CLINICS 2008;63(2):157-64

CLINICAL SCIENCE

PROGNOSTIC FACTORS AND EXPRESSION OF *MDM2* IN PATIENTS WITH PRIMARY EXTREMITY LIPOSARCOMA

Rosalvo Zósimo Bispo Júnior, Olavo Pires de Camargo, Cláudia Regina G. C. M. de Oliveira, Renée Zon Filippi, André Mathias Baptista, Marcelo Tadeu Caiero

Bispo Júnior RZ, Camargo OP de, Oliveira CRGCM de, Filippi RZ, Baptista AM, Caiero MT. Prognostic factors and expression of *MDM2* in patients with primary extremity liposarcoma. Clinics. 2008;63:157-64.

OBJECTIVE: The objective of this study was to investigate *MDM2* (murine double minute 2) protein expression and evaluate its relationship with some anatomical and pathological aspects, aiming also to identify prognostic factors concerning local recurrence-free survival, metastasis-free survival and overall survival in patients with primary liposarcomas of the extremities.
MATERIALS AND METHODS: Of 50 patients with primary liposarcomas of the extremities admitted to a Reference Service, between 1968 and 2004, 25 were enrolled in the study, following eligibility and exclusion criteria.
RESULTS: The adverse factors that influenced the risk for local recurrence in the univariant analysis included male sex ($P = 0.023$), pleomorphic histological subtype ($P = 0.027$), and high histological grade ($P = 0.007$). Concerning metastasis-free survival, age less than 50 years ($P = 0.040$), male sex ($P = 0.040$), pleomorphic subtype ($P < 0.001$), and high histological grade ($P = 0.003$) had a worse prognosis. Adverse factors for overall survival were age under 50 years ($P = 0.040$), male sex ($P = 0.040$), pleomorphic subtype ($P < 0.001$), and high histological grade ($P = 0.003$).
CONCLUSIONS: There was no correlation between immunohistochemically observed *MDM2* protein expressions and the anatomical and pathological variables studied. The immunohistochemical expression of *MDM2* protein was not considered to have a prognostic value for any of the surviving patients in this study (local recurrence-free survival, metastasis-free survival, or overall survival). The immunoexpression of *MDM2* protein was a frequent event in the different subtypes of liposarcomas.

KEYWORDS: Immunohistochemistry. Proto-oncogenic proteins. Prognosis. Liposarcoma/pathology. Liposarcoma/surgery.

INTRODUCTION

Soft tissue sarcomas are rare neoplasias; however, they may be present across all age groups and all body sites where soft tissues exist. In the United States, soft tissue sarcomas (STSs) occur in 0.7% of cancer patients aged 16 years and over.[1] In Brazil, the actual incidence of these neoplasias is difficult to determine due to lack of appropriate records.

Institute of Orthopedics and Traumatology, São Paulo University Medical School - São Paulo/SP - (IOT / HC / FMUSP), Brazil.
zosimo_jr@yahoo.com.br
Received for publication on 03/07/07
Accepted for publication on 07/07/07

Although being rare, STSs have a relatively high mortality rate. This high mortality can be attributed in part to aggressive local invasion, but mostly to frequent metastases.[2]

Liposarcomas can occur in any anatomical site; concerning the extremities, they occur more frequently in the thigh.[3-7]

Adipose tissue tumors represent a group of injuries whose classification is an issue of continuous debate.[8,9] Liposarcomas have been shown to possess many histological patterns.[10] Many changes have occurred over the years concerning the histological classification of this type of soft tissue sarcoma. Currently, according to the Classification of Soft Tissue and Bone Tumors of the World Health Organization, it is accepted that this sarcoma presents as one of 5

histological subtypes: well-differentiated, dedifferentiated, myxoid/round-cell, pleomorphic, and mixed.[11]

Most frequently, the proto-oncogene *MDM2* is amplified in soft tissue sarcomas. Most of the known oncogenes are changed by amplification, resulting in overexpression and, consequently, high levels of their protein products.

