Alabiad et al. Surgical and Experimental Pathology (2020) 3:13
https://doi.org/10.1186/s42047-020-00065-0

Surgical and Experimental Pathology

RESEARCH Open Access

The values of Transgelin, Stathmin, BCOR and Cyclin-D1 expression in differentiation between Uterine Leiomyosarcoma (ULMS) and Endometrial Stromal Sarcoma (ESS); diagnostic and prognostic implications

Mohamed Ali Alabiad[1], Ola A. Harb[1*], Mustafa Taha Abdelfattah[2], Basant Sh. El-Shafaay[3], Ahmed K. El-Taher[4] and Elsayed I. El-Hendawy[4]

Abstract

Background: Morphologic distinction between uterine leiomyosarcoma (ULMS) and endometrial stromal sarcoma (ESS) alone is not straightforward and has been shown to be challenging especially with poor differentiation, so immunohistochemistry (IHC) is often employed as an adjunct to morphology in uterine sarcoma.

Aim: We aimed to assess the diagnostic utility of Transgelin, Stathmin, BCOR and Cyclin-D1 separately and in-combinations in distinguishing ULMS from ESS, and to evaluate their prognostic value in patients with such sarcoma subtypes.

Material and Methods: We included 44 patients with uterine sarcoma. The diagnostic performances of Transgelin, Stathmin, BCOR and Cyclin-D1 were assessed in samples from all patients using immunohistochemistry.

Results: The combination of Stathmin and Transgelin expression has high sensitivity and specificity for diagnosis of LMS and differentiating it from ESS; 95.5% and associated with poor prognosis in LMS patients.
The combination of BCOR and Cyclin-D1 expression has high sensitivity and specificity for diagnosis of ESS and differentiating it from LMS; 90.9% and 95.5% respectively and associated with poor prognosis in ESS patients. The combination of Stathmin and Transgelin, BCOR and CyclinD1 expression has high sensitivity and specificity for diagnosis of LMS and differentiating it from ESS; 100%.

Conclusion: Combination of Stathmin, Transgelin, BCOR and Cycline-D1 raised the accuracy of differentiation between ULMS and ESS to 100% and has prognostic roles in such sarcomas.

Keywords: Uterine leiomyosarcoma, Endometrial Stromal Sarcoma, diagnosis, prognosis, immunohistochemistry

* Correspondence: olaharb2015@gmail.com
[1]Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Full list of author information is available at the end of the article

Introduction

Uterine sarcoma accounts for 3–7% of all primary uterine malignancies which carry a poor prognosis (Barral et al. 2017), as it is responsible for a large majority of uterine cancer–associated deaths with an overall 5-year survival rate ranging from 17.5 to 54.7% (Chern et al. 2017). Uterine sarcomas include many different histo-pathological subtypes (Benson and Miah 2017). Uterine leiomyosarcoma (ULMS) and endometrial stromal sarcoma (ESS) are among the most frequent uterine sarcomas, which, in adults, lead to fatal lung metastases and patients have an extremely poor prognosis (Tsuyoshi and Yoshida 2018). ULMS accounts for 60-70% of all uterine sarcoma and 1% of all uterine malignancies, but it is most dangerous due to their characteristic chemo-resistance and metastatic potential (Juhasz-Böss et al. 2018). Endometrial stromal sarcomas (ESS) account for < 10% of all uterine sarcomas and < 1% of all uterine malignancies. ESS has high incidence of local recurrence and distant metastasis even 20 years after initial diagnosis (Allen et al. 2017).

Because of the distinct difference in prognosis, management and treatment between ULMS and ESS, the need for an accurate diagnosis is imperative. Immunohistochemistry (IHC) is often employed as an adjunct to morphology in uterine sarcoma as morphologic distinction alone is not straightforward and has been shown to be challenging especially with poor differentiation or in cases with equivocal features (Hwang et al. 2015).

The routine immunomarker panel used by most surgical pathologist to distinguish ESS from ULMS consists of desmin, smooth muscle actin (SMA), h-caldesmon and CD10. Unfortunately, there is much overlap and both entities can be immunoreactive to the same antibodies (Hwang et al. 2015). So a novel marker combination is mandatory for accurate diagnosis (Mikami et al. 2002).

Stathmin1 (*STMN1)* is a cytoplasmic phosphoprotein known as oncoprotein 18 involved in the regulation of the cell cycle as the protein that destabilizes microtubules. It is also known as an activator of the PI3K-AKT-mTOR pathway. *STMN1* expression was associated with shorter progression-free survival and overall survival for all analyzed uterine cancer cases (Davidson and Micci 2017). STMN1 is over expressed across broad range of human malignancies (lymphoma, leukemia, ovarian, breast, uterus, prostate, and lung) (Rana et al. 2008). High expression of stathmin promotes cell proliferation, motility and metastasis of malignant tumors (Davidson and Micci 2017). Transgelin is an actin-binding gelling protein of the calponin family found on the membrane and in the cytoplasm of smooth muscle cells. Transgelin is one of the earliest markers of smooth muscle differentiation and stains visceral and vascular smooth muscle cells, in addition to myofibroblasts and related benign and malignant tumors (Tuffaha et al. 2018). BCOR (BCL6 co-repressor) is a protein coding gene and a transcriptional co-repressor that interacts with BCL6, which in turn, plays a critical role in the formation of germinal center and generation of memory T cells. Gene fusions involving BCOR resulting from chromosomal translocation of t(X; 22)(p11.4;q13.2) have been shown to be associated with a diverse range of human neoplasms (Nagaputra et al. 2019). Cyclin-D1 is one of Mammalian D cyclins (D1, D2, and D3) that coordinate cyclin-dependent kinase 4 (CDK4) and CDK6 to regulate cell cycle transition from G1 to S phase and its Overexpression results in dysregulated CDK activity, rapid cell growth under conditions of restricted mitogenic signaling, bypassing cellular checkpoints keys, and ultimately, neoplastic growth (Qie and Diehl 2016).

Previous studies assessed either the prognostic or the diagnostic values of Transgelin, Stathmin, BCOR and Cyclin-D1 separately in malignant tumors.

But in the current study we aimed to assess the diagnostic accuracy of Transgelin, Stathmin, BCOR and Cyclin-D1 separately and in-combinations in distinguishing ULMS from ESS, and to evaluate their prognostic value in patients with such sarcoma subtypes.

Methods

This retrospective study was approved by the institutional review board at Faculty of Medicine, Zagazig University. A total of 44 patients diagnosed in Pathology Department, Faculty of Medicine, Zagazig University between 2014 and 2018 were studied. These are composed of 22 LMS and 22 ESSs. Surgical management of patients was performed in Zagazig University hospitals; Department of Gynecology and Obstetrics and Department of General Surgery. Prognostic and clinical data of patients were collected from Departments of Clinical Oncology and Nuclear Medicine and Medical Oncology, Faculty of Medicine, Zagazig University.

We made the grading by those systems

ESS is classified by the World Health Organization 2014 into low-grade ESS (LG-ESS), high-grade ESS (HG-ESS), and undifferentiated uterine sarcoma (UUS) (Benson and Miah 2017). LG-ESS resembles proliferative-type endometrial stroma. It is composed of monotonous populations of small cells with round, oval to fusiform nuclei concentrically arranged around thin-walled spiral arterioles. They invade the myometrium in a characteristic fashion as Tongue-like infiltration of the myometrium and "worm-like" plugs in the uterine vessels. Mitotic activity is usually <5/10 high power fields (HPF). While (HG-ESS), is characterized by a proliferation of round cells with nested or pseudoglandular patterns separated by thin-walled vessels. The nuclei of the round cells are

larger and display more irregular nuclear contours. Mitotic activity is >10/10 HPF, and necrosis is common. UUS is considered a diagnosis of exclusion as it lacks stromal differentiation with pleomorphic nuclei. The tumor cells in UUS display severe nuclear atypia, brisk mitotic activity, and necrosis; it ported a dismal prognosis (Hoang et al. 2017). While Leiomyosarcoma is graded according to cellular differentiation, mitotic rate and amount of tumor necrosis into low, intermediate and high grade based on French Federation of Cancer Centers Sarcoma Group (FNCLCC) system (Coindre 2006). At diagnosis, tissue blocks containing the most representative and well-preserved tumor areas were selected for IHC analysis.

Immunohistochemistry

Immunohistochemistry was performed on tissue fixed with 10% neutral buffered formalin.

