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UTILITY OF IMMUNOHISTOCHEMICAL MARKERS IN THE DIAGNOSIS OF COMMON SOFT TISSUE SARCOMAS IN ADULTS: A BRIEF REVIEW

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ABSTRACT

Soft tissue sarcomas are a complex group of neoplasms arising from mesenchymal tissues. They present great difficulties for the practicing surgical pathologist due to their variety and several lines of differentiation. Their mimics include many pseudosarcomatous benign lesions and non-mesenchymal malignant lesions. The differential diagnostic considerations are many and special studies like immunohistochemistry are usually necessary to reach a definite conclusion. Immunohistochemistry is used as a routine diagnostic tool for soft tissue tumors. An extensive array of antibodies are available now to facilitate the characterization of soft tissue tumors. Because of its relatively low cost, simple technique and the availability of a large number of increasingly sensitive and /or specific antibodies, it has become the main diagnostic tool. Immunohistochemistry should be used as complement to morphological analysis. Use of single immunostain can lead to misdiagnosis and hence it is advised to use a panel of antibodies.

KEYWORDS: Sarcoma, Immunohistochemistry, Antibodies, Immunostain.

INTRODUCTION

Immunohistochemistry (IHC) is used as a routine diagnostic tool for soft tissue tumors.[1] Soft tissue sarcomas(STS) include numerous complex diagnostic entities and hence pose a major diagnostic challenge to the pathologist.[2] The problems include deceptive bland appearance in some tumors and the recognition of tumors of intermediate malignancy.[2] The expectations from a pathologist includes accurate tumor diagnosis, with site of origin, and sub-classification of the tumor type.[3] Soft tissue sarcomas predominantly occurs in adults and account for 1% of all malignancies. Histological diagnosis of soft tissue tumors is considered as one of the most difficult areas in routine histopathology practice, which is due to morphological overlap between various tumors and also a few reactive lesions.[4] Immuno histochemistry plays an important role in the diagnosis of soft tissue sarcomas.[5] It helps in the classification of a soft tissue tumor, assists in identifying their line of differentiation, and to a lesser degree, predicting their clinical behavior. Earlier days, IHC was considered as an ancillary technique along with routine light microscopy, but now many pathology laboratories have incorporated IHC as a routine procedure in the diagnosis of STS.[6]

REVIEW

Extensive online literature search was done on sites like Pubmed, Google scholar and Medline. Relevant articles about a few common adult soft tissue sarcomas were selected and reviewed. The following soft tissue sarcomas, which are relatively common in adults, are reviewed along with the role of specific IHC markers useful in the diagnosis of these tumors.

- Pleomorphic Rhabdomyosarcoma.
- Leiomyosarcoma of soft tissue.
- Synovial Sarcoma.
- Adult Fibrosarcoma.
- Malignant Peripheral Nerve Sheath Tumor(MPNST).
- Liposarcoma.
- Angiosarcoma.
- Alveolar Soft Part Sarcoma(ASPS).

Pleomorphic Rhabdomyosarcoma

It is a high-grade sarcoma occurring exclusively in adults. It consists of bizarre round and polygonal cells which show evidence of skeletal muscle differentiation.[7,8] Pleomorphic rhabdomyosarcoma arise in the older adults and morphologically are very similar to other pleomorphic sarcoma.[4]

Pleomorphic Rhabdomyosarcoma(Pleomorphic RMS) usually have diffuse desmin positivity and focal nuclear staining for myogenin. Another less sensitive marker in MyoD1.[9] Other markers include myoglobin and fast myosin.[7] Immunohistochemistry is used extensively to distinguish RMS from it mimics. Myogenin has been regarded as highly specific for myogenous differentiation.[10] Sensitivity for MyoD1 in RMS has ranged from 71% to 91% in formaldehyde fixed, paraffin-embedded tissue. Myogenin expression in non-neoplastic, atrophic or regenerative skeletal muscle is a potential source of diagnostic error.[10] Spindle cell rhabdomyosarcoma of the adult shows diffuse positivity for desmin. Also they show focal positivity of nuclei for myogenin and MyoD1. MyoD1 is less sensitive in spindle cell rhabdomyosarcomas whereas MyoD1 is more expressed than myogenin. One marker which is no longer specific or sensitive for skeletal muscle differentiation is myoglobin.[9]

Other markers like Vimentin and muscle specific actin have less optimal specificity for RMS and may not be expressed in the least differentiated tumor.[10]

