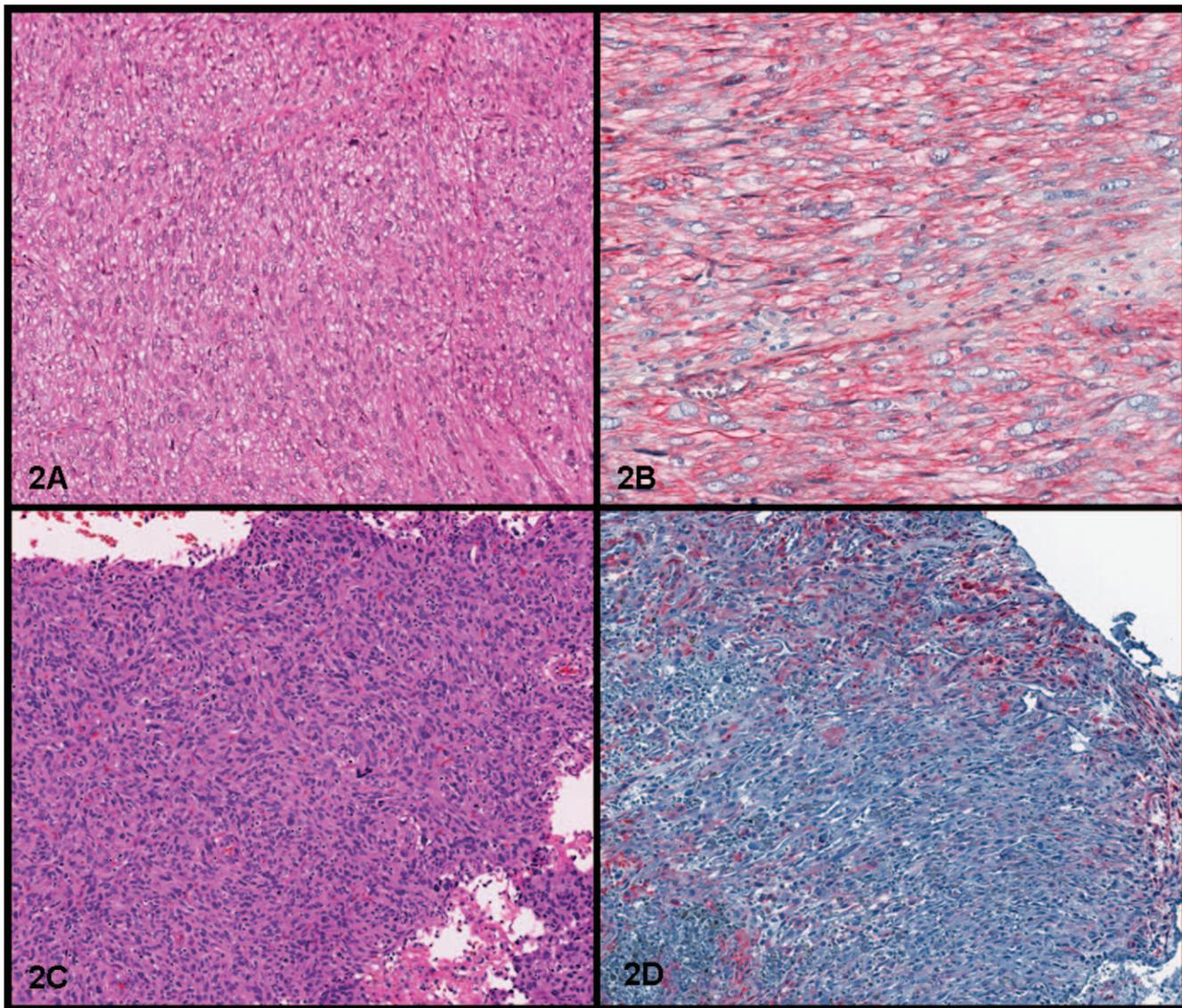


Figure 2. Although malignant fibrous histiocytomas (MFHs) may show patchy staining with smooth muscle markers, such as smooth muscle actin, leiomyosarcomas will show diffuse staining. A, Leiomyosarcoma by hematoxylin-eosin (original magnification $\times 10$). B, Smooth muscle actin stain of leiomyosarcoma (original magnification $\times 20$). C, MFH by hematoxylin-eosin (original magnification $\times 10$). D, Smooth muscle actin stain of MFH (original magnification $\times 10$).