Every tissue specimen was taken from the tumor focus, and was fixed in 10% buffered formalin, embedded in paraffin and sectioned into 4-μm slices. They were deparaffinized in xylene and then rehydrated through a graded alcohol series. 10 mM Citric acid buffer, pH 6.0 was used as standard microwave-based antigen retrieval methods (70°C for 15 minutes). The endogenous peroxidase activity of specimens was blocked by immersing the slides in a 3% hydrogen peroxide (H2O2) solution in methanol for 25 minutes at room temperature. Briefly, sections were blocked in bovine serum for 20 min to reduce non-specific background staining. The blocked sections were incubated with IHC analyses for Stathmin (Cell Signaling, Danvers, MA; Catalog #3352). Transgelin (Anti-SM22 alpha antibody (ab14106); pre-treatment: citrate antigen retrieval in the Biocare pressure cooker; dilution: 1:3000; source: abcam, Cambridge, Massachusetts), BCOR (clone C-10 (sc-514576; Santa Cruz, Dallas,TX)) and Cyclin-D1 ((Thermoscientific, clone SP4, rabbit monoclonal, 1:50)) were performed.

Positive IHC reactions were defined as dark brown reaction positive cytoplasmic staining for Stathmin and Transgelin and positive nuclear dark brown reaction for BCOR and Cyclin-D1 in tumor cells.

The diagnostic performances of Stathmin, Transgelin, BCOR and Cyclin-D1 were assessed in terms of sensitivity, specificity and accuracy. They were first evaluated for the diagnosis of ULMS and then were compared with ESS.

Evaluation of immunohistochemical expression:

- For Stathmin, cytoplasmic immunoreactivity was scored by its extent and intensity. Staining intensity was graded as follows19: negative (0), weak (1), moderate (2) and strong (3). Staining extent was rated according to the percentage of positive cells. Samples with no stained tumor cells were rated as 0, those with <10% of stained tumor cells were rated as 1, those 11%-50% of stained tumor cells were rated as 2, those with 50-75% of stained tumor cells were rated as 3 and those with >75% of stained tumor cells were rated as 4.19 The score of staining intensity multiplied by the score of extent equals an overall staining score. An overall staining score of 0, 1-3 and >3 were regarded as negative (-) and focal positive (+) and diffuse positive protein expression, respectively (Wang et al. 2014a).
- Transgelin Positivity was further divided into focal (<50% cells labeled) and diffuse (>50% cells labeled) patterns (Tawfik et al. 2014)
- Immunohistochemical staining for BCOR Intensity (strong, moderate, weak, and negative) and estimated percentage of positive tumor cells (nuclear staining only) were evaluated (Chiang et al. 2017).
- Cyclin-D1 immunostaining only nuclear staining was considered positive. Percentage of nuclei showing moderate to strong nuclear Cyclin-D1 staining was assessed by evaluating representative high-power fields (for whole tissue sections) or the entire tissue microarray cores. Tumors showing ≥ 70% moderate to strong nuclear staining for Cyclin-D1 were considered to be positive (Lee et al. 2012).

Statistical analysis

Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Shapiro-Wilk test. Kraskall Wallis H test was used to compare between more than two groups of normally distributed variables. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when was appropriate. Validity of immunohistochemical markers in diagnosis of histopathological type was calculated using diagnostic performance depend on sample 2x2 contingency tables generation. Sensitivity, specificity, positive predictive value, negative predicitive value, accuracy were calculated. All tests were two sided. A p-value < 0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

Results

Demographic results

Samples from 44 cases were included in our study and cases include 22 cases of LMS and 22 cases of ESS. The median age of all patients is 57 years ranged from 39 to

72 years. Clinical Data and pathological parameters are included in Table 1.

Histopathological results Figs. 1 and 2

LMS revealed a fascicular growth pattern (bundles intersect at right angles). Tumor cells merge with blood vessel walls, palisading of spindle cells with eosinophilic fibrillary cytoplasm, focal granularity. Nuclei are cigar-shaped and blunt-ended with variable atypia, often with cytoplasmic vacuoles at both ends of nuclei (unlike neural lesions). Mitotic figures are common, 18/22 cases showed myxoid changes. May have hemangiopericytoma-like vasculature, nuclear palisading, osteoclast-like giant cells, some may show extensive pleomorphism mimicking MFH often infiltrates into adjacent tissue they were graded according French Federation of Cancer Centers Sarcoma Group (FNCLCC) system (Coindre 2006).

Table 1 Comparison between included cases of leiomyosarcoma (LMS) and endometrial stromal sarcoma (ESS) regarding clinicopathological features

Characteristics	All (*N* = 44)	LMS (*N* = 22)	ESS (*N* = 22)
	No. (%)	No. (%)	No. (%)
Age (years)			
Mean ± SD	56.84 ±7.93	62.50 ±4.94	51.18 ±6.10
Median (Range)	57 (39-72)	62 (56-72)	50 (39-65)
≤ 55 years	18 (40.9%)	0 (0%)	18 (81.8%)
> 55 years	26 (59.1%)	22 (100%)	4 (18.2%)
Size			
<5 cm	6 (13.6%)	4 (18.2%)	2 (9.1%)
>5 cm	38 (86.4%)	18 (81.8%)	20 (90.9%)
Grade			
Grade I	9 (20.5%)	5 (22.7%)	4 (18.2%)
Grade II	12 (27.3%)	7 (31.8%)	5 (22.7%)
Grade III	23 (52.3%)	10 (45.5%)	13 (59.1%)
Extrauterine extension			
Absent	6 (13.6%)	4 (18.2%)	2 (9.1%)
Present	9 (20.5%)	5 (22.7%)	4 (18.2%)
N/A	29 (65.9%)	13 (59.1%)	16 (72.7%)
LVSI			
Absent	21 (47.7%)	14 (63.6%)	7 (31.8%)
Present	23 (52.3%)	8 (36.4%)	15 (68.2%)
Bladder/Rectum extension			
Absent	33 (75%)	18 (81.8%)	15 (68.2%)
Present	10 (22.7%)	4 (18.2%)	6 (27.3%)
N/A	1 (2.3%)	0 (0%)	1 (4.5%)
Other pelvic organ invasion			
Absent	15 (34.1%)	8 (36.4%)	7 (31.8%)
Present	29 (65.9%)	14 (63.6%)	15 (68.2%)
Adnexal invasion			
Absent	11 (25%)	7 (31.8%)	4 (18.2%)
Present	33 (75%)	15 (68.2%)	18 (81.8%)
Lymph node			
Negative	16 (36.4%)	10 (45.5%)	6 (27.3%)
Positive	28 (63.6%)	12 (54.5%)	16 (72.7%)
Distant metastasis			
Absent	29 (65.9%)	18 (81.8%)	11 (50%)
Present	15 (34.1%)	4 (18.2%)	11 (50%)

ESS revealed an infiltrative and diffuse proliferation of uniform round or oval cells, abundant small vessels, undifferentiated: pleomorphic round / oval to spindled cells, high mitotic rate, necrosis, monotonous ovoid to spindly cells with minimal cytoplasm intimately associated with prominent arterioles, closely resembles proliferative endometrial stroma. Up to 10 - 15 mitotic figures per 10 HPF in most active areas. Tongue-like infiltration between muscle bundles of myometrium Angiolymphatic invasion common. 17/22 cases showed abundant myxoid matrix, sporadic cases may show epithelioid and fibrous change, foam cells or hyalinization. They were graded according World Health Organization 2014.

Immunohistochemical findings

The detailed immunohistochemical characteristics are summarized in Tables 2, 3, 4, 5 and 6 and illustrated in Figs. 3 and 4.

- **Stathmin Expression**

Positive staining for Stathmin was observed in 100% (22/22) of LMS cases; 12 (54.5%) showed diffuse expressions, while the remaining 10(45.5%) cases showed focal expression. On the other hand, focal positivity was found in 2(9%) case and diffuse positivity in 1(4.5%) case of ESS.

The difference between Stathmin expression in the two groups was statistically highly significant ($p < 0.001$).

Diffuse strong positive Stathmin expression in ULMS was statistically significantly associated with large size of the tumor<5cm (13/18 cases)72.2%, higher grade (10/10 cases)100%, advanced stage (12/12cases)100%, (5/5cases)100% of extrauterine extension, adnexal invasion (13/15cases)86.8%, and the presence of lymphovascular invasion (8/8cases)100%, lymph node metastases (12/12cases)100%, (4/4cases)100% of presence of bladder,/rectum extension, and (4/4cases) 100% of the presence of distant metastases.

