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Immunohistochemical panel to differentiate endometrial stromal sarcoma, uterine leiomyosarcoma and leiomyoma: something old and something new

Helena Hwang[1], **Koji Matsuo**[2], **Kara Duncan**[3], **Elham Pakzamir**[4], **Huyen Q Pham**[2], **Adrian Correa**[3], **Alexander Fedenko**[3], **Paulette Mhawech-Fauceglia**[3]

[1]Department of Anatomic Pathology, University of Texas at Dallas, Dallas, Texas, USA

[2]Division of Gynecology Oncology, Departments of Obstetrics and Gynecology, University of Southern California, Los Angeles, California, USA

[3]Department of Surgical Pathology, University of Southern California, Los Angeles, California, USA

[4]Department of Preventive Medicine, University of Southern California, Los Angeles, California, USA

Abstract

Aims—To evaluate an immunohistochemical panel differentiating endometrial stromal sarcoma (ESS) from uterine leiomyosarcoma (ULMS) and leiomyoma (LM).

Methods—94 cases (28 ESS, 41 ULMS, 25 LM) were retrieved and arrayed. 10 immunomarkers (estrogen receptor (ER), progesterone receptor (PR), CD10, smooth muscle actin, desmin, h-caldesmon, transgelin, GEM, ASC1, stathmin1) were used. A predictive model was constructed and examined by receiver operating characteristics curve analysis to determine area under the curve (AUC).

Results—The combination of $ER^+/PR^+/CD10^+/GEM^-$/h-caldesmon$^-$/transgelin$^-$ can predict ESS versus ULMS with AUC predictive value of 0.872 (95% CI 0.784 to 0.961, $p<0.0001$). The combination of $ER^+/PR^+/CD10^+$/h-caldesmon$^-$/transgelin$^-$ can predict low grade (LG) ESS from 'LG' ULMS with AUC predictive value of 0.914 (95% CI 0.832 to 0.995, $p<0.0001$). Finally, ULMS and ESS, including the LGs, were more likely to be stathmin1$^+$ than LM.

Conclusions—Due to the different clinical course and management, adding novel antibodies (GEM, transgelin) to the well established immunohistochemistry panel seemed to be useful in

Correspondence to Professor Paulette Mhawech-Fauceglia, MD, Department of Pathology, University of Southern California, 1200 N. State Street. Room 7A116, Los Angeles, CA 90033-5000, USA; pfauceglia@hotmail.com.

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distinguishing ESS from ULMS and LG ESS from 'LG' ULMS. Finally, stathmin1 expression could be of value in differentiating LM from uterine sarcomas.

INTRODUCTION

Endometrial stromal sarcomas (ESSs) and uterine leiomyosarcomas (ULMSs) represent the majority of uterine mesenchymal tumours.[12] The new 2014 WHO classified ESS into low grade (LG) ESS, high grade ESS and undifferentiated endometrial sarcoma (UES).[3] LG ESSs are composed of a proliferation of cells reminiscent of endometrial stromal cells in proliferative phase. They invade the myometrium in a characteristic fashion and have a high frequency of lymphatic invasion. ESSs are low-malignant tumours with an indolent course and late recurrences. The standard treatment recommendation of ESSs is generally surgery (total hysterectomy with salpingo-oophorectomy) followed by progestin therapy in selected cases with excellent survival outcomes. The prognosis largely depends on the extent of disease at the initial diagnosis with 5-year survival rates of 90–100% for stage I-II and 60–70% for stage III-IV On the other hand, UESs, less common tumours than LG-ESSs, are malignant tumours that lack stromal differentiation. They are aggressive and most women are dead of disease at 2 years after diagnosis. The primary treatment is surgery followed by radiation therapy for local control and chemotherapy for systemic control.[4-7] ULMSs are also aggressive tumours with an overall poor prognosis, with 5-year survival of 15–25%. Tumour staging seems to be the most important prognostic factor, where stage I and II tumours have a better prognosis with 5-year survival of 25–70%. The main treatment of ULMS is surgery. Adjuvant therapy including chemotherapy/radiation therapy has been used to reduce recurrences, but its clinical efficacy is uncertain. Hormonal therapy is usually not used in patients with ULMS.[8-10] Because of the distinct difference in prognosis, management and treatment between ESS and ULMS, the need for an accurate diagnosis is imperative.

Immunohistochemistry (IHC) is often employed as an adjunct to morphology in uterine mesenchymal lesions, particularly in cases with equivocal features. The routine immunomarker panel used by most surgical pathologist to distinguish ESS from ULMS consists of estrogen receptor (ER), progesterone receptor (PR), desmin, smooth muscle actin (SMA), h-caldesmon and CD10.[11-19] Immunoprofiles such as $ER^+/PR^+/desmin^-/SMA^-$/h-$caldesmon^-/CD10^+$ usually support the diagnosis of ESS.[20] Unfortunately, however, there is much overlap and both entities can be immunoreactive to the same antibodies. New immunomarkers are thus needed to face this challenging problem.[21] Novel gene expression signatures differentiating ESS from ULMS, conducted by Davidson *et al*,[22] have recently emerged. The authors found that genes that were overexpressed in ESS were: *SLCA7A10/ASC1*, *EFNB3*, *CCND2*, *ECEL1*, *ITM2A*, *NPW*, *PLAG1 and GCGR*. Genes that were overexpressed in ULMS were: *CDKN2A*, *FABP3*, *TAGLN*, *JPH2*, *GEM*, *NAV2* and *RAB23*.[22] Of all of these proteins, transgelin (TAGLN) was the only antibody shown in a small number of uterine sarcomas and soft tissue sarcomas to have promising results.[2324]

The aim of this study is to evaluate antibodies that are routinely used such as ER, PR, desmin, SMA, h-caldesmon and CD10, as well as four novel antibodies, including stathmin

1, ASC1, GEM and transgelin, in series of uterine sarcomas and leiomyomas with the goal of incorporating these markers in the current IHC panel.