studies, but commercially available *PDGFRA* antibody does not yield reproducible results for clinical use at this time.¹²²

SMOOTH MUSCLE TUMORS

Leiomyoma and LMS

Leiomyoma and LMS are generally strongly and uniformly positive for SMA and HHF35 (Figure 2, A and B). Smooth muscle actin is more specific for smooth muscle than panactin HHF35. It is usually negative in skeletal muscle tumors in contrast to HHF35, which is frequently positive in these tumors. Desmin positivity varies with leiomyoma being usually at least focally positive, whereas LMS is positive in only 70% to 80% of cases, with less staining in the poorly differentiated examples.^{123,124} Calponin, a cytoskeleton-associated actin-binding protein, is frequently used as a myoepithelial marker but is also very useful in detecting smooth muscle differentiation in

STTs.¹²⁵ In addition to smooth muscle and myoepithelial cells, it is also expressed in myofibroblasts. It is consistently positive in leiomyoma, and it is positive in a high percentage of LMSs (90% in conventional LMS, 70% in pleomorphic LMS).¹²⁶ To define a poorly differentiated spindle cell sarcoma as LMS, at least 2 of 3 muscle markers (using for example SMA/desmin/HHF35 or SMA/desmin/calponin) should be positive, and the H&E appearance should be supportive as well. Markers specific for skeletal muscle differentiation (myoglobin, myogenin) are consistently negative in LMS. Keratin and EMA are positive in 10% to 30% of LMSs.^{127,128}

Tumors with myofibroblastic differentiation also stain with SMA and HHF35, at least focally, whereas desmin staining is less commonly observed. This may lead to potential confusion of myofibroblast-rich lesions, that is, desmoid fibromatosis with smooth muscle tumors. A less uniform staining pattern and recognition of appropriate mor-

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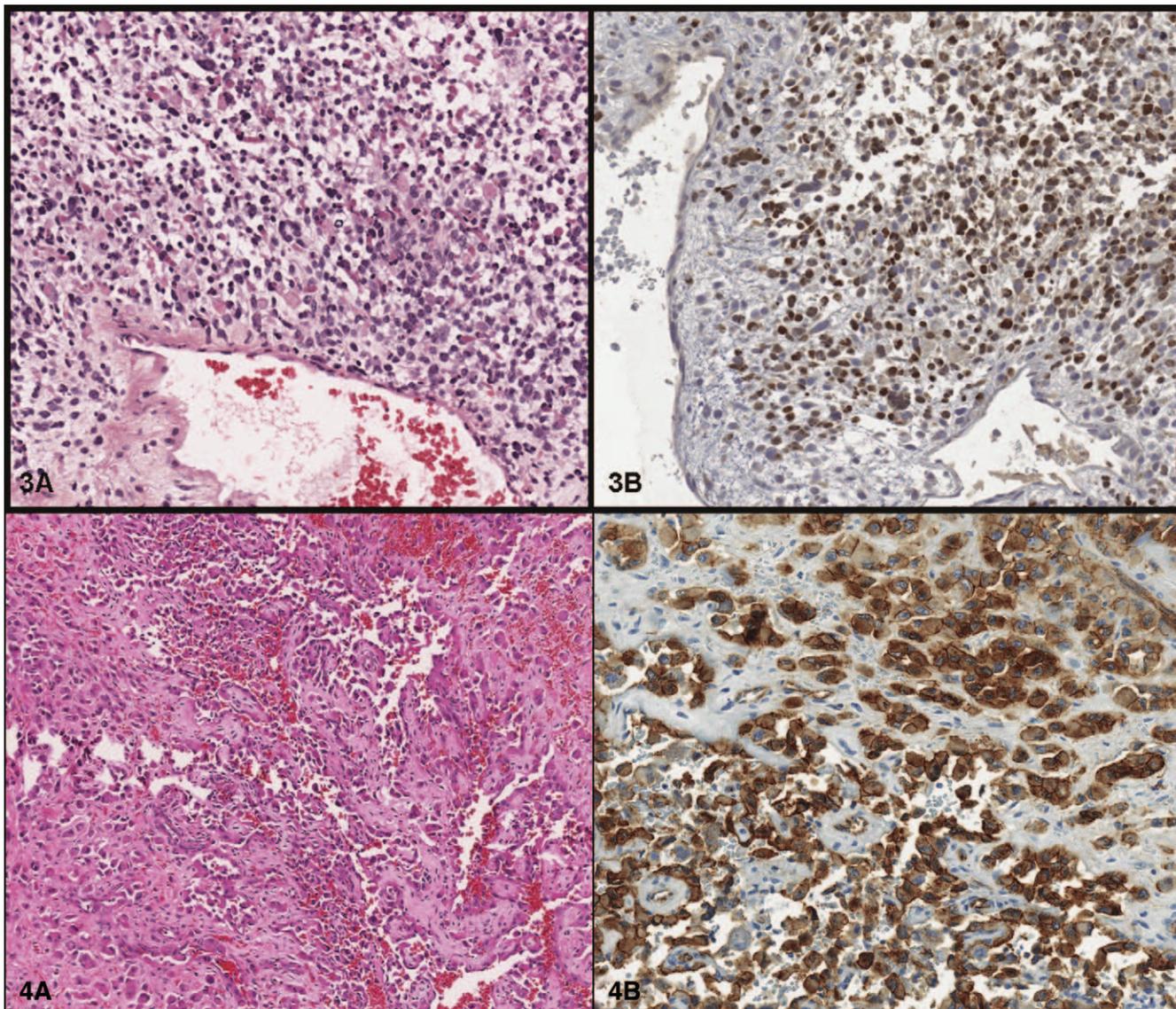


