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Immunohistochemistry in Diagnosis of Soft Tissue Tumours

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Abstract

Immunohistochemistry in soft tissue tumours, and especially sarcomas, is used to identify differentiation in the neoplastic cells. In some cases, specific antigens are expressed; however, an initial panel of antibodies is often required in order to establish the broad lineage, with a subsequent, more focused panel to allow classification. Immunohistochemical evaluation must be employed with the clinical picture, the morphology, and, when necessary, other ancillary techniques such as molecular genetics and cytogenetics.

While some diagnoses are evident on morphology, many soft tissue neoplasms are seen microscopically as spindle cell, epithelioid cell, small round cell or pleomorphic tumours which need further to be characterized. This article reviews selected applications of immunohistochemistry in diagnosis of each of the principal morphological groups, concentrating on areas of most use in daily practice.
Introduction

Soft tissue tumours comprise numerous different tumour types that are classified according to the type of mesenchymal tissue which they resemble. The main object of immunohistochemistry in soft tissue neoplasms, and especially sarcomas, is to identify differentiation in the neoplastic cells. This is straightforward for those tumours that correspond to normal tissue types, but less so for those sarcomas characterized by specific chromosomal translocations since they represent novel lineages. In some cases, specific antigens are expressed; however, an initial panel of antibodies is often required in order to establish the broad lineage, with a subsequent, more focused panel to allow precise classification.

Tumours with adipocytic or osteochondroid differentiation or vascular space formation can often be recognized from the light microscopic appearances. Many soft tissue neoplasms, however, show spindle cell, epithelioid cell, small round cell or pleomorphic morphology on initial examination and require further characterisation by immunohistochemistry, electron microscopy (although this now has a limited role\(^1\)), and genetic analysis. This article reviews selected applications of immunohistochemistry in diagnosis in each of the principal morphological groups, concentrating on areas of most use in daily practice. It is important to remember that immunohistochemical evaluation must be employed with the clinical picture, the morphology, and, when necessary, other ancillary techniques such as molecular genetics and cytogenetics.

Spindle Cell Tumours

BENIGN SPINDLE CELL NEOPLASMS
The differential diagnosis of the principal benign soft tissue tumours is straightforward when the lineage is obvious. Difficulty is sometimes encountered, especially in core biopsies, in assessing malignancy, the morphological criteria for which vary according to the tumour type. In specific situations, immunohistochemistry is occasionally of assistance in distinguishing benign from malignant lesions. For example, diffuse S100 protein positivity in a spindled nerve sheath tumour is more suggestive of schwannoma than malignant peripheral nerve sheath tumour (MPNST), especially if there are portions of capsule in the biopsy. Recently, it has been suggested that a rare CD34-positive cellular fibrous histiocytoma can be distinguished from the commonly CD34-positive dermatofibrosarcoma by the expression of podoplanin (D2-40) in the former but not the latter.\(^2\) Combinations of CD34 with other antibodies can be useful in selected circumstances (Table 1). Cell cycle-related and proliferation markers are of little use in individual cases. The proliferation index with Ki67 can be indicative of malignancy when high, but it can also be high in reactive lesions such as nodular fasciitis.

**INTERMEDIATE SPINDLE CELL NEOPLASMS**

*Fibromatosis* is a non-metastasizing lesion which is a consideration in the differential diagnostic of many types of spindle cell sarcoma. The lesional cells are myofibroblasts which are focally positive for smooth muscle actin (SMA) and occasionally for desmin in scattered cells. S100 protein can be focally positive but CD34 is negative. Nuclear immunoreactivity for beta-catenin is found in variable numbers of nuclei in about 80% of deep fibromatoses, but only 5% of superficial ones.\(^3\) Non-specific paranuclear staining is common in other myofibroblastic lesions and must not be misinterpreted. Nuclear
beta-catenin positivity can also be found in about 40% of solitary fibrous tumours, synovial sarcomas and endometrial stromal sarcomas, as well as in hypertrophic scars. It is absent from gastrointestinal stromal tumour, leiomyosarcoma, inflammatory myofibroblastic tumour and MPNST, which is of use in the differential diagnosis of intra-abdominal spindle cell neoplasms. Conversely, fibromatosis is negative for h-caldesmon, ALK, CD34, CD117 and DOG1.

