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DNA Content as a Predictor of Clinical Outcome in Soft Tissue Sarcoma Patients

W. Budach, V. Budach, B. Socha, M. Stuschke, C. Streffer and H.Sack

The prognostic relevance of cellular DNA content has been shown for a variety of human malignancies. However, only a few studies concerning soft tissue sarcomas have been published. Biopsies of 81 patients with soft tissue sarcomas, referred for primary or secondary surgery, were analysed by flow cytometry to determine cellular DNA content of tumours. Most patients (60/81) already had one or more local recurrences at the time of first presentation at Essen University. The median age of the patients was 45 years (range 14–79). 44 (54%) patients had euploid and 37 (46%) had aneuploid tumours. Age, sex, and tumour localisation (trunk versus extremity) were equally distributed between euploid and aneuploid sarcoma patients. The median follow-up was 69 months (range 9–312). The median survival time for euploid and aneuploid tumours was 84 and 30 months, respectively ($P < 0.0005$). In the univariate analysis, ploidy, S-phase percentage, localisation and tumour grading were significant predictors of survival, whereas in the multivariate analysis, only DNA content and tumour localisation were independent prognostic variables for survival.

Key words: sarcoma, ploidy, prognostic factor
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INTRODUCTION

HUMAN SOFT tissue sarcomas consist of a histologically and prognostically, very heterogeneous group of tumours. Distant metastases occur in approximately 50% of patients and remain the major cause of death in soft tissue sarcoma patients [1–5]. The considerable morbidity of currently available adjuvant chemotherapeutic regimens, including high doses of doxorubicin and ifosfamide, appears to be unreasonable in the subgroup of patients, who have a good prognosis by local treatment alone. Therefore, identifying patients with a high risk of distant

Table 1. Distribution of histological subtypes by stage (UICC 1987)

Histology	Stage IA		Stage IB		Stage IIA		Stage IIB		Stage IIIA		Stage IIIB		Stage IVA		Stage IVB		
	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E	Total
Liposarcoma			4	1		1	2	3	1	1	3	2	1				19
Malignant fibrous histiocytoma							1	4		3	5	1			2		16
Leiomyosarcoma				2			5		1		6	1					15
Undifferentiated sarcoma							3	1		1	1	2					8
Synovial sarcoma			1					2	1	1							5
Neurofibrosarcoma	1						1				2	1					5
Fibrosarcoma											3				1		4
Miscellaneous					1		2	2			1	1	1			1	9
Total	1	0	5	3	1	1	14	12	3	6	21	8	2	0	3	1	81

T, localised at the trunk; E, localised at an extremity.

disease, and selecting these patients for an intensive therapeutic approach would be beneficial for all patients. Classification of sarcomas into histogenetic subgroups, although improved by the use of immunohistochemical staining and electron microscopy, has failed to predict the clinical outcome of sarcoma patients. In larger clinical series, grade of malignancy could be identified as of prognostic relevance, and has led to a staging system based on tumour grading. However, different grading systems have been proposed [1, 3–5], and no general consensus regarding the morphological criteria that should be employed has been found. Furthermore, the results of adjuvant chemotherapy trials, with patient selection based on tumour grading, has not yielded satisfactory results [6–8]. Additional prognostic factors are needed to allow for a risk adapted therapy in soft tissue sarcoma patients.

The prognostic relevance of the DNA content of tumour cells has been well documented for a variety of human malignancies [9, 10]. The discrimination between euploid and aneuploid tumours can be easily achieved by flow cytometry from fresh biopsies or paraffin-embedded samples, and does not depend on subjective criteria. Some reports have suggested that DNA content is an important prognostic factor in soft tissue sarcoma [11–16]; however, the experience is still limited. In the present study, the prognostic relevance of DNA content in 81 soft tissue sarcoma patients was analysed, retrospectively.