Figure 3. Rhabdomyosarcoma (RMS) shows strong nuclear staining for muscle markers, such as MyoD1. A, Embryonal RMS by hematoxylin-eosin (original magnification $\times 20$). B, MyoD1 staining of embryonal RMS (original magnification $\times 20$). Courtesy of Victor A. Saldivar, MD, Christus Santa Rosa Children's Hospital, San Antonio, Tex.

Figure 4. Tumors of vascular origin show membranous staining with CD31. A, Angiosarcoma by hematoxylin-eosin (original magnification $\times 10$). B, CD31 staining of angiosarcoma (original magnification $\times 20$).

phologic and clinical features will be helpful in making this distinction.

SKELETAL MUSCLE TUMORS

Rhabdomyosarcoma

Rhabdomyosarcoma comprises a group of tumors with skeletal muscle differentiation. These tumors are divided into 3 main biologically distinct categories: embryonal rhabdomyosarcoma (ERMS), ARMS, and PRMS. The role of IHC in these tumors is mainly to confirm their skeletal muscle lineage, which may not be apparent on H&E morphology, especially in the more aggressive alveolar and pleomorphic types. The two overall most useful markers in RMS diagnosis are desmin and myogenin.¹²⁹

Desmin is highly sensitive for all tumors with skeletal differentiation¹³⁰ but somewhat nonspecific because it may also stain smooth muscle cells and occasionally even myo-

fibroblasts. It is also positive in desmoplastic small round cell tumor, IMT, and alveolar soft part sarcoma and should therefore never be used as the sole marker to diagnose RMS.^{131–133}

MyoD1 and myogenin are very specific for skeletal muscle differentiation with only rare reports of nuclear staining in nonrhabdomyosarcomatous tumors.¹³⁴ These antibodies react with nuclear transcription factors expressed early in skeletal muscle differentiation. Only nuclear staining should be considered as a positive result in both MyoD1 and myogenin (Figure 3, A and B). Myogenin is easier to use and more reliable than MyoD1 because of frequent cytoplasmic staining¹³⁴ and a tendency by the latter to fade.¹³⁵ Neither MyoD1 nor myogenin should be used on B5-fixed sections.¹³⁵

Although all types of RMS express these nuclear markers, there are relative differences in the amount and inten-

sity of staining. The most uniform and strongest staining pattern is seen in the alveolar type, including its solid variant. Staining is more patchy in ERMS, with islands of intensely positive cells alternating with fascicles of totally negative cells.^{135,136} Immunohistochemistry staining pattern is not reliable in subtyping RMS. Genetic markers are required for this distinction, in particular of ERMS from ARMS, given that these 2 types may have overlapping or mixed morphology.