Solitary fibrous tumour is diffusely positive for CD34 in 95% of cases, as well as for bcl-2, CD99 and beta-catenin. Occasional cells expressing cytokeratin (CK), epithelial membrane antigen (EMA), or S100 protein can be seen, which does not alter the diagnosis. Malignant solitary fibrous tumour, manifesting as spindle or polygonal cell sarcoma contiguous with (or recurrent at the site of a previous) typical solitary fibrous tumour, usually retains CD34 expression diffusely or focally.

Angiomatoid fibrous histiocytoma expresses desmin in about 50% of cases, but myogenin is negative. Other antigens that are sometimes positive are h-caldesmon, SMA (but the morphology is unlike that of smooth muscle tumours), CD99 and EMA. This tumour has three translocations resulting in the fusion genes EWSR1-CREB1 (the commonest), EWSR1-ATF1, or FUS-ATF1. The first two are shared with clear cell sarcoma (see below).

MALIGNANT SPINDLE CELL NEOPLASMS
These include synovial sarcoma, malignant peripheral nerve sheath tumour, leiomyosarcoma, myofibrosarcoma and fibrosarcoma, and spindle cell variants of rhabdomyosarcoma and angiosarcoma. Non-mesenchymal tumours such as sarcomatoid
carcinoma, melanoma and follicular dendritic cell sarcoma can present in soft tissue locations and thereby enter the differential diagnosis.

*Synovial sarcoma* is diagnostically obvious when biphasic. The monophasic form is a spindle cell sarcoma composed of closely packed uniform cells with overlapping nuclei and scanty cytoplasm, while poorly differentiated synovial sarcoma has polygonal or small round cells with high proliferation index. All types of synovial sarcoma exhibit epithelial differentiation with focal positivity for cytokeratins (including CK7 and CK19) and for EMA in most cases. Diffuse expression of bcl-2 and focal positivity for CD99, S100 protein, CD56 and calponin are also found in some, and other non-specific markers of occasional diagnostic value include beta-catenin (nuclear) and calretinin (nuclear). CD34 is almost always negative in synovial sarcoma which is useful in excluding solitary fibrous tumour, since the immunophenotype of these two tumours can otherwise overlap. TLE1, an antibody derived from gene expression profiling studies, is emerging as a highly sensitive (nuclear) marker for synovial sarcoma of all morphologic types. It is also not wholly specific, since occasional examples of nerve sheath tumours can display positivity. However, its sensitivity means that it is useful in excluding the diagnosis of synovial sarcoma when the result is negative. Reduced expression of INI1 (see below) has been described in some synovial sarcomas.

Synovial sarcoma is characterized by a specific chromosomal translocation t(X;18)(p11.2;q11.2), which results in fusion of the *SS18 (SYT)* gene from chromosome 18 with one of several variants of the *SSX* gene on the X chromosome. Recently, an antibody to the *SS18* gene product has been shown to be sensitive for identification of synovial sarcoma but it is not specific and has not found general application.
Malignant peripheral nerve sheath tumours (MPNST) have focal nuclear positivity for S100 protein in up to 67% of cases, which correlates with ultrastructural evidence of Schwann cell differentiation. There are no other ‘specific’ indicators of nerve sheath lineage (although electron microscopy can be contributory in S100 protein-negative examples) but many MPNST are focally positive for CD34, and a variable proportion of cases express GFAP, myelin basic protein, CD57 and nestin. Cytokeratins are occasionally positive but the presence of CK7 or CK19 is more indicative of synovial sarcoma than of MPNST. Immunoreactivity for NSE or PGP9.5 is sometimes found in MPNST but these markers are non-specific and of no value in diagnosis. EMA positivity is occasionally seen in MPNST with Schwannian differentiation, but is most useful in diagnosis of benign perineurioma and its rare malignant counterpart, which usually lack S100 protein. Another useful marker of perineurial cell differentiation is claudin-1 which, as a tight junction-associated protein, has a granular distribution in normal perineurial cells and in perineurioma. Claudin-1 has also been reported as positive in low-grade fibromyxoid sarcoma which can resemble perineurioma, especially in cutaneous lesions, so that genetic analysis (see below) can be required for diagnosis.