Desmin is highly sensitive for all tumors with skeletal muscle differentiation but it is somewhat nonspecific for skeletal muscle as it also stains smooth muscle cells and occasionally even stains myofibroblasts.[2] Desmin should never be used alone to diagnose RMS. In MyoD1 and myogenin negative tumors, Hematoxylin and Eosin morphology and ultrastructure are needed to classify a pleomorphic sarcoma as pleomorphic rhabdomyosarcoma[1,2,5]

Leiomyosarcoma

Soft tissue leiomyosarcomas (LMS) are malignant tumors arising from smooth muscle cells. They usually show cytological atypia, mitotic activity and necrosis [4]. They account for nearly 20% of all adult soft tissue sarcoma.[11]

Leiomyosarcoma, well differentiated type usually demonstrates a growth pattern of perpendicularly intersecting cellular fascicles composed of elongated spindle shaped tumor cells with abundant, brightly eosinophilic cytoplasm. In poorly differentiated tumors, classic features may be focal or difficult to appreciate due to increase in the number of pleomorphic cells.[11,19] Commonly used markers include Desmin, (Seen in all type of muscle) Smooth Muscle Actin(SMA), H-Caldesmon and smooth muscle myosin. Muscle markers are seen in poorly differentiated leiomyosarcoma.[11] Caldesmon is a useful marker expressed by smooth muscle cells in leiomyosarcomas. CD34 and CD117 are absent in LMS.[9] A spindle cell neoplasm which is positive for SMA but not for desmin or H-Caldesmon is unlikely to be a smooth muscle tumor and it is more likely to be myofibroblastic origin.[9] SMA negativity should be a reason to doubt a diagnosis of smooth muscle tumor.[1] The available markers for leiomyosarcomas can help to confirm the diagnosis but none of the markers alone is specific or sensitive. SMA is the most sensitive but not specific and it is also positive in many other tumors. Desmin is positive in only 50-70% of cases but is more specific and it is also positive in myofibroblastic lesions. H-caldesmon is specific, but depends upon the location and differentiation of tumor. Diagnostic confusion might arise due to unexpected positivity of cytokeratin and Epithelial membrane antigen (EMA) and this should be kept in mind.[5]

Liposarcoma

Liposarcomas are malignant adipocytic tumors accounting for approximately 20% of all sarcoma [12]**.** Four types of liposarcomas are described in the recent World health organization classification of soft tissue and bone tumors.[9] They are

(i) Atypical lipomatous tumor / well differentiated liposarcoma
(ii) De-differentiated liposarcoma.
(iii) Myxoid Liposarcoma.
(iv) Pleomorphic Liposarcoma.

All the types are associated with cytogenetic abnormalities. MDM2 immunohistochemistry is useful in atypical lipomatous tumor / well differentiated liposarcoma.[13] Dedifferentiated Liposarcoma is a morphological progression from well differentiated liposarcoma to a non lipogenic sarcoma. Significant genetic overlap is seen between atypical lipomatous tumor and de-differentiated liposarcoma. MDM2 and CDK4 are observed consistently in De-differentiated liposarcoma. This MDM2 immunopositivity is an extremely helpful diagnostic finding in dedifferentiated liposarcomas exhibiting myogenic differentiation. Myxoid Liposarcoma accounts for 30-35%of all liposarcomas. Molecular genetic studies are extremely useful in diagnosing myxoid liposarcomas and also to differentiate it from extraskeletal myxoid chondrosarcoma. IHC has got a limited role in diagnosing myxoid LPS.[13]

Pleomorphic liposarcoma is the the least common of all liposarcomas. It exhibits complex karyotypes and hence molecular genetics does not play a role in its diagnosis.[13] It is a high grade sarcoma and presence of lipoblasts is required for diagnosis on morphology[14] S-100 immunopositivity might be helpful in highlighting the presence of multivacuolated lipoblasts[13] MDM2, CDK4 and P16 immunomarkers together have increased diagnostic specificity and helps in differentiating atypical lipomatous tumor/well differentiated liposarcoma from de-differetiated liposarcoma and undifferentiated sarcoma.[15,16] Myxoid liposarcoma should be distinguished from myxofibrosarcoma and myxoid chondrosarcoma. S-100 is often expressed in myxoid liposarcomas whereas only vimentin and occasionally Pan-actin HHF35 are positive in myxofibrosarcomas. A

new marker SOX9 is expressed by myxoid chondrosarcoma.[6]

