Original paper

Comprehensive immunohistochemical analysis based on the origin of leiomyosarcoma

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Diagnostic criteria, biological behavior, and treatment approaches of leiomyosarcomas (LMS) may differ according to the origin of the tumor. This is important in terms of patient's management, especially in tumors located in the peritoneum and retroperitoneal sites. In our study, we aimed to demonstrate the immunophenotypic characteristics of uterine and extra-uterine LMS using a large antibody panel, and to determine whether they potentially play a role in the differences among these tumor groups.

Between 2006 and 2018, 29 uterine and 42 extra-uterine primary LMS were included in this study. Using tissue samples taken from the areas that best represented the tumor, an immunohistochemical study was performed on the blocks prepared by tissue micro-array method with estrogen and progesterone receptor (PR), WT-1, SMA, desmin, caldesmon, calponin, p16, p53, MDM2, CDK4, bcl-2, cyclin D1, fascin, EMMPRIN, FOXM1, c-erb-B2, c-Myc, PAX8, and CD117. Staining results of uterine and extra-uterine LMS were evaluated with these 20 antibodies.

In uterine LMS compared with extra-uterine LMS, estrogen receptor (48% vs. 12%), PR (62% vs. 21%), desmin (79% vs. 50%), and EMMPRIN (69% vs. 45%) staining rate was detected higher. In extra-uterine LMS, caldesmon (88% vs. 69%), c-Myc (33% vs. 10%), and cyclin D1 (52% vs. 28%) were stained higher than uterine LMS ($p < 0.05$). No significant staining difference was detected with other antibodies.

We concluded that estrogen receptor, PR, desmin, EMMPRIN, caldesmon, c-Myc, and cyclin D1 antibodies may help to determine primary origin of the tumor in LMS cases.

Key words: leiomyosarcoma, immunohistochemistry, uterine, extra-uterine.

Introduction

Leiomyosarcoma (LMS) is the most common sarcoma of the uterus, representing 40–50% of all sarcomas and 1–3% of all uterine malignancies [1–4]. It can occur in any part of the body, especially in the uterus, retroperitoneum, extremities, abdomen, and visceral regions [5]. It is stated to originate from smooth muscle cells or precursor mesenchymal stem cells, which differentiate into smooth muscle cells [6].

Diagnostic criteria, molecular features, prognosis, treatments, and sensitivity to treatment differ depending on uterine and extra-uterine locations of LMS [6–12]. For example, a smooth muscle tumor in soft tissue can be considered malignant due to cytological atypia or high mitotic activity, while the same finding can be interpreted as bizarre leiomyoma or mitotically active leiomyoma, which are benign leiomyoma variants of the uterus [11, 13–17]. Immuno-profile of tumors may be important for pa-

tients' treatment options. Some authors suggested aromatase inhibitors for the treatment of hormone receptor-positive LMS [18]. In addition, retroperitoneum and deep soft tissue tumors can reach large sizes and metastasize without symptoms, while those with subcutaneous localization are diagnosed early and almost never metastasize.

Leiomyosarcoma pathogenesis involves several functional gene families related to cell cycle regulation, cell homeostasis, signal transduction, growth factors, transcription factors, and oncogenes [3, 19–24]. Molecular sub-typing to determine the origin of these tumors has not yet been clarified, and the distinction in problem cases is largely based on clinical consensus [3]. Therefore, new auxiliary methods are needed to determine the origin of these tumors, which are genetically heterogeneous and complex. In this study, we aimed to investigate whether there is a difference in staining between LMS originating from the uterus and extra-uterine, and the value of detected data in the differentiation of these tumor groups by using a large immunohistochemical panel. The selection of immune markers primarily aimed to determine the origin of tumor. The panel was expanded with other antibodies, which were identified in previous studies and thought to may be significant in terms of tumor progression, differentiation, survival, and treatment efficacy. We also compared the immunohistochemical characteristics of uterine LMS with those of abdominal/ retroperitoneal origin, which are problematic in clinical recognition, and also have different diagnostic criteria and treatment protocols.

Material and methods

Case selection

Twenty males and 22 females with extra-uterine LMS (EU-LMS), and 29 uterine LMS (U-LMS) cases diagnosed with primary LMS between 2006 and 2018 at our pathology department were included in this study. Histopathological features of smooth muscle tumors, including cellularity, mitotic rate, cytological atypia, and tumor cell necrosis were analyzed and recorded. According to the current World Health Organization (WHO) diagnostic criteria, uterine LMS is diagnosed as spindle cell smooth muscle tumor when the tumor has at least two of the following criteria: marked cytological atypia (2+/3+), tumor cell necrosis, and high mitotic rate (≥ 4 mitoses/mm^2) [25]. Myxoid and epithelioid types of tumor were not included in the study. For extra-uterine LMS, the current WHO diagnostic criteria were used based on their localizations [26–28]. In all cases, sections stained with hematoxylin and eosin were re-examined, and the best samples were selected for tissue micro-array. Current medical records and clinical information of cases were scanned from computer records and reviewed. The study was granted approval by the relevant institutional ethics committee (approval number: OMU KAEK 2018/159).