MATERIALS AND METHODS

Case selection

After institutional review board approval was obtained, patients with a first time diagnosis of ESS, ULMS and leiomyoma (LM) were retrieved from the archives of the Departments of Pathology at the University of Southern California and the University of Texas at Dallas from 2005 to 2014. This was a retrospective study. Only cases with available paraffin-embedded tissue were included in the study. A total of 69 hysterectomy specimens with a diagnosis of uterine sarcomas were available; 28 cases were ESS (19 LG and 9 UES) and 41 cases were ULMS (28 'LG' and 13 'high grade'). Even though there is no clear-cut consensus on grading ULMS, a tumour was classified as 'LG ULMS' when there was mild cytological atypia and mitotic activity <20/high power filed (HPF) and as 'high grade ULMS' when there was moderate to severe atypia and mitotic activity ≥20/10HPF.[10] All histological diagnoses were made on hysterectomy specimens.

Tissue microarray building

For tumour microarray construction, paraffin-embedded tissues from these 94 cases were used as described previously.[25] Briefly, morphologically representative regions were carefully selected on each individual paraffin-embedded block (donor blocks) and a core tissue biopsy of 1 mm was punched and transferred to a composite paraffin-embedded block (receiver block). To account for tumour heterogeneity and tissue loss, three core biopsies were taken from different areas of each tumour. One section was stained with H&E to evaluate the presence of the tumour by light microscopy. Whole sections of 10 normal cases of endometrium/myometrium were also included.

Immunohistochemistry

For immunohistochemical (IHC) analysis, 4 μm thick sections were deparaffinised with xylene, and washed with ethanol. Sections were cooled for 20 min then incubated 10 min with 3% H_2O_2 to quench endogenous peroxidase activity. Blocking was performed using serum-free protein block, DakoCytomation (Carpenteria, California, USA), for 30 min. Ten antibodies were used (ER, PR, SMA, desmin, h-caldesmon, CD10, GEM, solute carrier family 7 (ASC1), transgelin, and stathmin1). The conditions of these antibodies are summarised in table 1. The evaluation of the antibodies was performed twice by two independent expert gynaecological pathologists, blinded of the original diagnosis, separated by a 1-month period. The percentage was assessed as follows: 0%, ≤10%, 11–50%, 51–75% and 76–100%; and the intensity as absent (0), weak (1+), moderate (2+) and strong (3+). Whenever there were discrepancies among the immunostaining evaluation in any given case, the higher intensity was taken as the final score. The immunostains evaluation of the first and second assessments was reviewed and when there was a discrepancy in scoring, a consensus was reached. The staining score was obtained by multiplying percentage with intensity and this score was used for our statistics analysis.

Statistical analysis

The primary interest of statistical comparison was to identify useful biomarkers to distinguish ESS from leiomyosarcoma (LMS). The secondary interest was to identify useful biomarkers to distinguish LG ESS from 'LG' ULMS. First, composition scores for the 10 biomarkers tested were determined based on IHC results (range 0–12) as intensity (range 0–3) multiplied by per cent expression (range 0–4). Then, expression patterns of the 10 biomarkers were examined, and the cut-off for composition score was determined based on the distribution of score across the histology subtype groups. Based on the cut-off score, univariate analysis with χ^2 test or Fisher's exact test was performed for each marker and the magnitude of statistical significance was expressed with OR and 95% CI. Sensitivity (Sen), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV) and accuracy were also determined. Among statistically significant biomarkers, receiver operating characteristics (ROC) curve analysis was performed based on descending order, adding each biomarker one by one, and the statistical significance was expressed with area under the curve (AUC) and 95% CI. ΔAUC change was calculated as interval AUC increment change by adding one additional biomarker. p Values less than 0.05 were considered statistically significant (all, two-tailed tests). SPSS (V.12.0, Chicago, Illinois, USA) was used for the statistical analysis.

RESULTS

The patterns of expression were as follows: nuclear for ER, PR; cytoplasmic for SMA, desmin, h-caldesmon, transgelin and GEM, and nuclear/cytoplasmic for stathmin1 and ASC1. The normal myometrium was moderately positive for ER and PR, strongly positive for desmin, SMA, h-caldesmon, ASC1, transgelin and GEM, weakly positive for stathmin1, and negative for CD10. The normal endometrium was strongly positive for ER, PR and CD10, weakly positive for transgelin, GEM, ASC1 and SMA, and negative for desmin, h-caldesmon and stathmin 1.

The cases were distributed as follows: 28 cases were ESS (19 LGs and 9 UES), 41 cases were ULMS (28 LGs and 13 high grades) and 25 cases were LM (10 regular LM, 10 cellular LM, 5 atypical LM). Expressions of each of the 10 antibodies in our series are summarised in table 2.

