Original paper

The role of histology, grading, location of tumour and ploidy in evaluation of outcome in patients with liposarcoma

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The review of literature indicates that several clinico-morphological factors such as location of the primary tumour as well as its size, histologic subtype, and grade or even selected molecular changes may significantly affect survival of liposarcoma (LPS) patients. Data concerning prognostic importance of DNA ploidy status in LPS cells are extremely limited and results of flow cytometry (FCM) studies have never been compiled with the current classification of malignant adipocytic tumours.

Based on evaluation of material from 54 liposarcomas which was available for both histological and FCM analysis, we distinguished four prognostic groups of patients. The best prognosis was noticed for diploid and grade G1 well-differentiated or myxoid liposarcomas localised on extremities. None of the patients with lipoma-like WDLPS and myxoid liposarcoma grade 1 metastasised, while metastases were observed among patients with dedifferentiated LPS (70% of 5-year MFS) and cellular myxoid or round cell liposarcoma (20% of 5-year MFS, only). The metastasis-free survival curves for the above mentioned groups of patients differed significantly ($p = 0.00001$)

Key words: liposarcoma, survival, tumour localization, ploidy, grading.

Introduction

Liposarcoma (LPS) accounts for approximately 20% of sarcomas in adults, being the most frequently encountered malignant soft tissue tumour in clinical practice. It is a complex neoplasm that is divided into different histological subtypes. The histological subgroups of LPS present different clinical characteristics and survival outcomes [1].

A review of survival data from the literature indicates that several clinico-morphological factors such as tumour size, histologic subtype [2], and grading [2, 3] or even selected molecular changes [4, 5, 6, 7] may significantly affect disease-free or/and overall survival of LPS patients.

What is more, according to the newest edition of the AJCC staging system [8] liposarcomas like other soft tissue sarcomas (STS) are no longer classified as a single malignancy; instead, new site-specific staging systems for STS of the trunk and extremities, retroperitoneum or head and neck have been developed. The peculiarity of STS is that the oncological outcome is strongly influenced by both histologic subtype and site of the tumour. This observation is reflected in some of the newer nomograms [9], including liposarcoma-specific nomogram introduced by the Memo-

rial Sloan-Kettering Cancer Centre (MSKCC) group in 2006 [10].

The data concerning the prognostic importance of DNA ploidy status in LPS cells are extremely limited [11], and results of flow cytometry (FCM) studies have never been compiled with the current classification of malignant adipocytic tumours [1].

The aim of this study was to determine the prognostic factors influencing the survival of patients affected by liposarcoma with consideration for location of the tumour, histological subtype and grade of LPS as well as ploidy of neoplastic cells. The mentioned characteristics were evaluated on the basis of retrospective analysis of overall- and metastasis-free survival of liposarcoma patients treated in a single oncological centre over 10 years.

Material and methods

Clinicopathological data were obtained from medical records collected in the archives of the Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology, Kraków, Poland. Paraffin blocks and haematoxylin and eosin-stained (HE) slides were retrieved from the Department of Tumour Pathology. Before embedding the material in paraffin blocks, the representative samples from each fresh tumour tissue were placed in 10% buffered formalin, and routinely processed. Additionally, small fragments of each tumour were placed in Hanks solution and PBS.

Pathological evaluation of HE slides was performed by two pathologists experienced in soft tissue pathology. Liposarcomas were classified into 4 subtypes according to the WHO classification. Tumour grade was evaluated according to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) system [1]. The final diagnosis in each problematic case was confirmed by MDM2 amplification evaluated by FISH (10 cases) or/and karyotype study (17 cases).

Flow cytometry

Assessment of DNA content was performed on the suspensions of cell nuclei from fresh tissue specimens. After mincing with scissors the tissue was disaggregated mechanically. Then aliquots of 1-2 $\times$ 10^6 cells were incubated with the staining solution (PI-Calbiochem 50 μg/ml, Nonidet P-40 and RNAse A-Sigma 1 mg/ml). The suspensions were sampled on FACSCalibur flow cytometer equipped with an argon laser (15 Mw, 488 nm). For each histogram at least 20 000 particles were analysed. For analysis of DNA histograms the ModFit software was used. The DNA histograms were classified according to principles adopted at the DNA Cytometry Consensus Conference 1992 [12]. Ploidy was expressed as DNA index (DI), and proliferation rate as percentage of S-phase fraction (SPF).

