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The Evaluation of Importance of New Immunohistochemical Markers for the Diagnosis and Differential Diagnosis of Mesenchymal Tumors

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Abstract

Objectives: The diagnosis of the mesenchymal neoplasms, due to their rarity and variations of their morphology, is challenging for even the most experienced pathologists. The aim of the study is to reevaluate the cases with previous diagnosis of sarcoma using newly found immunohistochemical markers and the 2020 World Health Organization (WHO) classsification.

Material and Methods: 183 cases who were diagnosed to have soft tissue sarcomas of the extremities between 2000-2015 were reevaluated using 2020 WHO classification. The histopathologic specimens were analyzed with the new immunohistochemical markers (TLE1, MUC4, MDM2, CDK4, TFE3, STAT6, INI1). The morphologic features and the ultimate diagnosis were compared with the previous histopathologic evaluation.

Results: The diagnosis was changed in 38 cases in this series after the application of the new immunohistochemical markers. The most remarkable alteration was detected in the groups of leiomyosarcoma and liposarcoma.

Conclusion: Soft tissue sarcomas exhibit difficulties during diagnosis even for experienced pathologists. This challenging process should be supported with the appropriate application of the immunohistochemical markers in order to decrease the rate of misdiagnosis. With newly developed immunohistochemistry markers, a detailed examination is required.

Key Words: Soft tissue sarcomas, Immunohistochemistry, WHO 2020

Mezenkimal Tümörlerin Tanı ve Ayırıcı Tanısında Yeni İmmünohistokimyasal Belirteçlerin Öneminin Değerlendirilmesi

Öz

Amaç: Mezenkimal neoplazmaların tanısı, nadir olmaları ve birbirinden farklı morfolojileri nedeniyle en deneyimli patologlar için bile zordur. Çalışmanın amacı, yeni bulunan immünohistokimyasal belirteçler ve 2020 Dünya Sağlık Örgütü (DSÖ) sınıflaması kullanılarak daha önce sarkom tanısı almış olguları yeniden değerlendirmektir.

Gereç ve Yöntemler: 2000-2015 yılları arasında ekstremitelerde yumuşak doku sarkomu tanısı

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alan 183 olgu, 2020 DSÖ sınıflaması kullanılarak yeniden değerlendirildi. Histopatolojik örnekler yeni immünohistokimyasal belirteçler (TLE1, MUC4, MDM2, CDK4, TFE3, STAT6, INI1) ile analiz edildi. Morfolojik özellikler ve nihai tanı önceki histopatolojik değerlendirme ile karşılaştırıldı.

Bulgular: Bu serideki 38 olguda yeni immünohistokimyasal belirteçler uygulandıktan sonra tanı değişti. En dikkat çekici değişiklik leiomyosarkom ve liposarkom gruplarında tespit edildi.

Sonuç: Yumuşak doku sarkomları, deneyimli patologlar için bile tanı sırasında güçlükler göstermektedir. Yanlış tanı oranını azaltmak için bu zorlu süreç, immünohistokimyasal belirteçlerin uygun şekilde uygulanmasıyla desteklenmelidir. Yeni gelişen immünhistokimyasal belirteçlerle dikkatli inceleme gereklidir.

Anahtar Kelimeler: Yumuşak doku sarkomu, İmmünohistokimyasal, DSÖ 2020

Introduction

Soft tissue tumors include a fairly heterogeneous group of neoplasms classified according to the origin of the tissue (1,2). Malignant soft tissue tumors make up fewer than 1% of all malignant neoplasms (3). The incidence of these tumors changes with age (4,5). The widespread use of modern techniques such as immunohistochemical (IHC) and molecular diagnostic methods has revolutionized the pathologic diagnosis. An immune panel is developed that is supported with the use of some clinical and histopathological clues. MUC4, TFE3, TLE1, STAT6, MDM2, CDK4, INI1, DOG1, and Brachyury are only a few of the biomarkers that have recently been discovered and are reported to be involved in diagnosis (6).