The q 13-15 region of chromosome 12 is complex and contains different genes, such as *MDM2,*[12] that are amplified or reorganized in lipomatous tumors, which can be demonstrated by immunohistochemistry.[13,14]

The number of publications on the molecular characteristics of STSs is constantly increasing, and research focuses on the search for additional prognostic factors, aiming to identify those related to risk of local recurrence, metastases, and death due to the disease, and to study their correlation with known clinical, anatomical, and pathological variables.[15]

The determination of molecular variables related to anatomical and pathological aspects and to the prognosis could help identify subgroups of patients with better or worse prognoses.[15] Thus, with more prognostic factors identified, it would be possible to select the risk patients and work toward improved therapeutic outcomes.

The objective of this study, which is part of a general survey on sarcomas,[16] was to investigate *MDM2* protein expression and evaluate its relationship with some anatomical and pathological aspects, aiming also to identify prognostic factors concerning local recurrence-free survival, metastasis-free survival, and overall survival in patients with primary liposarcomas of the extremities.

MATERIALS AND METHODS

A review was performed of the medical records of patients who were anatomically and pathologically diagnosed with liposarcoma of the extremities and were admitted to the Institute of Orthopedics and Traumatology, Faculty of Medicine, University of São Paulo (IOT/FMUSP), SP, Brazil, between 1968 and 2004. Based on these medical records and after a detailed anatomical and pathological evaluation from the paraffin blocks of specimens obtained in the original biopsy, we selected 50 patients. Of these 50 patients, 5 were excluded for presenting local recurrence in the first evaluation, and another 3 because they underwent biopsy only, with no treatment. Another 17 cases were excluded because their follow-up periods were less than 24 months. The analysis of variables was then performed for 25 patients (and their medical records) who underwent treatment at IOT-HC/FMUSP. The anatomical and pathological evaluation was made from paraffin blocks containing tumor fragments that were obtained from the surgical specimens.

The following variables were studied:

1. Clinical and epidemiological variables

Sex, age, and ethnicity

Of the 25 patients studied, 11 (44%) were men and 14 (56%) were women (ratio of 1:1.27).

The mean age was 52 ± 15 years (range, 20-86 years; median 53). Eleven patients (44%) were aged under 50 years, and 14 (56%) were 50 years old or over.

Caucasians were the most affected, with 22 (88%) subjects.

Anatomical site

Fifteen (60%) patients had tumor formation in the thigh. For the other patients, the leg was affected in 4 (16%) cases and the forearm in 2 (8%) cases. The arm, axilla, scapula, and foot were also affected (1 of each). The right side of the body exceeded its contralateral side in the 1.5:1 ratio.

Clinical condition and delay in seeking medical advice

The unanimous clinical complaint was the insidious onset of usually painless local tumors. The secondary symptom was pain, occurring in only 3 (12%) cases.

The period between the onset of symptoms and the seeking of medical advice was 2.9 months, ranging between 2 and 120 months (mean, 36.0 ± 41.3 months; median, 24.0 months).

Remote metastasis

Metastasis was identified in 3 (12%) patients, 1 with high-grade myxoid/round-cell histology and 2 with pleomorphic histology. All 3 patients developed an extrapulmonary metastasis, usually in the lumbar spine. Only 1 among the pleomorphic subtypes presented additional lung metastasis. The patient with lung metastasis died after a less than 7-month follow-up period.

Follow-up period

The average follow-up period for our patients with liposarcoma was 68.3 months (standard deviation, 47.4 months; median, 54.0 months; range, 8-184 months) (Table 1)

2. Anatomical and pathological variables

Tumor size and histology

All patients underwent surgical resection of the tumor. Variant myxoid/round-cell represents the most common histological type, affecting 13 (52%) of 25 cases. The case frequency by histological subtype of liposarcoma was distributed as follows: 9 (36%) well-differentiated, 13 (52%) myxoid/round-cell, and 3 (12%) pleomorphic. Using the

Table 1 - Distribution of clinical and epidemiological variables for the 25 patients with primary liposarcoma of the extremities

Variable	Category	n (%)
Age (years)	< 50	11 (44)
	≥ 50	14 (56)
Sex	Male	11 (44)
	Female	14 (56)
Site	Upper limb	5 (20)
	Lower limb	20 (80)
Side of body	Right	15 (60)
	Left	10 (40)
Delay in seeking medical advice (months)	< 24	12 (48)
	≥ 24	13 (52)

adopted selection criteria, we did not find cases of dedifferentiated and mixed liposarcomas.

