

Atypical Leiomyomas of the Uterus: A Clinicopathologic Study of 54 Cases and an Immunohistochemical Analysis of Ki-67 and p53

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ABSTRACT

Objective: Uterine atypical leiomyomas (ALs) are extremely rare and occur in an age group almost one decade earlier than that for leiomyosarcomas. According to the literature, extensive clinicopathologic studies on ALs were limited to only two studies (2, 8). Atypical leiomyomas of the uterus are well-defined neoplasms with smooth muscle cells. The aim of this study was to investigate clinicopathologic findings in 54 cases diagnosed with ALs as well as Ki-67 and p53 expressions immunohistochemically.

Methods: Fifty-four cases diagnosed between 2000 and 2013 were included. The histological and clinical features of the cases were reviewed, and their medical records were examined. Ki-67 and p53 were performed on all cases immunohistochemically.

Results: The average age of the patients was 41.8 years. The average clinical follow-up period was 57 months. Hysterectomy was performed in 31 patients, and myomectomy in 21 patients, while resection of the mass was performed in two patients due to the intraligamentary mass. The average size of the neoplasms was 6.2 cm. Severe cellular atypia was noticed in 25 patients. While the number of mitoses was 1/10 high power field in 30 patients, it was 4/10 high power field in six patients. Ki67 was found to be positive in 50 patients at the rate of 1–5% immunohistochemically, while p53 demonstrated staining at the ratio of 10–15% staining in four patients.

Conclusion: The differentiation of ALs from leiomyosarcomas is crucial. The recurrence rate after follow-up is 2%. In our opinion, the patients diagnosed with 'AL with limited experience' before should be correctly diagnosed as AL. We recommend that Ki-67 and p53 can be used as adjuvant markers immunohistochemically in the patients where a problem in differential diagnosis from leiomyosarcoma exists.

Keywords: Atypical, immunohistochemistry, Ki-67, leiomyomas, p53

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Leiomiomas atípicos del útero: un estudio clínico-patológico de 54 casos y un análisis inmunohistoquímico de Ki-67 y p53

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RESUMEN

Objetivo: Los leiomiomas atípicos uterinos (LA) son extremadamente raros y se presentan en un grupo de edad casi una década antes que los leiomyosarcomas. De acuerdo con la literatura, los extensos estudios clínico-patológicos en los LA se limitaron a sólo dos estudios (2, 8). Los leiomiomas atípicos del útero son neoplasmas bien definidos con células de músculo liso. El objetivo de este estudio fue investigar los hallazgos clínico-patológicos en 54 casos diagnosticados con LA, así como las expresiones Ki-67 y p53, de forma inmunohistoquímica.

Métodos: Se incluyeron cincuenta y cuatro casos diagnosticados entre 2000 y 2013. Se revisaron las características histológicas y clínicas de los casos y se examinaron sus historias clínicas. Ki-67 y p53 se realizaron en todos los casos de forma inmunohistoquímica.

Resultados: La edad promedio de los pacientes fue de 41.8 años. El período promedio de seguimiento clínico fue de 57 meses. Se realizaron histerectomías a 31 pacientes, y miomec-tomías a 21 pacientes, en tanto que a dos pacientes se les realizó resección de la masa debido a la situación intraligamentosa. El tamaño promedio de los neoplasmas fue de 6.2 cm. Se observó atipia celular severa en 25 pacientes. El número de mitosis fue de 1/10 campos de gran aumento en 30 pacientes, en contraste con el número de mitosis de 4/10 campos de gran aumento en seis pacientes. Se encontró que Ki67 fue positivo en 50 pacientes a razón de 1–5% inmunohistoquímicamente, mientras que p53 mostró tinción a razón de 10–15% de tinción en cuatro pacientes.

Conclusión: La diferenciación de LA entre los leiomyosarcomas es crucial. La tasa de recurrencia después del seguimiento es de 2%. En nuestra opinión, los pacientes diagnosticados con 'LA con experiencia limitada' antes, deben ser diagnosticados correctamente como LA. Recomendamos que Ki-67 y p53 sean usados como marcadores adyuvantes inmunohistoquímicamente en los pacientes con un diagnóstico diferencial de leiomyosarcoma.