Statistical analysis

The significance of differences between mean values was tested by Student's t-test. The significance of relation between categorical variables was tested by χ^2 test. The influence of factors on survival was assessed by log-rank test in univariate analysis and Cox proportional hazard model in multivariate analysis. A p-value of < 0.05 was considered statistically significant.

Results

Patients' characteristics

In total, 54 patients diagnosed with liposarcoma and available for FCM analysis were identified. The clinical characteristics of the enrolled patients are depicted in Table I. A. The studied group consisted of 26 women and 28 men, with a median age of 58 years (range 24-78 years). The tumours were located in retroperitoneal space (26 cases, 48.2%), extremities (25, 46.3%), and mediastinum, pelvis and abdominal cavity (one case, each). According to the newest WHO classification, the tumours were divided into four histologic subtypes: well differentiated (WD-LPS) (16 cases, 29.7%), dedifferentiated (DD-LPS) (17 cases, 31.5%) myxoid (M-LPS) (20 cases, 37.0%), and pleomorphic (P-LPS) (1 case) liposarcomas. Then, DD-LPS tumours were dichotomised into low-grade DD (8 cases, 14.8%) and high-grade DD (9 cases, 16.7%) variants. Myxoid LPS were also divided into classic (myxoid LPS G1; 5 cases, 9.2%) and non-classic (cellular myxoid and round cell; 15 cases, 27.8%) ones. There were 30 (55.6 %) patients with low-grade (G1) and 24 (44.4 %) patients with high-grade liposarcomas. In the latter group we distinguished 15 (27.8 %) and 9 (16.6 %) tumours with grade 2 and grade 3, respectively.

FCM data

The FCM characteristics of the enrolled patients are depicted in Table IB.

Interpretable DNA histograms were obtained in all cases. Most of the cases, 39/54 (72.2%), were diploid. Within 15 (27.8%) aneuploid samples the DI values were specified as: near-diploid (0.91-1.18) in 5 cases, aneuploid (1.52-1.8) in 4 cases, and near-tetraploid (2.02-2.12) in 5 cases; one case was defined as multiploid (1.11 +2.23).

Correlation between DI and SPF and variables studied was observed for histological type ($p = 0.0170$ and $p = 0.0352$, respectively) and localization ($p = 0.0344$ and $p = 0.0474$, respectively).

Table IA. Clinicopathological characteristics of 54 patients with liposarcoma

Parameter	WD-LPS n = 16	DD-LPS		M-LPS		P-LPS n = 1
		Low-grade n = 8	High-grade n = 9	Classic G1 n = 5	Cellular/round cell n = 15	
Female	8	5	5	2	6	1
Male	8	3	4	3	9	0
Age (years) median (range)	62 (35-78)	62 (35-71)	59 (39-68)	46 (24-68)	50 (37-64)	69
Location:						
Retroperitoneum and mediastinum, pelvis, and abdominal cavity	11	8	9	0	1	0
Extremity	5	0	0	5	14	1
Tumour size (mm) median (range)	175 (100-440)	300 (200-450)	250 (80-550)	80 (25-210)	170 (55-330)	180
Tumour grade						
G1	16	8	0	5	1	0
G2	0	0	6	0	9	0
G3	0	0	3	0	5	1
Number of patients with						
no recurrence	10	1	3	0	3	0
local recurrence	6	7	5	5	4	0
distal metastases	0	2	1	0	12	1
histolological progression	1	4	0	0	3	0
Patients' outcomes						
alive without symptoms	10	1	3	5	4	0
alive with disease	2	4	5	0	2	1
dead by disease	4	3	3	0	8	0
dead by other cause	0	0	1	0	1	0

WD-LPS – well differentiated liposarcoma; DD-LPS – dedifferentiated liposarcoma; M-LPS – myxoid liposarcoma; P-LPS – pleomorphic liposarcoma

