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Our experience in soft tissue tumours in correlation with Immunohistochemistry: A retrospective study

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Abstract

Introduction: The diagnosis and classification of soft tissue sarcomas has been a source of difficulty for surgical pathologists. This has been largely attributable to a significant degree of overlap in histologic features among different types of soft tissue sarcomas. The immunohistochemical identification of various cell and tissue markers specific for certain avenues of cell differentiation has permitted more accurate diagnosis and classification of these tumors.

Objective: The objective of this study was to evaluate soft tissue tumors which were unclassified on histopathology by IHC for typing the cell lineage.

Materials and Methods: We evaluated 61 cases of soft tissue tumours reported in a period of three years from April 2013 to March 2016 in which histopathology was inconclusive and IHC proved to be useful to reach to a definite diagnosis.

Conclusion: In our study, IHC contributed to the diagnosis in 80.3% cases where it helped to determine the soft tissue lineage. It was noncontributory in 13.1% cases where even after IHC the lineage could not be determined. In 6.6% cases the final diagnosis given after IHC was not correlating with the histopathological diagnosis. Hence we conclude that only histopathological examination is not sufficient at all times because there is a morphologic overlap of soft tissue sarcomas with each other and with some carcinomas and melanomas. Though immunohistochemistry is not a substitute for histopathological examination, it acts as an adjunct to arrive at definite diagnosis and hence proves to be the most important ancillary technique in the diagnosis of soft tissue tumors.

Keywords: Soft tissue tumors, Immunohistochemistry, Cell lineage, Hematoxylin and eosin, Diagnosis

Introduction

Soft tissue tumors (STT) pose a diagnostic challenge to general practicing pathologists because there is a huge overlap in the morphologic features. The usual approach is to diagnose by presumed cell lineage.(1) Because of the plethora of diagnoses and often subtle nature of diagnostic criteria, Immunohistochemistry (IHC) finds particular utility in soft tissue tumors.(2) Soft tissue is defined as non-epithelial extraskeletal tissue of the body exclusive of the reticuloendothelial system, glial and supportive tissue of various parenchymal organs. It includes smooth muscles, skeletal muscle, peripheral nervous system, adipose tissue, blood vessels, connective tissue and lymphatics. Soft tissue tumors account to 2% or less of surgical pathology cases.(3) IHC helps to identify the lineage of the tumour being mesenchymal or non mesenchymal and once mesenchymal lineage has been confirmed, various lineage specific markers are used for further histologic subtyping.(1) Immunohistochemistry is presently the most important adjunct tool in the evaluation of soft tissue tumors because it provides a practical approach in reaching to a definite diagnosis and is easily reproducible. Many diagnoses, such as those of fibrous, fibro-histiocytic and lipomatous tumors, are still based on histology. Despite of the development in IHC and other ancillary techniques, we fail to reach to a definite diagnosis in few cases of soft tissue tumours. There exists a complexity in patterns of expression of many antigens, hence a single antibody does not help. Consequently, the use of panel of antibodies becomes necessary.(4)

In this study, an attempt is made to evaluate all soft tissue tumors [including benign and malignant tumors] where histopathological examination [HPE] couldn't classify the tumors and IHC was required to give a definite diagnosis.

Materials and Methods

We evaluated 61 cases of soft tissue tumors retrospectively from March 2013 to April 2016 received in the department of pathology, in which HPE was inconclusive and a panel of antibodies was required to arrive at definite diagnosis. The clinical information regarding patient's age, sex, clinical history and the clinical diagnosis was extracted from the case file. A thorough gross examination of the specimen was done by histopathologist, taking into consideration gross areas of hemorrhage, necrosis and capsular invasion. Representative sections were taken, stained with haematoxylin and eosin and observed under microscope. A provisional diagnosis was given on HPE based on features like the capsular invasion, cell type, cellular arrangement, nuclear pleomorphism, necrosis, hemorrhage, number of mitotic figures per 10 high power field and tumor giant cells. The immunohistochemical markers for identification of various cell and tissue markers specific for certain avenues of cell differentiation was used for more accurate diagnosis and classification of these tumors.

