Original Article

Diagnostic Value of Immunohistochemistry in Soft Tissue Tumors

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ABSTRACT

Background: Diagnosis of soft tissue tumors is a great challenge to pathologist but with the help of immunohistochemistry (IHC), proper analysis and diagnosis of soft tissue tumours can be made easy. The main use of immunohistochemistry in soft tissue neoplasms especially in sarcomas is to identify differentiation in the neoplastic cells.IHC are used as a panel or as single marker depending on the tumor.

Methods: A total of 513 soft tissue tumor (STT) cases were collected and reviewed. The cases were separated into as benign, intermediate and malignant cases. In 90 cases of STT in which immunohistochemistry (IHC) were used was further analysed and classified depending on the various positivity and negativity of the marker used .The significance of IHC was also analysed.

Result: In our study of 513 cases of STT there were 380 benign cases, 90 malignant cases and 43 intermediate cases. A total of 90 cases of sarcomas were present out of which 54% cases required IHC, 20 % cases required IHC to support the diagnosis but 26% of cases did not require IHC, the diagnosis was made on haematoxylin and eosin (H&E). The IHC markers helped in correct diagnosis of STT cases.

Conclusion: Immunohistochemistry plays an important role in grading and giving precise diagnosis of soft tissue tumors. So it is important to use IHC to diagnose STT where haematoxylin and eosin did not give a precise diagnosis. Perfect diagnosis of STT helps in the correct therapeutic management of patients.

Keywords: Soft Tissue Tumors, Immunohistochemistry Markers, Soft Tissue Sarcomas, Diagnosis.

Introduction

Soft tissue tumours (STT) are composed of numerous different tumour types that are classifiedaccording to the type of mesenchymal tissue which they resemble.[1] Immunohistochemistry (IHC) plays an important role in STT diagnosis.[2] Immunohistochemistry has been introduced in the 80's and it is the application of immunologic principles to the study of cells and tissues.[3] IHC helps to rule out a non-mesenchymal tumor, followed by trying to define mesenchymal cell lineage. As there are many complex diagnostic entities recognized of an intermediate malignant category including some tumors with a deceptively bland histological appearance, it is important to diagnose these tumours.[4,5,6] The use of progressively small amounts of tissue for diagnosis highlights the importance of IHC. Immunohistochemistry will not be a substitute for skilled interpretation of conventionally stained microscopic specimens. As STT have several line of differentiation, numerous pseudo benign lesion and non mesenchymal malignant tumor are present which has to be differentiated for therapeutic reasons. So IHC is the major tool and plays an important role in determining the diagnosis of most of the soft tissue sarcomas. Panel of markers has to be used and quality of IHC technique and interpretation is also important.[2] But wide "random" panels can be misleading.

Because of the lack of sensitivity or specificity of markers, and of frequent "aberrant" immunoreactivities, the use of a single immunostain can lead to misdiagnoses. It is difficult to recommend panels as the choice of antibodies will change according to the specific clinicopathological differential diagnosis. However, a few "basic" panels can be suggested, depending on the morphological category of the tumor.

Histogenesis	Markers
Mesenchymal(general)	Vimentin
Epithelial	Cytokeratins (CK), epithelial membrane antigen (EMA)
Smooth Muscle	Desmin, smooth muscle actin (SMA)
Skeletal Muscle	Myoglobin
Fibrohistiocytic	CD68
Endothelial	CD34,CD31
Lipomatous	Immunohistochemistry generally not used
Neuroendocrine, Ewing's sarcoma/ PNET	Neuron-specific enolase, CD99

"Aberrant positivities", such as the frequent expression of cytokeratins in leiomyosarcomas, Ewing sarcomas or

epithelioidangiosarcomas should be known.[7] The type of positivity whether nuclear, cytoplasmic, membranous and expected extent like diffuse or focal positivity should also be known.The extent of the immunoreactivity is also important e.g. S100 will be diffusely positive in benign nerve sheath tumors but only focally positive in MPNSTs.