Table IB. FCM characteristics of 54 patients with liposarcoma

Parameter	WD-LPS n = 16	DD-LPS		M-LPS		P-LPS n = 1
		Low-grade n = 8	High-grade n = 9	Classic G1 n = 5	Cellular/round cell n = 15	
Diploid	12	5	4	5	12	**0**
Aneuploid	4	3	5	0	3	**1**
%SPF median (range)	1.2 (0.4-24.4)	3.0 (0.7-26.6)	6.8 (1.4-38.6)	1.8 (0.3-6.1)	1.9 (0.3-24.0)	**4.5**

WD-LPS – well differentiated liposarcoma; DD-LPS – dedifferentiated liposarcoma, M-LPS – myxoid liposarcoma; P-LPS- pleomorphic liposarcoma

Within tumours localised in extremities 4 (15.4%) had aneuploid cell line, in contrast to 11 (39.3%) retroperitoneal tumours.

Survival analyses

Follow-up data were available for 54 patients. Detailed data regarding clinical outcome of patients studied are depicted in Table IA.

Twenty-eight (52%) patients experienced at least one local recurrence. The total number of local recurrences ranged from 1 to 6 and median number of local recurrences was 2. Local recurrence-free survival ranged from 2 to 88 months (median 28 months). Distant metastases appeared in 16 patients. They were localized in lungs and liver (3 cases, each), abdominal cavity (2 cases), soft tissues (nine cases) and bones (one case).

The median overall survival was 65 months (ranged 4-393), and the median DFS and MFS survival was 35 months (ranged 0-114), and 54 months (1-393), respectively.

The median overall survival was significantly worse in the group of patients with high grade dedifferentiated liposarcoma (41 months). The median overall survival for WD liposarcoma, low-grade dedifferentiated liposarcoma, classic myxoid liposarcoma, and cellular myxoid/round cell liposarcoma cases was 69 (9-133), 46 (10-181), 123 (86-393), and 48 (12-114) months, respectively. The patient with pleomorphic LPS died after 41 months.

None of the three analysed variables (tumour location, grade, and ploidy) influenced alone the overall survival (Figs. 1A-C), however taking all these features together allowed us to draw OS curves, which differed significantly (Tables IIIA, B and Fig. 2A, B). It is worth pointing out that the prognostic group 0 (diploid and grade G1 tumours localised on extremities) consisted of 5 well-differentiated liposarcomas (lipoma-like variant) and 5 tumours with classic myxoid liposarcoma texture. All those patients have lived without signs of disease for 62-393 months (median OS: 108 months). The only exception in this group was a tumour presenting the histological picture typical for cellular myxoid liposarcoma. Its grade was estimated as G1 because of low mitotic activity and lack of necrosis of neoplastic tissue. This tumour progressed into round cell liposarcoma in the next relapse, and the patient died 20 months later.

The situation was quite different for patients from prognostic group no 2 (PG2). Half of the 22 patients of this group died of disease, five persons were alive with symptoms of clinical progression. The OS curves of PG0 and PG2 groups differ significantly ($p = 0.0336$). Clinicopathological characteristics of patients in the prognostic groups were summarized in Table II and their outcome was depicted in Tables IIIA, B.

On multivariate analysis, localisation of the tumour, grade and ploidy predicted overall survival. Combination of these three parameters allowed for distinction of the 4 prognostic groups of patients (Fig. 2A, B, Table IIIA, B).

Among liposarcomas localised in the retroperitoneal space, we could distinguish 11 cases with typical well-differentiated lipoma-like histological picture and 8 tumours presenting so-called non-classic texture (cellular or myxoid variants of WDLPS), which were named as low-grade dedifferentiated liposarcomas. The primary tumours in the low-grade dedifferentiated group were significantly larger ($p = 0.0167$) (Fig. 3). The mean diameter of low-grade dedifferentiated liposarcomas localised in the retroperitoneal space was 330 mm (SD: 260-400 mm), meanwhile the primary tumours of well differentiated liposarcoma measured an average of 210 mm (SD: 150-265 mm).

