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► **To cite this version:**

Solène-Florence Kammerer-Jacquet, Sixte Thierry, Florian Cabillic, Morgane Lannes, Florence Burtin, et al.. Differential diagnosis of atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma: utility of p16 in combination with MDM2 and CDK4 immunohistochemistry. *Human Pathology*, WB Saunders, 2017, 59, pp.34-40. 10.1016/j.humpath.2016.08.009 . hal-01439351

HAL Id: hal-01439351

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01439351>

Submitted on 18 May 2017

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TITLE PAGE

Differential diagnosis of atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma: utility of p16 in combination with MDM2 and CDK4 immunohistochemistry

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Running title: p16 in liposarcoma

Conflict of interest statement:

The authors of this article have no conflict of interest to declare.

Funding disclosure:

The authors of this article have no funding to declare.

Key Words:

Well-differentiated/dedifferentiated liposarcoma, differential diagnosis, p16, MDM2, CDK4, immunohistochemistry, Fluorescence in situ hybridization.

ABSTRACT:

Introduction: The differential diagnosis between atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS) and dedifferentiated liposarcoma (DDLPS) from their morphologic counterparts is challenging. Currently, the diagnosis is guided by MDM2 and CDK4 immunohistochemistry (IHC) and is confirmed by the amplification of the corresponding genes. Recently, p16 IHC has been proposed as a useful diagnostic biomarker.

Objective: To assess the utility of p16 IHC in the differential diagnosis of ALT/WDLPS and DDLPS.

Materials and methods: Our series included 101 tumors that were previously analyzed using fluorescence in situ hybridization (FISH) for *MDM2* and *CDK4* amplification. We compared sensitivity and specificity of p16 IHC to MDM2 and CDK4 IHC in the differential diagnosis of ALT-WDLPS (n=19) versus benign adipocytic tumors (n=44) and DDLPS (n=18) versus mimicking sarcomas (n=20).

Results: In the differential diagnosis of ALT-WDLPS, p16 had a sensitivity of 89.5% but a specificity of 68.2%, which was impaired by false-positive lipomas with secondary changes, especially in biopsies. Likewise, in the differential diagnosis of DDLPS, p16 had a sensitivity of 94.4% and a specificity of 70%, which hampered its use as a single marker. However, adding p16 to MDM2 and/or CDK4 increased diagnostic specificity. Indeed, MDM2+/p16+ tumors were all ALT-WDLPS and MDM2-/p16- tumors were all benign adipocytic tumors. Moreover, all MDM2+/CDK4+/p16+ tumors were DDLPS and the MDM2-/CDK4-/p16- tumor was an undifferentiated sarcoma.

Conclusion: Although the use of p16 as a single immunohistochemical marker is limited by its specificity, its combination with MDM2 and CDK4 IHC may help discriminate ALT-WDLPS/DDLPS.

TEXT**1. INTRODUCTION**

Adipocytic tumors represent the most common type of mesenchymal tumors [1]. According to its clinicopathological and molecular genetic characteristics, liposarcomas (LPS) are grouped into three categories: 1- atypical lipomatous tumor or well differentiated liposarcoma (ALT-WDLPS)/dedifferentiated liposarcoma (DDLPS), 2- myxoid/round cell liposarcoma and 3- pleiomorphic liposarcoma.

The differential diagnosis between ALT-WDLPS and benign adipocytic tumors or between DDLPS and other sarcomas is crucial because of differences in prognosis or treatment. Based on histological grounds, WDLPS can mimic normal fat tissue and lipomas. In WDLPS, fibrous septa containing atypical cells with enlarged, hyperchromatic nuclei may be poorly represented or absent, particularly in biopsy specimens. In lipomas such as ALT-WDLPS, fibrosis, necrosis and inflammatory cells may also be present. On the contrary, DDLPS, which occur mainly in the retroperitoneum or in the spermatic cord, can be confused with undifferentiated pleomorphic sarcoma, myxofibrosarcoma and also with myogenic sarcoma, chondrosarcoma and osteosarcoma in cases of heterologous differentiation. Finally, even if DDLPS are frequently described as non lipogenic sarcomas juxtaposed to WDLPS, such areas may be absent and the tumor may be completely dedifferentiated [2]. Thus, a diagnosis based on histological examination may be challenging and additional molecular analyses are often required.