The goal of the study is to use the WHO 2020 classification and IHC markers to reassess the diagnosis of patients previously diagnosed as sarcoma of the extremities, and to investigate the importance of these markers in mesenchymal tumor diagnosis (7).

Material and Methods

201 patients were diagnosed to have a mesenchymal tumor between 2000-2015 in a department of pathology of a university hospital. All mesenchymal tumor cases were located in the extremities. Slides and paraffin blocks of the cases were examined for this study.

Out of the 201 patients, those 183 with adequate material for further examination were included in this study. Malignant mesenchymal tumours comprised 167 of them and the remaining 16 were solitary fibrous tumors. During the initial diagnosis, all the sections and IHC-stained slides were reviewed by three pathologists, one of whom was experienced in soft tissue tumours.

The previous diagnosis of these cases was reassessed using MUC-4, STAT6, TLE1, TFE3, MDM2, CDK4, INI1 IHC. Other IHC markers were already studied while routine interpretation of these cases. The samples were introduced to a BenchMark XT device. MUC-4 (Santa Cruz 1:50), STAT6 (Santa Cruz 1:50), TLE1 (BioSB RTU), TFE3 (Cell marque 1:200), MDM2 (Santa Cruz 1:80), CDK4 (Santa Cruz 1:50), INI1 (Cell Marque RTU) antibodies were applied and staining was performed in the automated device subsequently and stained samples were covered using fluid-based material.

The study was approved by The Institution's Non-Interventional Clinical Research Ethics Committee (Protocol number: TTU20153799).

Results

There were 92 male and 91 female patients. The mean age in this series was 48 years, ranging between 1 and 89 years. Only 10% (n=19) of the cases were between 0-18 years old. The remaining 90% (n=164) were found to be older than 18 years.

The tumour location was the lower extremity in 116 patients (63%); the upper extremity in 38 (21%); the trunk in 14 cases (8%). The rare locations were the retroperitoneum in five; visceral in four; inguinal in four; head and neck in two of the patients. The most commonly made initial diagnosis in this series was undifferentiated pleomorphic sarcoma, which consisted of 41 cases (22%). Malignant peripheral nerve sheath tumor (MPNST) and myxofibrosarcoma were detected in 19 and 17 patients, respectively. The diagnostic distribution is presented in Table 1.

Table 1. Diagnostic distribution

Diagnosis of All Cases	Case Number
Undifferentiated pleomorphic sarcoma	41
Malignant peripheral nerve sheath tumor (MPNST)	19
Myxofibrosarcoma (MFS)	17
Synovial sarcoma (SS)	16
Solitary fibrous tumor (SFT)	16
Liposarcoma	13
Rhabdomyosarcoma (RMS)	13
Leiomyosarcoma	11
Fibrosarcoma	10
Clear-cell sarcoma	5
Low-grade fibromyxoid sarcoma (LGFMS)	3
Alveolar soft part sarcoma (ASPS)	3
Epithelioid sarcoma	2
Soft tissue osteosarcoma	2
Myxoinflammatory fibroblastic sarcoma	2
Mesenchymal chondrosarcoma	1
Epithelioid angiosarcoma	1
Sclerosing epithelioid fibrosarcoma (SEF)	1
Dermofibrosarcoma protuberans (DFSP), fibrosarcomatous variant	1
Epithelioid sarcoma–like hemangioendothelioma	1
Primitive neuroectodermal tumor	1
Inflammatory myofibroblastic tumor (IMT)	1
Myofibrosarcoma	1
Malignant mesenchymal tumor	2

After the ultimate evaluation using the WHO 2020 classification and new tumour markers, a change was detected in 61 patients of the series, yet the histopathological differences were found only in 38 of them. The remaining 23 cases were renamed according to the new nomenclature. Twenty of 23 cases was accepted to be undifferentiated PS, as the term "malignant fibrous histiocytoma (MFH)" was replaced by "undifferentiated PS". Moreover, three cases diagnosed with myxoid MFH previously were renamed as myxofibrosarcoma, because "myxofibrosarcoma" was included in new classification. The 38 patients in whom histopathological differences were detected with the newly applied markers are presented in Table 2.