Concerning histological grade, the selected cases were divided into 2 categories: low-grade and high-grade malignancies, corresponding to 18 (72%) and 7 (28%) cases, respectively.

Tumor sizes ranged between 3 and 37 cm (average. 17 ± 9 cm; median, 18 cm) as measured on the specimens after surgical resection. Later, the tumors were divided into 3 groups: < 5 cm (8%), between 5 and 10 cm (20%), and > 10 cm (72%). (Table 2)

3. Therapeutic variables

Surgical and adjuvant treatment

In all cases, the tumors were subjected to ample resection according to the Enneking classification.[17] Forty-eight percent of these patients underwent surgery only.

Thirteen (52%) patients received radiotherapy (RT) and/or chemotherapy (ChT) postoperatively. None of them received RT and/or ChT followed by surgery. Radiotherapy and/or ChT before and after surgery (both) were not instituted for any of the patients.

Local recurrence

Local recurrence was observed in 3 (12%) cases, 1 of them of the well-differentiated type and 2 of the myxoid/round-cell type (1 low-grade malignancy and 1 high-grade malignancy). Two low-grade cases presented 2 local recurrences each and had no adjuvant therapy. The patient with high-grade neoplasia developed only 1 episode of recurrence after receiving postoperative radiotherapy. None of the patients experiencing local recurrence presented remote metastasis.

Complications of treatment

Eleven (44%) of the 25 treated patients presented complications of 8 types, and some patients presented more than one type of associated form. The most frequent complications were 4 surface infections of the surgical wound, 4 peripheral nerve injuries, and 3 deep infections.

4. Immunohistochemical variable

MDM2 Expression

Of the 25 cases evaluated for *MDM2* protein expression, 22 (88%) presented positive indexes (≥ 10%) and 3 (12%) presented negative indexes (<10%).

INCLUSION CRITERIA

All patients included in this study met the following eligibility criteria: (a) having undergone surgery at IOT for local treatment of the primary tumor; (b) having anatomical and pathological confirmation of liposarcoma (all slides were reviewed by the same pathologist who is an expert in

Table 2 - Anatomical and pathological variables—overall survival (OS) at 2.5 and 10 years for the 25 patients with primary liposarcomas of the extremities

			OS			
Variable	Category	n	at 2 years	at 5 years	at 10 years	*P* **
Histological subtype	Well-differentiated	9	100 (0)	100 (0)	100 (0)	
	Myxoid/round-cell	13	92 (7)	92 (7)	-	0.001
	Pleomorphic	3	33 (27)	-	-	
Specimen size (cm)	< 5	2	100 (0)	100 (0)	-	
	Between 5 and 10	5	80 (18)	80 (18)	80 (18)	0.759
	> 10	18	94 (5)	89 (8)	89 (8)	
Grade	Low	18	100 (0)	100 (0)	100 (0)	0.003
	High	7	71 (17)	57 (19)	-	

* % values ± standard error; ** *P* values using the log rank test (Kaplan-Meyer curve)

musculoskeletal tissues); and (c) having a tumor located in the extremities only, as described in the medical record.

To study the *MDM2* nuclear expression marker, we selected the cases for which specimens had been preserved in paraffin blocks at the time of biopsy prior to any adjuvant therapy, and only primary tumors were included.

EXCLUSION CRITERIA

Patients were excluded from the study if they (a) presented metastases and/or local recurrence during the first evaluation; (b) had postoperative follow-up periods of less than 2 years, except for those who died of cancer before that period; (c) underwent any treatment prior to enrollment at IOT.

ASSESSMENT OF IMMUNOHISTOCHEMICAL POSITIVITY

The *MDM2* protein was classified as present or absent. For the immunohistochemical analysis, the tumors were classified as positive (positivity ≥ 10% of the sample) or negative (Figure 1).

Protein expression was assessed at 400x magnification, and the nuclei with unequivocally typical brown immunoexpression were considered positive. At least 400 nuclei of neoplastic cells were counted per case.

