

FIRST-LINE IMMUNOPHENOTYPING IN THE PATHOLOGIC DIAGNOSIS OF SOFT TISSUE TUMORS

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REZUMAT

Introducere și scop: Tumorile de țesuturi moi sunt relativ rare în patologia generală, dar diagnosticul este adesea dificil datorită histogenezei complexe și aspectelor microscopice similare. Scopul acestui studiu a fost de a investiga valoarea metodelor imunohistochimice de primă linie în tumorile de țesuturi moi.

Material și metodă: Au fost studiate 23 de cazuri internate cu tumori ale țesuturilor moi. Diagnosticul patologic de prezumție s-a stabilit pe baza examinării secțiunilor colorate de rutină cu hematoxilin-eozină-safranină. Secțiuni adiționale au fost prelucrate prin tehnica imunohistochimică, aplicând un spectru relativ larg de anticorpi, care au fost selecționați pentru imunofenotipizarea primară și secundară.

Rezultate: Diagnosticul morfologic a fost concordant cu rezultatele imunohistochimice la 12 cazuri (52,17%). Imunohistochimia a fost esențială pentru diagnosticul final la 9 cazuri (39,13%), și nu a fost utilă la alte două cazuri.

Concluzii: Rezultatele studiului demonstrează utilitatea imunohistochimiei pentru diagnosticul tumorilor de țesuturi moi, deși în unele cazuri markerii uzuali nu sunt suficient de specifici.

Cuvinte cheie: tumori de țesuturi moi, diagnostic patogenic, imunohistochimie, imunofenotipare

ABSTRACT

Background and Aims: Soft tissue tumors are relatively rare in general pathology, but in many cases the diagnosis is difficult because of the complex histogenesis and similar microscopic aspects. The purpose of this study was to evaluate the diagnostic value of the first-line immunohistochemical methods in soft tissue tumors.

Material and Methods: Twenty-three cases admitted with soft tissue tumors were investigated. The pathologic diagnosis of presumption was established on routine haematoxylin-eosin-safranin stained slides. Additional sections were submitted for immunohistochemistry using a relative large panel of antibodies, covering the field of primary and secondary immunophenotyping.

Results: The morphologic diagnosis matched with immunohistochemical results in 12 cases (52.17%). Immunohistochemistry was essential for the final diagnosis in 9 cases (39.13%), and it was not helpful in the 2 remaining cases.

Conclusion: Immunohistochemistry is extremely useful for the diagnosis of soft tissue tumors, although in some cases usual markers are not specific enough.

Key Words: soft tissue tumors, pathologic diagnosis, immunohistochemistry, immunophenotyping

INTRODUCTION

Soft tissue tumors are uncommonly found in general pathology and represent less than 5% from all

malignant tumors. They are rare tumors, but at the same time are responsible for 2% cancer-related deaths.¹ In many cases, the pathologic diagnosis is difficult because these tumors are extremely heterogeneous. As many authors previously showed, sarcomas are poorly understood, especially due to their histogenesis and behavior. As a consequence, their treatment is still poorly adjusted to the pathologic diagnosis and seems to be inadequate in the large majority of cases.

The role of the pathologist in the diagnosis of a soft tissue tumor is crucial, because she or he must decide if the lesion is reactive or a tumor, and if it is a tumor, to establish its benign or malignant character.

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The accuracy of the diagnosis is essential for the clinical behavior and therapy. On the other hand, the classic methods of pathology are seldom enough for the diagnosis. As mentioned by Enzinger and Weiss¹, less than 60% of cases are clarified if only haematoxylin-eosin stained slides are examined.

That is why, currently, immunohistochemistry is widely accepted as a useful method, not for the diagnosis of malignancy itself, but for the immunophenotyping of a soft tissue tumor. It is estimated that immunohistochemistry confirms the diagnosis in about 30 to 40% of cases, is useful to direct the diagnosis in 50 to 60% of cases, and it is non-contributive in 1-2% of cases.² The incidence of immunohistochemical procedures is significantly higher in soft tissue tumors than in carcinomas. This is why our aim was to investigate the value of primary immunophenotyping for the diagnosis in soft tissue tumors and to establish a specific diagnosis protocol.

MATERIAL AND METHODS

Patients. We retrospectively investigated specimens taken by open surgery from 23 patients, aged between 41 and 67 years, admitted in the County Hospital of Arad. The clinical diagnosis suspected a soft tissue tumor in all cases.