Table 2. Previous and ultimate diagnoses in 38 cases.

No	Age	Old diagnosis	New diagnosis	Immunohistochemistry (IHC)
1	69	LMS	DFSP, fibrosarcomatous	CD34 +
2	69	LMS	SFT, malign	STAT6 +, H-caldesmon -
3	54	LMS	Myxofibrosarcoma	H-caldesmon -
4	29	LMS	İMT	H-caldesmon -, ALK+
5	61	LMS	MPSKT	H-caldesmon -, S100 focal +
6	68	LMS	APS	H-caldesmon -
7	75	LMS	APS	H-caldesmon -
8	68	MFH	MPNST	S100 focal +
9	67	MFH	MPNST	S100 focal +
10	52	MFH	DDLPS	CDK4 +, MDM2 +
11	74	MFH	Fibrosarcoma	
12	72	MFH	Pl. LMS	H-caldesmon+
13	59	APS	DD Condrosarcoma	
14	58	APS	Mixoid LPS	CDK4 -, MDM2 -
15	16	MMT	SS	TLE1 +
16	76	MMT	DDLPS	CDK4 +, MDM2 +
17	72	MMT	SEF	MUC4 +, EMA+
18	46	MMT	SFT, malign	STAT6 +
19	53	MMT	APS	
20	22	MPNST	SS	TLE1 +
21	75	MPNST	SS	TLE1 +
22	57	MPNST	Myxofibrosarcoma	
23	54	MPNST	APS	S100 -
24	65	DDLPS	APS	CDK4 -, MDM2 -
25	80	DDLPS	APS	CDK4 -, MDM2 -
26	74	DDLPS	MİFS	CDK4 -, MDM2 -
27	53	DDLPS	Myxofibrosarcoma	CDK4 -, MDM2 -
28	70	Epithelioid S.	APS	INI1 +
29	50	Epithelioid S.	MMT	INI1 +
30	33	Myxoid LPS	MİFS	SMA focal+, ALK -
31	23	Myxoid LPS	SS	TLE1 +
32	60	ASPS	Clear cell sarcoma	TFE3 -
33	83	SS	LMS	H-caldesmon +, TLE1 -
34	73	Pl. RMS	APS	Desmin -, CD68 +, MDM2 -, CDK4 -
35	79	MERRT	A/AS Sarcoma	INI1 +
36	25	LGFMS	MPNST, low grade	MUC4 -, S100 fokal
37	28	A/AS Sarcoma	Myxofibrosarcoma	
38	28	SFT	Giant cell angiofibroma	STAT6 +

LMS: Leiomyosarcoma, MMT: Malignant mesenchymal tumor, MFH: Malignant Fibrous Histiocytoma, MPNST: Malignant peripheral nerve sheath tumor, ASPS: Alveolar soft part sarcoma, PL LPS: Pleomorphic liposarcoma, LMS: Leimyosarcoma, PL RMS: Pleomorphic rhabdomyosarcoma, SS: Sinovial sarcoma, DDLPS: Dedifferentiated liposarcoma, UPS:Undifferentiated pleomorphic sarcoma, LGFMS: Low grade fibromyxoid sarcoma, SFT:Solitary fibrous tumor, CCS: Clear cell sarcoma