Leiomyosarcoma is most often morphologically distinctive, with its non-tapered cells with eosinophilic cytoplasm and squared-off nuclei, arranged in cellular fascicles in a distinctive rectilinear pattern. Immunohistochemically, desmin, SMA, muscle specific actin (MSA), h-caldesmon, calponin and smooth muscle myosin (SMM) are expressed in the majority of leiomyosarcomas. Some examples co-express cytokeratins (frequently with dot pattern), EMA and S100 protein but usually only when the muscle-specific
antigens are present. CD99 can also be seen in a paranuclear dot pattern. CD34 expression is variable but generally negative in leiomyosarcoma, and CD117 is absent from smooth muscle tumours. A spindle cell neoplasm expressing SMA but not desmin or h-caldesmon is unlikely to be a smooth muscle tumour and is more likely to be myofibroblastic.\textsuperscript{31}

\textit{Myofibrosarcomas} are spindle cell or pleomorphic sarcomas which display myofibroblastic differentiation.\textsuperscript{32} Low grade myofibrosarcomas are composed of tapered cells containing ovoid nuclei and punctate nucleoli, arranged in sheets and fascicles.\textsuperscript{33} They are positive for SMA (with a ‘tram-track’ subplasmalemmal accentuation), and usually express calponin and less commonly desmin. H-caldesmon and SMM are negative, distinguishing myofibrosarcoma from leiomyosarcoma.\textsuperscript{34} CD34 and S100 protein are also negative; the combination of CD34 and desmin immunoreactivity is found in mammary-type myofibroblastomas,\textsuperscript{35} but their ultrastructure is more like that of smooth muscle cells than of true myofibroblasts.\textsuperscript{36} \textit{Inflammatory myofibroblastic tumour} (IMT) is a low-grade myofibroblastic malignancy with fasciitis-like, fascicular and sclerosing histological patterns.\textsuperscript{37} In addition to the usual myofibroblastic markers, about half of IMT, especially those in viscera and in children, express ALK, and recent evidence suggests that lack of ALK is prognostically adverse.\textsuperscript{38} In IMT, the ALK gene is rearranged with one of six partner genes, most of which result in cytoplasmic staining on immunohistochemistry, except for \textit{ALK-RANBP2}. The latter gives a distinctive nuclear membranous distribution as RANBP2 binding proteins are localised at the nuclear pore complex.\textsuperscript{39}
Adult fibrosarcoma is now a rare tumour which is by definition negative for all lineage markers (fibrosarcomas express vimentin but this is a wholly non-specific marker which need not usually be included in diagnostic panels). However, specific subtypes of fibrosarcoma are positive for CD34, including those arising in dermatofibrosarcoma, about half of myxofibrosarcomas, and a smaller proportion of myxoinflammatory fibroblastic sarcomas. Some superficial fibrosarcomas that are strongly CD34 positive but lack an apparent dermatofibrosarcomatous component have been shown to have the same COL1A1-PDGFRB fusion gene transcripts as dermatofibrosarcoma. In a CD34-negative fibrosarcoma, an occasional SMA-positive cell can sometimes be seen which is attributable to focal myofibroblastic differentiation, but diffuse or multifocal SMA staining indicates categorization as myofibrosarcoma.

Low grade fibromyxoid sarcoma occasionally has SMA or CD34 positivity (and, as previously noted, can express EMA and claudin-1), but the diagnosis is best confirmed by genetic demonstration of the t(7;16)(q33;p11) or FUS-CREB3L2 (or rarely FUS-CREB3L1) fusion gene, which has also been reported in some examples of sclerosing epithelioid fibrosarcoma.

Spindle cell rhabdomyosarcoma of juvenile (adolescent) or adult type is diffusely positive for desmin, and at least focally so in nuclei for myogenin and MyoD1. The latter is less sensitive, but in the rare sclerosing pseudovascular rhabdomyosarcoma, MyoD1 is more widely expressed than myogenin. It should be noted that these are nuclear antigens and that cytoplasmic staining for either is non-specific and does not denote rhabdomyoblastic differentiation. Myoglobin, a previously widely used
cytoplasmic marker for skeletal muscle differentiation, is of low sensitivity and specificity and is no longer recommended for routine use.