Material and methods. Specimens were processed in the same manner: fixation was performed in 10% formalin and/or Bouin solution and embedded in paraffin. Step sections were stained with routine haematoxylin-eosin- safranin and Masson's trichrome methods.

Immunohistochemistry. Additional sections (5 mm thick) were performed for immunohistochemistry. The working system was based on endogenous peroxidase inhibition with hydrogen peroxide 3%, antigen retrieval, labeled streptavidin biotin (LSAB2), and as chromogen was used diaminobenzidine dihydrochlorid. The final product of reaction was stained in brown. The spectrum of primary monoclonal and polyclonal antibodies (all ready-to-use) is shown in Table 1.

For each method, the internal positive control was represented by normal cells or structures, found close to the tumor. In the above-mentioned conditions, we considered that an external positive control was not necessary, excepting for vimentin V9 (marker of the correct primary processing of specimens). All reagents were from DakoCytomation (Denmark).

Table 1. Antibodies used in the study and their significance

Antibody	Significance
Actin (muscle cell), clone 1A4	Labels filaments of smooth muscle cells
Sarcomeric actin, clone alpha-Sr-1	Positive for striated derived muscle cells
CD30, clone Ber-H2	Cell of the anaplastic large cell lymphoma
CD31, clone JC70A	Recognizes an epitope of endothelin-1
CD34, clone QBEhd10	Positive for precursor of blood and endothelial cells
Cytokeratin, MNF116	Labels cells that are epithelial in origin
Cytokeratin, clone AE1/AE3,	Large spectrum cytokeratin
Desmin, clone D33	General marker for muscle cells
EMA, clone E29	Recognizes a membrane antigen on epithelial cells
FVIII, clone F8/86	Positive in cells that contain Weibel Palade bodies
Anti melanosoma, clone HMB45	Positive for cells that contain melanosomes
LCA, clone 2B11+PD7/26	Recognizes a membrane epitope of leukocytes
Neurofilament, clone 2F11	Positive for cells that are nervous in origin
NSE, clone BBS/NC/VI-H14	Positive for neural/neuroendocrine cells
S100 protein, polyclonal	Positive for supporting nervous cells, macrophages, adipose cells
Synaptophysin, clone SY38	Neural cells, neuroendocrine cells
Vimentin, clone V9	General marker of connective tissue cells

RESULTS

The morphologic analysis of usual stained sections allowed a presumptive diagnosis and classification of cases included in the study. We found 9 spindle cell tumors, 4 myxoid tumors, 4 round cell tumors, 5 pleomorphic tumors, and 1 case was classified as pseudoepithelial tumor. The next step was to investigate the features of the proliferating cells, the architecture of the tumor, stroma, and particularities of the tumor-associated blood vessels. Results of this analysis compared with data obtained from immunohistochemistry are shown in Table 2. A pathologic complete diagnosis based on conventional morphology was possible in only 9 cases. A diagnosis of probability was formulated in 7 cases, and in other 7 cases remained unclear.

The diagnosis was confirmed by immunohistochemistry in all tumors with typical aspect of conventional histology. Moreover, immunohistochemistry was helpful to establish the diagnosis in 12 uncertain cases (marked by ** in Table 2). Immunohistochemical techniques were useful in all undifferentiated tumors, without an evident connective nature. Results allowed the differential diagnosis between sarcoma versus melanoma, lymphoma or carcinoma. Even with immunohistochemistry, the final diagnosis was not possible in 2 cases (8.69%). The immunoreaction for vimentin was found positive in all cases. Specific positive reaction was noticed in 22 cases, demonstrating their mesenchymal origin.