Tissue micro-arrays and immunohistochemistry

Necrosis-free areas and parts that represented the tumor best in the hematoxylin and eosin stained slides were identified and removed from the block. Two samples for each case were embedded in 3 paraffin blocks with 60 cavities, each with a diameter of 1 mm. Immunohistochemical tests were applied to each block with 20 antibodies. Nuclear with estrogen receptor (ER), progesterone receptor (PR), FOXM1, CDK4, MDM2, c-Myc, PAX8, c-erb-B2, cyclin D1, p53, cytoplasmic and nuclear with WT-1, p16, bcl-2, membranous with EMMPRIN, and cytoplasmic staining with c-kit, fascin, calponin, SMA, desmin, and caldesmon were evaluated (Table 1). *P*-value < 5% staining was accepted as negative, and 6–100% staining was accepted as positive.

Statistical method

One-sample Kolmogorov-Smirnov and t-test were used to evaluate age distribution in statistical evaluation. Categorical data were compared with χ^2 test. The staining features of 20 immunohistochemical markers were compared in 2 groups with U-LMS and EU-LMS cases, and in 3 groups with U-LMS, females and males EU-LMS cases. *P*-value < 0.05 was considered significant.

Results

Demographic information

Of the 71 cases included in the study, 29 (41%) were U-LMS, and 42 (59%) were EU-LMS cases. Twenty-two (52%) of EU-LMS cases were females, and 20 (48%) were males. Fourteen of EU-LMS tumors were located in the extremities, 8 in the retroperitoneum, 6 in the abdomen, 6 in the skin, 4 in the intestine, 3 in the head and neck region, 3 in the testis, and 1 in the thorax. The mean age of all cases was 57.0 ±15.2 years (range, 18–79 years). The mean age of U-LMS cases was 52.5 ±9.8 years (range, 28–76 years), the mean age of EU-LMS cases was 60.0 ±17.5 years (range, 18–79 years), and the difference between the two groups was statistically significant, with $p = 0.026$. In EU-LMS cases, the age difference between female (59.0 ±15.8 years) and male (61.0 ±19.6 years) genders was not significant ($p > 0.05$) (Table 2).

Immunohistochemical findings

U-LMS cases were stained with ER, PR, desmin, and EMMPRIN at a higher rate than EU-LMS cases ($p < 0.05$) (Fig. 1). When EU-LMS cases were con-

Table 1. Antibodies used for immunohistochemistry

Marker	Clone and species	Company	Staining pattern (c, n, m, c–n)
Estrogen receptor	(SP1) rabbit monoclonal primary antibody	Ventana, Tucson, Arizona, USA	Nuclear
Progesterone receptor	Clone 16, NCL-L-PGR-312, mouse monoclonal antibody	Novacastra liquid, Leica, United Kingdom	Nuclear
WT-1	6F-H2, mouse monoclonal antibody	Cell Marque, Tucson, Arizona, USA	Nuclear and cytoplasmic
EMMPRIN	8D6: SC21746, mouse monoclonal IgG	Santa Cruz, Biotechnology, USA	Membranous
Caldesmon	E89, rabbit monoclonal antibody	Cell marque, USA	Cytoplasmic
Desmin	DE-R-11, mouse monoclonal antibody	Roche, Arizona, USA	Cytoplasmic
SMA	1A4, mouse monoclonal antibody	Roche, Arizona, USA	Cytoplasmic
Calponin	EP798Y, rabbit monoclonal antibody	Roche, Cell marque, USA	Cytoplasmic
C-Myc	EP121, rabbit monoclonal antibody	ZETA, USA	Nuclear
Cyclin D1	SP4, rabbit monoclonal antibody	Cell marque, USA	Nuclear
CDK4	DCS-31, mouse monoclonal antibody	ZETA, USA	Nuclear
P16	IHC016, mouse monoclonal antibody	GenomeMe, Canada	Nuclear and cytoplasmic
Bcl-2	SP66, rabbit monoclonal antibody	Ventana, Arizona, USA	Nuclear and cytoplasmic
P53	DO-7, mouse monoclonal antibody	Leica, United Kingdom	Nuclear
MDM2	BSB-2979, mouse monoclonal antibody	Bio-SB, Santa Barbara, USA	Nuclear
FOXM1	ABN286, rabbit polyclonal antibody	EMDMillipore, Temecula, USA	Nuclear
Fascin	FCN01(55K-2), mouse monoclonal antibody	Thermo Fisher Scientific, USA	Cytoplasmic
C-kit (CD117)	9.7, rabbit monoclonal antibody	Ventana, Arizona, USA	Cytoplasmic
C-erb-B2	4B5, rabbit monoclonal antibody	Roche, Tucson, Arizona, USA	Nuclear
PAX8	MRQ-50, mouse monoclonal antibody	Roche, Cell Marque, USA	Nuclear

Table 2. Age distribution of uterine and extra-uterine leiomyosarcomas

		Uterine ($n = 29$)	Extra-uterine ($n = 42$)	Total ($n = 71$)	*p*-value
Age at diagnosis (years)	Median	52.5 ±0.8	60.0 ±17.5	57.0 ±15.2	0.026
	Range	28–76	18–79	18–79	

sidered as two different groups according to female and male genders and three groups were evaluated separately, the U-LMS cases were stained more with ER, PR, desmin ($p < 0.05$) compared with EU-LMS cases. While the male and female EU-LMS cases were compared, no significant staining difference was found ($p > 0.05$). When EMMPRIN positivity was evaluated between the three groups, the difference in staining was not statistically significant ($p > 0.05$) (Table 3).