PATIENTS AND METHODS

Patients

The study was based upon 81 patients with soft tissue sarcomas treated at Essen University between 1980 and 1991. Biopsies for the assessment of DNA content of tumour cells were taken from the primary sarcoma lesions in 20 patients, from locally recurrent sarcomas in 50 patients, and from distant metastases in 11 patients. The median age of the patients was 45 years (range 14–79), 41 were male and 40 female. Table 1 summarises the histological subtypes of sarcomas according to the classification of Enzinger and associates [17] by tumour stage and localisation. The majority of patients had one or more known adverse prognostic factors, particularly stage IIB and IIIB soft tissue sarcomas, and most tumours (50/81) were located at the trunk. 11 patients (14%) had G1, 32 patients (40%) G2 and 38 patients (47%) G3 sarcomas.

Since biopsies for DNA measurements were only taken once during the course of disease, efforts were undertaken to evaluate the history of all patients retrospectively, from the date of first diagnostic biopsy (outside Essen University) to the time of biopsy at Essen University, and prospectively, from the time of DNA measurement until 1991. The history of the disease started in 62/81 patients 1–261 months before DNA measurements (median 22 months). The median follow-up from the time of diagnosis was 69 months (range 9–312), and the median follow-up from the assessment of DNA content was 38 months (range 1–137). Pathology and operation reports, as well as X-ray, computed tomography (CT) and magnetic resonance imaging (MRI) findings during the course of disease, were reviewed for all patients and questionnaires sent to the referring physicians in case of missing findings. TNM stage (including tumour grading) was defined according to the UICC classification (1987). Time of diagnosis was defined as the date of first diagnostic biopsy that revealed a soft tissue sarcoma. In case of mismatches ($n = 8$) of histological subtype of soft tissue sarcoma or grading between pathology report at the time of diagnosis (outside Essen University) and pathology report at the time DNA measurement (inside Essen University), the histopathological finding of Essen University as the local reference centre was used for classification. Metastatic disease was diagnosed based upon histopathological findings or based upon typically radiological findings in chest or bone X-rays or CT or MRI scans of the corresponding section of the body.

Treatments

All patients underwent surgery at the time of diagnosis, 39 (48%) received, in addition, postoperative radiation therapy. 60

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patients experienced from one up to a maximal nine (median one) further surgical procedures before referral to Essen University, which was followed by secondary radiation therapy in 30 patients. Definite surgery consisted of enucleation in 21 (26%) patients, wide local excision in 47 (58%) patients, radical (compartmental), local resections in 11 (14%) patients and amputation in 2 (2%) patients. Microscopically complete resections (R0) were achieved in 35 (43%) patients, microscopically residual disease (R1) remained in 10 (12%) patients and macroscopically residual disease (R2) in 28 (35%) patients. The high rate of R2 resections was a result of a high percentage of unfavourably located sarcomas of the trunk. In 8 (10%) patients, operation and pathological reports did not allow for a reliable estimate of residual disease. According to the surgical margins, 17/35 (49%), 4/10 (40%) and 14/28 (50%) of patients received radiotherapy after R0, R1 and R2 resections, respectively.

Assessment of ploidy

Fresh biopsy material was shredded by rubbing it through a 200-μm nylon mesh. The assay has been described in detail previously [10] but, briefly, the resulting aggregates of a few cells and single cells were suspended in 0.1 M Tris-buffer, 0.005 M EDTA and 0.07 M NaCl (pH = 7.4) and centrifuged at 600 rev/min for 5 min. The pellet was dissolved in 96% ethanol, incubated for 10 min with 0.5% pepsin solution (1000 U/g), centrifuged and then incubated with 1 mg/ml RNAse at a pH of 7.4 for 5 min. Following centrifugation, the resulting suspension of nuclei was stained with ethidium bromide (25 μM) and fluorescence of nuclei measured by the ICP 22 flow cytometer (Biophysik Company Phywe, Göttingen, Germany) and DNA histograms recorded. Human lymphocytes served as controls for diploid cells. Tumour cell populations with a G1 peak in the range of ±10% of human lymphocytes were considered euploid (diploid), all others aneuploid. In case of two G1 peaks ($n = 2$), one euploid and one aneuploid, the cell line was regarded as aneuploid. The distribution of cells in G1, G2/mitosis and S-phase was calculated by the 'Flow Analysis Data System' (Dr Ahrens Company, Bargteheide, Germany).