Results

Out of the total 61 cases that were evaluated, there were 28 females and 33 males. Table 1 shows the age distribution of the cases studied. The youngest patient was a female aged 2 years who was diagnosed of spindle cell haemangioma after IHC. Oldest patient was a female aged 80 years in whom, on HPE, a diagnosis of poorly differentiated malignancy was given. Table 2 describes various IHC markers used along with the results obtained. Despite the use of extensive antibody panel, IHC could not help reach to a definite diagnosis in this case and proved non-contributory. Overall, IHC contributed to the diagnosis in 49 cases where it helped to determine the soft tissue lineage. It was non-contributory in 8 cases where even after IHC the lineage could not be determined. In 4 cases the final diagnosis given after IHC was not correlating with the light microscopic diagnosis.(Table 3) Out of total 61 cases, maximum number of cases where IHC was required were of gastrointestinal stromal tumour(GIST).

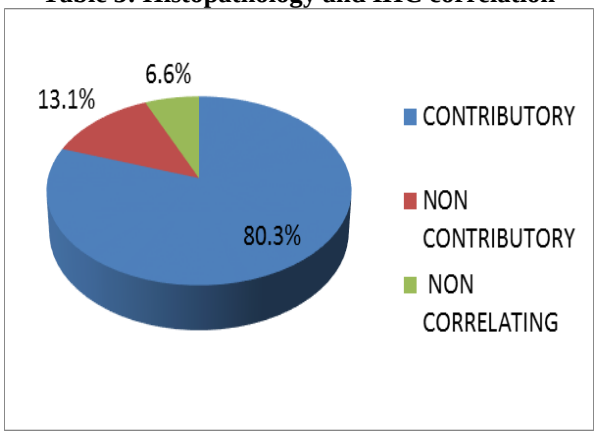
Table 1: Distribution of soft tissue tumours according to the age

Tumour Type	0-10 Y	11-20 Y	21-30 Y	31-40 Y	41-50 Y	51-60 Y	61-70 Y	71-80 Y	n
Embryonal rhabdomyosarcoma	-	2	-	1	1	-	-	-	4
	-	-	2	1	-	1	1	-	5
GIST	-	1	1	3	1	1	4	-	11
leiomyosarcoma	-	1	-	-	2	1	2	1	7
Ewings sarcoma	-	2	2	-	-	-	-	-	4
Schwanoma	-	-	2	-	-	-	-	-	2
MPNST	-	1	-	-	1	-	-	-	1
paraganglionoma	-	-	-	1	-	-	-	-	1
STUMP	-	-	-	-	-	-	1	-	1
Spindle cell haemangioma	1	-	-	-	-	-	-	-	1
Sclerosing stromal tumour	-	-	1	-	-	-	-	-	1
Desmoid fibromatosis	-	-	1	-	-	-	-	-	1
Synovial sarcoma	-	1	-	1	1	-	-	-	3
angiofibroma	-	1	-	-	-	-	-	-	1
Sarcoma	-	-	-	-	-	2	-	-	2
Myxoid neurofibroma	-	-	1	-	-	-	-	-	1
Mesenchymal hamartoma	1	-	-	-	-	-	-	-	1
SFT	-	-	-	-	-	-	1	-	1
DFSP	-	-	-	-	-	1	-	-	1
fibrosarcoma	-	1	-	-	-	-	-	-	1
Inflammatorory myfibroblastic tumour	-	-	-	-	-	1	-	-	1
Endometrial stromal sarcoma	-	-	-	1	1	-	-	-	2
angiosarcoma	1	-	-	-	-	-	-	-	1
High grade Serous carcinoma	-	-	-	-	-	1	-	-	1
Spindle cell tumour	-	-	-	-	-	1	-	-	1
Mixed endometrial and smooth muscle tumour	-	-	-	-	1	-	-	-	1
others	-	-	1	-	1	-	-	1	3
Total									61