As the treatment is different, it is extremely important to always rule out a non-sarcomatous tumor: carcinoma, melanoma, and lymphoma using adequate immunostains. Some of the commonly used markers are discussed below.

Epithelial markers Cytokeratins and EMA should be included in the immunohistochemical panel of most spindle and pleomorphic cell malignant tumors.

Lymphoid markers LCA, TdT should be included in the differential diagnosis of round cell neoplasms. Desmin is positive in rhabdomyosarcomas and in 70-90% of smooth muscle tumors. Caldesmon it is relatively specific for smooth muscle differentiation.[8,9]Myogenin is very specific in smooth muscle differentiation.

CD31is the most sensitive and specific vascular marker is, which is expressed by 95-100% of benign and malignant vascular tumors. However, the expression of CD31 by macrophages is a potential pitfall. CD68 is useful in the identification of histiocytic proliferations or reactive lesions. CD99 is expressed by most Ewing sarcomas, in a membranous fashion. FLI-1 expression in Ewing sarcoma / PNET is characteristic. Vimentin that is still widely used, although it does not provide any reliable diagnostic information. Vimentin is an important marker to detect the preservation of antigenicity of tissues. Ki 67 is the most commonly used proliferation marker. Ki-67staining is a more useful procedure than counting mitotic figures for assessing prognosis of the patients with sarcoma.

Materials and Methods

From January 2006 to June 2011 all soft tissue tumor diagnosed in the department of pathology, Sri Ramachandra Medical College and ResearchInstitute were retrieved from the surgical pathology files and analysed. A total of 513 cases were collected and reviewed. Out of these cases benign, intermediate and malignant cases were identified. The cases which lacked details of important clinical and pathological features were excluded from the study. The gross characteristics of the tumor which included the tumor location, size, necrosis, circumscription, cut section and secondary changes were obtained from the pathology report registers. Hematoxylin and eosin (H&E) stained sections of all the STT were reviewed and analysed. All the STT cases were analysed on the basis of cases which needed IHC for confirmation of the diagnosis, tumors which were diagnosed based only on H&E without IHC and cases which needed IHC for diagnosis. Most of the cases which needed IHC were malignant soft tissue tumor.

Result

Our study was a retrospective study in which we had analysed 513 cases of soft tissue tumours and retrieved cases which needed IHC in the period between January 2006 to June 2011, in the Department of Pathology, SRMC and RI.

There were 380 benign cases, 90 malignant cases and 43 intermediate cases. A total of 90 cases of sarcomas were present. 16 % of synovial sarcomas were common among STT sarcomas followed by 14% fibrosarcoma and 13 % leiomyosarcoma. (Fig: 1, Table: 1) Out of these, many STT cases needed IHC for diagnosing and confirmation. So the analysis of usefulness of IHC in sarcomas showed that it was not needed for the diagnosis of sarcomas in 26% of cases which was done on H&E. IHC was done to support the haematoxylin and eosin diagnosis in 20% of case and diagnosing 54% of sarcoma cases was possible only with IHC.

Discussion

Soft tissue sarcomas (STSs) are heterogeneous group of malignant tumors with a wide spectrum in terms of

Table: 1: Total number of histological types of STT.

S.NO	Histological type	Total cases
1.	Benign	380
2.	Intermediate	43
3.	Malignant	90
	Total cases	513

Table 2: Frequency of various histological types of sarcomas.

