

CyclinD1 Positive High-Grade Endometrial Stromal Sarcoma: A Fascinating Entity!

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ABSTRACT

The 2014 WHO classifies endometrial stromal tumours into endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS), high-grade endometrial stromal sarcoma (HGESS), and undifferentiated uterine sarcoma (UUS). LGESS and HGESS are histomorphologically, immunohistochemically and genetically distinct from each other.

A 51-year-old postmenopausal lady presented to us with vaginal bleeding. Radiological findings revealed a well defined heterogeneous lesion involving the whole of uterus. Hysterectomy revealed a large polypoidal tumour, occupying the entire uterine cavity. Microscopically, the tumor was predominantly composed of epithelioid cells with very few intervening spindle cell areas. Immunohistochemically, epithelioid cells were diffusely positive for cyclinD1, while were negative for CD10, ER, PR. Diagnosis of cyclinD1 positive HGESS was rendered. This case highlights the importance of performing cyclinD1 immunostaining in diagnosing HGESS.

Keywords: CyclinD1, Endometrial Stromal Sarcoma, High-grade

Introduction

Endometrial stromal tumours (EST) of the uterus are uncommon tumours, which account for less than 2% of all uterine tumours. The 2014 WHO classifies these tumours into endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS), high-grade endometrial stromal sarcoma (HGESS), and undifferentiated uterine sarcoma (UUS).^[1-3] Out of all these entities, HGESS is unique and has gone through several modifications since the earliest study by Norris and Taylor.^[4] This entity was removed from WHO 2003 classification, however, with progress in molecular understanding of ESSs, and identification of *YWHAE-NUTM2A/B* (previously known as *YWHAE-FAM22A/B*) gene fusion, HGESS has again been reintroduced in the updated 2014 WHO classification of uterine mesenchymal tumours. LGESS and HGESS are not only histomorphologically and genetically distinct but also express different immunomarkers. While LGESS usually expresses strong CD10, ER, PR; HGESS is typically negative for CD10, ER, PR.^[3] Although molecular confirmation (RT-PCR or FISH analysis) currently represents the standard to establish a definitive diagnosis of *YWHAE-NUTM2A/B* ESS, these tests are presently offered only at a few centers. Nevertheless, it is necessary to identify HGESS, as these patients may not respond to anti-estrogenic therapy unlike LGESS and the prognosis of *YWHAE*-rearranged cases is intermediate between LGESS and UUS.^[3] Lee et al has demonstrated consistent upregulation cyclinD1 in *YWHAE-NUTM2* ESS.^[5] Their study also revealed that *YWHAE*-

rearranged cases show diffuse ($\geq 70\%$) moderate to strong immunostaining for cyclinD1 in tumour cells. Thus they deduced that cyclin D1 can be used as a surrogate marker for identification of *YWHAE-NUTM2* ESS in appropriate setting.^[5] Here we present a case of HGESS, which was diagnosed based upon diffuse cyclinD1 positivity.

Case Report

A 51-year-old postmenopausal lady presented to us with history of off and on vaginal bleeding of 01 year duration. Per abdominal findings were a 12 weeks size mass which was firm, tender with smooth well defined borders.

Radiological Findings: She underwent transabdominal ultrasonography that revealed a bulky uterus measuring 11.5x10x 3 cm and showing heregenous echotexture. Right ovary was also bulky. She also underwent MRI pelvis which disclosed a well defined heterogeneous lesion involving the whole of uterus and measuring 8.3 x 5.9 x 7.5 cm. The lesion was predominantly soft with cystic areas. The lesion was extending upto uterocervical junction. Fat planes between the mass and urinary bladder were illdefined. The right adnexal mass measured 5.6 x 5.2 cm.

She underwent transabdominal hysterectomy with bilateral salpingoophrectomy. During intraoperative examination, a large uterine mass of the size of 12 weeks was noted, with extra uterine extension to right adnexa. The mass was adherent to the bladder and rectum. The mass could not be removed in toto.

