Int.j.pathol.2019;17(1): 176-185

Original Article

Prognostic Factors in Uterine Sarcomas: A Clinicopathologic and Immunohistochemical Study

Abdul Mohsen Al Kushi[*], Ahmad Omair[***], Haitham Arabi,[**] and Motasim Badri,[****]

[*]College of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.[**]Dept. of Pathology, King Abdulaziz Medical City, National Guard Health Affairs, Riyadh, Saudi Arabia.[***]Dept. of pathology, College of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.[****]Department of Epidemiology & Biostatistics, College of Public Health & Health Informatics, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

ABSTRACT

Background: Uterine sarcomas are rare malignant tumors histologically categorized into high-grade and low-grade sarcomas (HGS & LGS).

Objective: To examine the prognostic relevance of clinicopathological and immunohistochemical features for this rare group of tumors.

Methods: Clinicopathological data including age, follow-up, parity, tumor cell type, lymphovascular invasion, nuclear grade, stage and mitotic index was obtained for 28 cases treated at our institute. HGS (n=22) included 11 each of leiomyosarcoma (LMS), and carcinosarcoma (CS). LGS (n=6) included 3 each of Müllerien adenosarcoma (MAS) and endometrial stromal sarcoma (ESS). Sections were immunostained with antibodies for p53, Bcl-2, ER, HER-2 and c-Kit. The data was statistically analyzed for association between these factors and disease-free survival.

Results: Twelve (42.9%) patients with HGS died of the disease and none died among LGS. Descriptive analysis revealed a statistically significant association between death and HGS (p=0.024), sarcomas with nuclear grade 3 (p=0.029), mitotic index > 60 (p=0.016) and presence of lymphovascular invasion (p=0.028). More than 80% of the patients with recurrence were diagnosed with HGS. Median overall survival time was 70 months. The2, 5- and 10-year survival rates were 65%, 58% and 43% respectively. No statistically significant association was observed between survival times and histologic types of sarcoma (p=0.204) but stage 1 and 2 had a better survival compared to stage 3 and 4. Over expression of P53 was only found in 4 cases of CS; and complete membranous staining for Her-2 was also only observed in CS tumors (n=6). ER positive staining was found in all MAS and ESS tumors only. C-kit positive expression was observed in 8 cases, 7 of which were from HGS group.

Conclusion: This study reconfirms HGS being aggressive tumors with short survival rate. Greater mitotic index and nuclear grade, tumor cell type and vascular invasion are important prognostic indicators of survival.

Key Words: Uterine sarcomas; prognostic factors; immunohistochemistry, survival, mitotic index

Introduction

An estimated 11.4% of all new cases of female cancers in 2013 were of the genital tract, and less than 1% among these were observed to be uterine sarcomas.[1] They represent 3-7% of all uterine cancers globally with the highest incidence in Norway, where 9.7% of uterine malignancies were sarcomas.[2,3]

AUTHOR CORRESPONDENCE:
Dr. Ahmad Omair, M.B.B.S, Ph.D.
Assistant Professor Pathology, Dept. of Basic Sciences
College of Science and Health Professions
King Saud bin Abdulaziz University for Health Sciences
E.mail: omairah@ksau-hs.edu.sa,
dr.ahmad.omair@gmail.com

Uterine sarcomas are rare tumors, which makes their early diagnosis, treatment and prognosis more challenging and unpredictable. Uterine sarcomas can be histologically divided in to high grade and low-grade sarcomas. Subgroups of high grade are leiomyosarcomas (LMS), carcinosarcomas (CS) also known as malignant mixed Müllerien tumors (MMMT) and other rare undifferentiated uterine sarcomas. Similarly, the subgroups of low grade include endometrial stromal sarcomas (ESS), Müllerian adenosarcomas (AS) and other rare tumors. It has been reported that CS and LMS account for about 40% each of all cases, ESS for 10-15%, and undifferentiated sarcomas for 5-10% of cases.[4] LMS

and ESS are considered as monophasic pure sarcomas and CS (MMMT) as biphasic sarcomas with combined features of carcinoma and sarcoma.[5] Although it has been classified since 2002 as dedifferentiated subtype of endometrial carcinoma CS is still included in many recent sarcoma studies and review articles.[6,7] We also included CS in the high grade sarcoma group in this study. Traditionally, staging of uterine sarcomas follow the 1988 International Federation of Gynecology and Obstetrics (FIGO) staging system. However, a new international FIGO classification and staging system has been developed to take account of the differences of behavior between these tumor subtypes.[8]