HISTOLOGICAL TYPE	FREQUENCY	PERCENTAGE
Synovial Sarcoma	16	16.5%
Liposarcoma	8	8.2%

HISTOLOGICAL TYPE	FREQUENCY	PERCENTAGE
Fibrosarcoma	14	14.4%
Leiomyosarcoma	13	13.4%
Rhabdomyosarcoma	5	5.2%
MPNST	4	4.2%
Ewing's/PNET	5	5.2%
Angiosarcoma	1	1%
Alveolar sarcoma	1	1%
DSRCT	1	1%
Pleomorphic sarcoma	8	8.3%
ExtraskeletalChondrosarcoma	1	1.0%
SclerosingEpitheloidFibrosarcoma	2	2.1%
Low Grade Fibromyxoid Sarcoma	1	1%
DFSP	7	7.2%
High Grade STS	10	10.3%
Total	**97**	**100%**
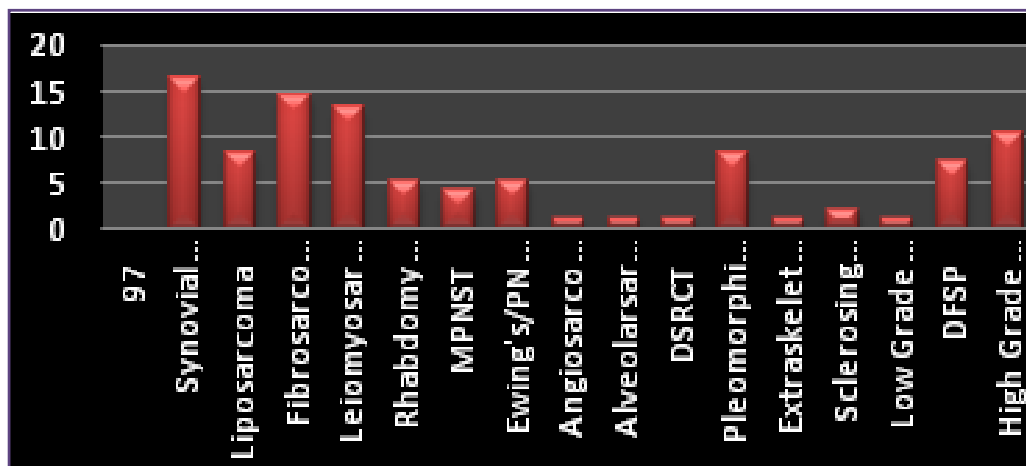
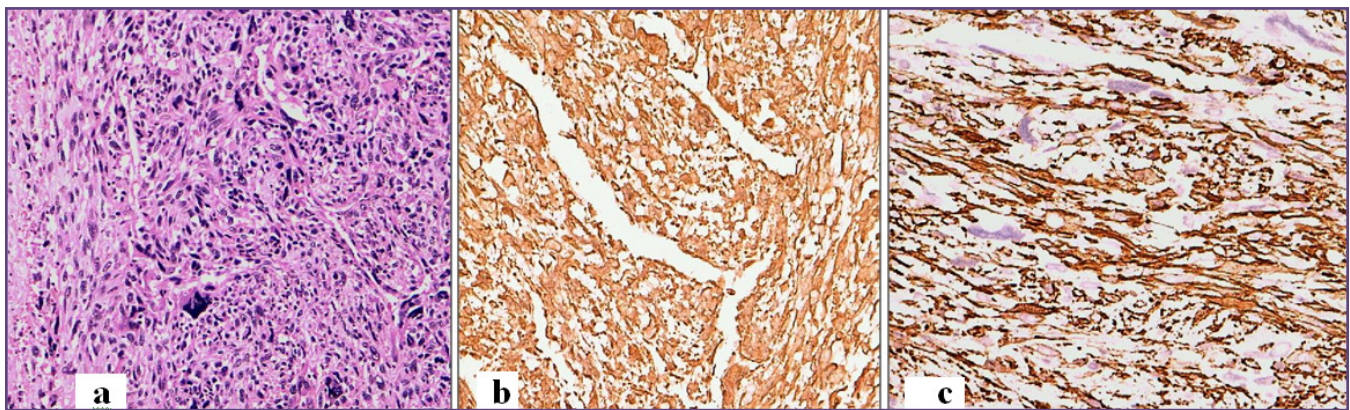**Fig. 1: Various histological subtypes of sarcomas.**