Table 2: Various IHC markers used for different soft tissue tumours

Tumour Type	Desmin	Myogenin	SMA	Vimentin	Synaptophysin	S 100	CD34	CD117	CD99	KI67(%)
GIST	NA	NA	-	+	NA	NA	focal +	strong +	NA	9,4
Leiomyosarcoma	+	-	+	+	NA	-	-	NA	NA	18,25,60,80
MFH	NA	-	Focal+	Diffuse +	NA	-	-	-	-	80/75
Ewings sarcoma	NA	-	NA	+/-	NA	NA	NA	NA	Membrane+	NA
Embryonal rhabdomyosarcom	Strong+	Strong+	NA	Diffuse+	NA	NA	NA	NA	-	NA
Synovial sarcoma	NA	NA	-	Strong+	NA	-	-	NA	Focal+	25
schwanoma	NA	NA	NA	NA	NA	Diffuse+	NA	NA	NA	1
MPNST	-	NA	-	Strong diffuse+	NA	-/+	-	NA	NA	NA
paraganglioma	NA	NA	NA	NA	Diffuse strong+	NA	NA	NA	NA	NA
angiofibroma	NA	NA	-	+	NA	NA	Blood vessel wall +	NA	NA	NA
Angiosarcoma	NA	NA	NA	NA	NA	NA	strong+	NA	NA	60
Inflammatory MFT	NA	NA	+	+	NA	NA	NA	NA	NA	NA
Desmoid fibromatosis	NA	NA	-	NA	NA	NA	-	NA	NA	NA
DFSP	NA	NA	NA	Diffuse+	NA	NA	Diffuse+	NA	NA	<10
fibrosarcoma	NA	NA	-	strong+	NA	-	-	NA	-	5

Table 3: Histopathology and IHC correlation



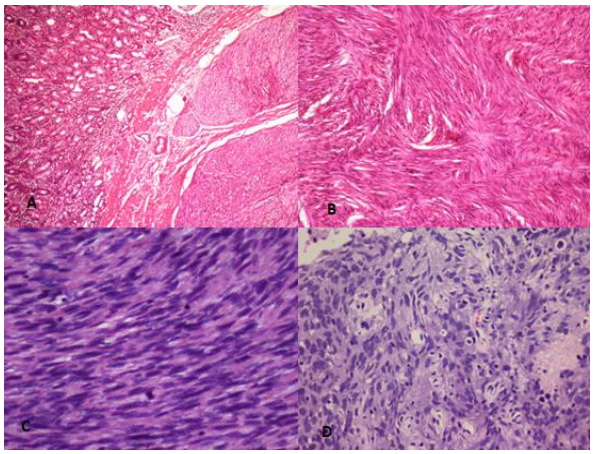


Fig. 1: (A, B) GIST characterized by bundles and fascicles of spindle shaped tumour cells.(H & E stain, 10X, 20X). (C) leiomyosarcoma characterized by spindle shaped tumour cells with hyperchromatic nuclei and numerous mitotic figures (H & E stain,40X). (D) pleomorphic high grade sarcoma (MFH) characterized by pleomorphic spindle cells (H & E, 20X)