Smooth muscle actin positivity is seen in a minority (approximately 10%) of RMSs.^{132,137} Aberrant marker expression for CK, S100, and neurofilament is rare.^{138,139}

Pleomorphic rhabdomyosarcoma is positive for desmin¹⁴⁰ but less consistently for MyoD1 and myogenin (~50%). In MyoD1/myogenin-negative tumors, other features of skeletal muscle differentiation (H&E morphology, ultrastructure) are needed to classify a pleomorphic sarcoma as PRMS.^{107,108}

VASCULAR TUMORS

Immunohistochemistry is very useful in determining vascular lineage, especially in poorly differentiated angiosarcoma and vascular tumors mimicking epithelial neoplasms (epithelioid hemangioendothelioma and epithelioid angiosarcoma). Commonly used vascular markers are factor VIII, CD34, CD31, and FLI-1 (Figure 4, A and B). Although factor VIII is highly specific for endothelium, it is less sensitive than CD31 and more difficult to interpret because of its presence in serum, which can result in high background staining in necrotic and hemorrhagic tissues.⁹ CD34, the hematopoietic progenitor cell antigen, is only positive in 50% of angiosarcoma.¹⁴¹⁻¹⁴⁴ The most sensitive marker for angiosarcoma is CD31, which is seen as a membranous staining pattern in more than 90% of cases.^{141,142} Specificity is excellent with only rare reports of CD31-positive carcinomas.¹⁴⁵ FLI-1 is a more recently introduced endothelial marker, which is also used in the diagnosis of Ewing/PNET (see later). Its specificity and sensitivity approach 100% when used in the differential diagnosis of vascular tumors.¹⁴⁶ Other FLI-1-positive tumors (Ewing/PNET and lymphomas) are morphologically quite distinct and are generally not confused with these vascular tumors.

TUMORS OF UNCERTAIN DIFFERENTIATION

These tumors comprise a heterogeneous group for which no definite histogenesis has been elucidated. Nevertheless, IHC is helpful in the diagnosis of many of these tumors, the most important ones being discussed in the following. Special emphasis is given to the immunohistochemical workup of Ewing sarcoma/PNET versus other primitive-appearing small round cell tumors.

Synovial Sarcoma

Synovial sarcoma is a unique soft tissue sarcoma showing both mesenchymal and epithelial differentiation. Despite its name, it is neither related to nor arising from synovial cells. Immunohistochemistry is particularly useful in the diagnosis of the monophasic spindle cell variant, which is more difficult to distinguish from other spindle cell sarcomas on morphologic grounds alone (Table 5). Vimentin is positive in both the epithelial and mesenchymal component.

Epithelial membrane antigen is the most sensitive marker to detect the epithelial component.^{147,148} A number of

Table 5. Differential Diagnosis of Nonpleomorphic Spindle Cell Sarcoma*

	S100	Desmin	Actin†	EMA	HMB-45
SS	-/+	-	-	+	-
MPNST	+/(-)	-	-	+	-
LMS	-	+	+	-/(+)	-
FS	-	-	-/(+)	-	-
CCS	+	-	-	-	+

* EMA indicates epithelial membrane antigen; SS, synovial sarcoma; -/+, positive in 30% of tumors; +, positive in more than 70%; -, negative in more than 70%; MPNST, malignant peripheral nerve sheath tumor; +/(-), between 50% and 70% positive, often weakly; LMS, leiomyosarcoma; -/(+), positive in up to 30%; FS, fibrosarcoma; and CCS, clear cell sarcoma.