Palabras clave: Atípico, inmunohistoquímica, Ki-67, leiomiomas, p53

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INTRODUCTION

Uterine smooth muscle tumours display a broad spectrum from benign conventional leiomyomas (LMs) to highly aggressive and malignant leiomyosarcomas (LMSs). Leiomyomas are mitotically inactive (10 or fewer mitoses generally in 10 high power field, HPF) and include insignificant cytological findings (1). While hyaline (ischaemic-type) necrosis typically exists in LMs, no coagulative tumour necrosis is observed (2). However, LMSs morphologically demonstrate at least two of the following criteria: cytologic atypia, increased mitotic rate (usually > 10/10 HPF), and coagulative tumour cell necrosis (2). Some of the entities between the two extremes of this diagnostic spectrum present

with a histopathological appearance that is similar to malignancy findings. These are entities with notable morphologic findings such as cellular LMs, epitheloid LMs, myxoid LMs, atypical leiomyomas (ALs) with low potential of recurrence, smooth muscle cell tumours with focal atypia and limited experience (coagulative-type necrosis exists, but these are findings without atypia and with < 10/10 number of mitosis per HPF), and LMs with a high mitotic index (> 20/10 mitosis per HPF exists, without atypia and necrosis). In tumour classification, the term 'limited experience' is avoided, and the term 'smooth muscle tumours of uncertain malignant potential (STUMP)' is typically added to the main category (2, 3).

The diagnosis of ALs is the one used for LMs with nuclear atypia (4). In the past, the terms 'symplastic LM' (5) and 'bizarre LM' (6, 7) have also been used instead of AL. However, such definitions are used less often at present. In 1994, Bell *et al* first defined ALs as neoplasms containing moderate or severe atypia identified with a low power field without accompanying increased mitotic activity and tumour cell necrosis (2). They stated that it would be appropriate to define smooth muscle tumours with only diffuse atypia as 'ALs', and those with multi-focal atypia as 'LMs with focal atypia and limited experience', because they found that only ALs with diffuse atypia in a large series of 213 cases had had a low potential of recurrence (1/46, 2%). In LMs with focal and multi-focal atypia, no recurrence (0/5) was observed (2). Ly *et al* also had similar findings in their clinicopathologic study of 51 cases (8). According to the literature, extensive clinicopathologic studies on ALs were limited to only these two studies (2, 8). In this study, we aimed to investigate Ki-67 and p53 expressions and detect their relationships with medical prognosis as well as identifying clinicopathologic features of ALs.

SUBJECTS AND METHODS

Fifty-four cases of ALs diagnosed between 2000 and 2013 were included in the study. Pathology reports and medical records of all cases were analysed. Clinical findings and follow-up results were obtained from follow-ups after surgery, clinicians of the patients and by direct phone calls to the patients. All case materials were reviewed microscopically. The age of patients at diagnosis, the diameter and locations of neoplasms, and surgical methods used were recorded. In total, histological sections of 54 ALs were reviewed. The relevancy of all neoplasms to the criteria of Bell *et al* (2) were evaluated. The following histological findings in ALs reviewed microscopically were noted: the distribution of atypical cells, the level of cytological atypia (moderate, severe), cellularity (mild, moderate, severe – in comparison to the neighbouring non-neoplastic myometrium), the highest number of mitoses in 10 HPF, the presence and type of necrosis (coagulative/ischaemic and unknown), degenerative findings, and margins of the tumour.

Immunohistochemical study

A block representing AL was chosen from each case and analysed using Ki-67 (clone SP6; Thermo Scientific; 1:100) and p53 (clone DO-7; Thermo Scientific; 1:200) by the avidin biotin peroxidase method immunohistochemically. Samples of 5 micron thickness taken

for immune staining were incubated for 45 minutes at 70°C for deparaffinization. Then, sections transferred to xylene were kept in an incubator for 30 minutes, and then kept for 10 more minutes outside the incubator. After the deparaffinization, sections were placed in alcohol (100% and 95%) and washed with distilled water. They were boiled in 10% citrate (pH 6) in a pressure cooker and then left chilling. They were kept for 15 minutes after hydrogen peroxide (3%) was added to them. The sections were washed with Tris Buffered Saline (TBS) solution (pH 7.4) and kept for 10 minutes in a super-block that interferes with the surface staining. After taking the super-blocks away, primary antibodies were dropped (Ki-67 and p53). After keeping the antibodies for two hours, they were washed with TBS, biotin was added and kept for 15 minutes. Horseradish peroxidase was dropped on the sections after washing with TBS and kept for 15 minutes. Additionally, DAB (Diaminobenzene) chromogen was applied for 10 minutes after washing with TBS for the last time. Sections were washed with tap water and taken to haematoxylin for counter staining. Immunohistochemical (IHC) inspection was carried out by two pathologists using a double-headed microscope. Only nuclear staining for Ki-67 and p53 was accepted as positive, and the per cent of positive cells was calculated.