Evaluation of prognostic factors

The following parameters were tested for prognostic significance in uni- and multivariate analysis: ploidy, S-phase, grading, localisation, T-stage, age, sex, surgical margins and radiotherapy. Histological subtype of sarcoma was not included in the analysis because only three histological subtypes, liposarcoma, leiomyosarcoma, and malignant fibrous histiocytoma, had sufficient numbers of cases to be analysed separately. Vascular invasion, amount of tumour necrosis and mitotic index, parameters which were of predictive value in some studies, were also not integrated in this series, since routine pathology records did not allow for a valid assessment of these parameters in approximately 50% of patients.

Statistics

Overall survival and disease-free survival were calculated by Kaplan and Meier statistical analysis. The significance of differences between survival curves were tested by the log rank test. For the multivariate analysis, the stepwise backwards proportional hazard regression method was used (Cox).

Table 2. Distribution of grading and ploidy

	Grade 1	Grade 2	Grade 3	Total
Euploid	8 (73%)	21 (66%)	15 (39%)	44
Aneuploid	3 (27%)	11 (34%)	23 (61%)	37
Total	11 (100%)	32 (100%)	38 (100%)	81

RESULTS

The DNA content of primary and recurrent soft tissue sarcomas range from 0.8–4.9-fold of the DNA content of normal lymphocytes. Euploid tumours were found in 44 (54%) patients, and aneuploid tumours in 37 patients (46%). The majority (29/37) of aneuploid tumours had a DNA content between 1.2 and 1.8, with four only having a DNA content above 1.8 and another four with a DNA content below 0.9. There was a significantly higher percentage of aneuploid tumours in histological grade 3 sarcomas as compared to grade 1 and grade 2 sarcomas (Table 2). The median percentage of tumour cells in S-phase was 16%. An S-phase proportion above the median was observed in 78% (29/37) of aneuploid tumours, and in 14% (6/44) of euploid tumours (χ^2: $P < 0.005$). The distribution of ploidy varied significantly between histologies. Whereas liposarcomas were almost always euploid, more than 80% of malignant fibrous histiocytomas were aneuploid (χ^2, $P < 0.001$). No significant differences were observed for the distribution of grading among histological subtypes. Location and tumour size were neither correlated with ploidy, nor with grading. Lymph node metastases were observed in 3 patients with aneuploid sarcomas during the course of disease, no lymph node involvement was seen in patients with euploid sarcomas.

Since the history of disease started in most patients months to years before biopsies for DNA measurements were taken, survival was analysed both from the day of diagnosis and from the day of biopsy for the assessment of DNA content. Comparing patients who underwent primary treatment at the time of DNA assessment to patients who had received surgery or/and radiation therapy earlier, no significant difference in distribution of stage, grading, location, age, sex, ploidy and tumour size were detectable. The number of high grade (G2 and G3) or aneuploid tumours did not increase with the number of local recurrences before the assessment of DNA content.

Survival analysis

The median survival from the time of diagnosis for all patients was 51 months, the 5-years actuarial survival rate was 43%. Five years after diagnosis, 60% of patients with euploid tumours and 19% of patients with aneuploid tumours were still alive ($P < 0.0005$). The median survival time from diagnosis was 84 months for euploid and 30 months for aneuploid tumours (Figure 1). Patients with grade 1 sarcomas had a significantly longer survival than patients with grade 2 and grade 3 sarcomas (Figure 2). Between grade 2 and grade 3 sarcomas, no significant difference was observed. The median survival was longer after microscopically complete resections (53 months) than after R1 or R2 resection (35 months), however this difference was not significant ($P = 0.2$).

The 5-years survival rates after the time of assessment of DNA content were 43% for euploid and 6% for aneuploid tumours ($P < 0.0005$). Patients with euploid and aneuploid tumours had

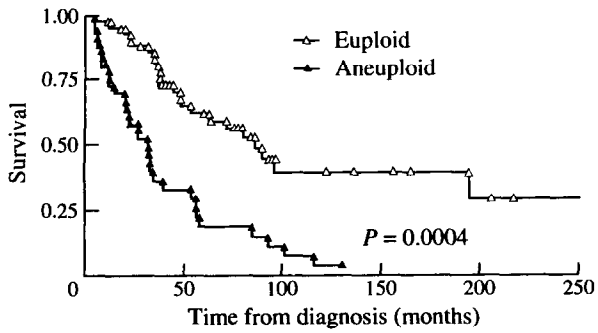

Figure 1. Time after diagnosis is plotted against actuarial survival probability for soft tissue sarcoma patients. Open and closed triangles indicate death or end of follow-up for patients with euploid and aneuploid sarcomas, respectively. *P* value: log rank test.

a median survival time of 47 months and 9 months, respectively, after the time of DNA measurement (Figure 3).