ALT-WDLPS and DDLPS share amplifications in the chromosomal region 12q13-15; these amplifications constantly affect *MDM2* (100%) and frequently *CDK4* (90%). These amplifications can be detected using fluorescence *in situ* hybridization (FISH), which is currently the gold standard method [3, 4], but FISH requires specific equipment that is only

available at expert medical centers. Alternatively, IHC might serve as an easier method to perform the diagnosis. Antibodies against MDM2 and CDK4 are a reliable way to detect protein overexpression that results from amplification of the corresponding genes [5]. Even if MDM2 and CDK4 immunostaining is correlated to gene amplification, MDM2 immunohistochemistry (IHC) suffers from a lack of specificity and may be difficult to interpret, especially if macrophages are present. Moreover, CDK4 IHC has a low sensitivity [6].

p16^{Ink4A} (p16), which is a transcript of the *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) gene, inhibits cell cycle progression by binding to CDK4 [7]. It was found to be overexpressed in ALT-WDLPS and DDLPS by both quantitative RT-PCR and IHC, and consequently, p16 has been proposed as a diagnostic marker [8, 9]. Interestingly, p16 antibodies, which are commonly used for the diagnosis of cervical dysplasia, are available in most pathology laboratories [10, 11].

This study proposed to focus on the differential diagnosis of these tumor types, which is a frequent challenge in the daily practice of pathologists. For this purpose, we evaluated the utility of p16 IHC, alone and in association with MDM2 and CDK4 IHC, in consecutive tumors using available FISH analysis data.

2. MATERIALS AND METHODS

2.1 Ethics statement

Institutional ethical guidelines were followed in this study. This study was approved by the institutional ethics committee of the Rennes University Hospital. The study was performed using routine FISH and IHC assays. The data collection and additional analyses, such as statistical tests, were performed anonymously.

2.2 Patients and tissue specimens

From 2010 to 2014, consecutive soft tissue tumors with contributive *MDM2* and *CDK4* FISH analysis performed at the time of diagnosis were retrospectively retrieved and reviewed. Intimal sarcomas and low-grade osteosarcomas were excluded from this study as they constitutively harbor amplifications of *MDM2* and *CDK4* (90%) [12, 13]. These cases originated from the routine pathology practice of the Rennes University Hospital.

According to the recommendations of international groups and the French Sarcoma Group as well as those found in published data, FISH analysis was performed in the following tumors to render a diagnosis of ALT-WDLPS as follows: in histologically suspected ALT-WDLPS, mature adipose tumors with positive *MDM2* and/or *CDK4* immunostaining, adipocytic tumors with questionable cytonuclear atypia, tumors greater than 10 cm in size, those with an unusual or deep location and recurrent lipomas. FISH was performed in the following tumors to render a diagnosis of DDLPS as follows: in poorly differentiated sarcomas with positive *MDM2* and/or *CDK4* immunostaining and tumors located in the retroperitoneum [3, 5, 14].

For each case, clinical and radiological features such as sex and age, tumor location and size were obtained from referring physicians and medical records. All cases were reviewed by 2 pathologists (SFKJ and ST) and a senior pathologist of the French Sarcoma Group (NS). All samples were formalin-fixed and paraffin-embedded (FFPE). Hematoxylin and eosin-stained sections as well as IHC slides were carefully reviewed. The tumors were classified according to the 2013 WHO classification for bone and soft tissue tumors [15]. Sarcomas were graded according to the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system, which is based on differentiation, number of mitotic figures and necrosis [16].