Pathological Findings: Grossly, the hysterectomy specimen revealed an enlarged uterus measuring 11.5 x 10 x 3.5

cm. On cutting open, entire uterine cavity was occupied by a large polypoid mass measuring 9x6 cm. The tumour seemed to be involving entire myometrium and overlying serosa. (Figure 1a) The endometrium varied in thickness from 0.1 to 0.2 cm. Cut surface of the tumour was soft and fleshy with few areas of hemorrhage and necrosis. Few areas showing cyst formation were also noted. Tumour grossly involved right parametrium and right adnexa.

Multiple sections from the tumour revealed predominantly large areas of monomorphic proliferation of epithelioid cells in vague nested pattern separated by delicate curvilinear vasculature. (Figure 1b) Few areas of spindle cell component with fibromyxoid stroma were also noted. (Figure 1c) The epithelioid cells showed moderate amount of eosinophilic cytoplasm with irregular nuclear contour, fine evenly dispersed chromatin with nuclear clearing and lack of prominent nucleoli. (Figure 1d) Mitotic count was

10-12/10 HPF. Areas of necrosis were seen. Extensive sampling (total 15 sections from tumour) did not reveal any carcinomatous component.

On Immunohistochemistry, epithelioid tumor cells were negative for CD10, broad-spectrum CK, EMA, desmin, smooth muscle actin (SMA), caldesmon, ER, PR, LCA, CD43, inhibin, CD99, WT-1, HMB-45, c-Kit/ CD117, DOG1, and p53. Epithelioid tumor cells exhibited diffuse nuclear staining for cyclinD1, which was negative in areas of spindle cell component (Figures 2a-d). However, spindle cell component exhibited positive staining for CD10.

Based upon histomorphology and immunohistochemistry, diagnosis of cyclinD1 positive high-grade endometrial stromal sarcoma (HGESS) was finally rendered. Post-operatively, the patient underwent 40Gy/28# of radiotherapy. She is presently doing well and is under follow-up.