Examples of expressions of different immunomarkers are illustrated in figures 1A-D and 2A-F. The Spe, Sen, PPV and NPV of each of the 10 immunomarkers to distinguish ESS from ULMS are illustrated in table 3. The three most specific immunomarkers to distinguish ESS from ULMS in descending order were; ER (Spe 97.6%, Sen 46.4%, PPV 92.9% and NPV 72.7%), PR (Spe 90.2%, Sen 57.1%, PPV 80% and NPV 75.5%) and CD10 (Spe 75.6%, Sen 59.3%, PPV 61.5% and NPV 73.8%). The three most sensitive immunomarkers in descending order were ASC1 (Sen 92.9%, Spe 17.5%, PPV 44.1% and NPV 77.8%), GEM (Sen 88.9%, Spe 35.9%, PPV 49% and NPV 82.4%) and h-caldesmon (Sen 70.4%, Spe 60%, PPV 54.3% and NPV 75%).

Figure 3 A shows the ROC curves with $ER^+/PR^+/CD10^+/GEM^-$/h-caldesmon$^-$/transgelin$^-$ being the best combination of markers for predicting ESS from ULMS with AUC 0.872

(95% CI 0.784 to 0.961, p<0.0001). The second best combination was $ER^+/PR^+/CD10^+/GEM^-$/h-caldesmon$^-$ with AUC 0.846 (95% CI 0.741 to 0.950, p<0.0001) (table 4).

On the other hand, when we evaluated the Sen, Spe, PPV and NPV in the 47 LG cases only (19 ESS and 28 ULMS), it appeared that the most specific markers to distinguish LG ESS from 'LG' ULMS in descending order were ER (Spe 96.4%, Sen 52.6%, PPV 90.9% and NPV 75%), PR (Spe 85.7%, Sen 73.7%, PPV 77.8% and NPV 82.8%) and CD10 (Spe 78.6%, Sen 61.1%, PPV 64.7% and NPV 75.9%). The most sensitive markers in descending order were GEM (Sen 94.4%, Spe 22.2%, PPV 44.7% and NPV 85.7%), ASC1 (Sen 89.5%, Spe 25%, PPV 44.7% and NPV 77.8%) and PR (Sen 73.7%, Spe 85.7%, PPV 77.8% and NPV 82.8%) (table 5).

Figure 3B shows the ROC curves with $ER^+/PR^+/CD10^+$/h-caldesmon$^-$/transgelin$^-$ to be the best combinations of markers to predict LG ESS from 'LG' ULMS with AUC 0.914 (95% CI 0.832 to 0.995, p<0.0001). The second best combination of makers would be $ER^{+/}$ $PR^+/CD10^+/$ h-caldesmon$^-$ with AUC 0.903 (95% CI 0.816 to 0.991, p<0.0001) (table 6).

Finally, we evaluated the expression of stathmin1 in all 25 cases of LM and 69 cases of ESS and ULMS. The data shows that sarcoma cases (ESS and ULMS) were more likely to express stathim1 than LM cases (ULMS 82.5%, ESS 77.8% and LM 40.0%, p=0.001). Also, stathmin 1 was valid in differentiating LM from LG ESS and 'LG' ULMS (n=45) (71.1% vs 40.0%, p=0.005).

DISCUSSION

Uterine sarcomas comprise less than 10% of uterine malignancies, with ULMS and ESS constituting the majority of cases.[12] ULMS and ESS can show LG and high grade features, a factor that can play a role in histological diagnosis, but is not necessarily used for classification.[689] Distinguishing ESS from ULMS, as well as LG tumours and LM from LG uterine sarcomas (ESS and ULMS), is usually straightforward, particularly on a hysterectomy specimen. But distinguishing ESS from ULMS can be very challenging on core biopsies or small excisional biopsies. Therefore, IHC must fulfil this purpose. Transgelin is an actin-binding protein of the calponin family and correlates with smooth muscle differentiation. Transgelin was found to be overexpressed in ULMS. A study was recently conducted by Robin *et al.*[23] to determine the value of transgelin as a smooth muscle immunomarker in soft tissue tumours, including high numbers of LMS cases. The authors found that, unlike h-caldesmon and desmin, which lack Sen to distinguish LMS from other soft tissue tumours (50% and 45%, respectively), transgelin emerged as the best diagnostic marker with high Sen (83%) and high Spe (83%). However, the authors failed to mention how many of those LMS cases were from uterine origin. A very recent small study by Tawfik *et al* using transgelin antibody on 13 cases of ESS and 8 of uterine LMS found that transgelin was 100% sensitive and specific in distinguishing LMS from ESS.[24] However in our series, transgelin seemed to have a more modest Sen and Spe of 59.3% and 69.2%, respectively. When distinguishing LG ULMS from LG ESS, transgelin proved to be 66.7% specific and 67.9% sensitive. The difference in results between our series and that above might due to our larger series of cases (69 vs 21) and the differing scoring systems used.

GEM is a Guanosine-5′- triphosphate (GTP)-binding mitogen-induced T cell protein. It is located on 8q22.1 and it is overexpressed in skeletal muscle.[26] It has been suggested that GEM might be a regulatory protein that participates in receptor mediated signal transduction at the plasma membrane.[27] The role of GEM in distinguishing ESS from ULMS has not yet been explored. In our series GEM proved to be a very sensitive immunomarker in distinguishing ESS from ULMS and also LG ESS from 'LG' ULMS (88.9% and 94.4%, respectively). However, GEM was lacking Spe in both cases.