The diagnosis of fibrosarcoma was established in only one case that showed positive reaction for vimentin and all other markers were negative. (Fig. 1)

Table 2. Primary diagnosis on conventional morphology versus data obtained from immunohistochemistry

No	Diagnosis on HE stained slides	IHC
1	Metastasis? Carcinoma?***	Ck+, EMA+
2	Fibrous histiocytoma	Vim+, Ck-
3	Round cell sarcoma. Merkel cell carcinoma?***	Ck20+, ChrA+
4	Spindle cell melanoma**	HMB45+, S100+
5	Pleomorphic sarcoma**	Vim+, fibrous histiocytoma
6	Myxosarcoma*, **	Vim+
7	Carcinoma? Malignant melanoma?***	Ck+, HMB45-, S100-
8	Rhabdomyosarcoma	Des+, Vim+, Ck-, S-Act+
9	Lymphoma? Round cell sarcoma?*, **	Vim+, ChrA-, Des-, CD45-, S100-, Ck-, EMA-, NSE-, Syn-
10	Myxoid liposarcoma	Vim+, S100+
11	Malignant fibrous histiocytoma-giant cell	Vim+, CD34+
12	Myxoid liposarcoma**	Vim+, S100+
13	Spindle cell sarcoma	Vim+, Act+ (leiomyosarcoma)
14	Epithelioid sarcoma**	Vim+, Ck-, CD34+, FVIII+ (angiosarcoma)
15	Leiomyoma	Vim+, Act+, S100-, Ck-
16	Leiomyosarcoma	Vim+, Act+
17	Lymphoma? Small cell carcinoma? Round cell sarcoma?***	Ck-, Vim+, LCA+ (malignant lymphoma)
18	Liposarcoma	Vim+, S100+
19	Anaplastic leiomyosarcoma** Fibrosarcoma? Malignant histiocytoma**	Vim+, Ck-, Act+, CD34- (leiomyosarcoma)
20	Dermatofibrosarcoma protuberans	Vim+, CD34+, HMB45-, S100-
21	Schwannoma	Vim+, S100+, NSE-, NFAP-
22	Schwannoma	Vim+, S100+, NSE-, NFAP-
23	Spindle cell sarcoma**	Vim+, Ck-, Act-, CD34-, S100-, Des-, FVIII- (fibrosarcoma)

IHC - immunohistochemical profile, Ck - cytokeratin, EMA - epithelial membrane antigen, Vim- vimentin, ChrA - chromogranin A, Des - desmin, Act - smooth muscle cell actin, S-Act - sarcomeric actin, NFAP - neurofilament associated protein, Syn - synaptophysin

*Diagnosis that remained uncertain after first line immunohistochemistry

**Cases with uncertain diagnosis after the morphologic diagnosis (12 cases)

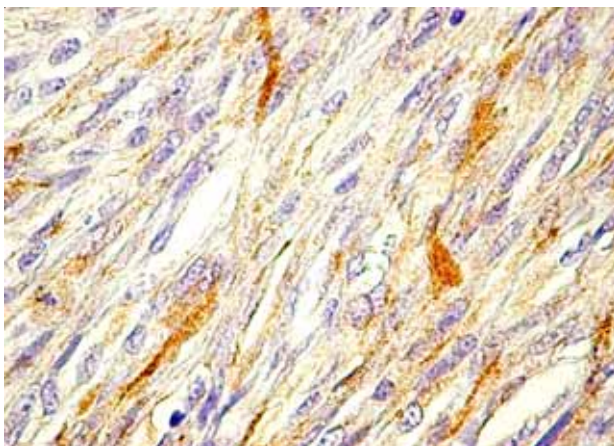


Figure 1. Vimentin. Fibrosarcoma. Positive immunoreaction for vimentin. Note the heterogeneous pattern that largely depends on the degree of differentiation

Vimentin was found to be less specific for the diagnosis, but the positive reaction correlates with the correct technical procedure. (Fig. 2) In rhabdo-

myosarcoma both desmin (diffuse pattern) and sarcomeric actin (isolated cells only) were positive. (Figs. 3 and 4)

S100 protein was constantly positive in schwannoma (Fig. 5) and liposarcoma.

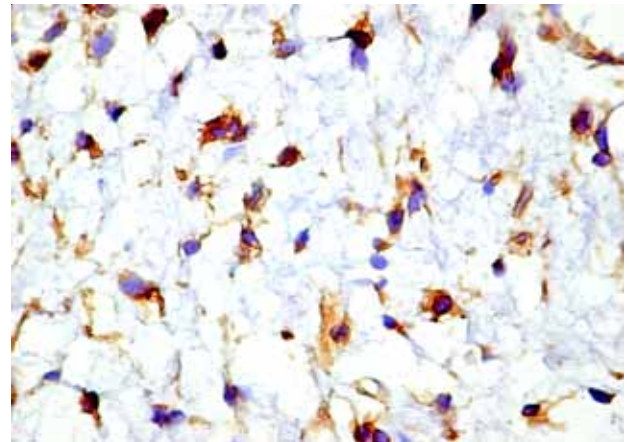


Figure 2. Vimentin. Myxosarcoma. Positive reaction for vimentin, limited at the cytoplasm of the malignant cells