Table 3 summarises the results of a univariate and a multivariate analysis of the impact of prognostic factors on survival. Whereas in the univariate analysis, a number of known prognostic factors were significant, in the multivariate analysis, DNA content and tumour localisation were the only significant predictors of survival.

Development of distant metastases

Five per cent (4/81) of patients, all with aneuploid tumours, had already distant disease at the time of diagnosis. At 5 years after diagnosis, this rate had increased to 64% of all patients. The median time to metastases was 33 months. Aneuploid tumours showed significantly earlier and more frequently distant spread than euploid tumours (Figure 4). Five years after diagnosis, 78% of patients with aneuploid tumours had developed distant disease as compared to 57% of patients with euploid tumours ($P < 0.01$). The median time to metastases was 23 months for aneuploid and 49 months for euploid tumours

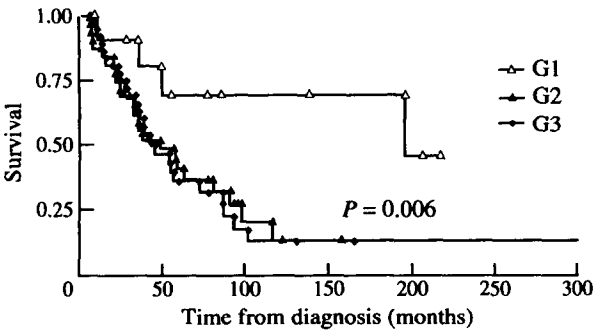

Figure 2. The time after diagnosis is plotted against actuarial survival probability for soft tissue sarcoma patients. Open triangles, closed triangles, and closed diamonds indicate death or end of follow-up for patients wih G1, G2, and G3 sarcomas, respectively. *P* value: log rank test (G1 against G2 and G3).

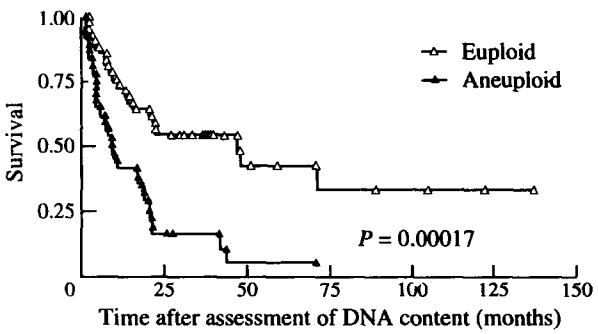

Figure 3. The time after assessment of DNA content is plotted against actuarial survival probability for soft tissue sarcoma patients. Open and closed triangles indicate death or end of follow-up for patients with eupoid and aneuploid sarcomas, respectively. *P* value: log rank test.

Table 3. Uni- and multivariate analysis of predictive factors of survival

	Univariate analysis: survival time (months)		Multivariate analysis	
Factor (a versus b)	Median time from diagnosis	Median time from DNA measurement	Median time from diagnosis	Median time from DNA measurement
Ploidy (euploid versus aneuploid)	a: 84, b: 30, $P < 0.0001$	a: 47, b: 9, $P < 0.0001$	$P < 0.001$	$P < 0.001$
S-phase (< 16 versus ≥ 16%)	a: 53, b: 27, $P < 0.001$	a: 21, b: 9, $P < 0.01$	n.s.	n.s.
Grading (G1 versus G2 + G3)	a: 75, b: 37, $P < 0.05$	a: 33, b: 14, $P < 0.05$	n.s.	n.s.
Localisation (trunk versus extremity)	a: 36, b: 40, $P = 0.059$	a: 9, b: 22, $P < 0.05$	$P = 0.05$	$P < 0.001$
T-stage (T1 versus T2)	a: 29, b: 44, n.s.	a: 6, b: 18, n.s.	n.s.	n.s.
Age (< 45 versus ≥ 45 years)	a: 35, b: 48, n.s.	a: 20, b: 11, n.s.	n.s.	n.s.
Sex (male versus female)	a: 40, b: 36, n.s.	a: 19, b: 11, n.s.	n.s.	n.s.
Irradiation (XRT versus no XRT)	a: 56, b: 53, n.s.	N.A.	n.s.	N.A.
Surgery (R0 versus R1/R2)	a: 53, b: 35, n.s.	N.A.	n.s.	N.A.