EU-LMS cases were stained with caldesmon, c-Myc, and cyclin D1 at a higher rate than U-LMS cases ($p < 0.05$) (Fig. 2). When EU-LMS cases were considered as two different groups according to female and male genders, the only difference in the c-Myc staining among these three antibodies was found statistically significant among the three groups ($p < 0.05$) (Table 3).

There was not any statistically significant differences between cases U-LMS and EU-LMS regarding WT-1, SMA, calponin, CDK4, bcl-2, p53, p16, FOXM1, fascin, and CD117 staining ($p > 0.05$) (Table 4). No staining was detected in any of the cases in the immunohistochemical evaluation with C-erb-b2, PAX8, and MDM2.

Staining of LMS located in the retroperitoneum ($n = 8$) and abdomen ($n = 6$), which are clinically more problematic due to their site in terms of differential diagnosis with U-LMS and staining differences with U-LMS, are summarized in Table 5. There was

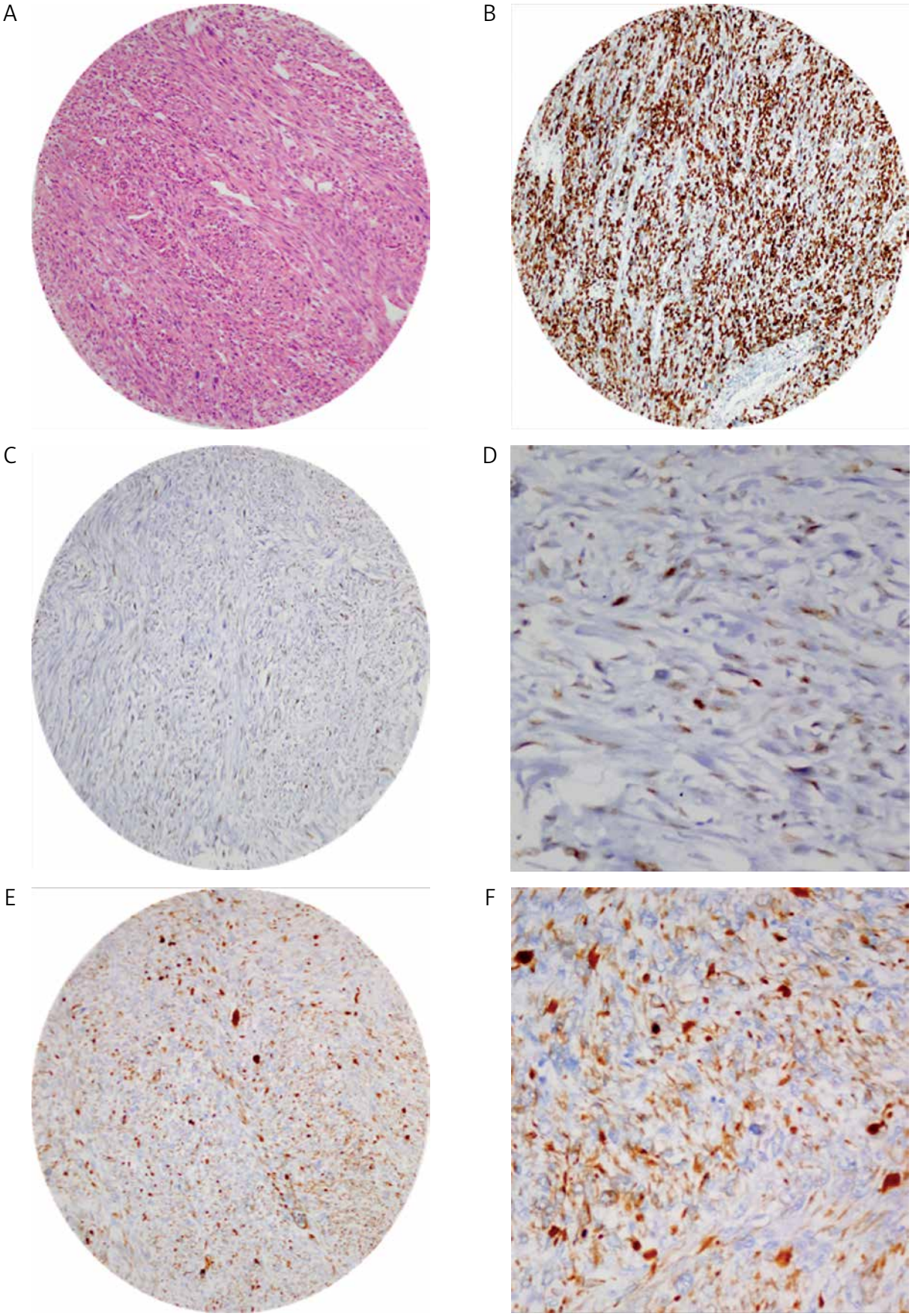

Fig. 1. In uterine leiomyosarcomas, estrogen receptor, progesterone receptor, desmin, and EMMPRIN staining rate was higher than extra-uterine leiomyosarcomas. A) Hematoxylin and eosin staining, 100×; B) estrogen receptor, 100×; C, D) progesterone receptor, 100×; E, F) desmin staining, 100×, 400×