Most uterine sarcomas present between the ages of 40 and 60 years. The majority of these neoplasms are aggressive with poor prognosis.[9] Various studies have shown an overall survival between 17.5 % and 54.7% at five years(6) and less than 50 % at 2 years even when they are detected at an early stage (stage I). The prognosis varies based on the histological subtypes as low-grade sarcomas are reported to be less aggressive than high grade, whereas undifferentiated tumors have very poor outcome. Additionally, despite being aggressive and associated with adverse prognosis, LMS has been associated with most unpredictable outcome.[10, 11]

The rarity and diversity of uterine sarcomas has led to identification of no clear prognostic factors that are predictive of outcome, particularly since prognosis varies considerably between the different subtypes. Clinicopathologic factors of tumor grade, cell type, mitotic index, and surgical stage have been reported to help predict the survival outcome but alone have proved to be insufficient to make prognosis more predictable. Immunohistochemistry (IHC) of biomarkers including P53 and Bcl-2, and receptor proteins (c-Kit, ER, and HER-2) have been analyzed for their association with overall survival of patients with uterine sarcomas. Expression of these biomarkers has been used in conjunction with pathological tumor classifications for predicting the behavior of the tumor, though the data is still not definitive. TP53 is the most important tumor suppressor gene, responsible for preventing tumor development by regulation of cell cycle. Mutant forms are responsible for inability to arrest cell cycle in case of DNA damage and leads to tumor development. This mutant form accumulates in the tumor cells and can easily be detected by IHC.[10,12] Bcl-2 (B-cell lymphoma 2) is a pro-apoptotic protein responsible for inducing apoptosis in cells with irreversibly damaged DNA. Mutant forms of coding genes lead to their inability to induce apoptosis and hence tumor development. Poor prognosis of LMS has been associated with over-expression of the P53 protein while Bcl-2 expression is correlated with a more favorable outcome.[9,13,14] The receptor proteins including estrogen receptor (ER), human epidermal growth factor receptor-2 (HER-2) and stem cell factor receptor (c-Kit/CD117) are responsible for growth of tumor cells and thus are ideal candidates for assessing prognosis and outcome.

As the prognosis has remained unchanged in recent decades, this retrospective study aimed at examining the prognostic relevance of clinical, pathological and immunohistochemical features for uterine sarcoma by assessing their correlation with overall and disease-free survival.

Methods

Data was collected from the database for a total of 30 patients with uterine sarcoma who were treated at our institute, between 1994 and 2006. Two patients were excluded due to wrong diagnosis and insufficient clinicopathologic data. The study was approved by the local ethical committee at our institute. The mean age of our sample was 54 years; median age was 58 years (range from 22 to 79 years) with 57.1% of the patients were menopausal at the time of diagnosis. The mean and median follow up time were 31 months and 11 months respectively (range from 1 to 120 months). The detailed clinical characteristics are listed in Table S1.

The final diagnosis was confirmed on surgical specimen for all patients. Twenty-four patients underwent staging laparotomy. The time of hysterectomy with staging or biopsy was considered as time of diagnosis. In our sample, there were 22 cases of high-grade sarcomas, including11 cases of leiomyosarcoma (LMS) and 11 cases of carcinosarcoma (CS). Six cases were of Low-grade sarcomas including 3mullerianadenosarcoma (MAS) and 3 endometrial stromal sarcoma (ESS). Tumor stage was determined using FIGO staging classification for uterine carcinomas.[15] Among our sample of 28 cases, 17 (60.7%) were of stage I and II and 11(39.3%) were of stage III and IV (Table 1).

Surgery was the first-line of treatment, with total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy (BSO) as the primary treatment for 50% of the patients, and with the additional removal of lymph node for 17.8% of the patients. 10.7% of patients had sub-total abdominal hysterectomy. Chemotherapy was given in 25% of the

patients, and radiotherapy in 39.2% of the cases (Table S1).