Synovial Sarcoma

Synovial Sarcoma is classified under the tumors of uncertain differentiation in the new WHO classification for tumors of soft tissue and bone.[7] This tumor arises in deep soft tissue of extremities and head and neck. Cytogenetic analysis shows a specific chromosomal translocation t(X;18)(p11;q11) which is present in more than 90% of cases.[4] Histologically, they are classified into biphasic, monophasic and poorly differentiated types.[17] Epithelial differentiation is exhibited in all types of synovial sarcomas. Focal positivity for CK7, CK19 and EMA is seen in most cases.[9] TLE1(transducin-like enhancer protein 1) is emerging as a potential marker for all types of Synovial Sarcoma with high nuclear sensitivity.[9] Hence, it is useful in excluding a diagnosis of synovial sarcoma when the result is negative. TLE1 is not specific for Synovial Sarcoma, but it also occur in solitary fibrous tumor and MPNST.[1] Another new marker INI1 is useful marker for poorly differentiated Synovial Sarcomas.[17,9] Its reduced expression is described in some Synovial sarcomas.[17] CD34 is usually negative. The use of both EMA and CK7 appear to yield the best possibility of detecting epithelial component in Synovial sarcoma. SMA and Desmin are usually negative. S-100 is not of much use.[2] One should be careful in overdiagnosing Synovial sarcomas due to keratin positive normal cells like endothelial cells.[1] Molecular confirmation in considered a diagnostic gold standard for synovial sarcoma, but a optimal IHC panel comprising EMA, BCL2, MIC2, CD34 and CK7 along with awareness of TLE1 expression in other tumor is also useful in the diagnosis.[18]

Malignant Peripheral Nerve Sheath Tumor

Malignant peripheral nerve sheath tumors (MPNSTs) are spindle cell sarcomas accounting for 3% to 10% of all soft-tissue sarcomas. It is a malignant tumor arising from a peripheral nerve, or from existing benign nerve sheath tumor or in patient with NF1 disease. A number of benign soft tissue tumors and other sarcomas and non-epithelial tumors appear similar to MPNST.[19,20]

Immunohistochemistry has an important role in differentiating MPNST from other mimics. Recently, new marker, SOX10 is reported to be a highly specific marker for MPNST when differentiation is required with a Synovial sarcoma.[21] Some of the markers which are positive in MPNST are CD34, S-100 and occasionally CK. MPNST have focal nuclear positivity for S-100 protein and many MPNST are focally positive for CD34 along with Glial Fibrillary Acidic Protein (GFAP), Myelin Basic Protein(MBP), CD57 and Nestin. Cytokeratins are occasionally positive but positivity for CK7 or CK19 should indicate more towards a Synovial sarcoma than MPNST.[9] S-100 is positive in 50-80% of MPNST and the positivity is usually focal and limited to a few tumor cells. Epithelioid MPNST shows diffuse positivity.[5] Desmin is positive in MPNST with rhabdomyoblastic differentiation.[5] S100 is also positive in metastatic melanoma hence S100 positive MPNST will be face a diagnostic difficulty in the absence of melanoma specific markers.[1]

Adult Fibrosarcoma

WHO has defined adult fibrosarcoma as a malignant tumor composed of fibroblasts with variable collagen and architecture resembling "herring bone". It is a very rare soft tissue sarcoma. They are composed of monotonous spindle cells with moderate pleomorphism. Tumors which shows greater degree of pleomorphism has been classified as undifferentiated pleomorphic sarcomas. By IHC, they express vimentin and occasionally may show positivity for SMA, indicating a myofibroblastic differentiation. A fibrosarcoma arising in a dermatofibrosarcoma protuberans are CD34 positive. Other spindle cell sarcomas may mimic adult fibrosarcoma like MPNST, Spindle cell variant of rhabdomyosarcoma and spindle cell variant of angiosarcoma. A panel of IHC markers which includes cytokeratins, S100 protein, CD34, SMA, Desmin, myogenin allows distinction.[22]

The diagnosis of fibrosarcoma is mostly by exclusion based upon the microscopic appearance and the absence of other markers except Vimentin.[6]

Alveolar soft part sarcoma (ASPS)

ASPS in a rare neoplasm arising in the soft tissues of lower limbs in adults, usually presenting as a slow growing mass.[23] Microscopy shows a nested or organoid pattern of growth, with uniform sized cells, polygonal to round with well-defined borders. Two markers which are highly sensitive for ASPS are TFE3 and Cathepsin K.[23] ASPS are consistently negative for keratin, EMA, Paired box 8 (PAX 8), and Human melanoma black (HMB-45). ASPS is also characterized by a chromosomal alteration which is specific for it. The unbalanced translocation is der (17) t (X; 17) (P11 ;q 25).[24]