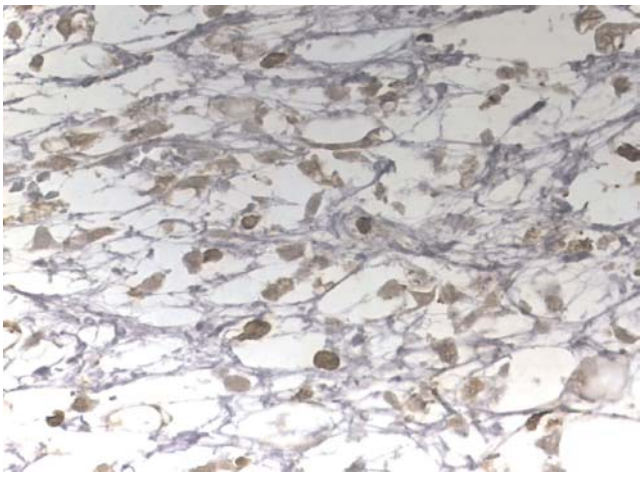

Figure 1 - Photomicrograph showing a positive immunohistochemical result for the presence of *MDM2* protein (magnification 400 x)

STATISTICAL ANALYSIS

The descriptive analysis of the sample was performed based on average, median, and percentage values. The simple frequencies of all variables studied were calculated. The accumulated survival probabilities were evaluated using the Kaplan-Meier method, and the survival curves were compared using the log rank test.

Positive or negative indexes for *MDM2* were evaluated as variables to determine their prognostic value and the value of the association with other (anatomical and pathological) variables. The associations were performed according to Fisher's exact test.

The rounding up of frequency and survival rates was used to simplify calculations. Statistical significance was defined for P values < 0.05.

RESULTS

The prognostic value of some important clinical, epidemiological, anatomical, pathologic, and immunohistochemical variables was also evaluated based on curves of local recurrence-free survival, metastasis-free survival, and overall survival.

Prognostic factors related to local recurrence-free survival

Male gender ($P = 0.023$) was considered an adverse factor; the pleomorphic subtype ($P = 0.027$) presented the highest index of local recurrence. High histological grade ($P = 0.007$) had the worst prognosis. The other factors investigated (age, site, side of body, delay in seeking medical advice, specimen size, or type if treatment performed) did not reach statistically significant levels. The presence or absence of *MDM2* did not reach statistically significant levels and was not considered a prognostic factor for local recurrence-free survival.

Prognostic factors related to metastasis-free survival

Age less than 50 years ($P = 0.040$), male sex ($P = 0.040$), pleomorphic subtype ($P < 0.001$), and high histological grade ($P = 0.003$) were considered adverse factors for metastasis-free survival (Figure 2). The other factors studied did not reach statistically significant levels (site, side of body, delay in seeking medical advice, specimen size, and type of treatment performed). Immunohistochemical evidence of the presence or absence of *MDM2* gene expression did not reach statistically significant levels and was not considered a prognostic factor for metastasis-free survival.

Prognostic factors related to overall survival

The overall survival curve for the 25 patients is shown in Figure 3. Age under 50 years ($P = 0.036$), male sex ($P = 0.043$), pleomorphic subtype ($P = 0.001$), and high histologi-

