APPENDIX

The Uppsala-Örebro Breast Cancer Study Group

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Grading of Soft Tissue Sarcomas: Experience of the EORTC Soft Tissue and Bone Sarcoma Group

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A practical grading system for soft tissue sarcomas was developed, based on 282 eligible patients entered in an EORTC adjuvant clinical trial. The primary tumours in this trial had to be adequately treated. Histopathological parameters, which appeared significant in two preceding studies, were tested. These parameters were differentiation of the tumour, presence and amount of necrosis, the presence and amount of myxoid areas and the number of mitoses. In addition, the size of the tumour was also analysed. The quantitative data (mitotic count and size of the tumour) were not *a priori* grouped, but were divided into categories based on the results of the statistical analysis. Based on a multivariate analysis only mitotic count, the presence or absence of necrosis and the size of the tumour were significantly correlated with the duration of survival or the time to distant metastases. Of these parameters, the mitotic count was the most important.
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INTRODUCTION

THE HISTOLOGICAL typing of soft tissue sarcomas gives only limited information about their clinical behaviour. Most soft tissue malignancies, and particularly the most frequent entities, cover a large spectrum of aggressiveness and metastatic potential [1].

Hence the grading of these tumours is an essential adjunct to the histological typing. It would be ideal if we could arrive at a grading system adapted to all known prognostic factors, such as the histological (sub)type, the localisation and the size of the tumour and its relation to vital structures. To obtain statistically meaningful figures in this way, one would have to study a very large number of uniformally treated patients. It is understood that this is hardly ever possible, taking into account the rarity of this group of malignancies. Moreover, the histogenetic typing of soft tissue sarcomas, even with the aid of modern immunochemical markers, still has a non-negligible degree of uncertainty.

The pathological subcommittee of the Soft Tissue and Bone Sarcoma Group of the EORTC was asked to develop a practical grading system which could be used in the framework of a multicentre clinical trial. Members of this subcommittee participated in two previous studies.

In the study of Albus-Lutter [2], based on 400 cases at the Netherlands Cancer Institute, soft tissue sarcomas were typed according to Enzinger and Weiss [1], and a grading system was worked out for the different histological types when the number of cases of a particular type was sufficient. Also, a grading system was developed irrespective of the histogenetic type of the tumour. The parameters tested were the number of mitoses, presence and degree of necrosis and myxoid components, grade of differentiation, localisation (superficial versus deep; anatomical localisation) and the size of the tumour. Also, patient

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characteristics such as age and sex were included. Based on a multivariate analysis of the study in which the histological diagnosis was not used, only the mitotic rate, the presence of necrosis, a myxoid component, the degree of differentiation, the size of the tumour and the localisation (superficial versus deep) retained their prognostic significance.

The other study comprised 155 patients from the French Cancer Centres [3]. The parameters tested in this study were: nuclear pleomorphism, the presence of malignant giant cells, vascular emboli, tumour cellularity, tumour differentiation, necrosis and the number of mitoses. In a multivariate analysis, only the degree of tumour differentiation, mitotic rate and the presence of necrosis retained their prognostic significance.

To test the applicability of a comparable grading system in the framework of a clinical trial, we evaluated the patients of an adjuvant soft tissue sarcoma trial of the EORTC.

PATIENTS AND METHODS

The patients of this study belonged to EORTC trial 62771. After curative local treatment in non-metastatic disease, the patients in this trial were randomised to receive adjuvant chemotherapy (CYVADIC) or no adjuvant chemotherapy. Patients aged 15–70 years with histologically proven soft tissue sarcoma were eligible for this study. They were required to have adequate haematological function (white blood cell count $> 4.0 \times 10^9$/l and platelet count $> 120 \times 10^9$/l) and no evidence of metastases, either haematological or in regional nodes. Also eligible were patients with locally recurrent tumours previously treated by surgery alone. Patients who had received prior chemotherapy or radiotherapy for other malignancies were excluded, as were those in poor physical or psychological condition. Other criteria for exclusion were severe hepatic dysfunction, bleeding disorders, significant symptomatic cardiac disease, serious infections and a history of other malignant disease, excluding basal cell skin cancer. All histological subtypes were included, with the exception of border-line tumours such as fibromatosis.