All cases of LMS were positive for Stathmin expression, whereas most ESS was negative for Stathmin expression. For each threshold for positivity, the sensitivity, specificity, positive predictive value, and negative

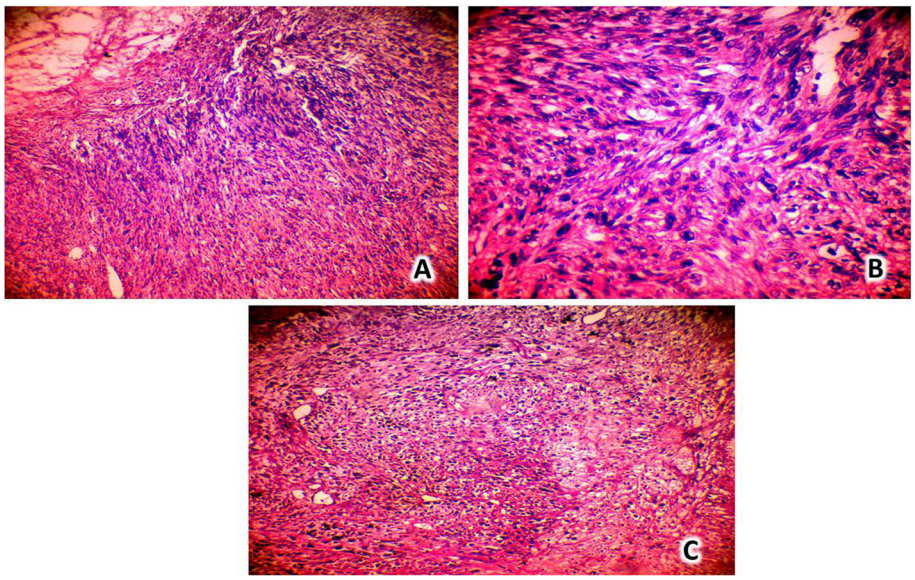

Fig. 1 Histophathological features of leiomyosarcoma (LMS). **a** & **b** revealed a fascicular growth pattern (bundles intersect at right angles), palisading of spindle cells with eosinophilic fibrillary cytoplasm, focal granularity. Nuclei are cigar-shaped and blunt-ended with variable atypia, often with cytoplasmic vacuoles at both ends of nuclei (unlike neural lesions). Mitotic figures are common, **c** Focal showed myxoid changes. Hematoxylin & Eosin stain; Magnification A original magnification x 100 B original magnification x 400 C original magnification x 200

predictive value of the Stathmin as a biomarker for predicting a LMS diagnosis and differentiating it from ESS was calculated.

The sensitivity of the Stathmin expression for diagnosis of LMS and differentiating it from ESS was 100% (95% confidence interval [CI] = 87-100%). However, the specificity was found to be 86.4 % (CI = 72-100%). The negative and positive predictive values for LMS were 100% (CI = 87-100%) and 88% (CI = 75.3-100%) respectively and the accuracy in diagnosis was 93.2%.

- **Transgelin Expression**

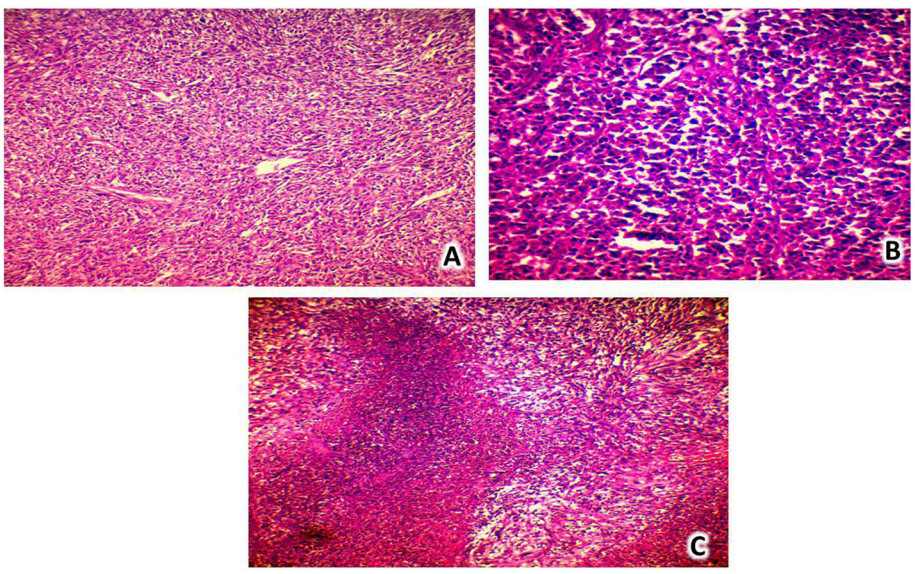

Fig. 2 Histophathological features of endometrial stromal sarcoma (ESS) (**a**& **b**) revealed an infiltrative and diffuse proliferation of uniform round or oval cells, abundant small vessels, undifferentiated: pleomorphic round / oval to spindled cells, high mitotic rate, necrosis, monotonous ovoid to spindly cells with minimal cytoplasm intimately associated with prominent arterioles, closely resembles proliferative endometrial stroma (**c**) ESS cases showed abundant myxoid matrix. Hematoxylin & Eosin stain; Magnification (**a**) original magnification x 100 (**b**) original magnification x 400 (**c**) original magnification x 200

Table 2 Correlations between clinicopathological features and Stathmin expression in all the included cases

Characteristics	All (*N* = 44)	Stathmin			*p*-value
		Negative (*N* = 19)	Focal weak +ve (*N* = 12)	Diffuse strong +ve (*N* = 13)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	56.84 ±7.93	51.31 ±6.30	62.33 ±6.56	59.84 ±6.16	<0.001*
Median (Range)	57 (39-72)	50 (39-65)	62.50 (52-72)	60 (44-70)	
≤ 55 years	18 (40.9%)	15 (83.3%)	2 (11.1%)	1 (5.6%)	<0.001‡
> 55 years	26 (59.1%)	4 (15.4%)	10 (38.5%)	12 (46.2%)	
Histopathology					
LMS	22 (50%)	0 (0%)	10 (45.5%)	12 (54.5%)	<0.001‡
ESS	22 (50%)	19 (86.4%)	2 (9.1%)	1 (4.5%)	
Size					
<5 cm	6 (13.6%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.046‡
>5 cm	38 (86.4%)	17 (44.7%)	8 (21.1%)	13 (34.2%)	
Grade					
Grade I	9 (20.5%)	4 (44.4%)	5 (55.6%)	0 (0%)	0.015‡
Grade II	12 (27.3%)	5 (41.7%)	5 (41.7%)	2 (16.7%)	
Grade III	23 (52.3%)	10 (43.5%)	2 (8.7%)	11 (47.8%)	
Extrauterine extension					
Absent	6 (13.6%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.003‡
Present	9 (20.5%)	4 (44.4%)	5 (55.6%)	0 (0%)	
N/A	29 (65.9%)	13 (44.8%)	3 (10.3%)	13 (44.8%)	
LVSI					
Absent	21 (47.7%)	7 (33.3%)	10 (47.6%)	4 (19%)	0.014‡
Present	23 (52.3%)	12 (52.2%)	2 (8.7%)	9 (39.1%)	
Adnexal invasion					
Absent	11 (25%)	4 (36.4%)	7 (63.6%)	0 (0%)	0.003‡
Present	33 (75%)	15 (45.5%)	5 (15.2%)	13 (39.4%)	
Lymph node					
Negative	16 (36.4%)	6 (37.5%)	10 (62.5%)	0 (0%)	<0.001‡
Positive	28 (63.6%)	13 (46.4%)	2 (7.1%)	13 (46.4%)	
FIGO Stage					
Stage I	6 (13.6%)	2 (33.3%)	4 (66.7%)	0 (0%)	0.001‡
Stage II	10 (22.7%)	4 (40%)	6 (60%)	0 (0%)	
Stage III	13 (29.5%)	5 (38.5%)	0 (0%)	8 (61.5%)	
Stage IV	15 (34.1%)	8 (53.3%)	2 (13.3%)	5 (33.3%)	
Transgelin					
Negative	25 (56.8%)	19 (76%)	5 (20%)	1 (4%)	<0.001‡
Focal weak +ve	6 (13.6%)	0 (0%)	6 (100%)	0 (0%0	
Diffuse strong +ve	13 (29.5%)	0 (0%)	1 (7.7%)	12 (92.3%)	
BCOR					
Negative	23 (52.3%)	4 (17.4%)	10 (43.5%)	9 (39.1%)	0.007‡
Focal weak +ve	7 (15.9%)	5 (71.4%)	0 (0%)	2 (28.6%)	
Diffuse strong +ve	14 (31.8%)	10 (71.4%)	2 (14.3%)	2 (14.3%)	
Cyclin-D1					
Negative	24 (54.5%)	2 (8.3%)	10 (41.7%)	12 (50%)	<0.001‡
Focal weak +ve	5 (11.4%)	5 (100%)	0 (0%)	0 (0%)	
Diffuse strong +ve	15 (34.1%)	12 (80%)	2 (13.3%)	1 (6.7%)	

* Kruskal Wallis H test; ‡ Chi-square test; $p < 0.05$ is significant

Table 3 Correlations between clinicopathological features and Transgelin expression in all the included cases