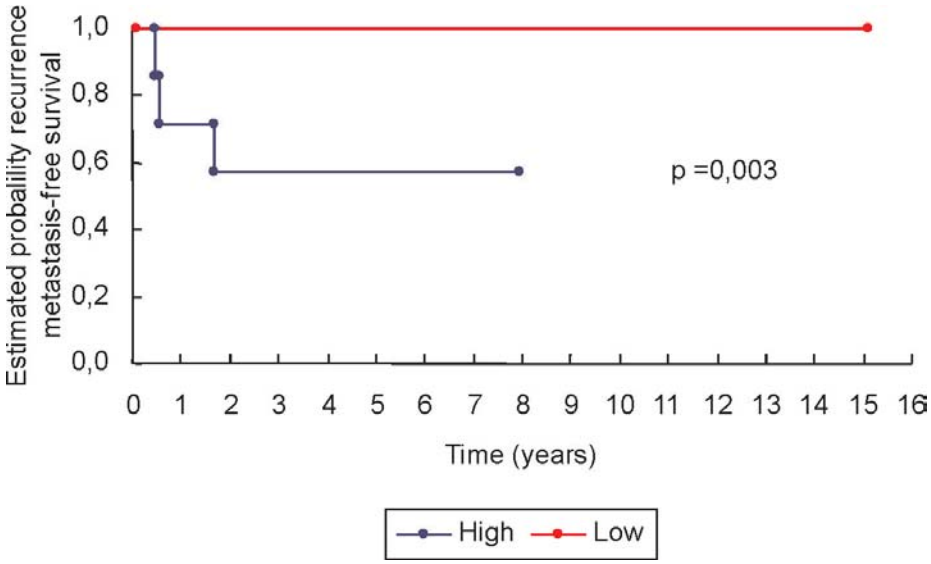

Figure 2 - Curve depicting metastasis-free survival (years) for the 25 patients with liposarcoma of the extremities according to the histological grade

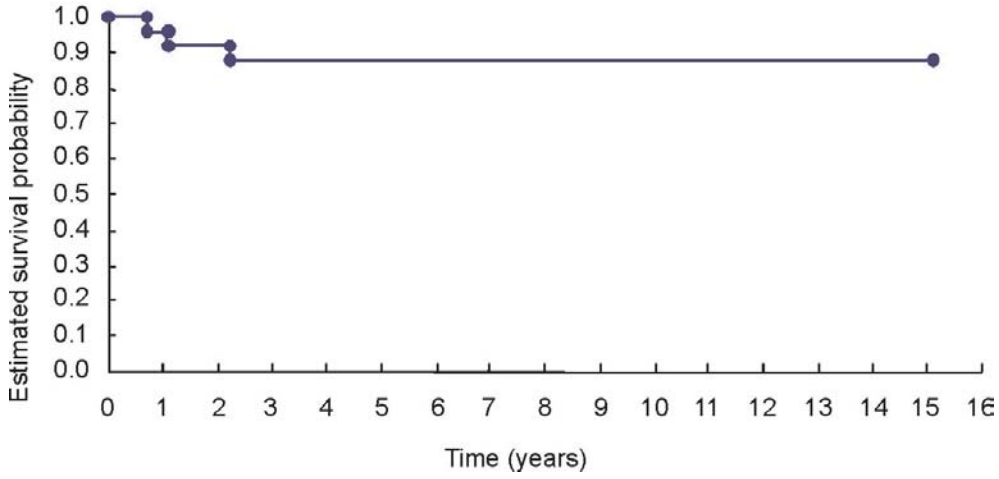

Figure 3 - Curve depicting overall survival (years) for the 25 patients with liposarcoma of the extremities

cal grade (P = 0.003) were also considered adverse factors. Site, side of body, delay in seeking medical advice, specimen size, and type of treatment performed did not reach statistically significant levels. Also, the presence or absence of *MDM2* protein expression did not influence the overall survival.

Analysis of the correlation between *MDM2* protein expression and anatomical and pathological variables

As shown in Table 3, there was no statistically significant association between the expression (or not) of *MDM2* protein and the following anatomical and pathological variables: subtype, grade, and size of tumors.

DISCUSSION

Although there are more than 30 histological types of soft tissue sarcomas, many of them are grouped for being similarly diagnosed, staged, and treated.[18] However, because these sarcomas include a large series of histological types, the clinical course of each type of sarcoma can be extremely different.[2]

Soft tissue sarcomas account for less than 1% of all malignant tumors.[19] As shown in many publications,[19-24] of this percentage, between 10% and 20% are liposarcomas, which are considered the second most frequent type.[20,23]

Concerning global survival, Reitan et al[5] asserted that younger patients presented better prognoses, which does not agree with our findings, where we found a significant difference regarding worse prognosis for patients under 50 years of age.