The traditional routine immunomarker panel used by most surgical pathologists to distinguish ESS from ULMS consists of ER, PR, desmin, SMA, h-caldesmon and CD10, with the immunoprofile $ER^+/PR^+/desmin^-/ SMA^-/$ h-caldesmon$^-/ CD10^+$ supporting the diagnosis of ESS.[20] However, in ULMS, wide ranges of ER and PR frequencies have been reported, varying from 20% to 87% for ER and 17% to 73% for PR.[11-14] Even though desmin and SMA are usually expressed in ULMS, they have also been reported to be positive in 10–40% of ESS cases.[28] Furthermore, positivity for CD10 ranged from 75% to 100% of ESS cases and from 0% to 60% of ULMS cases.[151929] Finally, even though h-caldesmon is very specific for ULMS, its Sen is only 50%.[23] High grade or more undifferentiated uterine sarcomas might lose expression of some of these proteins, making use of these immunomarkers somewhat limited. In our series, we found that only a few of these individual markers were either sensitive or specific in distinguishing ESS from ULMS; these included ER (Spe 97.6%, Sen 46.4%), PR (Spe 90.2%, Sen 57.1%), CD10 (Spe 75.6%, Sen 59.3%) and h-caldesmon (Sen 70.4%, Spe 60%).

As a general rule, in making an accurate diagnosis with high predictive value no one immunomarker is sensitive and specific enough to stand on its own. Therefore, surgical pathologists normally run an IHC panel to reach a diagnosis in their challenging cases. We found that a panel consisting of ER/PR/CD10/GEM/h-caldesmon was the best predictive panel in distinguishing ESS from ULMS; tumour cells that are ER, PR, CD10 positive and GEM, trangelin negative are most likely to be ESS. The best panel with high PV for a tumour to be LG ESS rather than 'LG' ULMS would be ER/PR/CD10/h-caldesmon/ transgelin; tumours ER, PR, CD10 positive and h-caldesmon and transgelin negative are more likely to be LG ESS than 'LG' LMS. This is a crucial distinction due to the differing prognosis and treatment, as LG ESSs have a better prognosis and are hormone-responsive, while 'LG' LMS are more aggressive, hormone-insensitive tumours with a questionable response to adjuvant therapy. ASC1 or SLCA7A10 (solute carrier family 7) is a 523 amino acid protein that has been found to be overexpressed in ESS.[23] However, in our series ASC1 failed to show any PV in distinguishing uterine sarcomas.

When high-grade uterine sarcomas exhibit a high mitotic rate and severe atypia, distinguishing them from LM is straightforward. LG tumours, however, can create a major diagnostic challenge, especially on core biopsies and small samples, where making the right diagnosis has a profound effect on patient management.[18] To distinguish LM from LG ESS and 'LG' ULMS, there is no established reliable immunomarker. The PI3k-AKT signalling pathway has been shown to play a critical role in the development of LMS and other malignancies.[3031] Stahmin1 is a candidate oncogene and seems to be a marker for the PI3 K pathway activation.[32] Stathmin1 is a major regulator of the microtubule dynamics and plays

a role in regulating cell division, motility and migration. In studying 25 cases of LM, of which 6 were atypical and 4 were cellular, stahmin1 seemed to be a good marker to distinguish ULMS and ESS from LMs. If a tumour expressed stahmin1, it was 36 times more likely to be malignant. All atypical and cellular LMs were negative for stahmin1. Even though our series has a limited number of atypical LMs, which create the most diagnostic challenges, these results are very promising and should be confirmed by larger studies.

The major shortcoming of our study is the lack of comparison between our results and others in the literature. The only published data are on transgelin; other antibodies are only mentioned in two very short abstracts with small numbers of cases (unpublished work). Therefore, our data is very promising and should be confirmed by others before being put into general use.

In summary, combining novel antibodies (GEM and transgelin) with the traditional markers (ER, PR, CD10, h-caldesmon) showed promising results in distinguishing ESS from ULMS. Furthermore, the novel antibody stahmin1 deserves future validation in differentiating LM from ESS and ULMS.

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Take home messages

- It is important to differentiate endometrial stromal sarcoma (ESS) from uterine leiomyosarcoma (ULMS).
- Adding novel antibodies (GEM, transgelin) to the established panel (estrogen receptor, progesterone receptor, CD10, smooth muscle actin, desmin, h-caldesmon) seemed to distinguish ESS from ULMS and low grade (LG) ESS from ‘LG’ ULMS which is vital due to their different clinical course and management.