RESULTS

Clinical findings

Clinicopathologic findings are summarized in the Table. The ages of the 54 patients varied between 26 and 58 years (41.8 years on average). The average clinical observation period was 57 months (between 3 and 141 months).

Table: Summary of clinical and pathologic features of atypical leiomyomas

Clinical and pathologic features	Results
Average age of patients	41.8 years
Average size of atypical leiomyomas	6.2 cm
Total abdominal hysterectomy	33 cases
Myomectomy	21 cases
With leiomyoma	24 cases
Solitary	30 cases
Intramuscular	38 cases
Well circumscribed	51 cases
Irregular borders	3 cases
Moderate atypia	29 cases
Severe atypia	25 cases
Diffuse atypia	22 cases
Coagulative type necrosis	–
Ischaemic type necrosis	20 cases
Average follow-up period	57 months

Major clinical symptoms of the cases were abnormal (severe and irregular) vaginal bleeding and abdominal pelvic pain. Nine cases were asymptomatic, and masses in these nine cases were localized during routine pelvic examinations. Hysterectomy was performed on one patient after conization because of cervical intraepithelial lesion. In another patient, hysterectomy was performed due to a persistent complete hydatiform mole. Overall, 31 patients had hysterectomy and 21 patients had myomectomy. Mass excision was performed in three patients due to the intraligamentary mass. Four patients had a history of pre-operative hormone therapy. One tumour was noted within the cervix, while three tumours had had intraligamentary localization. All the other tumours had limited view in the uterine corpus (intramural, submucosal, subserosal).

Pathologic findings

Macroscopic and microscopic findings are summarized in the Table. While ALs were solitary in 30 patients, ALs were noted in 24 patients with LMs. The average size of the neoplasms was 6.2 cm (between 1.5 cm and 13 cm). Three were subserosal, 38 were intramural and 13 were submucosal. Of these, 51 were well circumscribed, and three had irregular borders. Cystic degeneration was macroscopically noted in seven cases, while mucinous degeneration was seen in one patient and haemorrhage in two others. Section surfaces were usually solid, whorled, fibrous and cream-xantic.

During microscopic examinations, moderate atypia was seen in 29 cases (Fig. 1) and severe atypia in 25 cases (Fig. 2). Tumours with 'moderate atypia' demonstrated pleomorphism appreciable at low power and contained

easily found cells bearing nuclei that were large, plump and irregular contours, coarse chromatin, and prominent nucleoli. Tumours with 'severe atypia' comprised numerous such cells as well as cells with larger, more bizarre nuclei and pronounced chromatin clearing.

There was diffuse atypia in 22 cases (Fig. 3), and it was focal in the remaining patients (Fig. 4). While in 30 cases, the number of mitoses at 10 HPF was 1, in six cases, it was 4/10 HPF. In cases where neighbouring neoplastic tissue was noted, 51 tumours had pushing margins. In 20 of them, an ischaemic-type necrosis was present, and in such necrotic areas of inflammation, granulation and/or hyalinization were seen. Coagulative-type necrosis was not detected in any case. Some cystic and oedematous changes were noted. In nine cases, the cellularity was severe, whereas it was mild in 13 patients and moderate in others.

Clinic follow-ups

The average follow-up period was 57 months (between 3 and 141 months). The clinic follow-up was 0–24 months in 12 patients, 25–60 months in 20 patients, 61–120 months in 18 patients, and over 120 months in four patients. Intramuscular LM was found during the control in a 28-year-old patient who had mass excision due to intraligamentary mass, however. No recurrence was observed in other patients. One patient died because of lung cancer.