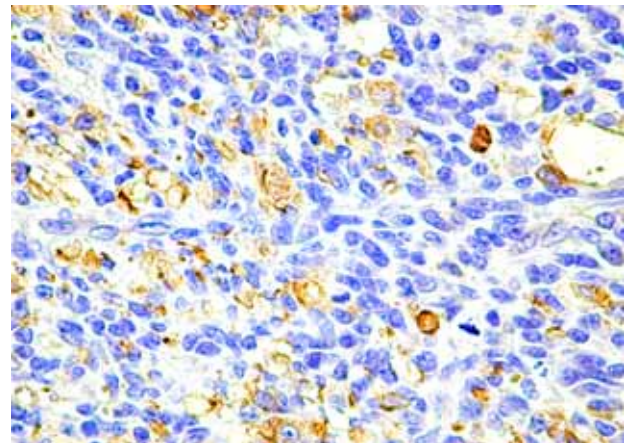


Figure 3. Sarcomeric actin. Rhabdomyosarcoma. Individual cell positive for sarcomeric actin

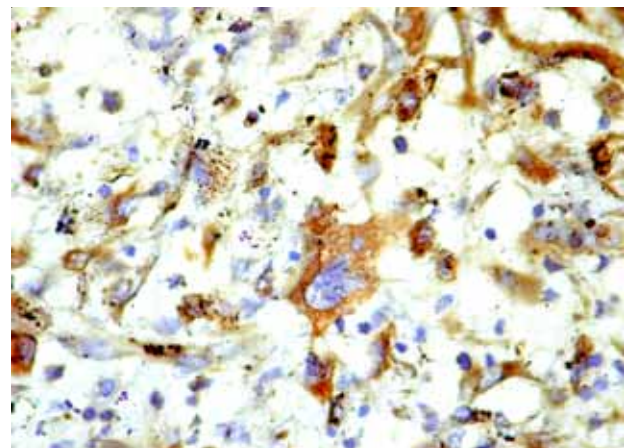


Figure 4. Desmin. Rhabdomyosarcoma. Malignant cells positive for desmin

The association with CD34 positive immunoreaction helped to emphasize the myxoid liposarcoma. Typically, CD34 was intense and diffuse

positive in dermatofibrosarcoma protuberans. (Fig. 6) The factor VIII was largely negative, excepting for one case (epitheloid sarcoma), positive for both factor VIII and CD31, indicating an unsuspected angiosarcoma. (Figs. 7 and 8)

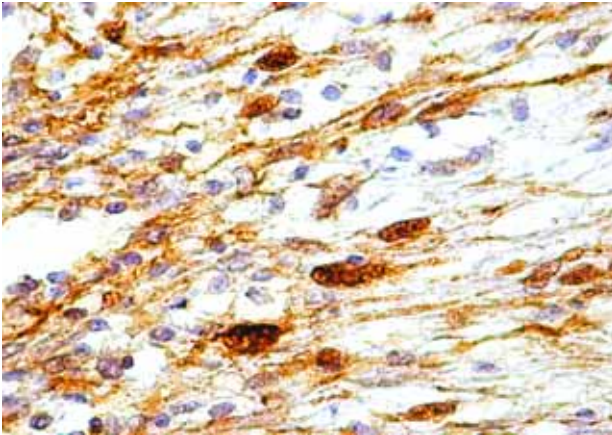


Figure 5. S100 Protein. Intense positive immunoreaction for S100 protein in schwannoma

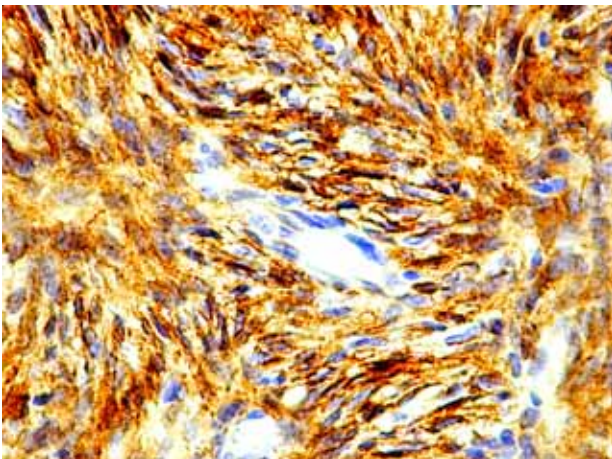


Figure 6. CD34. Dermatofibrosarcoma. All malignant cells are positive with anti CD34

