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ESTUDIS DE DOCTORAT EN MORFOLOGIA I PATOLOGIA ESTRUCTURAL I MOLECULAR

Tesi Doctoral

Uterine Sarcomas:
Immunohistochemical and Molecular Alterations with Prognostic Implications

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Cuando llegué por primera vez como residente visitante al Servicio de Patologia del Hospital de la Santa Creu i Sant Pau, en marzo de 2007, me ofrecieron la posibilidad de investigar los factores pronósticos en los sarcomas uterinos. Durante los 5 años siguientes (2008-2013) tuve la oportunidad de presentar los resultados de estas investigaciones en tres congresos de USCAP y 2 internacionales de oncología ginecológica siempre en forma de comunicaciones orales. Dichos resultados se publicaron a la vez en revistas de Patología y Oncología Ginecológica. Deseo manifestar aquí mi sincero agradecimiento a todas las personas que lo hicieron posible.

Barcelona, Noviembre 2013

ABBREVIATIONS

AR – Androgen Receptor

CDKN - Cyclin-dependent kinase

DNA – Deoxyribonucleic Acid

EGFR – Epidermal Growth Factor Receptor

EMA – Epithelial Membran Antigen

ER – Estrogen Receptor

ESN – Endometrial Stromal Nodule

ESS – Endometrial Stromal Sarcoma

EST – Endometrial stromal Tumor

FIGO – International Federation of Gynecology and Obstetrics

GnRH – Gonadotropin Realising Hormone

HDAC8 - Histone deacetylase 8

HG-ESS – High-grade Endometrial Stromal Sarcoma

HPFs – High Power Fields

LG-ESS – Low-grade Endometrial Stromal Sarcoma

LMS - Leiomyosarcoma

MF – Mitotic Figures

miRNA - MicroRNA

MMMT – Malignant Mixed Mullerian Tumor

PCNA - Proliferating cell nuclear antigen

PR – Progesteron Receptor

RNA – Ribonucleic Acid

STUMP – Smooth Muscle Tumor of Uncertain Malignant Potential

UES – Undifferentiated Endometrial Sarcoma

UTROSCT – Uterine Tumor Resembling Ovarian Sex Cord Tumor

VEGF – Vascular Endothelial Growth Factor

WHO – World Health Organization

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INTRODUCTION

Uterine sarcomas are rare tumors that account for approximately 1% of female genital tract malignancies and 3% to 7% of uterine cancers.[1] Although the aggressive behavior of most cases is well recognized, their rarity and histopathological diversity has contributed to the lack of consensus on risk factors for poor outcome and optimal treatment.[2]

Histologically, uterine sarcomas were first classified into carcinosarcomas, accounting for 40% of cases, leiomyosarcomas (40%), endometrial stromal sarcomas (10% to 15%), and undifferentiated sarcomas (5% to 10%). Recently, carcinosarcoma has been reclassified as a dedifferentiated or metaplastic form of endometrial carcinoma. Despite this, and probably because it behaves more aggressively than the ordinary endometrial carcinoma, carcinosarcoma is still included in most retrospective studies of uterine sarcomas, as well as in the 2003 World Health Organization (WHO) classification.[3]

The 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria for endometrial carcinoma have been used until now

to assign stages for uterine sarcomas in spite of the different biologic behavior of both tumor categories. Recently, however, a new FIGO classification and staging system has been specifically designed for uterine sarcomas in an attempt to reflect their different biologic behavior (Table 1).[4] Briefly, three new classifications have been developed: (1) staging for leiomyosarcomas and endometrial stromal sarcomas; (2) staging for adenosarcomas; and (3) staging for carcinosarcomas (MMMT). Whereas in the first classification stage I sarcomas are subdivided according to size, subdivision of stage I adenosarcomas takes into account myometrial invasion. On the other hand, carcinosarcomas will continue to be staged as endometrial carcinomas.

Table 1 FIGO staging for uterine sarcomas (2009).

(1) Leiomyosarcomas and endometrial stromal sarcomas (*)

Stage		Definition
I		Tumor limited to uterus
	IA	Less than or equal to 5 cm
	IB	More than 5 cm
II		Tumor extends beyond the uterus, within the pelvis
	IIA	Adnexal involvement
	IIB	Involvement of other pelvic tissues
III		Tumor invades abdominal tissues (not just protruding into the abdomen)
	IIIA	One site
	IIIB	More than one site
	IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	IVA	Tumor invades bladder and/or rectum
	IVB	Distant metastasis

(2) Adenosarcomas

Stage		Definition
I		Tumor limited to uterus
	IA	Tumor limited to endometrium/endocervix with no myometrial invasion
	IB	Less than or equal to half myometrial invasion
	IC	More than half myometrial invasion
II		Tumor extends beyond the uterus, within the pelvis
	IIA	Adnexal involvement
	IIB	Tumor extends to extrauterine pelvic tissue
III		Tumor invades abdominal tissues (not just protruding into the abdomen).
	IIIA	One site
	IIIB	More than one site
	IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	IVA	Tumor invades bladder and/or rectum
	IVB	Distant metastasis

(3) Carcinosarcomas

Carcinosarcomas should be staged as carcinomas of the endometrium.

* Note: Simultaneous endometrial stromal sarcomas of the uterine corpus and ovary/pelvis in association with ovarian/pelvic endometriosis should be classified as independent primary tumors.

1 LEIOMYOSARCOMAS

1.1 Clinical features

After excluding carcinosarcoma (MMMT), leiomyosarcoma has become the most common subtype of uterine sarcoma. However, it accounts for only 1-2% of uterine malignancies. The incidence of leiomyosarcoma is 0.3-0.4/100 000 women per year.[5] Approximately 1 of every 800 smooth muscle tumors of the uterus is a leiomyosarcoma.[5,6] The tumor is more common in black than in white women, but the difference is less than that estimated for leiomyoma.[7] The incidence in women who are on tamoxifen therapy for breast cancer is increased compared to those who are not.[7] Most leiomyosarcomas occur in women over 50 years of age who usually present with abnormal vaginal bleeding (56%), palpable pelvic mass (54%), and pelvic pain (22%). Signs and symptoms resemble those of the far more common leiomyoma and preoperative distinction between the two tumors may be difficult. Nevertheless, malignancy should be suspected by the presence of certain clinical behaviors, such as tumor growth in menopausal women who are not on hormonal replacement therapy.[8] Occasionally, the presenting manifestations are related to tumor rupture (hemoperitoneum),

extrauterine extension (one-third to one-half of cases), or metastases. Only very rarely does a leiomyosarcoma originate from a leiomyoma. Unlike carcinosarcoma, leiomyosarcoma is almost never associated with a history of pelvic radiation therapy.

1.2 Pathological features

Gross features

Leiomyosarcomas are either single masses or, when associated with leiomyomas, the largest mass. They are typically voluminous tumors with a mean diameter of 10 cm. Only 25% of cases are < 5 cm in size. About two-thirds of leiomyosarcomas are intramural, 1/5 submucosal, and 1/10 subserosal; 5% arise in the cervix. The cut surface is typically soft, bulging, fleshy, necrotic, and hemorrhagic (Figure 1) and lacks the prominent whorled appearance of leiomyomas. Leiomyosarcomas tend to be less circumscribed than leiomyomas and a sharp line of demarcation separating the tumor from the normal myometrium is not seen. The irregular margin denotes invasion and is not always so apparent in early tumors. When a myometrial tumor shows an unusual gross appearance, thorough sampling is recommended (at least one section per cm in diameter)

including the interface with the adjacent myometrium. However, there is overlap in appearance between leiomyosarcoma and ischemic degeneration of leiomyomas and most smooth muscle neoplasms that have a peculiar gross appearance are found to be benign.

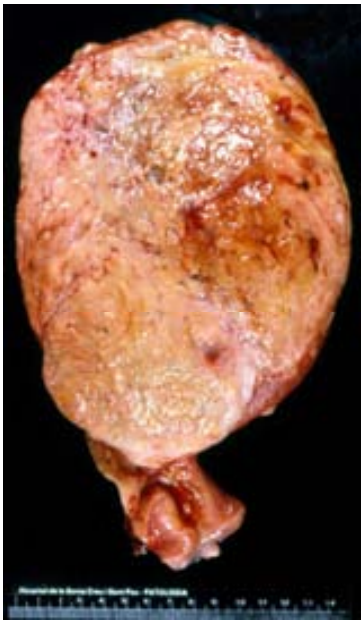


Fig. 1

Microscopic features

Most uterine leiomyosarcomas are high-grade and obviously malignant tumors. Compared with leiomyomas, they are usually more cellular, show moderate to severe nuclear atypia, and contain frequent mitotic figures (Figure 2).

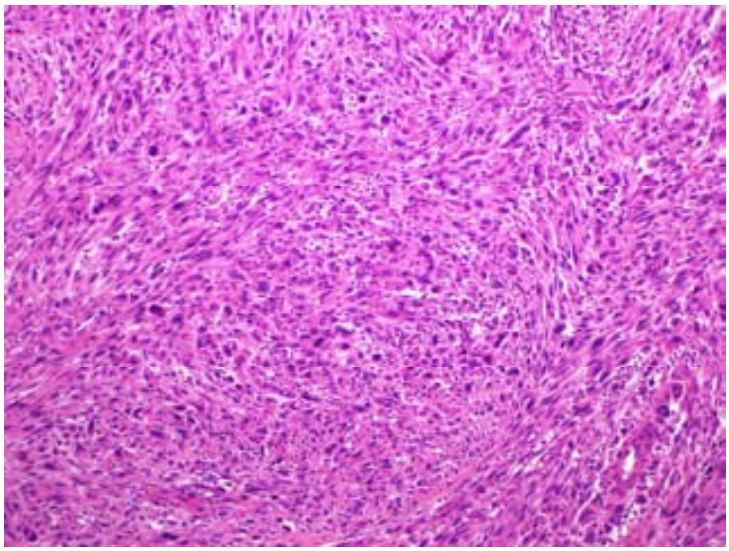


Fig. 2

The mitotic rate is usually of 10 or more MFs /10 HPFs, and over 90% of cases have more than 15 MFs/10 HPFs. The degree of smooth muscle differentiation varies both between tumors and within an individual leiomyosarcoma. Well-differentiated leiomyosarcomas consist of elongated smooth muscle cells with regular nuclei that may differ little from those of leiomyoma. At the other end of the spectrum, a poorly differentiated leiomyosarcoma shows rounded and pleomorphic cells that have virtually no resemblance to normal smooth muscle cells. Nuclear as well as cellular pleomorphism, nuclear hyperchromasia, and giant cells are indicative of increasing anaplasia. Multinucleated tumor cells are found in 50% of

leiomyosarcomas and osteoclasts-like cells are occasionally seen. Areas of tumor cell necrosis (Figure 3) and hemorrhage, which are already seen macroscopically, are common. Frequently, leiomyosarcomas have invaded the adjacent myometrial tissue at the time of diagnosis, even to the extent of breaking through the serosal surface of the uterus and involving other pelvic organs. Vascular invasion is found in 10-20% of cases.

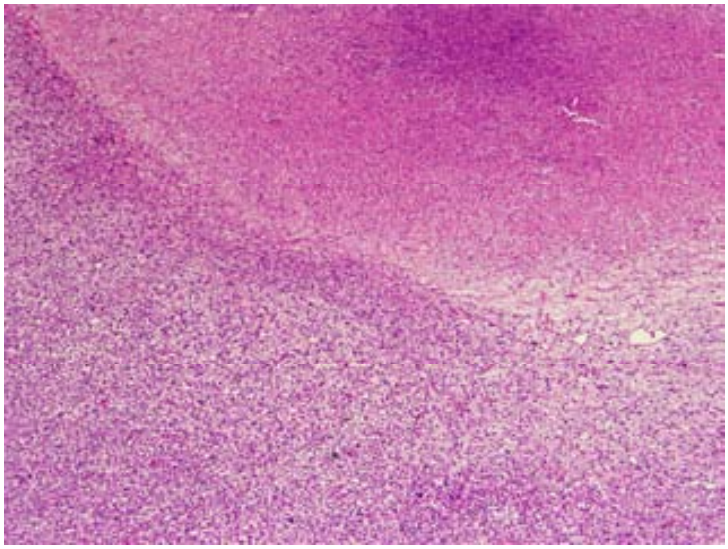


Fig. 3

Tumor cell necrosis is a characteristic feature of leiomyosarcoma, but its presence is not necessary for establishing the diagnosis. Nevertheless, tumor cell necrosis, nuclear atypia, and high mitotic

rate are thought to be the stronger histologic criteria and the presence of two of three is considered sufficient for the diagnosis.[9]

Epithelioid and myxoid leiomyosarcomas are two rare histological variants that lack the severe nuclear atypia and high mitotic activity of the usual spindle-shaped leiomyosarcomas.

Epithelioid leiomyosarcomas are composed predominantly or entirely of round or polygonal cells exhibiting eosinophilic or clear cytoplasm (Figure 4).[10,11] Tumor cells grow diffusely in nests, cords, or forming a plexiform pattern. Although nuclear pleomorphism is usually mild, some tumors show moderate to marked nuclear atypia. Mitotic rate is generally <3 MFs/10 HPFs. Most tumors infiltrate the adjacent myometrium but vascular invasion is rare. Tumor cell necrosis may be absent. Three of 26 tumors in one series recurred or metastasized.[10] The malignant tumors exhibited one or more of the following features: eosinophilic cells, infiltrating margins, necrosis, diameter greater than 6 cm, and absence of hyaline stroma.[10] In larger but still unpublished series of 80 cases, features indicative of malignancy were the presence of necrosis, vascular invasion,

significant nuclear pleomorphism, and a mitotic count of greater than 3 mitotic figures per 10 HPFs.[12] If none of these four features was present, the tumor behaved in a malignant fashion in under 10% of cases; however, if one, two or three features were identified, malignant behaviour was observed in 42%, 56%, and 88% of patients, respectively. Nearly 1 in 10 epithelioid tumors that showed no necrosis, no vascular invasion, no significant nuclear pleomorphism, and mitotic counts of less than 3 per 10 HPFs still behaved in a malignant fashion.

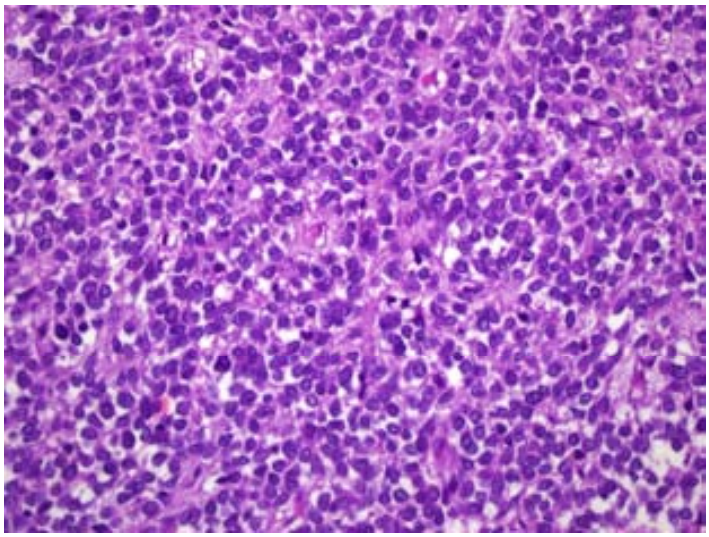


Fig. 4

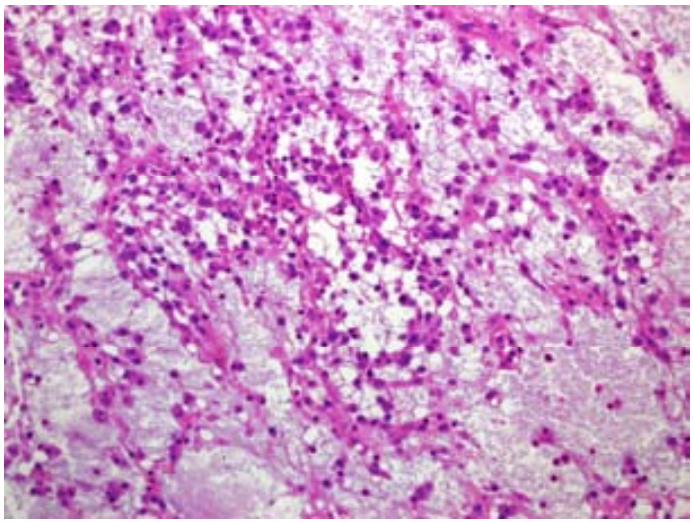


Fig. 5

Myxoid leiomyosarcomas are rare smooth muscle tumors with abundant myxoid stroma. On gross examination, the tumors are usually large, gelatinous, and apparently well-circumscribed (Figure 19.32). Microscopically, they differ from conventional leiomyosarcomas and have a hypocellular and myxoid appearance (Figure 5).[13] Despite low mitotic rates and bland nuclear features, myxoid leiomyosarcomas are almost always clinically malignant. Of the first six tumors reported,[13] all had mitotic indices from 0 to 2 per 10 HPFs but, in subsequent cases, about one-fourth contained 5 or more mitoses per 10 HPFs[14]. A single example had as many as 30 abnormal mitotic figures per 10 HPFs[15]. The typically low mitotic

count in these tumors is largely due to the separation of cells by the abundant myxoid stroma, so that there are few nuclei in each high-power field. Besides the myxoid appearance, other microscopic features that suggest the diagnosis of leiomyosarcoma include infiltrative margins and vascular-space invasion. The basophilic or eosinophilic myxoid matrix reacts strongly with alcian blue and colloidal iron. Smooth muscle markers are detected immunohistochemically in <25% of tumor cells.[16,17]

Myxoid tumors of the uterus must be regarded with suspicion, and any myxoid smooth muscle tumor with nuclear atypia, regardless of the mitotic activity or the presence or absence of necrosis should be diagnosed as leiomyosarcoma. Myxoid leiomyosarcoma should be distinguished from the far more common hydropic changes seen in degenerating leiomyomas The former tumor is histologically reminiscent of myxoid malignant fibrous histiocytoma of soft tissues.

HISTOLOGIC DIAGNOSIS OF LEIOMYOSARCOMA

As indicated above, the histologic features that play a role in the diagnosis of leiomyosarcoma and its distinction from leiomyoma.

include mitotic activity, nuclear atypia, tumor cell necrosis, degree of cellularity, degree of differentiation, presence of tumor giant cells, atypical mitotic figures, vascular invasion, and invasion of the surrounding myometrium. The last two are unquestionably diagnostic of malignancy (with the exception of intravenous leiomyomatosis, see above). If a smooth muscle tumor is well circumscribed, composed of cells that are uniform in size and shape, has no intravascular component, cytologic atypia and necrosis are lacking, and the mitotic index is less than 5 MF per 10 HPFs, then the tumor is a leiomyoma. On the other hand, if the tumor has infiltrative margins, intravascular growth, marked cytologic atypia and geographic tumor cell necrosis, a mitotic index greater than 10 MF per 10 HPFs, and abnormal mitotic figures, then it is an obvious leiomyosarcoma. It is when a smooth muscle neoplasm has features somewhere between these extremes that difficulty and controversy exists.

Initially, mitotic count, and specifically 10 MF per 10 HPFs, was the recommended threshold for the diagnosis of leiomyosarcoma[18] and, consequently, diagnosis was based almost exclusively on mitotic

count regardless of the degree of atypia.[19] However, it has become clear over the last three decades that mitotic activity is only one of several parameters to be evaluated when assessing the potential malignancy of smooth muscle tumors. From all histologic features, mitotic activity, degree of nuclear atypia, and the presence or absence of tumor cell necrosis emerged as the most important predictors of malignant behavior. By employing these three variables in the assessment of smooth muscle tumors, the diagnostic strategy moves away from complete dependence on mitotic count. This is shown graphically in Figure 6.

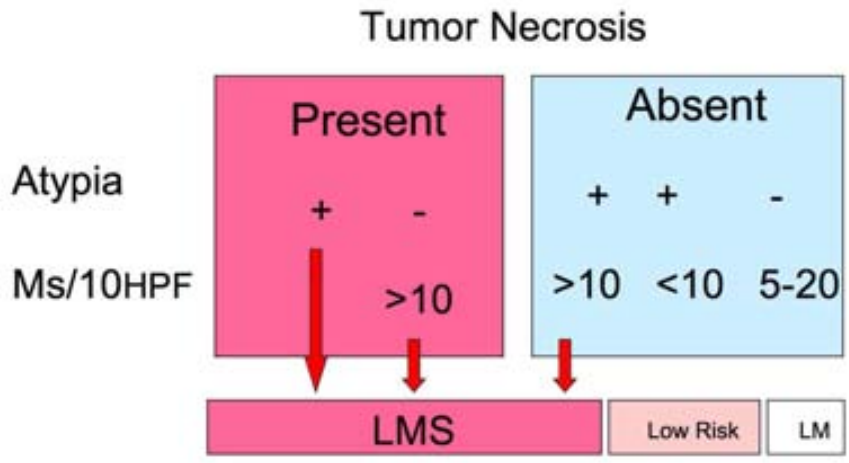


Fig. 6

DIAGNOSTIC CRITERIA

Mitotic activity

Mitotic counting (without rigor or standardization) is not reliable or reproducible, and could not be used as a precise basis for diagnosis, prognosis or treatment. There are many variables in mitotic counting: a) the number of sections taken from the tumor; b) the thickness of the sections; c) mitotic figures unrecognized or mistaken for pyknotic or otherwise degenerating nuclei; d) different number and size of high-power fields used: and e) the rapidity of fixation.

Only definite mitotic figures should be counted, while questionable figures should be ignored. Recent exposure to progestins can increase mitotic activity of smooth muscle tumors and this information should be sought from clinicians or the medical record in difficult cases. Likewise, ischemic change or proximity to an inflamed or ulcerated mucosa can induce a reactive increase in mitotic count. Atypical mitotic figures are often found in leiomyosarcomas (Figure 7). Examples of atypical mitotic figures include spindle poles in excess of two (i.e., tri- and tetrapolar metaphases), chromosomes lagging far behind the separating groups in later phases of division (as they may

be damaged by cycles of chromosomal fusion and subsequent breakage), and extreme polyploidy (which admittedly is a subjective appraisal as accurate enumeration requires other cytogenetic or molecular techniques). Each of these forms of atypia reflects cytogenetic aberrations that characterize malignant smooth muscle tumors and a mechanism to generate genomic instability.

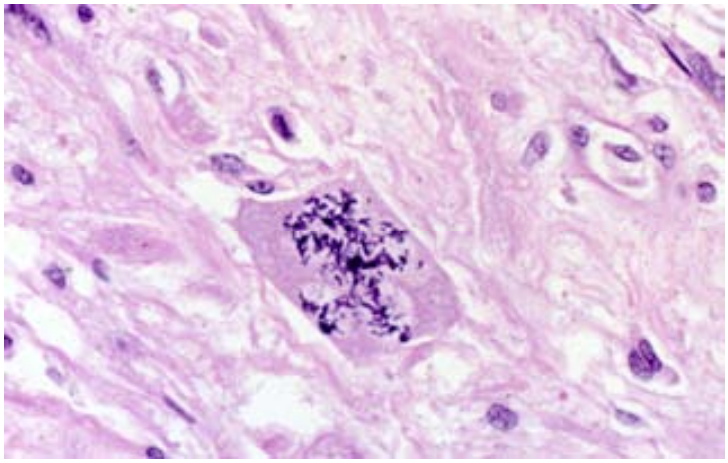


Fig. 7

Atypia

Paramount is nuclear pleomorphism, with a variable increase in nuclear size, irregularities of nuclear membrane, chromatin clumping, and prominent nucleoli also being taken into account. An increase in the number of nuclei, when none of these features is present, does not constitute atypia. Crowded normal nuclei are seen in a cellular

leiomyoma. Significant atypia (moderate or severe) can be identified readily under the low power of the microscope. Mild atypia is subtler, requires evaluation under a higher power, and does not carry the same diagnostic import, as do greater degrees of atypia. One difficulty in applying this approach occurs when the nuclear atypia is very uniform from tumor cell to tumor cell. The monomorphic quality of such tumors suggests that there is less intratumoral genetic heterogeneity than typical for leiomyosarcoma. This difficulty in recognizing nuclear atypia can be overcome when one compares the tumor nuclei to nuclei in the adjacent myometrium. This comparison reveals the increases in nuclear size, chromasia, and chromatin distribution in rare cases of low-grade or well-differentiated leiomyosarcomas.

Necrosis

Tumor cell necrosis is highly characteristic of leiomyosarcomas. It is characterized by an abrupt transition from the viable cells to the necrotic cells without an interposed zone of granulation tissue or fibrous tissue. Preserved nuclei with marked pleomorphism and hyperchromasia can still be seen within the necrotic areas and often

there is a perivascular growth of viable tumor cells. Tumor cell necrosis should be distinguished from infarct-type necrosis (which may be seen in benign or malignant smooth muscle tumors) and is characterized by a transition zone composed of granulation or fibrous (hyalinized) tissue depending upon the age of the infarct. The necrotic tissue has a mummified and homogeneous appearance, areas of hemorrhage are common, and no perivascular growth of tumor cells is seen. In some cases, distinguishing between tumor cell necrosis and infarct-type necrosis may be difficult. The 'geographic' tumor cell necrosis is often multifocal and distributed throughout the tumor. In contrast, benign necrosis typically consists of a single, often centrally located region with a simple, rounded border.

Coagulative necrosis is a feature of leiomyomas treated with GnRH analogues. The distinction between this type of necrosis and the necrosis found in leiomyosarcomas may be difficult. Treatment with GnRH analogues does not result in nuclear atypia elsewhere in the tumor and thick-walled blood vessels may be prominent.[20,21] Focused ultrasound effect, used in the ablation of 'fibroids', may mimic the pattern of necrosis found in leiomyosarcoma. Gross tissue hardening,

as well as histologic blandness and hypereosinophilia associated with thermal denaturation, should provide the clues needed to correlate with the clinical history and arrive at the correct diagnosis.

However, the histologic features typical of ischemic or hormonally induced degeneration and subsequent host tissue reaction found in leiomyomas may also occasionally be seen in leiomyosarcoma. Consequently, the presence of such benign degenerative changes cannot be used to exclude malignancy.

Other factors that favor malignancy

Besides high mitotic activity, significant nuclear atypia, and tumor cell necrosis, the finding of a tumor larger than 3 cm in diameter and, to a lesser extent, patient age over 50 years are factors associated with metastasis and mortality. Tumor under 3 cm almost never metastasize.[22]

1.3 Immunohistochemistry

Although immunohistochemistry is not necessary for the diagnosis of leiomyosarcoma, it may occasionally help to distinguish leiomyosarcoma from other uterine malignancies such as high-grade

endometrial sarcoma or sarcomatoid carcinoma.[16,23-31].

Leiomyosarcomas usually express smooth muscle markers such as desmin, h-caldesmon, smooth muscle actin, and histone deacetylase 8 (HDCA8). However, immunoreaction for one or more of these markers can be lost or may be weak in poorly differentiated leiomyosarcomas or in the epithelioid and myxoid variants. Also, leiomyosarcomas are often immunoreactive for CD10 and epithelial markers including keratin and EMA (the latter being more frequently positive in the epithelioid variant). Conventional leiomyosarcomas express estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) in 30-40% of cases. Whereas a variable proportion of uterine leiomyosarcomas has been reported as being immunoreactive for c-KIT, no c-KIT mutations have been identified.[32] Recent studies have shown statistically significant higher levels of Ki-67 in uterine leiomyosarcomas compared with benign smooth muscle tumors.[16,26-29] Mutation and overexpression of p53 have been described in a significant minority of uterine leiomyosarcomas (25-47%) but not in leiomyomas.[16,28,29] Intermediate rates have been found in bizarre and atypical (STUMP) leiomyomas. Overexpression of p16 has been described in uterine

leiomyosarcomas and may prove to be a useful adjunct immunomarker for distinguishing between benign and malignant uterine smooth muscle tumors[27-29].Strong and diffuse p16 immunoreaction, especially when accompanied by strong staining for p53 favors the diagnosis of leiomyosarcoma.

1.4 Molecular genetics

Although the vast majority of uterine leiomyosarcomas are sporadic, patients with germline mutations in fumarate hydratase are believed to be at increased risk for developing uterine leiomyosarcomas as well as uterine leiomyomas.[33,34] The oncogenic mechanisms underlying the development of uterine leiomyosarcomas remain elusive. Uterine leiomyosarcoma is a genetically unstable tumor that have complex structural chromosomal abnormalities and highly disturbed gene regulation which likely reflects the end-state of accumulation of multiple genetic defects. Extrapolating from experiences in soft tissue leiomyosarcomas, it is unlikely that recurrent disease-driven genetic aberrations (i.e. gene mutation or translocation events) will be found. In comparison with other more common uterine cancers, uterine leiomyosarcomas bear some

resemblance to type 2 endometrial carcinomas and high-grade serous carcinomas of ovary/fallopian tube origin, based on their genetic instability, frequent p53 abnormalities, aggressive behavior, and resistance to chemotherapy. Therefore, therapies that exploit the underlying genetic instability of uterine leiomyosarcomas may prove to be an effective therapeutic strategy.

Overexpression of the c-*myc* proto-oncogene occurs in about 50% of both leiomyomas and leiomyosarcomas, and does not correlate with survival.[35] *K-ras* is overexpressed in a small minority of leiomyomas but not at all in leiomyosarcomas.[36] The *MDM*2 gene, in contrast, is overexpressed in some leiomyosarcomas but not in leiomyomas.[36] The lack of gamma-smooth muscle isoactin gene, in a pilot study, correlated 100% with a histologic diagnosis of leiomyosarcoma.[37] Abnormalities of the retinoblastoma–cyclin D pathway have been found in about 90% of leiomyosarcomas,[38] which is not surprising considering that the retinoblastinoma gene is deleted in about three-fourths of leiomyosarcomas.[39] These different patterns of molecular alterations in leiomyomas and leiomyosarcomas may lead to the conclusion that they are different entities.[36]

Recently, p16, also known as INK4 or cyclin-dependent kinase inhibitor 2A (CDKN2A), has been implicated in the genesis of leiomyosarcoma.[25,40] P16 protein binds the CDK4–cyclin D complex and acts as a negative cell cycle regulator. Consequently, p16 deletion results in a loss of tumor suppression phenotype.

1.4.1 Proliferation markers

Proliferating cell nuclear antigen (PCNA) is a protein involved with copying DNA and therefore in cell division. It can be demonstrated immunohistochemically using the antibody PC10. The Ki-67 antigen identifies proliferating normal and neoplastic cells in histologic sections, using the MIB1 antibody. This is a more reliable indication of cell division and proliferation than the mitotic index. Recently, statistically significant higher levels of PCNA and Ki-67 have been shown in uterine leiomyosarcomas compared with leiomyomas.[41,42] In one study, the percentage of MIB1-positive tumor cells helped predict prognosis and extent of tumor spread.[41]

1.4.2 Flow cytometry, cytogenetics, and molecular genetics

Analysis of leiomyosarcomas by flow cytometry has produced mixed results. Studies show that between about 55 and 70% of the tumors are aneuploid.[42] While most studies report that neither ploidy nor S-phase fraction offers additional value to clinical and histologic factors already described,[43,44] one concluded that DNA ploidy helped identify cases that might have an adverse prognosis.[42]

Cytogenetic analyses show that leiomyosarcomas have both complex numerical and structural chromosomal aberrations.[45,46] The large variability in aberrations found among the metaphases from the same leiomyosarcoma also suggest, in contrast to benign leiomyoma, that genomic instability is a hallmark of malignancy in uterine smooth muscle tumors.[45] Loss of heterozygosity (LOH) analysis and comparative genomic hybridization (CGH), two different means to assess allelic imbalance, also detect complex genomic aberrations. In particular, frequent losses of 10q and 13q as well as occasional gain of 17p and losses of 2p and 16q have been observed.[39,47] At least some leiomyosarcomas have X inactivation that differs from their accompanying leiomyomas, suggesting that the benign and malignant

tumors arose from independent transformations and that the genesis of leiomyosarcoma occurs *de novo*. Whether malignant transformation of certain leiomyomas (e.g., bizarre leiomyoma) occurs, it remains to be proven fully.