A few investigators have asserted that the most common form of liposarcoma is the well-differentiated sarcoma.[25] The most frequent histological type found in our study was

Table 3 - Study of the correlation between the immunohistochemical evidence of *MDM2* expression and the anatomical and pathological variables in the 25 patients with primary liposarcoma of the extremities

Variable	Category	n	MDM2		*P**
			Negative n = 3	Positive n = 22	
Histological subtype	Well-differentiated	9	2 (67%)	7 (32%)	
	Myxoid/Round Cell	13	1 (33%)	12 (55%)	0.695
	Pleomorphic	3	0 (0%)	3 (14%)	
Specimen size (cm)	< 5	2	0 (0%)	2 (9%)	
	Between 5 and 10	5	0 (0%)	5 (22%)	1.000
	> 10	18	3 (100%)	15 (68%)	
Grade	Low	18	2 (67%)	16 (73%)	1.000
	High	7	1 (33%)	6 (27%)	

* *P* values using Fisher's exact test

the myxoid/round-cell type (52%), in agreement with other current studies.[23,26]

One of the most important factors influencing the survival rate seems to be the histological type of the tumor. In our case series, we found that the histological subtype of liposarcoma is significantly related with survival; that is, patients with pleomorphic liposarcoma are at higher risk for death than those who have myxoid/round cell or well-differentiated sarcomas. We also observed a significant risk of local recurrence and development of remote metastases and worse prognosis for patients with the pleomorphic histological type.

Tumor grade has been clearly recognized as a performance predictor, with high-grade tumors being associated with prognoses that are unfavorable for survival.[27] For liposarcomas of the extremities, this also appears to be the case.[2]

The high average value of tumor size seems to be directly related to the long evolution time presented by these neoplasias. However, in this series, we did not find that the dimension of the tumor was a prognostic factor in any of the surviving patients studied.

As a group, liposarcomas present a postoperative local recurrence rate of approximately 50%. However, these indexes are lower if only injuries of the extremities are considered.[28,29] In our case series, of the 25 patients undergoing ample surgical resection, 3 presented local recurrence during the follow-up period: 1 of the well-differentiated type and 2 of the myxoid/round-cell type (1 low-grade and 1 high-grade).

MDM2 gene amplification has been shown to occur at a high frequency in many studies on soft tissue sarcomas. The product of *MDM2* interacts with the p53 protein and inhibits its ability to regulate genetic expression as a transcription factor. Thus, amplification (and subsequent overexpression) of *MDM2* may have the same effect as that of mutations in p53.[30]

The changes in the *MDM2* gene are known to be a common mechanism in the genesis of liposarcomas.[31] The amplification of this gene and its mRNA overexpression can lead to overproduction of *MDM2* protein.[32] The immunohistochemical analysis reveals nuclear location and overexpression of *MDM2* in these tumors with amplification of the *MDM2* gene.[33]

In liposarcomas, amplification of the *MDM2* gene is observed only in high-frequency well-differentiated tumors, not in other tumor subtypes.[32] Another author[34] mentions that amplification of the *MDM2* gene is not also seen in myxoid liposarcomas, the largest subtype of sarcomas. On the other hand, Schneider-Stock et al[31] found amplification of the *MDM2* gene in the myxoid and pleomorphic variants, although the well-differentiated liposarcoma is characterized by an already known high frequency.

Although immunohistochemistry can be used to demonstrate *MDM2* overexpression, direct correlation between the gene amplification and protein overexpression is not the rule.[35] Nevertheless, mutations cause an irregular increase in stable proteins that can be detected by immunohistochemistry.[36]

In our case series, immunohistochemistry showed *MDM2* protein expression in 22 (88%) of the 25 cases. Of these 22 cases, 7 (32%) were of the well-differentiated subtype, 12 (54.5%) of the myxoid/round-cell type, and 3 (13.5%) of the pleomorphic type. Data from this study indicate a high frequency of the presence of *MDM2* protein in the different histological subtypes of liposarcomas, suggesting preliminarily a high sensitivity of this marker in this pathology. In spite of this, currently, the diagnosis of these neoplasms is based on morphology, not immunocytochemical tests.