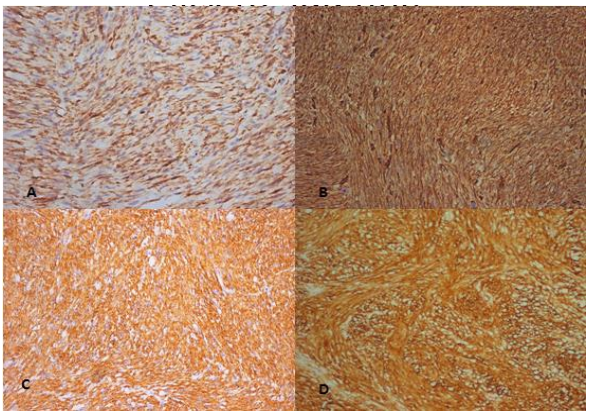


Fig. 2: (A) tumour cell of leiomyosarcoma s showing membrane and cytoplasmic positivity for desmin.(20X). (B) tumour cells of MPNST showing cytoplasmic and membrane positivity for vimentin.(20X). (C) tumour cells of leiomyosarcoma showing cytoplasmic and membrane positivity for SMA.(20X) (D) tumour cells of GIST showing cytoplasmic and membrane positivity for CD117.(20X)

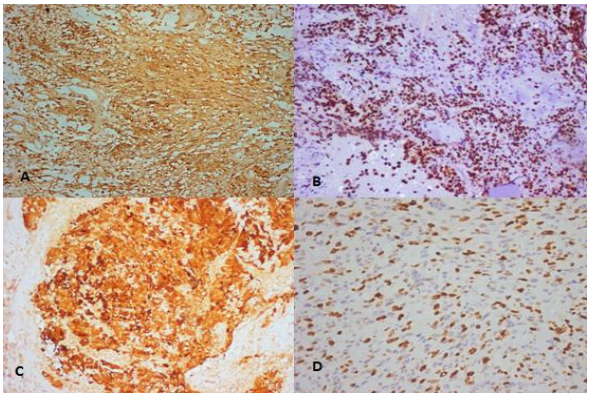


Fig. 3: (A) tumour cells of schwanoma showing cytoplasmic and membrane positivity for S100.(20X) (B) tumour cells of embryonal rhabdomyosarcoma showing nuclear positivity for myogenin.(20X). (C) tumour cells of synovial sarcoma showing cytoplasmic and membrane positivity for cytokeratin.(20X). (D) tumour cells of MFH showing both cytoplasmic membrane and nuclear positivity for Ki67 highlighting the mitotic figures.(20X)

Discussion

For any soft tissue tumor, the initial step of evaluation is to obtain material through incisional biopsy or fine needle aspiration. Light microscopic evaluation of hematoxylin–eosin-stained sections remains the standard technique for the initial diagnostic approach.[5] However, various ancillary techniques are used to increase the diagnostic accuracy and have become an indispensible part of the diagnosis of STT, this development applying both to adult and pediatric tumors.[6,7] These techniques include conventional special stains, electron microscopy, IHC, and molecular genetic analysis.

IHC plays an important role in STT diagnosis. The first approach consists of ruling out a non-mesenchymal tumor, followed by trying to define mesenchymal cell lineage. This is achieved with a panel of commonly used antibodies that helps narrow down the differential diagnoses 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.[8] In our study out of 61 cases IHC contributed significantly in 80% of the cases. There was a disparity between the HPE diagnosis and IHC diagnosis in 7% of the cases.

Our study is similar to the study done by Badwe et al,[9] in which out of the 301 cases of STTs reported over a period of 5 years, IHC was performed in 15

cases. Out of 15 cases, in 10 cases the HPE was confirmed on IHC, in 3 cases HPE was given as 'High grade sarcoma' and further typing was done on IHC and 2 cases showed disparity between the HPE and IHC diagnosis.

In our study, the HPE did not correlate with the IHC diagnosis in a case of GIST (Fig. 1 B) which was misdiagnosed as low grade leiomyosarcoma because of the site being the pelvis and spindle cell morphology on HPE. Similarly in a study done by Jha R.,[(1)] says before the application of IHC to detect the Kit protein expression, many GISTs were classified as smooth muscle tumors.

In our study, out of the 61 cases, the maximum number of cases where IHC was required were of GIST(18%) which is in contrast to the study done by Badwe et al[(9)] where adipocytic tumours (54.81%) formed the commonest group of STT and only 1 case of malignant GIST(0.33%) was reported.