1.5 Prognosis and treatment

Leiomyosarcomas are very aggressive tumors. It has become apparent that tumors diagnosed according to the 2003 WHO criteria are associated with poor prognosis even when confined to the uterus [30,48] and even when diagnosed at an early stage; recurrence rate has ranged from 53% to 71%.[1] First recurrences were in the lungs in 40% of patients and in the pelvis in only 13%. Overall survival rate ranged from 15% to 25% with a median survival of only 10 months in one study. In the Norwegian series[48] , patients with leiomyosarcomas limited to the uterus had poor prognosis with a 5-year overall survival of 51% at stage I and 25% at stage II (by the 1988 FIGO staging classification). All patients with spread outside the pelvis died within 5 years.

There has been no consistency among various studies regarding correlation between survival and patient age, clinical stage, tumor size, type of border (pushing versus infiltrative), presence or absence of necrosis, mitotic rate, degree of nuclear pleomorphism, and vascular invasion.[2,22,23,30,49-53] One study, however, found tumor size to be a major prognostic parameter[2]: five of 8 patients with tumors < 5 cm in diameter survived, whereas all patients with tumors > 5 cm in diameter died of tumor. In this study of 208 uterine leiomyosarcomas, the only other parameters predictive of prognosis were tumor grade and stage.[2] Histologic grade, however, has not been consistently identified as a significant prognostic parameter. In the report from Norway[48], including 245 leiomyosarcomas confined to the uterus, tumor size and mitotic index were significant prognostic factors and allowed for separation of patients into 3 risk groups with marked differences in prognosis. Ancillary parameters including p53, p16, Ki 67, and Bcl-2 have been used in leiomyosarcomas trying to predict outcome.[30] However, it is not clear whether they act independently of stage which still is the most significant prognostic factor for uterine sarcomas.

Treatment of leiomyosarcomas includes total abdominal hysterectomy and debulking of tumor if present outside the uterus. Removal of the ovaries and lymph node dissection remain controversial as metastases to these organs occur in a small percentage of cases and are frequently associated with intra-abdominal disease.[2] Ovarian preservation may be considered in premenopausal patients with early-stage leiomyosarcomas.[2] Lymph node metastases have been identified in 6.6% and 11% of two series of patients with leiomyosarcoma who underwent lymphadenectomy.[2,54] In the first series, the 5-year disease-specific survival rate was 26% in patients who had positive lymph nodes compared with 64.2% in patients who had negative lymph nodes ($p < 0.001$).[54] The influence of adjuvant therapy on survival is uncertain. Radiotherapy may be useful in controlling local recurrences and chemotherapy with doxorubicin or docetaxel/gemcitabine is now used for advanced or recurrent disease, with response rates ranging from 27% to 36%.[55,56,57] Some patients may respond to hormonal treatment. [58]

2 SMOOTH MUSCLE TUMORS OF UNCERTAIN MALIGNANT POTENTIAL (STUMP)

Uterine smooth muscle tumors that cannot be histologically diagnosed as unequivocally benign or malignant should be designated atypical leiomyomas.[9,59] This group is largely defined by the presence of nuclear atypia and <10 MFs/10 HPFs in absence of tumor cell necrosis. The accepted degree of nuclear atypia varies from mild[60] to moderate or severe[9] depending upon the mitotic index (5-9 or <10 MFs/10 HPFs, respectively). Furthermore, atypical leiomyomas were subdivided into three groups:[9]

a) Atypical leiomyoma with *low risk of recurrence*, which shows diffuse moderate to severe nuclear atypia, <10 MFs/ 10 HPFs, and no tumor cell necrosis. Only one of 46 such tumors was clinically malignant.[9]

b) Atypical leiomyoma *with limited experience,* characterized by focal moderate to severe nuclear atypia, <20 MFs/ 10 HPFs, and no tumor cell necrosis. All five cases in this group were clinically benign. Three of the five tumors had <5 MFs/ 10 HPFs and would be

considered leiomyomas with bizarre nuclei by most investigators. The other two tumors had 10-19 MFs/10 HPFs;[9] and

c) Smooth muscle tumors *of low malignant potential*, which had tumor cell necrosis, <10 MFs/ 10 HPFs, and none to mild nuclear atypia. One of four tumors in this group was clinically malignant, again underscoring the importance of tumor cell necrosis.

The unpredictable behavior of some of these tumors has led some to introduce the concept of the 'smooth muscle tumor of uncertain malignant potential' ("STUMP"), a term to be discouraged for the reasons given above. Nevertheless, most tumors classified as "STUMP" have been associated with favorable prognosis and, in these cases, only follow-up of the patients is recommended[174]. In two recent studies of 41 and 16 cases of "STUMP", only 3 (7%) and 2 (12%) patients developed recurrences, respectively. Recurrence occurred, several years after hysterectomy, in the form of "STUMP" in three cases and as leiomyosarcoma in the other two.[61,62] All five patients were alive and disease-free after prolonged follow-up. As indicated previously, when account is taken of mitotic count, myometrial invasion, nuclear atypia, tyumor cell necrosis, size of

tumor, and age of patient, tumors can be allocated to benign or malignant categories with greater certainty and the term ‘of uncertain malignancy’ can be avoided in most cases. [59]

3 ENDOMETRIAL STROMAL TUMOR

Endometrial stromal tumors are the second most common pure mesenchymal tumors of the uterus even though they account for less than 10% of all such tumors. According to the 2003 WHO classification[3], the term endometrial stromal tumor is applied to neoplasms typically composed of cells that resemble endometrial stromal cells of the proliferative endometrium.[3] They are divided into: endometrial stromal nodules, low-grade endometrial stromal sarcomas, and undifferentiated endometrial sarcomas.

3.1 Endometrial stromal nodule

These rare tumors are composed of cells reminiscent of proliferative-phase endometrial stromal cells. They occur at any age during reproductive or later years. Most are incidental findings in a hysterectomy specimen while others present with abnormal uterine bleeding.

The tumors are typically round and well-circumscribed but not encapsulated. They are usually solitary, ranging from under 1 to 22 (mean 7) cm. If located in the endometrium, they are frequently polypoid; however, they may be intramyometrial or subserosal. They have a uniform soft, yellow cut surface which does not show the whorled pattern characteristic of a leiomyoma. Cysts may be present.

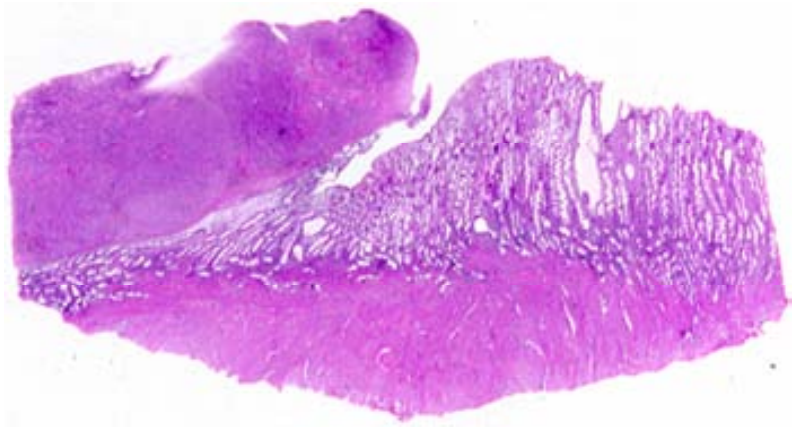


Fig. 8

The main distinguishing feature of endometrial stromal nodules is their expansile, non-infiltrating, smooth margin (Figure 8) that contrasts with the infiltrating irregular margin of stromal sarcomas.[63] Focal irregularities in the form of lobulated or finger-like projections

into the adjacent myometrium not exceeding 3 mm and not exceeding 3 in number may be seen.[64] Vascular invasion should not be present.

Endometrial stromal nodules have an excellent prognosis and patients are cured by hysterectomy.[65] Conservative treatment with excision of the mass is performed only when complete examination of the margins can be done which only occurs in rare instances.[66]

3.2 Low-grade endometrial stromal sarcoma

Endometrial stromal sarcomas account for approximately 0.2 % of all malignant uterine tumors and 10-15% of uterine malignancies with a mesenchymal component. They occur in women between 40 and 55 years of age. Some cases have been reported in patients with ovarian polycystic disease, after estrogen use, or tamoxifen therapy. Patients commonly present with abnormal uterine bleeding, pelvic pain, and dysmenorrhea but as many as 25% of them are asymptomatic.[67] At presentation, extrauterine pelvic extension, most commonly involving the ovary, is found in up to 1/3 of patients. Thus, when evaluating an ovarian tumor microscopically consistent with an endometrial stromal tumor, it is important to exclude a prior history of uterine

endometrial stromal tumor and to suggest inspection of the uterus, as the latter are far more common.

Grossly, there is irregular nodular growth involving the endometrium, myometrium, or both (Figure 9).

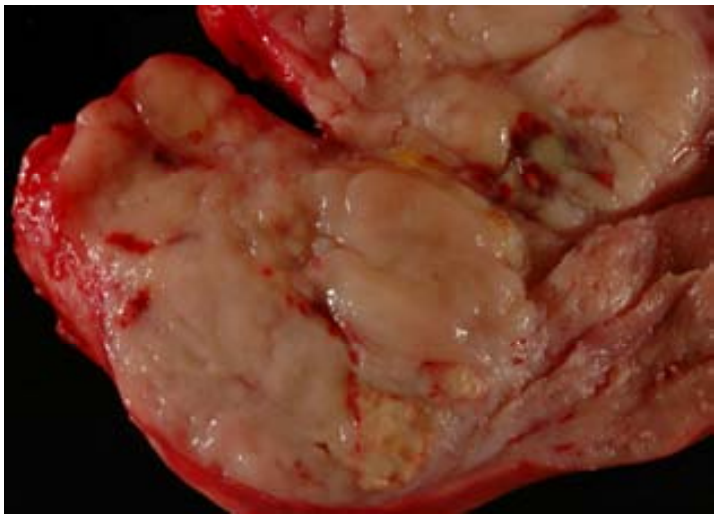


Fig. 9

The main mass is frequently associated with varying degrees of permeation of the myometrium, including worm-like plugs of tumor that fill and distend myometrial veins, frequently extending to parametrial veins and lymphatics (Figure 10). Microscopically, endometrial stromal sarcomas exhibit only mild nuclear atypia

(Figure 11). Mitotic activity is typically <5 MF/10 HPF. Necrosis is rarely seen.

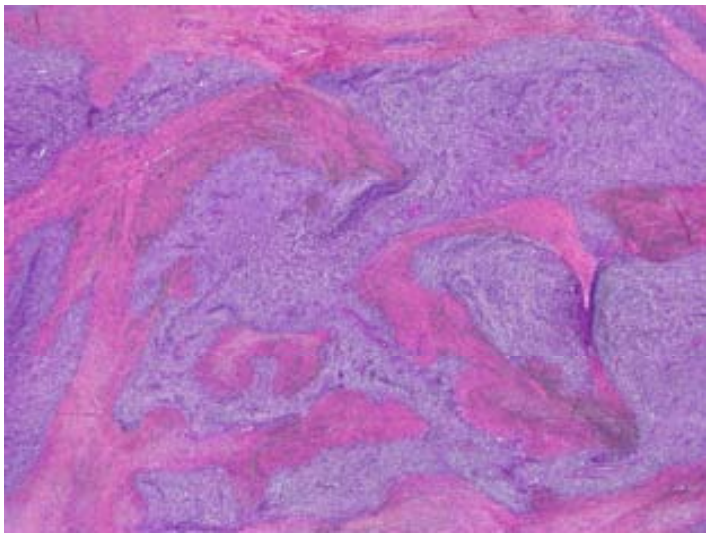


Fig. 10

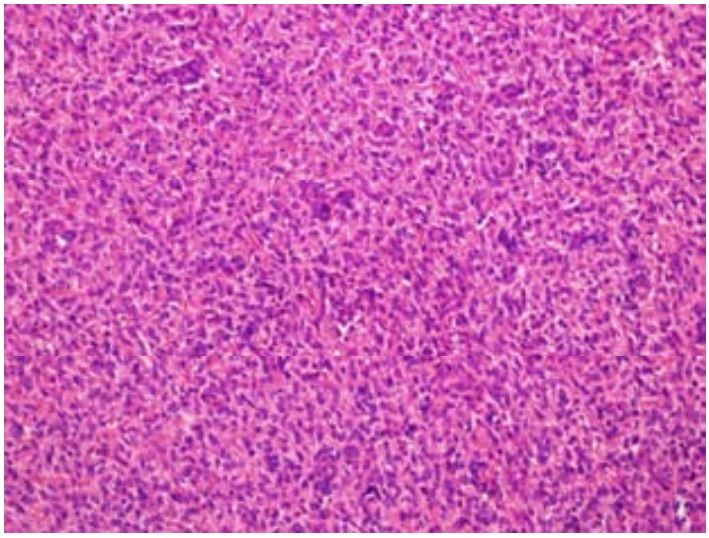


Fig. 11

3.2.1 Endometrial Stromal Variants

Both endometrial stromal nodule and endometrial stromal sarcoma display the following types of differentiation:

1. *Smooth muscle*

   This type of differentiation has been reported in the literature based on morphologic, immunohistochemical and ultrastructural studies.[65,68-77] As prognosis of these tumors relates to margin status of the endometrial stromal component, currently these tumors are defined as endometrial stromal neoplasms (nodule or sarcoma) in which the smooth muscle component accounts for >30% on hematoxylin-eosin evaluation. On gross examination, the smooth muscle component may be seen as whitish and firm areas (if prominent) in contrast to the endometrial stromal component (soft and yellow to tan to light brown). On microscopic examination, smooth muscle differentiation can be seen as pale to “pink” irregular islands of slightly epithelioid cells or much more characteristically as the so-called “starburst” pattern (Figure 12) present in a background of endometrial stromal neoplasia. The latter is characterized by a central area of hyalinization from which collagen bands radiate towards the

periphery and cells with an “epithelioid” appearance are embedded in between the collagen fibers. These epithelioid cells form short and non-organized fascicles of smooth muscle that in turn may transition into long and well formed fascicles of smooth muscle.[75] Often these “starburst” areas are seen at the periphery of the tumor and they may also be seen next to areas of “sex cord-like” differentiation in stromal tumors.[75] The smooth muscle cells are typically cytologic benign but rarely a malignant smooth muscle component has been reported. [78]

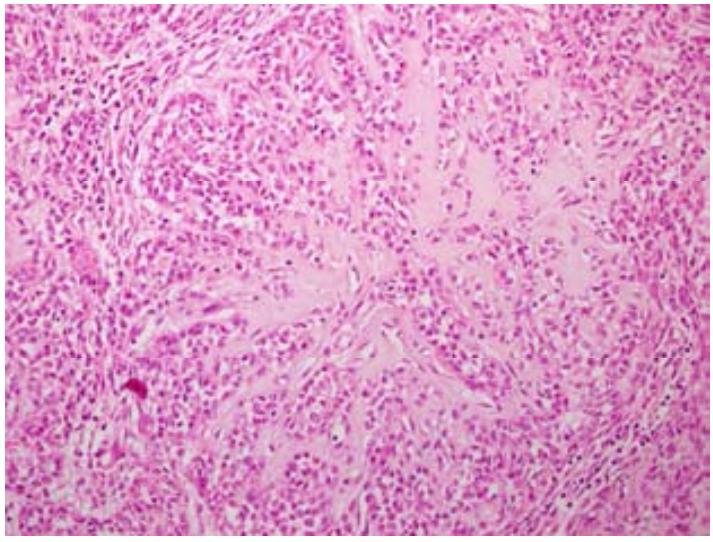


Fig. 12

2. *Myxoid and fibroblastic*:

These unusual types of differentiation in endometrial stromal tumors are characterized either by prominent myxoid or fibroblastic (loose collagen) background (Figure 13) imparting a hypocellular appearance in contrast to their typical hypercellular appearance.[79-81] On gross examination, they can have a gelatinous (if myxoid) or firm and white (if fibroblastic) cut surface. However, other characteristic morphologic features of endometrial stromal neoplasia are present including the typical "tongue" pattern of infiltration if low-grade sarcoma and arteriole-like vessels. Cells are typically small with scant cytoplasm and oval to spindle-shaped with minimal cytologic atypia and mitotic activity. Areas of conventional endometrial stromal neoplasm may be seen.

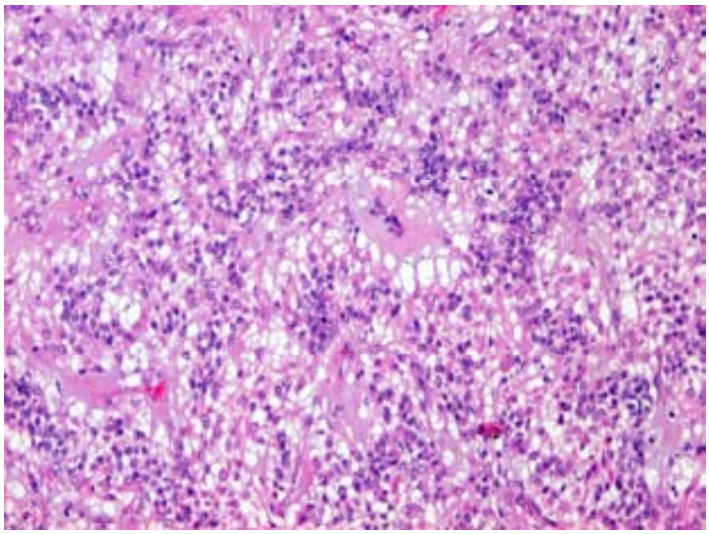


Fig. 13

3. *Sex-cord-like elements*

This variant has been reported with a variable frequency and extent in endometrial stromal tumors (up to 60%).[4,64,82-91] They consist of anastomosing cords, trabeculae, islands, small nests, tubules which may be retiform, or sheets of cells reminiscent of the patterns seen in granulosa and/or Sertoli cell tumors of the ovary (Figure 14). Cells typically have scant to abundant cytoplasm and round to oval nuclei, sometimes with grooves, indistinct or tiny nucleoli and minimal mitotic activity. These sex cord-like elements are present within a background of typical endometrial stromal neoplasia or in a loose hypocellular background. They not infrequently coexist with areas of

smooth muscle differentiation, and in these instances both elements often merge imperceptibly with one another.[78]

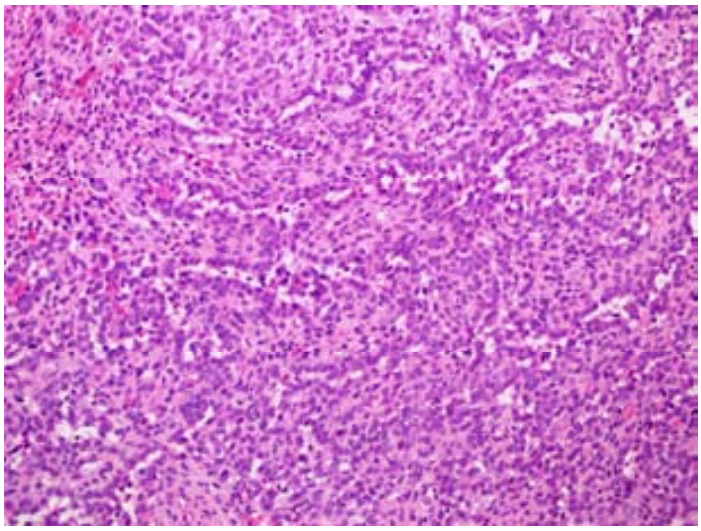


Fig. 14

4. *Glandular elements*

   They may vary in number, but can be quite extensive throughout the tumor. They can be seen in endometrial stromal nodules[65] but have been reported more frequently in endometrial stromal sarcomas.[74,92-96] Glands range from small and round (proliferative) to slightly irregular or cystically dilated (inactive). They have an endometrioid morphology (Figure 15)., lined by cuboidal to columnar cells with eosinophilic and rarely clear cytoplasm and pseudostratified nuclei that display a minimal

degree of cytologic atypia in most cases.[65,74,92-96] Foci of grade I endometrioid carcinoma have been occasionally reported.[92]

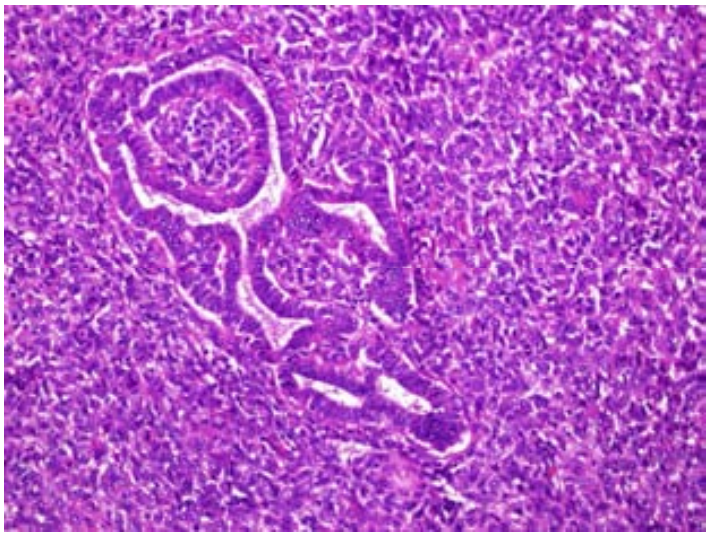


Fig. 15

5. *Skeletal muscle differentiation*

This type of differentiation may be seen as large cells with copious bright eosinophilic cytoplasm and abundant filament deposits typically wrapping around nuclei as well as cells having a strap-shaped morphology with cross striations. These cells are positive for myoglobin as well as myoD-1 and myogenin.[73,97]

6. *Epithelioid appearance*

This variant is defined as cells having abundant eosinophilic cytoplasm that confers an oval to polygonal appearance to the cells. This morphologic feature may be extensive within a given endometrial stromal tumor (up to 90% of cells).[64,98] Some of these cells may also have a granular quality of the cytoplasm.[98] This appearance has to be distinguished from pseudo-decidualization of neoplastic stromal cells that can occur in these tumors. A fibroblastic component has been reported in approximately 50% of endometrial stromal tumors with *t(10;17)* which are known to have a predominant epithelioid morphology associated with high-grade cytologic features and aggressive behavior (Figure 16).[99]

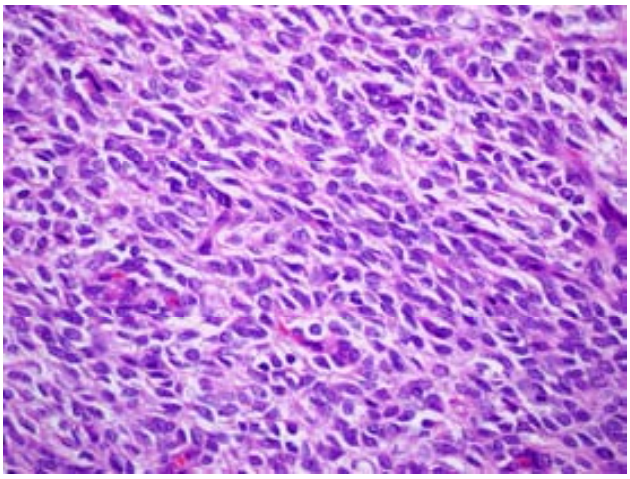


Fig. 16

7. *Rhabdoid morphology*

   It can be seen in endometrial stromal tumors more frequently in areas displaying sex cord-like differentiation. Cells characteristically have large eosinophilic hyaline cytoplasmic inclusions and eccentric vesicular nuclei often with prominent nucleoli.[71,86,89,100,101] By electronmicroscopy, abundant paranuclear deposits of intermediate filaments have been noted.[86]

8. *Cells with abundant clear cytoplasm*

   Cells with abundant clear cytoplasm may also rarely be seen[102] adding to the differential diagnosis of uterine mesenchymal tumors with clear cells.

9. *True papillae and pseudopapillae*

   True papillae and pseudopapillae have been reported in endometrial stromal tumors, mostly endometrial stromal sarcomas, either in a primary or metastatic setting. They may represent a focal or diffuse finding. Papillae or pseudopapillae are typically small with angulated outlines and may have a

glomeruloid appearance. They are lined by stromal cells which are also present within the vascular cores associated with small vessels.[103]

10. *Mature adipose tissue*

    Asipose tissue can occasionally be seen admixed with neoplastic endometrial stromal cells, either within the main tumor or within vascular spaces.[97]

11. *Cells with bizarre nuclei*

    As noted more commonly in benign smooth muscle tumors (leiomyomas with bizarre nuclei) can rarely occur. They may be mono- or multinucleated and typically have nuclei with smudged chromatin and occasional intranuclear cytoplasmic inclusions.[79,97,98,104]

12. *Osteoclast-type cells*

    They have also been reported in one endometrial stromal sarcoma.[105]

3.2.2 Immunohistochemistry

Endometrial stromal nodules and low-grade endometrial stromal sarcomas are typically immunoreactive for vimentin, muscle-specific actin, alpha-smooth muscle actin, and frequently keratin.[106-108]

Most endometrial stromal tumors as well as normal endometrial stromal cells stain for CD10. However, smooth muscle tumors, mixed mullerian tumors or even rhabdomyosarcomas may also be immunoreactive for CD10. [106-108] Thus, this antibody should not be used in isolation when evaluating the cell of origin in a uterine mesenchymal tumor. Not uncommonly, endometrial stromal tumors can exhibit diffuse alpha-smooth muscle actin reactivity, while desmin and h-caldesmon are generally negative or at most focally positive. [108] Other muscle markers including myosin and HDCA8 are also helpful in this differential diagnosis.[109] Areas of smooth muscle differentiation are reactive for all smooth muscle markers as well as for CD10. Areas of sex cord-like differentiation may be reactive for inhibin, calretinin, CD99, WT-1, and Melan A.[85] Endometrial stromal tumors frequently contain ER and PR and they also frequently express beta-catenin.[110] c-kit has been reported to be positive in a

small percentage of endometrial stromal tumors[111-114] but no mutation has been found.[115] It appears that c-kit may be more often positive in endometrial stromal sarcomas with high-grade cytologic features (especially if *t(10,17)*).

Nuclear beta-catenin expression has been shown in up to 40% of low-grade endometrial stromal sarcomas[116,117] as well as in high-grade endometrial stromal sarcomas, the latter frequently associated with cyclin D1 expression[117] p53 expression is typically absent in low-grade endometrial stromal sarcomas but it has been reported in high-grade/undifferentiated endometrial sarcomas (more often if pleomorphic subtype, as expected).[118,119] Cyclin D1 is mostly expressed in high-grade endometrial stromal sarcomas with *t(10;17)*[120] (Figure 17) although rarely it has been detected in endometrial stromal nodules, low-grade endometrial stromal sarcomas as well as undifferentiated endometrial sarcomas of uniform type.[117]

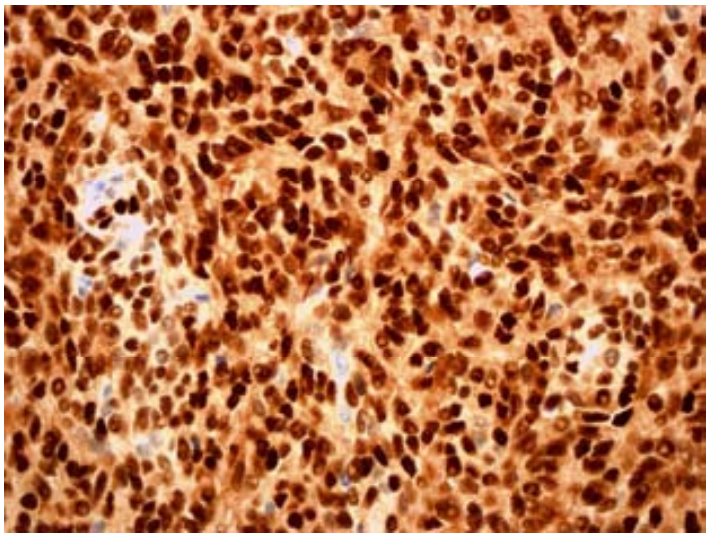


Fig. 17

3.2.3 Molecular biology

t(7,17)(p15;q21) is the most common chromosomal translocation in endometrial stromal tumors resulting in the *JAZF1-SUZ12* gene fusion.[121] It can be detected by cytogenetics, fluorescence in situ hybridization, or reverse-transcriptase polymerase chain reaction. It has been reported in the majority of endometrial stromal nodules, approximately 50% of low-grade endometrial stromal sarcomas and a minority of undifferentiated endometrial sarcomas, typically those classified as uniform type.[121-129] The presence of *JAZF1-SUZ12* gene fusion in at least 50% of endometrial stromal nodules and in a significant, but smaller, subset of low-grade endometrial stromal

sarcomas suggests that *JAZF1-SUZ12* fusion represents an early event in the development of endometrial stromal tumors and that additional events are necessary for tumor progression. The finding of identical translocations in occasional undifferentiated endometrial sarcomas and low-grade endometrial stromal sarcomas may indicate that at least some of the former tumors represent progression from low-grade tumors. The *JAZF1-SUZ12* cytogenetic abnormality has been also detected in morphologic variants of endometrial stromal tumors including those with smooth muscle, fibroblastic/myxoid, epithelioid, and sex cord-like differentiation, although to a less extent.[78,119,130,131] *t(6;17)(p21;p22)* and its variants represent the second most common cytogenetic abnormalities in endometrial stromal tumors.[126,132-135] A three way *t(6p;10q;10p)* has also been reported.[135] In general, it appears that there is no correlation between specific variant histologic subtypes and gene fusions among endometrial stromal tumors. However, very recently, a t*(10;17)(q22;p13)* has been reported in a subset of endometrial stromal sarcomas with high-grade epithelioid areas that in 50% of instances are associated with a fibroblastic variant of endometrial stromal neoplasm.[126,136,137]

3.2.4 Prognosis and treatment

Endometrial stromal sarcomas are indolent tumors with a favorable prognosis.[64] Tumor behavior is characterized by late recurrences even in patients with stage I disease; thus, long term follow-up is required. About one third of patients develop recurrences, most commonly in the pelvis and abdomen, and less frequently in the lung and vagina.[67]

The outcome in patients with endometrial stromal sarcomas depends largely on the extent of the tumor at the time of diagnosis. Surgical stage higher than I is a univariate predictor of unfavorable outcome. Generally endometrial stromal sarcomas have good prognosis, with 5- and 10-year actuarial survival for patients with stage I tumors of 98% and 89%, respectively.[67] Several other features may help predict outcome. Clinicopathologic factors reported in the older literature to be of potential prognostic importance included age, race, size, FIGO stage, depth of myometrial invasion, tumor grade, mitotic activity, and DNA ploidy.[138-141] However, in the largest study of low-grade endometrial stromal sarcomas, mitotic activity and cytologic atypia were not found to be predictive of tumor recurrence in stage I tumors (most common scenario), while size correlated

poorly with outcome as tumors <4 cm in diameter also recurred.[67] In another recent study,[48] prognosis of endometrial stromal sarcomas confined to the uterus (83 cases), was related to mitotic index and tumor cell necrosis.