2.3 Fluorescence *in situ* hybridization

FISH analyses were performed on 4- μ m-thick sections of FFPE tumor tissues with ZytoLight[®] SPEC MDM2 (12q15)/CEN 12 and ZytoLight[®] SPEC CDK4 (12q13-14)/CEN 12 Dual Color Probes (Zytovision, Bremerhaven, Germany) as previously described. All slides were analyzed by experienced cytogeneticists (FC and FD) [17]. At least 40 non-overlapping tumor nuclei were examined. Nuclei were considered amplified for *MDM2* or *CDK4* if the *MDM2*/CEN12 or *CDK4*/CEN12 ratios were > 2 . When ratios were < 2 , but more than 2 signals of the probe of interest were present, the nuclei were classified as polysomic for chromosome 12, as previously described [18].

FISH was considered positive if the tumor harbored at least *MDM2* amplification, which confirmed the diagnosis of ALT-WDLPS/DDLPS in the appropriate histological context. Conversely, a diagnosis of ALT-WDLPS/DDLPS was excluded if no *MDM2* amplification was found.

2.4 Immunohistochemistry

Four- μ m-thick sections of FFPE tumors were pretreated and immunostained using the Ventana Benchmark XT system. Primary antibodies for *MDM2* (clone IF2, Invitrogen, dilution 1/100), *CDK4* (clone DCS-31, Invitrogen, dilution 1/200), and p16 (clone E6H4, Roche, ready-diluted) were used as previously described [5,19-22].

The results were independently evaluated by 3 pathologists (NS, SFKJ and ST) who were blinded to the final diagnosis; discordant cases were reevaluated collegially. For each antibody, only well-defined nuclear reactivity was considered positive, whereas ill-defined, perinuclear and cytoplasmic staining were disregarded.

A tumor was considered positive for *MDM2* or *CDK4* when at least one tumor cell nucleus was stained per low power field, as previously described [5]. p16 IHC was semiquantitatively

evaluated as negative (0% of tumor cell nuclei stained), focal (1% to 10%), multifocal positive (11% to 50%) or diffuse (>50%), as proposed by Thway *et al.* [19]. Staining was considered positive when nuclear staining was observed in more than 10% of tumor cells according to Gonzalez *et al* [20].

2.5 Statistical Analysis

For each antibody and every combination of 2 or 3 antibodies, the sensitivity and specificity were calculated. This was performed for each differential diagnosis: ALT/WDLPS vs benign adipocytic tumors and DDLPS vs other sarcomas.

3. RESULTS

3.1 Patients and histological data

Among the 116 cases retrieved, 12 cases with non-interpretable FISH data (10.3%), 2 intimal sarcomas and one low-grade osteosarcoma were excluded. Consequently, 101 retrospective consecutive cases with FFPE material available from 95 patients were finally included in this study. Five patients had both biopsy and excision specimen analysis, and one patient presented with a local recurrence one year after the first resection. Tumor specimens included 42 biopsies and 59 excision specimens.

Patients were more frequently men (63.2%), and the median age at diagnosis was 61.3 years (range 19-89). The most frequent tumor locations were the thigh (29.5%), followed by the retroperitoneum (18.9%), the abdomen (7.3%) and the thorax (7.3%). The median size of the tumors was 13 cm (3-30 cm).

Based on clinical, morphological, immunohistochemical and cytogenetic considerations, the final diagnosis was benign adipocytic tumors in 44 cases, ALT-WDLPS in 19 cases, DDLPS in 18 cases and other sarcomas in 20 cases.

3.2 Differential diagnosis between ALT-WDLPS and benign adipocytic tumors

The histologic subtypes of the 19 ALT-WDLPS and the 44 lipomas are detailed in Table 1. Secondary changes defined by fat necrosis and inflammatory regions were observed in 25/44 (56.8%) cases.