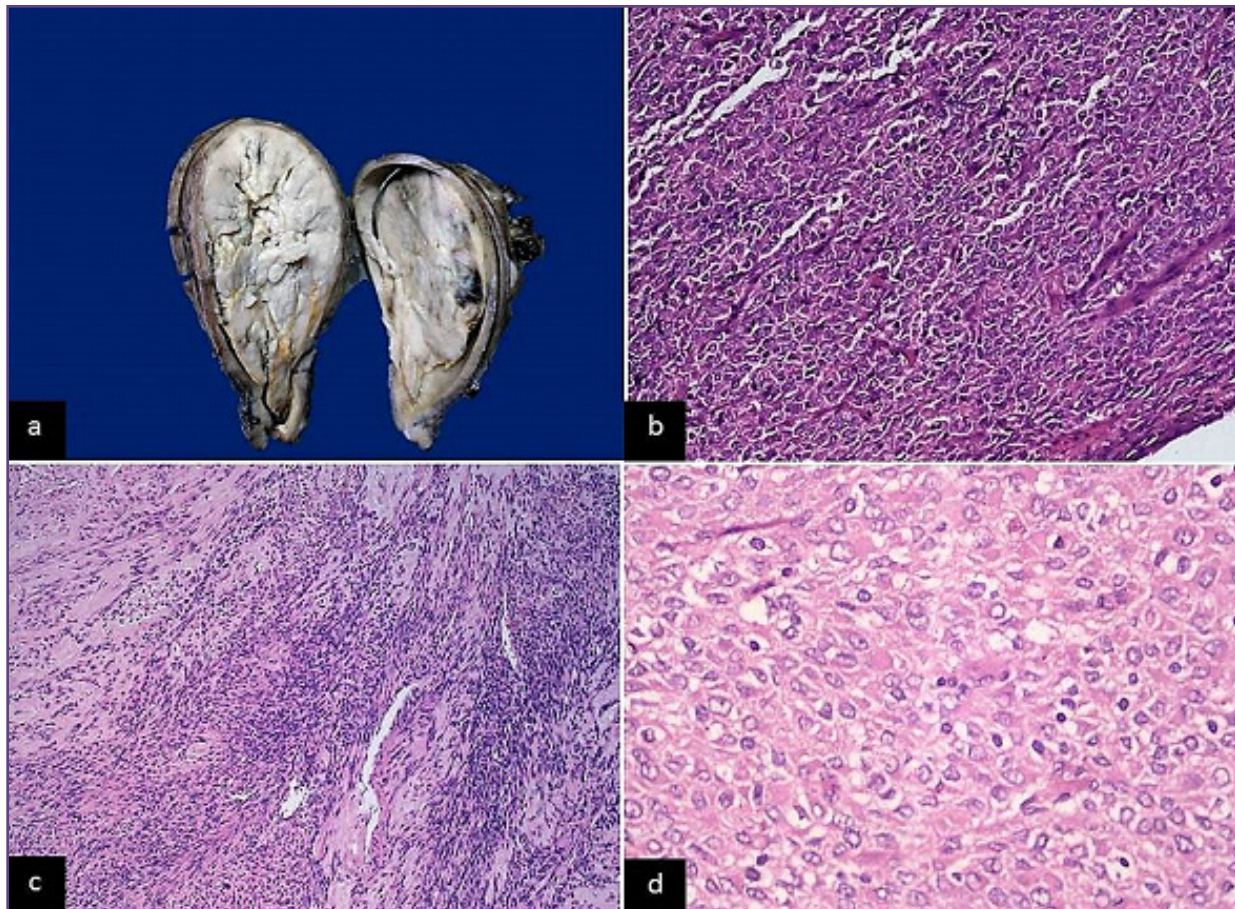


Fig. 1: Endometrial stromal sarcoma, high grade. (a) Gross examination revealed a large polypoid mass occupying the entire uterine cavity. (b) Infiltrating tumor composed of predominately nested growth of epithelioid cells separated by thin vascular channels. H and E, $\times 200$. (c) Few areas of spindle cell growth with fibromyxoid stroma were also seen. H and E, $\times 200$. (d) The epithelioid cells showed moderate amount of eosinophilic cytoplasm with irregular nuclear contour, fine evenly dispersed chromatin with nuclear clearing and lack of prominent nucleoli. H and E, $\times 400$.