Treatment of endometrial stromal sarcomas is largely surgical in the form of hysterectomy and bilateral salpingo-oophorectomy. These tumors are often sensitive to hormones and it has been stated that patients retaining their ovaries have a higher risk of recurrence[142]; however, there is no complete agreement on this issue.[139,142-145] Although lymph node metastases have been found in 7% of of 384 women with low-grade endometrial stromal sarcoma, this finding does not affect the excellent overall survival of these patients.[143] Patients may receive also adjuvant radiation or hormonal treatment with progestational agents or aromatase inhibitors.[146-147] High-grade endometrial stromal sarcomas with *t(10;17)* should be treated aggressively with combination of radiation and chemotherapy as they do not respond to conventional treatment for low-grade endometrial stromal sarcoma.[99,136]

3.3 Undifferentiated endometrial sarcoma

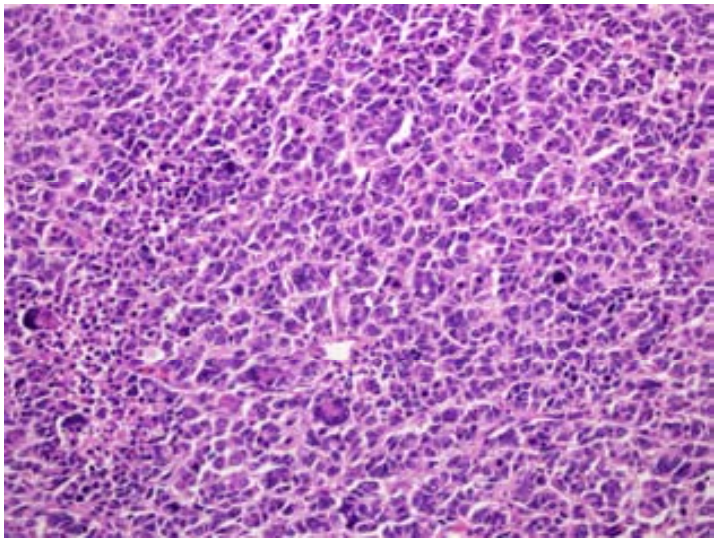
This is a high-grade uterine sarcoma bearing little or no resemblance to proliferative-phase endometrium.[3] However, it is hypothesized that it has an endometrial origin (at least in most cases) by virtue of its topographical location as well as the existence of composite tumors where low-grade endometrial stromal sarcomas coexist with a high-grade or even undifferentiated sarcoma.[117] Furthermore, it has been shown that some undifferentiated endometrial sarcomas share the same immunohistochemical and molecular profile than low-grade endometrial stromal sarcomas supporting this hypothesis.[117] These tumors are rare and they only should be diagnosed when a low-grade component is seen in its vicinity of after excluding other more common uterine sarcomas including leiomyosarcoma, sarcomatous overgrowth in a müllerian adenosarcoma, malignant mixed müllerian tumor or rhabdomyosarcoma.

3.3.1 Clinicopathological features

The diagnosis of undifferentiated endometrial sarcoma is applied to tumors that exhibit myometrial invasion, severe nuclear pleomorphism, high mitotic activity, and/or tumor cell necrosis, and

lack smooth muscle or endometrial stromal differentiation.[3] Grossly, they are often polypoid and show a fleshy, gray to white cut surface and prominent areas of hemorrhage and necrosis. On microscopic examination, there is destructive myometrial invasion while the intravascular worm-like plugs characteristic of low-grade endometrial stromal sarcomas are typically absent. They have marked cellular pleomorphism and brisk mitotic activity, almost always exceeding 10 MF/10HPF and sometimes approaching 50 MF/10HPF. Extensive necrosis is frequently present (Figure 18). These tumors should be diagnosed only after extensive sampling has excluded smooth or skeletal muscle differentiation or even small foci of carcinoma, as this finding would result in a diagnosis of carcinosarcoma. The histological appearance of this tumor is more like the mesenchymal elements of a carcinosarcoma than a typical endometrial stromal tumor.[3] Occasional tumors have a component of low-grade endometrial stromal sarcoma indicating that the high-grade component is presumably of endometrial stromal derivation. A recent study [119] has divided high-grade tumors into two categories based on nuclear uniformity and has proposed that undifferentiated endometrial sarcomas showing nuclear regularity may represent an

intermediate subcategory of endometrial stromal tumors (formerly classified as high-grade endometrial stromal sarcomas) that shares some immunohistochemical and molecular features with low-grade endometrial stromal sarcoma and is associated with better outcome than undifferentiated sarcomas exhibiting nuclear pleomorphism.[119]



3.3.2 Immunohistochemistry and somatic genetics

These tumors may show variable CD10 expression.[148] Some UES-U share with low-grade endometrial stromal sarcoma ER and PR expression, ß-catenin mutations, lack of p53 mutations and detection of JAZF1-JJAZ1 fusion gene.[119] In a more recent study, Kurihara and colleagues noted that these tumors expressed cyclin D1.[117] In

contrast, UES-P show the opposite profile being ER, PR and ß-catenin negative, with no associated JAZF1-JJAZ1 fusion gene but harbored complex karyotypes and high frequency of p53 mutations.[119] Tumors with uniform features are cyclin D-1 positive in contrast to those with pleomorphic features.[117] Recent analysis of MiRNA target gene pathways has shown several altered miRNA in low-grade endometrial stromal sarcoma as well as undifferentiated endometrial sarcoma involving Wnt, VEGF and EGFR signaling pathways. All these findings may suggest that at least in some instances, undifferentiated endometrial sarcoma and low-grade endometrial stromal sarcoma may be related.[117]

3.3.3 Prognosis and treatment

Undifferentiated endometrial sarcomas have very poor prognosis and most patients die of disease within two years of the diagnosis. In a recent study [48], vascular invasion was the only statistically significant prognostic factor, with a 5-year crude survival of 83% and 17% when vascular invasion was absent or present, respectively (P=0.02). Local recurrences and distant metastases are common and are associated with a high mortality. Treatment is primarily surgical with or without

addition of adjuvant radiotherapy or chemotherapy.[150,151] Adjuvant therapy does not appear to affect prognosis.[152]

OBJECTIVES

Background

Evaluation of conventional pathological parameters alone has been insufficient for predicting the behavior of uterine leiomyosarcomas and immunohistochemical analysis of various oncoproteins has been used. Several studies have shown that uterine leiomyosarcomas have significantly higher Ki67 index and p53 and p16 expression levels than benign leiomyomas. Also, it has been reported that leiomyosarcomas overexpressing bcl-2 show less lymphovascular space involvement and are associated with prolonged survival. Recently, study of tumor microenvironment has revealed an interaction between tumor cells and their surrounding stroma. We have previously showed that a stromal signature derived from a macrophage response occurs in a subset of uterine leiomyosarcomas associated with adverse outcome.

Objective

1. In an attempt to further increase prognostic accuracy of uterine leiomyosarcomas, we investigated conventional clinicopathologic parameters together with the expression of biomarkers Ki67, p53,

p16, Bcl-2, and CD163 (tumor associated macrophages) in a series of 84 uterine leiomyosarcomas.

Background

Endometrial stromal tumors (ESTs) may pose diagnostic challenges particularly when they exhibit variant histologic appearances, involve extrauterine sites, or present as metastatic disease. In such cases, immunohistochemical markers as well as identification of specific nonrandom chromosomal rearrangements may be helpful. Over the last decade, fluorescence in situ hybridization (FISH) has been progressively incorporated as a diagnostic tool for the evaluation of ESTs.

Objective

2. The purpose of this study was to review a series of endometrial stromal tumors and compare the results of FISH analysis with the clinicopathological characteristics. We investigated the frequency of rearrangements involving JAZF1, SUZ12, EPC1, and PHF1 in a series of 23 endometrial stromal tumors including cases with

classic and variant morphology trying to find out the value of FISH analysis in difficult cases.

RESULTS

MANUSCRIPT 1

RESULTS

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Review

Uterine sarcomas: A review

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ABSTRACT

Objective. Uterine sarcomas are rare tumors that account for 3% of uterine cancers. Their histopathologic classification was revised by the World Health Organization (WHO) in 2003. A new staging system has been recently designed by the International Federation of Gynecology and Obstetrics (FIGO). Currently, there is no consensus on risk factors for adverse outcome. This review summarizes the available clinicopathological data on uterine sarcomas classified by the WHO diagnostic criteria.

Methods. Medline was searched between 1976 and 2009 for all publications in English where the studied population included women diagnosed of uterine sarcomas.

Results. Since carcinosarcomas (malignant mixed mesodermal tumors or MMMT) are currently classified as metaplastic carcinomas, leiomyosarcomas remain the most common uterine sarcomas. Exclusion of several histologic variants of leiomyoma, as well as "smooth muscle tumors of uncertain malignant potential," frequently misdiagnosed as sarcomas, has made apparent that leiomyosarcomas are associated with poor prognosis even when seemingly confined to the uterus. Endometrial stromal sarcomas are indolent tumors associated with long-term survival. Undifferentiated endometrial sarcomas exhibiting nuclear pleomorphism behave more aggressively than tumors showing nuclear uniformity. Adenosarcomas have a favorable prognosis except for tumors showing myometrial invasion or sarcomatous overgrowth. Adenofibromas may represent well-differentiated adenosarcomas. The prognosis of carcinosarcomas (which are considered here in a post-script fashion) is usually worse than that of grade 3 endometrial carcinomas. Immunohistochemical expression of Ki67, p53, and p16 is significantly higher in leiomyosarcomas and undifferentiated endometrial sarcomas than in endometrial stromal sarcomas.

Conclusions. Evaluation of H&E stained sections has been equivocal in the prediction of behavior of uterine sarcomas. Immunohistochemical studies of oncoproteins as well as molecular analysis of non-random translocations will undoubtedly lead to an accurate and prognostically relevant classification of these rare tumors.

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Contents

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Introduction

Uterine sarcomas are rare tumors that account for approximately 1% of female genital tract malignancies and 3% to 7% of uterine cancers [1]. Although the aggressive behavior of most cases is well recognized, their rarity and histopathological diversity has contributed to the lack of consensus on risk factors for poor outcome and optimal treatment [2].

Histologically, uterine sarcomas were first classified into carcinosarcomas, accounting for 40% of cases, leiomyosarcomas (40%), endometrial stromal sarcomas (10% to 15%), and undifferentiated sarcomas (5% to 10%). Recently, carcinosarcoma has been reclassified as a dedifferentiated or metaplastic form of endometrial carcinoma. Despite this, and probably because it behaves more aggressively than the ordinary endometrial carcinoma, carcinosarcoma is still included in most retrospective studies of uterine sarcomas, as well as in the 2003 World Health Organization (WHO) classification [3].

The 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria for endometrial carcinoma have been used until now to assign stages for uterine sarcomas in spite of the different biologic behavior of both tumor categories. Recently, however, a new FIGO classification and staging system has been specifically designed for uterine sarcomas in an attempt to reflect their different biologic behavior (Table 1) [4]. Briefly, three new classifications have been developed: (1) staging for leiomyosarcomas and endometrial stromal sarcomas; (2) staging for adenosarcomas; and (3) staging for carcinosarcomas (MMMT). Whereas in the first classification stage I sarcomas are subdivided according to size, subdivision of stage I adenosarcomas takes into account myometrial invasion. On the other hand, carcinosarcomas will continue to be staged as endometrial carcinomas.

Table 1
FIGO staging for uterine sarcomas (2009).

Stage	Definition
(1) Leiomyosarcomas and endometrial stromal sarcomas[a]	
I	Tumor limited to uterus
IA	Less than or equal to 5 cm
IB	More than 5 cm
II	Tumor extends beyond the uterus, within the pelvis
IIA	Adnexal involvement
IIB	Involvement of other pelvic tissues
III	Tumor invades abdominal tissues (not just protruding into the abdomen)
IIIA	One site
IIIB	More than one site
IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	
IVA	Tumor invades bladder and/or rectum
IVB	Distant metastasis
(2) Adenosarcomas	
I	Tumor limited to uterus
IA	Tumor limited to endometrium/endocervix with no myometrial invasion
IB	Less than or equal to half myometrial invasion
IC	More than half myometrial invasion
II	Tumor extends beyond the uterus, within the pelvis
IIA	Adnexal involvement
IIB	Tumor extends to extrauterine pelvic tissue
III	Tumor invades abdominal tissues (not just protruding into the abdomen).
IIIA	One site
IIIB	More than one site
IIIC	Metastasis to pelvic and/or para-aortic lymph nodes
IV	
IVA	Tumor invades bladder and/or rectum
IVB	Distant metastasis
(3) Carcinosarcomas	
Carcinosarcomas should be staged as carcinomas of the endometrium.	

[a] *Note*. Simultaneous endometrial stromal sarcomas of the uterine corpus and ovary/pelvis in association with ovarian/pelvic endometriosis should be classified as independent primary tumors.

Leiomyosarcoma

Clinical features

After excluding carcinosarcoma (MMMT), leiomyosarcoma has become the most common subtype of uterine sarcoma. However, it accounts for only 1–2% of uterine malignancies. Most occur in women over 40 years of age who usually present with abnormal vaginal bleeding (56%), palpable pelvic mass (54%), and pelvic pain (22%). Signs and symptoms resemble those of the far more common leiomyoma and preoperative distinction between the two tumors may be difficult. Nevertheless, malignancy should be suspected by the presence of certain clinical behaviors, such as tumor growth in menopausal women who are not on hormonal replacement therapy [5]. Occasionally, the presenting manifestations are related to tumor rupture (hemoperitoneum), extrauterine extension (one-third to one-half of cases), or metastases. Only very rarely does a leiomyosarcoma originate from a leiomyoma.

Pathological features

The histopathologic diagnosis of uterine leiomyosarcoma is usually straightforward since most clinically malignant smooth muscle tumors of the uterus show the microscopic constellation of hypercellularity, severe nuclear atypia, and high mitotic rate generally exceeding 15 mitotic figures per 10 high-power-fields (MF/10 HPF) [6–7] (Fig. 1a). Moreover, one or more supportive clinicopathologic features such as peri- or postmenopausal age, extrauterine extension, large size (over 10 cm), infiltrating border, necrosis, and atypical mitotic figures are frequently present [8].

Epithelioid and myxoid leiomyosarcomas, however, are two rare variants which may be difficult to recognize microscopically as their pathologic features differ from those of ordinary spindle cell leiomyosarcomas. In fact, nuclear atypia is usually mild in both tumor

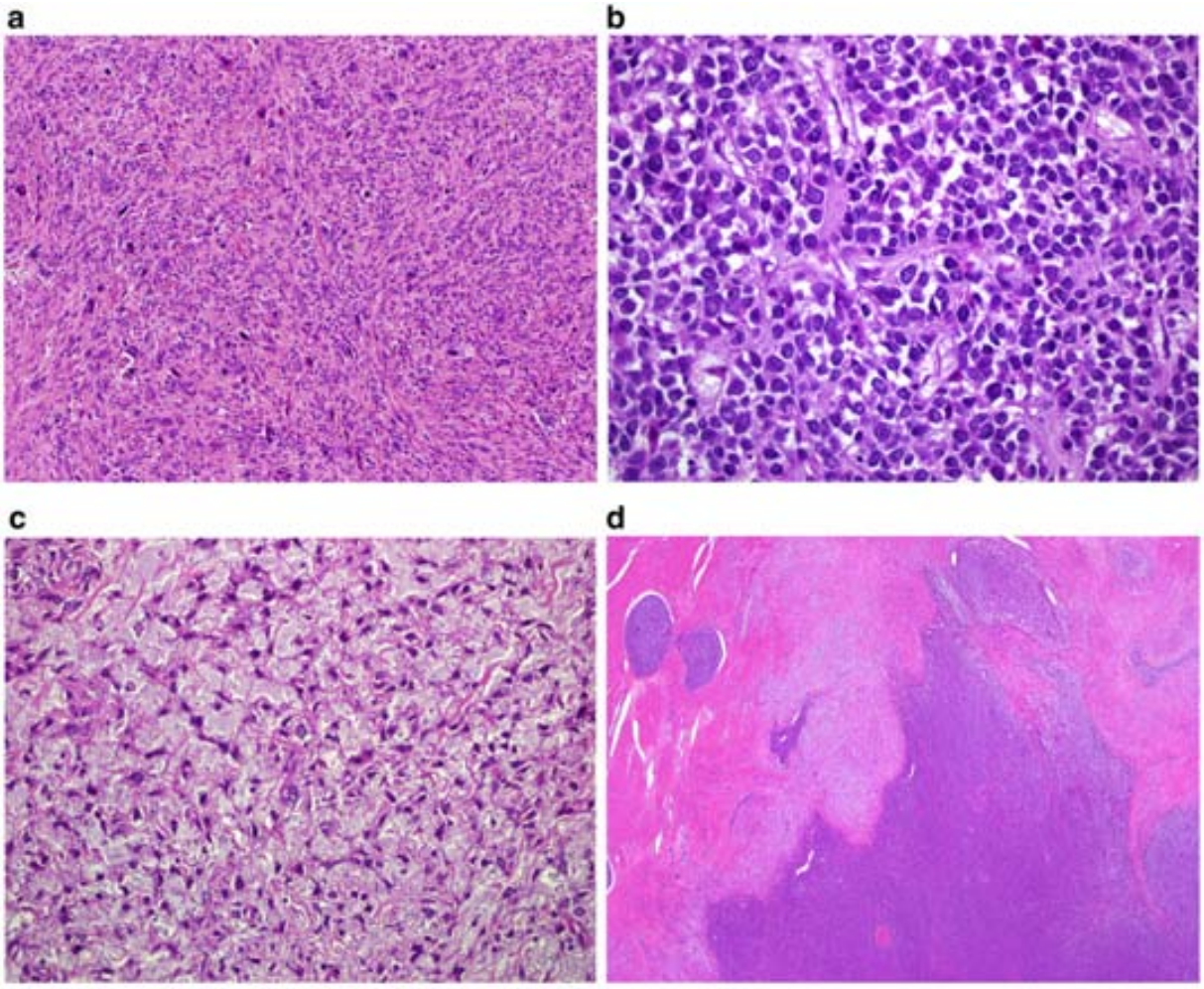

Fig. 1. (a) Leiomyosarcoma, spindle-cell variant; (b) myxoid leiomyosarcoma; (c) epithelioid leiomyosarcoma; (d) endometrial stromal sarcoma.

types and the mitotic rate is often <3 MF/10 HPF [9] (Figs. 1b, c). In epithelioid leiomyosarcomas, necrosis may be absent and myxoid leiomyosarcomas are often hypocellular. In the absence of severe cytologic atypia and high mitotic activity, both tumors are diagnosed as sarcomas based on their infiltrative borders [10].

The minimal pathological criteria for the diagnosis of leiomyosarcoma are more problematic and, in such cases, the differential diagnosis has to be made, not only with a variety of benign smooth muscle tumors that exhibit atypical histologic features and unusual growth patterns (Table 2), but also with smooth muscle tumors of uncertain malignant potential (STUMP) (Table 3). Application of the 2003 WHO diagnostic criteria [4] has allowed distinguishing these unusual histologic variants of leiomyoma frequently misdiagnosed as well-differentiated or low-grade leiomyosarcomas in the past. Indeed, in a recent population-based study of uterine sarcomas from Norway [11], of 356 tumors classified initially as leiomyosarcomas, diagnosis was confirmed in only 259 cases (73%), whereas 97 (27%) were excluded on review and reclassified as leiomyomas or leiomyoma variants. Follow-up information, however, revealed that 4 of 48 excluded tumors (1 cellular leiomyoma and 3 STUMPs) developed metastases.

Table 2
Benign smooth muscle tumors of the uterus.

Leiomyoma variants that may mimic malignancy	Smooth muscle proliferations with unusual growth patterns
• Mitotically active leiomyoma	• Disseminated peritoneal leiomyomatosis
• Cellular leiomyoma	• Benign metastasizing leiomyoma
• Hemorrhagic leiomyoma and hormone-induced changes	• Intravenous leiomyomatosis
• Leiomyoma with bizarre nuclei (atypical leiomyoma)	• Lymphangioleiomyomatosis
• Myxoid leiomyoma	
• Epithelioid leiomyoma	
• Leiomyoma with massive lymphoid infiltration	

Immunohistochemistry and molecular biology

Recently, several immunohistochemical and molecular genetic studies on uterine leiomyosarcomas have been reported [12,13–19]. Leiomyosarcomas usually express smooth muscle markers such as desmin, h-caldesmon, smooth muscle actin, and histone deacetylase 8 (HDCA8). However, it is important to keep in mind that epithelioid and myxoid leiomyosarcomas may show lesser degrees of immunoreaction for these markers. Also, leiomyosarcomas are often immunoreactive for CD10 and epithelial markers including keratin and EMA

Table 3
Smooth muscle tumors of uncertain malignant potential (STUMP).

Pathologic criteria
• Tumor cell necrosis in a typical leiomyoma
• Necrosis of uncertain type with ≥10 MF / 10 HPFs, or marked diffuse atypia
• Marked diffuse or focal atypia with borderline mitotic counts
• Necrosis difficult to classify

(the latter being more frequently positive in the epithelioid variant). Conventional leiomyosarcomas express estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) in 30-40% of cases. Whereas a variable proportion of uterine leiomyosarcomas has been reported as being immunoreactive for c-KIT, no c-KIT mutations have been identified [20].

Recent studies have shown statistically significant higher levels of Ki67 in uterine leiomyosarcomas compared with benign smooth muscle tumors [15–19]. Mutation and overexpression of p53 have been described in a significant minority of uterine leiomyosarcomas (25-47%) but not in leiomyomas [15,18,19]. Intermediate rates have been found in atypical leiomyomas and STUMPs. Overexpression of p16 has been described in uterine leiomyosarcomas and may prove to be a useful adjunct immunomarker for distinguishing between benign and malignant uterine smooth muscle tumors [13–15].

The vast majority of uterine leiomyosarcomas are sporadic. Patients with germline mutations in fumarate hydratase are believed to be at increased risk for developing uterine leiomyosarcomas as well as uterine leiomyomas [21,22]. The oncogenic mechanisms underlying the development of uterine leiomyosarcomas remain elusive. Overall, uterine leiomyosarcoma is a genetically unstable tumor that demonstrates complex structural chromosomal abnormalities and highly disturbed gene regulation which likely reflects the end-state of accumulation of multiple genetic defects. Extrapolating from experiences in soft tissue leiomyosarcomas, it is unlikely that recurrent disease-driven genetic aberrations (i.e. gene mutation or translocation events) will be uncovered. In comparison with other more common uterine malignancies, uterine leiomyosarcomas bear some resemblance to type 2 endometrial carcinomas and high-grade serous carcinomas of ovary/fallopian tube origin, based on their genetic instability, frequent p53 abnormalities, aggressive behavior, and resistance to chemotherapy. Therefore, therapies that exploit the underlying genetic instability of uterine leiomyosarcomas may prove to be an effective therapeutic strategy.

Prognosis and treatment

Leiomyosarcomas are very aggressive tumors. It has become apparent that tumors diagnosed according to the 2003 WHO criteria are associated with poor prognosis even when confined to the uterus [11,23] and even when diagnosed at an early stage; recurrence rate has ranged from 53% to 71% [1]. First recurrences were in the lungs in 40% of patients and in the pelvis in only 13%. Overall survival rate ranged from 15% to 25% with a median survival of only 10 months in one study. In the Norwegian series [11], patients with leiomyosarcomas limited to the uterus had poor prognosis with a 5-year overall survival of 51% at stage I and 25% at stage II (by the 1988 FIGO staging classification). All patients with spread outside the pelvis died within 5 years.

There has been no consistency among various studies regarding correlation between survival and patient age, clinical stage, tumor size, type of border (pushing versus infiltrative), presence or absence of necrosis, mitotic rate, degree of nuclear pleomorphism, and vascular invasion [2,12,23–29]. One study, however, found tumor size to be a major prognostic parameter [2]: five of 8 patients with tumors <5 cm in diameter survived, whereas all patients with tumors >5 cm in diameter died of tumor. In this study of 208 uterine leiomyosarcomas, the only other parameters predictive of prognosis were tumor grade and stage [2]. Histologic grade, however, has not been consistently identified as a significant prognostic parameter. In the report from Norway [11], including 245 leiomyosarcomas confined to the uterus, tumor size and mitotic index were significant prognostic factors and allowed for separation of patients into 3 risk groups with marked differences in prognosis. Ancillary parameters including p53, p16, Ki 67, and Bcl-2 have been used in leiomyosarcomas trying to predict outcome [23]. However, it is not clear whether they act independently of stage which still is the most significant prognostic factor for uterine sarcomas.

Treatment of leiomyosarcomas includes total abdominal hysterectomy and debulking of tumor if present outside the uterus. Removal of the ovaries and lymph node dissection remain controversial as metastases to these organs occur in a small percentage of cases and are frequently associated with intra-abdominal disease [2]. Ovarian preservation may be considered in premenopausal patients with early-stage leiomyosarcomas [2]. Lymph node metastases have been identified in 6.6% and 11% of two series of patients with leiomyosarcoma who underwent lymphadenectomy [2,30]. In the first series, the 5-year disease-specific survival rate was 26% in patients who had positive lymph nodes compared with 64.2% in patients who had negative lymph nodes ($p<0.001$) [30]. The influence of adjuvant therapy on survival is uncertain. Radiotherapy may be useful in controlling local recurrences and chemotherapy with doxorubicin or docetaxel/gemcitabine is now used for advanced or recurrent disease, with response rates ranging from 27% to 36% [31,32]. Some patients may respond to hormonal treatment [33].

Smooth muscle tumors of uncertain malignant potential (STUMP)

Uterine smooth muscle tumors that show some worrisome histological features (i.e., necrosis, nuclear atypia, or mitoses), but do not meet all diagnostic criteria for leiomyosarcoma, fall into the category of STUMP (Table 3) [3,34]. The diagnosis of STUMP, however, should be used most sparingly and every effort should be made to classify a smooth muscle tumor into a specific category [3,34]. Most tumors classified as STUMP have been associated with favorable prognosis and, in these cases, only follow-up of the patients is recommended [35]. In fact, in a recent study of 41 cases of STUMP, the recurrence rate was 7%. One of the two recurrences was in the form of STUMP and the other as leiomyosarcoma [36].

Endometrial stromal tumor

Endometrial stromal tumors are the second most common pure mesenchymal tumors of the uterus even though they account for less than 10% of all such tumors. According to the latest WHO classification [3], the term endometrial stromal tumor is applied to neoplasms typically composed of cells that resemble endometrial stromal cells of the proliferative endometrium [3]. They are divided into: endometrial stromal nodules, low-grade endometrial stromal sarcomas, and undifferentiated endometrial sarcomas.

Endometrial stromal nodule

These rare tumors are composed of cells reminiscent of proliferative-phase endometrial stromal cells. They occur at any age during reproductive or later years. Most are incidental findings in a hysterectomy specimen while others present with abnormal uterine bleeding.

The tumors are typically round and well-circumscribed but not encapsulated. They are usually solitary, ranging from under 1 to 22 (mean 7) cm. If located in the endometrium, they are frequently polypoid; however, they may be intramyometrial or subserosal. They have a uniform soft, yellow cut surface which does not show the whorled pattern characteristic of a leiomyoma. Cysts may be present.

The main distinguishing feature of endometrial stromal nodules is their expansile, non-infiltrating, smooth margin that contrasts with the infiltrating irregular margin of stromal sarcomas [37]. Focal irregularities in the form of lobulated or finger-like projections into the adjacent myometrium not exceeding 3 mm and not exceeding 3 in number may be seen [38]. Vascular invasion should not be present.

Endometrial stromal nodules have an excellent prognosis and patients are cured by hysterectomy [39]. Conservative treatment with

excision of the mass is performed only when complete examination of the margins can be done which only occurs in rare instances [40].

Low-grade endometrial stromal sarcoma

Endometrial stromal sarcomas account for approximately 0.2% of all malignant uterine tumors and 10-15% of uterine malignancies with a mesenchymal component. They occur in women between 40 and 55 years of age. Some cases have been reported in patients with ovarian polycystic disease, after estrogen use, or tamoxifen therapy. Patients commonly present with abnormal uterine bleeding, pelvic pain, and dysmenorrhea but as many as 25% of them are asymptomatic [41]. At presentation, extrauterine pelvic extension, most commonly involving the ovary, is found in up to 1/3 of patients. Thus, when evaluating an ovarian tumor microscopically consistent with an endometrial stromal tumor, it is important to exclude a prior history of uterine endometrial stromal tumor and to suggest inspection of the uterus, as the latter are far more common.

Grossly, there is irregular nodular growth involving the endometrium, myometrium, or both. The main mass is frequently associated with varying degrees of permeation of the myometrium, including worm-like plugs of tumor that fill and distend myometrial veins, frequently extending to parametrial veins and lymphatics. Microscopically, endometrial stromal sarcomas exhibit only mild nuclear atypia. Mitotic activity is typically <5 MF/10 HPF. Necrosis is rarely seen (Fig. 1d).

Immunohistochemistry and molecular biology

Endometrial stromal nodules and low-grade endometrial stromal sarcomas are typically immunoreactive for vimentin, muscle-specific actin, alpha-smooth muscle actin, and frequently keratin [42–44]. Most endometrial stromal tumors as well as normal endometrial stromal cells stain for CD10. However, smooth muscle tumors, mixed mullerian tumors or even rhabdomyosarcomas may also be immunoreactive for CD10 [42–44]. Thus, this antibody should not be used in isolation when evaluating the cell of origin in a uterine mesenchymal tumor. Not uncommonly, endometrial stromal tumors can exhibit diffuse alpha-smooth muscle actin reactivity, while desmin and h-caldesmon are generally negative or at most focally positive [44]. Other muscle markers including myosin and HDCA8 are also helpful in this differential diagnosis [45]. Areas of smooth muscle differentiation are reactive for all smooth muscle markers as well as for CD10. Areas of sex cord-like differentiation may be reactive for inhibin, calretinin, CD99, WT-1, and Melan A. [46] Endometrial stromal tumors frequently contain ER and PR and they also frequently express beta-catenin [47]. Endometrial stromal sarcomas often carry the translocation t(7;17) with involvement of two zinc finger genes, JAZF1 and JJAZ1, suggesting a genetic basis for tumor development [48].

Prognosis and treatment

Endometrial stromal sarcomas are indolent tumors with a favorable prognosis [38]. Tumor behavior is characterized by late recurrences even in patients with stage I disease; thus, long term follow-up is required. About one third of patients develop recurrences, most commonly in the pelvis and abdomen, and less frequently in the lung and vagina [41].

The outcome in patients with endometrial stromal sarcomas depends largely on the extent of the tumor at the time of diagnosis. Surgical stage higher than I is a univariate predictor of unfavorable outcome. Generally endometrial stromal sarcomas have good prognosis, with 5- and 10-year actuarial survival for patients with stage I tumors of 98% and 89%, respectively [41]. Several other features may help predict outcome. Clinicopathologic factors reported in the older literature to be of potential prognostic importance included age, race, size, FIGO stage, depth of myometrial invasion, tumor grade, mitotic activity, and DNA ploidy [49–52]. However, in the largest study of low-grade endometrial stromal sarcomas, mitotic activity and cytologic atypia were not found to be predictive of tumor recurrence in stage I tumors (most common scenario), while size correlated poorly with outcome as tumors <4 cm in diameter also recurred [41]. In another recent study [11], prognosis of endometrial stromal sarcomas confined to the uterus (83 cases) was related to mitotic index and tumor cell necrosis.