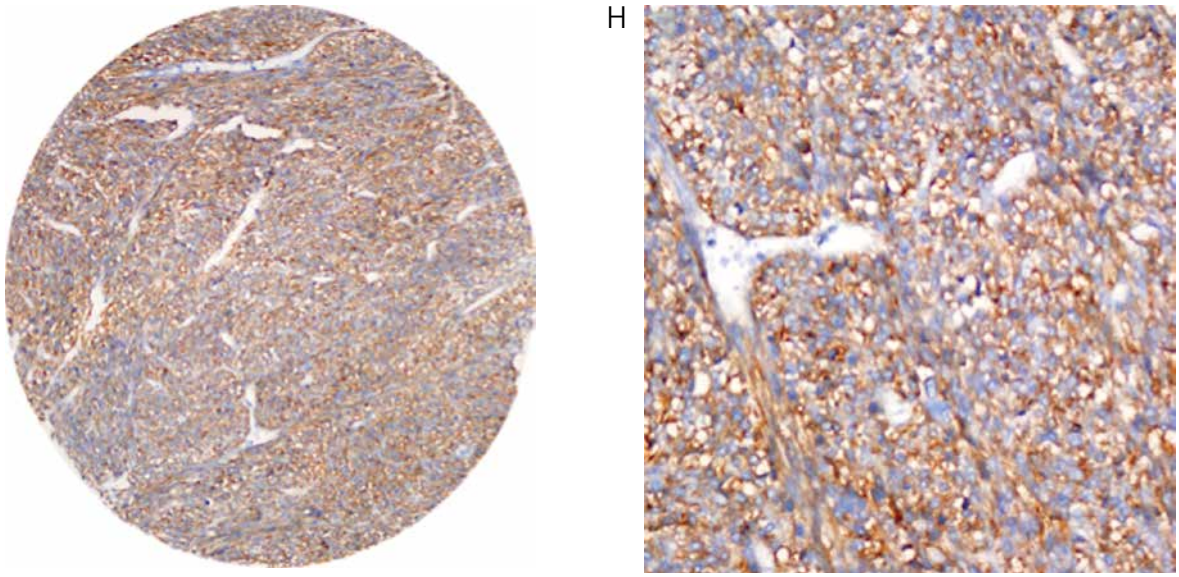

Fig. 1. Cont. G, H) EMMPRIN staining, 400×

no statistically significant differences between the groups ($p > 0.05$).

Discussion

Many immunohistochemical markers have been previously investigated to determine the histopathological diagnosis, origin, and behavior of LMS [13–16, 22, 29–38]. In the selection of immunomarkers, the primary aim was to determine the origin of the tumor. In addition, it was aimed to establish the staining status and the presence of differences in U-LMS and EU-LMS of the markers discussed in various studies in terms of tumor progression, survival indicator, and treatment efficacy. Steroid hormone receptors (ER, PR), mullerian transcription markers (WT1, PAX8), smooth muscle markers (SMA, desmin, caldesmon, and calponin), markers that may contribute to treatment efficacy (CD117), and markers effective in tumor progression (EMMPRIN, fascin, c-Myc, MDM2, FOXM1, cyclin D1, CDK4, bcl-2, p16, p53, and c-erb-B2) were selected for the current study.

Steroid hormone receptors are one of the most commonly used markers in routine practice to differentiate U-LMS and EU-LMS. Statistically significant results were obtained in previous studies evaluating ER and PR positivity rates in U-LMS and EU-LMS. Rao *et al*. reported ER positivity in 12.5% of EU-LMS cases (2 of 16 tumors) and 71% of U-LMS cases (10 of 14 tumors). It was reported that a stained case in the EU-LMS group was localized in the retroperitoneum, and stained focal and weakly positive. The other case was located in the upper extremity, and stained focal and edge only [39]. Kelley *et al*. reported ER positivity in 87% of cases (13 of 15 tumors), and PR positivity in 80% (2 of 16 tumors) of U-LMS. Whereas ER positivity in 25% (4 of 16 tumors, 3 of which were female cases), and PR in 13% (2 of 16 tumors) of EU-LMS were detected. They observed that all EU-LMS cases showed only weak (1+: 1 to 25% of nuclei stained) ER and PR immunoreactivity, except for one case, which was stained intensely (4+: 76–100% of nuclei stained). The relatively higher staining rate of EU-LMS cases compared with other studies can be explained by taking the cut-off value as 1% in this study. Intense (4+) ER and PR staining was detected in a 61-year-old female patient, with a solitary tumor in the lower thoracic vertebral region and no primary U-LMS. Although the authors thought that this case may be an undiagnosed U-LMS due to previous hysterectomy for uterine fibroid, it was reported that hematoxylin-eosin-stained sections taken from the hysterectomy sample were leiomyoma only, and did not show any recurrence [40]. In Carvalho *et al*. study, ER positivity in 63% (19 of 30 tumors) and PR positivity in 73% (22 of 30 tumors) of U-LMS cases were reported. While 23% (11 of 48 tumors) of EU-LMS cases were ER-positive, PR positivity was reported in 40% cases (19 of 48 tumors), with a higher rate compared with the literature. It was stated that significant ratio of positively stained cases were weakly positive [41]. Lee *et al*. reported ER positivity in 50% (51 of 102 tumors) of U-LMS and 3% (4 of 140 tumors) of EU-LMS cases. In that study [9], the locations of ER-positive EU-LMS included one in male's genital region, one in male's rectal region, and two in female's abdominal/pelvic region. In the current study, 48% (14 of 29 tumors) of U-LMS cases and 12% (5 of 42 tumors) of EU-LMS cases were stained positively with ER, similar to previous studies. Two of the ER-positive EU-LMS cases were females, and three were males. Two of these tumors were of abdominal origin. 62% of U-LMS (18 of 29 tumors) and 21% of EU-LMS (9 of 42 tumors)