According to the FISH analysis, the 19 ALT-WDLPS harbored an *MDM2* amplification with a *CDK4* co-amplification in 14 cases. According to the IHC analysis, *MDM2* and *CDK4* expression was present in scattered nuclei. As previously described, the staining of some nuclei of macrophages might be confusing [5]. In ALT-WDLPS, *MDM2* was positive in 17/19 cases (sensitivity = 89.5%) and *CDK4* was positive in 13/19 cases (sensitivity = 68.4%). In benign adipocytic tumors, the immunohistochemical expression of *MDM2* and *CDK4* was negative in 43/44 cases (same specificity = 97.7%). Our results revealed some discordance between the FISH and IHC analyses. For *MDM2*, 3 cases were discordant: 1 lipoma was IHC+/FISH- and 2 ALT-WDLPS were IHC-/FISH+. For *CDK4*, 4 cases were discordant: 1 ALT-WDLPS and 1 spindle cell lipoma were IHC+/FISH- and 2 ALT-WDLPS were IHC-/FISH+.

Then, we evaluated the utility of p16 immunostaining to improve IHC screening. The staining in tumor cells was nuclear and cytoplasmic with a moderate to strong intensity (Figure 1). Nuclear p16 staining should be distinguished from the cytoplasmic expression of p16, which occurs in endothelial, perivascular and inflammatory cells. In ALT-WDLPS, p16 was positive in 17/19 cases (sensitivity = 89.5%) including the 2 *MDM2*-negative tumors (Table 2A). In

benign adipocytic tumors, p16 was negative in 30/44 cases (specificity = 68.2%). The 14 cases with p16 positivity corresponded to conventional lipomas (n = 10) with secondary changes in 8 cases; the remainder were intra muscular lipomas (n = 2), a spindle cell lipoma (n = 1) and a pleomorphic lipoma (n = 1). In biopsies (n = 28), p16 had a sensitivity of 83.3% and a specificity of 43.8%, whereas in excision specimens (n = 35), p16 had a sensitivity of 100.0% and a specificity of 82.1%. Moreover, we tested the utility of p16 in combination with MDM2 and CDK4. Interestingly, whereas the MDM2-/CDK4- phenotype resulted in 2 missed ALT-WDLPS, all MDM2+/p16+ tumors (n=15) were ALT-WDLPS and all MDM2-/p16- tumors (n=29) were benign adipocytic tumors.

3.3 Differential diagnosis between DDLPS and other sarcomas

The histologic subtypes of the 18 DDLPS and the 20 other sarcomas and their FNCLCC grades are presented in Table 1.

According to the FISH analysis, the 18 DDLPS harbored *MDM2* amplification, and 16 of these, harbored co-amplification of *CDK4*. According to IHC, MDM2 and CDK4 staining was more diffuse and intense than that observed in ALT-WDLPS. In terms of DDLPS, 18/18 cases were positive for MDM2 (MDM2 sensitivity = 100%) and 15/18 cases were positive for CDK4 (CDK4 sensitivity = 83.3%). In terms of other sarcomas, 6/20 cases were negative for MDM2 (MDM2 specificity = 30%) and 19/20 cases were negative for CDK4 (CDK4 specificity = 95%). For MDM2, 14 cases demonstrated discordance (FISH-/IHC+), which may be partially explained by chromosome 12 polysomy (n = 6). For CDK4, 4 cases demonstrated discordant FISH/IHC results as follows: 1 DDLPS and 1 undifferentiated sarcoma not otherwise specified (NOS) were IHC+/FISH-, whereas 2 DDLPS were IHC-/FISH+.