Chemotherapy in this trial did not influence survival (or the appearance of distant metastases). The total number of patients entered in this trial was 468, of which 317 patients were eligible. There were 32 institutions participating in this study; 10 institutions entered 80% of the total number of patients and 85% of the eligible patients. Slides of 282 eligible patients were submitted for pathological review to the pathological subcommittee of the EORTC Soft Tissue and Bone Sarcoma Group. The median age of the patients was 43 years. After local surgery, i.e. local resection in 79% and amputation in 21% (31% of those with limb sarcomas), radiotherapy was applied in 59% of these patients. Local recurrences at entry in the trial were observed in 20%.

After pathological review the histopathological diagnoses of these sarcomas were: malignant fibrous histiocytoma (MFH) 22%, synovial sarcoma 16%, liposarcoma 15%, leiomyosarcoma 12%, neurogenic sarcoma 8%, fibrosarcoma 6%, angiosarcoma 3%, rhabdomyosarcoma 2%, miscellaneous sarcoma 7.5%, undifferentiated or unclassifiable sarcoma 8.5%.

The histopathological parameters which appeared significant in the previously mentioned studies were tested. These were differentiation of the tumour, presence and amount of necrosis (if absent, present less than 10%, between 10–50%, more than 50%), the presence of myxoid areas (if absent, present less than 50%, more than 50%) and the number of mitoses. The mitoses were counted in 10 consecutive high power fields (10 HPF, HPF are approximately 1.5 mm^2). In contrast to the previous studies,

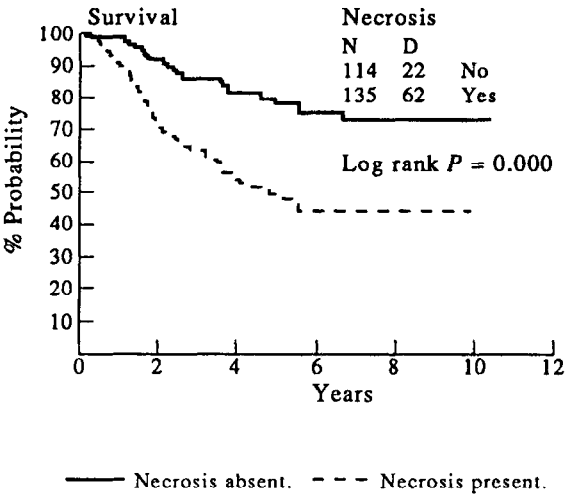

Fig. 1. Survival related to necrosis. N, number of patients; D, number of deceased patients.

the quantitative parameters were not *a priori* grouped but divided into categories based on the current data. The size of the tumour was also analysed.

Curves giving the duration of survival and time to distant metastases were calculated using the Kaplan–Meier method and compared using the log rank test. The relative importance of the various factors was assessed using Cox's proportional hazards regression model.

Patients were excluded from the analysis if data for a particular parameter were not available. Patient numbers for each analysis are given in Figs 1–8.

RESULTS

Duration of survival

All histological parameters examined were correlated with the duration of survival. Figures 1–4 give the duration of survival according to the presence of necrosis, the number of mitoses, presence of a myxoid component, and the degree of differentiation.