Table 1: Clinicopathologic characteristics of uterine sarcomas in 28 patients

Characteristics	All (n = 28)	LMS (n = 11)	CS (n = 11)	MAS (n = 3)	ESS (n = 3)
FIGO Stage					
I	16 (57.1%)	6 (54.5%)	5 (45.5%)	3 (100%)	2 (66.7%)
II	1 (3.6%)	0	1 (9.1%)	0	0
III	3 (10.7%)	3 (27.3%)	0	0	0
IV	8 (28.6%)	2 (27.3%)	5 (45.5%)	0	1 (33.3%)
Tumor Grade					
Low Grade	6 (21.4%)	0	0	3 (100 %)	3 (100%)
High Grade	22 (78.6%)	11 (100%)	11 (100%)	0	0
Lymphovascular invasion					
Yes	14 (50%)	6 (54.5%)	5(45.5%)	0	3(100%)
No	9 (32.1%)	4(36.4%)	3(27.3%)	2 (66.7%)	0
Not reported	5 (17.9%)	1(9.1%)	3(27.3%)	1 (33.3%)	0
Mitotic index					
0 – 10	6 (21.4%)	0	0	3 (100%)	3 (100%)
>10	22 (78.6%)	11(100%)	11(100%)	0	0

CS, carcinosarcoma; ESS, endometrial stromal sarcoma; FIGO, International Federation of Gynecology and Obstetrics; LMS, leiomyosarcoma; MAS, müllerien adenosarcoma

Out of the 28 Patients, paraffin blocks for immunohistochemical analysis were only available for 24 tumors. Sections of paraffin blocks were immunostained with antibody clones for biomarkers P53 (DO-7 from Dako, CA USA), Bcl-2(124 from Dako, CA USA) and receptor proteins c-Kit (9-7 from Ventana, Roche diagnostics USA), ER (SP1 from Ventana, Roche diagnostics USA), and HER-2 (4B5 from Ventana, Roche diagnostics USA). For P53, staining was considered negative if less than 10% of nuclei are stained and wild type expression if weak or patchy staining of 10%-50% are stained. Overexpression was identified when there was diffuse strong nuclear staining of more than 50% nuclei.[12] For Bcl-2 expression, it was considered positive if cytoplasmic immunoreaction was observed in 50% or more of tumor cell.[14] Her-2 immunostaining was considered negative (0 and 1+) if there was no staining or weak staining involving only part of membrane in more than 10% cells. It was considered positive if there was weak to moderate staining of complete membrane (2+) or if there was strong staining of the complete membrane (3+)(16).For ER, no or weak to moderate staining (0 to 2) was considered negative while strong and diffuse staining (3) was considered positive.[17] For c-Kit expression was classified from 0 to 2 if there was no staining or mild to moderate and 3 if there was strong staining.[18]

Data are presented as proportions and were compared using the χ^2 or Fischer's exact test. Primary outcomes were disease recurrence and overall survival. Kaplan-Meier method was used to construct survival curves which were compared using the log-rank test. Variables included in the statistical analysis were age, duration of follow-up, parity, menopausal status, tumor cell type, lymphovascular invasion, nuclear grade, stage and mitotic index, along with immunohistochemistry results for biomarkers (p53, Bcl-2, c-Kit, ER, and HER-2). All tests were two-sided and a p value of < 0.05 was considered statistically significant.

Results

For 28 patients diagnosed with uterine sarcoma the mean follow-up time was 30 months (range from 1 to 120 months). However, for those who survived, the mean follow-up time was 35 months (range from 1 to 79 months). In our sample, 39.3 % of the cases were leiomyosarcoma (LMS), 39.3 %carcinosarcoma (CS), 10.7 % müllerien adenosarcoma (MAS) and 10.7 % endometrial stromal sarcoma (ESS). Sixteen patients were at stage I, 1 at stage II, 3 patients were stage III and 8 patients had stage IV tumor. The further

stratification of tumor stage based upon histological subtypes are given in Table 1. High grade tumors were identified in 78.6% patients and 21.4% had low grade tumors. All patients with LMS and CS had high grade tumors, while patients with MAS and ESS had low grade tumors. More than 75% cases had mitotic index greater than 10 per 10 HPF and all patients belonged to high grade sarcoma group (LMS and CS). Contrary to this, all patients from low grade sarcoma group (MAS and ESS) had mitotic index less than 10 per 10 HPF. Lymphovascular invasion was found in half of our sample, involving 54.5% of LMS patients, in 45.5% of CS patients, and in all ESS patients. However, no lymphovascular invasion was found in any of the MAS patients. (Table 1)

Descriptive statistical analysis of death and recurrence revealed a statistically significant association between high grade sarcomas (p=0.024), sarcomas with nuclear grade 3(p=0.029), mitotic index > 60 (p=0.016) and presence of lymphovascular invasion (p=0.028) with death in patients with uterine sarcoma. Table S2 gives a detailed description of the death and recurrence descriptive analysis. No significant association of any variable was found with recurrence.