Next, we evaluated the utility of p16 IHC to improve the diagnosis. The p16 staining was nuclear and cytoplasmic with a moderate to strong intensity (Figure 2). In DDLPS, 17/18 cases were positive for p16 (sensitivity = 94.4%), and the sensitivity of p16 was similar to that of MDM2 but higher than that of CDK4 (Table 2B). In other sarcomas, 14/20 cases were negative for p16 (specificity = 70%), and the specificity of p16 in this case was higher than that of MDM2 but lower than that of CDK4. Interestingly, the 4 myxofibrosarcomas that were positive for MDM2 were all negative for p16. In biopsies (n = 14), p16 had a sensitivity of 100.0% and a specificity of 37.5%, whereas in excision specimens (n = 24), p16 had a sensitivity and a specificity of 91.7%. We also studied the utility of p16 in combination with MDM2 and CDK4 IHC. Interestingly, unlike the MDM2+/CDK4+ combination, which resulted in the misdiagnosis of 1 undifferentiated sarcoma NOS, all MDM2+/CDK4+/p16+ tumors (n=15) were DDLPS, while the MDM2-/CDK4-/p16- tumor was determined to be an undifferentiated sarcoma NOS.

4. DISCUSSION

The differential diagnosis of ALT-WDLPS and DDLPS is a frequent challenge in the daily practice of pathologists. MDM2 and CDK4 IHC provide clues that help with the diagnosis, but pathologists are impeded by pitfalls regarding their interpretation. MDM2 staining tends to be weak and scattered in ALT-WDLPS, and this might be misinterpreted by pathologists who are unaware of lipomas with secondary changes because inflammatory cells may also stain positive for MDM2 [5]. In addition, MDM2 positivity by IHC is not a specific indicator of *MDM2* amplification because MDM positivity is observed in cases of polysomy, as seen in our study of poorly differentiated sarcomas. CDK4 staining is more diffuse and more intense but is less sensitive than MDM2 staining. This is partially explained by

approximately 10% of ALT-WDLPS/DDLPS that do not possess amplification of *CDK4* [9, 21]. Thus, a validation by FISH, which is currently the gold standard method, is mandatory in many cases. Unfortunately, FISH is a time-consuming assay that requires specific equipment and experienced cytogeneticists who are only available in expert medical centers. FISH is also characterized by a significant level of non-contributive results. For these reasons, improvement of the IHC screening panel could be useful to better select the cases that might require confirmation by FISH.

p16 and p14^{ARF} (p14) are tumor suppressor proteins encoded by *CDKN2A* that are expressed through an alternative splicing of the first exon [7]. Indeed, p14 binds to MDM2 and inhibits its ubiquitin ligase activity, which in turn increases the levels of the anti-apoptotic p53 protein. p16 inhibits cell cycle progression by binding to the CDK4/cyclin D1 complex. It has been proposed that MDM2 and CDK4 overexpression triggers a feedback on *CDKN2A*, which induces the transcription of both p14 and p16 proteins that in turn could reduce cell proliferation. Thus, the p16 level is supposed to be correlated with the levels of MDM2 and CDK4 and appears to be a promising marker for the diagnosis of liposarcomas [8]. In the literature, p14 and p16 were both found to be overexpressed in ALT-WDLPS/DDLPS according to quantitative RT-PCR even in tumors without amplification of *CDK4* [8, 9].

To gain insight into the value and limitations of p16 IHC in the differential diagnosis of ALT-WDLPS and DDLPS, we retrieved a large series of 101 consecutive tumors analyzed by FISH. p16 IHC was performed according to the recommendation of previous studies that used the same antibody clone and dilution [19, 20, 22]. In regards to the interpretation of p16 staining, pathologists should be aware of and disregard cytoplasmic staining that may be observed in endothelial and inflammatory cells [22, 23].

In the differential diagnosis of ALT-WDLPS and benign adipocytic tumors, p16 had a sensitivity of 89.5% and a specificity of 68.2%. This sensitivity is similar to that in published

studies [19, 22]. The lower specificity may be explained by the high number of cases of lipomas with secondary changes that were included in our study. This is in agreement with data from Gonzalez *et al.* and Ng *et al.*, who also reported adipocytic nuclear p16 staining in these regions [20, 24]. Likewise, in the differential diagnosis of DDLPS and mimicking sarcomas, p16 had a good sensitivity (94.4%) but a lower specificity (70%), particularly in grade 3 (FNCLCC) sarcomas, which are complex tumors with potential numerous genomic alterations that lead to p16 overexpression. Interestingly, myxofibrosarcomas, which are frequently found to be MDM2-positive, were all negative for p16 by IHC [5]. Our results are consistent with those in a recent publication that evaluated p16 utility in the differential diagnosis of DDLPS, although that study was limited to tumors localized to the retroperitoneum [25].