The most discriminating results were obtained by grouping

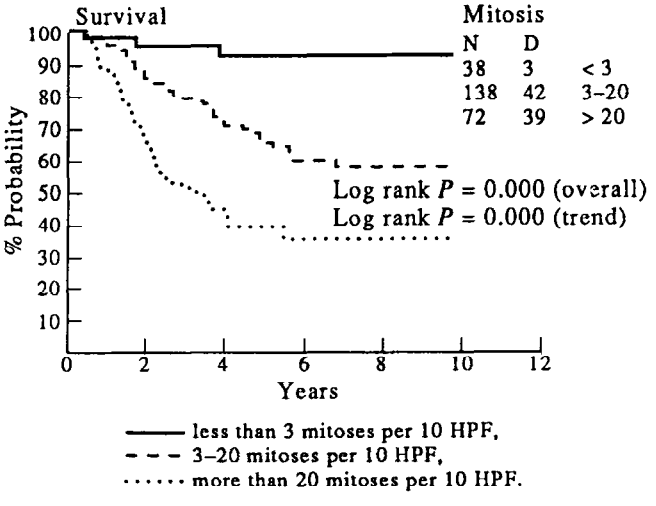

Fig. 2. Survival related to the number of mitoses. N, number of patients; D, number of deceased patients.

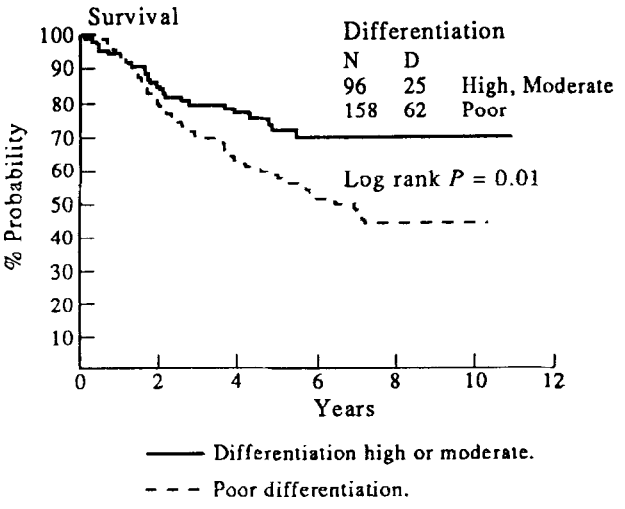

Fig. 3. Survival related to a myxoid component. N, number of patients; D, number of deceased patients.

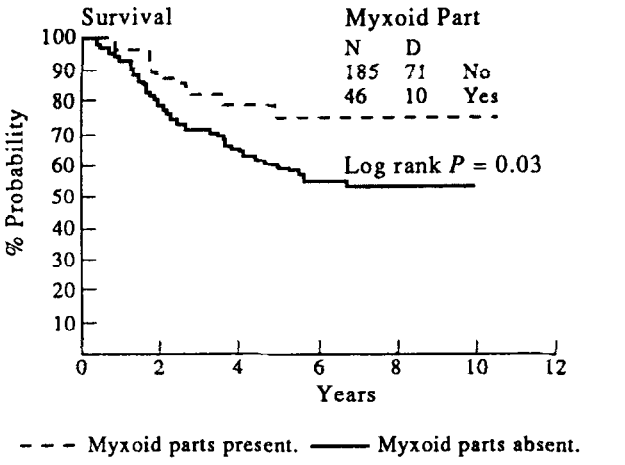

Fig. 4. Survival related to the degree of differentiation. N, number of patients; D, number of deceased patients.

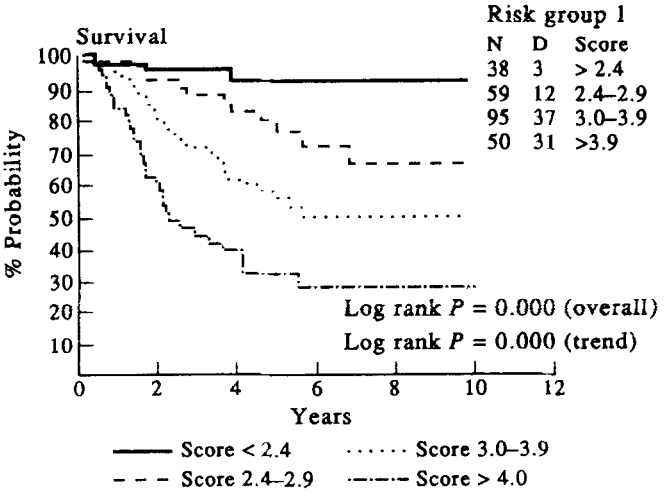

Fig. 5. Survival related to the number of mitoses and necrosis. N, number of patients; D, number of deceased patients.