*Spindle cell angiosarcoma* is an uncommon soft tissue sarcoma of skin or soft tissue in which diagnostic vasoformative areas can be present only focally and therefore absent from a core biopsy. The tumour can be identified by its expression of CD31, and less commonly of CD34 and other endothelial markers (see discussion of epithelioid endothelial neoplasms below). CD117 positivity has also been reported in angiosarcoma\(^52\) and in Kaposi sarcoma (KS). The latter, however, demonstrates highly specific immunoreactivity for HHV8 (KSHV) in the nuclei of its spindled and endothelial cells, which is not found in other endothelial neoplasms.\(^53\) Podoplanin (D2-40) is a lymphatic endothelial marker that has a membranous staining pattern in Kaposi sarcoma, but also in some angiosarcomas and gastrointestinal stromal tumours (which also display CD34 and CD117, as in angiosarcoma).\(^54\) Antibodies to vascular endothelial growth factor and its receptor (such as VEGF-C and VEGFR-3) are also immunoreactive in KS.\(^55, 56\)

*Follicular dendritic cell sarcoma (FDCS)*, which sometimes arises in soft tissue sites,\(^57-59\) is characterised by fascicles and whorls of cells in which the nuclei typically display prominent membranes and a speckled chromatin pattern. Immunoreactivity for CD21, CD35 (the two are more sensitive when used together as a cocktail \(^60\) and CD23 is diagnostic, and other markers reportedly helpful in identifying FDCS include fascin, clusterin\(^61\) and D2-40.\(^62\) Many examples are also positive for S100 protein and EMA.\(^63\)

*Sarcomatoid carcinoma* can express cytokeratins focally, especially those of high molecular weight, but can also be negative.\(^64\) The diagnosis can be facilitated by finding
areas of epithelial differentiation or surface dysplasia, or a nested reticulin pattern. Spindle cell carcinoma can also express SMA, but desmin, h-caldesmon, CD34 and S100 protein are absent. SMA-positive/CK-negative sarcomatoid carcinoma, which is usually a pleomorphic neoplasm, can be difficult to distinguish from pleomorphic myofibrosarcoma without knowledge of the history and the location of the tumour.

**Gastrointestinal stromal tumours (GIST)** can arise not only in the gastrointestinal tract but also in extra-gastrointestinal locations, especially omentum, mesentery and retroperitoneum. The two predominant patterns are spindled or epithelioid, which have a similar immunophenotype. GIST typically express CD117, the product of the *KIT* gene, which encodes a receptor tyrosine kinase, and which in GIST shows a variety of prognosis-related mutations.⁶⁵-⁶⁷ A small number of GIST are CD117-negative, and some of these have mutations in the gene which encodes platelet-derived growth factor receptor A (PDGFRA).⁶⁷ These are often epithelioid gastric GIST. The antibody DOG1, identified by gene expression profiling, has high sensitivity and specificity for GIST and can identify most KIT-negative tumours.⁶⁸, ⁶⁹ Other antigens found in GIST include CD34 in 50-100% of tumours (varying with site⁷⁰), SMA (varying inversely with CD34 expression), h-caldesmon, bcl-2 and, less commonly, desmin or S100 protein. Beta-catenin is negative in nuclei, in contrast to fibromatosis.⁵

**Epithelioid and clear cell tumours**

Epithelioid soft tissue neoplasms include epithelioid sarcoma, and epithelioid variants of MPNST, leiomyosarcoma, and fibrosarcoma, as well as clear sarcoma, alveolar soft part sarcoma, PEComa (Table 2), and melanoma and carcinoma when presenting in soft tissue locations.
Epithelioid sarcoma (EpS), of both classical and proximal types, is positive for the epithelial markers cytokeratins and EMA and, in about 50% of cases for CD34. This is particularly useful in making the distinction from primary or metastatic carcinomas, which are CD34 negative. In addition, CK5/6, a useful marker for carcinomas especially those with squamous differentiation, is detectable in only a small proportion of epithelioid sarcomas. EpS generally lacks desmin, S100 protein and FLI-1, which help in diagnosis from rhabdomyosarcoma, melanoma, and epithelioid angiosarcoma respectively. Antibodies to INI1 are negative in about 90% of EpS, in malignant rhabdoid tumours (MRT), in about half the cases of epithelioid MPNST, and in some extraskeletal myxoid chondrosarcomas with rhabdoid cytomorphology. This relates to the location of the SMARC/INI gene on chromosome 22q, which is mutated or deleted in MRT and EpS. Absence of INI1 is extremely useful for diagnosis of these tumours since all other epithelioid or polygonal cell tumours in the differential diagnosis display nuclear positivity for this marker. MRT can be distinguished from EpS by absence of CD34, although clinical data are required to distinguish MRT from CD34-negative EpS.