Out of eleven patients with lipoma-like WD-LPS, five alive without symptoms 52-82 months (median

Table II. Clinicopathological characteristics of 54 patients with liposarcoma based on the prognostic groups

Parameter	PG0 n = 11	PG1 n = 11	PG2 n = 22	PG3 n = 10
Female	5	5	12	5
Male	6	6	10	5
Age (years) median (range)	58 (24-71)	61 (45-78)	55 (35-77)	59 (39-68)
Location				
Retroperitoneum and mediastinum, pelvis, and abdominal cavity	0	7	12	10
Extremity	11	4	10	0
Tumour size (mm) median (range)	170 (25-350)	175 (55-420)	200 (100-450)	225 (80-550)
Tumour grade				
G1	11	7	12	0
G2	0	2	6	7
G3	0	2	4	3
Diploid	11	0	22	5
Aneuploid	0	11	0	5
%SPF median (range)	1.6 (0.3-6.1)	4.5 (1.9-26.6)	1.1 (0.3-6.5)	5.7 (1.4-38.6)
Noumber of patients with				
no recurrence	3	5	6	3
local recurrence	8	3	10	6
distal metastases	1	3	10	2
histologic progression	2	1	4	1
Patients' outcomes				
alive without symptoms	10	4	6	3
alive with disease	0	4	5	2
dead by disease	1	3	11	3
dead by other cause	0	0	0	2

PG0 – prognostic group 0; PG1 – prognostic group 1; PG2 – prognostic group 2; PG3 – prognostic group 3

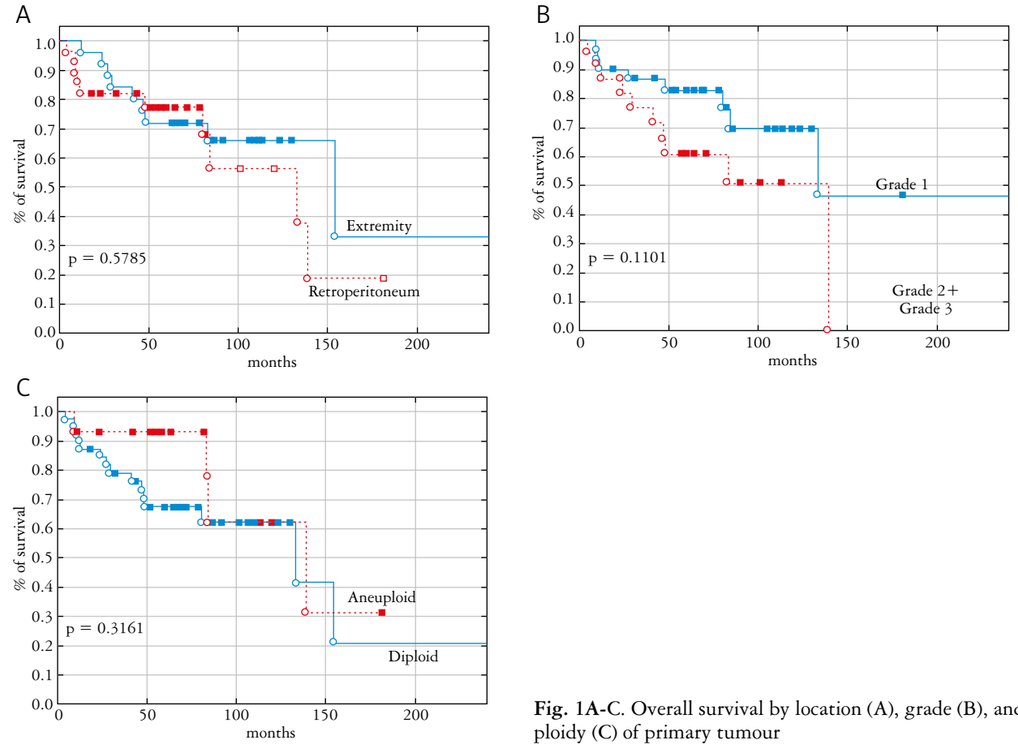

Fig. 1A-C. Overall survival by location (A), grade (B), and ploidy (C) of primary tumour