Treatment of endometrial stromal sarcomas is largely surgical in the form of hysterectomy and bilateral salpingo-oophorectomy. These tumors are often sensitive to hormones and it has been stated that patients retaining their ovaries have a higher risk of recurrence [53]; however, there is no complete agreement on this issue [50,53–56]. Although lymph node metastases have been found in 7% of 384 women with low-grade endometrial stromal sarcoma, this finding does not affect the excellent overall survival of these patients [54]. Patients may receive also adjuvant radiation or hormonal treatment with progestational agents or aromatase inhibitors [57,58].

Undifferentiated endometrial sarcoma

Clinicopathological features

The diagnosis of undifferentiated endometrial sarcoma is applied to tumors that exhibit myometrial invasion, severe nuclear pleomorphism, high mitotic activity, and/or tumor cell necrosis, and lack smooth muscle or endometrial stromal differentiation [3]. Grossly, they are often polypoid and show a fleshy, gray to white cut surface and prominent areas of hemorrhage and necrosis. On microscopic examination, there is destructive myometrial invasion while the intravascular worm-like plugs characteristic of low-grade endometrial stromal sarcomas are typically absent. They have marked cellular pleomorphism and brisk mitotic activity, almost always exceeding 10 MF/10HPF and sometimes approaching 50 MF/10HPF. Extensive necrosis is frequently present. These tumors should be diagnosed only after extensive sampling has excluded smooth or skeletal muscle differentiation or even small foci of carcinoma, as this finding would result in a diagnosis of carcinosarcoma. The histological appearance of this tumor is more like the mesenchymal elements of a carcinosarcoma than a typical endometrial stromal tumor [3]. Occasional tumors have a component of low-grade endometrial stromal sarcoma indicating that the high-grade component is presumably of endometrial stromal derivation. A recent study [59] has divided high-grade tumors into two categories based on nuclear uniformity and has proposed that undifferentiated endometrial sarcomas showing nuclear regularity may represent an intermediate subcategory of endometrial stromal tumors (formerly classified as high-grade endometrial stromal sarcomas) that shares some immunohistochemical and molecular features with low-grade endometrial stromal sarcoma and is associated with better outcome than undifferentiated sarcomas exhibiting nuclear pleomorphism [59].

Immunohistochemistry

Undifferentiated endometrial sarcomas lack immunoreaction for ER and PR, but a high proportion is EGFR immunoreactive [3]. CD10 expression is not helpful in the differential diagnosis with other uterine sarcomas because undifferentiated endometrial sarcoma as well as leiomyosarcoma, rhabdomyosarcoma, and carcinosarcoma may express this marker. Smooth muscle markers and myogenin or myoD1 may be used to rule out leiomyosarcoma or rhabdomyosarcoma respectively, or to identify a rhabdomyosarcomatous component of a carcinosarcoma.

Prognosis and treatment

Undifferentiated endometrial sarcomas have very poor prognosis and most patients die of disease within two years of the diagnosis. In a recent study [11], vascular invasion was the only statistically significant prognostic factor, with a 5-year crude survival of 83% and 17% when vascular invasion was absent or present, respectively ($P=0.02$). Local recurrences and distant metastases are associated with a high mortality. Treatment is primarily surgical with or without addition of adjuvant radiotherapy or chemotherapy [60,61].

Adenosarcoma

Clinical features

The rare mullerian adenosarcoma is a mixed tumor of low malignant potential with distinctive clinicopathologic features [62]. It occurs mainly in the uterus of postmenopausal women but also in adolescents and young adults and in extrauterine locations [62]. The most common presenting symptom is abnormal vaginal bleeding but some patients present with pelvic pain, an abdominal mass or vaginal discharge. Some patients have taken tamoxifen therapy or have had prior radiation therapy. Most commonly, adenosarcomas arise from the endometrium, including the lower uterine segment, but rare tumors arise in the endocervix and within the myometrium, probably from adenomyosis. Rarely, adenosarcomas have an extrauterine location and involve the ovary, pelvic tissues, or intestinal serosa.

Pathological features

The uterine cavity is typically filled and distended by a soft polypoid and sometimes large mass which may project through the cervical os. The cut surface may show variably sized cysts or clefts. There is often focal hemorrhage and necrosis. The margin of the tumor is usually well defined.

Microscopically, it shows an intimate admixture of benign but sometimes atypical glandular epithelium and low-grade sarcoma, usually of endometrial stromal type. Typically, the glands are cystic and the stroma concentrates around them forming periglandular cuffs (Figs. 2a, b). The histologic picture is reminiscent of a phyllodes tumor of the breast. Although the mean mitotic rate is 9 MF/10 HPF [62], in the presence of hypercellular periglandular cuffs, only 2 MF/10 HPF are enough for the diagnosis [62]. Most adenosarcomas show only mild to moderate nuclear atypia in the stromal component. Heterologous mesenchymal elements (usually rhabdomyosarcoma, but also cartilage, fat, and other elements) are found in 10–15% of cases. Vaginal or pelvic recurrence, estimated to occur in about 25–30% of cases at 5 years, is associated almost exclusively with myometrial invasion and sarcomatous overgrowth [62]. Myometrial invasion is found in 15% of cases, but deep invasion in only 5%. Sarcomatous overgrowth defined as the presence of pure sarcoma, usually of high-grade and without a glandular component, occupying

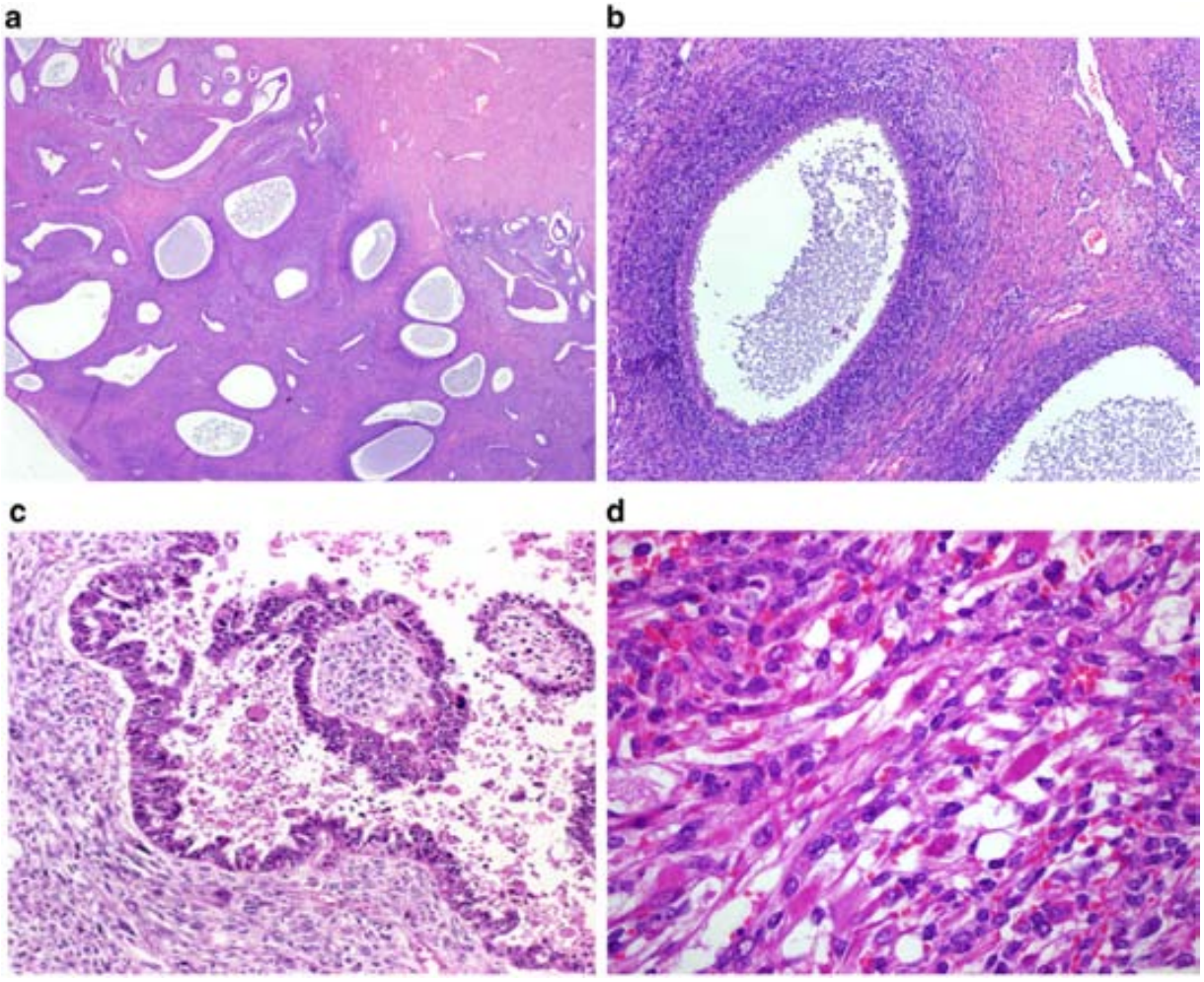

Fig. 2. (a) Adenosarcoma; (b) periglandular cuffing in adenosarcoma; (c) carcinosarcoma; (d) Rhabdomyosarcoma component in carcinosarcoma.

at least 25% of the tumor, has been reported in 8–54% of uterine and 30% of ovarian adenosarcomas [62].

Adenosarcoma versus adenofibroma

Adenosarcomas are low-grade neoplasms classified halfway along the spectrum of mixed mullerian tumors, with adenofibromas at one end and carcinosarcomas at the other. Whereas the histologic diagnosis of the latter is usually straightforward, distinction between adenosarcoma and its rarer benign counterpart, the adenofibroma, may be difficult. A recent study [63] has shown that some clinically malignant tumors without sarcomatous overgrowth may exhibit only moderate stromal cellularity with focal periglandular cuffs, low mitotic count (<2 MFs/10 HPF) and mild nuclear atypia. The finding of such cases raise the question whether or not adenofibroma exists as a tumor entity. In this study, immunoreaction for several tumor markers was similar both in typical adenosarcomas and adenofibromas associated with favorable outcome. Thus, it was suggested that some of so-called "adenofibromas" may in fact represent exceedingly well differentiated adenosarcomas [63].

Immunohistochemistry

In most adenosarcomas without sarcomatous overgrowth, the immunophenotype of the stromal component resembles that of an endometrial stromal sarcoma. In cases with sarcomatous overgrowth, the mesenchymal component exhibits a higher Ki-67 proliferation, p53 immunoreaction and there is usually loss of expression of ER, PR and CD10. The immunophenotype is similar to that of a high-grade uterine sarcoma [63,64] and DNA is aneuploid [65].

Prognosis and treatment

Except when associated with myometrial invasion or sarcomatous overgrowth, the prognosis of adenosarcoma is far more favorable than that of carcinosarcoma; however, about 25% of patients with adenosarcoma ultimately die of their disease [62]. Recurrences usually occur in the vagina, pelvis, or abdomen. They may be late, for which reason long-term follow-up is needed. Local recurrences and distant metastases, which occur in 5% of cases, are almost always composed of pure sarcoma (70%). Treatment of choice is total abdominal hysterectomy with bilateral salpingo-oophorectomy. In the series from Norway [11], which included 23 adenosarcomas, tumor cell necrosis was the strongest prognostic factor ($P = 0.006$).

Carcinosarcoma (malignant mixed mullerian tumor)

Clinical features

Carcinosarcoma, also referred to as "malignant mixed mullerian tumor," is a biphasic neoplasm composed of distinctive and separate, but admixed, malignant-appearing epithelial and mesenchymal elements (Fig. 2c). It accounts for almost half of all uterine sarcomas [65,66]. Although they occur typically in post-menopausal women, a small number has been reported in patients less than 40 years of age. Most women present with abnormal vaginal bleeding and uterine enlargement. The serum level of CA125 is elevated in most cases. At presentation, extrauterine spread (stages III–IV) is found in up to 1/3 of cases. Up to 37% of patients with carcinosarcomas have a history of pelvic irradiation. These tumors tend to occur in younger women, often contain heterologous elements, and are found at advanced stage [67].

Pathological features

Carcinosarcomas are typically large, bulky polypoid masses, filling the uterine cavity and prolapsing through the cervical os. The cut surface is usually fleshy and often shows areas of hemorrhage, necrosis, and cystic change. Myometrial invasion is frequently seen. Rare tumors may arise in the uterine cervix. On microscopic examination, the carcinomatous component is usually serous (two-thirds of cases) or endometrioid (one-third) but, rarely, it may be clear cell, mucinous, or squamous cell carcinoma. In a recent study, 10% of the carcinomatous components were FIGO grade 1, 10% grade 2, and 80% grade 3 [66]. The sarcomatous components are heterogeneous. The homologous components of carcinosarcoma are usually spindle cell sarcoma without obvious differentiation; many resemble fibrosarcomas or pleomorphic sarcomas. Almost all are high grade sarcomas. The most common heterologous elements are malignant skeletal muscle or cartilage resembling either pleomorphic rhabdomyosarcoma or embryonal rhabdomyosarcoma [66] (Fig. 2d).

Histogenesis

Recently, it has been proposed that carcinosarcomas may represent metaplastic carcinomas [66–68]. Findings that support this hypothesis include: (a) frequent association of carcinosarcomas with otherwise typical endometrial adenocarcinomas within the same hysterectomy specimen; (b) frequent recurrence of carcinosarcomas as pure adenocarcinomas; (c) occasional recurrence of apparently pure endometrial adenocarcinomas as carcinosarcomas; and (d) similar metastatic pattern of carcinosarcomas and endometrial adenocarcinomas. Nevertheless, from a managerial viewpoint, it should be emphasize that carcinosarcomas have distinctive clinical and pathological features which warrant their separation from endometrial carcinomas; i.e., they are highly aggressive tumors and fatal in the vast majority of cases. Unlike metaplastic carcinomas in other sites, there is usually no merging of the two components of carcinosarcomas at either histological or ultrastructural [69] levels and heterologous mesenchymal elements are common.

Immunohistochemistry

The immunophenotype parallels that of the individual elements; i.e., the serous component should express cytokeratins, epithelial membrane antigen (EMA), and p53, while the rhabdomyoblastic elements should express desmin, myogenin, or MyoD1. However, it is well known that the sarcomatous component can express cytokeratins (as in leiomyosarcomas) and the epithelial component is often immunoreactive for vimentin (as in endometrial carcinomas). Such findings reflect the common mesodermal origin of these tumors. The homologous component can also express CD10, a marker used initially for the diagnosis of endometrial stromal tumors. In most cases, immunohistochemistry is not needed for diagnosis and should only be used to confirm the presence of rhabdomyoblasts.

Prognosis and treatment

Carcinosarcomas are highly aggressive tumors, far more aggressive than usual endometrial carcinomas. The overall 5-year survival for patients with carcinosarcoma is around 30% and for those with stage I (confined to the corpus) approximately 50% [1,66–69]. This is in contrast with that of other high grade endometrial cancers for which 5-year survival in stage I disease is approximately 80% or better [70,71]. Surgical stage and, particularly, depth of myometrial invasion are the most important prognostic indicators. Myometrial invasion beyond the inner third is seen in 80% of tumors and 40% show deep myometrial invasion. However, confinement to an endometrial polyp in absence of myometrial invasion does not preclude extrauterine spread. Lymphatic and blood vessel invasion are found in most cases. Metastatic and recurrent tumors may exclusively be carcinomatous, sarcomatous, or mixed, but they are often predominantly carcinomatous [66,69]. Tumors containing serous and clear cell carcinoma are

thought to be associated with higher frequency of metastases, deep myometrial invasion, lymphatic or vascular space invasion, and cervical involvement [68]. In common with the older literature, a recent study has found that the presence of heterologous elements is a statistically significant poor prognostic factor in stage I patients [66].

Appropriate treatment includes total abdominal hysterectomy with bilateral salpingo-oophorectomy, removal of pelvic and aortic lymph nodes, omentectomy, and peritoneal cytology. The role of adjuvant radiotherapy and chemotherapy is uncertain but some studies have demonstrated the advantage of radiotherapy for disease-specific survival in early-stage tumors as well as local control in advanced-stage tumors. Taxanes and cisplatin-based chemotherapy as well as ifosphamide, along with whole pelvic irradiation, may lead to increased survival in patients with metastatic carcinosarcomas [72–74].

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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MANUSCRIPT 2

RESULTS

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Uterine leiomyosarcomas: Tumor size, mitotic index, and biomarkers Ki67, and Bcl-2 identify two groups with different prognosis ☆

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ABSTRACT

Background. Prognostic factors for uterine leiomyosarcomas are not well established. Although most tumors are associated with poor prognosis even when apparently confined to the uterus (stage I), some cases that exhibited morphologic features of malignancy had prolonged survival.

Methods. Using tissue microarrays of 84 uterine leiomyosarcomas, we investigated conventional clinicopathologic parameters, including International Federation of Gynecology and Obstetrics (FIGO) stage, together with expression of Ki67, p53, p16, and Bcl-2, attempting to distinguish leiomyosarcomas with different prognosis. The rate of CD163 immunoreactive tumor macrophages was also investigated.

Results. Tumor size and mitotic index were significant prognostic factors by univariate ($p=0.018$ and $p=0.003$, respectively) and multivariate ($p=0.006$ and $p=0.001$) analyses. Of the biomarkers investigated, only Ki67 immunoreaction was significant by univariate analysis and was associated with adverse prognosis ($p=0.01$). However, combination of tumor size, mitotic index, Ki67, and Bcl-2 worked even better. Using these 4 parameters, unsupervised hierarchical clustering identified 2 groups of tumors with different prognosis ($p=0.001$): group 1 consisted mostly of smaller leiomyosarcomas (<10 cm) with mitotic index <20 MF/10 HPF, negative Ki67, and positive or negative Bcl-2 immunostaining. These tumors were associated with better prognosis. In contrast, group 2 leiomyosarcomas which were mostly ≥10 cm in diameter had higher mitotic index (≥ 20 MF/10 HPF), and were positive for Ki67 and negative for Bcl-2 had worse prognosis. Also, the number of CD163-macrophages was greater in group 2 than group 1 ($p=0.007$).

Conclusions. Tumor size and mitotic index are morphologic predictors of malignancy in uterine leiomyosarcomas. Combination of tumor size, mitotic index, Ki67, and Bcl-2 protein expression allows distinguishing 2 groups of leiomyosarcomas with different survival. Leiomyosarcomas associated with poor outcome had a higher number of CD163 stromal macrophages.

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Introduction

Uterine sarcomas are rare tumors accounting for 3% of uterine cancers [1,2]. After excluding carcinosarcoma (malignant mixed mesodermal tumor), currently classified as metaplastic carcinoma [3], leiomyosarcoma has become the most common subtype of uterine sarcomas. The histopathologic criteria for the diagnosis of leiomyosarcoma have evolved over the last four decades. The value of nuclear atypia and mitotic activity has been reconsidered and tumor necrosis has emerged as an important predictor of malignancy [4]. Application of the 2003 World Health Organization (WHO) diagnostic criteria [5] has allowed distinguishing rare variants of leiomyoma frequently misdiagnosed as well-differentiated or low-grade leiomyosarcomas in the past. After excluding these mimics, most tumors left are leiomyosarcomas exhibiting moderate to severe nuclear atypia, high mitotic index, and/or tumor cell necrosis. These tumors are aggressive neoplasms associated with poor prognosis, even if surgically treated at early stage (stage I) [6]. The minimal pathological criteria for the diagnosis of leiomyosarcoma are more problematic and, in such cases, the term smooth muscle tumors of uncertain malignant potential (STUMP) has been used [4,5]. However, most tumors classified as STUMP have been associated with favorable prognosis and, in these cases, only follow-up of the patients is recommended [7,8].

The prognosis of uterine sarcomas varies considerably according to their histologic type. Whereas low-grade endometrial stromal sarcomas are commonly associated with a favorable outcome, and undifferentiated sarcomas usually have an adverse prognosis, the

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behavior of leiomyosarcomas is less predictable [9-13]. Although most leiomyosarcomas are very aggressive tumors associated with poor prognosis, some cases that exhibited the morphologic features of malignancy have been associated with prolonged survival [6].

The rarity of uterine leiomyosarcomas has hampered the development of a specific staging system. In fact, a modification of the 1988 FIGO staging system developed primarily for endometrial adenocarcinomas has been used until recently. However, a new FIGO classification specifically designed for uterine leiomyosarcomas, including variables such as tumor size, extra-uterine spread, and invasion of abdominal tissues, was proposed in 2009 [14]. Nevertheless, since uterine leiomyosarcomas are most frequently diagnosed while still apparently confined to the uterus even if they are predominantly high grade, FIGO staging system tends to group most patients within stage I, falsely suggesting prognostic homogeneity in a highly heterogeneous group of patients. Thus, there is an urgent need for a stronger prognostic model in these patients.

Evaluation of conventional pathological parameters alone has been insufficient for predicting the behavior of uterine leiomyosarcomas and immunohistochemical analysis of various oncoproteins has been used. Several studies have shown that uterine leiomyosarcomas have significantly higher Ki67 index and p53 and p16 expression levels than benign leiomyomas. Also, it has been reported that leiomyosarcomas overexpressing bcl-2 show less lymphovascular space involvement and are associated with prolonged survival [6,15-22]. Recently, study of tumor microenvironment has revealed an interaction between tumor cells and their surrounding stroma [23,24]. We have previously shown that a stromal signature derived from a macrophage response occurs in a subset of uterine leiomyosarcomas associated with adverse outcome [25].

In an attempt to further increase prognostic accuracy, we investigated conventional clinicopathologic parameters together with the expression of biomarkers Ki67, p53, p16, Bcl-2, and CD163 (tumor associated macrophages) in a series of 84 uterine leiomyosarcomas.

Materials and methods

Case selection

Eighty-four leiomyosarcomas diagnosed from 1978 to 2008 were retrieved from the Surgical Pathology data base of two medical centers (Vancouver General Hospital, Vancouver, B.C., Canada and Hospital de la Santa Creu i Sant Pau, Barcelona, Spain) and the consultation files of one of the authors (JP). The pathology slides were reviewed by four of the authors (CHL, EDA, IE, and JP). Only spindle cell leiomyosarcomas were included. Rare variants such as epithelioid and myxoid leiomyosarcomas were not analyzed. Diagnostically equivocal categories such as smooth muscle tumors of uncertain malignant potential (STUMP) were not included [5,7,8]. Clinical and pathologic information regarding patient's age, tumor size, gross and histological features, tumor stage, treatment, and follow-up were obtained from hospital charts and pathology reports. Twenty leiomyosarcomas from Hospital de la Santa Creu i Sant Pau, Barcelona, which were also investigated for Ki67, p53, p16, and Bcl-2 and had been previously reported [6], were excluded from this study.

Microscopically, the tumors were classified according to the 2003 WHO classification system [5]. The diagnosis of leiomyosarcoma was based on the following features: infiltrating border, hypercellularity, moderate to severe nuclear atypia, high mitotic rate generally exceeding 10 mitotic figures (MF)/10 high power field (HPF), and/or tumor necrosis (coagulative necrosis). The diagnosis was established when the tumor had two or more of the following features: mitotic index ≥ 10/10 HPFs, moderate/severe cytologic atypia, and/or tumor necrosis [4]. Tumors were originally staged using the 1988 FIGO staging system designed for carcinomas of the uterine corpus. However, for the purpose of this study, we converted the original staging into the new 2009 FIGO staging system specifically constructed for uterine sarcomas [14].

Mitotic activity was assessed by counting MFs in four sets of 10 HPFs (HPF = 0.196 mm^2) in the most cellular areas. The highest mitotic index, defined as the highest count in any one set, was recorded. The degree of nuclear atypia was determined on the basis of nuclear enlargement, pleomorphism, and hyperchromasia. Atypia was considered absent or mild if it was similar to that of a leiomyoma, and severe if it was obvious at low-power magnification (×40). Clinical endpoint was defined as dead of tumor (disease specific survival).

Tissue microarrays

Paraffin blocks were available in all 84 tumors. Areas showing tumor elements were selected on H&E slides and marked on the corresponding paraffin blocks. Two representative 0.6-mm tissue cores were obtained from each selected zone. Tissue cores were precisely arrayed in a paraffin block using a tissue microarray (TMA) workstation (Beecher Instruments, Silver Spring, MD) as previously described [26]. An H&E-stained section was made to confirm the presence of the original areas selected from each tumor. Subsequently, serial-sectioned slides were obtained. Each TMA slide allowed the analysis of 30 samples at a time, minimizing variation during the staining process.

Immunohistochemistry

The TMAs were immunostained for 5 selected proteins involved in cell proliferation (Ki67, p53, p16), apoptosis (Bcl-2), and macrophage differentiation (CD163). The antibody clone names, sources, dilutions, and antigen pretreatment are listed in Table 1. TMAs were sectioned at 4 μm and mounted on charged slides, deparaffinized in xylene, and rehydrated through a graded alcohol series to distilled water. Endogenous peroxidase activity was blocked and heat-induced antigen retrieval was carried out by immersion of the sections in sodium-citrate buffer (0.01 M sodium-citrate monohydrate, pH 6.0) or ethylene diamine tetra acetate buffer (pH 8.0) and incubation in an autoclave (Matachana, Barcelona, Spain) at 120 °C for 10 minutes. Immunohistochemical stainings were performed using the Dako Autostainer (Dako Cytomation, Carpinteria, CA). The slides were incubated with the primary antibodies using optimized protocols. The peroxidase-labeled polymer was applied for 30 minutes at room temperature. The detection system used was EnVision (Dako, Glostrup, Denmark) with diaminobenzidine as the chromogen. The slides were subsequently washed in water, counterstained with hematoxylin, dehydrated, and mounted. Adequate immunoreactive tissue samples were used as positive controls for each antibody. Negative controls were produced by omission of the primary antibodies.

Interpretation and scoring of immunohistochemical preparations

For p53, positive immunoreaction was defined as any nuclear staining in ≥ 50% of tumor cells. Positivity for p16 was restricted to tumors exhibiting a positive cytoplasmic and/or nuclear

Table. 1
Primary antibodies, dilutions, and antigen retrieval method used.

Antibody	Clone	Dilution	Antigen retrieval	Vendor
Bcl-2	100/D5 (124)	1:1	low PH	Master Diagnostica
CD163	10/D6	1:100	high PH	Novocastra
Ki-67	MIB-1	1:1	low PH	Dako
p16	INK4a	1:1	high PH	MTM CINtec
p53	BP53-12-1	1:150	low PH	Biogenex

immunoreaction in ≥ 50% of tumor cells. Positivity for Bcl-2 was considered in cases showing cytoplasmic immunoreaction in ≥ 50% of tumor cells. Positive Ki67 immunoreaction was defined as nuclear staining occurring in more than 10% of tumor cells. The criteria for positive immunoreactions are based on previous studies [19]. A tumor infiltrating macrophage identified by CD163 was defined as a cell with abundant cytoplasm with dendritic pattern, and a round or oval nucleus. Quantification for CD163 immunostaining was done as follows: score 0 (sparse infiltrates) for <25 positively stained macrophages per 0.6 mm tumor core, and score 1 (dense infiltrates) for ≥ 25 positively stained macrophages per 0.6 mm tumor core.

Statistical analysis

The Kaplan–Meier method was used to calculate disease specific survival, defined as time beginning from the date of diagnosis until date of death. The following variables were introduced: age, tumor size, nuclear atypia, tumor necrosis, mitotic count, and expression of biomarkers p53, p16, Ki67, Bcl-2, and CD163. Factors that appeared to affect survival on the basis of univariate analysis were considered for the multivariate Cox proportional hazards model. They included: tumor size, mitotic index, and expression of Ki67 and Bcl-2. For all analyses, $p<0.05$ was considered statistically significant. Data were stored and analyzed using the SPSS 18.0 statistical software (Chicago, IL). Hierarchical clustering analysis of the immunohistochemical results were performed using Deconvoluter 6 and TMA-Combiner 7 programs [27,28]. An agglomerative method (average linkage) was used.

Results

Clinical and operative findings

The clinicopathologic features are summarized in Table 2. The patients were 29 to 67 years of age (mean: 51 years; median: 53) at the time of diagnosis. Of the 77 leiomyosarcomas with staging information available, 8 were stage IA, 63 stage IB, 2 stage IIIA, 1 stage IIIB, 1 stage IVA, and 2 stage IVB. The initial treatment was known in all 84 patients: 4 underwent tumorectomy with preservation of the uterus, and 80 had total abdominal hysterectomy, with bilateral salpingo-oophorectomy in 68. Eighteen patients received chemotherapy (doxorubicin/epidoxorubicin and ifosfamide, or docetaxel/gemcitabine) and 13 had radiation therapy.

Table 2
Clinicopathologic features of 84 leiomyosarcomas.

Age (years)	51 (29–67)
Size (cm)	11 (3–35)
Stage	
IA	8/77 (10%)
IB	63/77 (82%)
IIIA	2/77 (3%)
IIIB	1/77 (1%)
IVA	1/77 (1%)
IVB	2/77 (3%)
Mitoses	
5–19	52/84 (62%)
≥ 20	32/84 (38%)
Nuclear atypia	
Low	20/84 (24%)
Moderate	22/84 (26%)
Severe	42/84 (50%)
Tumor necrosis	39/84 (46%)
Treatment	
TAH-BSO	68/84 (81%)
TAH	12/84 (14%)
Resection	4/84 (5%)
Chemotherapy	18/84 (21%)
Radiotherapy	13/84 (15%)
Mean follow-up (years)	3 (0.2-8)
Recurrences	54/84 (64%)
Outcome	
DOT	21/82 (26%)
AWD	39/82 (47%)
NED	22/82 (27%)

AWD: alive with disease; BSO, bilateral salpingo-oophorectomy; DOT: dead of tumor; NED: no evidence of disease; TAH, total abdominal hysterectomy.

Pathologic findings

Most leiomyosarcomas were large (mean tumor diameter: 11 cm; median: 12.5; range: 3 to 35 cm), solitary masses with a fleshy consistency. The cut surfaces were described as having a variegated appearance ranging from tan to grey with foci of hemorrhage and necrosis. Microscopically the tumors showed intersecting bundles of spindle-shaped cells with abundant fibrillary eosinophilic cytoplasm. The hyperchromatic nuclei were fusiform, had rounded ends, and contained coarse chromatin and prominent nucleoli. Nuclear atypia was severe in 42 cases, moderate in 22 and mild in 20. In 52 tumors (52/84; 62%) mitotic count varied from 5 to 19/10 HPF (only two tumors had less than 10/10 HPF), and in 32 (32/84; 38%) was equal or more than 20/10 HPF. The maximum number of mitoses was 47 per 10 HPF. Atypical mitoses were seen. Tumor necrosis was identified in 39 leiomyosarcomas (46%).

Follow-up

Follow-up (disease specific survival), ranging from 3 months to 8 years (average 3 years; median 3.5 years), was available in 82 cases. Fifty-four patients (64%) developed metastases (timing unknown) and in 7 cases the primary uterine tumor measured 5 cm or less. Site of pelvic metastases included peritoneum, retroperitoneum, cul-de-sac, ovaries, mesosalpynx, and bladder. The most common site of distant metastases was the lung (37 cases; bilateral in 14). Other sites included large and small bowel (9 cases), liver (8 cases), kidney, breast, brain, and bone. Of the 84 patients, 21 (25%) died of tumor (1 stage IA, 17 stage IB, 1 stage IVA, 2 stage IVB), 39 were alive with tumor (1 stage IA, 28 stage IB, 2 stage IIIA, 1 stage IIIB), and 22 patients were alive without evidence of disease (all with stage I tumors).

Relationship of clinicopathologic parameters with survival

Both tumor size and mitotic count were statistically significant findings of prognostic value by univariate ($p=0.018$ and $p=0.003$) and multivariate ($p=0.006$ and $p=0.001$) analyses. Tumors size equal or larger than 10 cm in diameter and mitotic index equal or higher than 20 MF/10 HPF were associated with poor prognosis. All other factors including age of the patient, tumor stage, tumor necrosis, nuclear atypia, and postoperative treatment did not show any significant correlation with survival.