Epithelioid MPNST differs from its spindle cell counterpart in displaying diffuse S100 protein expression in most cases. It differs from melanoma in having more uniform cytological features, frequent association with a typical spindle cell nerve sheath component, and absence of melanoma-specific antigens. INI-negative cases should not be misdiagnosed as epithelioid sarcoma, or INI-positive cases as epithelioid angiosarcoma, since both of these tumour types are S100 protein negative.
Epithelioid endothelial neoplasms manifest varying combinations of vascular endothelial cell markers. These include CD34, CD31, FLI-1 (in nuclei), FVIIIIRAg, thrombomodulin and CD117. In epithelioid angiosarcoma and malignant variants of epithelioid haemangiendothelioma these antigens are selectively expressed so that not all are detectable by immunohistochemistry. CD31 and FVIIIIRAg are the most specific, although the latter is not very sensitive. These tumours show diffuse nuclear immunoreactivity for INI1, and D2-40 is positive in some cases. In addition, epithelioid angiosarcoma is frequently cytokeratin-positive, while usually lacking EMA. Epithelioid sarcoma, especially the proximal variant, can be morphologically similar to epithelioid angiosarcoma and can also co-express CD34 and cytokeratins, but lacks CD31, FVIIIIRAg, FLI-1, and INI1. Epithelioid sarcoma-like haemangiendothelioma is a very rare low-grade spindle cell neoplasm that displays positivity for cytokeratins, CD31, and FL1-1, but lacks CD34 (Table 1).

PEComa is the term used for a group of clinically disparate tumours composed of supposed perivascular epithelioid cells – spindle or polygonal cells with clear or granular cytoplasm and uniform nuclei, which are arranged in nests around sinusoidal vessels. Members of the PEComa ‘family’ include angiomylipoma, lymphangioleiomyomatosis, clear cell ‘sugar’ tumour of lung and other sites, and clear cell myomelanocytic tumour. PEComas co-express SMA and the melanocytic antigens HMB-45, melan-A, and microphthalmia transcription factor (MITF). It has become recognized that PEComas can also focally express S100 protein, desmin and h-caldesmon as well as TFE3 in some cases. Most PEComas are benign but malignant examples are increasingly reported. The principal differential diagnosis (Table 2) is with clear sarcoma of tendons and
aponeuroses which also expresses melanoma-specific antigens, but which has diffuse S100 protein positivity, lacks SMA and h-caldesmon, and is characterized by a specific translocation t(12;22)(q13;q12) with fusion of EWS and ATF-1 genes. The morphologically similar clear cell sarcoma of the gastrointestinal tract with osteoclast-like giant cells is also S100 protein positive but lacks melan-A and HMB45 expression, and usually has t(2;22)(q33;q12) with EWS-CREB1 fusion. However, examples of both fusions have been described at both sites. The same fusions are also found in angiomatoid fibrous histiocytoma, representing one of several examples of promiscuity among gene fusions.

Alveolar soft part sarcoma has long been an enigmatic tumour in which the occasional report of immunoreactivity for desmin and, for a time, MyoD1, led to the assumption that the tumour showed skeletal muscle differentiation. This tumour, however, is now known to be a translocation-associated sarcoma characterized by t(X;17)(p11;q25) with fusion of TFE3 and ASPL genes. Antibodies to TFE3 are available which demonstrate nuclear positivity in alveolar soft part sarcoma; similar staining is also found in some translocation-associated carcinomas of kidney, in some PEComas and in examples of granular cell tumour.

**Pleomorphic tumours**

Pleomorphic sarcomas include liposarcoma (pleomorphic and dedifferentiated), rhabdomyosarcoma, MPNST, leiomyosarcoma, myofibrosarcoma, and undifferentiated pleomorphic sarcoma (also known as pleomorphic malignant fibrous histiocytoma or
MFH). Undifferentiated or sarcomatoid carcinoma, melanoma and rarely lymphoma can also have similar microscopic appearances.