Characteristics	All (*N* = 44)	Transgelin			*p*-value
		Negative (*N* = 25)	Focal weak +ve (*N* = 6)	Diffuse strong +ve (*N* = 13)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	56.84 ±7.93	52.84 ±7.56	65 ±4.97	60.76 ±4.16	<0.001*
Median (Range)	57 (39-72)	51 (39-72)	63.50 (59-72)	60 (56-70)	
≤ 55 years	18 (40.9%)	18 (100%)	0 (0%)	0 (0%)	<0.001‡
> 55 years	26 (59.1%)	7 (26.9%)	6 (23.1%)	13 (50%)	
Histopathology					
LMS	22 (50%)	3 (13.6%)	6 (27.3%)	13 (59.1%)	<0.001‡
ESS	22 (50%)	22 (100%)	0 (0%)	0 (0%)	
Grade					
Grade I	9 (20.5%)	7 (77.8%)	2 (22.2%)	0 (0%)	0.014‡
Grade II	12 (27.3%)	5 (41.7%)	4 (33.3%)	3 (25%)	
Grade III	23 (52.3%)	13 (56.5%)	0 (0%)	10 (43.5%)	
Extrauterine extension					
Absent	6 (13.6%)	5 (83.3%)	1 (16.7%)	0 (0%)	<0.001‡
Present	9 (20.5%)	4 (44.4%)	5 (55.6%)	0 (0%)	
N/A	29 (65.9%)	16 (55.2%)	0 (0%)	13 (44.8%)	
LVSI					
Absent	21 (47.7%)	10 (47.6%)	6 (28.6%)	5 (23.8%)	0.022‡
Present	23 (52.3%)	15 (65.2%)	0 (0%)	8 (34.8%)	
Adnexal invasion					
Absent	11 (25%)	7 (63.6%)	4 (36.4%)	0 (0%)	0.007‡
Present	33 (75%)	18 (54.5%)	2 (6.1%)	13 (39.4%)	
Lymph node					
Negative	16 (36.4%)	9 (56.2%)	6 (37.5%)	1 (6.2%)	0.001‡
Positive	28 (63.6%)	16 (57.1%)	0 (0%)	12 (42.9%)	
FIGO Stage					
Stage I	6 (13.6%)	5 (83.3%)	1 (16.7%)	0 (0%)	0.001‡
Stage II	10 (22.7%)	4 (40%)	5 (50%)	1 (10%)	
Stage III	13 (29.5%)	5 (38.5%)	0 (0%)	8 (61.5%)	
Stage IV	15 (34.1%)	11 (73.3%)	0 (0%)	4 (26.7%)	
Stathmin					
Negative	19 (43.2%)	19 (100%)	0 (0%)	0 (0%)	<0.001‡
Focal weak +ve	12 (27.3%)	5 (41.7%)	6 (50%)	1 (8.3%)	
Diffuse strong +ve	13 (29.5%)	1 (7.7%)	0 (0%)	12 (92.3%)	
BCOR					
Negative	23 (52.3%)	7 (30.4%)	6 (26.1%)	10 (43.5%)	0.004‡
Focal weak +ve	7 (15.9%)	5 (71.4%)	0 (0%)	2 (28.6%)	
Diffuse strong +ve	14 (31.8%)	13 (92.9%)	0 (0%)	1 (7.1%)	
Cyclin-D1					
Negative	24 (54.5%)	5 (20.8%)	6 (25%)	13 (54.2%)	<0.001‡
Focal weak +ve	5 (11.4%)	5 (100%)	0 (0%)	0 (0%)	
Diffuse strong +ve	15 (34.1%)	15 (100%)	0 (0%)	0 (0%)	

* Kruskal Wallis H test; ‡ Chi-square test; $p < 0.05$ is significant

Table 4 Correlations between clinicopathological features and BCOR expression in all the included cases

Characteristics	All (*N* = 44)	BCOR			
		Negative (*N* = 23)	Focal weak +ve (*N* = 7)	Diffuse strong +ve (*N* = 14)	*p*-value
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	56.84 ±7.93	62.52 ±4.98	51 ±7.85	50.42 ±4.23	<0.001*
Median (Range)	57 (39-72)	62 (56-72)	51 (39-60)	50 (44-60)	
≤ 55 years	18 (40.9%)	0 (0%)	5 (27.8%)	13 (72.2%)	<0.001‡
> 55 years	26 (59.1%)	23 (88.5%)	2 (7.7%)	1 (3.8%)	
Histopathology					
LMS	22 (50%)	19 (86.4%)	2 (9.1%)	1 (4.5%)	<0.001‡
ESS	22 (50%)	4 (18.2%)	5 (22.7%)	13 (59.1%)	
Grade					
Grade I	9 (20.5%)	9 (100%)	0 (0%)	0 (0%)	<0.001‡
Grade II	12 (27.3%)	6 (50%)	6 (50%)	0 (0%)	
Grade III	23 (52.3%)	8 (34.8%)	1 (4.3%)	14 (60.9%)	
Extrauterine extension					
Absent	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	0.007‡
Present	9 (20.5%)	7 (77.8%)	2 (22.2%)	0 (0%)	
N/A	29 (65.9%)	10 (34.5%)	5 (17.2%)	14 (48.3%)	
LVSI					
Absent	21 (47.7%)	15 (71.4%)	5 (23.8%)	1 (4.8%)	0.001‡
Present	23 (52.3%)	8 (34.8%)	2 (8.7%)	13 (56.5%)	
Other pelvic organ invasion					
Absent	15 (34.1%)	9 (60%)	5 (33.3%)	1 (6.7%)	0.010‡
Present	29 (65.9%)	14 (48.3%)	2 (6.9%)	13 (44.8%)	
Adnexal invasion					
Absent	11 (25%)	11 (100%)	0 (0%)	0 (0%)	0.001‡
Present	33 (75%)	12 (36.4%)	7 (21.2%)	14 (42.4%)	
Lymph node					
Negative	16 (36.4%)	14 (87.5%)	2 (12.5%)	0 (0%)	0.001‡
Positive	28 (63.6%)	9 (32.1%)	5 (17.9%)	14 (50%)	
Distant metastasis					
Absent	29 (65.9%)	19 (65.5%)	7 (24.1%)	3 (10.3%)	<0.001‡
Present	15 (34.1%)	4 (26.7%)	0 (0%)	11 (73.3%)	
FIGO Stage					
Stage I	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	<0.001‡
Stage II	10 (22.7%)	8 (80%)	2 (20%)	0 (0%)	
Stage III	13 (29.5%)	5 (38.5%)	5 (38.5%)	3 (23.1%)	
Stage IV	15 (34.1%)	4 (26.7%)	0 (0%)	11 (73.3%)	
Stathmin					
Negative	19 (43.2%)	4 (21.1%)	5 (26.3%)	10 (52.6%)	0.007‡
Focal weak +ve	12 (27.3%)	10 (83.3%)	0 (0%)	2 (16.7%)	
Diffuse strong +ve	13 (29.5%)	9 (69.2%)	2 (15.4%)	2 (15.4%)	
Transgelin					
Negative	25 (56.8%)	7 (28%)	5 (20%)	13 (52%)	0.004‡

Table 4 Correlations between clinicopathological features and BCOR expression in all the included cases *(Continued)*

Characteristics	All (*N* = 44)	BCOR			
		Negative (*N* = 23)	Focal weak +ve (*N* = 7)	Diffuse strong +ve (*N* = 14)	*p*-value
	No. (%)	No. (%)	No. (%)	No. (%)	
Focal weak +ve	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	
Diffuse strong +ve	13 (29.5%)	10 (76.9%)	2 (15.4%)	1 (7.7%)	
Cyclin-D1					
Negative	24 (54.5%)	21 (87.5%)	2 (8.3%)	1 (4.2%)	<0.001‡
Focal weak +ve	5 (11.4%)	2 (40%)	3 (60%)	0 (0%)	
Diffuse strong +ve	15 (34.1%)	0 (0%)	2 (13.3%)	13 (86.7%)	

* Kruskal Wallis H test; ‡ Chi-square test; $p < 0.05$ is significant

Positive staining for Transgelin was observed in19/22 (96.4 %) of LMS cases; 13 (59.1%) showed diffuse expressions, while 6(27.3%) cases showed focal expression. On the other hand, negative expression was found in all cases of ESS.

The difference between Transgelin expression in the two groups was statistically highly significant ($p < 0.001$).

Diffuse strong positive Transgelin expression in ULMS was statistically significantly associated with large size of the tumor<5cm (13/18 cases)72.2%, higher grade (10/10 cases)100%, advanced stage (12/12cases)100%, (5/5cases) 100%of extrauterine extension, adnexal invasion (13/15 cases)86,6%, and (8/8cases)100% of the presence of lymphovascular invasion, (12/12cases) 100% of lymph node metastases, (4/4cases) 100% of presence of bladder, rectum extension, and (4/4cases) 100%of the presence of distant metastases.

All cases of LMS were positive for Transgelin expression, whereas most ESS were negative for Transgelin expression.