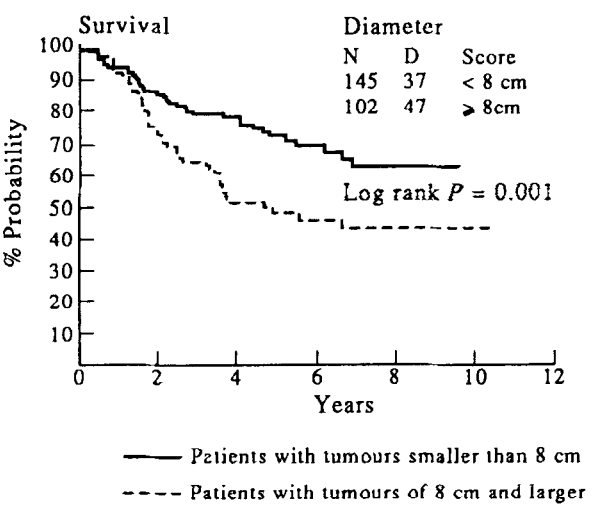

Fig. 6. Survival related to size. N, number of patients; D, number of deceased patients.

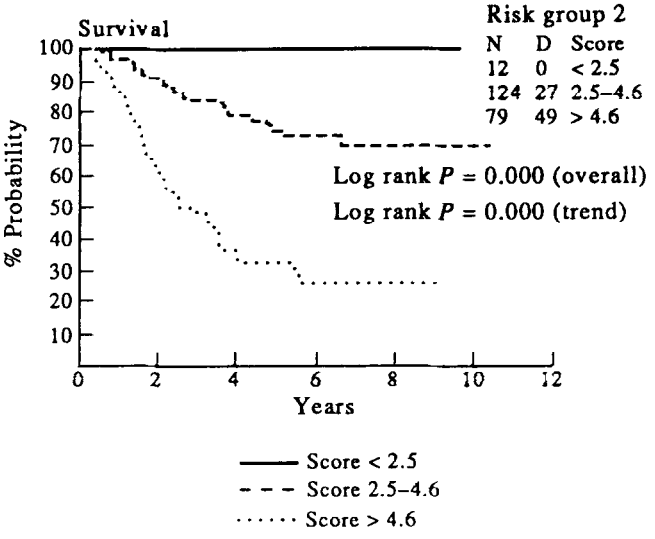

Fig. 7. Survival related to the number of mitoses, necrosis and size. N, number of patients; D, number of deceased patients.

the number of mitoses into three categories: less than three per 10 HPF, three to 20 per 10 HPF and more than 20 per 10 HPF. When necrosis or a myxoid component was present, the actual percentage was not of prognostic importance. Based on a multivariate analysis, using Cox's proportional hazards regression model, only the mitotic rate ($P < 0.0001$) and the presence of necrosis ($P = 0.004$) retained their significance, of which the mitotic rate was by far the most important. The following score function was obtained: score S1 = 0.732 × necrosis + 0.873 × mitoses, where necrosis is 1 if there is no necrosis and 2 if some necrosis is found. Mitoses is 1 if less than three mitoses per 10 HPF, 2 if three to 20 mitoses per 10 HPF and 3 if > 20 mitoses per 10 HPF, and where the multiplication factors for necrosis and mitoses are the coefficients derived from the multivariate model.

Survival curves based on this score function are illustrated in Fig. 5 and are defined as follows:

Curve I if score is <2.4 38 patients.
Curve II if score is 2.4–2.9 59 patients.