Pleomorphic neoplasms are composed predominantly of pleomorphic spindle, polygonal and giant cells with mitoses, necrosis and inflammation. In addition the specific subtypes have foci of differentiation perceived either morphologically (lipoblasts) or immunohistochemically (skeletal, smooth muscle, melanocytic or epithelial antigens). In many cases without apparent lineage, the pleomorphic cells have ultrastructural features of fibroblasts with a variable proportion of cells showing myofibroblastic differentiation. Thus, MFH and undifferentiated areas of other pleomorphic sarcomas can display focal SMA positivity, with a characteristic subplasmalemmal linear distribution imparting a ‘tram-track’ appearance. Desmin positivity is occasionally seen but h-caldesmon is absent. It is important to identify pleomorphic sarcomas with any type of myogenic differentiation at immunohistochemical level since, counter-intuitively, they have a worse prognosis than undifferentiated pleomorphic sarcomas. Pleomorphic rhabdomyosarcoma usually has diffuse desmin positivity and focal nuclear staining for myogenin. MyoD1 is a less sensitive marker except in sclerosing pseudovascular rhabdomyosarcoma. In pleomorphic leiomyosarcoma, typical fascicular areas occupying <25% of the pleomorphic tumour are usually required for the diagnosis. However, immunohistochemical evidence of smooth muscle differentiation (desmin, SMA and h-caldesmon) without corresponding morphological features is associated with a worse prognosis. MFH-like undifferentiated sarcomas with widespread SMA positivity can be termed pleomorphic myofibrosarcomas; the distinction is important since
myofibroblastic differentiation, although less well-studied than myoid differentiation, also appears prognostically unfavorable.

*Dedifferentiated liposarcoma*, like well differentiated liposarcoma, has *MDM2* and *CDK4* amplification, detectable by fluorescence *in situ* hybridization (FISH) and also by immunohistochemistry. Most retroperitoneal (and some extremity) undifferentiated pleomorphic sarcomas (MFH) and myxofibrosarcomas express these markers (in nuclei) and are now considered to represent dedifferentiated liposarcomas even when no well-differentiated component remains. In addition, dedifferentiated liposarcoma can express actin, desmin and CD34 focally in the pleomorphic areas, which should not be misinterpreted as leiomyosarcoma.

*Sarcomatoid carcinoma* can appear as a pleomorphic neoplasm. This usually expresses cytokeratins in a multifocal or diffuse distribution that is best demonstrated with a broad spectrum antibody or ‘cytokeratin cocktail’. Even very focal cytokeratin expression should prompt consideration of a primary carcinoma, and it can be accepted as evidence of epithelial differentiation in an organ-based (visceral) pleomorphic neoplasm. It should be remembered, however, that some undifferentiated pleomorphic sarcomas in soft tissue have (aberrant) expression of cytokeratin, usually in a few scattered cells. Also, as previously noted, spindled carcinomas can acquire myofibroblastic differentiation and thereby be focally positive for SMA (but not desmin or h-caldesmon). As always, the final diagnosis must summate all data including clinical and radiological findings.

**Small round cell tumours**
This group of soft tissue neoplasms includes Ewing sarcoma/primitive neuroectodermal tumour (ES), desmoplastic small round cell tumour (DSRCT), alveolar rhabdomyosarcoma (ARMS), neuroblastoma (NB), and poorly differentiated synovial sarcoma (PDSS). Most of these occur predominantly in childhood and adolescence, but are also seen sporadically in adults.\(^\text{106}\) Several of these sarcoma subtypes have specific, translocations and consequent fusion genes that allow precise diagnosis, and in some instances provide prognostic information, using FISH or PCR-based methods.\(^\text{90, 107}\) A molecular pathology service is not always readily available, but the immunophenotypes of these small round cell sarcomas differ sufficiently to enable diagnosis in many cases. Lymphomas, especially of lymphoblastic type, must also be considered in the differential diagnosis, and TdT should be part of the diagnostic panel in the appropriate clinical setting. Similarly leukaemic deposits, and small cell carcinomas might need exclusion by immunostaining for myeloid markers or cytokeratins, neuroendocrine antigens and TTF1 respectively.