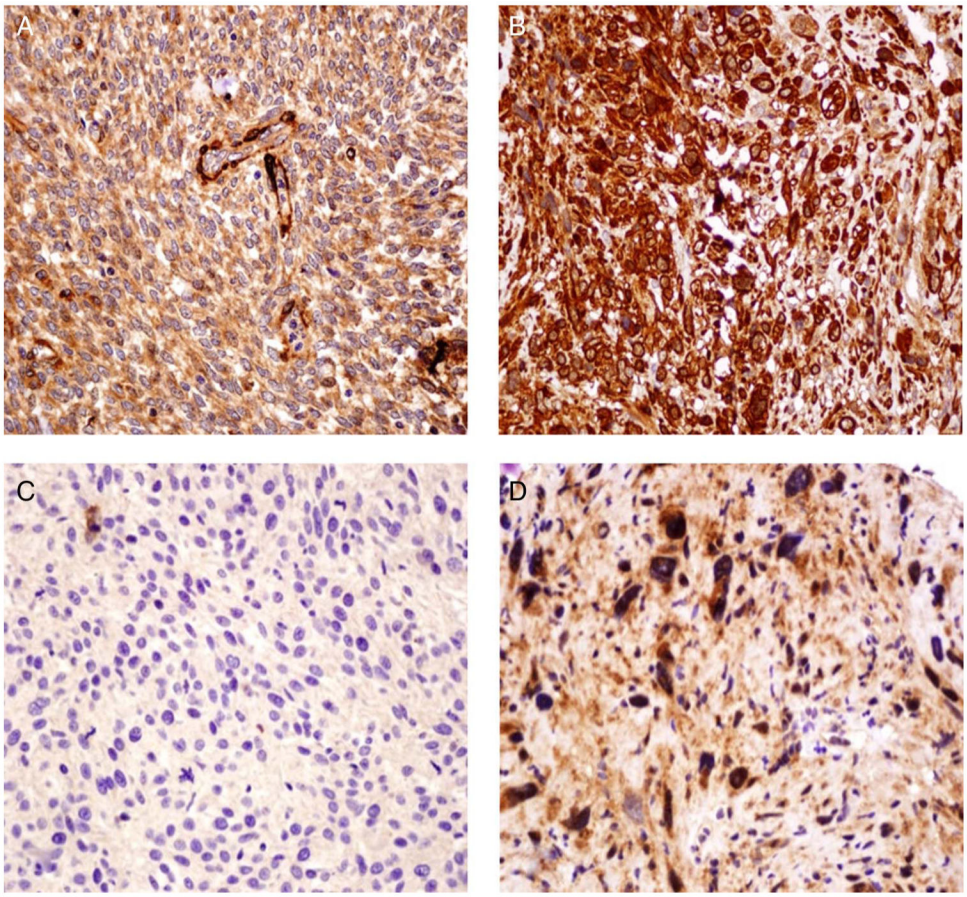

Figure 1.

(A). Negative expression of transgelin in a case of endometrial stromal sarcoma (ESS) (×40). (B) A case of uterine leiomyosarcoma (ULMS) strongly positive for transgelin. The expression was cytoplasmic in pattern. (C) high grade endometrial stromal sarcoma (HGESS) negative for Guanosine-5′- triphosphate (GTP)-binding protein overexpressed in skeletal muscle (GEM). (D) 'HG' leiomyosarcoma strongly positive for GEM. GEM was expressed in a cytoplasmic pattern.

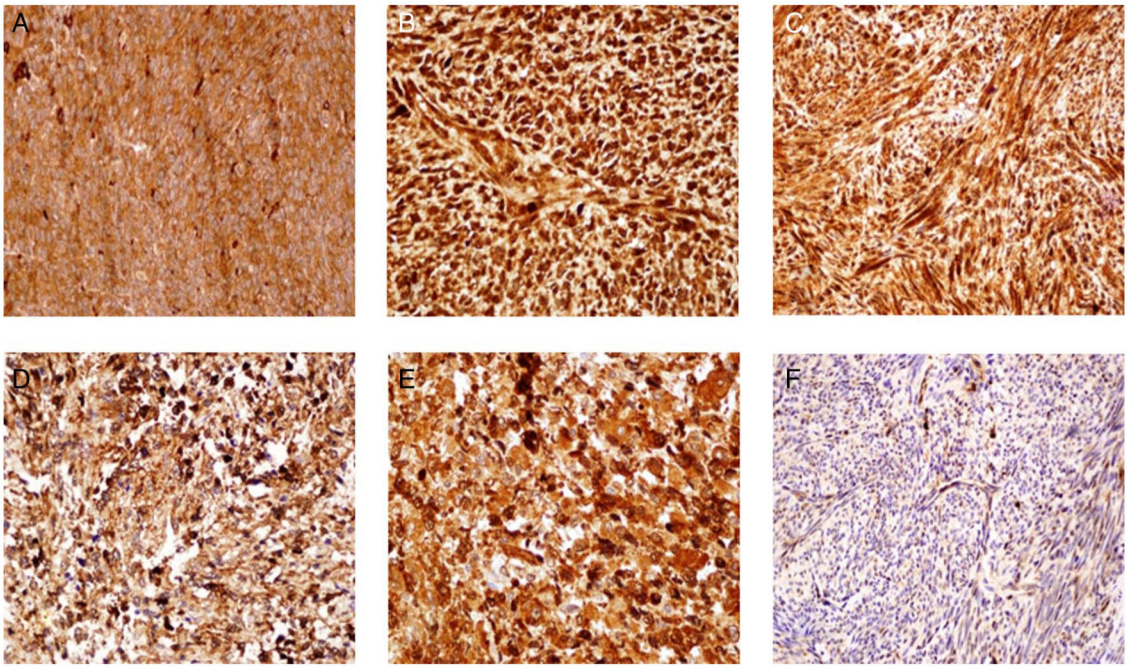

Figure 2.