The most common change in diagnosis was detected in the leiomyosarcoma (LMS) cases. There were seven cases priorly diagnosed LMS (Cases 1-7). After further evaluation, we observed that H-caldesmon was negative in Cases 1-7. Case 1 with spindle cells was initially diagnosed as leiomyosarcoma of the knee based on focal

weak positivity of SMA, yet the diagnosis was revised as DFSP fibrosarcomatous variant owing to focal CD34 positivity. While the diagnosis of Case 2 was revised as malignant SFT based on STAT6 positivity. Case 3 was changed to MFS due to the presence of myxoid areas, and curvilinear vasculature. The diagnosis of Case 4 was revised as inflammatory myofibroblastic sarcoma due to ALK positivity, focal SMA positivity, and the presence of extensive inflammatory cells. The diagnosis of Case 5 was changed to MPNST due to the focal positivity of S100, and morphologically fluctuating fascicular pattern. Absence of fascicular pattern, and presence of extensive pleomorphic cells led to revision of the diagnosis from LMS to undifferentiated PS in Cases 6 and 7.

There were seven cases priorly diagnosed with MFH or UPS (Cases 8-14). The diagnoses of Cases 8 and 9 were revised as MPNST due to the fascicular pattern, monotonous cell appearance, absence of pleomorphic cells, and heterogeneous positivity of S100. Case 10 was redefined as dedifferentiated liposarcoma due to the positivity of CDK4 and MDM2. The diagnosis of Case 11 was changed to fibrosarcoma owing to the staghorn pattern, and the negative results obtained with all markers applied. The diagnosis of Case 12 was changed to leiomyosarcoma based on H-caldesmon positivity, and the presence of pleomorphic cells. Case 13 was redefined as dedifferentiated chondrosarcoma due to S100-positive cartilage islets. The diagnosis of Case 14 was revised as myxoid/round-cell LPS due to the presence of plexiform capillary vasculature, sparse lipoblasts, and MDM2 negativity.

There were five priorly diagnosed MMT (Cases 15-19). Case 15 was diagnosed as synovial sarcoma (SS) with diffuse positivity of TLE1. Although unclassified initially, Case 16 was later diagnosed as DDLPS owing to the positivity of CDK4 and MDM2. The diagnosis of Case 17 was changed to sclerosing epithelioid fibrosarcoma due to the positivity of MUC4, EMA and S100, and the presence of cells with epithelioid morphology on sclerotic collagenous stroma. Case 18 was diagnosed as malignant SFT based on the positivity of STAT6. Case 19 was diagnosed as undifferentiated PS because of pleomorphic cells and negative IHC markers.

There were four priorly diagnosed MPSNT (Cases 20-23). Cases 20 and 21 were redefined as SS based on S100 negativity, and diffuse TLE1 positivity. The diagnosis of Case 22 was revised as MFS based on the plexiform pattern, curvilinear vascular structures, and the negative IHC markers. Due to the pleomorphic morphology, extensive atypical cells, and S100 negativity, the diagnosis of Case 23 was changed as undifferentiated PS.

There were four priorly diagnosed DDLPS (Cases 24-27). CDK4 and MDM 2 were negative in all these four cases. While Case 24 was deemed as S100 positive DDLPS, the diagnosis was later revised as inflammatory PS. Case 25 was redefined as undifferentiated PS based on the morphological features. The diagnosis of Case 26 was revised as acral fibroblastic sarcoma due to the distal location in the extremity, composition of cells with prominent nucleoli, and presence of inflammatory cells in the background. The

diagnosis of Case 27 was changed to MFS based on the presence of myxoid areas, and absence of lipoblasts.

The initial diagnosis of Case 28 was epithelioid sarcoma, which was later revised as undifferentiated PS due to the absence of INI1 loss, advanced age of the patient, and the absence of a nodular growth pattern.