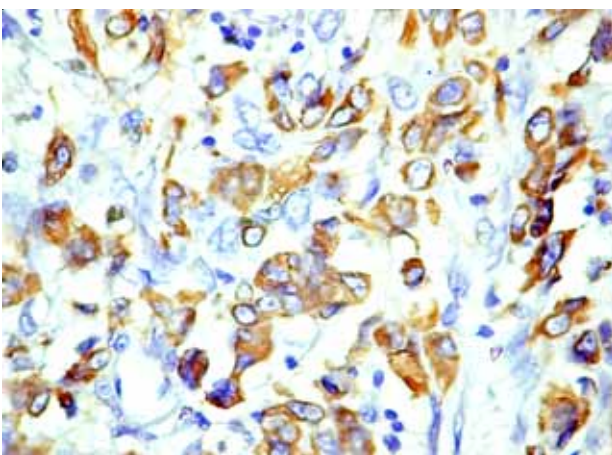


Figure 7. CD31. Angiosarcoma. Malignant cells are intensely positive for the highly specific marker of the endothelium, CD31

Smooth muscle cell actin was positive in malignant cells in 7 from the 23 cases included in the study. The

only case with malignant cells that were negative for vimentin showed positive reaction for pan-cytokeratin and epithelial membrane antigen, and therefore, it proved the metastasis already suspected by morphology. After immunophenotyping two cases remained unsolved, and eventually the diagnosis round cell sarcoma and myxosarcoma was formulated.

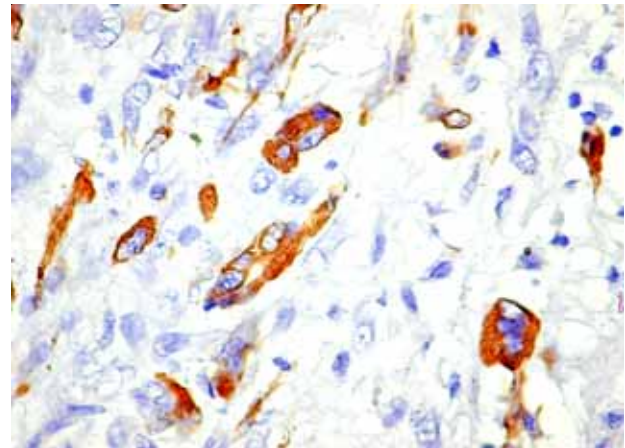


Figure 8. Factor VIII. Angiosarcoma. Individual cells, epitheloid in appearance, positive for factor VIII

DISCUSSION

Immunohistochemistry is a very useful method in the study of soft tissue tumors, for the diagnosis and histogenesis as well. Antigens that usually are made evident in such tumors are classified as components of the cytoskeleton, enzymes, lectins, hormone receptors, and tissue specific antigens. Markers that are most frequently used for diagnosis are general markers of soft tissue tumors, endothelial, muscle, nervous, and epithelial.³ The vimentin is strongly expressed by the large majority of soft tissue tumors. On one hand from this point of view, the diagnostic value of vimentin is limited. On the other hand, expression of vimentin informs about the proper fixation and embedding, because the epitope recognized by the monoclonal antibody V9 is temperature-sensitive.⁴

Many years ago fibrosarcoma was the most frequent diagnosis in soft tissue tumors. In the age of immunohistochemistry it was demonstrated that fibrosarcoma is a rare tumor, and the diagnosis is based on negative immunoreactions except for vimentin. We may say that the final diagnosis for fibrosarcoma is established by excluding other tumors. In cases included in this study only one fibrosarcoma was demonstrated. Co-expression of vimentin and cytokeratin is found in synovial sarcoma,⁵ and epitheloid sarcomas. In the diagnosis of vascular tumors the most useful markers are CD31, factor VIII, and CD34. The most sensitive and specific marker of

normal and malignant endothelial cells is CD31, positive in over 90% of cases with angiosarcoma.^{6,7} The anti-CD34 antibody is highly sensitive, but less specific because it is also expressed in solitary fibrous tumors and benign nerve sheath tumors. On the other hand, CD34 is helpful in the differential diagnosis between dermatofibrosarcoma and malignant fibrous histiocytoma.^{8,9}