Table 3. Statistically significant immunohistochemical staining of uterine and extra-uterine leiomyosarcomas

Marker	Origin		Negative, *n* (%)	Positive, *n* (%)	*p*-value
Estrogen receptor	Uterine		15/29 (52.0)	14/29 (48.0)	0.00053
	Extra-uterine		38/42 (88.0)	5/42 (12.0)	
	Uterine		15/29 (52.0)	14/29 (48.0)	0.00278
	Extra-uterine	Female	20/22 (91.0)	2/22 (9.0)	
		Male	17/20 (85.0)	3/20 (15.0)	
Progesterone receptor	Uterine		11/29 (38.0)	18/29 (62.0)	0.00052
	Extra-uterine		33/42 (79.0)	9/42 (21.0)	
	Uterine		11/29 (38.0)	18/29 (62.0)	0.00221
	Extra-uterine	Female	18/22 (82.0)	4/22 (18.0)	
		Male	15/20 (75.0)	5/20 (25.0)	
EMMPRIN	Uterine		9/29 (31.0)	20/29 (69.0)	0.04825
	Extra-uterine		23/42 (55.0)	19/42 (45)	
	Uterine		9/29 (31.0)	20/29 (69.0)	0.14213
	Extra-uterine	Female	12/22 (55.0)	10/22 (45.0)	
		Male	11/20 (55.0)	9/20 (45.0)	
Desmin	Uterine		6/29 (21.0)	23/29 (79.0)	0.01239
	Extra-uterine		21/42 (50.0)	21/42 (50.0)	
	Uterine		6/29 (21.0)	23/29 (79.0)	0.03581
	Extra-uterine	Female	12/22 (55.0)	10/22 (45.0)	
		Male	9/20 (45.0)	11/20 (55.0)	
Caldesmon	Uterine		9/29 (31.0)	20/29 (69.0)	0.04643
	Extra-uterine		5/42 (12.0)	37/42 (88.0)	
	Uterine		9/29 (31.0)	20/29 (69.0)	0.12265
	Extra-uterine	Female	2/22 (9.0)	20/22 (91.0)	
		Male	3/20 (15.0)	17/20 (85.0)	
C-Myc	Uterine		26/29 (90.0)	3/29 (10.0)	0.02566
	Extra-uterine		28/42 (67.0)	14/42 (33.0)	
	Uterine		26/29 (90.0)	3/29 (10.0)	0.00756
	Extra-uterine	Female	12/22 (55.0)	10/22 (45.0)	
		Male	11/20 (55.0)	9/20 (45.0)	
Cyclin D1	Uterine		21/29 (72.0)	8/29 (28.0)	0.03761
	Extra-uterine		20/42 (48.0)	22/42 (52.0)	
	Uterine		21/29 (72.0)	8/29 (28.0)	0.07521
	Extra-uterine	Female	9/22 (41.0)	13/22 (59.0)	
		Male	11/20 (55.0)	9/20 (45.0)	

cases were positive for PR in our cohort. In a study by Carvalho *et al*., ER or PR positivity in EU-LMS cases was more common in female cases (15 of 24 female cases (63%) vs. 6 of 24 male cases (25%)) [41]. In the present study, there was no significant difference in ER and PR staining when male and female cases of EU-LMS groups were compared ($p > 0.05$). The ER staining percentage of extra-uterine tumors was always above 70% in males, and the staining intensity varied as weak, moderate, and high. In females, LMS in the pelvic region was stained at high intensity and high rate, while the weak intensity and low rate ER staining in the calf was stained. It was observed that the case of LMS located in the chest wall in a male patient was stained with diffuse strong PR, and in a female patient, LMS located in the retroperitone-