For each threshold for positivity, the sensitivity, specificity, positive predictive value, and negative predictive value of the Transgelin as a biomarker for predicting a LMS diagnosis and differentiating it from ESS was calculated.

The sensitivity of the Transgelin expression for diagnosis of LMS and differentiating it for ESS was 86.4 % (95% confidence interval [CI] = 72-100%). However, the specificity was found to be 100 % (CI = 43-68%). The negative and positive predictive values for LMS were 100% (CI = 87-100%) and 88% (CI = 75-100%) respectively and the accuracy in diagnosis was 93.2%.

- **BCOR Expression**

Positive staining for BCOR was observed in 81.8 % (18/22) of ESS cases; 13(59.1%) showed diffuse expressions, while 5 (22.7%) cases showed focal expression. On the other hand, negative expression was found in 19(86.4%) cases of LMS. Focal positivity was found in 2 (9.1%) cases and diffuse positivity in 1(4.5%) case of ESS.

The difference between BCOR expression in the two groups was statistically highly significant ($p < 0.001$).

Diffuse strong positive BCOR expression in ESS was statistically significantly associated with large size of the tumor<5cm (14/20 cases)70%, higher grade (13/13 cases)100%, advanced stage (14/16cases)87.5%, (4/4cases)100% of extrauterine extension, adnexal invasion (14/18 cases)77.7%, and (14/15cases)93.3% of the presence of lymphovascular invasion, (14/16cases)87.5% of lymph node metastases, (6/6cases)100% of presence of bladder, rectum extension, and (11/11cases)100% of the presence of distant metastases. For each threshold for positivity, the sensitivity, specificity, positive predictive value, and negative predictive value of the BCOR as a biomarker for predicting a LMS diagnosis and differentiating it from ESS was calculated.

The sensitivity of the BCOR expression for diagnosis of ESS and differentiating it from LMS was 86.4 % (95% confidence interval [CI] = 72-100%). The specificity was found to be 81.8 % (CI = 65.7-100%). The negative and positive predictive values for LMS were 85.7% (CI = 70.7-100%) and 82.6% (CI = 67.1-100%) respectively and the accuracy in diagnosis was 84.1%.

- **Cyclin-D1 Expression**

Positive staining for Cyclin-D1 was observed in 20/22 (90.9%) of ESS cases; 15(68.2%) showed diffuse expressions, while 5 (22.7%) cases showed focal expression. On the other hand, negative expression was found in all the 22 cases of LMS.

The difference between Cyclin-D1 expression in the two groups was statistically highly significant ($p < 0.001$).

Diffuse strong positive Cyclin-D1 expression in ESS was statistically significantly associated with large size of the tumor<5cm (15/20 cases)75%, higher grade (13/13 cases)100%, advanced stage (15/16cases)93.7%, (4/4cases)100% of extrauterine extension, adnexal invasion (15/18cases)83.3%, and (15/15cases)100% of the presence of lymphovascular invasion, (15/16cases)93.7% of

Table 5 Correlations between clinicopathological features and Cyclin D1 expression in all the included cases

Characteristics	All (*N* = 44)	Cyclin D1			*p*-value
		Negative (*N* = 24)	Focal weak +ve (*N* = 5)	Diffuse strong +ve (*N* = 15)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Age (years)					
Mean ± SD	56.84 ±7.93	62.16 ±4.91	51.40 ±11.01	50.13 ±3.39	<0.001*
Median (Range)	57 (39-72)	61.50 (56-72)	50 (39-65)	50 (44-55)	
≤ 55 years	18 (40.9%)	0 (0%)	3 (16.7%)	15 (83.3%)	<0.001‡
> 55 years	26 (59.1%)	24 (92.3%)	2 (7.7%)	0 (0%)	
Histopathology					
LMS	22 (50%)	22 (100%)	0 (0%)	0 (0%)	<0.001‡
ESS	22 (50%)	2 (9.1%)	5 (22.7%)	15 (68.2%)	
Grade					
Grade I	9 (20.5%)	7 (77.8%)	2 (22.2%)	0 (0%)	0.006‡
Grade II	12 (27.3%)	7 (58.3%)	3 (25%)	2 (16.7%)	
Grade III	23 (52.3%)	10 (43.5%)	0 (0%)	13 (56.5%)	
Extrauterine extension					
Absent	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	<0.001‡
Present	9 (20.5%)	5 (55.6%)	4 (44.4%)	0 (0%)	
N/A	29 (65.9%)	13 (44.8%)	1 (3.4%)	15 (51.7%)	
LVSI					
Absent	21 (47.7%)	16 (76.2%)	5 (23.8%)	0 (0%)	<0.001‡
Present	23 (52.3%)	8 (34.8%)	0 (0%)	15 (65.2%)	
Bladder/Rectum extension					
Absent	33 (75%)	20 (60.6%)	4 (12.1%)	9 (27.3%)	0.017‡
Present	10 (22.7%)	4 (40%)	0 (0%)	6 (60%)	
N/A	1 (2.3%)	0 (0%)	1 (100%)	0 (0%)	
Adnexal invasion					
Absent	11 (25%)	9 (81.8%)	2 (18.2%)	0 (0%)	0.022‡
Present	33 (75%)	15 (45.5%)	3 (9.1%)	15 (45.5%)	
Lymph node					
Negative	16 (36.4%)	12 (75%)	4 (25%)	0 (0%)	0.001‡
Positive	28 (63.6%)	12 (42.9%)	1 (3.6%)	15 (53.6%)	
Distant metastasis					
Absent	29 (65.9%)	20 (69%)	5 (17.2%)	4 (13.8%)	<0.001‡
Present	15 (34.1%)	4 (26.7%)	0 (0%)	11 (73.3%)	
FIGO Stage					
Stage I	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	<0.001‡
Stage II	10 (22.7%)	6 (60%)	4 (40%)	0 (0%)	
Stage III	13 (29.5%)	8 (61.5%)	1 (7.7%)	4 (30.8%)	
Stage IV	15 (34.1%)	4 (26.7%)	0 (0%)	11 (73.3%)	
Stathmin					
Negative	19 (43.2%)	2 (10.5%)	5 (26.3%)	12 (63.2%)	<0.001‡
Focal weak +ve	12 (27.3%)	10 (83.3%)	0 (0%)	2 (16.7%)	
Diffuse strong +ve	13 (29.5%)	12 (92.3%)	0 (0%)	1 (7.7%)	

Table 5 Correlations between clinicopathological features and Cyclin D1 expression in all the included cases *(Continued)*

Characteristics	All (*N* = 44)	Cyclin D1			*p*-value
		Negative (*N* = 24)	Focal weak +ve (*N* = 5)	Diffuse strong +ve (*N* = 15)	
	No. (%)	No. (%)	No. (%)	No. (%)	
Transgelin					
Negative	25 (56.8%)	5 (20%)	5 (20%)	15 (60%)	<0.001‡
Focal weak +ve	6 (13.6%)	6 (100%)	0 (0%)	0 (0%)	
Diffuse strong +ve	13 (29.5%)	13 (100%)	0 (0%)	0 (0%)	
BCOR					
Negative	23 (52.3%)	21 (91.3%)	2 (8.7%)	0 (0%)	<0.001‡
Focal weak +ve	7 (15.9%)	2 (28.6%)	3 (42.9%)	2 (28.6%)	
Diffuse strong +ve	14 (31.8%)	1 (7.1%)	0 (0%)	13 (92.9%)	

* Kruskal Wallis H test; ‡ Chi-square test; $p < 0.05$ is significant

lymph node metastases, (6/6cases)100% of presence of bladder, rectum extension, and (11/11cases)100% of the presence of distant metastases.

For each threshold for positivity, the sensitivity, specificity, positive predictive value, and negative predictive value of the Cyclin-D1 as a biomarker for predicting a LMS diagnosis and differentiating it from ESS was calculated.

The sensitivity of the Cyclin-D1 expression for diagnosis of ESS and differentiating it from LMS was 100 % (95% confidence interval [CI] = 87-100%). The specificity was found to be 90.9 % (CI = 78.9-100%). The negative and positive predictive values for LMS were 100 % (CI = 87-100%) and 91.7% (CI = 80.6-100%) respectively and the accuracy in diagnosis was 95.5%.

Diagnostic performance of markers combinations

The sensitivity, specificity, accuracy, negative and positive predictive values of the combination of Stathmin and Transgelin expression for diagnosis of LMS and differentiating it from ESS was 95.5% (95% confidence interval [CI] = 86.8-100%).

The sensitivity was 90.9% specificity 95.5% accuracy 39.2%, negative and positive predictive values (91.3%,and 95.2% of the combination of BCOR and CyclinD1 expression for diagnosis of ESS and differentiating it from LMS (95% confidence interval.

The sensitivity, specificity, accuracy, negative and positive predictive values of the combination of Stathmin and Transgelin, BCOR and Cyclin-D1 expression for diagnosis of LMS and differentiating it from ESS was 100% (95% confidence interval).