Immunohistochemical results

In one patient, p53 was positive with 40% staining and in another, with 30% staining. In 24 cases, p53 was 1–5% positive; in 14 cases (Fig. 5), p53 was 5–10% positive;

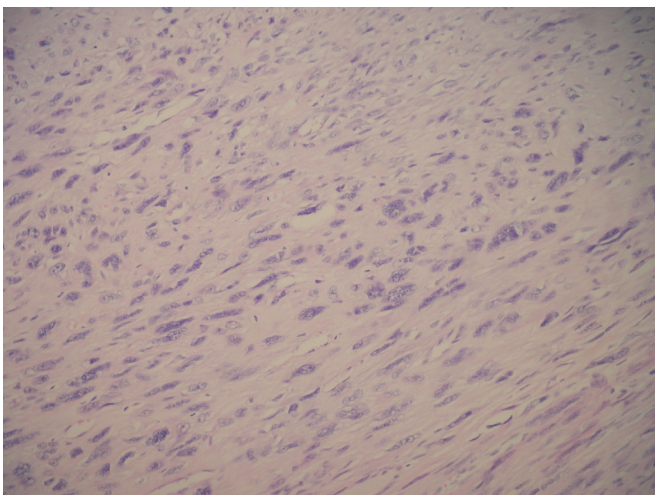


Fig. 1: Moderate atypia in the cells (x 200).

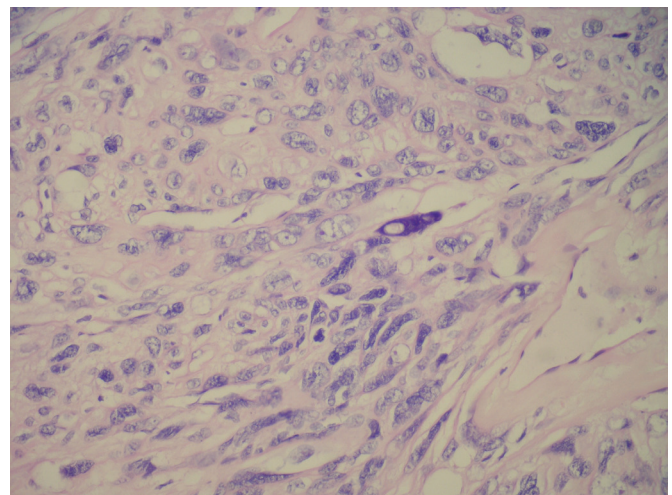


Fig. 2: Severe atypia in the cells (x 400).

in other cases, it was negative. Also, in 50 cases, Ki-67 was 1–5% positive (Fig. 6); in four cases, it was 10–15% positive.

DISCUSSION

Uterine ALs are extremely rare and occur in an age group almost one decade earlier than that for LMSs (1). While the average age of diagnosis of LMSs is 50–55 years, that of ALs was reported as 42–43 years (1). In this study, the average age of the patients was 41.8 years, and this finding is relevant to the literature. Because of their benign nature, it is important to differentiate ALs from LMSs (1). The severe atypical findings in ALs may be distributed throughout the LM (diffuse) or they may be present focally/multifocally. The number of mitosis is 10/10 HPF or less, and there is no tumour cell necrosis (8). If the number of mitosis is more than 10/10 HPF,

then these tumours should be considered as LMSs (9). Although both ALs and LMSs show severe nuclear atypia, they have quite different prognoses. In this study, we focussed especially on the classical AL. These are neoplasms which have morphologies earlier defined as ‘AL with diffuse atypia’ and ‘limited experience with focal or multifocal atypia’.

Diagnostically, the morphological criteria determined by Bell *et al* (2) were used as a baseline. Atypical cells are thought to be degenerated cells which have lost their division capacity and show some abortive mitotic figures (6). There is no tumour cell necrosis in ALs (10). The existence of tumour necrosis in uterine smooth muscle cell tumours is associated with malignant behaviour (2). Coagulative tumour necrosis observed in LMSs was not seen in any of our cases. However, in 20 cases, there was granulation tissue and/or fibrous tissue around