For the first time, we provide results according to the type of specimen when we refer to previous studies in the field [20-22,26]. Indeed, we demonstrated that p16 specificity was particularly low in biopsies compared with excision specimens, which limits its individual use. The interpretation of biopsies is affected by limited samples and the heterogeneity of tumors. This might have an impact on p16 utility because biopsy specimens are the most challenging.

Altogether our results show that p16 IHC is characterized by a low specificity that hampered its use as a single IHC marker, especially in biopsy specimens. The adding of p16 to MDM2 and/or CDK4 might be useful to increase diagnostic specificity. Indeed, the combination of p16 and MDM2 was successful in the discrimination of all the ALT-WDLPS from benign adipocytic tumors. Likewise, positivity for p16, MDM2 and CDK4 led to the correct identification of DDLPS, whereas undifferentiated sarcomas were negative for all three markers. Prospective and multicenter studies are needed to confirm these results, and if successful, p16 IHC may be added to the MDM2 and CDK4 IHC panel.

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FIGURES AND TABLES LEGENDS

Figure 1: Representative p16 immunohistochemistry in WDLPS and lipoma with secondary changes.

In WDLPS (A, HES x100), p16 was observed to be diffusely and strongly expressed (B, IHC x200). In lipoma with secondary changes (C, HES x100), p16 expression in adipocytic nuclei may be confusing (D, IHC x200).

Figure 2: Representative p16 immunohistochemistry in DDLPS and myxofibrosarcoma.

In DDLPS (A, HES x100), p16 was diffusely expressed (B, IHC x200) whereas in myxofibrosarcoma (C, HES x100), no expression of p16 was observed (D, IHC x200).

Table 1. Immunohistochemistry results for MDM2, CDK4, p16 and combination of antibodies

Table 2. Sensitivity and specificity of markers or combination of markers in immunohistochemistry.