Discussion

Endometrial stromal sarcomas (ESS) and uterine leiomyosarcomas (ULMS) represent the majority of uterine mesenchymal tumours and the differentiation between them remains a difficult challenge (Tawfik et al. 2014).

The routine differentiation between ESS from ULMS depend on a panel of (SMA – CD10- Desmin-H-caldesmon), mainly positive CD10 and negative smooth muscle actin (SMA) for ESS and positive SMA, negative CD10 for ULMS. But this panel has been shown to be not entirely specific and less helpful in this regard (Hwang et al. 2015).

As SMA and Desmin are generally expressed in many different types of skeletal muscle, smooth muscle, myofibroblastic and myoepithelial tumors, as well as in other lesions, H-caldesmon also is reputedly specific to smooth muscle differentiation but lacks sensitivity (Robin et al. 2013) in addition ESS immune-profile may have a positive expression of both SMA and Desmin (Chu et al. 2001).

In addition endometrial stromal sarcoma variants lose CD10 immunoreactivity especially in high grade and undifferentiated types (Bhargava et al. 2005; Sciallis et al. 2014), on the other hand High-grade leiomyosarcomas exhibit distinct and extensive immunoreactivity for CD10 and different studies have shown that a small population of leiomyoma and leiomyosarcoma are focally positive for CD10. So CD10 expression is not contributory in distinguishing between ESS and high-grade leiomyosarcoma (Mikami et al. 2002).

Stathmin-1 is a cytoplasmic phosphoprotein that regulates microtubules motion during mitosis and interphases hence its importance in oncogenesis and considered a significant indicator of activation of the oncogenic phosphatidylinositol 3-kinase-AKT-mammlian target of rapamycin pathway (PI3K-AKT-mTOR) pathway, a pathway proposed to be centrally associated with smooth muscle proliferation and highly activated in both uterine and extra uterine leiomyosarcoma (Shahin et al. 2019)

In the present study we have found that Stathmin-1 has high significant differentiation between the two groups with specific positivity to leiomyosarcoma, as it was positive in all cases of ULMS and positive only in 3/

Table 6 Diagnostic performance of Stathmin, Transgelin, BCOR and Cyclin-D1 expressions in differentiation between Leiomyosarcoma (LMS) and Endometrial stromal sarcoma (ESS)

Markers	TP	FP	TN	FN	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy (95% CI)
Stathmin	22	3	19	0	100%	86.4% (72-100)	88% (75.3-100)	100%	93.2% (85.()100)
Transgelin	19	0	22	3	86.4% (72-100)	100%	100%	88% (75.()100)	93.2% (85.7-100)
BCOR	19	4	18	3	86.4% (72-100)	81.8% (65.7-100)	82.6% (67.1-100)	85.7% (70.7-100)	84.1% (73.3-94.9)
Cyclin D1	22	2	20	0	100%	90.9% (78.()100)	91.7% (80.()100)	100%	95.5% (89.()100)
Stathmin & Transgelin	21	1	21	1	95.5% (86.()100)	95.5% (86.()100)	95.5% (86.()100)	95.5% (86.()100)	95.5% (89.()100)
Stathmin & BCOR	22	3	19	0	100%	86.4% (72-100)	88% (75.()100)	100%	93.2% (85.()100)
Stathmin & Cyclin D1	22	2	20	0	100%	90.9% (78.()100)	91.7% (80.()100)	100%	95.5% (89.()100)
Transgelin & BCOR	20	1	21	2	90.9% (78.()100)	95.5% (86.()100)	95.2% (86.()100)	91.3% (79.()100)	93.2% (85.()100)
Transgelin & Cyclin D1	22	1	21	0	100%	95.5% (86.()100)	95.7% (87.()100)	100%	97.7% (93.()100)
BCOR & Cyclin D1	20	1	21	2	90.9% (78.()100)	95.5% (86.()100)	95.2% (86.()100)	91.3% (79.()100)	93.2% (85.()100)
Stathmin, Transgelin & BCOR	21	1	21	1	95.5% (86.()100)	95.5% (86.()100)	95.5% (86.()100)	95.5% (86.()100)	95.5% (89.()100)
Stathmin, Transgelin & Cyclin D1	22	1	21	0	100%	95.5% (86.()100)	95.7% (87.()100)	100%	97.7% (93.()100)
Transgelin, BCOR & Cyclin D1	20	0	22	2	90.9% (78.()100)	100%	100%	91.7% (80.()100)	95.5% (89.()100)
Stathmin, BCOR & Cyclin D1	21	0	22	1	95.5% (86.()100)	100%	100%	95.7% (87.()100)	97.7% (93.()100)
Stathmin, Transgelin, BCOR & Cyclin D1	22	0	22	0	100%	100%	100%	100%	100%

TP True positive, *FP* False positive, *TN* True negative, *FN* False negative, *SN* Sensitivity, *SP* Specificity, *PPV* Positive Predictive Value, *NPV* Negative Predictive Value, *95%CI* 95% Confidence Interval; $p < 0.05$ is significant

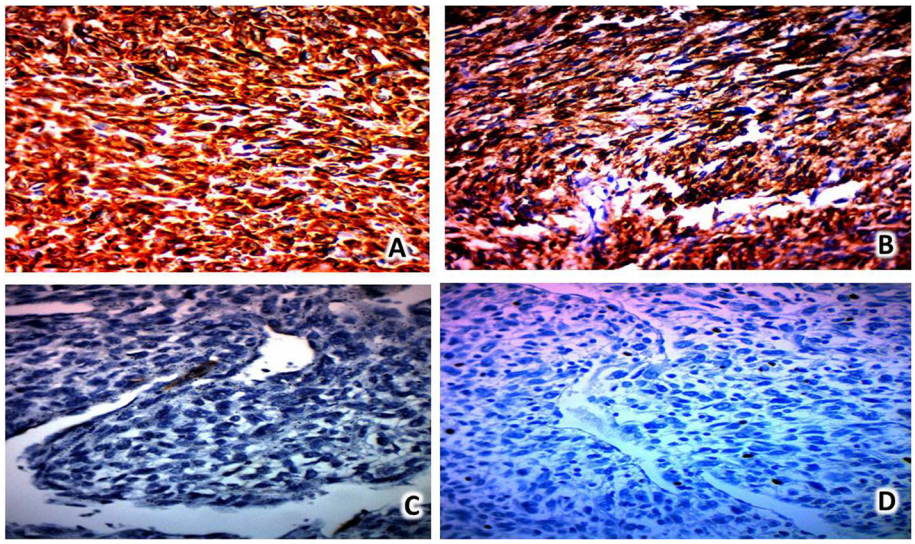

Fig. 3 Immunohistochemical profile of leiomyosarcoma (LMS) (**a**) diffuse strong cytoplasmic positivity for Stathmin (**b**) Diffuse strong cytoplasmic positivity for Transgelin. (**c**) Diffuse nuclear negativity for BCOR (**d**) Diffuse nuclear negativity for Cyclin-D1. Streptavidine biotin method. Magnification (**a**) original magnification x 400 (**b**) original magnification x 400 (**c**) original magnification x 400 (**d**) Original magnification x 200

22 of ESS cases, two of them were focally positive, whereas the sensitivity of the Stathmin-1 expression was 100%. However, the specificity was found to be only 86.4%; the positive and negative predictive values were 88% and 100% respectively.

That was similar to results of (Allen et al. 2015) Stathmin-1 sensitivity, specificity, positive predictive values and negative predictive values were 100 % 69 %, 67% and 100 % respectively.

Similarly, (Shahin et al. 2019) who found Stathmin-1 sensitivity, specificity, positive predictive values and negative predictive values were 80 % 66 %, 60% and 84 % respectively.