Fig. 2a: H&E.10x-Pleomorphic Leiomyosarcoma: Highly pleomorphic cells with giant cells.; 2b: 20X - Immunohistochemical stains for Vimentin and 2c: SMA shows diffuse cytoplasmic positivity in the tumour cells.
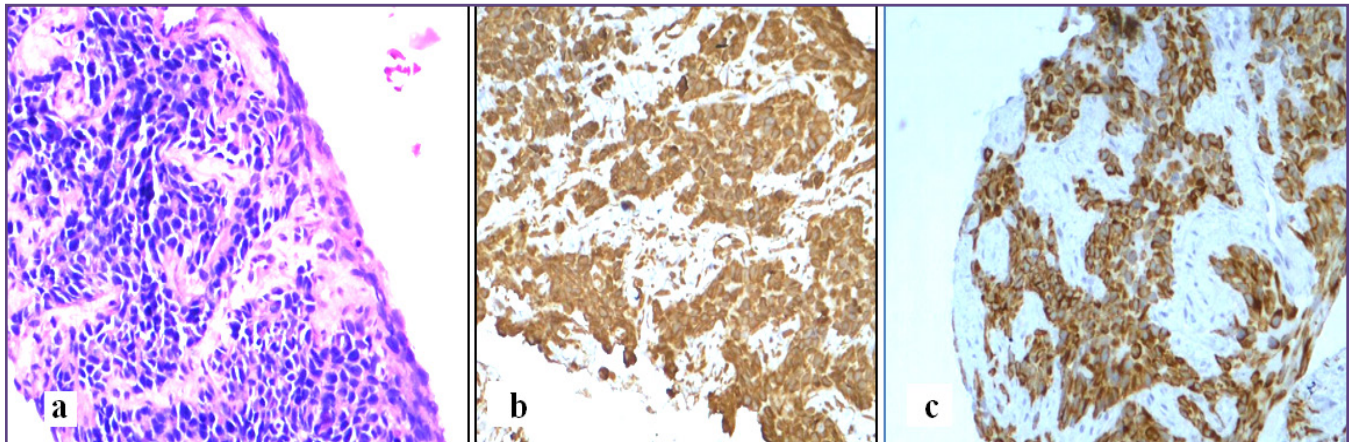
Fig. 3a: H&E. 10x -Rhabdomyosarcoma :Small round blue cells with moderate amount of eosinophilic cytoplasm. 3b: 20x -Immunohistochemical stains for Vimentin and 3c :Desmin shows cytoplasmic positivity in the tumor cells.
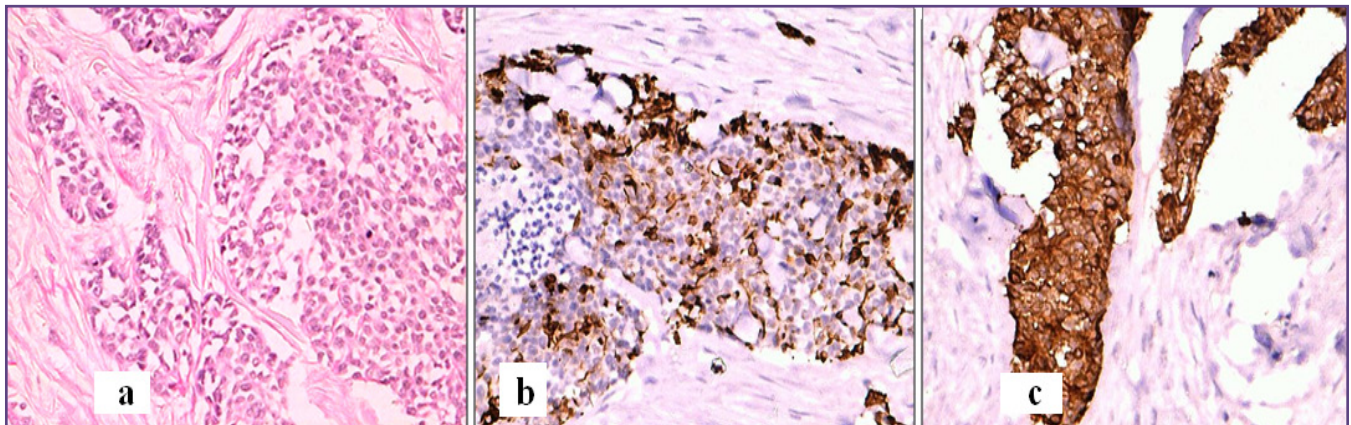