† Various actin stains include smooth muscle actin, muscle-specific actin, and HHF35.

keratins including AE1/AE3 cocktail, CK7, CK8, CK18, and CK19¹⁴⁹ stain the epithelial component in the biphasic tumors. Monophasic tumors stain only focally and not as consistently with these keratins. The use of both EMA and CK7 appears to yield the best chance of detecting epithelial differentiation in synovial sarcoma.¹⁵⁰ Other commonly positive epithelial markers are BerEp4 and E-cadherin. Calretinin staining is found in about 70%.¹⁵¹

S100 positivity is seen in 30% of synovial sarcomas.¹⁵² It is therefore not helpful in distinguishing it from malignant peripheral nerve sheath tumor. CD34 is only rarely positive, whereas SMA and desmin are usually negative. The spindle cells in synovial sarcoma are usually positive for Bcl-2, a marker of limited usefulness given its lack of specificity.¹⁵³ Another frequently positive stain is CD99, either in a strong membranous or cytoplasmic staining pattern.¹⁵⁴

Poorly differentiated synovial sarcomas are less consistently positive for CKs (40%–50%), whereas they still retain EMA expression in 90%. In such cases, cytogenetic or molecular techniques to detect the characteristic *SYT-SSX* fusion are needed to arrive at the correct diagnosis.

Clear Cell Sarcoma of Soft Tissue

Despite their similar IHC staining profiles, clear cell sarcoma of soft tissue is clinically and genetically distinct from cutaneous melanoma.¹⁵⁵ Like the latter, it is positive for melanocytic markers S100, HMB-45, and less consistently for melan (Mart-1).¹⁵⁶⁻¹⁵⁸ Other positive markers include neuron-specific enolase, CD57, and vimentin. Variable results are seen with c-Kit (CD117).¹⁵⁹ Negative markers include keratins, EMA, SMA, and desmin. Although melanocytic stains are helpful in separating clear cell sarcoma from other soft tissue sarcomas, they cannot distinguish clear cell sarcoma from cutaneous melanoma. This distinction is based on tumor morphology, location, or genetic confirmation of the *EWSR1-ATF* fusion gene, which has been found in clear cell sarcoma but never in cutaneous melanoma.¹⁶⁰

Epithelioid Sarcoma

Epithelioid sarcoma is a rare soft tissue sarcoma showing epithelial differentiation (Figure 5, A and B). Cytokeratins and EMA are strongly positive in almost all cases, mostly coexpressed with vimentin.^{161,162} CD34 is positive in about 50% with a strong membranous staining pattern.¹⁶³ When positive, this stain is helpful in excluding carcinoma because carcinomas are almost always CD34