Positive expression of ASC1 in (A) low grade (LG) endometrial stromal sarcoma (ESS), (B) 'LG' uterine leiomyosarcoma (ULMS), (C) leiomyoma (LM). ASC1 was strongly expressed in cytoplasmic and nuclear patterns. Positive expression of stathmin1 in (D) in ESS, (E) leiomyosarcoma and (F) LM. Stathmin1 was strongly expressed in cytoplasmic and nuclear patterns.

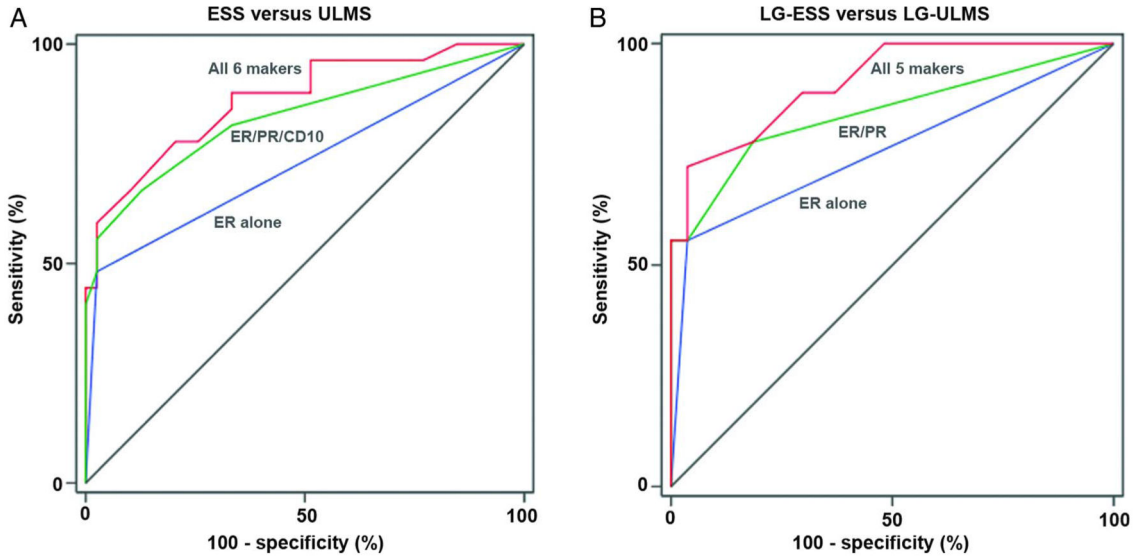

Figure 3.

Receiver operating characteristics curves for prediction of endometrial stromal sarcoma (ESS) and low grade (LG) ESS. (A) Comparison of ESS (n=28) and uterine leiomyosarcoma (ULMS) (n=41). All 6 markers include ER, PR, CD10, GEM, h-caldesmon and transgelin. (B) Comparison of LG ESS (n=19) and 'LG' ULMS (n=28). All 5 markers include ER, PR, CD10, h-caldesmon and transgelin. Abbreviations: LG-ESS, low-grade endometrial stromal sarcoma; LG-ULMS, low-grade uterine leiomyosarcoma; ER, estrogen receptor; PR, progesterone receptor; and CD10, cluster of differentiation 10.

Table 1

Conditions and titrations of the 10 antibodies

Antibodies	Company	Dilution	Condition	Control tissue
ER	ABCAM (SP1)	1:100	PH 8.0 20 min	Breast CA
Stathmin1	GENE TEX	1:500	PH 8.0 20 min	Breast CA
GEM	NOVUS	1:50	PH 8.0 20 min	Normal skin
Transgelin	GENE TEX	1:25	PH 8.0 20 min	Head and neck SCC
PR	NOVOCASTRA	1:200	PH 6.0 20 min	Breast
CD10	LEICA (56C6)	ready to use	PH 8.0 20 min	Tonsil
SMA	LEICA (αSM-1)	ready to use	PH 6.0 20 min	Ovary
Desmin	LEICA (DE-R-11)	ready to use	PH 8.0 20 min	striated muscle
h-caldesmon	Cell Marque (E89)	1:50	PH 6.0 20 min	striated muscle
ASC1	Novus	1:100	PH 8.0 20 min	liver

ASC1, solute carrier family 7; CA, California; CD10, cluster of differentiation 10; ER, estrogen receptor; PR, progesterone receptor; SMA, smooth muscle actin.