CONCLUSIONS

1) There was no correlation between the *MDM2* protein expression as observed by immunohistochemistry and the anatomical and pathological variables studied; additionally, there was no relationship between those variables and the different prognostic factors in primary liposarcomas of the extremities. **2**) Factors such as male sex, pleomorphic histological subtype, and high grade of malignancy are unfavorable for local recurrence-free survival. **3**) Factors such as male sex, age under 50 years, pleomorphic subtype, and high histological grade are adverse for metastasis-free survival and overall survival. **4**) *MDM2* expression was a frequent event in the different subtypes of liposarcomas.

REFERENCES

1. Yang JC, Rosemberg SA. Surgical treatment of soft tissue sarcomas of the extremities. In: Sugarbaker PH, Malawe MM, editors. Musculoskeletal surgery for cancer. New York: Thieme Medical Publishers; 1992. p 1-11.
2. Chang HR, Gaynor J, Tan C, Hajdu SI, Brennan MF. Multifactorial analysis of survival in primary extremity liposarcoma. World J Surg. 1990;14:610-18.
3. Huebert HT. Liposarcoma: the Manitoba experience. Can J Surg. 1981;24:391-6.
4. Daniell SJN. Liposarcoma: a ten-year experience. Int Orthop. 1985;9:55-8.
5. Reitan JB, Kaalhus O, Brennhovd IO, Sager EM, Stenwig AE, Talle K. Prognostic factors in liposarcoma. Cancer. 1985; 55:2482-90.
6. Gustafson P, Rydholm A, Willen H, Baldetorp B, Ferno M, Akerman M. Liposarcoma: a population based epidemiologic and prognostic study of features of 43 patients, including tumor DNA content. Int J Cancer. 1993;55:541-6.
7. Kulhavy M, Uijs RR, Sur RK, Donde B, Nayler S, Sur M, et al. Symmetrical multifocal liposarcoma. S Afr J Surg. 1997;35:68-9.
8. Boltze C, Schneider-Stock R, Jäger V, Roessner A. Distinction between lipoma and liposarcoma by MDM2 alterations: a case report of simultaneously occurring tumors and review of the literature. Pathol Res Pract. 2001;197:563-8.
9. Forus A, Bjerkehagen B, Sirvent N, Meza-Zepeda LA, Coindre JM, Berner JM, et al. A well-differentiated liposarcoma with a new type of chromosome 12-derived markers. Cancer Genet Cytogenet. 2001;131:13-8.
10. Evans HL. Liposarcoma: a study of 55 cases with a reassessment of its classification. Am J Surg Pathol. 1979;3:507-23.
11. Fletcher CDM, Unni KK, Mertens F. Pathology and genetics of tumours of soft tissue and bone – WHO – Lion: IARC Press;2002.
12. Forus A, Florenes VA, Maelandsmo GM, Meltzer PS, Fodstad O, Myklebost O. Mapping of the amplification units in the q13-14 region of chromosome 12 in human sarcomas: some amplica do not include MDM2. Cell Growth Differ. 1993;4:1065-70.
13. Pilotti S, Della Torre G, Lavarino C, Di Palma S, Sozzi G, Minoletti F, et al. Distinct MDM2/p53 expression patterns in liposarcoma subgroups: implications for different pathogenetic mechanisms. J Pathol. 1997;181:14-24.
14. Dei Tos AP, Doglioni C, Piccinin S, Sciot R, Furlanetto A, Boiocchi M, et al. Coordinated expression and amplification of the MDM2, CDK4, and HMGI-C genes in atypical lipomatous tumours. J Pathol. 2000;190:531-6.
15. Ferreira FO. Estudo de fatores prognósticos e da expressão histoquímica de AgNOR e imunoistoquímica de KI-67 e p53 em pacientes adultos portadores de lipossarcomas das extremidades [thesis]. Sao Paulo: Faculdade de Medicina, Universidade de Sao Paulo; 1999.
16. Baptista AM, Camargo OP, Croci AT, Oliveira CRGCM, Azevedo Neto RS, Giannotti MA, et al. Synovial sarcoma of the extremities: prognostic factors for 20 nonmetastatic cases and a new histologic grading system with prognostic significance. Clinics. 2006;61:381-386.
17. Enneking WF, Spanier SS, Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. 1980. Clin Orthop Relat Res. 2003;415:4-18.
18. Singer S, Corson JM, Gonin R, Labow B, Eberlein TJ. Prognostic factors predictive of survival and local recurrence for extremity soft tissue sarcoma. Ann Surg. 1994;219:165-73.
19. Stout AO. Sarcomas of the soft tissues. Cancer. 1961;11:210-8.
20. Enzinger FM, Weiss, SW. Soft tissue tumours. 2nd ed. St. Louis: CV Mosby; 1988.
21. Mack TM. Sarcomas and other malignancies of soft tissue, retroperitoneum, peritoneum, pleura, heart, mediastinum and spleen. Cancer. 1995; 75:211-44.
22. Daugaard S. Current soft-tissue sarcoma classifications. Eur J Cancer. 2004;40:543-8.
23. Lehnhardt M, Kuhnen C, Drücke D, Homann H-H, Joneidi Jafari H, Steinau HU. Liposarkome der extremitäten. Aktuelle aspekte zur chirurgischen terapie-eine analyse von 167 patienten. Der Chirurg. 2004;75:1182-90.
24. Moureau-Zabotto L, Thomas L, Bui BN, Chevreau C, Stockle E, Martel P, et al. Management of soft tissue sarcomas (STS) in first isolated local recurrence: a retrospective study of 83 cases. Radiother Oncol. 2004;73:313-19.
25. Dei Tos AP, Pedeutour F. Atypical lipomatous tumour/well-differentiated liposarcoma. *In*: Fletcher CDM, Unni KK, Mertens F. Pathology and genetics of tumours of soft tissue and bone – WHO – Lion: IARCPress, 2002;35-7.
26. Weiss SW, Goldblum JR. Enzinger and Weiss's soft tissue tumours. 4th ed. St. Louis: CV Mosby; 2001.