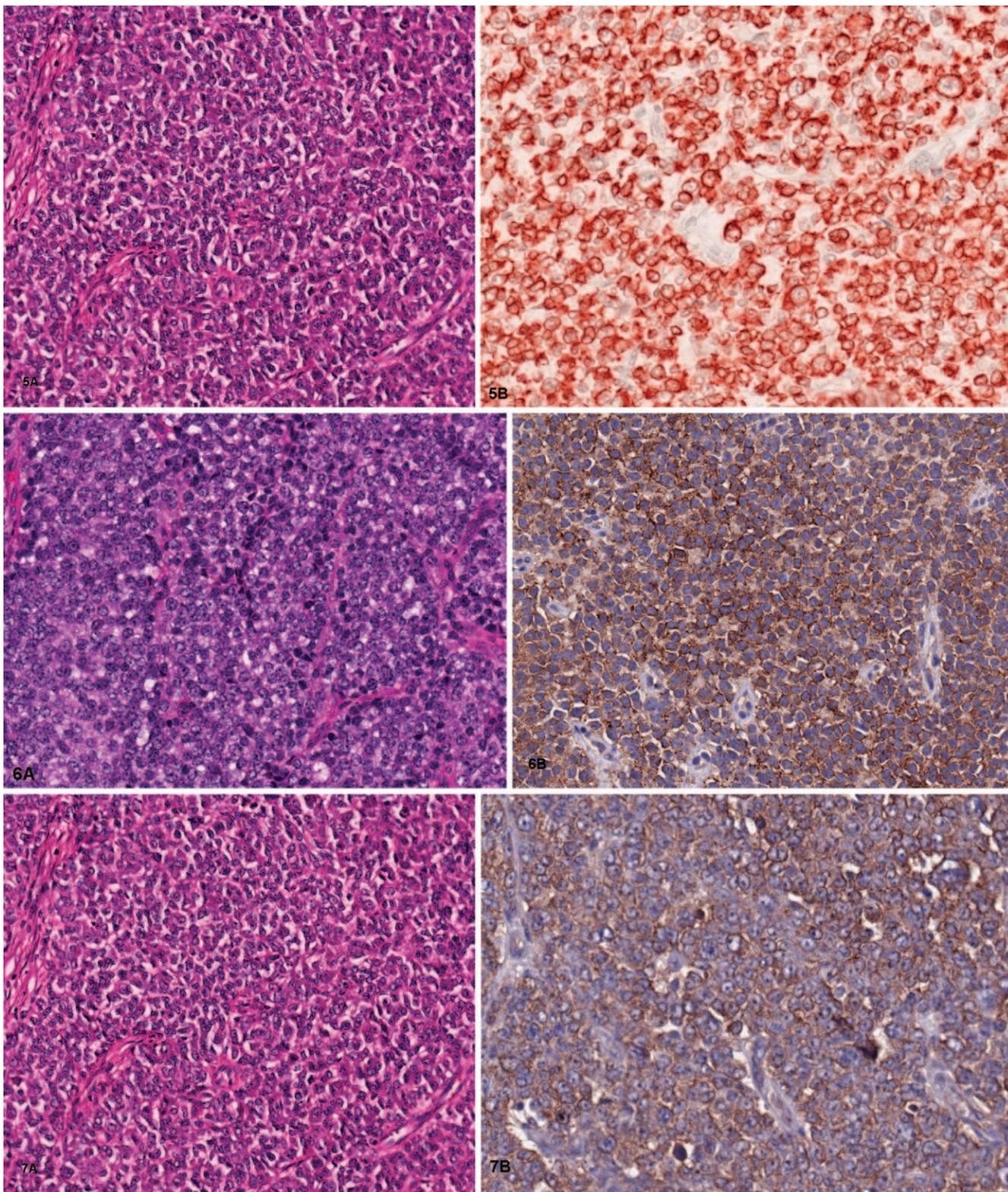


Figure 5. Epithelioid sarcoma shows epithelial differentiation by immunohistochemistry. *A*, Epithelioid sarcoma by hematoxylin-eosin (original magnification $\times 20$). *B*, Pankeratin (AE1/AE3) staining of epithelioid sarcoma (original magnification $\times 20$).

Figure 6. Ewing sarcoma shows membranous staining with CD99. *A*, Ewing sarcoma by hematoxylin-eosin (original magnification $\times 20$). *B*, Membranous CD99 staining of Ewing sarcoma (original magnification $\times 40$).

Figure 7. CD99 staining is not specific for Ewing sarcoma as shown by this case of epithelioid sarcoma. *A*, Epithelioid sarcoma by hematoxylin-eosin (original magnification $\times 20$). *B*, Membranous CD99 staining of epithelioid sarcoma (original magnification $\times 40$).

Table 6. Immunoprofile of Small Round Cell Tumors of Soft Tissue*

	EWS/PNET	RMS	SS, Round Cell Type	Epithelioid Sarcoma	Lymphoma/Leukemia
Lymphoid marker†	—	—	—	—	+
CD99	+	—	+	—	+/-
FLI-1	+	—	—	—	+/-
CK/EMA	-/(+)	—	+/(-)	++	—
Desmin	—	+	—	—	—
Myogenin	—	+	—	—	—
S100	-/(+)	—	-/+	—	—
CD34	—	—	—	+/-	—

* EWS/PNET indicates Ewing sarcoma/primitive neuroectodermal tumor; RMS, rhabdomyosarcoma; SS, synovial sarcoma; —, negative in more than 70% of tumors; +, positive in more than 70%; +/-, variable; CK/EMA, cytokeratin/epithelial membrane antigen; -/(+), mostly negative but may be positive in up to 30%; +/(-), keratin positive in 40% to 50%, EMA in 90%; ++, strongly positive in more than 70%; and -/+, variable but more often negative.