Table 2

Expressions of the 10 antibodies in 94 cases of leiomyoma, ESS and ULMS

		Uterine leiomyosarcoma		Endometrial stromal sarcoma		
	Leiomyoma	**Low grade**	**High grade**	**Low grade**	**High grade**	**p Value**
Total	n=25	n=28	n=13	n=19	n=9	
ER	%	%	%	%	%	<0.0001
Score 0	12.0	71.4	92.3	21.1	55.6	
Score 1–7	12.0	25.0	7.7	26.3	11.1	
Score 8–12	76.0	3.6	0	52.6	3 (33.3)	
PR						<0.0001
Score 0	8.0	57.1	100	26.3	66.7	
Score 1–7	12.0	28.6	0	0	11.1	
Score 8–12	80.0	14.3	0	73.7	22.2	
Desmin						0.004
Score 0	4.0	39.3	38.5	55.6	37.5	
Score 1–7	0	0	7.7	11.1	12.5	
Score 8–12	96.0	60.7	53.8	33.3	50.0	
SMA *						0.001
Score 0	0	18.5	38.5	28.8	55.6	
Score 1–7	4.0	0	0	22.2	11.1	
Score 8–12	96.0	81.5%)	61.5	50.0	33.3	
CD10 **						0.005
Score 0	64.0	60.7	38.5	27.8	33.3	
Score 1–7	32.0	17.9	30.8	11.1	11.1	
Score 8–12	4.0	21.4	30.8	61.1	55.6	
h-caldesmon *						0.005
Score 0	8.0%	25.9	23.1	55.6	55.6	
Score 1–7	4.0	3.7	15.4	16.7	0	
Score 8–12	88.0	70.4	61.5	27.8	44.4	
Transgelin *						0.036
Score 0	0	10.7	8.3	11.1	11.1	

		Uterine leiomyosarcoma		Endometrial stromal sarcoma		
	Leiomyoma	**Low grade**	**High grade**	**Low grade**	**High grade**	**p Value**
Score 1–7	0	17.9	8.3	33.3	0	
Score 8–12	100	71.4	83.3	55.6	88.9	
GEM ***						0.056
Score 0	20.0	44.4	25.0	44.4	11.1	
Score 1–7	28.0	25.9	0	33.3	33.3	
Score 8–12	52.0	29.6	75.0	22.2	55.6	
ASC1 **						0.30
Score 0	0	7.1	0	5.3	0	
Score 1–7	20.0	17.9	0	5.3	0	
Score 8–12	80.0	75.0	100	89.5	100	
Stathmin1 *						0.001
Score 0	8.0	14.8	0	11.1	0	
Score 1–7	52.0	11.1	0	22.2	0	
Score 8–12	40.0	74.1	100	66.7	100	

Number (%) is shown. Score represents composite score based on intensity and % expression. χ^2 test for p values

* 2 missing

** 1 missing

*** 3 missing.

CD10, cluster of differentiation 10; ER, estrogen receptor; ESS, endometrial stromal sarcoma; GEM, Guanosine-5′- triphosphate (GTP)-binding protein overexpressed in skeletal muscle; and ASC1 solute carrier family 7; PR, progesterone receptor; SMA, smooth muscle actin; ULMS, uterine leiomyosarcoma.

Table 3

Comparison of biomarker expressions between all ESS and ULMS

	Score	(%)	OR (95% CI)	p Value	Sen (%)	Spe (%)	PPV (%)	NPV (%)	Acc (%)
(%) ER				<0.0001					
LMS	≥8	27.30	1						
ESS	≥8	92.90	34.7 (4.17 to 288)		46.40	97.60	92.90	72.70	76.80
PR				<0.0001					
LMS	≥8	24.50	1						
ESS	≥8	80.00	12.3 (3.45 to 44.1)		57.10	90.20	80.00	75.50	76.80
CD10				0.005					
LMS	≥8	26.20	1						
ESS	≥8	61.50	4.51 (1.58 to 12.9)		59.30	75.60	61.50	73.80	69.10
GEM				0.043					
LMS	≤8	17.60	1						
ESS	≤8	49.00	4.48 (1.14 to 17.6)		88.90	35.90	49.00	82.40	57.60
h-caldesmon				0.024					
LMS	≤8	25.00	1						
ESS	≤8	54.30	3.56 (1.26 to 10.1)		70.40	60.00	54.30	75.00	64.20
Trangelin				0.044					
LMS	≤8	28.90	1						
ESS	≤8	55.20	3.02 (1.10 to 8.32)		59.30	69.20	57.10	71.10	65.20
SMA				0.045					
LMS	≤8	30.00	1						
ESS	≤8	55.60	2.92 (1.06 to 8.06)		55.60	70.00	55.60	70.00	64.20
ASC1				0.29					
LMS	≥8	22.20	1						
ESS	≥8	44.10	2.76 (0.53 to 14.4)		92.90	17.50	44.10	77.80	48.50
Desmin				0.21					
LMS	≤8	20.30	1						
ESS	≤8	47.10	2.04 (0.75 to 5.57)		61.50	56.10	47.10	69.70	58.20
Stathmin1				0.43					

	Score	(%)	OR (95% CI)	p Value	Sen (%)	Spe (%)	PPV (%)	NPV (%)	Acc (%)
LMS	≤8	37.00	1						
ESS	≤8	47.60	1.55 (0.55 to 4.41)		37.00	72.50	47.60%	63.00	58.20

χ^2 test for p values. Score represents composition score based on intensity and % expression.

Acc, accuracy; CD10, cluster of differentiation 10; ER, estrogen receptor; ESS, endometrial stromal sarcoma; GEM, Guanosine-5′- triphosphate (GTP)-binding protein overexpressed in skeletal muscle; and ASC1, solute carrier family 7; LMS, leiomyosarcoma; NPV, negative predictive value; PPV, positive predictive value; PR, progesterone receptor; Sen, sensitivity; SMA, smooth muscle actin; Spe, specificity; ULMS, uterine leiomyosarcoma.