Survival times were assessed both from the date of first histopathological diagnosis and from the date of biopsy for the measurement of DNA content. Median time from diagnosis to time of DNA measurement was 22 months. For uni- and multivariate analysis, patients were divided into two groups for each tested factor as indicated in parenthesis. Median survival in months is given for each subgroup of the tested factors in the univariate analysis. N.A., not available; n.s., non-significant.

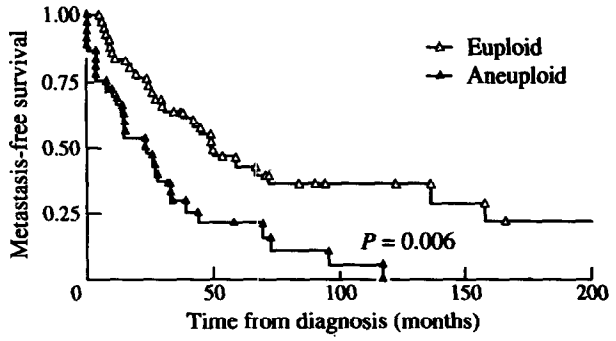

Figure 4. The time after diagnosis is plotted against the actuarial probability to develop distant disease for soft tissue sarcoma patients. Open and closed triangles indicate occurrence of distant disease or end of follow-up for patients with euploid and aneuploid sarcomas, respectively. *P* value: log rank test.

($P < 0.01$). Patients with grade 1 sarcomas developed significantly fewer metastases as compared to patients with grade 2 or grade 3 sarcomas.

At the time of assessment of DNA content, 40% (32/81) of patients, 32% (14/44) with euploid and 49% (18/37) with aneuploid tumours (χ^2 n.s.) had already developed distant disease. This rate increased to 70% within 2 years. The median time to metastases was 6 months. Two years after the time of assessment of DNA content distant metastases had occurred in 59% of patients with euploid tumours and in 84% of patients with aneuploid tumours ($P < 0.005$). The median time to distant spread was 13 months for euploid and 1 month for aneuploid tumours, after the time of assessment of DNA content.

Excluding patients from the analysis who had distant metastases at the time of DNA measurement, gave similar results: distant spread occurred within 2 years in 51% of all patients, in 40% of patients with euploid, and in 84% of patients with aneuploid tumour ($P < 0.01$). The respective median time intervals were 18, 36 and 11 months. Ploidy and tumour grading were significant predictors in the univariate analysis, whereas in the multivariate analysis, ploidy alone was an independent predictor of metastases (Table 4).

Local tumour control

Since accurate data on the extent of surgery and histopathological findings at the margins of resection were not available for all surgical procedures outside Essen University, analysis was based on the remaining 73 patients. Five years after surgery, 27% of patients with R0 resections continued to be free of local relapse as compared to 4% after R1 or R2 resections ($P = 0.05$). In uni- and multivariate analyses, high malignancy (G2 and G3), residual disease after surgery (R1, R2 resections) and omission of postoperative radiotherapy were independent prognostic factors associated with poor local control (Table 5). DNA content was without predictive value for local tumour control.