All patients (12), who died had high grade sarcoma (HGS) and among living patients (16), ten were HGS. Similarly, more than 80% of the patients with recurrence were diagnosed with HGS. It was also observed that all dead patients had grade 3 sarcomas and most of the patients in which disease recurred also had grade 3 tumors. Among dead patients most were stage 4 followed by stage 1, while among living patients' stage 1 was the predominant followed by stage 4. Recurrence was equal among these two stages. Most of the dead cases had mitotic index > 30 and most of the living patients had mitotic index < 30. Most of the patients with no recurrence also had mitotic index < 30. Lymphovascular invasion was predominant among patients with recurrence or death.

For all patients the median overall survival time was 70 months. The 2, 5 and 10-year survival rates were 65%, 58% and 43% respectively. There was no statistically significant association between survival times and histologic types of sarcoma (p=0.204). There was no association between survival time and stages of sarcomas (p=0.266), however, stage 1 and 2 had a better survival (70 months) compared to stage 3 and 4 (14 months); Figure 1 depicts this relationship, employing Kaplan/Meier curve. The median time to recurrence in our sample was 7 months.

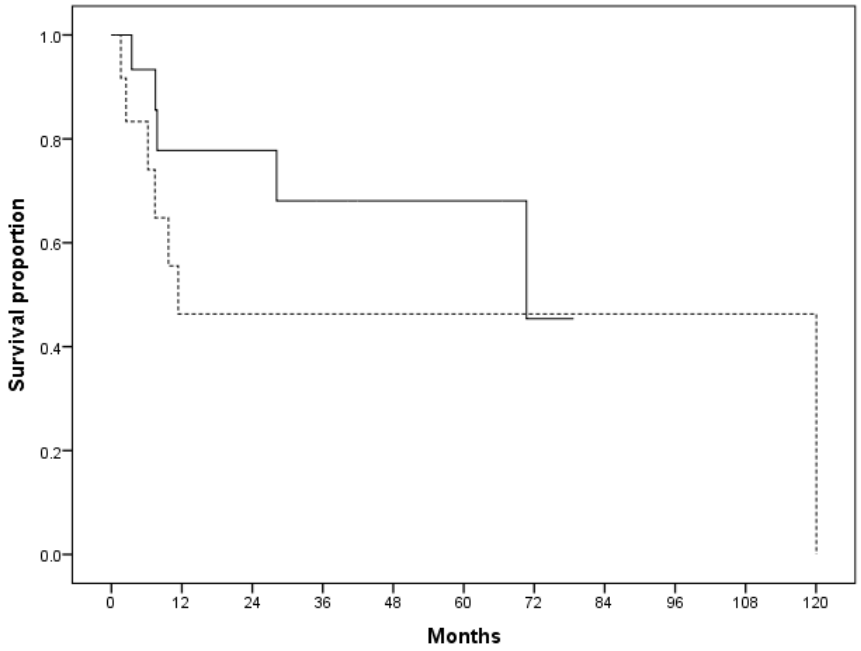

Figure 1. Kaplan/Meier curve for survival rate and times stratified according to Stage of disease (Solid line: stage 1 or 2, dotted line stage 3 or 4)

Immunohistochemistry data was available for twenty-four of the patients, and the biomarkers p53, Bcl-2, ER, c-kit and Her2 were analyzed in this study and are presented in Table S3. Positive overexpression of P53 was only found in 4 cases of CS; there was no P53 overexpression (wild type expression) seen in any of the other tumor subtypes. Complete membranous staining for Her-2 (3+) was only observed in CS tumors (6 cases in total). ER positive staining was found in all MAS and ESS tumors (total of 3 and 2 cases, respectively), however no ER staining was seen in LMS or CS tumors. However, positive Bcl-2 expression was seen in six cases of CS, four cases of LMS, three cases of MAS, and one case of ESS. For c-kit, positive expression was observed in 8 cases, 7of which were from high grade sarcoma group (4 from CS and 3 from LMS tumors). Only one tumor from ESS stained positive to C-Kit and none from MAS.

No significant association was observed between the immunohistochemical expression and recurrence and survival.

Discussion

Uterine sarcomas are rare, aggressive and heterogenous malignant tumors with poor prognosis and[14] Sarcomas have been reported to be a rare entity even among populations with a high incidence of uterine malignancies.[19] This rarity and heterogeneity have led to difficulty in identification of prognostic markers. Therefore, studies have been focused on establishing a consensus on these risk factors, in order to make the outcome more predictable. This study was another effort in this direction, as it evaluated prognostic relevance of prospective clinical, pathological and immunohistochemical risk factors and their influence on survival.