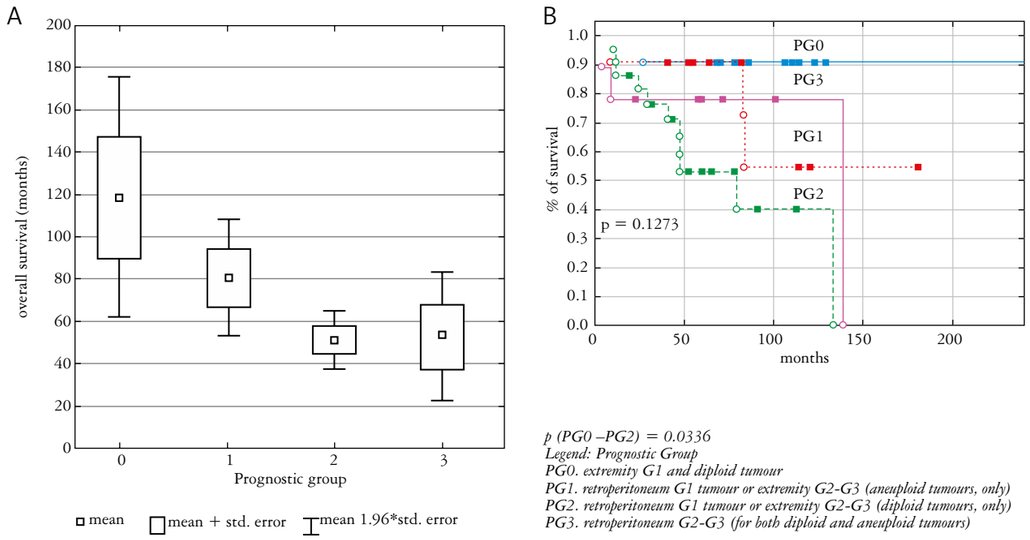

Fig. 2A, B. Overall survival in the four prognostic groups of liposarcoma patients: box plot (A) and curves (B)

Table IIIA. Clinical outcome based on combination of histological grade, localization, and ploidy in 54 cases of liposarcoma

Prognostic Group	N	Overall survival Median (range)	Metastasis No of cases (%)	Metastasis-free survival Median (range)
0. extremity, G1 and diploid tumour	11	106 (27-393)	1 (9.1%)	(5-393)
1. retroperitoneum G1 or extremity G2-G3 tumour (aneuploid tumours only)	11	82 (9-181)	3 (27.3%)	55 (9-181)
2. retroperitoneum G1 or extremity G2-G3 tumour (diploid tumours only)	22	47 (10-133)	10 (45.5%)	29 (1-133)
3. retroperitoneum G2-3, (both diploid and aneuploid tumours)	10	40 (1-139)	2 (20%)	22 (1-139)

Table IIIB. Clinical outcome based on combination of histological grade, localization, and ploidy in 54 cases of liposarcoma

Prognostic Group Number of cases	0 N = 11	1 N = 11	2 N = 22	3 N = 10
Without recurrence or metastases	3 (27.3%)	5 (45.5%)	6 (27.3%)	3 (30.0%)
Recurrences	8 (72.7%)	3 (27.3%)	10 (45.5%)	6 (60.0%)
Metastases	1 (9.1%)	3 (27.3%)	10 (45.5%)	2 (20.0%)
Histologic progression	2 (18.2%)	1 (9.1%)	4 (18.2%)	1 (10.0%)
Alive without symptoms	10 (90.9%)	4 (36.4%)	6 (27.3%)	3 (30.0%)
Alive with disease	0	4 (36.4%)	5 (22.7%)	2 (20.0%)
Dead by disease	1 (9.1%)	3 (27.3%)	11 (50.0%)	3 (30.0%)
Dead by other cause				2 (20.0%)

60), four patients died of disease. Among them were two patients with huge and not removable retroperitoneal tumours. Conversely, in the second group (LG-dedifferentiated liposarcomas) only 1 patient lived without symptoms of disease (120 months). Four patients lived with signs of disease (19-181 months, median: 38.0 months), and three patients died (after 10-84 months, median 48 months).

The overall survival of patients with classic form of myxoid liposarcoma (myxoid liposarcoma G1) and patients with tumours presenting non-classic texture (cellular myxoid liposarcomas and round cell liposarcomas) also differed (p = 0.0233) (Fig. 4). None of the patients with myxoid liposarcoma G1 died because of neoplastic disease.