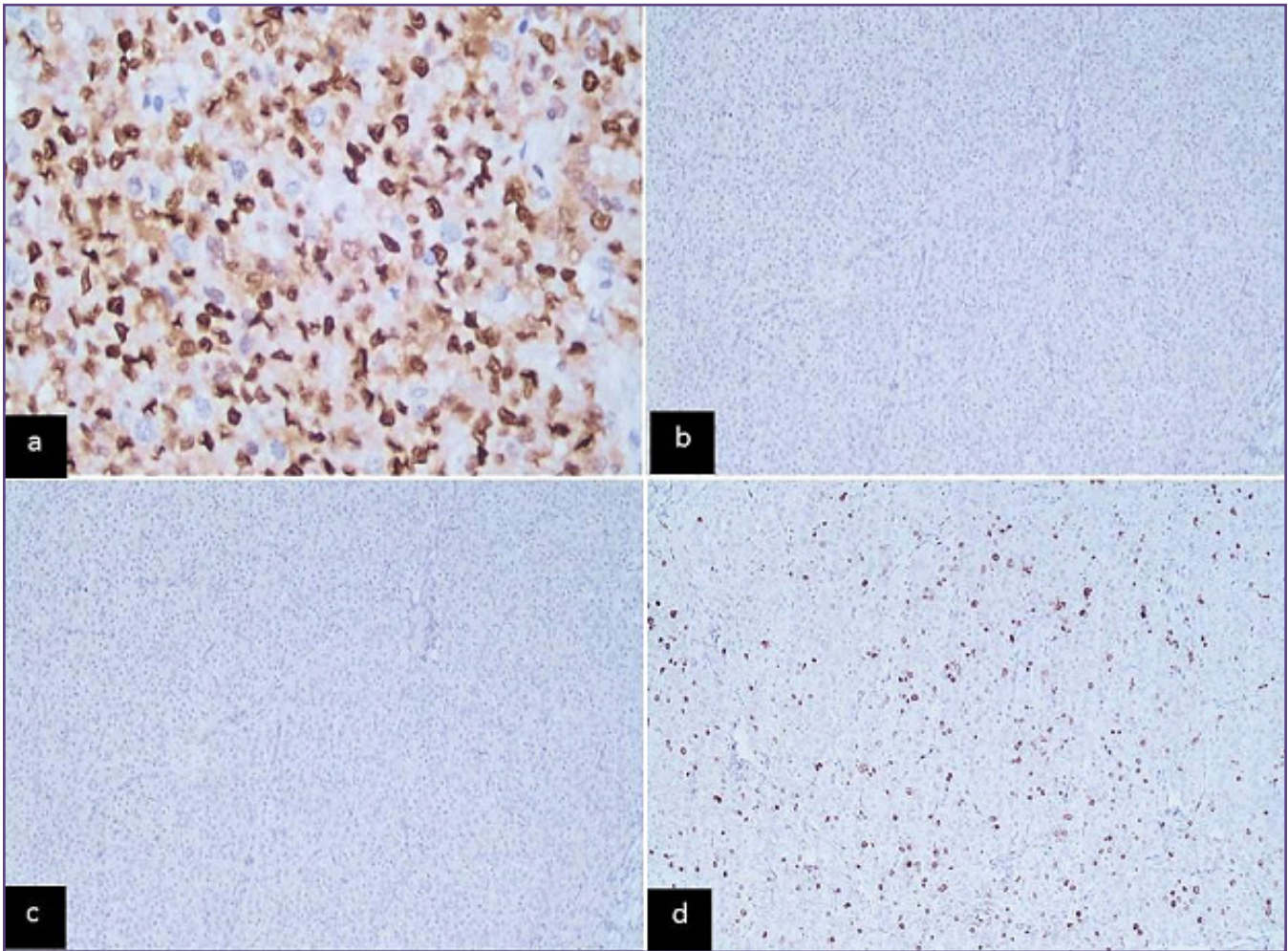


Fig. 2: Immunohistochemistry results of epithelioid component. (a) Epithelioid tumour cells showed strong and diffuse positivity for cyclinD1. Diaminobenzidine (DAB), $\times 200$. (b) CD10 negativity in epithelioid cells. DAB, $\times 200$. (c) ER negativity in epithelioid cells. DAB, $\times 200$. (d) Mib-1 labelling index was 20-25%. Diaminobenzidine (DAB), $\times 200$.

Discussion

Endometrial stromal sarcomas accounts for approximately 0.2% of all malignant uterine tumors and 10–15% of uterine sarcomas. These tumours frequently occur in women between 40 and 55 years of age, as seen in the present case.^[6]

Norris and Taylor, in 1966 first classified EST into ESN, LGESS, and HGESS.^[4] The subdivision into low-grade (< 10 mitosis/10HPF) and high-grade (≥ 10 mitosis/10 HPF) was based on mitotic count. They studied necrosis and cytological atypia but found these to be prognostically not relevant. However, further studies confirmed that even mitotic count was not prognostically significant. Consequent to this, the 2003 WHO classification removed the category of high grade ESS and reclassified these tumours into ‘ESS’ (low-grade tumours with histological resemblance

to proliferative endometrial stroma) and ‘undifferentiated endometrial sarcoma (UES)’ (pleomorphic tumours with no resemblance to endometrial stroma). Problem with the 2003 WHO classification was that not only UES was a heterogeneous category comprising of tumours with different clinical behavior but it was also silent on categorization of tumours with components of high-grade and low-grade ESS. Further molecular studies showed that ESSs was characterized by translocation involving chromosomes 7 and 17 [t(7; 17)(p15; q21)], leading to fusion of JAZF1/JJAZ1/SUZ12, however, only 50-60% of UES cases demonstrated this translocation.^[6,7] Lee et al in 2012 described a novel genetic fusion between *YWHAE* and *FMS22A/B* (now *NUTM2A/B*) in ESS harbouring translocation involving chromosome 10 and 17 [t(10;17)(q22;p13)] and associated clinicopathological features.^[8] Of the 11 *YWHAE*-rearranged primary uterine tumors