The specificity of markers for malignant fibrous histiocytoma remains a problem of debate. CD68 is expressed in about half of cases and some of them are positive with factor XIIIa, but both are nonspecific for the diagnosis.¹⁰ The most sensitive marker for sarcomas that are muscular in origin is desmin. About 90-95% of the rhabdomyosarcomas express desmin, but the pattern of the final reaction product is variable.¹¹ Smooth muscle cell actin and muscle specific actin HHF35 are the most sensitive markers for leiomyosarcomas that were demonstrated in 3 cases.

S100 protein is a nonspecific marker. A large panel of tumors, including 50% of malignant schwannomas, liposarcomas, chondrosarcomas, clear cell sarcomas of the tendons and chordomas expresses it.^{12,13} Expression of epithelial markers is usually found in some sarcomas mentioned above. Aberrant expression of cytokeratin was mainly noticed in leiomyosarcoma and malignant fibrous histiocytoma.

Can we speak about an immunohistochemical protocol in the study of soft tissue tumors? The answer is yes, based on data published by other authors and confirmed by our results. (Table 3)

Table 3. Spectrum of positive markers in main categories of soft tissue tumors^{2,3,10}

Tumor	Main markers	Other markers
Solitary fibrous tumor	>90% CD34+, CD99+	Rare case + for desmin
Dermatofibrosarcoma	>90% CD34+	-
Leiomyosarcoma	90% actin and HHF35 + 79% Desmin +	10% cytokeratin + S100 + on occasion
Rhabdomyosarcoma	95% desmin + 90% HHF35 + 30-40% Myoglobin +	Sarcomeric actin Rare Cytokeratin, NSE, neurofilament, S100 +
Angiosarcoma	90% CD31 + 80% CD34 + 60% factor VIII +	Ulex agglutinin Epitheloid: cytokeratin+ Rare: EMA +
Synovial sarcoma	> 90% EMA + 60-70% Cytokeratin +	30% S100 + 60% CD99 +
Malignant peripheral nerve sheath tumor	50% S100 +	CD57, PGP9.5, basic myelin protein + Rare: cytokeratin, HHF35 +
Ewing/PNET	95% CD99+ 75% NSE, PGP9.5+	Synaptophysin, S100, neurofilament + <10% cytokeratin +
Epitheloid sarcoma	>90% EMA, cytokeratin +	50% CD34 + Rare: S100 +
Clear cell sarcoma	>90% S100 +, HMB45 +	NSE + in majority of cases
Alveolar sarcoma	Desmin, sarcomeric actin +	Some cases: NSE, S100 +

In cases with soft tissue tumors with unclear diagnosis immunohistochemistry is extremely contributive for the final diagnosis. Nowadays, classification of soft tissue tumors is largely based on immunohistochemical profile, and moreover, it allows the differential diagnosis between sarcomas and lymphoma, malignant melanoma or carcinoma. As the connective origin of the tumor is evident on routine stained slides, immunohistochemistry becomes helpful for the cell-origin of the proliferation. Some tumors with well-established diagnostic criteria do not have a characteristic immunohistochemical profile (malignant fibrous histiocytoma, haemangiopericytoma, and fibrosarcoma). Unexpected immunohistochemical results do not minimize the value of the method, but shows that application of a limited panel of antibodies is of reduced diagnostic value. Similar to other studies, we have had cases without a specific diagnosis after first-line immunohistochemistry. For such cases, additional markers and probably the molecular pathology are helpful for the diagnosis.

CONCLUSION

The immunohistochemical study of 23 cases with soft tissue tumors revealed the possibility to confirm the morphologic finding in 12 cases (52.17%). Immunohistochemical results changed the initial morphologic diagnosis in 9 cases (39.13%), and were not helpful in 2 cases (8.69%) that remained unsolved. Our results sustain the value of the first-line immunohistochemistry as a confirmative and diagnostic method in soft tissue tumors.

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