This study included 28 patients who were treated at our hospital during a period of 12 years. The distribution of different histological types in our sample was similar to what has been reported earlier by Koivisto-Korander et al.[6] On the other hand studies have reported increased frequency of LMS compared to other sub types (12, 20).The histologic diagnosis in this study was initially made or suspected on biopsy specimen for most of the patients but final diagnosis was confirmed on surgical specimen for all patients. Twenty four out of 28 patients also underwent staging laparotomy.

At diagnosis, the median age of our sample (58 ears) was almost the same as reported in similar studies by Koivisto-Koranderet al and Denschlag et al. On the other hand, the median follow up period in this study (11 months) was significantly shorter than what has been reported by Koivisto-Korander et al which was 41 months. This may indicate a strict follow up regimen. This may be the reason for a lower recurrence rate in our cohort (21.4%) compared to Koivisto-Koranderet al, who reported a recurrence rate of 50% (6). Sagae et al reported a greater recurrence rate among LMS (60%) followed by the CS (43.5%) and least among ESS (30%) (21). Contrary to these reported findings, our study observed highest recurrence among ESS (33.3%) followed by CS (27.3%) and least recurrence was observed for LMS (18.2%). About 54.5 % of LMS tumors were stage 1 compared to 45.5 % among CS and only 27.3 % of LMS tumors were stage 4 compared to 45.5 % of CS tumors. This may explain the greater incidence of recurrence among CS compared to LMS. On the other hand, the highest incidence of recurrence among EES tumors is an interesting finding as only 1 out of 4 tumors were stage 4 and remaining 2 were stage 1. This may be a result of genetic and molecular aberrations in this tumor as reported by Tsuyoshi et al.[22] A smaller sample size with only 3 EES tumors in a sample of 28 can be another reason and this finding may be incidental. In our sample of 28 tumors, 61 % were stage 1 and 2, while 39 % were stage 3 and 4 tumors. Despite having a significant percentage of tumors belonging to stage 3 and 4, the recurrence rate of 21.4 % indicates better diagnosis, appropriate treatment and strict compliance of follow up. Surgery was the main form of treatment in our patients (78.5 %), along with it 25 % underwent chemotherapy and 39.2 % radiotherapy. Among the patients in which recurrence occurred, all underwent surgery, with 3 treated with additional radiotherapy and 1 with chemotherapy.

Although majority of our patients were alive with disease, a substantial number died as well (42.8%) and among these were 63.6 % of all CS patients, 45.5 % of all LMS patients. None of the patients with ESS or MAS died. Although our recurrence rate was low, death percentage was significant. The aggressive nature of the disease is evident from the fact that a significant percentage died due to disease. Studies which have reported a better survival rates, also had a greater proportion of tumors belonging to stage 1 or 2 (77.8 %) (21). In our study comparatively a lower percentage was stage 1 or 2. This variation and heterogeneity of these tumors make the outcome unpredictable and therefore identification and consensus on prognostic factors is extremely important.

We report an improved 2, 5- and 10-years overall survival rates (65%, 58% and 43% respectively) in this study compared to 62%, 51% and 38% as reported by Koivisto-Korander et al. The median survival time although was not significantly different among the two studies (70 months and 65 months respectively). We did not observe a statistically significant influence of histologic types and stage of disease on the overall survival, but it was observed that median survival for LMS (120 month) was much greater than median survival for CS (30 months) (Figure 1). This finding is in line with the study by Kim et al who also reported better survival with LMS tumors and contradict to Evan at al.[23] Similarly, we report that median survival of stage 1 and 2 tumors is 5 times better than that of stage 3 and 4 tumors (Figure S2).

The importance of age, duration of follow-up, parity, menopausal status, tumor cell type, lymphovascular invasion, nuclear grade, stage and mitotic index, immunohistochemical biomarkers (p53, Bcl-2, c-Kit, ER, and HER-2) has already been established as important prognostic factors.[14,24,25] Our analysis of these factors revealed significant association of high nuclear grade, mitotic index greater than 60, and lymphovascular invasion with poor survival. Kim et al also reported association of mitotic index and tumor grade with overall survival.[12] None of the Immunohistochemical markers were found to be associated with overall survival. TP53 is a tumor suppressor gene and Cancer Genome Atlas (TCGA) Research Network has reported mutations and deletions in TP53.[26] Kim at al has also reported association of poor survival with overexpression of P53 protein. In our study only 16.7% tumors were positive for P53 over-expression and all of them were CS tumors. On the other hand, Bcl-2 has been reported to be associated with better outcome.[14] In our study 58.3% of all tumors stained positive for Bcl-2 and 100% of the MAS tumors were positive. The protective pro-apoptotic effect Bcl-2 may be an explanation of our observation that MAS tumors were all low grade, stage 1, without lymphovascular invasion and none of these tumors recurred nor any of MAS patients died.