DISCUSSION

Assessment of ploidy was an independent, highly significant predictor of survival and likelihood of metastases in the 81 adult soft tissue sarcoma patients studied. The median survival after diagnosis for the whole group was in the same range as reported from other series [12, 18–20], although some accumulation of known adverse prognostic factors, such as location at the trunk and high malignancy, was observed (Tables 1, 2). Patients with euploid tumours survived from the day of diagnosis almost three times longer and had a 2-fold lower risk of distant disease as compared to patients with aneuploid tumours (Figure 1). A better prognosis of patients with euploid soft tissue sarcomas has been found in most published studies [11–16], although the difference did not reach significance in some smaller series [21–24]. As an only exception, Dias and colleagues [25] reported a better prognosis of aneuploid tumours in rhabdomyosarcomas of childhood. Gustafson and associates [14] observed a favourable outcome of patients with tetraploid sarcomas as compared to other aneuploid tumours. In the present study, none of the tumours presented a tetraploid DNA content.

In the univariate analysis, besides DNA content, S-phase fraction and tumour grading were predictive parameters for the development of distant disease and overall survival. Tumour

Table 4. Uni- and multivariate analysis of predictive factors of distant metastases

	Univariate analysis: time to metastases (months)		Multivariate analysis	
Factor: (a versus b)	Median time from diagnosis	Median time from DNA measurement	Median time from diagnosis	Median time from DNA measurement
Ploidy (euploid versus aneuploid)	a: 49, b: 23, $P < 0.01$	a: 13, b: 1, $P < 0.001$	$P < 0.05$	$P < 0.01$
S-phase (< 16 versus ≥ 16%)	a: 36, b: 15, $P < 0.01$	a: 4, b: 3, n.s.	n.s.	n.s.
Grading (G1 versus G2 + G3)	a: 49, b: 25, $P < 0.01$	a: 17, b: 4, $P < 0.05$	n.s.	n.s.
Localisation (trunk versus extremity)	a: 25, b: 32, n.s.	a: 3, b: 11, n.s.	n.s.	n.s.
T-stage (T1 versus T2)	a: 17, b: 28, n.s.	a: 3, b: 4, n.s.	n.s.	n.s.
Age (< 45 versus ≥ 45 years)	a: 26, b: 27, n.s.	a: 4, b: 2, n.s.	n.s.	n.s.
Sex (male versus female)	a: 27, b: 27, n.s.	a: 5, b: 3, n.s.	n.s.	n.s.
Irradiation (XRT versus no XRT)	a: 42, b: 30, n.s.	N.A.	n.s.	N.A.
Surgery (R0 versus R1/R2)	a: 39, b: 30, n.s.	N.A.	n.s.	N.A.

Time to development of metastases was assessed both from the date of first histopathological diagnosis and from the date of biopsy for the measurement of DNA content. Median time from diagnosis to time of DNA measurement was 22 months. For uni- and multivariate analysis, patients were divided into two groups for each tested factor as indicated in parenthesis. Median time to development of metastases in months is given for each subgroup of the tested factors in the univariate analysis. N.A., not available; n.s., non-significant.

Table 5. Uni- and multivariate analysis of predictive factors of local tumour control

Factor (a versus b)	Univariate analysis: time to local recurrence (months) Median time from diagnosis	Multivariate analysis
Ploidy (euploid versus aneuploid)	a: 12, b: 12, n.s.	n.s.
S-phase (< 16 versus ≥ 16%)	a: 13, b: 9, n.s.	n.s.
Grading (G1 versus G2 + G3)	a: 21, b: 12, $P < 0.05$	$P < 0.05$
Localisation (trunk versus extremity)	a: 12, b: 12, n.s.	n.s.
T-stage (T1 versus T2)	a: 8, b: 12, n.s.	n.s.
Age (< 45 versus ≥ 45 years)	a: 12, b: 12, n.s.	n.s.
Sex (male versus female)	a: 12, b: 13, n.s.	n.s.
Irradiation (XRT versus no XRT)	a: 16, b: 13, n.s.	$P < 0.05$
Surgery (R0 versus R1/R2)	a: 13, b: 8, $P < 0.05$	$P < 0.05$

For uni- and multivariate analysis patients were divided into two groups for each tested factor as indicated in parentheses. Median time to local recurrence in months is given for each subgroup of the tested factors in the univariate analysis. n.s., non-significant.

localisation was an additional prognostic factor for overall survival, whereas tumour size was neither significant for overall survival, nor for the development of distant metastases. The latter might be a consequence of the small number of patients with T1 tumours ($n = 11$), precluding the detection of significant differences.