The diagnosis of Case 29 was initially epithelioid sarcoma; however, it could not be classified due to the absence of INI1 loss or any significant result with other IHC methods applied, the diagnosis was therefore revised as malignant mesenchymal tumor. Case 30 was initially diagnosed as myxoid liposarcoma, then the diagnosis was revised as myxoinflammatory fibroblastic sarcoma owing to the presentation in the finger, absence of lipoblasts, and presence of cells with prominent nucleoli. The diagnosis of Case 31 was myxoid/round-cell LPS, which was later changed to SS due to the negativity of S100, and positivity of TLE1. Case 32 was initially diagnosed as ASPS, then the diagnosis was redefined as clear-cell sarcoma due to TFE3 negativity, and diffuse positivity of NSE and S100. The diagnosis of Case 33 was monophasic SS, which was then revised as leiomyosarcoma based on TLE1 negativity, and H-caldesmon positivity. Case 34 was initially considered as pleomorphic RMS, then this diagnosis was revised as undifferentiated PS due to negative staining with desmin, MDM2 and CDK4, and positive staining with CD68. The diagnosis of Case 35 was changed from MERRT to undifferentiated/unclassified sarcoma since INI1 was found to be positive, and no further identification could be made with other IHC methods. Case 36 was initially diagnosed as LGFMS, which was later revised as MPNST since MUC4 was negative and S100 was focal positive. While the diagnosis of Case 37 was initially undifferentiated/unclassified sarcoma, it was later revised as myxofibrosarcoma due to the presence of myxoid areas, and curvilinear vascular structures. The diagnosis of Case 38 was changed from SFT to a subtype of SFT, namely giant-cell angiofibroma, upon observation of the giant cells.

Discussion

Sarcomas are rare, heterogeneous, hard-to-classify tumors that account for 1% of adult tumors, and have more than 50 histologic subtypes. In addition, benign lesions with more than 100 different morphological structures are included in the group of mesenchymal tumors. It is difficult to perform diagnosis in these tumors showing so many different morphologies. A reason behind the poor diagnosis is that benign and malignant entities often show similar morphologies. For soft tissue tumors, up to 27% incompatibility has been reported among pathologists in the literature (1). In the literature, there are only small case series which were studied the misdiagnosis of sarcomas unlike our large series of sarcomas. The diagnosis was changed in 38 cases (21%) in our series by the new IHC markers. The incompatibility ratio reduced and was contributed to confirmation of the diagnosis by receiving a second opinion and with the help of supportive IHC and molecular methods. In the general approach to these tumors, the mesenchymal origin should be determined in the biopsy specimen, and the diagnosis of lymphoma, melanoma, and carcinoma should be ruled out.

With this study, we would like to emphasize that soft tissue sarcomas exhibit difficulties in diagnosis, the misdiagnosis may occur frequently even though for experienced

pathologists, thus careful examination with IHC markers is required. We also think that it will contribute to the literature as a guide to pathologists in diagnostic traps by reminding important clues in differential diagnosis. Surgery is the main treatment method in soft tissue sarcoma, and the role of radiotherapy and chemotherapy is still controversial. The type of surgery depends on the tumor size, location, and histological grade of the tumor. For high grade sarcomas, there are several treatment approaches that are based on not only achieving good local control but also reducing the risk of developing subsequent systemic metastasis. The value of systemic chemotherapy depends on the specific histological subset of the sarcoma (7). It is important to determine the histological subtype**,** for instance SS and myxoid LPS are more likely to have tendency to respond to the systemic chemotherapy. Most of our cases are composed of high grade sarcomas, and among them, the diagnosis were changed into the same tumor differentiation score.

As a result, since even the benign–malignant differentiation is a problem, the morphological evaluation of this broad range of soft tissue tumor is the gold standard. However, IHC is indispensable in diagnosing and subtyping of soft tissue tumors. It is necessary to create a panel related to morphology by going step by step in a certain algorithm. To know which IHC marker neglect or replaceis applied to which soft tissue tumor in which pattern and to analyze dyes correctly are important for a correct definitive diagnosis. Antibodies are never 100% specific or sensitive. Therefore, diagnosis should not be made according to a single marker, and positive and negative staining should be considered. No findings should neglect or replace morphological features and clinical findings. It is important to understand not only the diagnostic utility of these recent technologies but also their potential limits and pitfalls. Clinical and radiologic correlation is still a must to render accurate diagnostic, prognostic, and therapeutic information to guide patient care.

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