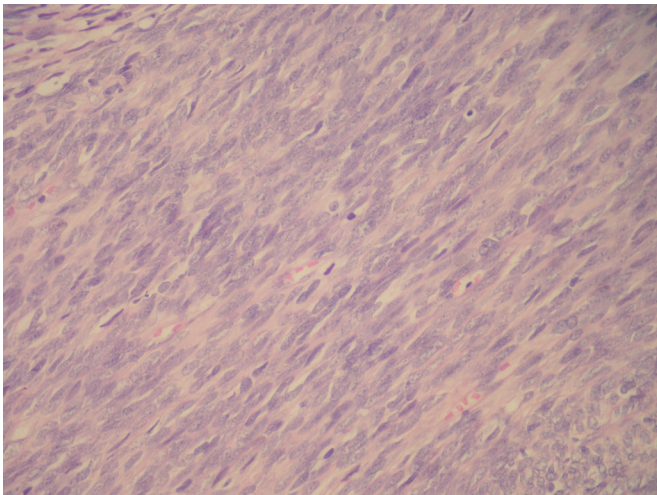


Fig. 3: Diffusely distributed atypical cells (x 200).

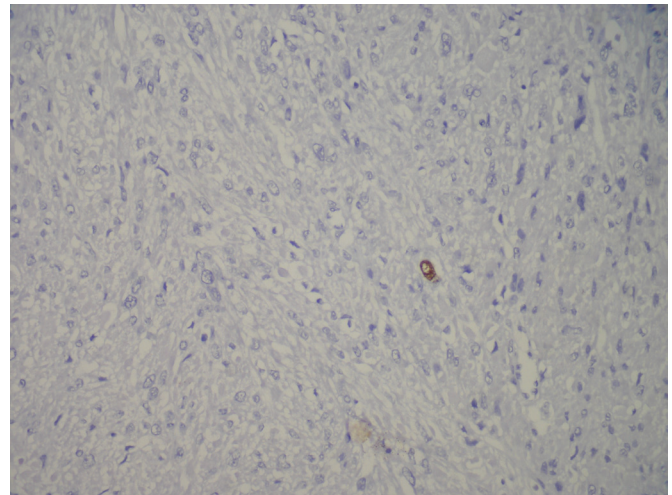


Fig. 5: P53 positivity in atypical cells (x 200).

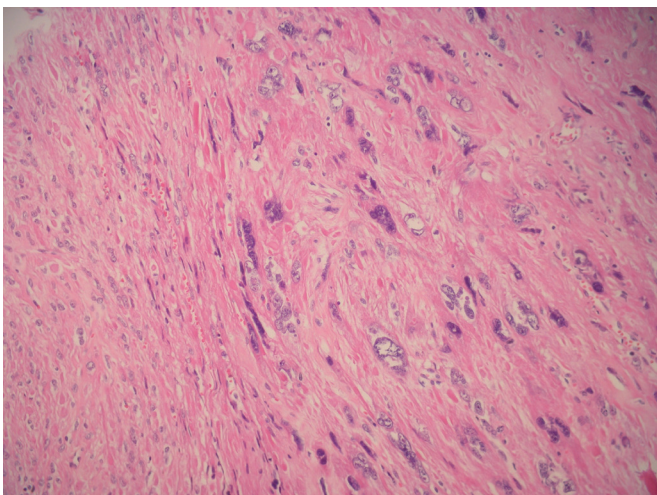


Fig. 4: Focally distributed severe atypical cells (x 200).

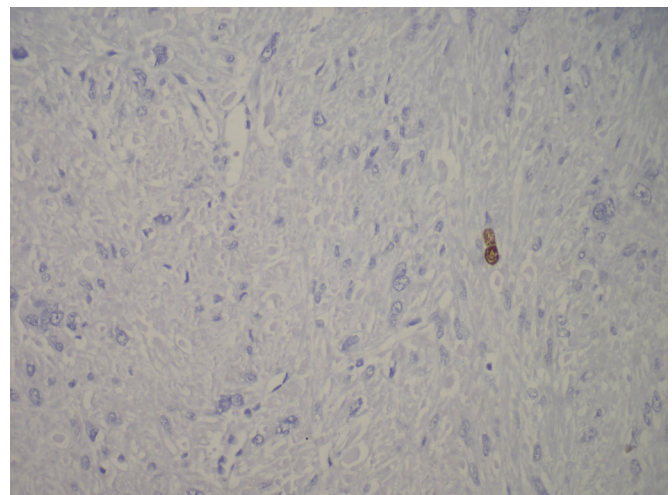


Fig. 6: Ki-67 positivity in atypical leiomyoma (x 200).

the necrotic area separating the living tissue and creating a zonation effect developed as a result of infarction.