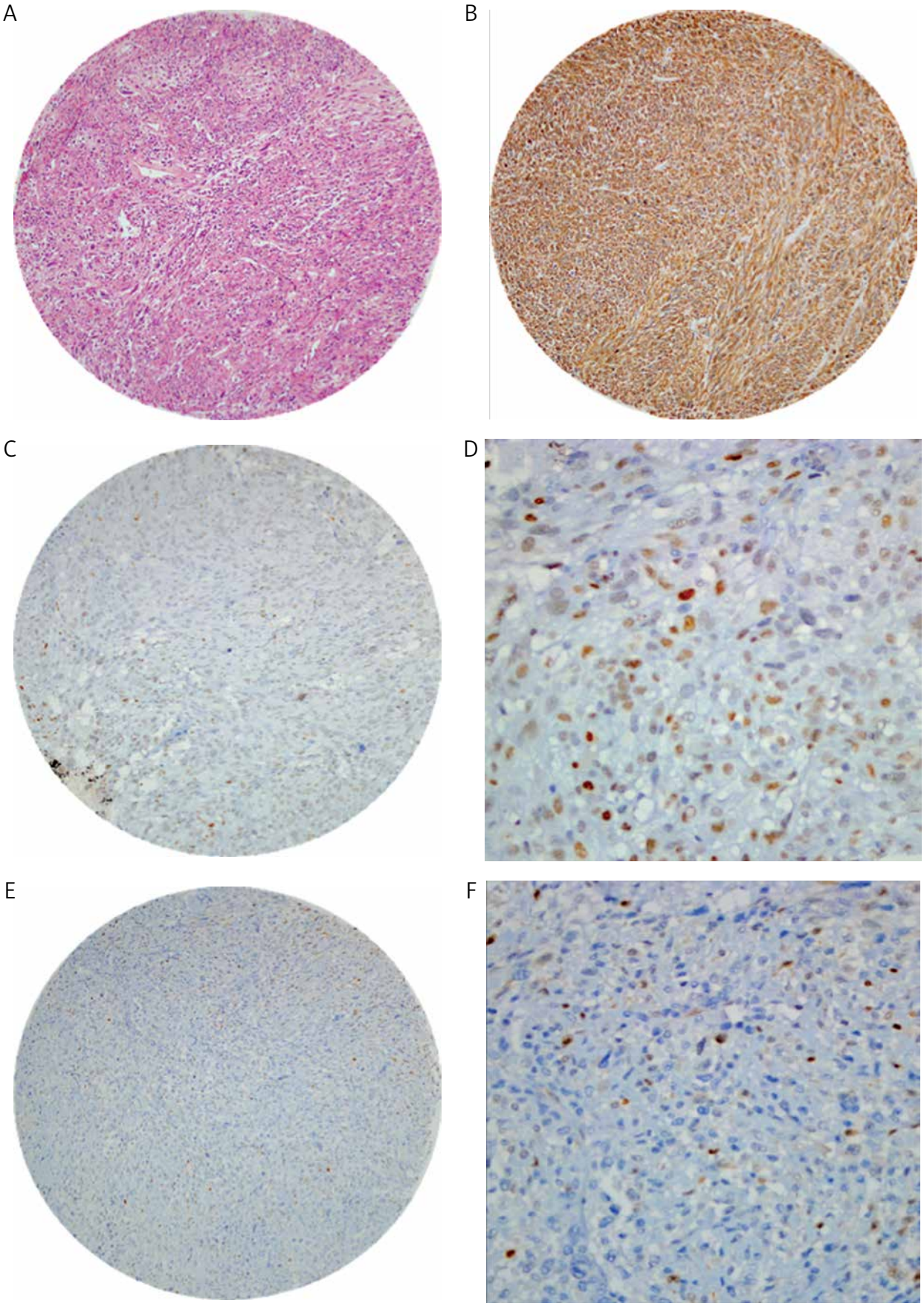

Fig. 2. Extra-uterine leiomyosarcomas were stained at higher rate with caldesmon, c-Myc, and cyclin D1 when compared with uterine leiomyosarcomas. A) Hematoxylin and eosin staining, 100×; B) caldesmon staining, 100×; C, D) c-Myc staining, 100×, 400×; E, F) cyclin D1 staining, 100×, 400×

Table 4. Statistically insignificant immunohistochemical staining of uterine and extra-uterine leiomyosarcomas

Marker	Origin	Negative, *n* (%)	Positive, *n* (%)	*p*-value
WT-1	Uterine	19/29 (66.0)	10/29 (34.0)	0.9149522
	Extra-uterine	27/42 (64.0)	15/42 (36.0)	
SMA	Uterine	1/29 (4.0)	28/29 (96.0)	0.2079416
	Extra-uterine	5/42 (12.0)	37/42 (88.0)	
Calponin	Uterine	10/29 (35.0)	19/29 (65.0)	0.0602755
	Extra-uterine	24/42 (57.0)	18/42 (43.0)	
CDK4	Uterine	24/29 (83.0)	5/29 (17.0)	0.5051350
	Extra-uterine	32/42 (76.0)	10/42 (24.0)	
P16	Uterine	13/29 (45.0)	16/29 (55.0)	0.0628564
	Extra-uterine	10/42 (24.0)	32/42 (76.0)	
Bcl-2	Uterine	9/29 (31.0)	20/29 (69.0)	0.0746420
	Extra-uterine	22/42 (52.0)	20/42 (48.0)	
P53	Uterine	25/29 (86.0)	4/29 (14.0)	0.7422097
	Extra-uterine	35/42 (83.0)	7/42 (17.0)	
FOX-M1	Uterine	6/29 (21.0)	23/29 (79.0)	0.1836920
	Extra-uterine	4/42 (9.0)	38/42 (91.0)	
Fascin	Uterine	11/29 (38.0)	18/29 (62.0)	0.2930035
	Extra-uterine	11/42 (26.0)	31/42 (74)	
CD117	Uterine	25/29 (86.0)	4/29 (14)	0.5760020
	Extra-uterine	38/42 (90.0)	4/42 (10)	