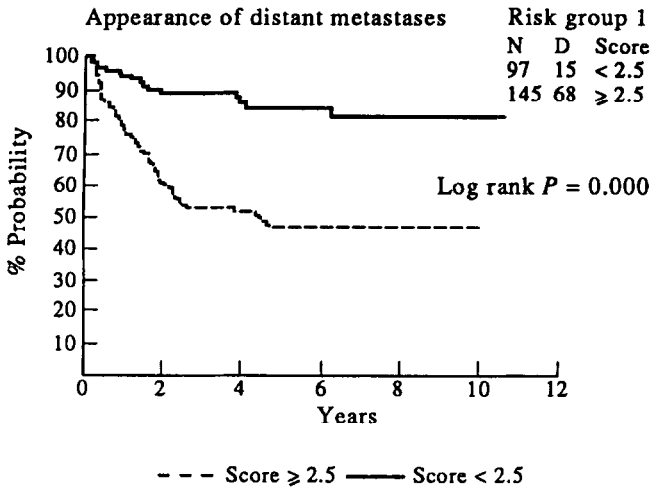

Fig. 8. Time to distant metastases related to the number of mitoses and necrosis. N, number of patients; D, number of patients with distant metastases. Score based on mitoses and necrosis.

Curve III if score is 3.0–3.9 95 patients.
Curve IV if score is >3.9 50 patients.

In a simplified form, the same curves can be defined as in Table 1.

Regarding the size of the tumour, the best correlation with survival was obtained by dividing the patients into two categories: those with a tumour diameter less than 8 cm and those with a diameter of 8 cm or larger (Fig. 6).

The addition of the size of the tumour to the score function S1 improves the prediction of survival ($P = 0.0007$). Adding the tumour size, the score function becomes: score S2 = 0.748 × necrosis + 0.911 × mitosis + 0.801 × tumour diameter, where the tumour size is 1 if diameter <8 cm and 2 if diameter ≥ 8 cm.

In Fig. 7, survival curves for three risk groups are defined as:
Curve I if score is <2.5 12 patients.
Curve II if score is 2.5–4.6 124 patients.
Curve III if score is >4.6 79 patients.

Time to distant metastases

For the time to the appearance of distant metastases, the diameter of the tumour was not statistically significant. Based on mitotic rate ($P < 0.0001$) and necrosis ($P = 0.01$), the score M1 can be calculated based on the following formula: score M1 = 0.617 × necrosis + 0.750 × mitoses in which necrosis and mitotic rate are defined as before.

The curves for the two groups are illustrated in Fig. 8 and defined as:

Table 1. Definitions of survival curves

Mitoses/ 10 HPF	Necrosis	
1	1	38 patients, curve 1
1	2	Excellent prognosis
2	1	59 patients, curve 2
		Rather good prognosis
2	2	95 patients, curve 3
3	1	Rather poor prognosis
3	2	50 patients, curve 4
		Poor prognosis

Table 2. Simplified version of Fig. 8.

Mitoses	Necrosis	
1	1	97 patients. Good prognosis regarding
1	2	appearance of distant metastases
2	1	
2	2	145 patients. Poor prognosis regarding
3	1	appearance of distant metastases.
3	2	

Curve I if score <2.5 97 patients.
Curve II if score ≥2.5 45 patients.

These groups can also be given as in Table 2.

DISCUSSION

A large number of histopathological parameters in soft tissue sarcomas are related to prognosis in terms of survival and the appearance of distant metastases [2–12]. Multivariate analysis enables us to sort out the minimum set of factors which give the best prognostic information. Previous studies of the same kind stress without exception the significance of the mitotic count [2–4, 6, 10–12].

The fact that in this study the cut off levels for the quantitative parameters (mitotic rate, size of the tumour) were chosen based on data from the trial may have had a particular bearing on the results. The mitotic count, the categories of which were determined by the data from this study, outweighs the other histological parameters to such a degree that, apart from the number of mitoses, only the presence of necrosis retained significance.