The strength of this study includes precise diagnosis on surgical specimen from all patients, use of 5 immunohistochemical markers along with clinicopathologic parameters and analysis of overall survival along with death and recurrence statistics. The limitation of this study is small sample size despite of retrospective analysis of uterine sarcoma cases over a period of 12 years. This shows the rare nature of this neoplasm.

We conclude that this study confirms the previously reported findings of high-grade uterine sarcomas being aggressive tumors with relatively short survival rate. Greater mitotic index and nuclear grade, tumor cell type and vascular invasion are the most important prognostic indicators of survival. Though not observed to be significant in this study, ER, p53, c-Kit, and HER 2 can be useful ancillary tools to discriminate between high grade and low-grade uterine sarcoma in difficult cases and their analysis in larger study samples is highly recommended.

Conflict of Interest: Authors declare no conflict of interest.

Grant Support & Financial Disclosures: None.

Table S1: Clinical characteristics of 28 uterine sarcoma patients

Characteristics	All (n = 28)	LMS (n = 11)	CS (n = 11)	MAS (n = 3)	ESS (n = 3)
Mean age in years (SD)	54 (16.2)	50 (14.2)	65 (11)	47 (22.2)	36 (11.6)
Menstrual status					
Menopause	16 (57.1%)	4 (36.4%)	10 (90.9%)	2 (66.7%)	0
Pre-menopause	2 (7.1%)	2 (18.2%)	0	0	0
Menorrhagia	7 (25%)	3 (27.3%)	1 (9.1%)	1 (33.3%)	2 (66.7%)
Post-partum	1 (3.6%)	1 (9.1%)	0	0	0
Unknown	2 (7.1%)	1 (9.1%)	0	0	1 (33.3%)
Parity					
0	3(10.7%)	2(18.2%)	0	0	1(33.3%)
1-4	5(17.9%)	0	3(27.3%)	1(33.3%)	1(33.3%)
≥5	20(71.4%)	9(81.8%)	8(72.7%)	2(66.7%)	1(33.3%)
Surgery					
TAH & BSO	14 (50%)	5 (45.5%)	5 (45.5%)	2 (66.7%)	2 (66.7%)
TAH, BSO, & LN	5 (17.8%)	3 (27.3%)	2 (18.2%)	0	0
Sub TAH	3 (10.7%)	2 (18.2%)	1 (9.1%)	0	0
Biopsy only	6 (21.5%)	1 (9.0%)	3 (27.3%)	1 (33.3%)	1 (33.3%)
Chemotherapy					
Yes	7 (25%)	3(27.3%)	3 (27.3%)	0	1 (33.3%)
No	19 (68%)	7(63.6%)	8 (72.7%)	3 (100%)	1 (33.3%)
Unknown	2 (7.1%)	1 (9.1%)	0	0	1 (33.3%)
Radiotherapy					
Yes	11 (39.2%)	4 (36.4%)	6 (54.5%)	1 (33.3%)	0
No	15 (53.6%)	6(54.5%)	5 (45.5%)	2 (66.7%)	2 (66.7%)
Unknown	2 (7.1%)	1 (9.1%)	0	0	1 (33.3%)
Follow up					
Mean time (month)	31	27	27	48	35
Outcome					
DOD	12 (42.8%)	5 (45.5%)	7 (63.6%)	0	0
AWD	16 (57.1%)	6 (54.5%)	4 (36.4%)	3 (100%)	3 (100%)
Recurrence					
Yes	6 (21.4%)	2 (18.2%)	3 (27.3%)	0	1 (33.3%)
No	18 (64.3 %)	6 (54.5%)	8 (72.7%)	3 (100%)	2 (66.7%)
Unknown	4 (14.3%)	3 (27.3%)	0	0	0

AWD, alive with disease; BSO, bilateral salpingo-oophorectomy; CS, carcinosarcoma; DOD, died of disease; ESS, endometrial stromal sarcoma; LMS, leiomyosarcoma; LN, lymph nodes; MAS, müllerien adenosarcoma; SD, standard deviation; TAH, total abdominal hysterectomy;

Table S2: Descriptive analysis of clinicopathologic parameters with death and recurrence in 28 Sarcoma patients