A. In the diagnosis of ALT-WDLPS

B. In the diagnosis of DDLPS

Figures

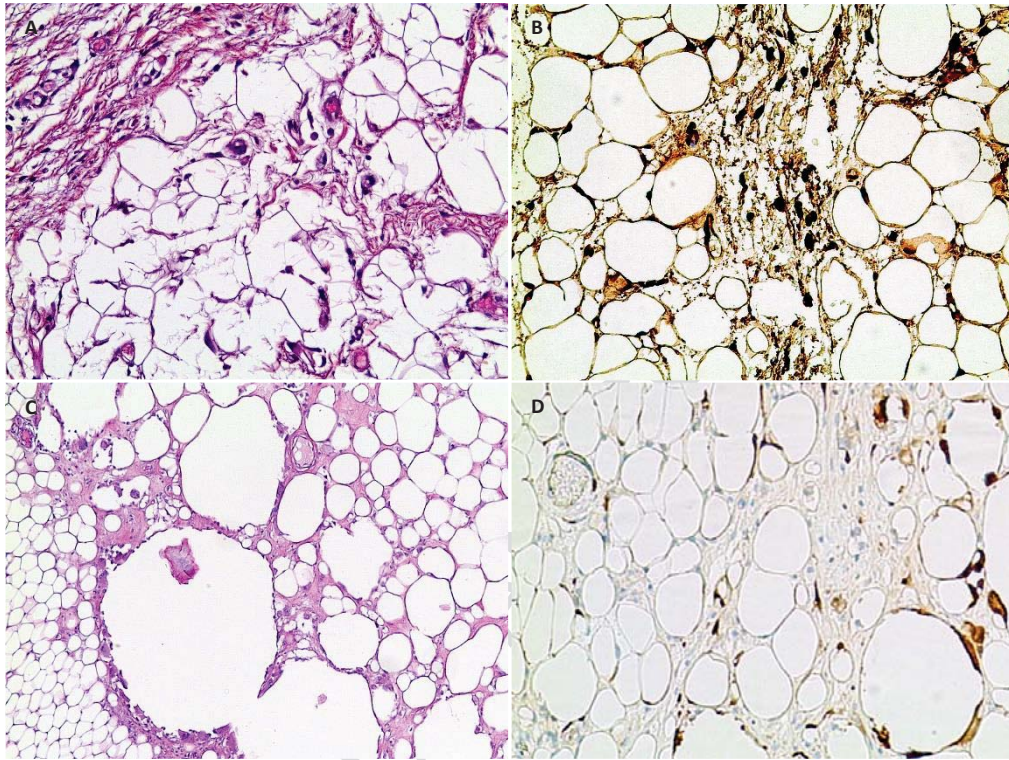


Figure 1: Representative p16 immunohistochemistry in WDLPS and lipoma with secondary changes. In WDLPS (A, HES x100), p16 was diffusely and strongly observed (B, IHC x200). In lipoma with secondary changes (C, HES x100), p16 expression in adipocytic nuclei may be confusing (D, IHC x200).

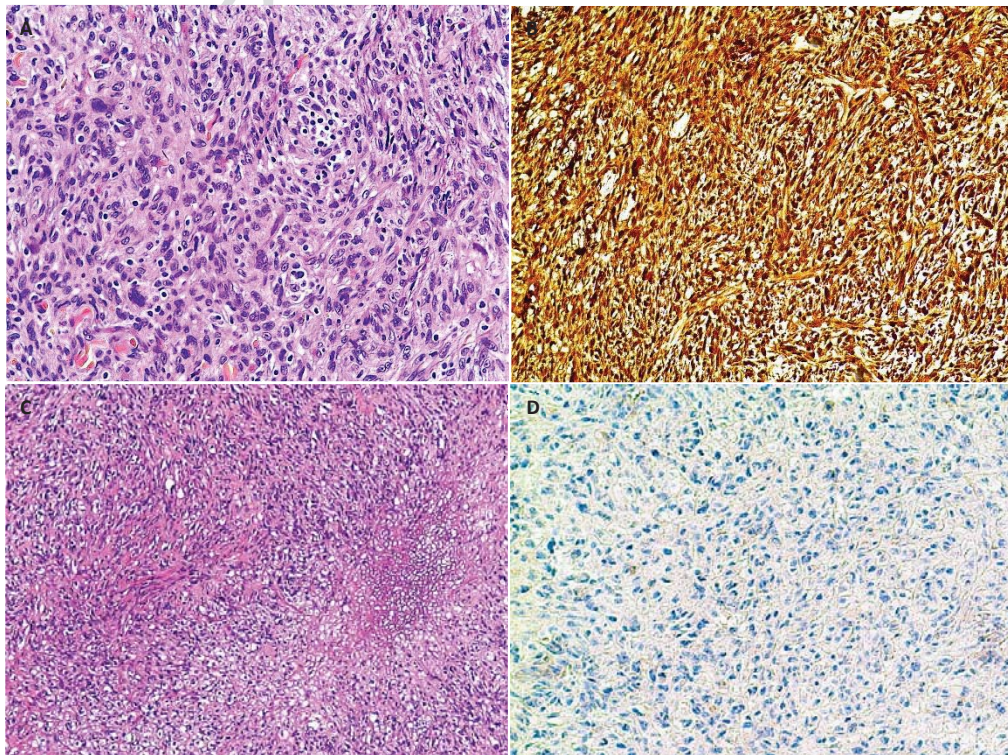


Figure 2: Representative p16 immunohistochemistry in DDLPS and myxofibrosarcoma. In DDLPS (A, HES x100), p16 was diffusely expressed (B, IHC x200) whereas in myxofibrosarcoma (C, HES x100), no expression of p16 was observed (D, IHC x200).