Fig. 4a: H&E. 100x - DSCRT : Sheets of small round cells surrounded by desmoplastic stroma.4b: 100x :Desmin shows diffuse cytoplasmic. 4c: EMA membrane positivity in the tumour cells.
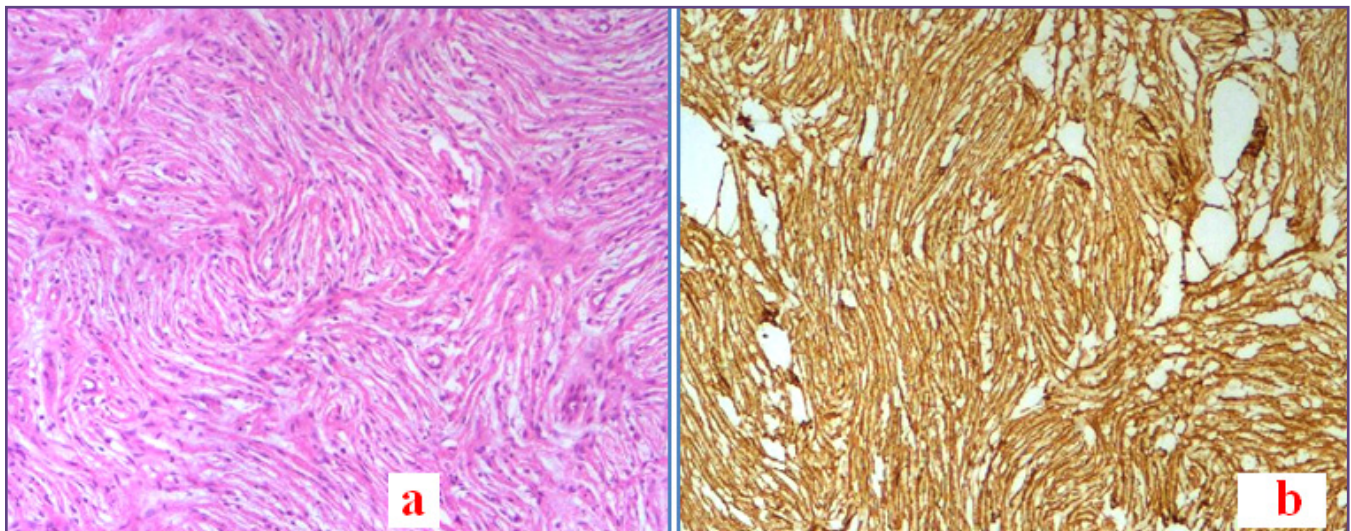

Fig. 5a: H&E. 100X-DFSP :Spindle shaped cells arranged in storiform pattern. 5b:200x CD34 shows cytoplasmic and membrane positivity in the tumour cells.