(Hwang et al. 2015) Found different results for us as he found Stathmin-1 sensitivity, specificity, negative predictive values and positive predictive values were 37%, 72.5.%, 47.6% and 63% respectively, The difference in results between our series and that might due to different

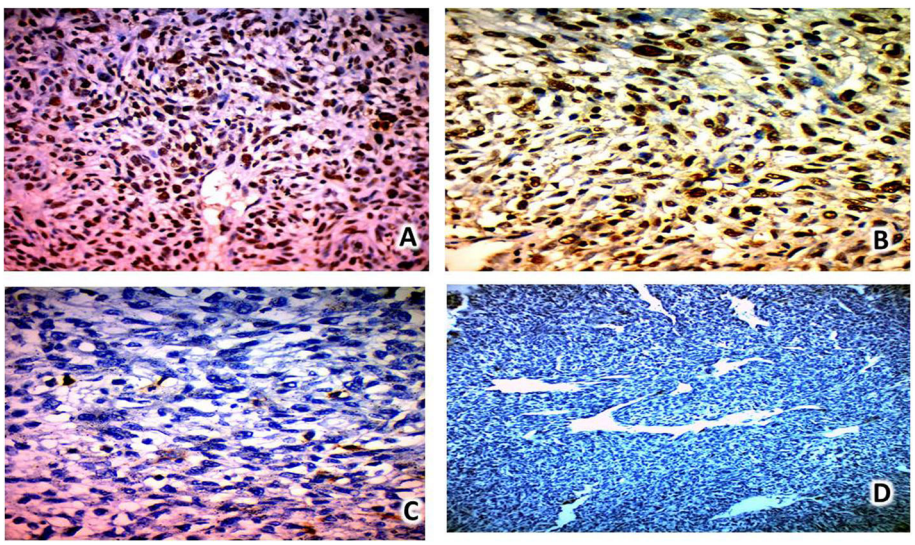

Fig. 4 Immunohistochemical profile of endometrial stromal sarcoma (ESS) (**a**) diffuse strong nuclear positivity for BCOR (**b**) Diffuse strong nuclear positivity for Cyclin-D1 (**c**) Diffuse cytoplasmic negativity for Stathmin. (**d**) Diffuse cytoplasmic negativity for Transgelin; Streptavidin biotin method. Magnification; (**a**) original magnification x 400 (**b**) original magnification x 400 (**c**) original magnification x 400. **d** Original magnification x 100

in number of cases and the differing scoring systems used.

As we proved that high Stathmin-1 expression was correlated significantly with poor clinicopathological outcomes where it was its strong positivity significantly correlated with all the prognostic parameters as (high grade III, large size cases, extra-uterine extension, lymph-vascular space invasion, bladder/rectum extension, adnexal invasion, lymph node metastasis, distant metastasis, and advanced FIGO stage. This agree with results found by (Shahin et al. 2019) whom found that Stathmin-1 expression was strong positive in 5 out of 6 cases with recurrence/distant metastasis, Stathmin-1 over expression is a good indicator for poor prognosis.

Also Satathemin-1 had a strong expression correlation with poor prognostic clinico-pathological parameters in esophageal carcinoma (Wang et al. 2014b), cholangiocarcinoma (Watanabe et al. 2014) and bladder cancer (Hemdan et al. 2014).

As construction of cancer specific effector drugs will change the strategy and tactic of cancer therapy. STMN1 proved to have stronger and a significant therapeutic target efficacy with a high affinity and potency (Hemdan et al. 2014). Target-specific anti-Stathmin drugs have been demonstrated to reduce cell proliferation, clonal growth, cell motility, distant metastasis, and increase apoptosis of malignant tumors (Biaoxue et al. 2016). Also it is a promising molecular target for controlling cancer progression and chemotherapeutic resistance via p27 regulation (Watanabe et al. 2014).

In our study we have found that Transgelin has high significant differentiation between ULMS and ESS with specific positivity to ULMS as it was positive in 19/22 (96.4 %) cases of ULMS and negative in all cases of ESS, , whereas Transgelin sensitivity, specificity, positive predictive values and negative predictive values were 86.4%, 100%, 100% and 88% respectively.

Near to our results, were results of (Robin et al. 2013) who found Transgelin sensitivity, specificity, positive predictive values and negative predictive values were 83 % 82 %, 67% and 92 % respectively. Also (Tawfik et al. 2014) found that both sensitivity and specificity of Transgelin were 100%.

Different from our results Hwang et al., (Hwang et al. 2015) who found Transgelin sensitivity, specificity, positive predictive values and negative predictive values were 59.3 %, 69.2 %, 57.1% and 71.1 % respectively. The difference in results between our series and that might due to different in number of cases and the differing scoring systems used.

Also we proved that high Transgelin expression was correlated significantly with poor clinicopathological outcomes where it was its strong positivity significantly correlated with all the prognostic parameters as (high grade III, large size cases, extra-uterine extension, lymph-vascular space invasion, bladder/rectum extension, adnexal invasion, lymph node metastasis, distant metastasis, and advanced FIGO stage, Transgelin is a good prognostic indicator marker.

In our study we have found that BCOR has high significant differentiation between ULMS and ESS with specific positivity towards ESS, as it was positive in 81.8 % (18/22) cases of ESS and negative in 19(86.4%) cases of ULMS, where the sensitivity, specificity, positive predictive values and negative predictive values were 86.4%, 81.8%, 82.6.7% and 85.7% respectively.

Near to our results Chiang et al., (Chiang et al. 2017) and [Hoang et al., (Hoang et al. 2017) whom found that BCOR sensitivity was 100%, and (Lewis et al. 2018) Found that BCOR sensitivity was 78.5%.

As we proved that high BCOR expression was found to be correlated significantly with poor clinicopathological outcomes, where it was its strong positivity significantly correlated with all the prognostic parameters as (high grade III, large size cases, extra-uterine extension, lymph-vascular space invasion, bladder/rectum extension, adnexal invasion, lymph node metastasis, distant metastasis, and advanced FIGO stage.

This agree with results found by (Chiang et al. 2017) who found high BCOR expression found in all high grade and all metastatic cases and (Lewis et al. 2018) found high expression of BCOR in all cases of lymph node metastasis and high FIGO stage, also (Hoang et al. 2017) found high BCOR expression in all cases associated with an aggressive clinical course, including (multisite bony metastases, progressive peritoneal disease and metastases to the lung and skin). (Mariño-Enriquez et al. 2018) reported high expression of BCOR in cases with high grade and lymph-vascular invasion.

In the present study we have found that Cyclin-D1 has high significant differentiation between the two groups with specific positivity to endometrial stromal sarcoma as as it was positive in 20/22 (90.9%), cases of ESS and negative in all cases of ULMS, whereas, the sensitivity, specificity, positive predictive values and negative predictive values were 100%, 90.9%, 91.7% and 100% respectively.

That was similar to (Lee et al. 2012) who found Cyclin-D1 sensitivity; specificity, positive predictive values and negative predictive values were 100 % 99 %, 80% and 100 % respectively.

Near to our results (Shelly et al. 2017) who stated that the sensitivity and specificity of Cycline-D1, in high grade ESS were 100% and 99%, respectively and (Nagaputra et al. 2019) and (Sciallis et al. 2014) whom found sensitivity of Cycline-D1, in high grade ESS was 88% and 83.3% respectively.

(Kurihara et al. 2010) Found different results for us as he found that the sensitivity and specificity of cycline-

D1, in high grade ESS were 61% and100%, respectively, on the other hand (Sciallis et al. 2014) stated that cycline-D1 had high sensitivity but low specificity to ESS.

Cyclin D1 overexpression correlates with tumor metastasis and poor prognosis in a series of human cancers as pancreatic adenocarcinoma, cutaneous melanoma, endometrial cancer, colorectal carcinoma, and MCL, as it influences local invasion, metastasis, and patients' prognosis (Qie and Diehl 2016).

We proved in this study that high Cyclin D1 expression was found to be correlated significantly with poor clinicopathological outcomes, where it was its strong positivity significantly correlated with all the prognostic parameters as (high grade III, large size cases, extra-uterine extension, lymph-vascular space invasion, bladder/rectum extension, adnexal invasion, lymph node metastasis, distant metastasis, and advanced FIGO stage.

This agrees with results found by (Sciallis et al. 2014) who proved that high expression of Cycline-D1 often present in cases with high stages and those whom behaved aggressively.

As Cyclin-D1-CDK4 has a critical role in regulating cell cycle progression, this emphasizes the potential role of CDK inhibitors as a target therapy and highlights the importance of cyclin-D1 expression in ESS. In addition Cyclin-D1 does not possess enzymatic activity, making it a challenging therapeutic target (Qie and Diehl 2016).

We proved in our study that the combination of the four markers (Stathmin, Transgelin, BCOR & Cyclin D1) gives the best results rather than the combination of two or three markers wherese it raised all parameters including (the sensitivity, specificity, positive predictive value, negative predictive value and accuracy) up to 100%.

Conclusion

Combination of Stathmin-1 and Transgelin are highly sensitive and specific diagnostic and prognostic markers for ULMS, whereas combination of BCOR and Cycline-D1 are highly sensitive and specific diagnostic and prognostic markers for ESS and the combination of Transgelin, BCOR, Cycline-D1, and Stathmin-1 raised the accuracy of differentiation between ULMS and ESS to 100%.

Abbreviations

ULMS: uterine leiomyosarcoma; ESS: endometrial stromal sarcoma; IHC: immunohistochemistry; *STMN1*: Stathmin1; BCOR: BCL6 co-repressor; CDK4: cyclin-dependent kinase 4

Acknowledgements

NA

Authors' contributions

All authors share in data collection, processing, writing and revision of the manuscript before publications. The author(s) read and approved the final manuscript.

Authors' information

NA

Funding

No Funds were received

Availability of data and materials

All data are available

Ethics approval and consent to participate

Ethics approval and consent to participate has been obtained from the local ethical committee of faculty of medicine, Zagazig University.