Table 4

Predictive model of ESS over ULMS

Combination of immunomarker expressions	AUC (95% CI)	ΔAUC change	p Value
ER alone	0.728 (0.595 to 0.861)		0.002
ER+PR	0.797 (0.677 to 0.917)	0.069	<0.0001
ER+PR+CD10	0.831 (0.722 to 0.939)	0.034	<0.0001
ER+PR+CD10+GEM	0.823 (0.706 to 0.940)	−0.008	<0.0001
ER+PR+CD10+GEM+h-Caldesmon	0.846 (0.741 to 0.950)	0.023	<0.0001
ER+PR+CD10+GEM+h-Caldesmon+Transgelin	0.872 (0.784 to 0.961)	0.026	<0.0001

Using the six biomarkers shown to be significant in univariate analysis in table 3, receiver operating characteristics curve analysis was performed based on the magnitude of significance (OR) for ESS over ULMS. Delta AUC change represents interval AUC increment change by adding one additional biomarker.

AUC, area under the curve; C-cal, C-caldesmon; CD10, cluster of differentiation 10; ER, estrogen receptor; ESS, endometrial stromal sarcoma; GEM, Guanosine-5′- triphosphate (GTP)-binding protein overexpressed in skeletal muscle; LMS, leiomyosarcoma; PR, progesterone receptor; ULMS, uterine leiomyosarcoma.

Table 5

Comparison of biomarker expressions between LG ESS and 'LG' ULMS

	Score	(%)	OR (95% CI)	p Value	Sen (%)	Spe (%)	PPV (%)	NPV (%)	Acc (%)
ER				<0.0001					
'LG' ULMS	≥8	25.00	1						
LG ESS	≥8	90.90	30.0 (3.36 to 268)		52.60	96.40	90.90	75.00	78.70
PR				<0.0001					
'LG' ULMS	≥8	17.20	1						
LG ESS	≥8	77.80	16.8 (3.86 to 73.1)		73.70	85.70	77.80	82.80	80.90
CD10				0.012					
'LG' ULMS	≥8	24.10	1						
LG ESS	≥8	64.70	5.76 (1.56 to 21.3)		61.10	78.60	64.70	75.90	71.70
GEM				0.22					
'LG ULMS	≤8	14.30	1						
LG ESS	≤8	44.70	4.86 (0.53 to 44.3)		94.40	22.20	44.70	85.70	51.10
C-caldesmon				0.033					
'LG' ULMS	≤8	22.70	1						
LG ESS	≤8	56.50	4.42 (1.21 to 16.1)		72.20	63.00	56.50	77.30	66.70
Transgelin				0.034					
'LG' ULMS	≤8	24.00	1						
LG ESS	≤8	57.10	4.22 (1.20 to 14.9)		66.70	67.90	57.10	76.00	67.40
SMA				0.11					
'LG' ULMS	≤8	30.00	1						
LG ESS	≤8	60.00	3.50 (0.96 to 12.8)		50.00	77.80	60.00	70.00	66.70
ASC1				0.28					
'LG' ULMS	≥8	22.20	1						
LG ESS	≥8	44.70	2.83 (0.52 to 15.5)		89.50	25.00	44.70	77.80	51.10
Desmin				0.14					
'LG' ULMS	≤8	27.30	1						
LG ESS	≤8	50.00	2.67 (0.78 to 9.15)		66.70	57.10	50.00	72.70	60.90
Stathmin1				0.76					

	Score	(%)	OR (95% CI)	p Value	Sen (%)	Spe (%)	PPV (%)	NPV (%)	Acc (%)
'LG' ULMS	≤8	37.00	1						
LG ESS	≤8	44.40	1.36 (0.40 to 4.58)		44.40	63.00%	44.40	63.00	55.60

χ^2 test for p values. Score represents composition score based on intensity and % expression.

Acc, accuracy; CD10, cluster of differentiation 10; ER, estrogen receptor; GEM, Guanosine-5′- triphosphate (GTP)-binding protein overexpressed in skeletal muscle; and ASC1, solute carrier family 7; LG ESS, low-grade endometrial stromal sarcoma; NPV, negative predictive value; PPV, positive predictive value; PR, progesterone receptor; Sen, sensitivity; SMA, smooth muscle actin; Spe, specificity; ULMS, uterine leiomyosarcoma.

Table 6

Predictive model of LG ESS over ‘LG’ ULMS

Combination of antibodies expressions	AUC (95% CI)	ΔAUC change	p Value
ER alone	0.759 (0.602 to 0.916)		0.004
ER+PR	0.844 (0.713 to 0.974)	0.085	<0.0001
ER+PR+CD10	0.877 (0.763 to 0.990)	0.033	<0.0001
ER+PR+CD10+h-Cal	0.903 (0.816 to 0.991)	0.026	<0.0001
ER+PR+CD10+h-Cal+Trang	0.914 (0.832 to 0.995)	0.011	<0.0001

Using the five significant biomarkers shown to be significant in univariate analysis in table 5, receiver operating characteristics curve analysis was performed based on the magnitude of significance (OR) for LG ESS over ‘LG’ ULMS. Delta AUC change represents interval AUC change by adding one additional biomarker.

AUC, area under the curve; h-Cal, h-caldesmon; CD10, cluster of differentiation 10; ER, estrogen receptor; LG ESS, low-grade endometrial stromal sarcoma; PR, progesterone receptor; Trang, Transgelin; ULMS, uterine leiomyosarcoma.