There was a statistically significant difference between survival of the patients with and without metastases in each histological subtype of tumour. The increased risk of metastasis correlated with histologic subtype (p = 0.0002) and grade of tumour (p = 0.0007). None of the patients with lipoma-like WD-LPS and myxoid liposarcoma grade 1 developed metastasis, while metastases were observed among patients with dedifferentiated LPS and cellular myxoid or round cell liposarcoma. The metastasis-free survival curves for above mentioned groups of patients differed significantly (p = 0.00001) (Fig. 5). The increased risk of metastasis correlated also with primary tumour site (p = 0.0394), and age of patients (p= 0.0037). The metastases were noticed in 4/29 (13.8%) patients with retroperitoneal tumours and 13/26 (50.0%) patients with tumours located on the extremities. Ploidy and SPF of tumour cells did not influence the risk of metastases.

Discussion

According to the current WHO classification malignant adipocytic tumours are categorised into four subtypes based on histologic findings: atypical lipomatous tumour/well differentiated liposarcoma, dedifferentiated LPS, myxoid LPS and pleomorphic LPS. Well differentiated LPS displays the prototypic characteristics of adipocytic neoplasm, namely adult fat and additionally present scattered hyperchromatic, often multinucleated stromal cells, and fibrillary, collagenous zones ranging from minimal (lipoma-like WD-LPS) to dominant (sclerosing variant of WD-LPS). Dedifferentiated liposarcoma is defined by a combination of WD-LPS texture and cellular, nonlipogenic sarcoma of variable histological grade [1]. In 1997, Henricks *et al*. proposed the concept of low-grade dedifferentiation in reference to fibrous components of WDLPS that had cellularity reminiscent of fibromatosis or low-grade fibrosarcoma [13]. Elgar and Goldblum, in their report on 20 cases of well-dedifferentiated and dedifferentiated liposar-

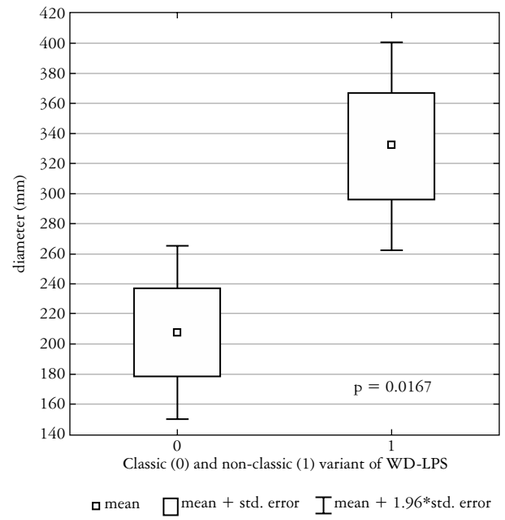

Fig. 3. The mean size of well-differentiated (lipoma-like) and low-grade dedifferentiated liposarcoma localised in the retroperitoneal space

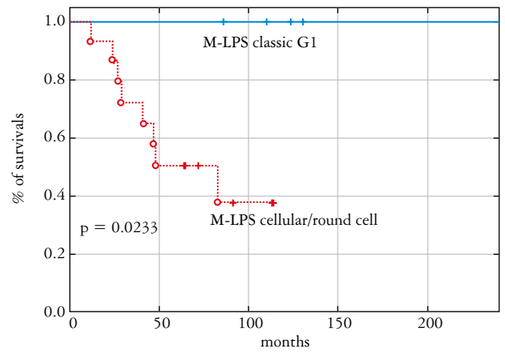

Fig. 4. Overall survival curves for patients with myxoid/round cell liposarcomas

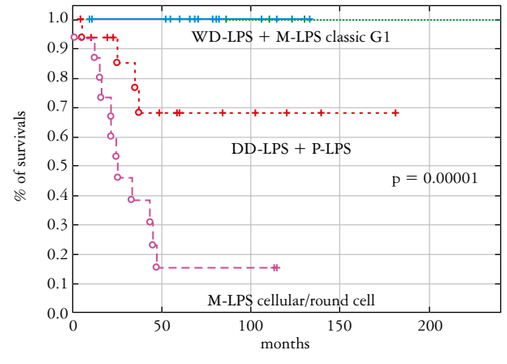

Fig. 5. Metastasis-free survival based on the histological type and grade of primary tumour