In a case of a tumor from a wrist joint, a diagnosis of glomus tumor was made on HPE which was diagnosed as synovial sarcoma on IHC because the tumour cells showed strong positivity to pancytokeratin, bcl-2 and were negative for CD34. This has also been stated in a review of literature done by Daraji et al[(4)] where they have stated that both the epithelial and the spindle cell components of biphasic synovial sarcoma show pancytokeratin (panCK) and EMA positivity. In monophasic tumors, scattered spindle cells are positive for both markers. In contrast to haemangiopericytoma/solitary fibrous tumor CD34 is consistently negative.

The other two cases which showed disparity between the HPE and the IHC were a case of angiofibroma and mesenchymal hamartoma with myofibroblastic differentiation which were misdiagnosed as high grade undifferentiated sarcoma and neurofibroma respectively, on HPE.

IHC could not contribute significantly in 8 cases wherein we came across 5 cases of pleomorphic high grade sarcoma (malignant fibrous histiocytoma) which showed positivity only to vimentin and were negative for S100, CD34, desmin, Smooth Muscle Actin (SMA), panCK, LCA and myogenin (Fig. 1 D). Despite the use of an extensive antibody panel we could not reclassify these sarcomas into specific type of sarcomas. This is similar to the study done by Thway et al,[(10)] in which out of the 29 minor diagnostic discrepancies, most frequently the diagnoses was changed to pleomorphic sarcoma (so-called malignant fibrous histiocytoma). This is in contrast to the study done by Coindre et al[(11)] which showed that among 25 tumors initially diagnosed as retroperitoneal malignant fibrous histiocytoma (MFH), 17 were reclassified as dedifferentiated liposarcoma by extensive sampling followed by IHC and comparative genomic hybridization. It is important to distinguish these tumours because of their variable prognostic implications.[(12)]

However, a study done by Bhagat et al[(13,14,15)] states that the term MFH is preferably replaced by pleomorphic high grade sarcoma, not further specified, or pleomorphic (myo) fibrosarcoma. It is best defined immunophenotypically by the presence of vimentin in the absence of any lineage-specific markers. Similarly in a study done by Bahrami et al,[(16)] it was stated that pleomorphic MFH or undifferentiated high-grade pleomorphic sarcoma is a group of pleomorphic sarcomas that do not demonstrate a definitive line of differentiation even with the application of immunohistochemistry. MFH is therefore a diagnosis of exclusion, which is made in the absence of reaction with any lineage selective markers in a high-grade pleomorphic sarcoma.

Vimentin expression also serves as an internal control to assess antigenic preservation as its antigenicity is best preserved in frozen and formalin-fixed tissues and to select fields optimal for the expression of other markers. Hence, it is used as positive internal control in all cases.[(13,15,16,17)]

In undifferentiated carcinomas, vimentin is still reactive because, as the cells become dedifferentiated, they lose the characteristics of the origin tissue.[(13,18)]

In our case of a tumor in the mandible, after an extensive use of antibody panel we could rule out neural tumors, solitary fibrous tumors, poorly differentiated spindle cell carcinoma, leiomyosarcoma, rhabdomyosarcoma and vascular tumours but could not reach to a definite diagnosis.

Conclusion

In the field of soft-tissue pathology, IHC, on the one hand, has confirmed the diagnostic accuracy of previous generations of pathologists who, based on morphology alone, have made sense of a frightening number of entities. On the other hand, it has revealed the inherent capabilities of histogenetically unrelated tumors to adopt variable and often overlapping morphological features. Today, the role of IHC is so firmly established that pathologists often tend to rely on its results in detriment of careful histopathological analysis. The increase in the number of applications and the use of an unnecessarily large number of markers may lead to unnecessary costs. These can be reduced by gaining a good knowledge of the sensitivity, specificity, and potential pitfalls of reagents and by optimizing the application and proper interpretation of results. In general, it is advisable to use a limited number of markers as a first step and then expand the number of tests accordingly. Also IHC should be used as an ancillary techniques and morphology being the gold standard.

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