27. Collin C, Friedrich C, Godbold J, Hadju SI, Brennan MF. Prognostic factors for local recurrence and survival in patients with localized extremity soft tissue sarcoma. Semin Surg Oncol. 1988;4:30-7.

28. Chang HR, Hajdu SI, Collin C, Brennan MF. The prognostic value of histologic subtypes in primary extremity liposarcoma. Cancer. 1989;64:1514-20.

29. Nascimento AG, Oliveira AM. Patologia geral. In: Lopes A. Sarcomas de partes moles. Rio de Janeiro: Medsi; 1999. p 41-68.

30. Simpson AJG. A genética molecular de sarcomas de partes moles and sua aplicação à clínica. In: Lopes A. Sarcoma de partes moles. Rio de Janeiro: Medsi; 1999. p 19-30.

31. Schneider-Stock R, Walter H, Radig K, Rys J, Bosse A, Kuhnen C, et al. MDM2 amplification and loss of heterozygosity at rb and p53 genes: no simultaneous alterations in the oncogenesis of liposarcomas. J Cancer Res Clin Oncol. 1998;124:532-40.

32. Nakayama T, Toguchida J, Wadayama B, Kanoe H, Kotoura Y, Sasaki MS. MDM2 gene amplification in bone and soft-tissue tumors: association with tumor progression in differentiated adipose tissue tumors. Int J Cancer. 1995;64:342-6.

33. Leach FS, Tokino T, Meltzer P, Burrell M, Oliner JD, Smith S, et al. p53 mutation and MDM2 amplification in human soft tissue sarcomas. Cancer Res. 1993;53:2231-4.

34. Nilbert M, Rydholm A, Willen H, Mitelman F, Mandahl N. MDM2 gene amplification correlates with ring chromosomes in soft tissue tumors. Genes Chrom Cancer. 1994;9:261-5.

35. Holstein I, Pelmus M, Aurias A, Pedeutour F, Mathoulin-Pélissier S, Coindre JM. Evaluation of MDM2 and cdk4 amplification by real-time PCR on paraffin wax-embedded material: a potential tool for the diagnosis of atypical lipomatous tumours/well-differentiated liposarcomas. J Pathol. 2004;202:95-102.

36. Bodner SM, Minna JD, Jensen SM. Expression of mutant p53 proteins in lung cancer correlates with the class of p53 gene mutation. Oncogene. 1992;7:743-9.