Variable	Death			Recurrence		
	Dead n=12 (42.9%)	Alive n=16 (57.1%)	p-value	Yes n=6 (21.4%)	No n=22 (78.6%)	p-value
Sarcoma Grade						
High	12(100%)	10(62.5%)	0.024	5(83.3%)	17(77.3%)	1.0
Low	0	6(37.5%)		1(16.7%)	5(22.7%)	
Nuclear Grade						
1	0	5(31.3%)		1(16.7%)	4(18.2%)	
2	0	1(6.2%)	0.029	0	1(4.5%)	1.0
3	12(100%)	10(62.5%)		5(83.3%)	17(77.3%)	
Stage						
1	4(33.3%)	11(68.8%)		2 (33.3%)	14(63.6%)	
2	1(8.3%)	0		1(16.7%)	0	0.22
3	1(8.3%)	2(12.5%)	0.12	1(16.7%)	2(9.1%)	
4	6(50%)	3(18.7%)		2 (33.3%)	6(27.3%)	
Mitotic Index						
<30	5(41.7%)	12(75%)		3 (50%)	14(63.6%)	
30-60	2(16.6%)	4(25%)	0.016	2 (33.3%)	4(18.2%)	0.8
>60	5(41.7%)	0		1(16.7%)	4(18.2%)	
Lymphovascular invasion						
Yes	7(58.3%)	7(43.8%)		5(83.3%)	9(40.1%)	
No	1(8.3%)	8(50%)	0.028	1(16.7%)	8(36.3%)	0.25
Unknown	4(33.3%)	1(6.2%)		0	5(22.6%)	
Parity						
<5	3(25%)	4(25%)	1.0	2(33.3%)	4(18.2%)	1.0
>5	9(75%)	12(75%)		4(66.7%)	16(72.7%)	
Unknown	0	0		0	2(9.1%)	

Table S3: Immunohistochemical analysis of patients among the different types of uterine sarcomas

Biomarker	All (n = 24)	CS (n = 10)	LMS (n = 9)	MAS (n = 3)	ESS (n = 2)
p53					
Over Expression	4 (16.7%)	4(40%)	0	0	0
Negative	20(83.3%)	6(60%)	9(100%)	3(100%)	2(100%)
ER					
Positive	5(20.8%)	0	0	3(100%)	2(100%)
Negative	19(79.2%)	10(100%)	9(100%)	0	0
Bcl-2					
Positive	14(58.3%)	6(60%)	4(44.4%)	3(100%)	1(50%)
Negative	10(41.7%)	4(40%)	5(55.6%)	0	1(50%)
Her2					
Positive (3+)	6(27%)	6(60%)	0	0	0
Negative	18(75%)	4(40%)	9(100%)	3(100%)	2(100%)
C-kit					
Positive	8 (33.3%)	4 (40%)	3 (33.3%)	0	1 (50%)
Negative	16 (66.7%)	6 (60%)	6 (66.7%)	3 (100%)	1 (50%)

LMS, leiomyosarcoma; CS, carcinosarcoma; ESS, endometrial stromal sarcoma; MAS, müllerien adenosarcoma