ASPS needs to be differentiated from other potential mimickers like Clear cell sarcoma and Melanoma. ASPS is strongly positive for both TFE3 and Cathepsin K, whereas TFE3 is negative for both melanoma and clear cell sarcoma.[23]

Angiosarcoma

Angiosarcomas are vascular tumors which arises from the endothelial cells of blood vessels and lymphatics.[25] They produce a diagnostic difficulty especially a non or poorly formed vasculature or vasoformative cases. In such cases it is impossible on morphologic ground to differentiate it from undifferentiated carcinoma or other sarcomas. The markers useful for angiosarcoma diagnosis are Factor VIII related antigen (FVIII RA), Ulex europeus lectin type 1 (UEA-1), and CD31. Other vascular markers used are CD34, FLI-1[26]. Factor VIII

is highly specific for endothelium but it is less sensitive than CD31. Most sensitive marker for angiosarcoma is CD31 which show a membranous positivity in more than 90% of cases.[2] ERG (ETS related gene) is a good diagnostic novel endothelial marker for angiosarcoma[1] Some IHC markers have great diagnostic utility in few tumors. They are Desmin and Myogenin for Rhabdomyosarcoma; CD34 and CD31 for Epithelioid Angiosarcoma; Cytokeratin, EMA and CD34(-ve) for Synovial Sarcoma(Spindle cell variant); CD117 for GIST; Cytokeratin, EMA and CD34 for Epitheliod sarcoma; S100 and HMB45 for Clear Cell Sarcoma, and lastly, Cytokeratin, Desmin and WT1 for Desmoplastic Small Round Cell Tumor.[6] There are some IHC markers which lack diagnostic specificity and hence they are not recommended in diagnostic practice. Vimentin is present in most mesenchymal cells and generally useless for specific diagnosis. Myoglobin antibodies which are available, frequently leads to incorrect conclusion of skeletal muscle phenotype. Bcl2, present in diverse type of tumors lacks lineage specificity. Similarly, alpha-1 antitrypsin and alpha-1 antichymotrypsin also lacks lineage specificity.[1]

Table 1. IHC markers useful for selected soft tissue sarcomas.[6]

	TUMOR	**IHC Markers**
Spindle cell Sarcomas	MPNST	S100 HNK 1 Vimentin PGP 9.5
	Synovial Sarcoma	Vimentin EMA Cytokeratin CD99
	Leiomyosarcoma	SMA Desmin Pan Actin Caldesmon
	Fibrosarcoma	Vimentin
Pleomorphic Sarcomas	Pleomorphic Rhabdomyosarcomas	Actin (HHF 35) Desmin Myogenin MyoD1
	Pleomorphic Leiomyosarcoma	Desmin Caldesmon SMA Actin (HHF 35)
	Pleomorphic Liposarcoma	Vimentin S-100 (Occasional
Myxoid Soft Tissue Sarcomas	Myxoid Liposarcoma	Vimentin S100
	Myxoid Chondrosarcoma	Vimentin S-100 (variably) HNK-1 SOX9
	Myxofibrosarcoma	Vimentin Actin (variably) CD68
	Myxoid Leiomyosarcoma	Actin Desmin Caldesmon SMA

CONCLUSION

IHC is an invaluable tool as an ancillary technique. It provides good information to the pathologist in establishing a diagnosis. Some of the immunomarkers also provides relevant prognostic and therapeutic information. Microarray technology promises a better future for IHC that will select new useful proteins for diagnosis, prognosis, and treatment decision making. IHC has a proven utility and is an integral component in proper analysis of soft tissue tumors. A simple panel of 6 markers which include CD34, Desmin, EMA, keratin cocktail AE1/AE3, S100 protein alpha SMA can be used initially. This panel helps in differential diagnosis of fibroblastic, myoid, nerve sheath & perineural cells, Synovial sarcoma, Epitheliod sarcoma and others. However this panel must be supplemented with well

selected additional markers. However, one should also know that no single marker is totally monospecific for a given tumor type. Another pitfall is that, differentiation between benign and malignant proliferation cannot be done exclusively by IHC and histological assistance is mandatory. IHC, when used in the context of careful histological evaluation is a very useful and efficient tool in the hands of experienced pathologist who is aware of all diagnostic entities.

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