In the pathology literature much is written about the exactness of mitotic count, i.e. the number of mitoses per HPF [13–16]. In a recent article [17] this method was heavily criticised. Many factors can influence the mitotic count, for instance, interobserver variation, size of microscopic field, representativeness of the sample, section thickness and size of tumour cells. In our experience from this study, in agreement with Coindre *et al.* [18], mitotic count appeared to be a reproducible parameter, at least in this group of malignancies. It has to be stressed that mitotic count is also inherently a measure of cellularity. Cellularity may vary widely in soft tissue sarcomas; myxoid tumours, for instance, have only a few cells per HPF. Cellularity, like mitotic count, is inversely correlated with prognosis. In this way, mitotic count may owe its strong predictive value in soft tissue sarcomas to the fact that it encompasses two variables pointing in the same direction. Hence, it is not surprising that cellularity as an independent factor disappeared after multivariate analysis in the study of Trojani *et al.* [3]. The mitotic count may also vary in different parts of a sarcoma. We did not carry out a systematic study but from a pilot study, we have the impression that the largest number of mitoses is generally found in the periphery of the tumour, the area most often biopsied or taken out for microscopic examination by the pathologist. Nevertheless, it may be possible that the number of mitoses is underestimated and, as a result, a particular tumour may be undergraded.

The dominant significance of the mitotic count for prognosis stresses the desirability of identifying proliferation markers which can provide a better measure of the percentage of proliferating cells. An example is the Ki-67 monoclonal antibody [19],

which has been applied to these tumours [20, 21]. The use of this antibody requires fresh frozen tissue. In contrast to this monoclonal antibody, the more recently described PC10 monoclonal antibody can be used on paraffin-embedded archival material [22]. This antibody recognises proliferating cell nuclear antigen (PCNA) and has already proven its value in a soft tissue tumour [23].

The degree of necrosis was estimated on the microscope slides, a method which can only give a very rough approximation. The pathologist is inclined to select the more viable parts for microscopic examination. This is probably the reason that we, in contrast with other investigators [2, 3], could only relate prognosis to the presence of necrosis and not with the amount of necrosis.

Some parameters generally used in the grading of soft tissue sarcomas are clearly subjective and hamper reproducibility. Among these parameters are qualifications such as degree of pleomorphism, grade of differentiation, etc. This is illustrated by the fact that in the course of this study it became apparent that in the work of Trojani *et al.* [3], an alternative interpretation of the degree of differentiation was used compared with the study of Albus-Lutter [2]. We tested both interpretations of differentiation and both were related to prognosis but in a slightly different way. After multivariate analysis, this debatable parameter lost its significance in both interpretations.

Modern statistical methods not only enable us to define the minimum set of relevant prognostic factors, but also give us the opportunity to reassess the importance of the various prognostic categories. If the number of patients allows such a procedure, it is possible to define groups of patients with a specified survival rate or risk of metastases. In this sense, it is not necessary to fix beforehand the number of "grades of malignancy". They can be based on the results of a particular clinical trial or therapeutic regimen and re-evaluated in prospective trials.

A grading system of soft tissue sarcomas, which does not take into account the histogenetic diagnosis, has the inherent disadvantage that the entities which are present in only a small number of patients have an inadequate influence upon the final conclusions. The tumour types taken together belong in this group, in this study as "miscellaneous", with the addition of the angiosarcomas and the poorly represented rhabdomyosarcomas. The application of the results derived from all tumours may lead to erroneous conclusions in some small subgroups, if some of these entities have a different natural history. So, it seems desirable not to employ too rigorously the general parameters for these categories but to take into consideration their behaviour according to data from literature.

It should also be mentioned that the patients included in the present study were candidates for an adjuvant trial and were treated according to a standard protocol. For this study, generally only a few or even just one slide were available for review as they were provided by a large number of participating centres. Consequently, parameters such as the amount of necrosis, as measured in the total tumour mass, the adequacy of the excision and the type of tumour margins (well circumscribed versus invasive), which appeared significant in a rather recent study [24], were not taken into account. However, the results of this study are relevant if the tumour can be treated locally in an adequate fashion. In this case, these results can be used for defining the entry criteria in clinical trial protocols, to stratify patients prospectively in order to adjust for prognostic factors, and to categorise patients after biopsy according to the probable aggressiveness of behaviour, which could determine further treatment.

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