Immunohistochemical results

Ki67, p53, p16, Bcl-2, and CD163

The immunohistochemical results are shown in Table 3. A positive Ki67 nuclear immunoreaction was observed in 52 of 84 (62%) uterine leiomyosarcomas. A positive p53 nuclear immunoreaction was found in 19 of 84 (23%) cases. Positive p16 nuclear and/or cytoplasmic immunoreaction was present in 60 of 84 (71%) tumors. Bcl-2

Table 3
Immunohistochemical analysis of 84 leiomyosarcomas.

Ki67	p53	p16	Bcl-2	CD163
52/84 (62%)	19/84 (23%)	60/84 (71%)	30/84 (36%)	40/84 (48%)

Immunoreactive cases for Ki67, p53, p16, Bcl-2, and CD163.

immunoreaction (defined as cytoplasmic staining) was present in 30 (30/84; 36%) cases. Nearly all leiomyosarcomas contained CD163-positive macrophages in their stroma. Forty of 84 (48%) showed a dense macrophage infiltration (score 1).

Association of Ki67, p53, p16, Bcl-2, and CD163 with survival

By univariate analysis, uterine leiomyosarcomas exhibiting Ki67 immunoreaction were associated with poor prognosis ($p = 0.01$). P53, p16, and CD163 however, did not show any significant correlation with survival. On the other hand, Bcl-2 immunoreaction tended to be associated with a favorable outcome ($p = 0.1$).

Hierarchical clustering analysis

Tumor size, mitotic index, Ki67, and Bcl-2 correlated better with survival and were selected for hierarchical clustering analysis (Fig. 1A). Using the four variables, two groups of uterine leiomyosarcomas with different prognosis were identified ($p = 0.001$) (Fig. 1B): group 1 ($n = 38$) consisted mostly of smaller leiomyosarcomas (< 10 cm in size) with lower mitotic index (< 20 MF/10 HPF), negative Ki67, and positive or negative Bcl-2 immunostainings (Fig. 2A). These tumors were associated with favorable prognosis (5-year survival: 80%). In contrast, group 2 tumors ($n = 44$) were mostly ≥ 10 cm in diameter with higher mitotic index (≥ 20 MF/10 HPF), positive for Ki67, and negative for Bcl-2 (Fig. 2B). These tumors were associated with poor prognosis (5-year survival: 40%). Furthermore, group 2 leiomyosarcomas showed higher number of CD163-macrophages than group 1 leiomyosarcomas ($p = 0.007$) (Figs. 1A and 2C).

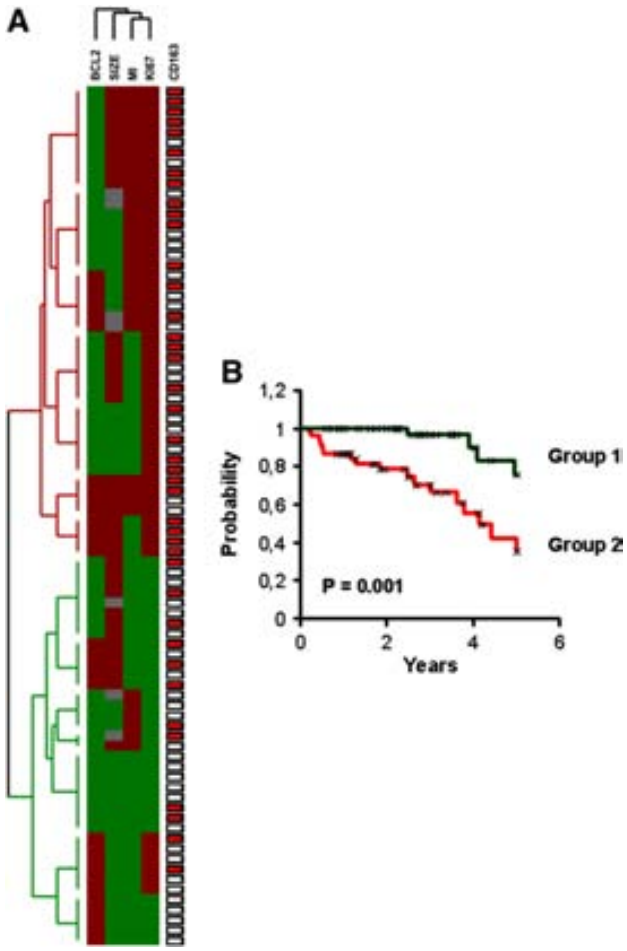

Fig. 1. A. Unsupervised hierarchical clustering analysis of 82 leiomyosarcomas. In the heat map, each row represents a tumor and each column a single parameter. For tumor size and mitotic index, red indicates ≥ 10 cm and ≥ 20 MF/10 HPF, respectively; green indicates <10 cm and <20 MF/10HPF. For Ki67 and Bcl-2, red indicates higher-than-average expression and green indicates lack of expression. In both cases, grey indicates missing data. The dendrogram shows the proximity of samples. Enclosed in the clustering image, CD163 immunoreaction is graphically represented for each case. B. Kaplan–Meier's survival analysis for leiomyosarcomas. The difference in survival between the two groups was statistically significant ($p = 0.001$).

Discussion

The prognosis of uterine sarcomas varies considerably according to their histologic type. Whereas low-grade endometrial stromal sarcomas are commonly associated with a favorable outcome, and undifferentiated sarcomas usually have an adverse prognosis, the behavior of leiomyosarcomas is less predictable [9-13,29-31]. Even if tumor stage is thought to be an important prognostic factor, tumors apparently confined to the uterus (stage I) still develop recurrence and metastasis [6,9]. In our series, the 5-year overall survival for patients with stage I leiomyosarcoma was only 53%. On the other hand, we have recently reported some bona fide stage I leiomyosarcomas that were associated with prolonged survival [6].

In spite of their different nature, uterine leiomyosarcomas had been staged until recently according to the criteria applied for endometrial carcinomas. In 2008, however, a new FIGO classification and staging system was elaborated for uterine sarcomas which are now staged independently [14]. In the new system, myometrial invasion and cervical involvement are replaced by tumor size. Tumor size has been found to be of prognostic value in stage I disease and both 5 cm and 10 cm thresholds have been proposed [9,11,32-34] even if rare cases of metastasizing leiomyosarcomas less than 5 cm in diameter have occurred. For risk stratification, a recent analysis of 819 stage I leiomyosarcomas revealed that tumor size is better than myometrial invasion (5-year overall survival in stage IA [5 cm or less] and IB [more than 5 cm] were 76.6% vs. 48.4%, $p = 0.001$) [35]. In our study, tumor size (less or more than 10 cm; all stages included) was found to be of prognostic value both by univariate and multivariate analyses; yet 7 of the 54 leiomyosarcomas that metastasized measured 5 cm or less and 1 had an aggressive behavior. The patient was a 51-year-old woman whose tumor was only 4 cm in diameter, but showed severe cytologic atypia and 19 MF/10 HPF. She died with vaginal recurrence in 2 months.

Other pathologic features thought to be prognostically relevant include nuclear atypia, mitotic activity, tumor necrosis (or *coagulative* necrosis), and vascular space invasion. Even if most of these features are required for the diagnosis of leiomyosarcoma and, thus, are found in all cases, their quantitative variations have been analyzed in an attempt to predict prognosis [5].

Does low-grade leiomyosarcoma ever occur? Histologic grade has not been consistently identified as a significant prognostic parameter in leiomyosarcoma and there is no universally accepted grading system. In fact, application of more restrictive diagnostic criteria (WHO, 2003) has allowed the exclusion of unusual histologic variants of leiomyoma frequently misdiagnosed as well-differentiated or low-grade leiomyosarcomas in the past. Consequently, it has become apparent that most leiomyosarcomas are high grade tumors. Nevertheless, two recent investigations including 208 and 1396 leiomyosarcomas, reported that high nuclear atypia had an adverse effect on survival [11,29], thus implying that low-grade leiomyosarcomas are associated with more favorable prognosis. In our series, however, nuclear atypia did not show any significant correlation with survival. Indeed, six of 20 patients with leiomyosarcomas exhibiting only mild to moderate atypia, which had been classified as low-grade or well-differentiated leiomyosarcomas, died of tumor. On the other hand, our results do not support that tumor necrosis (coagulative

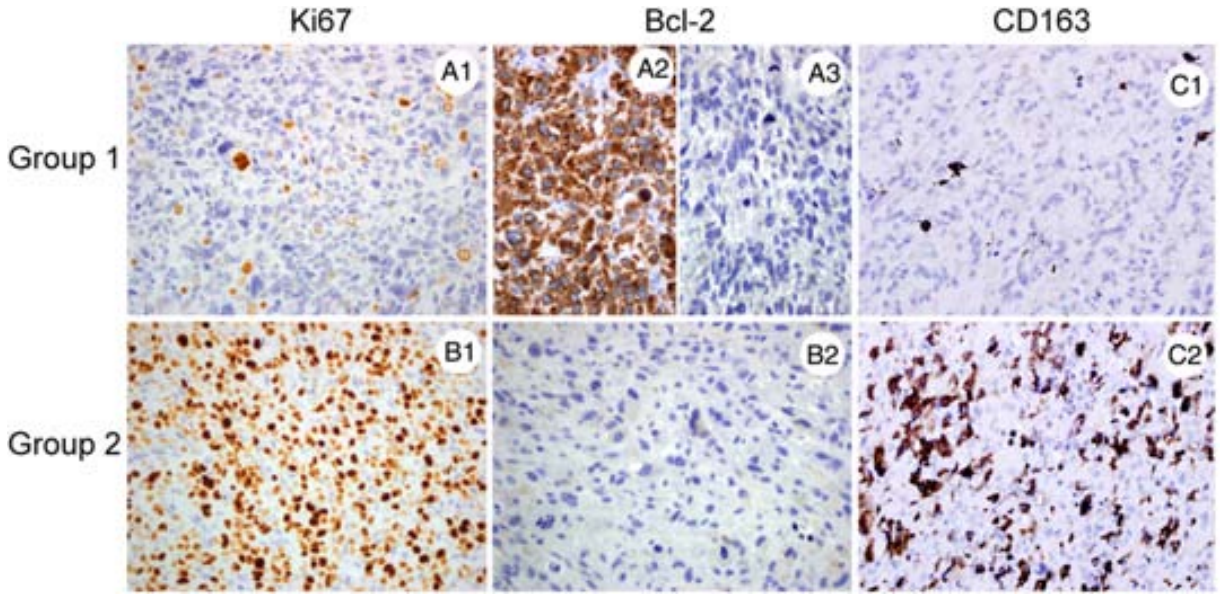

Fig. 2. Immunoreactions for Ki67, Bcl-2, and CD163.

necrosis) is by itself an indicator of malignancy. In our series, only 46% of leiomyosarcomas had tumor necrosis and, yet, 31 of 45 (69%) leiomyosarcomas lacking tumor necrosis developed metastases. Even if vascular invasion (which was not available in our series) has been reported in 10–30% of cases [6,30,34,36] its presence is often difficult to prove.

The prognostic value of the mitotic index is also controversial. In a recent study [9] of 245 leiomyosarcomas confined to the uterus, tumor size and mitotic index were the only significant prognostic factors on multivariate analysis and allowed stratification of patients into three risk groups with marked differences in prognosis. Also on multivariate analysis, another study of 78 uterine leiomyosarcomas [37] showed that mitotic index was, after stage, the second best predictor of prognosis. However, a third study of 71 uterine sarcomas [30] failed to demonstrate a relationship between mitotic index and survival. In our series, high mitotic count was prognostically significant both by univariate and multivariate analyses. Reasons for such discrepancies may include differences in handling of the surgical specimens, thickness of sections, size of the HPF, and the interpretation of mitotic figures.

Thus, even if most leiomyosarcomas are thought to be high-grade tumors by morphologic analysis, rare low-grade cases capable of recurrence and metastasis do occur. On the other hand, the outcome of patients with high-grade leiomyosarcoma is not uniform. Thus, we can conclude that evaluation of conventional pathologic parameters is less than optimal for predicting prognosis and the use of biomarkers is indicated.

Expression of Ki67, p53, p16, and Bcl-2 has been used in leiomyosarcomas trying to predict outcome [6,38]. However, it is not clear whether they act independently of clinicopathological parameters, particularly stage. Recently, we reported that 3 of 15 stage I leiomyosarcomas that exhibited the morphologic features of malignancy (moderate to severe nuclear atypia, 6–20 MF/10 HPF, and tumor necrosis) and showed a strong immunoreaction for Bcl-2, were associated with prolonged survival suggesting that Bcl-2 might possibly be involved in the inhibition of tumor progression or spread [6,38]. Noteworthy, 8 of the 15 patients with Bcl-2-negative tumors died of disease. To validate these results, as well as to explore the utility of additional biomarkers we have studied the expression of 4 selected proteins involved in cell proliferation (Ki67, p53, p16) and apoptosis (Bcl-2) in a series of 84 leiomyosarcomas. Univariate analysis revealed that, except for Ki67, none of the markers per se had any influence on disease specific survival. However, combination of tumor size, mitotic index, Ki67, and Bcl-2, identified two groups of leiomyosarcomas with different prognosis.

We have recently shown that colony-stimulating factor-1 expression by leiomyosarcoma cells and stromal macrophage (CD163) infiltrates are both features associated with poor prognosis in leiomyosarcomas [25]. In the current study, tumors exhibiting large size, high mitotic count, and strong Ki67 immunoreaction often had a dense CD163 macrophage infiltration.

In summary, our study confirms the prognostic significance of tumor size and mitotic index as morphologic predictors of malignancy in uterine leiomyosarcomas, regardless of the presence of tumor necrosis. We have also shown that the only prognostically significant biomarker for leiomyosarcomas is Ki67. Nevertheless, combination of tumor size, mitotic index, Bcl-2 and Ki67 immunoreactions helps to identify leiomyosarcomas with different outcome. This prognostic model seems to be more accurate than traditional staging systems because it accounts for heterogeneity in tumor histology and clinical features.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

Acknowledgments

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MANUSCRIPT 3

RESULTS

Endometrial Stromal Sarcomas With Sex Cord Differentiation Are Associated With *PHF1* Rearrangement

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Abstract: Endometrial stromal tumors may pose diagnostic challenges particularly when they exhibit variant histologic appearances, involve extrauterine sites, or present as metastatic disease. In such cases, use of immunohistochemical markers and identification of specific nonrandom chromosomal rearrangements may be helpful. Over the last decade, fluorescence in situ hybridization (FISH) has been progressively incorporated as a diagnostic tool for the evaluation of endometrial stromal tumors. The purpose of this study was to review a series of these tumors and compare the results of FISH analysis with the clinicopathologic characteristics. Three endometrial stromal nodules (ESNs), 13 endometrial stromal sarcomas (ESSs), and 7 undifferentiated endometrial sarcomas (UESs) were reviewed. Three metastases from 1 of the ESS cases were also analyzed. Nine of these tumors (1 ESN, 8 ESSs, and 1 UES) exhibited unusual histologic features, including smooth muscle (3), sex cord (7), epithelioid (1), fibromyxoid (1), and skeletal muscle (2) differentiation. A tissue microarray was prepared, and FISH analysis was performed using break-apart and fusion probes for *JAZF1, SUZ12, EPC1,* and *PHF1* genes. FISH was successful in 22 cases, and rearrangements involving *JAZF1, SUZ12, EPC1,* and *PHF1* genes were detected in 10 of the 22 (45%) uterine tumors, including 2 of the 3 ESNs and 8 of 12 ESSs. Genetic rearrangements were found neither in the 3 metastases of the ESS nor in any of the UESs. It is noteworthy that a correlation between sex cord differentiation and *PHF1* rearrangement was encountered in ESSs ($P = 0.008$). In our series, all ESSs showing sex cords had *PHF1* genetic rearrangement, suggesting that such rearrangements may induce sex cord differentiation.

Key Words: endometrial stromal nodules, endometrial stromal sarcomas, undifferentiated endometrial sarcomas, sex cord, FISH, *JAZF1*, *SUZ12*, *EPC1*, *PHF1*

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Endometrial stromal tumors (ESTs) are the second most common pure mesenchymal tumors of the uterus, even though they account for < 10% of these tumors. According to the World Health Organization classification, the term EST is applied to neoplasms typically composed of uniform cells resembling the stromal cells of the proliferative endometrium.[1] They are predominantly or exclusively intramural neoplasms and are divided into benign and malignant categories on the basis of the type of tumor margin. Well circumscribed tumors are benign stromal nodules, whereas those exhibiting myometrial invasion and, typically, finger-like permeation of lymphovascular spaces are designated as stromal sarcomas.[1]

Traditionally, endometrial stromal sarcomas (ESSs) were subdivided into low-grade and high-grade forms on the basis of the mitotic count—that is, < 10 mitotic figures (MF)/10 high-power fields (HPF) and 10 or more MF/10 HPF, respectively.[2] Whereas low-grade ESSs were originally described as clinically indolent tumors compatible with long survival even if extrauterine spread had occurred,[3–6] patients with high-grade ESSs usually developed widespread metastases and died of tumor within 2 or 3 years.[7–11] Later, it was claimed that many of the tumors reported as "high-grade ESSs" were in fact poorly differentiated uterine sarcomas composed of pleomorphic cells that bear no resemblance to endometrial stromal cells and were similar to the sarcomatous component of a malignant mixed mullerian tumor.[12] Thus, the classification proposed by the World Health Organization in 2003[1] restricts the term ESS to low-grade (nuclear grade 1) tumors resembling endometrial stromal cells, regardless of their mitotic index (MI).[13] Tumors exhibiting nuclear grade 2 or 3 (formerly high-grade ESSs) are now classified as undifferentiated endometrial sarcomas (UESs).

Besides ESTs with classic morphologic features, several histologic variants of these tumors have recently

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been described—that is, ESTs may contain endometrial-type glands in up to 40% of cases and sex cord–like structures in 15% to 20% of cases; moreover, fibromyxoid, or smooth muscle differentiation (spindle or epithelioid), or cells with ambiguous differentiation between stromal and smooth muscle cells may develop in these neoplasms. Rarely, skeletal muscle cells may also be found in ESTs.[14]

The diagnosis of histologic variants of ESS is not always straightforward. Although immunohistochemistry may be useful in the distinction of ESTs from highly cellular leiomyomas (ie, markers of differentiation, such as h-caldesmon and CD10),[15] it is less useful for the identification of endometrial stromal variants in problematic cases. ESTs with sex cord elements merge almost imperceptibly with uterine tumors resembling ovarian sex cord tumors (UTROSCTs) and together represent the most common uterine neoplasms showing sex cord–like features; however, this tumor variant can be misinterpreted as epithelioid leiomyosarcoma, and in such cases a molecular marker specific for ESTs is needed.

Over the last decade, specific molecular genetic alterations have been identified in ESTs. For example, endometrial stromal nodules (ESNs) and low-grade ESSs often carry chromosomal rearrangements involving JAZF1 and members of the polycomb complex gene (*SUZ12, PHF1, EPC1*) most frequently resulting from a chromosomal translocation t(7;17).[16,17] Recently, a novel genetic fusion YWHAE-FAM22A/B resulting from translocation t(10; 17) (q22; p13) has been described in a subset of ESSs, which are histologically of higher grade and clinically more aggressive than the JAZF1-rearranged ESS.[18,19] These tumors, which show only modest endometrial stromal differentiation, lack the nuclear pleomorphism of undifferentiated sarcomas and most likely represent the old "high-grade ESSs."

We investigated the frequency of rearrangements involving *JAZF1, SUZ12, EPC1,* and *PHF1* in a series of ESTs including cases with classic morphologic features and histologic variants.

MATERIALS AND METHODS

Case Selection

Twenty-six ESTs, diagnosed from 1988 to 2010, were collected from the archives of the Department of Pathology of the Hospital de la Santa Creu i Sant Pau, Barcelona (6 cases) and the consultation files of one of the authors (J.P.; 20 cases). The cases included 3 ESNs, 13 ESSs, 3 metastases from one of the ESSs, and 7 UESs (3 with uniform and 4 with pleomorphic nuclei). Histologic variants, including smooth muscle (3), sex cord (7), epithelioid (1), fibromyxoid (1), and skeletal muscle (2) differentiation, were encountered in 1 ESN, 7 ESSs, and 1 UES. Clinical and pathologic information regarding the patient's age, clinical symptoms, tumor size, gross features, stage, treatment, and follow-up were obtained from hospital charts and pathology reports.

The term EST was applied to neoplasms typically composed of cells that resemble endometrial stromal cells of the proliferative endometrium.[20] They were classified into noninvasive (stromal nodules) and invasive (stromal sarcomas). ESSs exhibited no or only mild nuclear atypia and characteristically invaded the myometrium and lymphovascular spaces. An arborizing vascular pattern was found in all cases. No tumor cell necrosis was seen. The diagnosis of UES was applied to cases that lacked smooth muscle or endometrial stromal differentiation and exhibited destructive myometrial invasion, moderate to severe nuclear atypia, high mitotic activity, and/or tumor cell necrosis.

Mitotic activity was assessed by counting MFs in 4 sets of 10 HPF (HPF = 0.196 mm^2) in the most cellular areas. The highest MI, defined as the highest count in any 1 set, was recorded. The degree of nuclear atypia was determined on the basis of nuclear enlargement, pleomorphism, and hyperchromasia. Atypia was considered absent or mild if it was similar to that of a low-grade ESS and severe if it was obvious at low-power magnification ($\times 40$).

The tumors were staged by the criteria proposed recently by the International Federation of Gynecology and Obstetrics for uterine sarcomas.[21] Recurrent tumor was defined as a tumor found at an interval after the apparently complete removal of an adequately staged tumor.

Tissue Microarrays

Paraffin blocks were available in all 26 tumors. Areas showing tumor elements were selected on hematoxylin and eosin slides and marked on the corresponding paraffin blocks. Two representative 0.6-mm-thick tissue cores were obtained from each selected zone. Tissue cores were precisely arrayed in a paraffin block using a tissue microarray (TMA) workstation (Beecher Instruments, Silver Spring, MD) as previously described.[22] A hematoxylin and eosin-stained section was used to confirm the presence of the original areas selected from each tumor. Subsequently, serial-sectioned slides were obtained. Each TMA slide allowed the analysis of 30 samples at a time, minimizing variation during the staining process.

Fluorescence In Situ Hybridization

Bacterial artificial chromosome (BAC) clones obtained using the University of California Santa Cruz genome browser to design fluorescence in situ hybridization (FISH) probes flanking known translocation break points in *JAZF1, SUZ12, PHF1,* and *EPC1* were selected. The following BAC clones (Children's Hospital Oakland Research Institute, Oakland, CA) were obtained: RP11-597H8, RP11-78F4, RP11-466B23, RP11-945M23, RP11-55J8, RP11-299H3, RP11-398A1, RP11-112D12, RP11-94D23, RP11-242N19, RP11-908F2, RP11-983E11, RP11-108P17, RP11-104F5, and RP11-73A23. For each gene, up to 2 probes were used on either side of the break point to increase the strength of the FISH signals. BAC clones were grown on lysogeny broth agar supplemented

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with 12.5 mg/mL chloramphenicol. DNA was isolated from bacteria using a rapid alkaline lysis miniprep method and labeled with a nick translation kit (Vysis, Downer's Grove, IL) with Spectrum Orange-11-dUTP or Spectrum Green-11-dUTP. We performed 2-color FISH on 6-mm-thick TMA sections by first using 4 break-apart probe assays to detect rearrangements of *JAZF1, SUZ12, PHF1,* and *EPC1* genes and subsequently using 3 fusion probe assays to detect JAZF1-SUZ12, JAZF1-PHF1, and EPC1-PHF1 gene fusions. Cells were counterstained with 4′,6-diamidino-2-phenylindole. In each tissue core containing at least 200 tumor nuclei, FISH signals were analyzed and scored in 100 tumor diploid nuclei in all break-apart and fusion assays as previously described. Foci of nuclear crowding and overlap were avoided. In all break-apart assays, "positive" for gene rearrangement was defined as unpaired signals observed in $\geq 30\%$ of tumor nuclei; "equivocal" was defined as unpaired signals in ≥ 10 and $< 30\%$ of tumor nuclei; and "negative" was defined a unpaired signals in $< 10\%$ of tumor nuclei. For fusion assays, only cases that were "equivocal" or "positive" for gene rearrangement by break-apart FISH were evaluated. "Positive" for gene fusion was defined as paired signals observed in $\geq 50\%$ of nuclei, whereas "negative" for gene fusion was defined as paired signals in $< 50\%$ of nuclei.

Statistical Analysis

Qualitative variables were examined using the Pearson χ^2 test. Disease-free survival and overall survival were defined as time from the date of diagnosis until the date of recurrence and death, respectively. Actuarial survival rates were calculated according to the product-limit method of Kaplan and Meier and compared using the log rank test. For all analyses, $P < 0.05$ was considered statistically significant. Data were stored and analyzed using the SPSS 18.0 statistical software (Chicago, IL).

RESULTS

Clinical and Pathologic Findings

The clinicopathologic features of all cases are summarized in Table 1.

Endometrial Stromal Nodules

The patients were 29 to 41 years of age (mean: 35 y) at the time of diagnosis. All 3 patients were asymptomatic; 2 underwent tumorectomy, and the other had a hysterectomy. The tumors were grossly and microscopically well circumscribed without vascular invasion. Histologically, the tumor cells resembled endometrial stromal cells and lacked significant nuclear atypia and mitotic activity. In addition, 1 nodule contained intermixed bundles of well-differentiated smooth muscle, isolated skeletal muscle cells, and sex cord–like structures. None of the patients with ESN presented local recurrences or metastases.

Endometrial Stromal Sarcomas

Thirteen primary uterine tumors and 3 metastases from one of the cases were studied. The patients were 19 to 65 years of age (mean: 44 y) at the time of diagnosis. Presenting manifestations included abdominal pain or discomfort (10 cases), irregular vaginal bleeding (11 cases), and abdominal distension (4 cases). Two patients were asymptomatic. Treatment was known in all patients: although initially 3 underwent tumorectomy, eventually all 13 had total abdominal hysterectomy, 8 of them with bilateral salpingo-oophorectomy. Postoperatively, 2 patients received anthracycline-based chemotherapy (doxorubicin/epidoxorubicin and ifosfamide), and one of them also had radiation therapy. One patient received hormonal therapy. Eight patients presented with stage I tumor (1 IA, and 7 IB), whereas 3 patients had stages IIB, 1 IIIB, and 1 IIIC tumors involving ovaries, peritoneum, and para-aortic lymph nodes.

Grossly, the ESSs appeared as solitary, nodular, and predominantly intramural masses with a mean diameter of 14 cm (range: 4 to 50 cm). Their cut surfaces were described as fleshy, bulging, and tan to yellow. There was extensive permeation of the myometrium in the form of numerous tongue-like projections. Histologically, the tumors were densely cellular and showed uniform, oval to spindle cells of endometrial stromal type. Nuclear atypia was only mild. A rich network of delicate small arterioles resembling those of the late secretory endometrium was seen in all cases. Clear lymphovascular space invasion was seen in all tumors. Occasionally, scattered nests of foam cells were encountered. Sex cord–like differentiation was found in 6 tumors and smooth muscle in 2. Tumor cell necrosis was absent. Mitotic count varied from 1 to 8/10 HPF (mean mitotic count, 4/10 HPF).

Three metastatic tumors, all from the same patient, were resected from the pelvic and abdominal cavity 2, 34, and 36 months after the initial surgery. Compared with the classic primary tumor, the metastases contained epithelioid tumor cells that exhibited higher nuclear atypicality and mitotic activity.

Undifferentiated Endometrial Sarcomas

The patients were 46 to 85 years of age (mean: 58 y) at the time of diagnosis. Presenting manifestations included irregular vaginal bleeding (7 cases) and abdominal distension (5 cases). All 7 patients had total abdominal hysterectomy with bilateral salpingo-oophorectomy. Four patients presented with stage IB tumor, 2 with stage IIB, and 1 with stage IIIC. Three patients received anthracycline-based chemotherapy (doxorubicin/epidoxorubicin and ifosfamide) and radiation therapy.

Grossly, the UESs had a mean diameter of 18 cm (range: 8 to 30 cm). Microscopically, they showed infiltrative margins, nuclear hyperchromasia, and tumor necrosis. Whereas 4 of the 7 tumors showed marked nuclear pleomorphism, the other 3 exhibited nuclear uniformity and had foci reminiscent of low-grade ESS. The mitotic count varied from 10 to 25/10 HPF (mean: 16/10 HPF).

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TABLE 1. Clinicopathologic Features and Gene Rearrangement Status of 23 ESTs

Case	Histologic Diagnosis	Age	Size	Mitosis	Stage	Distant Metastases	Patient Status	FISH
1	ESN	35	8	0	IB	No	NED at 4 y	*JAZF1; SUZ12*
2	ESN	41	10	0	IB	No	NED at 12 y	JAZF1-SUZ12
3	ESN smooth muscle + skeletal muscle + sex cord	29	8	2	IB	No	NED at 20 y	Negative
4	ESS classic	47	7	1	IB	Yes	NED at 11 y	Nonconclusive
5	ESS classic	42	8	2	IB	Yes	NED at 12 y	*JAZF1*
6	ESS classic	19	3.5	5	IA	Yes	AWT at 4 y	Negative
7	ESS classic	41	11	3	IIIB	No	AWT at 6 y	Negative
8	ESS classic	40	12	5	IIB	No	AWT at 5.5 y	Negative
9	ESS classic + epithelioid	39	15	8	IIB	Yes	DOC at 11 y	Negative
10	ESS sex cord	49	9	5	IIB	Yes	AWT at 6.2 y	*PHF1*
11	ESS sex cord	53	50	3	IB	No	NED at 6 y	*PHF1; EPC1*
12	ESS sex cord	56	25	4	IB	No	NED at 4.5 y	*PHF1*
13	ESS sex cord	48	7	5	IIIC	Yes	AWT at 6 y	*PHF1*
14	ESS sex cord + fibromyxoid	65	8.2	4	IB	No	NED at 5 y	*PHF1*
15	ESS sex cord + smooth muscle	34	8	2	IB	No	NED at 12 y	*JAZF1; PHF1;* JAZF1-PHF1
16	ESS smooth muscle	44	15	3	IB	No	NED at 12 y	*PHF1*
17	UES uniform	55	14	15	IIB	Yes	AWT at 4 y	Negative
18	UES uniform	54	22	22	IB	No	NED at 3 y	Negative
19	UES uniform	46	29	13	IIB	Yes	AWT at 9 y	Negative
20	UES pleomorphic	84	11	19	IB	Yes	DOT at 0.9 y	Negative
21	UES pleomorphic	65	8	20	IB	Yes	DOT at 0.1 y	Negative
22	UES pleomorphic	48	20	15	IIIC	Yes	DOT at 0.2 y	Negative
23	UES pleomorphic + skeletal muscle	53	9	7	IB	Yes	DOT at 0.6 y	Negative

AWT indicates alive with tumor; DOC, died of other causes; DOT, died of tumor; NED, no evidence of disease.

One case showed skeletal muscle differentiation, and 5 showed lymphovascular space invasion.