Consent for publication

Consent for publication has been obtained

Competing interests

No conflicts of interest were obtained

Author details

[1]Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. [2]Gynecology and Obstetrics Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. [3]Clinical oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. [4]General Surgery Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Received: 24 December 2019 Accepted: 20 May 2020
Published online: 11 June 2020

References

Allen AJ, Ali SM, Gowen K, Elvin JA, Pejovic T (2017) A recurrent endometrial stromal sarcoma harbors the novel fusion JAZF1-BCORL1. Gynecologic Oncol Reports 20:51

Allen MML, Douds JJ, Liang SX, Desouki MM, Parkash V, Fadare O (2015) An immunohistochemical analysis of stathmin 1 expression in uterine smooth muscle tumors: differential expression in leiomyosarcomas and leiomyomas. Int J Clin Exp Pathol 8(3):2795

Barral M, Placé V, Dautry R, Bendavid S, Cornelis F, Foucher R, Guerrache Y, Soyer P (2017) Magnetic resonance imaging features of uterine sarcoma and mimickers. Abdominal Radiol 42(6):1762–1772

Benson C, Miah AB (2017) Uterine sarcoma–current perspectives. Int J Womens Health 9:597

Bhargava R, Shia J, Hummer AJ, Thaler HT, Tornos C, Soslow RA (2005) Distinction of endometrial stromal sarcomas from 'hemangiopericytomatous' tumors using a panel of immunohistochemical stains. Mod Pathol 18(1):40

Biaoxue R, Xiguang C, Hua L, Shuanying Y (2016) Stathmin-dependent molecular targeting therapy for malignant tumor: the latest 5 years' discoveries and developments. J Transl Med 14(1):279

Chern JY, Boyd SLR, Stephanie V, Blank SV (2017) Uterine Sarcomas: The Latest Approaches for These Rare but Potentially Deadly Tumors. Oncology 31(3):235-46

Chiang S, Lee CH, Stewart CJ, Oliva E, Hoang LN, Ali RH, Hensley ML, Arias-Stella JA III, Frosina D, Jungbluth AA, Benayed R (2017) BCOR is a robust diagnostic immunohistochemical marker of genetically diverse high-grade endometrial stromal sarcoma, including tumors exhibiting variant morphology. Mod Pathol 30(9):1251

Chu PG, Arber DA, Weiss LM, Chang KL (2001) Utility of CD10 in distinguishing between endometrial stromal sarcoma and uterine smooth muscle tumors: an immunohistochemical comparison of 34 cases. Mod Pathol 14(5):465

Coindre JM (2006) Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med 130(10):1448–1453

Davidson B, Micci F (2017) Molecular characteristics of uterine sarcomas. Expert Rev Mol Diagn 17(5):515–522

Hemdan T, Lindén M, Lind SB, Namuduri AV, Sjöstedt E, De Ståhl TD, Asplund A, Malmström PU, Segersten U (2014) The prognostic value and therapeutic target role of stathmin-1 in urinary bladder cancer. Br J Cancer 111(6):1180

Hoang LN, Aneja A, Conlon N, Delair DF, Middha S, Benayed R, Hensley ML, Park KJ, Hollmann TJ, Hameed MR, Antonescu CR (2017) Novel high-grade endometrial stromal sarcoma: a morphologic mimicker of myxoid leiomyosarcoma. Am J Surg Pathol 41(1):12

Hwang H, Matsuo K, Duncan K, Pakzamir E, Pham HQ, Correa A, Fedenko A, Mhawech-Fauceglia P (2015) Immunohistochemical panel to differentiate endometrial stromal sarcoma, uterine leiomyosarcoma and leiomyoma: something old and something new. J Clin Pathol 68(9):710–717
Juhasz-Böss I, Gabriel L, Bohle RM, Horn LC, Solomayer EF, Breitbach GP (2018) Uterine leiomyosarcoma. Oncol Res Treatment 41
Kurihara S, Oda Y, Ohishi Y, Kaneki E, Kobayashi H, Wake N, Tsuneyoshi M (2010) Coincident expression of β-catenin and cyclin D1 in endometrial stromal tumors and related high-grade sarcomas. Mod Pathol 23(2):225
Lee CH, Ali RH, Rouzbahman M, Marino-Enriquez A, Zhu M, Guo X, Brunner AL, Chiang S, Leung S, Nelnyk N, Huntsman DG (2012) Cyclin D1 as a diagnostic immunomarker for endometrial stromal sarcoma. Am J Surg Pathol 36(10):1562
Lewis N, Soslow RA, Delair DF, Park KJ, Murali R, Hollmann TJ, Davidson B, Micci F, Panagopoulos I, Hoang LN, Arias-Stella JA III (2018) ZC3H7B-BCOR high-grade endometrial stromal sarcomas: a report of 17 cases of a newly defined entity. Mod Pathol 31(4):674
Mariño-Enriquez A, Lauria A, Przybyl J, Ng TL, Kowalewska M, Debiec-Rychter M, Ganesan R, Sumathi V, George S, McCluggage WG, Nucci MR (2018) BCOR internal tandem duplication in high-grade uterine sarcomas. Am J Surg Pathol 42(3):335–341
Mikami Y, Hata S, Kiyokawa T, Manabe T (2002) Expression of CD10 in malignant Müllerian mixed tumors and adenosarcomas: an immunohistochemical study. Mod Pathol 15(9):923
Nagaputra JC, Goh RCH, Kuick CH, Chang KTE, Sittampalam K (2019) ZC3H7B-BCOR high-grade endometrial stromal sarcoma with osseous metaplasia: Unique feature in a recently defined entity. Human Pathol Case Reports 15:54–58
Qie S, Diehl JA (2016) Cyclin D1, cancer progression, and opportunities in cancer treatment. J Mol Med 94(12):1313–1326
Rana S, Maples PB, Senzer N, Nemunaitis J (2008) Stathmin 1: a novel therapeutic target for anticancer activity. Expert Rev Anticancer Ther 8(9): 1461–1470
Robin YM, Penel N, Pérot G, Neuville A, Vélasco V, Ranchere-Vince D, Terrier P, Coindre JM (2013) Transgelin is a novel marker of smooth muscle differentiation that improves diagnostic accuracy of leiomyosarcomas: a comparative immunohistochemical reappraisal of myogenic markers in 900 soft tissue tumors. Mod Pathol 26(4):502
Sciallis AP, Bedroske PP, Schoolmeester JK, Sukov WR, Keeney GL, Hodge JC, Bell DA (2014) High-grade endometrial stromal sarcomas: a clinicopathologic study of a group of tumors with heterogenous morphologic and genetic features. Am J Surg Pathol 38(9):1161–1172
Shahin NA, Al-khader A, Alsayed M, Aljarrah D, Khader M (2019) Differential Expression, Diagnostic and Prognostic Significance of Stathmin-1 and CD147 in Various Uterine Smooth Muscle Tumors. Int J Morphol 37(1):163-71
Shelly D, Ahmed I, Srinivasagowda SK, Bharadwaj R (2017) CyclinD1 Positive High-Grade Endometrial Stromal Sarcoma: a Fascinating Entity! Ann Pathol Laboratory Med 4(5):160–164
Tawfik O, Rao D, Nothnick WB, Graham A, Mau B, Fan F (2014) Transgelin, a novel marker of smooth muscle differentiation, effectively distinguishes endometrial stromal tumors from uterine smooth muscle tumors. Int J Gynecol Obstetrical Reproductive Med Res 1(1):26
Tsuyoshi H, Yoshida Y (2018) Molecular biomarkers for uterine leiomyosarcoma and endometrial stromal sarcoma. Cancer Sci 109(6):1743
Tuffaha MS, Guski H, Kristiansen G (2018) Markers and Immunoprofile of Muscle Tumors. In Immunohistochemistry in Tumor Diagnostics. Springer, Cham, pp 209–216
Wang, F., Xuan, X.Y., Yang, X., Cao, L., Pang, L.N., Zhou, R., Fan, Q.X. and Wang, L.X. ,. Stathmin is a marker of progression and poor prognosis in esophageal carcinoma. Asian Pac J Cancer Prev, 2014a; 15(8) : 3613-8.
Wang, F., Xuan, X.Y., Yang, X., Cao, L., Pang, L.N., Zhou, R., Fan, Q.X. and Wang, L.X. Stathmin is a marker of progression and poor prognosis in esophageal carcinoma. Asian Pac J Cancer Prev, 2014b; 15(8):.3613-8.
Watanabe A, Suzuki H, Yokobori T, Tsukagoshi M, Altan B, Kubo N, Suzuki S, Araki K, Wada S, Kashiwabara K, Hosouchi Y (2014) Stathmin1 regulates p27 expression, proliferation and drug resistance, resulting in poor clinical prognosis in cholangiocarcinoma. Cancer Sci 105(6):690–696

Publisher's Note