histologic type and prognosis.[1] STT has been classified according to the WHO as benign, intermediate and malignant. It can occur in any age group. Histological grade of sarcomas is the most important prognostic factors. A total of 513 cases were studied, of which 380 cases were benign, 43 cases were intermediate and 90 cases were malignant. The study was undertaken to analyze the importance of immunohistochemistry (IHC) in soft tissue tumours. Among the immunohistochemical analysis of malignant tumours 54% of cases required immunohistochemistry (IHC) for the diagnosis, were as in 20% of cases IHC was useful to support the diagnosis. Immunohistochemistry is the most valuable adjuvant to hematoxylin and eosin staining in diagnostic histopathology. It is important in diagnosis of STT because of their variety with several lines of differentiation and the frequent difficulty of diagnosis with numerous psedosarcomatous benign lesion and non mesenchymal tumours. JM Coindre et al has shown that IHC is part of the definition of the tumours and is determinant for the diagnosis of rabdomyosarcoma (RMS), desmoplastic small round cell tumour (DSRCT) and synovial sarcoma.(Fig:2,3,4) Desmin and myogenin will be positive in the diagnosis of RMS.[10] Our study also showed that these sarcomas needed IHC for the diagnosis. The desmin marker was useful in our study to identify all the RMS. Truong lo et al has also shown the same utility of desmin in RMS. As myogenin is more specific to skeletal muscle differentiation it is useful in diagnosing alveolar RMS.[11] Malignant vascular tumours like angiosarcoma also need IHC to diagnose. CD 31 is the best marker for vascular lesion. In our study synovial sarcomas with biphasic pattern, IHC was useful to confirm the diagnosis. But in monophasic and poorly differentiated subtype IHC has to be done to diagnose the sarcomas. Folpe Al and Lopes JM et al studies showed that IHC is useful for the diagnosis of monophasic and poorly differentiated synovial sarcomas[12,13] which correlated with our study. For the synovial sarcoma cases for which Bcl-2 was needed, it was done and positive reaction was present. Suster S et al has also shown that the Bcl2 is positive in synovial sarcomas but it is a non specific marker.[14]A DSRCT was present among the sarcomas in our study which showed desmin, CK and EMA positive with SMA positivity in the stroma. Ordonez NG et al IHC finding of DSRCT with 90% positivity for CK, EMA and desmin with dot like positivity.

JM Coindre et al studies also showed that IHC may be helpful in the diagnosis of sarcomas like Ewing's sarcoma, Leiomyosarcoma, Dermatofibrosarcoma protuberance (DFSP) and myxoid liposarcoma (Fig:5) and it is also useful to support the diagnosis. For low grade sarcomas like DFSP IHC CD34 is positiveand it is done to rule out benign fibrous histiocytoma.[15] The DFSP cases in our study showed storiform pattern of cell arrangement but CD 34 stain was done to support the diagnosis. Del Tos AP et al study showed the diagnostic utility of S100 in myxoid Liposarcoma which can be positive in some cases. Few of our myxoid liposarcoma (LPS) also showed S100 focal positivity.

Sarcomas with no specific immmunohistochemical profile is seen in fibrosarcoma, pleomorphic malignant fibrous histiocytoma (MFH), these sarcomas will not display any specific marker.JM Coinder study showed IHC may be useful to rule out other sarcomas with a specific line of differentiation. The nuclear proliferative antigen Ki67 is identified to correlate with the prognosis of the patients to predict the outcome and distant metastasis of sarcoma patients. A significant correlation has been reported between Ki67 expression and mitotic rate in sarcomas.[16] A correlation between ki67 reactivity and tumour grade in sarcomas has been detected in different retrospective studies. The findings in our study indicate that precise use of immunohistochemical stain can be a valuable adjunct to routine histopathology, exact grading of the sarcomas and in treatment management of patients.

Conclusion
Our study concludes that immunohistochemistry is an important tool in giving precise diagnosis of soft tissue tumours and it should be done as a panel to diagnose cell of origin.IHC can be done on routine basis to diagnose undifferentiated STT, confirm the diagnosis and to accurately grade the sarcomas so that it will be beneficial for the management of the patient and preoperative and postoperative treatment planning would be possible.

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