Table 5. Immunohistochemical staining of retroperitoneal, abdominal, and uterine leiomyosarcomas

Parameters	Retro-peritoneal, *n* (%)	Abdominal, *n* (%)	Uterine, *n* (%)
ER	0/8 (0.0)	2/6 (33.3)	14/29 (48.0)
WT-1	3/8 (30.0)	3/6 (50.0)	10/29 (34.0)
PR	0/8 (0.0)	1/6 (16.6)	18/29 (62.0)
Caldesmon	7/8 (87.5)	6/6 (100.0)	20/29 (69.0)
Desmin	5/8 (62.5)	2/6 (33.3)	23/29 (79.0)
C-Myc	2/8 (25.0)	2/6 (33.3)	3/29 (10.0)
EMMPRIN	5/8 (62.5)	3/6 (50.0)	20/29 (69.0)
Cyclin D1	5/8 (62.5)	2/6 (33.3)	8/29 (28.0)

um was stained in weak intensity and low intensity. These findings suggested that hormone receptor positivity in LMS is a feature related to the tumor itself rather than gender.

WT-1, the transcription factor that plays a role in the development of the genitourinary system, has recently been defined as a guide in Müllerian differentiation. For this reason, its' usability in differentiation of U-LMS and EU-LMS has been investigated by various authors. Lee *et al*. reported nuclear WT-1 staining of 8% (8 of 98 tumors) for U-LMS, whereas none for EU-LMS. The authors observed cytoplasmic WT-1 immunostaining in 55% (54 of 98 tumors) of U-LMS and 52% (68 of 131 tumors) of EU-LMS cases [9]. In Carvalho *et al*. study evaluating 30 cases of U-LMS and 48 cases of EU-LMS, nuclear WT-1 staining was detected in 11% of all tumors. 23% of U-LMS (7 of 30 tumors) and 6% of EU-LMS (3 of 48 tumors) were stained nuclear positive for WT-1. In this study, WT-1 positivity was found only in female cases and in ER-positive retroperitoneum and uterine tumors. Therefore, nuclear WT-1 expression was thought to identify a common subset of tumors that were likely Müllerian in a particular group of tumors in female cases [41]. Bing *et al*. also reported cytoplasmic WT-1 staining in 64% (16 of 25 tumors) of U-LMS in their

cohort [42]. In the current study, 14% of U-LMS cases (4 of 29 tumors) and 5% of EU-LMS cases (2 of 42 tumors) were nuclear-positive for WT-1. One of the cases with positive nuclear staining in the EU-LMS group was in the retroperitoneum and the other was in the para-testicular location. Although nuclear WT-1 positivity was observed more frequently in U-LMS, this difference was not statistically significant ($p < 0.05$). In our study, all of the cases that demonstrated nuclear staining showed cytoplasmic staining as well. When nuclear and cytoplasmic staining are evaluated together, 34% (10 of 29 tumors) of U-LMS and 36% (15 of 42 tumors) of EU-LMS cases were stained for WT-1. There was no statistically significant staining between these two groups, both nuclear and cytoplasmic WT-1-positive.

EMMPRIN, also known as CD147, is a 58 kDa weight transmembrane protein that is encoded by BSG gene [17]. EMMPRIN activates the induction of matrix metalloproteinases, which are important for extra-cellular matrix degradation and tumor progression. Although its' use in the differentiation of uterine smooth muscle tumors has been investigated, its' place in differential diagnosis of U-LMS and EU-LMS has not been investigated before [31]. Although the difference in EMMPRIN staining between U-LMS and EU-LMS was significant in our study (69% vs. 45% positive, respectively; $p = 0.04825$), the difference in staining in U-LMS and female EU-LMS was insignificant, suggesting that EMMPRIN cannot be a reliable marker when distinguishing U-LMS and EU-LMS cases in routine practice.

In previous studies, staining frequencies of LMS with SMA, caldesmon, desmin, and calponin were investigated [43–45]. In Carvalho's study, it was stated that EU-LMS were stained with SMA at a higher rate than U-LMS (100% vs. 87%, respectively). Higher rates of desmin staining were detected in uterine tumors (83%) and retroperitoneal tumors in female cases (86%), and caldesmon was stained at higher rates in retroperitoneal tumors, in all females (94%, 15 of 16 tumors). With calponin, a positivity of 87% (26 of 30 tumors) was detected in U-LMS and 92% (44 of 48 tumors) in EU-LMS [41]. In our study, the percentage of staining with SMA, desmin, caldesmon, and calponin was 96%, 79%, 69%, and 65% for U-LMS, and 88%, 50%, 88%, and 43% for EU-LMS, respectively (Tables 3, 4). It was thought that the statistically significant staining difference between these two groups resulted from the high desmin staining rate of U-LMS, similar to Carvalho's research. In our study, when compared with Carvalho's study, it was noted that staining with caldesmon in EU-LMS cases did not make any difference in female gender, but it was stained at a higher rate in EU-LMS. Demicco *et al*. in their studies on 203 EU-LMS and 181 U-LMS cases, indicated that loss of expression in muscle markers could be observed in LMS, associated with loss of differentiation [46]. In our study, it was thought that the reason for no staining with muscle markers in some LMS cases were poorly differentiated tumor areas or limited sampling with the tissue micro-arrays.