coma of the retroperitoneum, included also a low-grade dedifferentiated category that was defined in a manner similar to that proposed by Henricks *et al*. Interestingly, Elgar and Goldblum did not find a significant difference in survival among patients with conventional well differentiated LPS, low-grade dedifferentiated liposarcoma, and high-grade dedifferentiated liposarcoma [14]. What is more, Hisaoka *et al*. described eight cases of unusual liposarcoma with combined well-differentiated and myxoid areas resembling the texture of myxoid malignant fibrous histiocytoma or myxoid liposarcoma. However, none of these tumours with myxoid MFH-like features had TLS/FUS-CHOP fusion transcripts characteristic of myxoid and round cell liposarcomas. On the contrary, one case with the myxoid lesions had nonrandom chromosomal aberrations, such as ring and marker chromosomes, characteristic of a well-differentiated variant of liposarcoma. Hisaoka *et al.* suggested that myxoid changes in WDLPS might represent a form of dedifferentiation [15]. Dedifferentiated liposarcomas associated with myxoid MFH-like or myxoid liposarcoma-like component were described subsequently by Hasegawa *et al.* [16], and Huang *et al*. [17].

Taking into consideration the above-mentioned findings, we stratified our well-differentiated and dedifferentiated liposarcomas according to the WHO classification, but we distinguished additionally a low-grade LPS group with histological picture corresponding to the tumours that Evans named as cellular atypical lipomatous tumours or low-grade dedifferentiated liposarcoma [18]. In this group we included five cases characterised by significant amounts of myxoid stroma. Two of them presented the histologic picture imitating myxoid liposarcoma texture. The final diagnoses of those cases was confirmed by both cytogenetic and molecular studies (MDM2 amplification by FISH). Our classic variant of WDLPS corresponds to Evans' conventional atypical lipomatous tumour.

Initially we stratified myxoid liposarcomas into three groups, the same as Dalal *et al.* [10], according to the percentage of round cells in the tumour texture. However, we did not find the difference in overall- and metastasis-free survivals between so-called cellular/transitional myxoid LPS and round cell LPS, so finally we put these two groups together. In this way myxoid liposarcomas were divided into classic myxoid LPS grade 1 and the cellular/ round cell LPS.

For the presented group of LPS patients the median overall survival was 65 months (ranged 4-393 months), and the median DFS and MFS survival was 35 months (ranged 0-114 months), and 54 months (1-393), respectively. None of the three analyzed variables (tumour location, grade, and ploidy) influenced alone the overall survival, however taking all these features together allowed us to draw OS curves, which differed significantly. Among the four prognostic groups the patients with myxoid LPS grade 1 or/and well differentiated LPS (lipoma-like variant) localised on extremities had the most favorable prognosis. Almost none of the patients developed distant metastasis nor died of disease in 20 years of observation. What is more, we found statistically significant difference in the metastasis-free survival curves between tumours localized in the retroperitoneum and diagnosed as WDLPS grade 1 and both low and/or high grade dedifferentiated liposarcomas. The worse value of metastasis free survival was noticed for patients with LPS tumours built of cellular myxoid and/or round cell texture.

Conclusions

1. Patients with myxoid liposarcoma grade 1 or/and well-differentiated liposarcoma (lipoma-like variant) localised on extremities have the most favorable prognosis. None of those patients died of disease in 20 years of observation. One exception in this group was a female patient with myxoid liposarcoma, graded G1 apart from focally cellular texture, who developed histologic progression (G1 - > G2) and subsequent tumour spread and death.

2. The lowest proportion of overall survival (50% of 5-year survival and 40% of 10-year survival) was noted in diploid liposarcomas localised of the extremities (G2 or G3 tumous) or in the retroperitoneal space.

3. The increased risk of metastasis correlated with histologic type and grade of tumour. None of the patients with lipoma-like WDLPS and myxoid liposarcoma grade 1 developed metastasis, while metastases were observed among patients with dedifferentiated LPS (70% of 5-year MFS) and cellular myxoid or round cell liposarcoma (20% of 5-year MFS, only). The metastasis-free survival (MFS) curves for the above-mentioned groups of patients differed significantly ($p = 0.00001$)

The authors declare no conflict of interest.

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