Ernst *et al* stated that global DNA methylation had increased and, due to an accumulation of extremely high levels of inactive DNA, severe nuclear atypia occurred in the samples obtained from ALs (11). In addition, it is known that when cells transform into neoplastic cells, more genes are methylated (12) and high levels of DNA methylation can be observed in immortal cells. However, so many other studies have determined that cytological atypia does not increase the risk of recurrence or metastasis (8, 13–15). To be able to make the distinction between these abnormal and abortive mitotic forms, researchers have conducted some studies on whether Ki-67 could be used as a potential indicator instead of mitotic count in uterine smooth muscle cell tumours (13, 16–18).

Ly *et al* found Ki-67 2% positive in ALs and 25% positive in LMSs (8). Besides, Sun and Mittal showed that PHH3 could give more useful results for mitotic figure counting than Ki-67 (13). In 48% of the bizarre LMs, they also found positivity in more than 10% of tumour cells with Ki-67 (13). Sun and Mittal detected Ki-67 positivity in 5–25% of cells in just three of their series with 13 cases (13). With these findings, it was concluded that there was not any helpful IHC indicator for a definite distinction and histological criteria were the gold standards (5). In this study, Ki-67 positivity was found in four cases in 10–15% of the cells. In 50 cases, 1–5% staining was obtained. Thus, we believe that IHC Ki-67 staining would help to make the differential diagnosis with LMSs in cases which have histologically severe nuclear atypia but mitotic figure counting is difficult because of nuclear findings. Mutations of p53, a tumour suppressor gene, are seen quite often in human malignancies. In the previous studies, any significant differences between LMSs and LMs were revealed in terms of p53 (16, 19–21). Whereas Sun and Mittal detected 38.5% positivity in ALs, they also did not find any significant differences of p53 staining between atypical and non-atypical cells (13). Additionally, Ly *et al* reported a strong p53 positivity at the rate of 7% in ALs (8). In our study, positive p53 staining was highly variable. There was strong positivity at 30% in one case and 40% in another. While p53 was 1–5% positive in 24 cases, it was 5–10% in 14 cases. In other cases, there was no p53 staining. Our findings supported the fact that p53 is not a reliable IHC marker in the differential diagnosis of AL from LMS.

Our study confirmed the criteria determined by Bell *et al*. Histologically, there was diffuse and severe atypia

in one case showing recurrence. This tumour was 6 cm in diameter with an intraligamentary placement. Ki-67 was 10–15% positive. As Ly *et al* have suggested, we agree that these tumours should be gathered under the title of ALs instead of using the term ‘LMs with focal atypia, limited experience’. Besides, our findings make us think that these tumours show a better prognosis when compared to those which do not contain diffuse atypia. We believe that myomectomy is a safe operative procedure for ALs because no recurrence was observed in 21 patients who had had myomectomy. Even so, it is important to be careful about tumour number, tumour size and positive margins in patients having myomectomy.

There is a concern that smooth muscle tumours localized in the cervix are more aggressive. Only a few atypical smooth muscle cell tumours localized in the cervix were reported in the literature (22, 23). In the present study, only one case of AL was localized in the cervix, and no recurrence was detected during follow-up.

In conclusion, in this study, we have presented our experience with ALs in the most extensive case series in the literature including the clinicopathologic correlation. Although ALs are extremely rare, differential diagnosis from LMSs is necessary. Our findings support the prognostic parameters determined by Bell *et al*, and ALs show benign clinical progress. We believe that the patients who had been diagnosed as ‘AL with focal atypia-limited experience’ should be considered under the AL title. We also emphasize that Ki-67 IHC staining contributes to the diagnosis greatly in histologically problematic cases.

REFERENCES

1. Zaludek CJ, Norris HJ. Mesenchymal tumors of the uterus. In: Kurman RJ, ed. Blaustein's pathology of the female genital tract. 5th ed. New York: Springer-Verlag; 2002: 561–615.
2. Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasm. A clinicopathologic study of 213 cases. *Am J Surg Pathol* 1994; **18**: 535–8.
3. Longacre TA, Hendrickson MR, Kempson RL. Predicting clinical outcome for uterine smooth muscle neoplasms with reasonable degree of certainty. *Ad Anat Pathol* 1997; **4**: 95–104.
4. Hertig A, Gore H. Tumors of the vulva, vagina and uterus. Washington, DC: Armed Forces Institute of Pathology; 1960.
5. Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkinson EJ, eds. Histological typing of female genital tract tumors. New York: Springer-Verlag; 1994.
6. Downes KA, Hart WR. Bizarre leiomyomas of the uterus: a comprehensive pathologic study of 24 cases with long-term follow-up. *Am J Surg Pathol* 1997; **21**: 1261–70.
7. Fukunaga M, Ushigome S. Uterine bizarre lipoleiomyoma. *Pathol Int* 1998; **48**: 562–5.
8. Ly A, Mills AM, McKenney JK, Balzer BL, Kempson RL, Hendrickson MR et al. Atypical leiomyomas of the uterus: a clinicopathologic study of 51 cases. *Am J Surg Pathol* 2013; **37**: 643–9.