**Ewing sarcoma** is a translocation-associated tumour, which can arise in bone or extraskeletal sites; examples with evidence of neural differentiation are termed PNET.\(^\text{90}\) The large majority of ES display diffuse membranous staining for CD99, and some express neurofilament proteins and NSE. About 70% show nuclear immunoreactivity for FLI-1.\(^\text{108}\) A small number are also positive for S100 protein or display dot positivity with broad spectrum anticytokeratin antibodies and membranous staining for EMA. CD56 is usually negative, unlike in small cell neuroendocrine carcinoma and PDSS.\(^\text{109}\) The latter also expresses TLE1, but some cases have a similar immunophenotype to ES and although there are subtle morphological differences (ES tends to be more uniform and
lacks intercellular reticulin), genetic analysis may be required to make the diagnosis. The morphologic spectrum of ES is expanding due to increased recognition of atypical variants aided, in part, by molecular studies.\textsuperscript{110, 111} Although there is a wide range of available antibodies at least three of a panel composed of CD99, FLI-1, HNK1, and caveolin-1 reportedly show immunoreactivity in all cases with caveolin-1 being positive in CD99-negative cases.\textsuperscript{111}

*Desmoplastic small round cell tumour* was originally described in the abdomen and pelvis in adolescent males, and is now recognized in other locations. It shows presumed primitive mesothelial differentiation, and has a characteristic t(11;22)(p13;q12) rearrangement with formation of an *EWS-WTI* fusion gene. Immunohistochemically, DSRCT shows nuclear staining with antibodies to the C-terminal end of WT1 as well as multilineage differentiation with dot positivity for desmin, cytokeratins (CK20 and CK5/6 are negative), and neural markers including chromogranin A, synaptophysin, neurofilaments, and CD56.\textsuperscript{112, 113} However, not all lineage markers are expressed in every case.\textsuperscript{114} DSRCT is usually negative for CD99 but 10-20\% (and especially those with variant translocations) can be positive which does not therefore exclude the diagnosis.\textsuperscript{115, 116}

*Alveolar rhabdomyosarcoma*, as with other rhabdomyosarcoma subtypes, has diffuse cytoplasmic staining for desmin. Myogenin is expressed in a majority of tumour cell nuclei and is more sensitive than MyoD1.\textsuperscript{117} CD56 is also positive as in other types of rhabdomyosarcoma. 70-90\% of ARMS have either t(2;13)(q35;q14) creating a *PAX3-FOXO1* fusion gene or t(1;13)(p36;q14) with resulting *PAX7-FOXO1* fusion.\textsuperscript{118}
Immunohistochemistry with an antibody to PAX5 can detect all translocation-positive cases of either type.\textsuperscript{119}

*Neuroblastoma* can be recognized not only by its clinical context (the vast majority are in patients <4 years old and arise in adrenal or other sympathetic nervous system locations), but also by its expression of NB84, NSE, CD56 and neurofilament proteins.\textsuperscript{120} Antibodies to these markers, which can be used as a panel, are particularly useful in detection of residual disease in bone marrow specimens taken after chemotherapy. Neuroblastoma is negative for cytokeratins, desmin and CD99, which can be particularly helpful in diagnosis of rare examples in adults.\textsuperscript{121}

**Adipose tumours**

The distinction between lipoma and atypical lipomatous tumour (ALT)/well differentiated liposarcoma can be difficult since it depends on focal morphologic subtleties which are not always represented in the samples taken. The diagnosis is facilitated by the demonstration of immunohistochemical positivity for products of the murine double minute type 2 (*MDM2*) and cyclin-dependent kinase 4 (*CDK4*) genes which are overexpressed in ALT as a result of amplification in the 12q14-15 chromosomal region. All ALT are immunoreactive in nuclei for MDM2 (often focally) and 91\% for CDK4 (more diffusely).\textsuperscript{99} These have application not only in the differential diagnosis of lipoma and ALT, but also in determining whether the fatty tissue at the margin of excision of ALT is neoplastic or not. As mentioned above, in dedifferentiated liposarcoma (DDL), which is genetically related to ALT, *CDK4* and *MDM2* are similarly overexpressed.\textsuperscript{102, 122} Their specificity in DDL is reduced since a small proportion of
MPNST, myxofibrosarcomas and embryonal rhabdomyosarcomas are immunoreactive with both but, apart from morphological considerations, the diagnosis of DDL is supported by positivity for these two markers in the differentiated adipose tissue adjacent to the pleomorphic neoplasm.