† Lymphoid markers: terminal deoxyribonucleotide transferase, leukocyte common antigen, CD79a, CD19, and CD3.

negative.¹⁶¹ S100 and desmin are only rarely positive.¹⁶⁴ E-cadherin is negative in contrast to many carcinomas.¹⁶⁵

Alveolar Soft Part Sarcoma

This rare tumor containing periodic acid-Schiff–positive crystalline inclusions was thought to be of myogenic origin. Subsequent studies did not confirm this theory and histogenesis remains unclear to this day. A relationship to muscle was theorized because of frequent desmin positivity in alveolar soft part sarcoma. Percentages of positivity vary between studies (~50% on average).^{166,167} Smooth muscle actin staining is sometimes also seen, whereas nuclear staining for MyoD1 and myogenin is consistently absent.^{133,168} A unique feature of this sarcoma is its weak or even negative staining with vimentin. S100 staining is sometimes present, whereas other neural markers and keratins are negative.¹⁶⁸ In recent years, a characteristic translocation (X;17) resulting in a *ASPL-TFE3* fusion gene has been found in alveolar soft part sarcoma.^{169,170} This fusion gene is not exclusive to alveolar soft part sarcoma but is also present in a pediatric variant of renal cell carcinoma.^{171,172} A new immunohistochemical stain for TFE3 protein has been developed, which detects the overexpressed TFE3 protein in alveolar soft part sarcoma and in other *ASPL-TFE3* fusion gene–positive tumors.¹⁷³

Ewing Sarcoma/PNET and “Small Round Cell Tumors”

Ewing sarcoma and PNET are now considered members of the Ewing family of tumors. They are highly aggressive primitive round cell tumor of uncertain histogenesis with variable degrees of neural differentiation.

Immunohistochemical stains are negative with most mesenchymal markers with the exception of vimentin.¹⁷⁴ Neural markers S100, CD56, chromogranin, and synaptophysin may be positive but are often only focally or weakly.¹⁷⁵ Cytokeratin positivity is not uncommon (up to 20%) and may be focal or diffuse.¹⁷⁶ The most helpful marker is CD99 (O13, HBA71), which is positive in more than 90% of PNET with a characteristic membranous staining pattern^{177,178} (Figure 6, A and B). After the discovery of the Ewing-specific tumor translocations t(11;22)(q24;q12) and respective *EWS/ETS* fusion oncogene, the immunohistochemical stain FLI-1 has proven to be more specific for Ewing/PNET than CD99 with somewhat inferior sensitivity.^{179–181} Specificity is limited by cross reactivity with acute lymphoblastic leukemia, non-Hodgkin lymphoma, and endothelial cells¹⁸² (Figure 7, A and B). Sensitivity is limited by the occurrence of variant translocations that do not involve the *FLI* gene or by low ex-

pression of FLI-1. Given the lack of specificity of CD99 and more limited sensitivity of FLI-1, these stains have to be interpreted in the context of a comprehensive panel of immunohistochemical stains aimed at ruling out other tumors with small round cell phenotype (Table 6). Examples of such tumors occurring predominantly in young individuals are leukemia/lymphoma, solid variant of ARMS, mesenchymal chondrosarcoma, desmoplastic round cell tumor, poorly differentiated synovial sarcoma, metastatic neuroblastoma and metastatic Wilms tumor, mesenchymal chondrosarcoma, extrarenal rhabdoid tumor, and proximal type epithelioid sarcoma. In adults, metastatic melanoma and metastatic carcinomas (poorly differentiated, ie, small cell carcinoma) have to be considered as well. A comprehensive IHC panel should include hematology markers able to identify cells at various stages of lymphocyte differentiation (terminal deoxyribonucleotide transferase, CD79a, CD19, CD3, and leukocyte common antigen), vimentin, muscle markers desmin and myogenin, pankeratin, and S100 with the possible addition of neural markers chromogranin, synaptophysin, and CD57. When metastatic melanoma is a consideration, additional melanocytic markers should be added. Suspicion of metastatic carcinoma is supported by positive CK that can then be followed by the appropriate IHC panel to define the specific type and most likely primary site.

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