The expression of c-Myc in LMS cases has been investigated in several studies. Jeffers *et al*. reported c-Myc over-expression in 11 of 23 U-LMS cases, but this did not correlate with survival [47]. Tsiatis *et al*. detected nuclear c-Myc expression in 15 of 28 soft tissue LMS cases, and they reported that cases with c-Myc-positive tumors had significantly shorter metastasis-free survival intervals than cases with c-Myc-negative tumors [48]. Its' place in the differentiation of U-LMS and EU-LMS has not been investigated before. In the present study, nuclear c-Myc expression was found at a higher rate in EU-LMS cases (10% vs. 33%), and this difference was statistically significant ($p = 0.02$).

Cyclin D1 and CDK4 amplification have been reported to be observed in various tumors, including sarcomas [49–52]. Rb-cyclin D1 pathway is thought to be a specific target for molecular abnormalities in soft tissue LMS, and cyclin D1 over-staining may indicate an alternative mechanism to bypass Rb-mediated inhibition of cell proliferation [53]. Lee *et al*. showed that cyclin D1 is known to be a sensitive and specific diagnostic immunomarker for the histologically higher-grade and clinically more aggressive endometrial stromal sarcoma (ESS) sub-type YWHAE-FAM22 ESS [54]. In our study, a higher rate of cyclin D1 staining was observed in EU-LMS cases compared with U-LMS (52% vs. 28%, respectively; $p = 0.037$). In the current study, as with other antibodies, cyclin D1-positive was evaluated as $> 5\%$. As in a study of Lee *et al*., when $\geq 70\%$ moderate to strong nuclear positivity was taken as a cut-off, positivity was observed in 2 U-LMS and 6 EU-LMS in our series. There was no significant difference between the two groups in regard to CDK4 [54].

Bcl-2 expression, which was previously investigated as an indicator of tumor behavior, prognosis, and survival for LMS showed a similar staining percentage in U-LMS and EU-LMS cases in the present study ($p > 0.05$) [29, 30, 38]. p53 gene mutation observed mostly in advanced stage and high-risk soft tissue sarcomas has been also investigated in U-LMS and EU-LMS in the literature, and was found to be stained relatively more frequently in U-LMS cases [54–60]. In a study of Lee *et al*., p53 was stained positively in 29% (25 of 87 tumors) of U-LMS cases and 22% (21 of 96 tumors) of EU-LMS cases [9]. It was thought that MDM2 could act independently and through p53 gene mutation, while MDM2 expression was found in U-LMS cases in several studies [39]. Hall *et al*. reported MDM2 over-expression of 13% (3 of

23 tumors), while Blom *et al*. reported 8% (4 of 49 tumors) in U-LMS cases [56–58]. In a study by Rao *et al*., it was stated that MDM2 amplification was more frequently seen in EU-LMS cases [39]. In our study, the staining difference in bcl-2, p53, and MDM2 was not significant in distinguishing U-LMS and EU-LMS cases ($p > 0.05$). This suggests that even though they are involved in oncogenesis, it will not contribute to differentiating them from other soft tissue sarcomas as well as determining the origin of U-LMS and EU-LMS.

Although most of the tumors outside the uterus are thought to be of vascular smooth muscle origin, Posligua *et al*. in their study conducted on 19 low-grade and 31 high-grade LMS cases shown that smooth muscle tumors observed in the peritoneum and retroperitoneum may be a second primary associated with secondary mullerian system rather than recurrence in follow-up of low-grade tumors. Staining differences supporting the possibility of independent tumors between U-LMS and EU-LMS were detected in 7 out of 10 cases with immunohistochemical markers (ER, WT1) [61]. When we compared U-LMS cases with retroperitoneal and abdominal LMS cases, we observed that ER, PR, and WT-1 were stained at a relatively lower rate in retroperitoneal LMS, while ER and WT-1 were stained in similar rates in abdominal LMS compared with U-LMS. Unlike Posligua's study, we did not classify tumors as low- and high-grade in our study. However, as proposed by Posligua *et al*., it should be kept in mind that the possibility of retroperitoneal and peritoneal low-grade LMS may arise from a secondary Müllerian system, and they may have similar immuno-profile as uterine tumors.

Conclusions

In the current study conducted on 71 LMS cases using the tissue micro-array method, it was concluded that staining with ER, PR, desmin, and EMMPRIN might support the uterine origin, while caldesmon, c-Myc, and cyclin D1 may support the extra-uterine origin. The representation of a small part of the tumor due to the use of the tissue micro-array method in our study may have affected the staining results, especially for tumors with heterogeneous differentiation areas. Nevertheless, we think our study results should be considered in cost-effective planning while making differential diagnoses of U-LMS and EU-LMS using immunohistochemical method.

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