Follow-up

Information on follow-up from 1 month to 20 years (average: 6.8 y) was available in all patients (Table 1). Of the 13 patients with ESS, 7 (all stage IB) were alive without evidence of disease with a mean follow-up of 8 years; 5 [stages IA, IIB (2), IIIB, and IIIC] were alive with tumor at 4, 5.5, 6.2, 6, and 6 years, respectively; and 1 (stage IIB) died of septic shock, after 5 pelvic recurrences, 11 years after diagnosis. Four of the 7 patients with UES (1 stage IIIC and 3 stage IB) died within 1 year with widespread tumor, 2 (stage IIB) are alive with tumor at 4 and 9 years, and the other (stage IB) is clinically free of disease 3 years postoperatively. Local and distal recurrences occurred in 6 of 13 ESSs and 6 of 7 UESs and involved lung (4), peritoneum (8), and para-aortic lymph nodes (2).

Relationship of Clinicopathologic Parameters With Survival

Clinicopathologic parameters, including the patient's age, tumor stage, tumor size, histologic type, mitotic count, tumor necrosis, nuclear atypia, and adjuvant treatment, did not show any significant correlation with survival; however, all 4 patients with UES showing marked nuclear pleomorphism died of tumor in < 1 year. In contrast, the other 3 patients who had tumors exhibiting nuclear uniformity and focal resemblance to endometrial stroma had a better outcome; 2 of these patients, who had stage IIB tumors, are alive with disease at 4 and 9 years, and the other is clinically free of tumor 3 years postoperatively. There was a significant difference in overall survival between patients with UESs (median: 1 y) and patients with ESSs (median: 11 y) ($P = 0.02$).

FISH Analysis

The results of FISH analysis for all cases are shown in Table 1. FISH was successful in 25 of 26 samples (85%). Rearrangements involving *JAZF1, SUZ12, EPC1,* and *PHF1* genes were detected in 10 tumors, including 2/3 ESNs and 8/13 ESSs. Rearrangements were found neither in the metastatic ESS nor in any of the UESs. JAZF1-SUZ12 gene fusion was found in 1 ESN, whereas JAZF1-PHF1 gene fusion was encountered in 1 ESS. In addition, a variety of genetic rearrangements with unknown partners were identified: *PHF1* gene rearrangements were found in 7 ESSs (6 with sex cord–like and 1 with smooth muscle differentiation); gene rearrangements of *JAZF1* were found in 2 ESNs and 2 ESSs, one classic and the other with smooth muscle component; gene rearrangements of *SUZ12* were found in 2 ESNs; and *EPC1* gene rearrangement was found in a single ESS with sex cord–like differentiation. A correlation between sex cord differentiation and specific gene rearrangements of *PHF1* was found (confidence coefficient = 0.591; $P = 0.008$). None of the 7 UESs showed evidence for the investigated genetic rearrangements. No significant differences in patient outcome or other clinicopathologic features were found between FISH-positive and FISH-negative cases.

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FISH analysis was crucial for establishing the correct diagnosis in one of the ESSs with sex cord–like and fibromyxoid differentiation, which had been interpreted initially as leiomyosarcoma with epithelioid and myxoid features (Figs. 1A, B) (Table 1, case 14). The tumor cells were medium sized with central nuclei and abundant clear to eosinophilic cytoplasm; there was only moderate nuclear atypia, and 4 to 5 MF/10 HPF (Fig. 1C). The tumor showed a strong immunoreaction for caldesmon (Fig. 1D) and CD10 (Fig. 1E); however, the Ki67 index was low (Fig. 1F), and the immunoreaction for p53 was negative. FISH analysis revealed *PHF1* rearrangement. The tumor was reclassified as ESS with sex cord differentiation, and the patient is alive and well 5 years postoperatively.

FISH was also determinant for the diagnosis in a second case of ESS containing both sex cord–like and smooth muscle elements (Figs. 2A, B) (Table 1, case 15). Whereas the smooth muscle component showed a starburst appearance with central hyalinization and blunt radiations, the sex cord–like foci suggested a UTROSCT. In this histologically complex case, the value of immunohistochemistry was limited as the tumor cells reacted for both desmin and CD10. However, break-apart FISH assays revealed *JAZF1* (Fig. 2C) and *PHF1* rearrangements. In addition, JAZF1-PHF1 fusion (Fig. 2D) was identified by FISH. These findings confirmed the endometrial stromal nature of the neoplasm. The patient is alive and well 12 years postoperatively.

DISCUSSION

ESTs are rare mesenchymal neoplasms difficult to diagnose when exhibiting an unusual morphology, such as fibromyxoid, epithelioid, sex cord, smooth muscle, and/or skeletal muscle differentiation. Recently, specific genetic alterations—rearrangements and gene fusions—have been identified in these tumors. To determine the

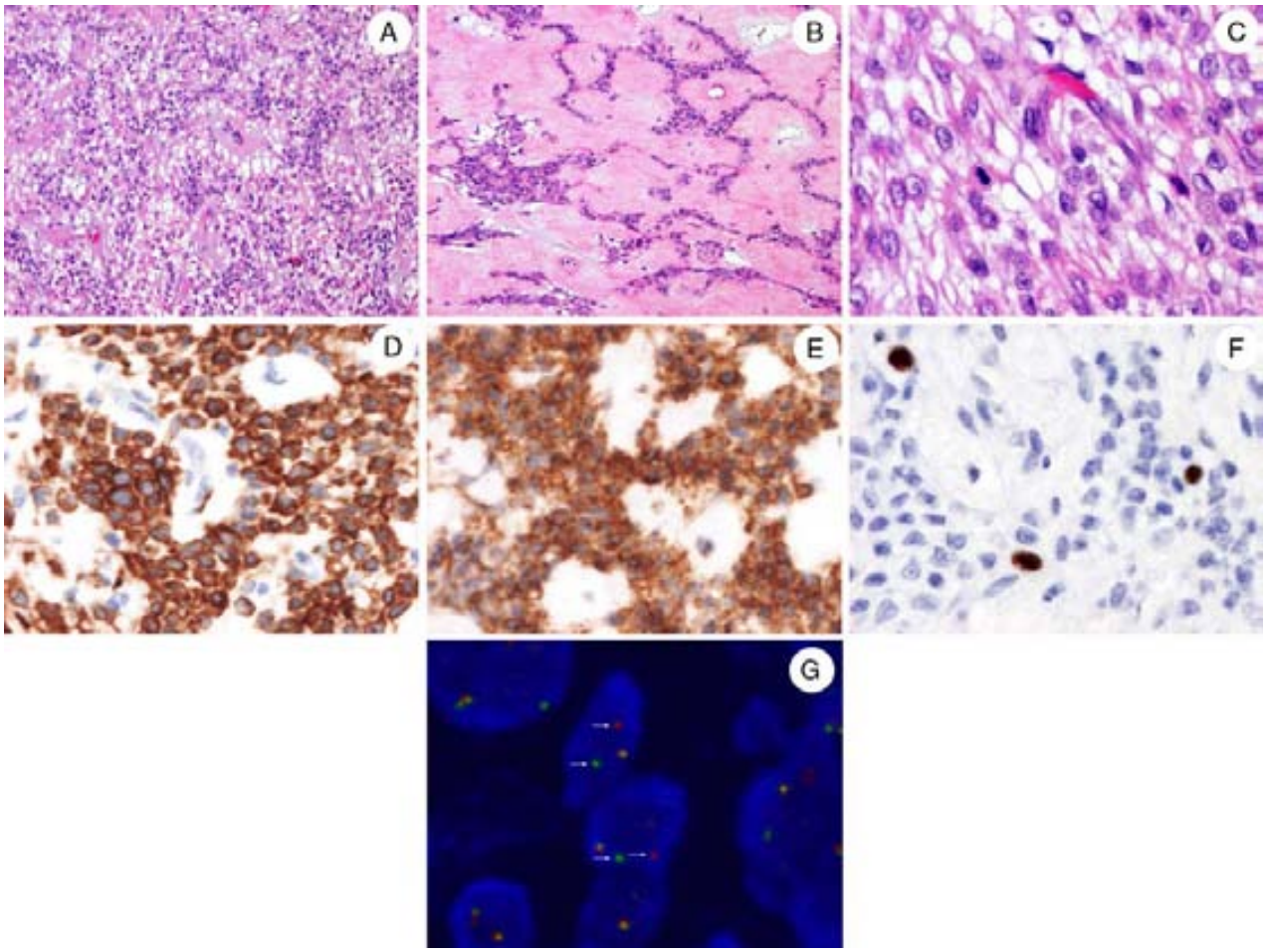

FIGURE 1. ESS with sex cord–like and fibromyxoid differentiation. A, The medium-sized tumor cells are distributed in anastomosing cords and show round to oval nuclei with eosinophilic cytoplasms. The cords are intersected by fibromyxoid stroma. B, Elongated cords of tumor cells separated by hyalinized fibrous stroma. C, The tumor cells exhibit only mild nuclear atypia. An MF is seen. D, Strong caldesmon immunoreaction. E, Strong CD10 immunoreaction. F, Low Ki67 index. G, PHF1 rearrangement by break-apart FISH assay (green centromeric and red telomeric PHF1 probes). The presence of separate green and red signals indicates rearrangement in this region (arrows).

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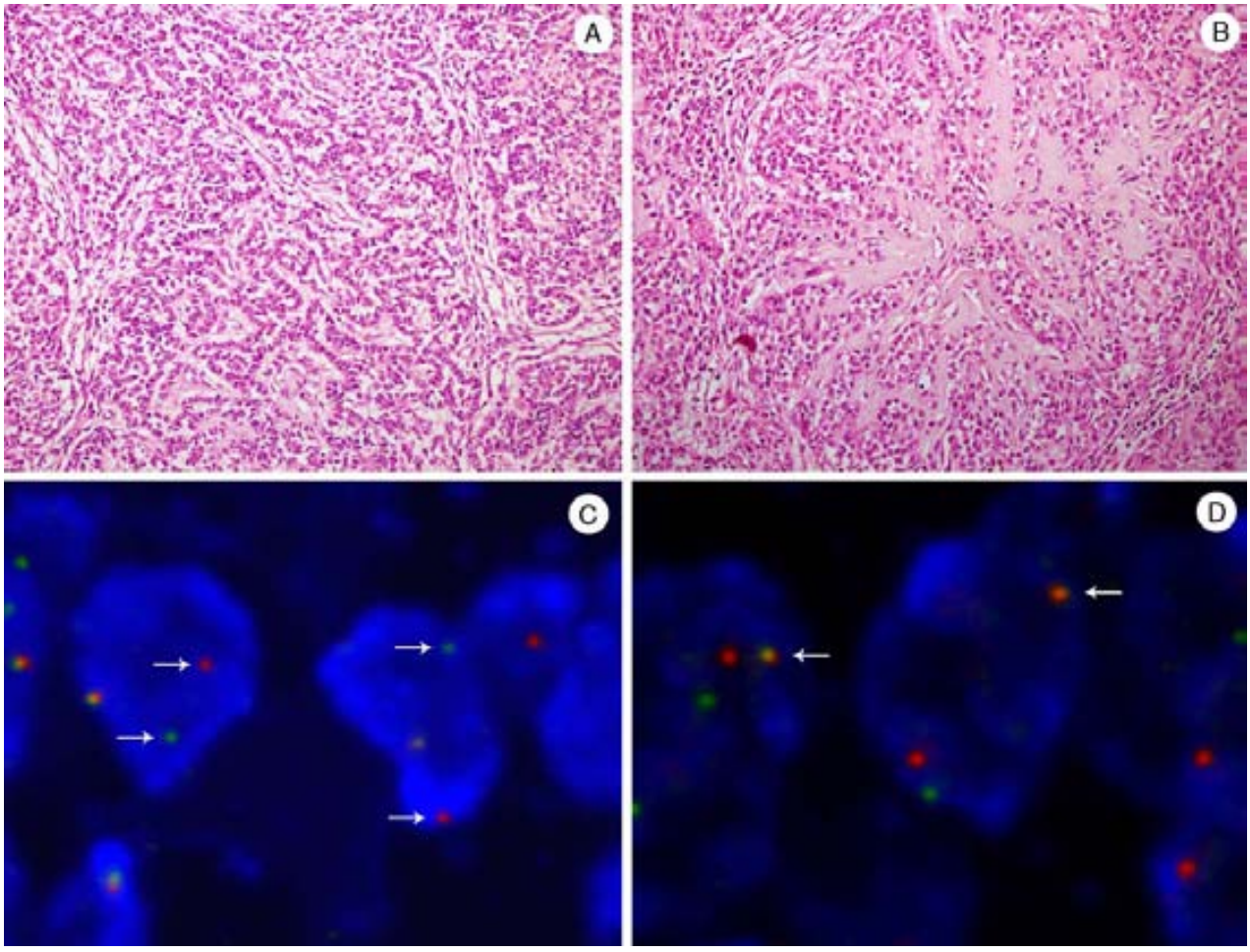

FIGURE 2. ESS with sex cord (A) and smooth muscle (starburst) (B) differentiation. C, JAZF1 rearrangement by break-apart FISH assay (green telomeric and red centromeric JAZF1 probes). The presence of separate green and red signals indicates rearrangement in this region (arrows). D, JAZF1-PHF1 gene fusion confirmed by fusion FISH assay (red centromeric JAZF1 and green centromeric PHF1 probes). The presence of adjacent green and red signals indicates fusion of both genes (arrows).

utility of FISH analysis as a diagnostic tool in difficult cases, we investigated the frequency of chromosomal rearrangements in a series of 23 ESTs, including ESNs, ESSs, and UESs, 10 of them with a variant histology. Our study showed gene rearrangements in nearly half of the uterine ESTs, with *PHF1* rearrangement being the most common. We found *PHF1* rearrangement in 54% (7 of 13) ESSs, *JAZF1* in 66% (2 of 3) ESNs and 17% (2 of 12) ESSs, and *EPC1* in 8% (1 of 13) ESSs. No rearrangements were detected in UESs.

Cytogenetic studies of ESTs have recognized a number of reciprocal translocations that correlate with specific tumor types. The presence of the t(7;17) translocation and JAZF1-SUZ12 gene fusion has been described in a number of classic-type ESTs and less often in morphologic variants. This gene fusion has been found in 92% of classic ESNs and 70% of classic ESSs by FISH and reverse transcription polymerase chain reaction[16,17,23–29] but only in 56% and 15% of ESNs and ESSs morphologic variants, respectively.[17,23,24,26–28] It has been stated[17] that the presence of the t(7;17) translocation and JAZF1-SUZ12 gene fusion in most ESNs and a large number of ESSs supports that these tumors share a common genetic pathway and suggests that gene fusion is an early event in the development of ESTs. Of the 3 ESNs in our series, 2 had the *JJAZ1* rearrangement and 1 of them the JAZF1-SUZ12 gene fusion; however, only 1 of 12 ESSs and none of the 7 UESs showed the JAZF1-SUZ12 gene fusion. The low frequency of this gene fusion in the ESSs could be explained by a greater prevalence of histologic variants (mainly tumors with sex cord and smooth muscle differentiation) in our series.

Several other genetic changes have been described sporadically in ESTs, such as translocations involving the *PHF1* gene on 6p21,[30,31] the third most common chromosomal band rearranged in ESTs. The regular involvement of *PHF1*, recombined with *JAZF1* or with *EPC1*, suggests that this gene has a possible role in the development of a subset of ESTs distinct from those harboring the t(7;17) translocation. In our study, there was a

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correlation between ESSs with sex cord differentiation and the occurrence of *PHF1* rearrangement. Furthermore, the frequencies of *PHF1* rearrangements and sex cord differentiation observed in our series are higher than those documented in the literature. The high number of sex cord ESSs in our study (77%) almost certainly reflects referral bias, as most cases were seen in consultation. Overall, these observations suggest that genetic fusion involving *PHF1* may preferentially induce sex cord differentiation in ESSs.

Our findings provide additional evidence that the diagnosis of histologic variants of ESTs is not always straightforward. ESTs with sex cord elements can be misinterpreted as epithelioid or myxoid leiomyosarcomas, and the identification of rearrangements by FISH may be diagnostically and prognostically important. In fact, one of the ESSs from our series exhibited both sex cord–like and fibromyxoid differentiation as well as strong immunoreactivity for caldesmon and CD10. This tumor, which had been initially diagnosed as leiomyosarcoma with epithelioid and myxoid features, even when Ki67 index was low, was found to carry *PHF1* rearrangement and was subsequently reclassified as ESS with sex cord differentiation.

Another interesting case from our series was an ESS containing both sex cord–like and smooth muscle elements in a manner similar to the so-called UTROSCT. In this case, the value of immunohistochemistry was also limited, as the tumor cells reacted for both desmin and CD10. However, break-apart FISH assays revealed *JAZF1* and *PHF1* rearrangements, and JAZF1-PHF1 fusion was also identified. These findings confirmed the endometrial stromal nature of the neoplasm.

Our findings also confirm that UESs showing uniform nuclei represent an intermediate subcategory of ESTs associated with better outcome compared with UESs.[2] In fact, whereas all 4 patients with UESs lacking endometrial stromal features and exhibiting marked nuclear pleomorphism died of tumor within 1 year, none of the other 3 patients whose tumors focally resembled ESS and showed nuclear uniformity died of tumor. Therefore, as recently pointed out by Kurihara et al,[23,24] it appears that an intermediate group of uterine sarcomas with only modest endometrial stromal differentiation, higher MI, and greater nuclear atypia compared with low-grade ESSs but lacking the nuclear pleomorphism of undifferentiated sarcomas also occur.

Recently, a novel genetic fusion YWHAE-FAM22A/B resulting from translocation t(10;17)(q22;p13) has been described in a subset of ESSs that show fibrous, epithelioid, or round cell differentiation and high-grade histologic features such as uniform and at least moderate nuclear atypia and increased mitotic activity. These tumors are clinically more aggressive than *JAZF1*-rearranged ESS and typically show upregulation of cyclin D1.[18,19,32] Incidentally, all ESTs in our series lacked cyclin D1 immunoreactivity (data not shown). Although investigation of the genetic fusion YWHAE-FAM22A/B or translocation t(10;17)(q22;p13) was not carried out in the intermediate group of the series of Kurihara and colleagues, it is likely that some of the tumors included in this group were YWHAE-FAM22 ESSs and probably correspond to the old "high-grade ESSs." It would appear that we have come full circle, and perhaps the pathology community was too quick to abandon the term "high-grade ESS."

In summary, ancillary techniques such as detection of gene rearrangements by FISH may be diagnostically important in the diagnosis of ESTs with classic and variant morphology. All ESTs with sex cord–like histology showed evidence of *PHF1* genetic rearrangement indicating that this genetic fusion may preferentially induce sex cord differentiation in ESTs. However, the number of ESTs with sex cord differentiation in this study is too small to draw a definitive conclusion.

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DISCUSSION

The prognosis of uterine sarcomas varies considerably according to their histologic type. Whereas low-grade endometrial stromal sarcomas are commonly associated with a favorable outcome, and undifferentiated sarcomas usually have an adverse prognosis, the behavior of leiomyosarcomas is less predictable.[2,23,48-51,54,153] Even if tumor stage is thought to be an important prognostic factor, tumors apparently confined to the uterus (stage I) still develop recurrence and metastasis.[30,48] In our series, the 5-year overall survival for patients with stage I leiomyosarcoma was only 53%. On the other hand, we have recently reported some bona fide stage I leiomyosarcomas that were associated with prolonged survival.[30]

In spite of their different nature, uterine leiomyosarcomas had been staged until recently according to the criteria applied for endometrial carcinomas. In 2008, however, a new FIGO classification and staging system was elaborated for uterine sarcomas which are now staged independently.[4] In the new system, myometrial invasion and cervical involvement are replaced by tumor size. Tumor size has been found to be of prognostic value in stage I disease and both 5 cm and 10 cm thresholds have been proposed [2,48,52,53,154] even if rare cases of

metastasizing leiomyosarcomas less than 5 cm in diameter have occurred. For risk stratification, a recent analysis of 819 stage I leiomyosarcomas revealed that tumor size is better than myometrial invasion (5-year overall survival in stage IA [5 cm or less] and IB [more than 5 cm] were 76.6% vs. 48.4%, p=0.001).[155] In our study, tumor size (less or more than 10 cm; all stages included) was found to be of prognostic value both by univariate and multivariate analyses; yet 7 of the 54 leiomyosarcomas that metastasized measured 5 cm or less and 1 had an aggressive behavior. The patient was a 51-year-old woman whose tumor was only 4 cm in diameter, but showed severe cytologic atypia and 19 MF/10 HPF. She died with vaginal recurrence in 2 months.

Other pathologic features thought to be prognostically relevant include nuclear atypia, mitotic activity, tumor necrosis (or *coagulative* necrosis), and vascular space invasion. Even if most of these features are required for the diagnosis of leiomyosarcoma and, thus, are found in all cases, their quantitative variations have been analyzed in an attempt to predict prognosis.[3]

Does low-grade leiomyosarcoma ever occur? Histologic grade has not been consistently identified as a significant prognostic parameter in leiomyosarcoma and there is no universally accepted grading system. In fact, application of more restrictive diagnostic criteria (WHO, 2003) has allowed the exclusion of unusual histologic variants of leiomyoma frequently misdiagnosed as well-differentiated or low-grade leiomyosarcomas in the past. Consequently, it has become apparent that most leiomyosarcomas are high grade tumors. Nevertheless, two recent investigations including 208 and 1396 leiomyosarcomas, reported that high nuclear atypia had an adverse effect on survival[2,54], thus implying that low-grade leiomyosarcomas are associated with more favorable prognosis. In our series, however, nuclear atypia did not show any significant correlation with survival. Indeed, six of 20 patients with leiomyosarcomas exhibiting only mild to moderate atypia, which had been classified as low-grade or well-differentiated leiomyosarcomas, died of tumor. On the other hand, our results do not support that tumor necrosis (coagulative necrosis) is by itself an indicator of malignancy. In our series, only 46% of leiomyosarcomas had tumor necrosis and, yet, 31 of 45

(69%) leiomyosarcomas lacking tumor necrosis developed metastases. Even if vascular invasion (which was not available in our series) has been reported in 10-30% of cases[1,23,30,53] its presence is often difficult to prove.

The prognostic value of the mitotic index is also controversial. In a recent study[48] of 245 leiomyosarcomas confined to the uterus, tumor size and mitotic index were the only significant prognostic factors on multivariate analysis and allowed stratification of patients into three risk groups with marked differences in prognosis. Also on multivariate analysis, another study of 78 uterine leiomyosarcomas[156] showed that mitotic index was, after stage, the second best predictor of prognosis. However, a third study of 71 uterine sarcomas[23] failed to demonstrate a relationship between mitotic index and survival. In our series, high mitotic count was prognostically significant both by univariate and multivariate analyses. Reasons for such discrepancies may include differences in handling of the surgical specimens, thickness of sections, size of the HPF, and the interpretation of mitotic figures.

Thus, even if most leiomyosarcomas are thought to be high-grade tumors by morphologic analysis, rare low-grade cases capable of recurrence and metastasis do occur. On the other hand, the outcome of patients with high-grade leiomyosarcoma is not uniform. Thus, we can conclude that evaluation of conventional pathologic parameters is less than optimal for predicting prognosis and the use of biomarkers is indicated.

Expression of Ki67, p53, p16, and Bcl-2 has been used in leiomyosarcomas trying to predict outcome.[30,157] However, it is not clear whether they act independently of clinicopathological parameters, particularly stage. Recently, we reported that 3 of 15 stage I leiomyosarcomas that exhibited the morphologic features of malignancy (moderate to severe nuclear atypia, 6-20 MF/10 HPF, and tumor necrosis) and showed a strong immunoreaction for Bcl-2, were associated with prolonged survival suggesting that Bcl-2 might possibly be involved in the inhibition of tumor progression or spread.[30,157] Noteworthy, 8 of the 15 patients with Bcl-2-negative tumors died of disease. To validate these results, as well as to explore the utility of additional biomarkers we have studied the expression of

4 selected proteins involved in cell proliferation (Ki67, p53, p16) and apoptosis (Bcl-2) in a series of 84 leiomyosarcomas. Univariate analysis revealed that, except for Ki67, none of the markers per se had any influence on disease specific survival. However, combination of tumor size, mitotic index, Ki67, and Bcl-2, identified two groups of leiomyosarcomas with different prognosis.

We have recently shown that colony-stimulating factor-1 expression by leiomyosarcoma cells and stromal macrophage (CD163) infiltrates are both features associated with poor prognosis in leiomyosarcomas.[158] In the current study, tumors exhibiting large size, high mitotic count, and strong Ki67 immunoreaction, often had a dense CD163 macrophage infiltration.

In summary, our study confirms the prognostic significance of tumor size and mitotic index as morphologic predictors of malignancy in uterine leiomyosarcomas, regardless of the presence of tumor necrosis. We have also shown that the only prognostically significant biomarker for leiomyosarcomas is Ki67. Nevertheless, combination of tumor size, mitotic index, Bcl-2 and Ki67 immunoreactions helps

to identify leiomyosarcomas with different outcome. This prognostic model seems to be more accurate than traditional staging systems because it accounts for heterogeneity in tumor histology and clinical features.

Although there is universal agreement that endometrial stromal sarcomas resembling the stroma of the proliferative endometrium and showing only mild nuclear atypia are indolent tumors associated with long-term survival[67], classification and terminology of high-grade endometrial sarcomas showing significant nuclear atypia has been controversial.[119] Traditionally, endometrial stromal sarcomas were subdivided into low- and high-grade forms based on mitotic count; i.e., less than 10 mitotic figures [MF]/10 high power fields [HPF] and 10 or more MF/10 HPF, respectively.[159] Whereas low-grade endometrial stromal sarcomas were originally described as clinically indolent tumors compatible with long survival even if extrauterine spread had occurred [68,160-162], patients with high-grade endometrial stromal sarcomas usually developed widespread metastases and died of tumor within two or three years.[163-167] Later, it was claimed that many of the tumors reported as “high-grade endometrial stromal

sarcomas" were in fact poorly differentiated uterine sarcomas composed of pleomorphic cells that bear no resemblance to endometrial stromal cells and were similar to the sarcomatous component of a malignant mixed mullerian tumor (MMMT).[168] Thus, the classification proposed by the WHO in 2003[3] restricts the term endometrial stromal sarcoma to low-grade (nuclear grade 1) tumors resembling endometrial stromal cells, regardless of their mitotic index.[67] Tumors exhibiting nuclear grade 2 or 3 (formerly high-grade endometrial stromal sarcomas) are now classified as undifferentiated endometrial sarcomas.

Besides ESTs with classic morphological features, several histological variants of these tumors have recently been described; i.e., ESTs may contain endometrial type glands in up to 40% of cases and sex cord-like structures in 15-20% of cases; also, fibromyxoid, or smooth muscle differentiation (spindle or epithelioid), or cells with ambiguous differentiation between stromal and smooth muscle cells may develop in these neoplasms. Rarely, skeletal muscle cells may also be found in ESTs.[73]

The diagnosis of histologic variants of ESS is not always straightforward. Although immunohistochemistry may be useful in the distinction of ESTs from highly cellular leiomyomas (i.e., markers of differentiation, such as h-caldesmon, and CD10)[108], it is less useful for the identification of endometrial stromal variants in problematic cases. Endometrial stromal tumors with sex cord elements merge almost imperceptibly with uterine tumors resembling ovarian sex cord tumors (UTROSCTs), and together represent the most common uterine neoplasms showing sex cord-like features; however, this tumor variant can be misinterpreted as epithelioid leiomyosarcoma and, in such cases, a molecular marker specific for ESTs is needed.

Thus, ESTs are rare mesenchymal neoplasms difficult to diagnose when exhibiting an unusual morphology, such as fibromyxoid, epithelioid, sex cord, smooth-muscle, and/or skeletal muscle differentiation. Recently, specific genetic alterations –rearrangements and gene fusions- have been identified in these tumors. To determine the utility of FISH analysis as a diagnostic tool in difficult cases, we investigated the frequency of chromosomal rearrangements in a series of 23 ESTs, including ESNs, ESSs, and UESs, 10 of them with a variant histology. Our study showed gene rearrangements in nearly half of uterine ESTs, with PHF1 rearrangement being the most common. We found PHF1 rearrangement in 54% (7 of 13) ESSs, JAZF1 in 66% (2 of 3) ESNs and 17% (2 of 12) ESSs; and EPC1 in 8% (1 of 13) ESSs. No rearrangements were detected in UESs.

Cytogenetic studies of endometrial stromal tumors have recognized a number of reciprocal translocations that correlate with specific tumor types. The presence of the t(7;17) translocation and JAZF1- SUZ12 gene fusion has been described in a number of classic-type ESTs and less often in morphologic variants. This gene fusion has been found in 92% of classic ESNs and 70% of classic ESSs by FISH and RT-PCR[78,119,117,121,125,128,130,131,169] but only in 56% and 15% of ESNs and ESSs morphologic variants, respectively.[78,117,119,130,131,169] It has been stated[169] that the presence of the t(7;17) translocation and JAZF1- SUZ12 gene fusion in most ESNs and a large number of ESSs supports that these tumors share a common genetic pathway and suggest that gene fusion is an early event in the development of ESTs. Of the 3 ESNs in our series, 2 had the JJAZ1 rearrangement and 1 of them the JAZF1-SUZ12 gene fusion; however, only 1 of 12 ESS and none of the 7 UESs showed the JAZF1- SUZ12 gene fusion. The low frequency of this gene fusion in the ESSs could be explained by a greater prevalence of histologic variants (mainly tumors with sex cord and smooth muscle differentiation) in our series.

Several other genetic changes have been described sporadically in EST, such as translocations involving the PHF1 gene on 6p21[126,135], the third most common chromosomal band rearranged in ESTs. The regular involvement of PHF1, recombined with JAZF1 or with EPC1, suggests that this gene has a possible role in the development of a subset of ESTs distinct from those harboring the t(7;17) translocation. In our study, there was a correlation between ESSs with sex cord differentiation and the occurrence of PHF1 rearrangement. Furthermore, the

frequencies of PHF1 rearrangements and sex cord differentiation observed in our series are higher than those documented in the literature. The high number of sex cord ESSs in our study (77%) almost certainly reflects referral bias since most cases were seen in consultation. Overall, these observations suggest that genetic fusion involving PHF1 may preferentially induced sex cord differentiation in ESSs.

Our findings provide additional evidence that the diagnosis of histologic variants of ESTs is not always straightforward. Endometrial stromal tumors with sex cord elements can be misinterpreted as epithelioid or myxoid leiomyosarcomas and the identification of rearrangements by FISH may be diagnostically and prognostically important. In fact, one of the ESSs from our series exhibited both sex cord-like and fibromyxoid differentiation as well as strong immunoreactivity for caldesmon and CD10. This tumor, which had been initially diagnosed as leiomyosarcoma with epithelioid and myxoid features, even if Ki67 index was low, was found to carry PHF1 rearrangement and subsequently reclassified as ESS with sex cord differentiation.

Another interesting case from our series was an endometrial stromal sarcoma containing both sex cord-like and smooth muscle elements in a manner similar to the so-called uterine tumor resembling ovarian sex cord tumor (UTROSCT). In this case, the value of immunohistochemistry was also limited since the tumor cells reacted for both, desmin and CD10. However, breakapart FISH assays

revealed JAZF1 and PHF1 rearrangements and JAZF1-PHF1 fusion was also identified. These findings confirmed the endometrial stromal nature of the neoplasm.

Our findings also confirm that undifferentiated endometrial sarcomas showing uniform nuclei represent an intermediate subcategory of endometrial stromal tumors associated with better outcome than undifferentiated endometrial sarcomas.[159] In fact, whereas all 4 patients with undifferentiated endometrial sarcomas lacking endometrial stromal features and exhibiting marked nuclear pleomorphism died of tumor within 1 year, none of the other 3 patients whose tumors focally resembled endometrial stromal sarcoma and showed nuclear uniformity died of tumor. Therefore, as recently pointed out by Kurihara et al.[117,119], it seems that an intermediate group of uterine sarcomas with only modest endometrial stromal differentiation, higher mitotic rate, and greater nuclear atypia than low-grade endometrial stromal sarcomas but lacking the nuclear pleomorphism of undifferentiated sarcomas also occur.