Conclusion

Existing antibody panels, when correctly selected, have a major role in diagnosis of soft tissue sarcomas. Diagnostically useful new antibodies, such as DOG1 and TLE1, are emerging from gene profiling studies, and in addition to the usual reagents, antibodies to fusion gene products might theoretically be of value in detecting translocation sarcomas. For example, antibodies raised against TLS/EWS-CHOP chimeric oncoproteins have shown high specificity and sensitivity for myxoid and round cell liposarcoma. These approaches can be expected to lead to improved diagnosis of soft tissue tumours and perhaps to more efficient use of immunohistochemistry with limited and therefore less expensive panels.

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Table 1. CD34 and other markers in selected spindle and epithelioid soft tissue tumours

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<td>Epithelioid angiosarcoma</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>CD31, INI1</td>
</tr>
<tr>
<td>Epithelioid sarcoma</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>INI1 negative</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumour</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td></td>
<td>CD117, DOG1, h-caldesmon</td>
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<tr>
<td>Mammary type myofibroblastoma</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Neurofibroma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>EMA, claudin-1</td>
</tr>
<tr>
<td>Perineurioma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>EMA, claudin-1</td>
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<tr>
<td>Peripheral nerve sheath tumour, malignant</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>+ focal</td>
<td>+ (Triton)</td>
<td></td>
</tr>
<tr>
<td>Schwannoma</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>+ diffuse</td>
<td>-</td>
<td>GFAP</td>
</tr>
<tr>
<td>Solitary fibrous tumour</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Epithelioid malignant peripheral nerve sheath tumor</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>INI1 (50% negative)</td>
</tr>
<tr>
<td>Epithelioid sarcoma-like haemangioendothelioma</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>CD31+, FLI1+</td>
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<tr>
<td>Fibromatosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>Beta-catenin</td>
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<tr>
<td>Tumour Type</td>
<td>ALK</td>
<td>Calponin, h-caldesmon</td>
<td>IgG4/IgG Ratio</td>
<td>h-caldesmon, SMM</td>
<td>EMA, CK5/6, CK34betaE12</td>
<td>TLE1, CD99, CD56</td>
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<td>------------------</td>
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<tr>
<td>Inflammatory myofibroblastic tumour</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td></td>
<td>ALK (50%)</td>
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<tr>
<td>Myofibrosarcoma</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>±</td>
<td>Calponin, h-caldesmon negative</td>
</tr>
<tr>
<td>Retroperitoneal fibrosis</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>IgG4/IgG ratio</td>
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<tr>
<td>Smooth muscle tumours</td>
<td>−</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td></td>
<td>h-caldesmon, SMM</td>
</tr>
<tr>
<td>Spindle cell carcinoma</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>EMA, CK5/6, CK34betaE12</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>±</td>
<td>−</td>
<td>TLE1, CD99, CD56</td>
</tr>
</tbody>
</table>

± = in some cases,  CK = cytokeratin. EMA = epithelial membrane antigen, GFAP = glial fibrillary acidic protein, SMM = smooth muscle myosin.
Table 2. Differential diagnosis of soft tissue tumours with clear cells

<table>
<thead>
<tr>
<th></th>
<th>S100 protein</th>
<th>HMB45</th>
<th>Melan-A</th>
<th>SMA</th>
<th>Desmin</th>
<th>TFE3</th>
<th>Other diagnostic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell sarcoma (soft tissue)</td>
<td>+ (diffuse)</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>t(12;22)(q13;q12), EWSR1-ATF1</td>
</tr>
<tr>
<td>Clear cell sarcoma (GIT)</td>
<td>+ (diffuse)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>t(2;22)(q33;q12), EWSR1-CREB1</td>
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<tr>
<td>PEComa</td>
<td>± (focal)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td></td>
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<tr>
<td>Paraganglioma</td>
<td>- (except sustentacular cells)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CD56+, NF+, CG+</td>
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<tr>
<td>Alveolar soft part sarcoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>+</td>
<td>t(X;17)(p11;q25), ASPL-TFE3</td>
</tr>
</tbody>
</table>

± = in some cases, GIT = gastrointestinal tract, SMA = smooth muscle actin, NF = neurofilament, CG = chromogranin,