described by them, 7 contained a mixture of round cell and spindle cell areas, whereas 3 and 1 showed a purely round cell and purely spindle cell appearance, respectively. The round cell component described was highly cellular, and the tumor cells were typically arranged in a vaguely nested growth pattern, with the nests being separated by a delicate stromal capillary network. The round cells were epithelioid in appearance with scant to moderate amount of eosinophilic cytoplasm, had irregular nuclear contours with inconspicuous nucleoli. These tumours showed brisk mitosis and areas of necrosis. Our case also demonstrated high - grade round cell component with characteristic nuclear features, brisk mitosis and areas of necrosis.

Immunohistochemically, the high-grade round cell component of the tumour shows diffuse strong nuclear staining for cyclinD1, lack of CD10, and weak or absent staining for ER and PR.^[6] This is in contrast to LGESS which characteristically show diffuse CD10, ER, PR positivity and weak/patchy cyclinD1 staining. Our case also showed strong nuclear staining for cyclinD1 in more than 70% of epithelioid tumour cells and negative staining for ER, PR, and CD10. Spindle cell component showed CD10 positivity and cyclin D1 negativity.

YWHAE-NUTM2A/B (previously *YWHAE-FMS22A/B*) rearrangement needs to be confirmed by molecular tests such as reverse transcription polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH) analysis. However, in limited resource settings, these tests may not be available at all the centers. In this scenario, the distinctive morphological and immunohistochemical features of HGESS are generally good surrogate markers for *YWHAE*-rearrangement. In fact, Lee et al observed diffuse cyclinD1 positivity in *YWHAE*-rearranged ESS cases with sensitivity of 100% and specificity of 99%.^[5] Genetic analysis could not be performed in our case due to non-availability of the test in our laboratory.

Differential diagnosis in our case included epithelioid leiomyosarcoma, malignant perivascular epithelioid cell tumour (PEComa), undifferentiated uterine sarcoma, and undifferentiated uterine carcinoma. No conventional areas of leiomyosarcoma were seen and tumour cells were negative for SMA, desmin, and caldesmon. PEComa was excluded by absence of immunoeexpression of muscle and melanocytic markers. Undifferentiated uterine carcinoma (UUC) can occur in pure form or in combination with low-grade endometrioid adenocarcinoma and show diffuse positivity for cyclinD1. However, they usually show focal/patchy positivity for EMA/ broad-spectrum CK. In our

case, no areas of low-grade endometrioid adenocarcinoma were identified despite of extensive sampling (total 15 sections studied of tumour). The tumour cells showed no immunostaining with EMA/ CK. Adequate tumour sampling is of paramount importance in the cases where UUC and HGESS are in differential diagnosis. This fact was highlighted by Shah et al, in their study of cyclinD1 expression in ten cases of UUC.^[10] All the tumours showed cyclinD1 expression, with six cases showing diffuse and strong staining and four cases with patchy staining. Thus, adequate sampling and staining for EMA/broad-spectrum CK cannot be overemphasized to rule out any carcinomatous component.

UUS is a high-grade sarcoma that lacks a specific line of differentiation. Histologically, these have been classified into uniform (UUS-u) and pleomorphic (UUS-p) type.^[9] Out of these, UUS-u can show morphological and immunohistochemical overlap with HGESS. However, finding of CD10 positivity in UUS-u usually excludes HGESS.^[4]

It is important to diagnose HGESS, because these patients usually present with advanced stage disease (stages II–IV) and frequently have recurrences, usually within a few years after initial surgery. Anti-estrogenic therapy is likely ineffective given the lack of ER and PR immunopositivity in the high-grade component. Furthermore, although experience is limited, adjuvant therapy may provide survival benefit.^[3]

Conclusion

In high grade uterine mesenchymal tumours showing round cell component in isolation or with spindle cell component, revealing absent CD10 immunoeexpression, an extended panel of immunohistochemistry, including cyclinD1 should be added, before labelling the tumour as undifferentiated uterine sarcoma. This has prognostic as well as therapeutic implications.

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