9. Mittal K, Joutoovsky A. Areas with benign morphologic and immunohistochemical features are associated with some uterine leiomyosarcomas. *Gynecol Oncol* 2007; **104**: 362–5.
10. Tavassoli FA, Devilee P. World Health Organization classification of tumors. Pathology and genetics of tumors of the breast and female genital organs. Lyon: IARC Press; 2003.
11. Ernst LM, Luo FF, Parkash V, Zheng W. Increased global DNA methylation in symplastic leiomyomas. *Mod Pathol* 2002; **15**: 196A.
12. Liu L, Zhang J, Bates S, Li JJ, Peehl DM, Rhim JS et al. A methylation profile of in vitro immortalized human cell lines. *Int J Oncol* 2005; **26**: 275–85.
13. Sun X, Mittal K. MIB-1 (Ki-67), estrogen receptor, progesteron receptor, and p53 expression in atypical cells in uterine symplastic leiomyomas. *Int J Gynecol Pathol* 2009; **29**: 51–4.
14. Evans HL, Chawla SP, Simpson C, Finn KP. Smooth muscle neoplasm of the uterus other than ordinary leiomyoma. A study of 46 cases, with emphasis on diagnostic criteria and prognostic factors. *Cancer* 1988; **62**: 2239–47.
15. Deodhar KK, Goyal P, Rekhi B, Menon S, Maheshwari A, Kerkar R et al. Uterine smooth muscle tumors of uncertain malignant potential and atypical leiomyoma: a morphological study of these grey zones with clinical correlation. *Indian J Pathol Microbiol* 2011; **54**: 706–11.
16. O'Neill CJ, McBride HA, Connolly LE, McCluggage WG. Uterine leiomyosarcomas are characterized by high p16, p53 and MIB-1 expression in comparison with usual leiomyomas, leiomyoma variants and smooth muscle tumours of uncertain malignant potential. *Histopathology* 2007; **50**: 851–8.
17. Mayerhofer K, Obermair A, Windbichler G, Petru E, Kaider A, Hefler L et al. Leiomyosarcoma of the uterus: a clinicopathologic multicenter study of 71 cases. *Gynecol Oncol* 1999; **74**: 196–201.
18. Zhai YL, Kobayashi Y, Mori A, Orii A, Nikaido T, Konishi I et al. Expression of steroid receptors. Ki-67 and p53 in uterine leiomyosarcomas. *Int J Gynecol Pathol* 1999; **18**: 20–8.
19. Niemann TH, Raab SS, Lenel JC, Rodgers JR, Robinson RA. p53 protein overexpression in smooth muscle tumors of the uterus. *Hum Pathol* 1995; **26**: 375–9.
20. Blom R, Guerrieri C, Stal O, Malmstrom H, Simonsen E. Leiomyosarcoma of the uterus: a clinicopathologic, DNA flow cytometric, p53, and mdm-2 analysis of 49 cases. *Gynecol Oncol* 1998; **68**: 54–61.
21. Layfield LJ, Liu K, Dodge R, Barsky SH. Uterine smooth muscle tumors: utility of classification by proliferation, ploidy, and prognostic markers versus traditional histopathology. *Arch Pathol Lab Med* 2000; **124**: 221–7.
22. Tiltman AJ. Leiomyomas of the uterine cervix: a study of frequency. *Int J Gynecol Pathol* 1998; **17**: 231–4.
23. Ito H, Sasaki N, Miyagawa K, Tahara E. Bizarre leiomyoblastoma of the cervix uteri. Immunohistochemical and ultrastructural study. *Acta Pathol Jpn* 1986; **36**: 1737–45.