Table 1. Immunohistochemistry results for MDM2, CDK4, p16 and combination of antibodies

Type of Tumor	MDM2+	CDK4+	p16+	MDM2+CDK4+	MDM2+p16+	CDK4+p16+	MDM2+CDK4+P16+
ATL-WDLPS (n=19)	17	13	17	13	15	13	13
lipoma like (12)	10	7	10	7	8	7	7
sclerosing (6)	6	5	6	5	6	5	5
inflammatory (1)	1	1	1	1	1	1	1
Lipoma (n=44)	1	1	14	0	0	1	0
conventional lipoma (36)	1	0	10	0	0	0	0
intramuscular lipoma (3)	0	0	2	0	0	0	0
spindle cell lipoma (2)	0	1	1	0	0	1	0
pleomorphic lipoma (1)	0	0	1	0	0	0	0
lipomatosis (2)	0	0	0	0	0	0	0
DDLPS (n=18)	18	15	17	15	17	15	15
Grade 2 (FNLCC) (12)	12	10	12	10	12	10	10
Grade 3 (FNLCC) (6)	6	5	5	5	5	5	5
Other Sarcomas (n=20)	14	1	6	1	1	0	0
undifferentiated sarcoma NOS (9)	6	1	3	1	1	0	0
undifferentiated pleomorphic sarcoma (5)	3	0	2	0	0	0	0
myxofibrosarcoma (4)	4	0	0	0	0	0	0
conventional osteoblastic osteosarcoma (1)	0	0	1	0	0	0	0
MPNST (1)	1	0	0	0	0	0	0
Grade 2 (FNLCC) (6)	5	1	0	1	0	0	0
Grade 3 (FNLCC) (14)	9	0	6	0	1	0	0

NOS: Not Otherwise Specified, MPNST: Malignant Peripheral Nerve Sheath Tumor, FNLCC: Fédération Nationale des Centres de Lutte Contre le Cancer grading system

Table 2. Sensitivity and specificity of markers or combination of markers in immunohistochemistry**A. In the diagnosis of ALT-WDLPS**

	Sensitivity	Specificity
MDM2+	89.5%	97.7%
CDK4+	68.4%	97.7%
p16+	89.5%	68.2%
Any 2 positive	78.9%	97.7%
MDM2+CDK4+	68.4%	100.0%
MDM2-CDK4-	95.5%	89.5%
MDM2+p16+	78.9%	100.0%
MDM2-p16-	65.9%	100.0%
CDK4+p16+	68.4%	97.7%
CDK4-p16-	68.2%	89.5%
MDM2+CDK4+p16+	68.4%	100%
MDM2-CDK4-p16-	65.9%	100.0%

B. In the diagnosis of DDLPS

	Sensitivity	Specificity
MDM2+	100%	30.0%
CDK4+	83.3%	95.0%
p16+	94.4%	70.0%
Any 2 positive	94.7%	90.0%
MDM2+CDK4+	83.3%	95.0%
MDM2-CDK4-	30.0%	100.0%
MDM2+p16+	94.4%	95%
MDM2-p16-	5.0%	100.0%
CDK4+p16+	83.3%	100.0%
CDK4-p16-	65.0%	94.4%
MDM2+CDK4+p16+	83.3%	100%
MDM2-CDK4-p16-	5.0%	100.0%

Highlights:

- The diagnosis of well-differentiated/dedifferentiated liposarcoma may be difficult.
- In this indication, p16 immunohistochemistry had a low specificity, especially in biopsies, when used alone.
- Added to MDM2 and CDK4, could help discriminate well differentiated/dedifferentiated liposarcoma.

ACCEPTED MANUSCRIPT