Recently, a novel genetic fusion YWHAE-FAM22A/B resulting from translocation t(10;17)(q22;p13) has been described in a subset of ESSs that show fibrous, epithelioid, or round cell differentiation and high-grade histologic features such as uniform and at least moderate nuclear atypia and increased mitotic activity. These tumors are

clinically more aggressive than JAZF1-rearranged ESS and typically show upregulation of cyclin D1.[99,137,170] Incidentally, all ESTs in our series lacked cyclin D1 immunoreactivity (data not shown). Although investigation of the genetic fusion YWHAE-FAM22A/B or translocation t(10;17)(q22;p13) was not done in the intermediate group of the series of Kurihara et al., it is likely that some of the tumors included in this group were YWHAE-FAM22 ESSs and probably correspond to the old “high-grade endometrial stromal sarcomas”. It would appear that we have come full circle, and perhaps the pathology community was too quick to abandon the term “high-grade endometrial stromal sarcoma”

In summary, ancillary techniques such as detection of gene rearrangements by FISH may be diagnostically important in the diagnosis of ESTs with classic and variant morphology. All ESTs with sex cord-like histology showed evidence of PHF1 genetic rearrangement indicating that this genetic fusion may preferentially induce sex cord differentiation in ESTs. However, the number of ESTs with sex cord differentiation in this study is too small to draw a definitive conclusion.

CONCLUSIONS

CONCLUSIONS

1. Most uterine sarcomas are high-grade leiomyosarcomas exhibiting atypia, high mitotic index, and tumor necrosis.
2. Uterine sarcomas of all types are associated with poor prognosis as indicated by their high frequency of recurrence and metastasis.
3. Uterine sarcomas are aggressive neoplasms, even if treated surgically at an early stage (Stage I).
4. In leiomyosarcomas, tumor size over 10 cm and MI higher then 20/10 HPF are the most important pathological prognostic factors, regardless of the presence of tumor necrosis.
5. Immunohistochemical study including P16, P53, Ki67, and bcl-2 revealed that only Ki67 per se has prognostic significance.
6. Combination of tumor size, mitotic index, Bcl-2 and Ki67 immunoreactions helps to identify leiomyosarcomas with different outcome. This prognostic model seems to be more accurate than traditional staging systems because it accounts for heterogeneity in tumor histology and clinical features.
7. In our series of ESTs there was a high frequency of ESSs with sex cord differentiation and all cases had PHF1 genetic

rearrangement suggesting that this rearrangement may preferentially induce sex cord differentiation.

8. Fluorescence in situ hybridization assay may support the diagnosis of ESTs with classic and variant morphology by demonstrating the presence of specific rearrangements.
9. Undifferentiated endometrial sarcomas with nuclear uniformity may represent an intermediate category equivalent to tumors formerly called “high-grade endometrial stromal sarcomas”.

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APPENDIX

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Original contribution

Comparative clinicopathologic and immunohistochemical analysis of uterine sarcomas diagnosed using the World Health Organization classification system☆

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Summary Uterine sarcomas are rare tumors that account for 3% to 7% of uterine cancers. Their histopathologic classification was revised by the World Health Organization (WHO) in 2003. The objectives of this study were to determine the frequency of different subtypes of uterine sarcoma applying the WHO criteria to a series of cases, compare the outcome of patients with different subtypes, and compare their immunoprofiles using a panel of immunomarkers. Thirty-four uterine sarcomas were identified for a 20-year period (1988-2008). Eighteen benign tumors of smooth muscle or endometrial stromal origin served as a comparison group. A tissue microarray was prepared and immunostaining performed for 10 selected oncoproteins involved in cell proliferation (Ki-67, P53, p16, and phosphatase and tensin homolog [PTEN]), cell differentiation (CD10, h-caldesmon, estrogen receptor, and progesterone receptor), and apoptosis (bcl-2 and Twist). Hierarchical clustering analysis of the immunohistochemical results was performed. The uterine sarcomas were classified as follows: 20 leiomyosarcomas, 9 endometrial stromal sarcomas, and 5 undifferentiated endometrial sarcomas. The outcome for patients with uterine sarcoma was poor, irrespective of histologic type, even for those with stage I tumors. Of the patients with follow-up available, 12 (67%) of 18 with leiomyosarcoma, 4 of 5 with undifferentiated sarcoma, and 4 of 7 with endometrial stromal sarcoma experienced recurrence and 8 patients with high-grade sarcomas died of tumor. In our series, most uterine sarcomas were leiomyosarcomas. Comparison was made between leiomyosarcomas that recurred and those with a favorable outcome and 3 patients with leiomyosarcoma without evidence of recurrence on long-term follow-up had tumors that were negative/low expressors of Ki-67, p53, p16, and Twist, with strong expression of bcl-2. A subset of undifferentiated endometrial sarcomas composed of cells with uniform nuclei may be a separate entity from those with nuclear anaplasia and may be related to low-grade endometrial stromal sarcomas. It may be possible to identify a subset of leiomyosarcomas with a favorable prognosis based on staining with a panel of immunomarkers for cell proliferation and apoptosis.
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1. Introduction

Uterine sarcomas are rare tumors that account for approximately 1% of female genital tract malignancies and 3% to 7% of uterine cancers [1-3]. Because of their rarity and histopathologic diversity, there is as of yet no consensus on the risk factors for poor outcome and on their optimal treatment [4].

Traditionally, uterine sarcomas were classified histologically into carcinosarcomas (malignant mixed mesodermal tumors), accounting for 40% of cases, leiomyosarcomas (40%), endometrial stromal sarcomas (15%), and undifferentiated sarcomas (5%). However, carcinosarcomas are no longer considered uterine sarcomas but metaplastic forms of endometrial carcinomas or tumors undergoing divergent differentiation [5]. Most of the reported series of pure uterine sarcomas have focused on specific histologic types, such as leiomyosarcomas [6-8] or endometrial stromal sarcomas [9], and very few comparative studies including all major clinicopathologic categories have been reported [10,11].

For the last decade, the value of nuclear atypia and mitotic activity for the diagnosis of leiomyosarcoma has been reconsidered and tumor cell necrosis has emerged as an important predictor of malignant behavior [6]. Histologic grade, however, has not been consistently identified as a significant prognostic parameter of leiomyosarcoma [7,12,13]. Although there is universal agreement that endometrial stromal sarcomas resembling the stroma of the proliferative endometrium and showing only mild nuclear atypia are indolent tumors associated with long-term survival [9], classification and terminology of high-grade endometrial sarcomas showing significant nuclear atypia has been controversial [14].

Until recently, the 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria for endometrial carcinoma were used to assign stages for uterine sarcomas. The 2008 FIGO staging system specifically designed for uterine leiomyosarcomas is shown in Table 1 [15]. In patients with leiomyosarcomas, there is almost general agreement that tumor stage is the most significant predictor for overall survival; however, high frequency of recurrence and metastasis has been reported in patients with stage I tumors making its predictive value less than optimal.

The histopathologic classification of uterine sarcomas was revised by the WHO in 2003. Application of the more restrictive diagnostic criteria allows for the exclusion of smooth muscle tumors of uncertain malignant potential (STUMP), as well as several leiomyoma variants (eg, cellular, mitotically active, and atypical leiomyomas) all often misdiagnosed as leiomyosarcomas in the past. The objectives of this study were to determine the frequency of different types of uterine sarcoma applying the WHO criteria to a series of cases and to compare the outcome of patients with different categories of these tumors. Simple morphologic evaluation of hematoxylin and eosin (H&E) sections has been equivocal in the prediction of behavior and various ancillary techniques have been used to improve prognostic accuracy [16-26]. Thus, we have performed an immunohistochemical study of several prognostic markers of cell proliferation, cell differentiation, and apoptosis, and compared results with patient survival.

Table 1 2008 FIGO staging of uterine sarcomas (leiomyosarcomas and endometrial stromal sarcomas)

Stage	Definition
I [a]	Tumor limited to uterus
IA	<5 cm
IB	>5 cm
II	Tumor extends beyond the uterus, within the pelvis
IIA	Adnexal involvement
IIB	Involvement of other pelvic tissues
III	Tumor invades abdominal tissues (not just protruding into the abdomen)
IIIA	One site
IIIB	>1 site
IIIC	Metastasis to pelvic and/or paraaortic lymph nodes
IV	Tumor invades bladder and/or rectum and/or distant metastasis
IVA	Tumor invades bladder and/or rectum
IVB	Distant metastasis

[a] Two different substaging for leiomyosarcomas/endometrial stromal sarcomas.

2. Materials and methods

2.1. Case selection

Fifty-two mesenchymal uterine tumors diagnosed from the year 1988 to 2008 were retrieved from the Surgical Pathology database of the Hospital de la Santa Creu i Sant Pau, Barcelona (27 cases) and the consultation files of one of the authors (JP; 25 cases). The cases included 20 leiomyosarcomas, 5 undifferentiated endometrial sarcomas, 12 endometrial stromal tumors (9 endometrial stromal sarcomas and 3 endometrial stromal nodules), and 15 leiomyomas (9 usual and 6 unusual variants). Diagnostically equivocal categories were not included. All slides from the original surgery were examined. The number of H&E-stained sections per tumor ranged from 1 to 35 (mean, 7.2 slides). One to 22 slides (mean, 10.6 slides) from 5 metastatic tumors of 2 patients with progressive disease were also available for examination. Clinical and pathologic information regarding patient's age, clinical symptoms, tumor size, gross features, stage, treatment, and follow-up were obtained from hospital charts and pathologic reports, or by written or spoken communication with pathologists and gynecologists. The study was approved by the Hospital de la Santa Creu i Sant Pau Institutional Review Board.

Microscopically, the tumors were classified according to the 2003 WHO classification system [27]. Tumors exhibiting smooth muscle differentiation were diagnosed as leiomyosarcomas based on the following features: infiltrating border, hypercellularity, moderate to severe nuclear atypia, high mitotic rate generally exceeding 10 mitotic figures (MF)/10 high power field (HPF), and/or tumor cell necrosis. Leiomyosarcomas were subclassified into spindle cell (usual), epithelioid, myxoid, or mixed types. The diagnosis of spindle cell leiomyosarcoma was made when the tumor had at least 2 of the following 3 features: nuclear atypia, mitotic index more than 10/10 HPF, and/or tumor cell necrosis [6,27]. A tumor was designated as epithelioid leiomyosarcoma if it showed more than 50% of epithelioid cells, a mitotic count of more than 5/10 HPF, and/or nuclear atypia or tumor cell necrosis. Large, gelatinous tumors with a prominent myxoid extracellular matrix were diagnosed as myxoid leiomyosarcomas if they showed myometrial invasion or significant nuclear atypia, more than 2 MF/10 HPF, or tumor cell necrosis [28]. Tumors exhibiting various components of similar proportions were subclassified as mixed leiomyosarcomas.

The term *endometrial stromal tumor* was applied to neoplasms typically composed of cells that resemble endometrial stromal cells of the proliferative endometrium [27]. They were classified into noninvasive (stromal nodules) and invasive (stromal sarcomas). Endometrial stromal sarcomas exhibited only mild nuclear atypia and characteristically invaded the myometrium and lymphovascular spaces. An arborizing vascular pattern was found in all cases. No tumor cell necrosis was seen. The diagnosis of undifferentiated endometrial sarcoma was applied to cases that lacked smooth muscle or endometrial stromal differentiation and exhibited myometrial invasion, severe nuclear pleomorphism, high mitotic activity, and/or tumor cell necrosis. Both, leiomyosarcomas and undifferentiated endometrial sarcomas were considered high-grade sarcomas.

Mitotic activity was assessed by counting MFs in 4 sets of 10 HPFs (HPF = 0.196 mm^2) in the most cellular areas. The highest mitotic index (MI), defined as the highest count in any one set, was recorded. The degree of nuclear atypia was determined on the basis of nuclear enlargement, pleomorphism, and hyperchromasia. Atypia was considered absent or mild if it was similar to that of a leiomyoma or a low-grade endometrial stromal sarcoma and severe if it was obvious at low-power magnification (×40).

The tumors were staged by the criteria proposed recently by the FIGO for uterine sarcomas (Table 1) [15]. A stage was assigned retrospectively on the basis of the recorded intraoperative and pathologic findings if it had not been assigned initially or the results of the initial staging were unavailable. *Recurrent tumor* was defined as tumor found at an interval after the apparently complete removal of an adequately staged tumor. *Poor outcome* was defined as either dead of tumor or alive with tumor.

2.2. Tissue microarrays

Paraffin blocks were available in all 52 primary uterine tumors and 5 metastases. As controls, we used 14 cases of endometriosis and 10 samples of normal myometrium. Areas showing tumor elements were selected on H&E slides and marked on the corresponding paraffin blocks. Two representative 0.6-mm tissue cores were obtained from each selected zone. Tissue cores were precisely arrayed in a paraffin block using a tissue microarray (TMA) workstation (Beecher Instruments, Silver Spring, MD) as previously described [29]. An H&E-stained section was made to confirm the presence of the original areas selected from each tumor. Subsequently, serial-sectioned slides were obtained. Each TMA slide allowed the analysis of 30 samples at a time, minimizing variation during the staining process.

2.3. Immunohistochemistry

The tissue microarrays were immunostained for 10 selected proteins involved in cell proliferation (Ki-67, P53p16, and phosphatase and tensin homolog [PTEN]), cell differentiation (CD10, h-caldesmon, estrogen receptor [ER], and progesterone receptor [PR]), and apoptosis (bcl-2 and Twist). The antibody clone names, sources, dilutions, and antigen pretreatment are listed in Table 2. Tissue microarrays were sectioned at 4 μm and mounted on charged slides, deparaffinized in xylene, and rehydrated through a graded alcohol series to distilled water. Endogenous peroxidase activity was blocked, and heat-induced antigen retrieval was carried out by immersion of the sections in sodium citrate buffer (0.01 mol/L sodium citrate monohydrate, pH 6.0) or ethylene diamine tetraacetate buffer (pH 8.0) and incubation in an autoclave (Matachana, Barcelona, Spain) at 120°C for 10 minutes. Immunohistochemical stainings were performed using the Dako Autostainer (DakoCytomation, Carpinteria, CA). The slides were incubated with the primary antibodies using optimized protocols. The peroxidase-labeled polymer was applied for 30 minutes at room temperature. The detection system used was EnVision (Dako, Glostrup, Denmark) with diaminobenzidine as the chromogen. The slides were subsequently washed in water, counterstained with hematoxylin, dehydrated, and mounted. Adequate immunoreactive tissue samples were used as positive controls for each antibody. Negative controls were produced by omission of the primary antibodies.

2.4. Interpretation and scoring of immunohistochemical preparations

Appropriate immunostaining patterns for each antibody (ie, membranous, cytoplasmic, or nuclear) were assessed by a semiquantitative system. Both extent (percentage of positive cells) and intensity of immunostaining were evaluated. The resulting score (H-score) was calculated by

Table 2 Primary antibodies, dilutions, and antigen retrieval method used

Antibody	Clone	Dilution	Antigen retrieval	Vendor
Bcl-2	100/D5 (124)	1:1	ACL low pH	Master Diagnostica
h-Caldesmon	h-CD1	1:50	ACL pH 9	Dako
CD10	56C6	1:50	ACL pH 9	Novocastra
Her2 neu	P	1:100	ACL low pH	Dako
c-Kit	P	1:100	ACL pH 9	Dako
Cyclin D1	Sp4	1:50	ACL pH 9	Neomarkers
EGFR	Kit K1494			Dako
ER	6F11	1:40	ACL low pH	Novocastra
HMB-45	HMB-45	1:2	ACL low pH	Dako
Ki-67	MIB-1	1:1	ACL low pH	Dako
p16	Kit K 5336		Bath 98°C 10 min	Dako
p53	BP53-12-1	1:150	ACL low PH	Biogenex
PR	16	1:200	ACL low PH	Novocastra
PTEN	6H2.1	1:50 overnight	Bath 98°C 40 min low PH	Cascade Biosciences
Twist	P	1:1000	ACL pH 9	Abcam
WT1	6F-H2	1:50	ACL low pH	Dako

Abbreviations: ACL indicates autoclave; EGFR, epidermal growth factor receptor.

multiplying the staining intensity (0 = no staining; 1 = mild staining; 2 = moderate staining; and 3 = strong staining) by the percentage of immunoreactive cells (0-100). The H-score (0-300) was obtained on the 2 tissue cores from each selected area, and the mean value was considered the final H-score. The cutoff value for positivity was based on normal tissue staining. The immunostaining was considered negative when the H-score was less than 10; weak, 100 or less; moderate, 101 to 200; and strong, 201 to 300. Median values were calculated for all variables, and in cases with dispersion of data, mean values were also obtained.

2.5. Hierarchical clustering analysis

Hierarchical clustering analysis of the immunohistochemical results were performed using Deconvoluter 6 and TMA-Combiner 7 programs [30,31].

2.6. Statistical analysis

Comparisons of numerical data (H-scores) were performed with the nonparametric Mann-Whitney test. Qualitative variables were examined using Pearson χ^2 test. Disease-free survival and overall survival were defined as time beginning from the date of diagnosis until date of recurrence or death, respectively. Actuarial survival rates were calculated according to the product-limit method of Kaplan and Meier and compared using the log-rank test. Factors that appeared to affect survival on the basis of univariate analysis were considered for the multivariate Cox proportional hazards model. For all analyses, $P < .05$ was considered statistically significant. Data were stored and analyzed using the SPSS 15.0 statistical software (SPSS Inc, Chicago, IL).

3. Results

3.1. Clinical and operative findings

The clinicopathologic features are summarized in Table 3. The patients were 19 to 85 years of age (mean, 49 years) at the time of diagnosis. The presenting manifestations included abdominal pain or discomfort (19 cases), irregular vaginal bleeding (16 cases), and abdominal distension (10 cases). Seven patients were asymptomatic. The initial treatment was known in all patients as follows: 6 underwent tumorectomy and 46 had total abdominal hysterectomy, 34 of them with bilateral salpingo-oophorectomy. Seventeen patients received anthracycline-based chemotherapy (doxorubicin/epidoxorubicin and ifosfamide), and 12 of them also had radiation therapy. One patient received hormonal therapy.

Of the 20 leiomyosarcomas, 15 were stage IB, 2 stage IIB, 1 stage IIIA, 1 stage IIIB, and 1 stage IVB. Four undifferentiated endometrial sarcomas were stage IB and the other, stage IIB. Of the 9 endometrial stromal sarcomas, 1 was stage IA, 5 stage IB, 2 stage IIB, and 1 stage IIIC.

3.2. Pathologic findings

Most leiomyosarcomas were large (mean tumor diameter, 18.5 cm; range, 7-30 cm), solitary masses with a fleshy consistency. The cut surfaces were described as displaying a variegated appearance ranging from tan to gray with foci of hemorrhage and necrosis. Microscopically, all tumors showed infiltrative margins. Fifteen tumors were leiomyosarcomas of the usual spindle cell type, 3 were predominantly myxoid, and the other 2 mixed, epithelioid and myxoid. The usual leiomyosarcomas were composed of

Table 3 Mesenchymal uterine tumors: clinicopathologic features of 52 cases

Diagnosis	No.	Histologic subtypes and features	Mean age (range) (y)	Clinical symptoms (no. of pts)	Mean size (range) (cm)	Tumor cell necrosis	Lymphovascular invasion	Nuclear atypia	Mean mitotic count/10 HPFs (range)	FIGO stage	Treatment	Recurrence	Follow-up
Leiomyosarcoma	20	Spindle, 15 Myxoid, 3 Epithel-myxoid, 2	59.6 (37-85)	AUB, 9 AP, 7 AD, 11	18.5 (7-30)	19/20	6/15	Moderate, 8 Severe, 12	15.7 (10-30) Myxoid LMS 3.75 (1-5)	IB, 15 IIB, 2 IIIA, 1 IIIB, 1 IVB, 1	TAH-BSO, 16 TAH, 3 Resection, 1 Chemo, 10 Rad, 7 BrachyTx, 2	11/16	DOT, 8 AWT, 4 NED, 5 DOC, 1 LFU, 2
Undifferentiated endometrial sarcoma	5	Rhabdoid, sex cords, 1	61 (53-84)	AUB, 2 AP, 3	14.15 (8-22)	5/5	2/3	Severe, 3 Moderate, 2	16 (7-22)	IB, 4 IIB, 1	TAH-BSO, 5 Chemo, 2 Rad, 2		DOT, 3 AWT, 1 NED, 1
Endometrial stromal sarcoma	9	Sex cords, 3	40.6 (19-49)	AUB, 3 AP, 4 AD, 1 None, 1	7.7 (4-15)	0/9	9/9	Mild, 9	3.7 (1-8)	IA, 1 IB, 5 IIB, 2 IIIC, 1	TAH-BSO, 5 TAH, 4 Chemo, 3 Rad, 1 BrachyTx, 1	4/6	AWT, 3 NED, 3 LFU, 2 DOC, 1
Endometrial stromal nodule	3		35 (29-41)	AUB, 1 AP, 1 None, 1	8.6 (8-10)	0/3	0/3	None, 3	0.6 (0-2)		TAH, 1 Resection, 2		NED, 2 LFU, 1
Leiomyoma	15	Cellular, 3 Epithel, 1 Myxoid, 1 Atypical, 1 Usual, 9	42.9 (23-57)	AUB, 1 AP, 4 None, 11	7 (1-21)	0/15	0/15	None, 10 Mild, 1 Moderate, 4	1 (1-5)		TAH-BSO, 8 TAH, 4 Resection, 3		NED, 15

Abbreviations: AD indicates abdominal distension; AP, abdominal pain; AUB, abnormal uterine bleeding; AWT, alive with tumor; BrachyTx, brachytherapy; BSO, bilateral salpingo-oophorectomy; chemo, chemotherapy; DOC, died of other causes; DOT, died of tumor; epithel, epithelioid; FIGO, International Federation of Gynecology and Obstetrics; hormon, hormonal therapy; LFU, lost to follow-up; LMS, leiomyosarcoma; pts, patients; NED, no evidence of disease; rad, radiation therapy; RC, recent case; TAH, total abdominal hysterectomy.

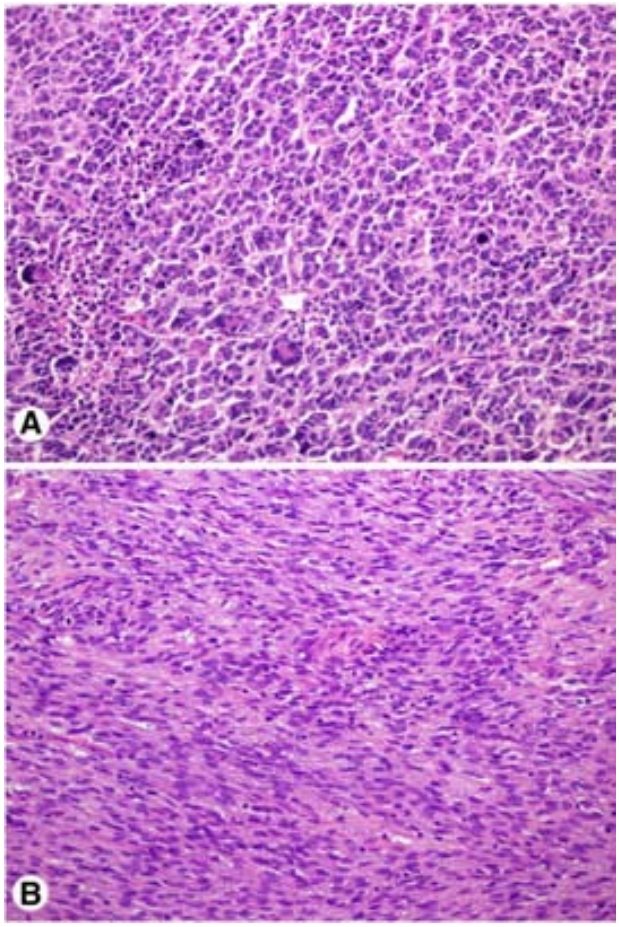


Fig. 1 Undifferentiated endometrial sarcomas showing marked nuclear pleomorphism (A) and nuclear uniformity (B).

intersecting bundles of spindle-shaped cells with abundant fibrillary eosinophilic cytoplasm. The hyperchromatic nuclei were fusiform, had rounded ends, and contained coarse chromatin and prominent nucleoli. In the myxoid leiomyosarcomas, the smooth muscle cells were widely separated by myxoid material, and the tumors appeared hypocellular. Leiomyosarcomas with an epithelioid component showed rounded polygonal epithelial-like cells. Overall, nuclear atypia was severe in 12 cases and moderate in 8. Mitotic count varied from 10 to 30/10 HPFs (mean, 15.7/10 HPFs). In the myxoid leiomyosarcomas, mitotic activity varied from low (mean, 3.75/10 HPFs), in hypocellular areas, to 4 to 7 MF/10 HPF in more cellular fields. Tumor cell necrosis was identified in 19 leiomyosarcomas including all 3 myxoid tumors and 1 of the 2 mixed epithelioid and myxoid leiomyosarcomas. Vascular invasion was found in only 6 cases (30%).

The undifferentiated sarcomas had a mean diameter of 14.15 cm (range, 8-22 cm). Microscopically, they showed infiltrative margins, nuclear hyperchromasia, and tumor necrosis. Whereas 3 of the 5 tumors exhibited marked nuclear pleomorphism, the other 2 showed nuclear uniformity and had foci reminiscent of low-grade endometrial stromal sarcoma (Fig. 1). The mitotic count varied from 7 to 22/10 HPF (mean, 16/10 HPF). One case showed sex cordlike elements, 1 had rhabdoid differentiation, and 2 showed lymphovascular invasion.

The endometrial stromal sarcomas appeared as solitary, nodular, and predominantly intramural masses with a mean diameter of 7.7 cm (range, 4-15 cm). Their cut surfaces were described as fleshy, bulging, and tan to yellow. There was extensive permeation of the myometrium in the form of numerous tonguelike projections. Histologically, the tumors were densely cellular and showed uniform, oval to spindled cells of endometrial stromal type. Nuclear atypia was only mild. A rich network of delicate small arterioles resembling those of the late secretory endometrium was seen in all cases. Clear space lymphovascular invasion was seen in all cases. Occasionally, scattered nests of foam cells were encountered. Sex cordlike differentiation was found in 4 tumors and smooth muscle in 2. Tumor cell necrosis was absent. Mitotic count varied from 1 to 8/10 HPF (mean mitotic count, 3.7/10 HPF). The 3 endometrial stromal nodules were grossly and microscopically well circumscribed without vascular invasion. The tumor cells lacked significant nuclear atypia and mitotic activity.

The uterine leiomyomas were intramural tumors. They were typically solid and well-circumscribed masses ranging from 1 to 21 cm (mean diameter, 7.0 cm). The sectioned surfaces of 9 tumors showed the white-gray color and whorled appearance typical of leiomyomas; however, in the remaining 6 tumors, the cut surfaces were described as yellow-tan and one was focally hemorrhagic. Microscopically, the former tumors were usual leiomyomas, whereas the latter were leiomyoma variants, including 3 cellular, 1 myxoid, 1 epithelioid, and 1 atypical (pleomorphic) leiomyoma.

3.3. Follow-up

Follow-up from 1 month to 12 years (average, 3.7 years) was available in 47 patients (Table 3). Of the 18 patients with leiomyosarcomas and follow-up available, 8 died of tumor (3 stage IB, 2 stage IIB, 1 stage IIIA, 1 stage IIIB, and 1 stage IVB), 4 were alive with tumor (all stage IB), 1 died of squamous cell carcinoma of cervix, and 2 patients who had been observed for less than 1 year are clinically free of tumor; only 3 patients are alive without evidence of disease (all with stage IB tumors) at 8, 8, and 3 years postoperatively (Table 4). Of the 5 patients with undifferentiated endometrial sarcomas (all stage IB), 3 died of tumor within 1 year; one (stage IIB) is alive with tumor at 9 years, and the other (stage IB) is clinically free of disease 1 year postoperatively. Of the 7 patients with endometrial stromal sarcomas and follow-up available, 3 (all stage IB) were alive without evidence of disease at 8 months, 5.0 years, and 5.0 years; 3 (stages IA, IIB, and IIIC) were alive with tumor at 5.5, 6.2, and 6 years, respectively; and 1 (stage IIB) died of septic shock, after 5 pelvic recurrences, 11 years after diagnosis.

Table 4 Clinicopathologic and immunohistochemical features of 3 leiomyosarcomas with favorable outcome

Case No.	Age (y)	Size (cm)	Stage	Nuclear atypia	Tumor necrosis	Mitoses MF x10HPF	Treatment	Follow-up	p16 (H-score)	p53 (H-score)	Ki 67 (H-score)	Twist (H-score)	Bcl-2 (H-score)
17	73	13	IB	Moderate	+	15	TAH-BSO Chemotherapy	NED, 8 y	5	3	5	35	160
7	47	25	IB	Severe	+	6	TAH-BSO Chemotherapy Pelvic irradiation	NED, 8 y	10	0	10	8	270
14	65	23	IB	Severe	+	20	TAH-BSO Pelvic irradiation	NED, 3 y	0	0	15	2	100

Eleven patients with leiomyosarcomas developed metastases that were synchronous with the primary uterine tumors in 3 cases and metachronous in the other 8. The latter were found from 1 month to 8 years after the initial diagnosis. Sites of pelvic metastases included peritoneum, retroperitoneum, cul-de-sac, ovaries, mesosalpynx, and bladder. The most common site of distant metastases was the lung (4 cases). Other sites included large and small bowel, liver, brain, and bone. Average disease-free survival for leiomyosarcomas was 2 years. The shortest interval occurred in a patient, 37 years of age, who had a myxoid leiomyosarcoma, developed peritoneal spread, and died of tumor 1 month postoperatively.

Of the 5 patients with undifferentiated endometrial sarcomas, one whose tumor had rhabdoid features developed distant metastases to lymph nodes, lung, brain, and bone 8 months postoperatively. Another patient, whose tumor had extended to the adnexae and pelvic wall, developed abdominal and pelvic recurrences postoperatively at 6 and 9 years, respectively. A patient with endometrial stromal sarcoma who had paraaortic lymph node metastases at the time of diagnosis developed recurrences in the abdominal peritoneum at 5 and 6 years postoperatively. Of the other 5 patients with endometrial stromal sarcoma and available follow-up, 3 developed metastases from 3 months to 4 years after the initial diagnosis.

3.4. Relationship of clinicopathologic parameters with survival

Tumor stage and patient's age were the only statistically significant parameters of prognostic value for leiomyosarcoma by univariate analysis (P = .05 and P = .00, respectively). Tumor stage was also confirmed as a significant prognostic indicator by multivariate analysis (P = .05). However, 7 (54%) of 13 stage IB leiomyosarcomas recurred, and 4 patients died of tumor. In patients with high-grade sarcomas, lymphovascular invasion, significantly influenced disease-free survival by univariate analysis (P = .03) (multivariate analysis, P = .06). All other factors including tumor size, histologic type, mitotic count, tumor cell necrosis, and nuclear atypia did not show any significant correlation with survival. However, all 3 patients with stage IB undifferentiated endometrial sarcoma showing marked nuclear pleomorphism died of tumor in less than 1 year. In contrast, the other 2 patients, who had tumors exhibiting nuclear uniformity and focal resemblance to endometrial stroma had better outcome; one of them, who had a stage IIB tumor, is alive with disease at 9 years and the other (stage IB) is clinically free of tumor 2 year postoperatively. There was a significant difference in overall survival between patients with high-grade sarcomas (leiomyosarcomas and undifferentiated endometrial sarcomas) (median, 1 year) and patients with endometrial stromal sarcomas (median, 11 years) (P = .02) (Fig. 2).