.References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11-30.
2. Platz CE, Benda JA. Female genital tract cancer. Cancer. 1995;75(1 Suppl):270-94.
3. Nordal RR, Thoresen SO. Uterine sarcomas in Norway 1956-1992: incidence, survival and mortality. Eur J Cancer. 1997;33(6):907-11.
4. Pautier P, Genestie C, Rey A, Morice P, Roche B, Lhomme C, et al. Analysis of clinicopathologic prognostic factors for 157 uterine sarcomas and evaluation of a grading score validated for soft tissue sarcoma. Cancer. 2000;88(6):1425-31.
5. Denschlag D, Masoud I, Stanimir G, Gilbert L. Prognostic factors and outcome in women with uterine sarcoma. Eur J Surg Oncol. 2007;33(1):91-5.
6. Koivisto-Korander R, Butzow R, Koivisto AM, Leminen A. Clinical outcome and prognostic factors in 100 cases of uterine sarcoma: experience in Helsinki University Central Hospital 1990-2001. Gynecol Oncol. 2008;111(1):74-81.
7. Gadducci A, Cosio S, Romanini A, Genazzani AR. The management of patients with uterine sarcoma: a debated clinical challenge. Crit Rev Oncol Hematol. 2008;65(2):129-42.
8. Prat J. FIGO staging for uterine sarcomas. Int J Gynaecol Obstet. 2009;104(3):177-8.
9. D'Angelo E, Spagnoli LG, Prat J. Comparative clinicopathologic and immunohistochemical analysis of uterine sarcomas diagnosed using the World Health Organization classification system. Hum Pathol. 2009;40(11):1571-85.
10. Major FJ, Blessing JA, Silverberg SG, Morrow CP, Creasman WT, Currie JL, et al. Prognostic factors in early-stage uterine sarcoma. A Gynecologic Oncology Group study. Cancer. 1993;71(4 Suppl):1702-9.
11. Abeler VM, Royne O, Thoresen S, Danielsen HE, Nesland JM, Kristensen GB. Uterine sarcomas in Norway. A histopathological and prognostic survey of a total population from 1970 to 2000 including 419 patients. Histopathology. 2009;54(3):355-64.
12. Kim SH, Kim JW, Kim YT, Kim JH, Yoon BS, Ryu HS. Prognostic factors and expression of p53 and mdm-2 in uterine sarcomas. Int J Gynaecol Obstet. 2006;95(3):272-7.
13. 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(1):54-61.
14. D'Angelo E, Espinosa I, Ali R, Gilks CB, Rijn M, Lee CH, et al. Uterine leiomyosarcomas: tumor size, mitotic index, and biomarkers Ki67, and Bcl-2 identify two groups with different prognosis. Gynecol Oncol. 2011;121(2):328-33.
15. FIGO (International Federation of Gynecology and Obstetrics) 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006;95 Suppl 1:S1-257.
16. Yang W, Wei B, Chen M, Bu H. [Evaluation of immunohistochemistry HER2 results interpretation in invasive micropapillary carcinoma of the breast]. Zhonghua Bing Li Xue Za Zhi. 2015;44(1):48-52.
17. Mukherjee T. Interpretation of ER and Her2neu hormonal receptor in breast cancer. Med J Armed Forces India. 2016;72(1):99.
18. Pilloni L, Bianco P, Difelice E, Cabras S, Castellanos ME, Atzori L, et al. The usefulness of c-Kit in the immunohistochemical assessment of melanocytic lesions. Eur J Histochem. 2011;55(2):e20.
19. Kostun J, Bouda J, Vlasak P, Bartakova A, Berezovskiy D, Weinberger V, et al. Incidence of uterine sarcomas in West Bohemia, at the Department of Gynaecology and Obstetrics, University Hospital in Pilsen, Czech Republic. Ceska Gynekol. 2017;82(6):436-42.
20. Livi L, Paiar F, Meldolesi E, Simontacchi G, Amunni G, Barca R, et al. Treatment of uterine sarcoma at the University of Florence from 1980-2001. Tumori. 2005;91(2):139-43.
21. Sagae S, Yamashita K, Ishioka S, Nishioka Y, Terasawa K, Mori M, et al. Preoperative diagnosis and treatment results in 106 patients with uterine sarcoma in Hokkaido, Japan. Oncology. 2004;67(1):33-9.
22. Tsuyoshi H, Yoshida Y. Molecular biomarkers for uterine leiomyosarcoma and endometrial stromal sarcoma. Cancer Sci. 2018.
23. Evans HL. Endometrial stromal sarcoma and poorly differentiated endometrial sarcoma. Cancer. 1982;50(10):2170-82.
24. Terek MC, Akman L, Hursitoglu BS, Sanli UA, Ozsaran Z, Tekindal MA, et al. The retrospective analysis of patients with uterine sarcomas: A single-center experience. J Cancer Res Ther. 2016;12(1):309-13.
25. Wolfson AH, Wolfson DJ, Sittler SY, Breton L, Markoe AM, Schwade JG, et al. A multivariate analysis of clinicopathologic factors for predicting outcome in uterine sarcomas. Gynecol Oncol. 1994;52(1):56-62.
26. Cancer Genome Atlas Research Network. Electronic address edsc, Cancer Genome Atlas Research N. Comprehensive and Integrated Genomic Characterization of Adult Soft Tissue Sarcomas. Cell. 2017;171(4):950-65 e28.

Authors' Contribution:

AMAK: conceived the study, designed it, analysed the data and contributed in manuscript writing.

AO: Study conception, study conduction, manuscript writing

HA: Data analysis and interpretation

MB: Data analysis and interpretation

Int.j.pathol.2019;17(1): 176-185

HISTORY	
Date Received	12-03-2019
Date sent for Reviewer	19-03-2019
Date Received Reviewer's Comments	24-05-2019
Date Received Revised Manuscript	10-06-2019
Date Accepted	11-06-2019