Local tumour control was significantly lower in patients with high grade sarcomas, in patients with microscopical or macroscopical residual disease after surgery and in patients who did not receive postoperative radiotherapy. Although survival was 18 months longer in patients with microscopically complete resections, this difference did not reach statistical significance. However, while the majority of patients with locally recurrent tumours of the limb will be locally salvaged by secondary surgery, the data support the concept that all efforts should be undertaken to achieve local control during the primary therapy. Neither tumour size nor DNA content was predictive for local tumour control. The latter indicates that the observed survival difference between patients with euploid and aneuploid tumours is predominantly the result of a different probability of developing distant disease. The lack of a significant influence of tumour size on local control might indicate that, after adequate survival procedures, expected differences between small and large tumours are obscured.

Biopsies for DNA measurements were only taken once during the course of disease. In approximately 75% of patients, the history of the disease started months to years before the DNA measurements. However, the predictive value of ploidy in the uni- and multivariate analyses remained unchanged, whether the day of diagnosis or the day of DNA measurement was used as the starting point of analyses. This finding is contrary to the notion that DNA content might change towards aneuploidy during the natural course of disease, and is indirect evidence that the DNA content is stable in the majority of patients.

Several authors have investigated the prognostic value of tumour grading in sarcoma patients, and in all larger series, a clear impact could be demonstrated. Using univariate analysis, the impact of tumour grading was also evident in the present study. However, in the multivariate analysis, ploidy and not tumour grading was found to be an independent prognostic factor for survival or development of distant metastases. Only a few studies have been reported [11, 12, 14, 15] evaluating simultaneously the prognostic impact of ploidy and grading in sarcoma patients using a multivariate analysis. In the largest series on 148 patients, published by the Scandinavian Sarcoma Group [11] both ploidy and tumour grading were found to be independent prognostic parameters for survival and metastases, in addition to tumour size and vascular invasion. In the multivariate analysis of Bauer and associates [12] on 102 sarcoma patients, only ploidy and tumour size were significant parameters, with tumour grading not reaching significance, although this was highly significant in the univariate analysis. The same finding was made by Malmström and colleagues on 37 uterine sarcomas [15], whereas for the data of Gustafson and associates [14] on 48 sarcoma patients, neither ploidy nor tumour grading were independent parameters in the multivariate analysis, although they were significant in the univariate analysis.

The observation that DNA content but not tumour grading was an independent prognostic factor in some studies might be a result of a covariation between these parameters. In most studies, some degree of relationship between ploidy and grading has been reported, although not a simple one to one correlation. As shown in Table 2, 39% of grade 3 tumours were euploid and more than 20% of grade 1 tumours were aneuploid in our series. Thus, it appears that measurement of DNA content does not identify the same subgroup of patients as grading does, but provides additional prognostically relevant information. Interestingly enough, after exclusion of DNA content from the multivariate analysis in our series, grading became an independent prognostic factor (data not shown). Therefore, our results and the data of Bauer and associates [12] are not in disagreement with the larger studies that identified grading as an independent prognostic parameter, but did not look at DNA content.

Tumour grading is influenced by the experience of the investigator and by the use of different grading systems, contributing to substantial variation between pathologists. There is still no consensus regarding the morphological criteria and the grading system to be employed in soft tissue sarcomas. Whereas Hajdu [4] and Enneking [3] proposed two levels of malignancy, Russell and associates [5] proposed three levels and Rööser [1] four levels of malignancy. Using the latter system, a discrimination between grade 3 and grade 4 proved to be most relevant. In the present study, a distinction between grade 1, according to the system of Russels and colleagues [5], and grades 2 and 3 was identified as being prognostically relevant (Figure 2). Regardless

of the grading system, it is an inherent problem that the assessment of prognostic factors based on histological slides leaves room for subjective interpretation. In contrast, measurement of DNA content has the advantage of being an objective method, free of observers' bias.

In conclusion, there is overwhelming evidence that ploidy is an important prognostic factor in soft tissue sarcomas of adults. In the present study and some other series, ploidy proved to be an even better predictor of clinical outcome of soft tissue sarcoma patients than tumour grading. However, a conclusive statement of whether both ploidy and grading or only one of these parameters should be used to select high risk patients requires still more data.

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