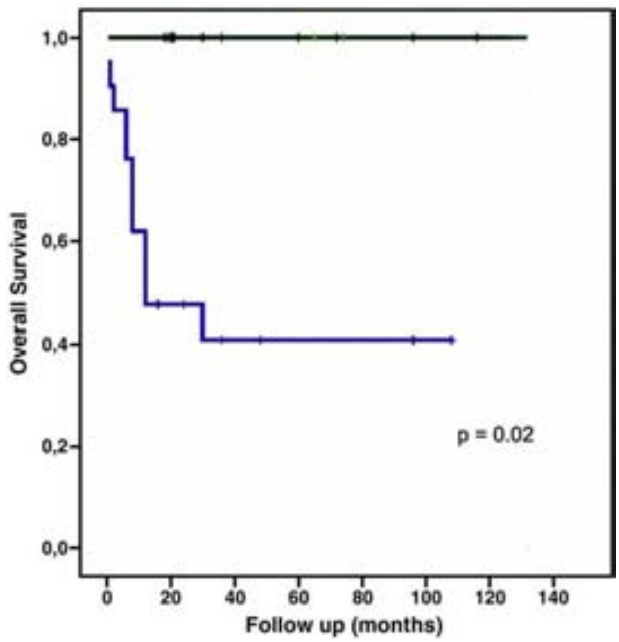


Fig. 2 Kaplan-Meier's survival analysis for patients with endometrial stromal sarcomas and patients with high-grade uterine sarcomas (leiomyosarcomas and undifferentiated endometrial sarcomas). Survival differences between both groups were statistically significant (P = 0.02).

3.5. Immunohistochemical results

The immunohistochemical results are shown in Figs. 3 to 5 and in Table 5.

3.6. Ki67, p53, and p16

The immunoreactions for Ki67, p53, and p16 had a similar staining distribution and intensity in leiomyosarcomas and undifferentiated endometrial sarcomas. Both tumor types showed stronger and more diffuse immunostaining for the 3 markers, with H-scores significantly higher than those obtained for endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas. Moderate to strong immunostaining for p53 and p16 (H-score, 101-300) was found in 40% and 75% of leiomyosarcomas, respectively, and 3 of 5 undifferentiated endometrial sarcomas. Of the 3 myxoid leiomyosarcomas, 2 had lower H-scores for Ki67, p53, and p16 than the spindled leiomyosarcomas. Noteworthy, the only 3 leiomyosarcomas associated with favorable outcome showed weak or negative immunostaining for Ki67, p53, and p16 (Table 4). Compared with the primary tumors, the metastases of a leiomyosarcoma and an endometrial stromal sarcoma showed much stronger immunostainings for p53 and p16, whereas Ki67 immunoreaction was similar. Ten of 14 cases of endometriosis and all samples of normal myometrium showed low or negative immunostaining for all 3 markers. The remaining 4 cases of endometriosis showed a moderate to strong immunoreaction for p16, and one of them also exhibited moderate immunostaining for Ki67.

3.7. Twist

Immunoreaction for Twist was strong in the high-grade sarcomas and was even stronger than that found for p53. In fact, high expression of Twist was seen in 16 (80%) of 20 leiomyosarcomas (mean H-score, 208) and in all 5 undifferentiated endometrial sarcomas. Again, the 3 leiomyosarcomas associated with favorable outcome showed very weak or negative Twist immunostaining (Table 4). Also, endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas showed Twist immunoreactions that were predominantly weak to moderate. However, an endometrial stromal sarcoma and 2 unusual leiomyomas (a cellular leiomyoma and an epithelioid leiomyoma) showed strong Twist immunoreactions (H-scores, 297, 224, and 275, respectively). Twist immunoreaction was negative in endometriosis and normal myometrium.

3.8. Bcl-2

Bcl-2 was expressed more frequently and strongly in endometrial stromal sarcomas, endometrial stromal nodules, and particularly in leiomyomas than in leiomyosarcomas (mean H-score, 35) and undifferentiated endometrial sarcomas. However, the 3 leiomyosarcomas associated with favorable outcome showed stronger bcl-2 immunoreactions (H-scores, 160, 270, and 100) (Table 4). Bcl-2 immunoreaction was predominantly negative or weak in endometriosis and in all but 2 of the 10 samples of normal myometrium.

3.9. Estrogen receptor and PR

Both ER and PR immunoreactions were very weak in leiomyosarcomas and undifferentiated endometrial sarcomas. In contrast, endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas showed strong ER and mainly PR immunoreactions. Worth noting, the metastases of a leiomyosarcoma had moderate to strong immunoreactions for ER and PR that were absent in the primary tumor. The samples of endometriosis and normal myometrium showed moderate to strong immunostainings.

3.10. H-caldesmon and CD10

Both leiomyomas and leiomyosarcomas showed a strong reactivity for h-caldesmon (mean H-scores, 230 and 135, respectively). In contrast, h-caldesmon expression was absent in all endometrial stromal tumors, except for 2 cases with focal smooth muscle differentiation. All endometrial stromal tumors were strongly reactive for CD10 (mean H-score, 158.6). Of 20 leiomyosarcomas, 5 showed focal and weak CD10 immunostaining (mean H-score, 22).

3.11. PTEN

There were no statistically significant differences between H-scores of PTEN in uterine sarcomas and benign related tumors.

3.12. Hierarchical clustering analysis

Hierarchical clustering analysis of the immunohistochemical results (Fig. 4) revealed 3 distinct clusters as follows: (*a*) high-grade sarcomas that reacted strongly for Ki67, p53, p16, and Twist; (*b*) endometrial stromal sarcomas and endometriosis showing strong immunoreaction for CD10, PTEN, ER, and PR; and (*c*) leiomyomas and samples of normal myometrium exhibiting caldesmon, bcl-2, PTEN, and PR immunoreactions. The 3 leiomyosarcomas associated with bcl-2 overexpression and prolonged survival clustered with benign leiomyomas. On the other hand, 3 metastases from an endometrial stromal sarcoma clustered with the high-grade sarcomas.

3.13. Relationship of immunohistochemical results with survival

In leiomyosarcomas, p53 immunoreaction related inversely with overall survival by univariate and multivariate

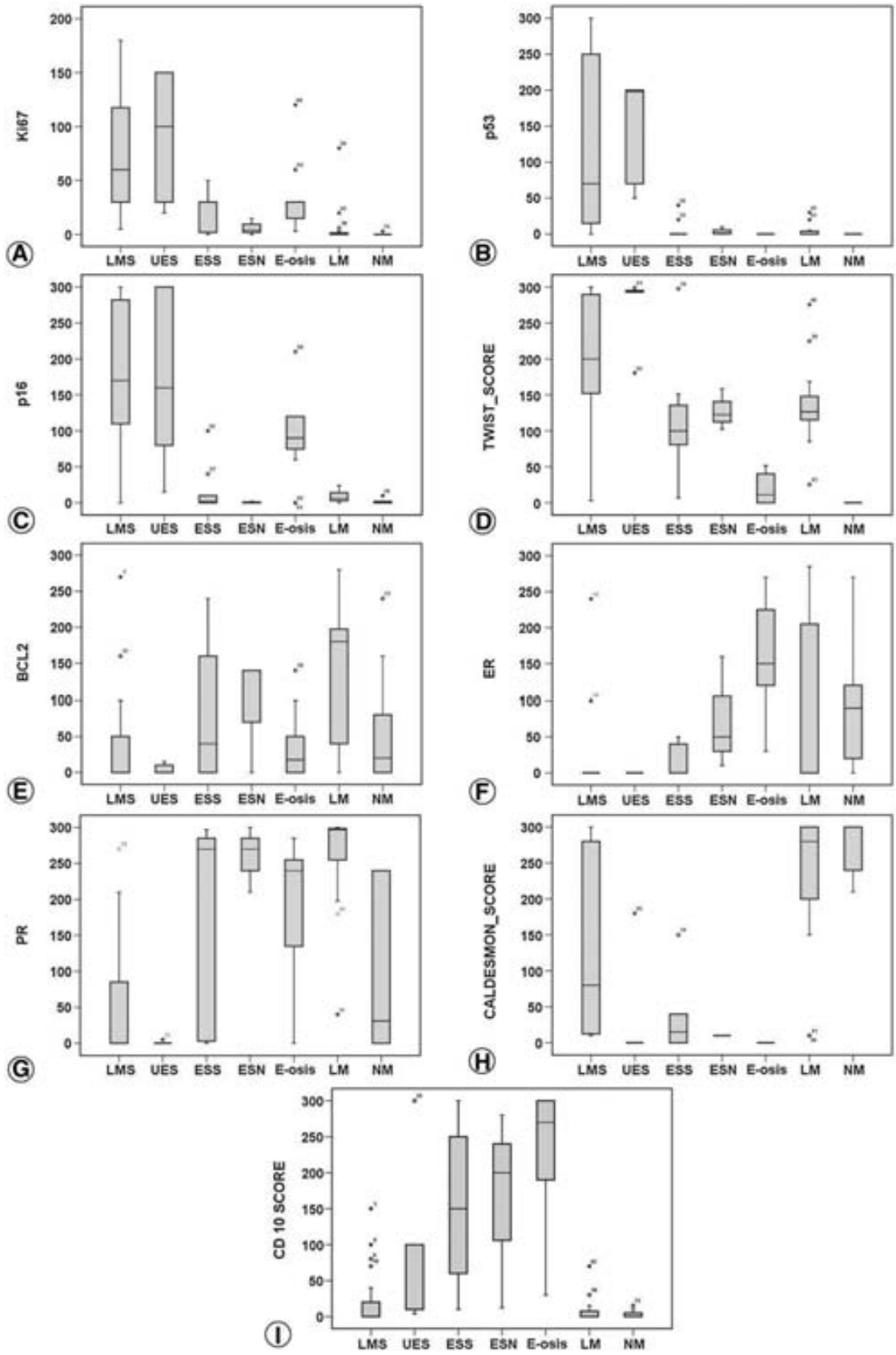
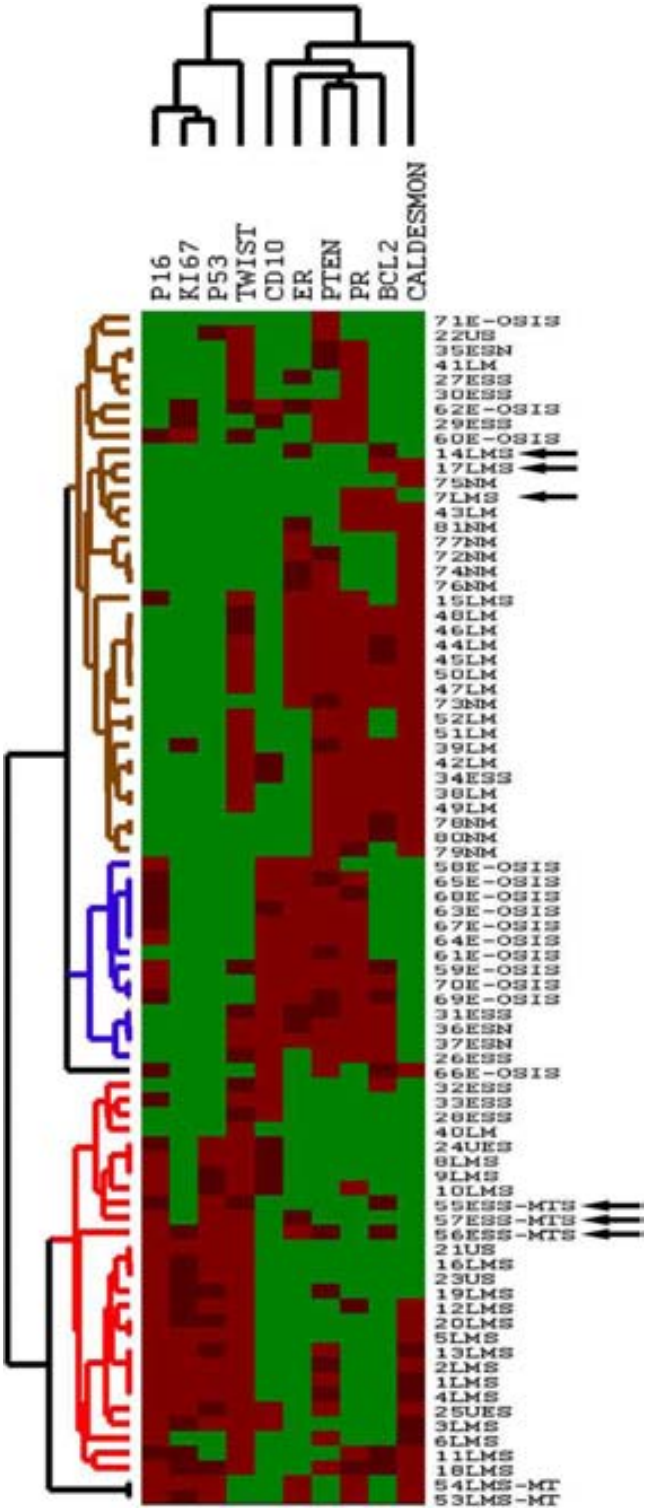


Fig. 3 Plot box graphs representing immunohistochemical values for Ki67 (A), p53 (B), p16 (C), Twist (D), bcl-2 (E), ERs (F), PRs (G), caldesmon (H), and CD10 (I) immunoreactions in leiomyosarcomas (LMS), undifferentiated endometrial sarcomas (UES), endometrial stromal sarcomas (ESS), endometrial stromal nodules (ESN), endometriosis (E-osis), leiomyomas (LM), and normal myometrium (NM).

analysis (HR, 1.006; $P = .05$). Twist expression also showed an inverse relationship with overall survival by univariate analysis (HR, 1.009; $P = .07$). Similarly, immunoreactions for p16 and Ki67 had a negative effect on disease-free survival (HR, 1.010; $P = .007$ and HR, 1.012; $P = .021$, respectively). On the other hand, leiomyosarcomas exhibiting strong bcl-2 immunostaining had longer overall disease-free survival by univariate ($P = .03$) and multivariate analyses ($P = .001$). Moreover, a similar relationship between bcl-2 expression and disease-free survival was obtained for all uterine sarcomas from this series by multivariate (HR, 0.984; $P = .002$) analysis. Although the high-grade sarcomas showed low or negative ER and PR immunostaining, no statistically significant relation with survival was found. No relationship was found between CD10 and PTEN immunoreactions and survival.



4. Discussion

Most series of uterine sarcomas have focused on specific histologic types [6,7,12,19], and only a few comparative studies including all major categories have been reported [10,11,13]. We have performed a clinicopathologic and immunohistochemical analysis of 34 uterine sarcomas of all types and 18 benign related tumors classified as per the criteria recommended by the 2003 WHO classification system. In our series, diagnostically equivocal categories, such as STUMP, were not included. Rather, all leiomyosarcomas showed the histologic features of malignancy including diffuse moderate-to-severe nuclear atypia, and high mitotic rate. Tumor cell necrosis was found in all but 1 case and vascular invasion in 30% of cases.

Factors associated with poor prognosis have not been consistent in different series of uterine leiomyosarcomas [7,8,10,22,32]. However, in a recent study of 245 leiomyosarcomas confined to the uterus [11], tumor size and mitotic index were found to be significant prognostic factors and allowed for separation of patients into 3 risk groups with marked differences in prognosis. In our study, FIGO stage and patient's age were the only statistically significant parameters of prognostic value by univariate analysis. Tumor stage was also significant by multivariate analysis. However, although patients often presented with early stage tumors, recurrence and metastases still occurred. In fact, 13 (72%) of 18 leiomyosarcomas with follow-up available were appar-

Fig. 4 Unsupervised hierarchical clustering analysis of 34 primary uterine sarcomas, 5 metastases from 2 of them, 18 benign mesenchymal uterine tumors, and 24 controls (14 cases of endometriosis and 10 samples of normal myometrium). In the heat map, each row represents a tumor o control and each column a single protein. Red indicates higher-than-average expression; brown indicates lower-than-average expression; and green indicates lack of expression. The dendrogram shows the proximity of samples. E-osis indicates endometriosis; ESN, endometrial stromal nodule; ESS, endometrial stromal sarcoma; ESS-MT, metastatic endometrial stromal sarcoma (from ESS 33); LM, leiomyoma; LMS, leiomyosarcoma; LMS-MT, metastatic leiomyosarcoma (from LMS 20); NM, normal myometrium; UES, undifferentiated endometrial sarcoma. Three LMSs associated with bcl-2 overexpression (cases 7, 14, and 17) clustered with LMs (←). Three metastases from an ESS clustered with the high-grade sarcomas (←).

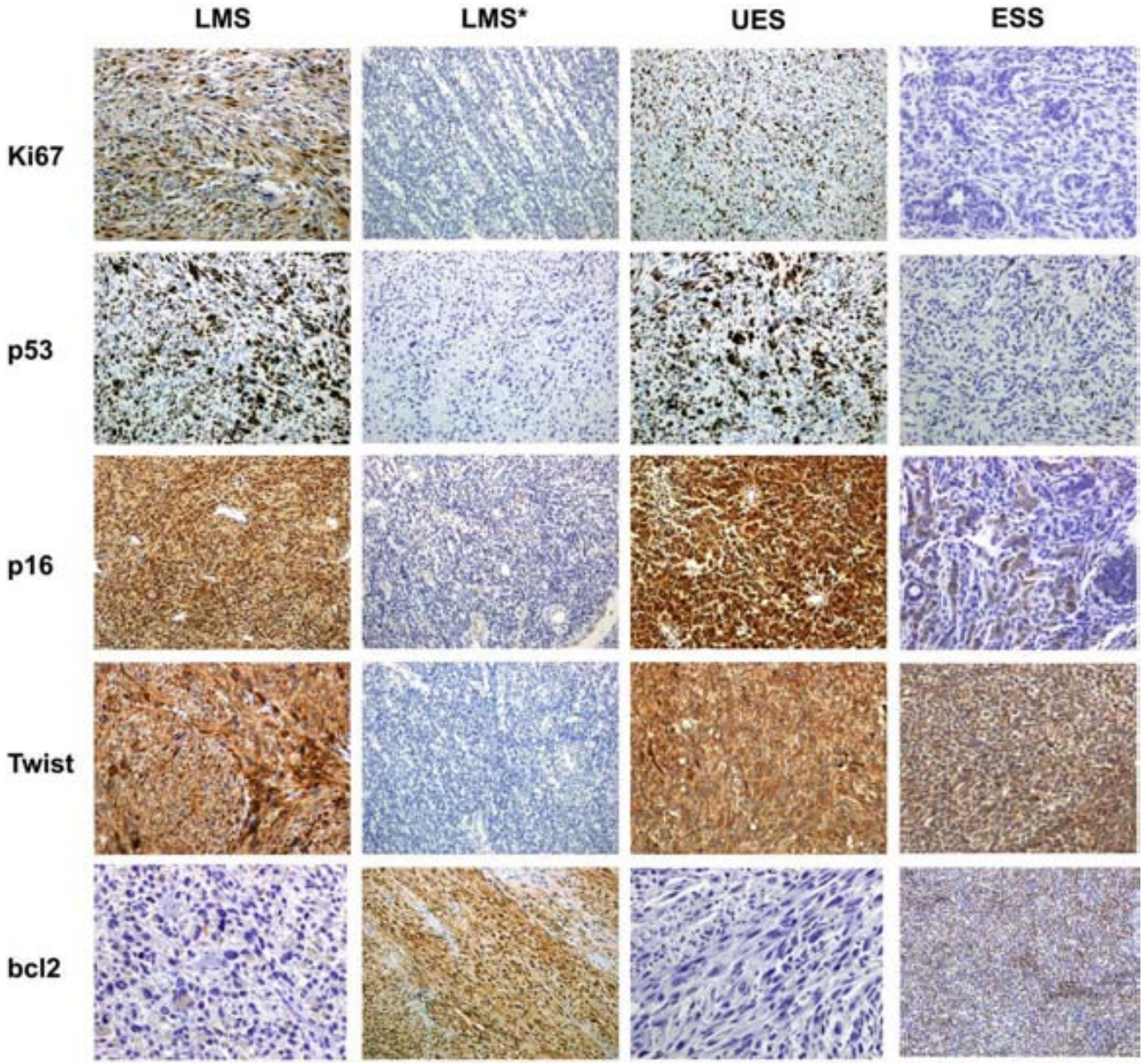


Fig. 5 Uterine sarcomas. Immunoreactions for Ki67, p53, p16, Twist, and bcl-2. ESS indicates endometrial stromal sarcomas; LMS, leiomyosarcomas; LMS*, leiomyosarcomas associated with prolonged survival; UES, undifferentiated endometrial sarcomas.

ently confined to the uterus (stage I) at initial surgery, and yet, 7 of these tumors (54%) recurred. In addition, lymphovascular space invasion influenced disease-free survival by univariate analysis. All other factors including tumor size, histologic subtype, mitotic count, tumor necrosis, and nuclear atypia did not show any significant relation with survival. Average disease-free survival was 2 years.

High nuclear atypia has been found to have an adverse effect on survival in 2 recent investigations [7,12], including 208 and 1396 leiomyosarcomas, respectively. Discrepancy with our findings may be explained by the smaller size of our series and that all our cases exhibited either moderate or severe nuclear atypia. Actually, the application of the 2003 WHO criteria excludes variants of leiomyoma, such as cellular, mitotically active, and atypical leiomyomas, all frequently misdiagnosed as leiomyosarcomas in the past [11]. Consequently, most leiomyosarcomas in our series were associated with adverse prognosis. In fact, 12 (67%) of the 18 patients either died of tumor or are alive with progressive disease.

Whereas all 3 patients with undifferentiated endometrial sarcomas (all stage IB), lacking endometrial stromal features and exhibiting marked nuclear pleomorphism, died of tumor within 1 year, none of the other 2 patients whose tumors focally resembled endometrial stromal sarcoma and showed nuclear uniformity died of tumor. A recent study has pointed out that undifferentiated endometrial sarcoma showing nuclear regularity represents an intermediate subcategory

Table 5 Immunohistochemical analysis of 34 uterine sarcomas and 18 benign related tumors

Diagnosis	Ki67 H-score (min-max)	p53 H-score (min-max)	p16 H-score (min-max)	Twist H-score (min-max)	Bcl-2 H-score (min-max)	ER H-score (min-max)	PR H-score (min-max)	Caldesmon H-score (min-max)	CD10 H-score (min-max)
Leiomyosarcoma (n = 20)	75 (5-180)	116 (0-300)	165 (0-300)	202 (2-299)	36 (0-270)	17 (0-100)	51 (0-210)	137 (80-300)	22 (0-100)
Undifferentiated endometrial sarcoma (n = 5)	90 (20-150)	144 (50-200)	171 (15-300)	272 (180-298)	5 (0-15)	0	1 (0-5)	36 (0-180)	80 (4-300)
Endometrial stromal sarcoma (n = 9)	22 (0-50)	7 (0-40)	18 (0-100)	114 (6-297)	86 (0-240)	16 (0-50)	176 (0-297)	30 (0-150)	152 (80-300)
Endometrial stromal nodule (n = 3)	6 (0-15)	4 (0-10)	1 (0-2)	128 (102-158)	94 (0-140)	73 (10-160)	260 (210-300)	0	164 (12-280)
Endometriosis (n = 15)	33 (3-120)	0	90 (0-210)	19 (0-52)	35 (0-140)	164 (30-270)	195 (0-285)	0	229 (30-300)
Leiomyoma (n = 15)	7 (0-80)	4 (0-30)	9 (0-24)	135 (25-275)	133 (0-280)	93 (0-285)	258 (40-300)	230 (10-300)	9 (0-70)
Normal myometrium (n = 10)	0 (0-3)	0	2 (0-10)	0	58 (0-240)	97 (0-270)	99 (0-240)	276 (210-300)	3 (0-15)

of endometrial stromal tumors (formerly classified as high-grade endometrial stromal sarcomas) that shares some molecular genetic and immunohistochemical features with endometrial stromal sarcomas and is associated with better outcome [14]. In another recent study [11], prognosis of endometrial stromal sarcomas confined to the uterus (83 cases) was related to mitotic index and tumor cell necrosis. Combination of these 2 parameters allowed for separation of patients into 3 risk groups with marked differences in prognosis. As expected, patients with high-grade sarcomas in our series had a shorter overall survival (median, 1 year) than patients with endometrial stromal sarcomas (median, 11 years), and the difference was statistically significant. That is, none of the 6 patients with endometrial stromal sarcoma and follow-up available has died of tumor even if 4 of them developed distant metastases.

The use of immunohistochemistry in the diagnosis of uterine sarcomas is quite limited, with the exception of specific markers of differentiation (ie, h-caldesmon and CD10) that can be applied in problematic cases. Unexpectedly, however, in the course of this study we found 3 leiomyosarcomas exhibiting the morphologic features of malignancy that were associated with prolonged survival. The finding of such cases prompted us to perform a comparative immunohistochemical analysis of 10 selected oncoproteins involved in cell proliferation, cell differentiation, and apoptosis on pure uterine sarcomas, including all major histologic types, and other benign related lesions such as leiomyomas, endometrial stromal nodules, and endometriosis.

Several studies have shown that uterine leiomyosarcomas have significantly higher Ki67 index and p53 expression levels than benign smooth muscle tumors [20-23,25,26,33]. In our series, 25% of leiomyosarcomas showed moderate Ki67 immunoreactions and 40% exhibited moderate to strong p53 immunostaining. Similar scores were obtained in 3 of 5 undifferentiated endometrial sarcomas. In contrast, endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas exhibited weak or negative immunostainings. Worth noting, the metastases of a leiomyosarcoma and an endometrial stromal sarcoma showed much stronger immunostaining than the corresponding primary tumors. In leiomyosarcomas, high Ki67 index and p53 overexpression had a negative effect on disease-free survival by univariate and multivariate analysis.

p16 is a tumor suppressor protein that negatively regulates the cell cycle. Abnormal expression of p16 has been described in various tumors, including cervical cancer. Although high p16 expression in carcinoma of the cervix and its precursors is the surrogate marker for hPV infection, overexpression of p16 in other tumors is not necessarily associated with hPV. Overexpression of p16 has recently been described in uterine leiomyosarcomas and found to be higher than in leiomyomas [17,19,20,25,34]. In the former tumors, its reported frequency ranged from 57% to 100% and immunoreaction was found in from more than 25% to more than 50% of tumor cells. Contrarywise, 13% or less of uterine

leiomyomas showed p16 immunoreaction [19,20,25]. In one study, however, up to 60% of atypical (bizarre) leiomyomas showed immunostaining for p16 [20]. Another study revealed a correlation between p16 overexpression and poor outcome [17]. In our series, 15 (75%) of 20 leiomyosarcomas showed moderate to strong p16 immunoexpression. Similarly, p16 was overexpressed in 3 of 5 undifferentiated endometrial sarcomas. In contrast, endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas showed negative or weak immunostaining. In leiomyosarcomas, p16 overexpression had a negative effect on disease-free survival.

Twist is an oncogene that inhibits apoptosis and appears indistinguishable from bcl-2 [35]. A major obstacle to the expansion of tumor cells is the induction of programmed cell death. Therefore, oncogene-driven proliferation must be associated with inhibition of apoptosis to allow malignant overgrowth. The oncogenic cooperation between *myc* and *Twist* illustrates such a process. Myc oncoproteins act both as growth promoting factors and apoptosis promoters [36]. Apoptosis is mainly triggered by the ARF-p53 pathway [37]. Thus, *myc*-induced apoptosis is p53-dependent. *Twist* counteracts *myc* proapoptotic properties by knocking down the *p53* pathway. In fact, it has been recently demonstrated that tumor cell growth is modulated by the molecular interaction between Twist and p53 [38]. Overexpression of *myc* has been reported in 50% of uterine leiomyosarcomas (6/12) and uterine leiomyomas (11/23) [39]. The same percentage of leiomyosarcomas (50%; 29/57) showed p53 overexpression, whereas leiomyomas lacked this feature [21,24]. In our study, immunoreaction for Twist was strong in the high-grade sarcomas and was even stronger than that found for p53. Endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas also exhibited Twist immunoreaction, but it was predominantly moderate to weak.

Although expression of bcl-2 is usually stronger in uterine leiomyomas than in leiomyosarcomas [18], it has been recently reported that patients with leiomyosarcomas overexpressing bcl-2 have less lymphovascular space involvement and longer overall survival [18]. In our series, bcl-2 was expressed more frequently and strongly in endometrial stromal sarcomas, endometrial stromal nodules, and particularly in leiomyomas than in leiomyosarcomas and undifferentiated endometrial sarcomas. Bcl-2 expression had a positive effect on disease-free survival by univariate analysis. In fact, the 3 leiomyosarcomas associated with favorable outcome showed stronger bcl-2 immunoreactions. Noteworthy, these tumors, which exhibited severe nuclear atypia, high mitotic index, and tumor cell necrosis, showed weak or negative immunostaining for Ki67, p53, p16, and Twist. They could represent a subset of indolent leiomyosarcomas associated with specific molecular genetic changes and inhibition of apoptosis.

As expected, both ER and PR immunoreactions were weaker in leiomyosarcomas [16,26] and undifferentiated endometrial sarcomas than in endometrial stromal sarcomas, endometrial stromal nodules, and leiomyomas. However, no statistically significant correlations with survival were encountered. Worthy of note, the metastases of a leiomyosarcoma and an endometrial stromal sarcoma showed moderate to strong immunoreactions for ER and PR that were absent in the corresponding primaries. As for markers of differentiation, both leiomyomas and leiomyosarcomas showed intense reactivity for h-caldesmon [40], whereas endometrial stromal tumors were strongly reactive for CD10 [41].

In summary, the 2003 WHO classification criteria have allowed the diagnosis of a variety of mostly benign smooth muscle tumors of the uterus such as STUMPs and specific variants of leiomyoma, formerly diagnosed as well-differentiated or low-grade leiomyosarcoma. Furthermore, carcinosarcoma has been removed from the category of uterine sarcoma and is now considered a metaplastic carcinoma. Our study confirms that most tumors remaining in the category of uterine sarcoma, based on the 2003 WHO criteria, are leiomyosarcomas exhibiting moderate to severe nuclear atypia, high mitotic index, and/or tumor cell necrosis. These tumors, such as the far less common undifferentiated endometrial sarcomas, are aggressive neoplasms associated with poor prognosis, even if surgically treated at early stage (stage I). Consequently, these patients require adjuvant therapy. In contrast, endometrial stromal sarcomas showing only mild nuclear atypia, low mitotic activity, and usually lacking tumor cell necrosis are indolent tumors associated with prolonged survival despite recurrence and metastasis. However, within the category of undifferentiated endometrial sarcomas, tumors exhibiting nuclear pleomorphism seem to behave more aggressively than tumors showing nuclear uniformity. The latter tumors may represent an intermediate category equivalent to what were formerly called *high-grade endometrial stromal sarcomas*. Immunohistochemically, all 3 markers of proliferation— Ki67, p53, and p16—are significantly higher in leiomyosarcomas and undifferentiated endometrial sarcomas than in endometrial stromal sarcomas and benign tumors. The antiapoptotic marker Twist is expressed both in benign and malignant tumors, but the immunoreaction is stronger in the high-grade sarcomas. In contrast, bcl-2 immunostaining is stronger in endometrial stromal sarcomas and benign tumors. However, leiomyosarcomas that are negative or low expressors for markers of cell proliferation and show a strong immunoreaction for bcl-2 seem to be associated with prolonged survival. These tumors could represent a favorable form of leiomyosarcoma associated with specific molecular genetic alterations.

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