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Papers

Histopathological Grade and Response to Chemotherapy in Advanced Soft Tissue Sarcomas

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In a retrospective analysis, we evaluated the possible significance of histopathological grade with regard to response to chemotherapy in advanced soft tissue sarcomas. In three EORTC protocols, the same dose-schedule was used for patients randomised to treatment with doxorubicin as a single agent (75 mg/m^2 every third week). The submitted pathological slides from 94 of these patients were reviewed and graded. The following parameters were subjectively graded (+/++/+++): nuclear pleomorphism, necrosis, cellularity and vascularity. Mitoses were counted in 20 high-power fields, and a final grade assigned as I, II, IIIA or IIIB. The results were tested both with regard to response (complete response + partial response vs. no change + progressive disease) and survival. However, no statistically significant correlations or trends could be demonstrated. Thus, tumour grade, although a prognostic factor by itself, does not seem to be able to predict response to chemotherapy in the advanced stage.
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INTRODUCTION

LOCALLY RECURRING or metastasising soft tissue sarcomas present a significant therapeutic challenge, especially when surgical and radiotherapeutic treatment options are exhausted. Results with chemotherapy are only moderate; doxorubicin and ifosfamide remain the most effective single agents with average response rates of approximately 20–30% [1, 2]. Ongoing research includes analyses of the dose/response and schedule/response relationships, as well as the testing of new drugs for activity against sarcomas [2].

Sarcomas are heterogenous, morphologically as well as clinically, but so far it has not been possible to identify factors that could predict the probability of response to chemotherapy. One reason is their rarity, which yields inadequate numbers for analysing the importance of individual histological types, even in multi-centre trials. In addition, diagnostic criteria and classifications are changing continuously through the introduction of new entities (e.g. malignant fibrous histiocytoma) and techniques (especially immunohistochemistry). More feasible is correlation of tumour grade to response, but rather few studies have reported their results of such an analysis [3–6].

The EORTC Soft Tissue and Bone Sarcoma Group has used single-agent doxorubicin as a standard for comparison with

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carminomycin and epirubicin, and with combination regimens (CYVADIC, and doxorubicin + ifosfamide). The results of these trials have been reported elsewhere [7–9].

We decided to use this material in order to analyse the possible significance of the histological grade, as well as individual histological parameters with regard to response to chemotherapy and survival in advanced soft tissue sarcomas.

MATERIALS AND METHODS

For patients entering the EORTC trials, representative tissue slides from their tumours have to be sent for review and grading by a sub-committee of pathologists. The slides from patients treated according to the EORTC protocols 62801, 62802 and 62851 were kept at two centres, one in The Netherlands and one in France. Using the material stored in the Dutch centre in Utrecht (chairman: Dr J. van Unnik), from 94 patients randomised to single agent doxorubicin (75 mg/m^2 every third week), a subjective grading was performed of the following parameters: necrosis (I: none; II: ≤ 50%; III: > 50%), nuclear pleomorphism (I: slight; II: moderate; III: pronounced), cellularity in relation to stroma (I: low; II: moderate; III: high), and vascularity (I: low; II: moderate; III: high).

Moreover, mitotic figures were counted in at least 20 high-power fields (HPF), depending on the heterogeneity of the tumour and the amount of tissue available. In our microscopes, 10 HPF correspond to 2.5 mm^2. A grade was assigned using the system of Myhre Jensen *et al.* [10, 11]; it is based on the number of mitoses (grade 1 = less than 1 mitosis/10 HPF; grade 2 = 1–5 mitoses/10 HPF; grade 3A = 6–20 mitoses/10 HPF; grade 3B = more than 20 mitoses/10 HPF), but also takes into account cellularity, pleomorphism, necrosis and histogenetic type and subtype (for details, see [11]). The presence of areas with a lower grade was also noted.

The results were analysed in the EORTC Data Center and correlated to response and survival. In the response analysis, patients with complete or partial remission (CR + PR, respectively) were pooled and considered as responders, while no change (NC) and progressive disease (PD) were regarded as failures. Duration of survival was calculated from the day the patient entered the trial until death.

Table 1. Pathological review of 94 advanced soft tissue sarcomas: histological diagnoses and grade

Histological type	Grade I	II	IIIA	IIIB	All
Leiomyosarcoma	0	8	18	10	36
Malignant fibrous histiocytoma	0	1	4	8	13
Synovial sarcoma	0	0	4	1	5
Neurogenic sarcoma	0	0	2	3	5
Liposarcoma*	1	1	1	1	4
Rhabdomyosarcoma†	0	1	1	1	3
Fibrosarcoma	0	1	0	1	2
Angiosarcoma	0	2	0	0	2
Miscellaneous sarcoma‡	0	1	3	3	7
Unclassified or undifferentiated	0	5	7	5	17
Total	1	20	40	33	94

*Well-differentiated sclerosing 1 (grade I), mixed 2 (grade II and IIIA), pleomorphic 1 (grade IIIB).
†All three adult (pleomorphic) type.
‡Mixed mesodermal 4, haemangiopericytomas 2, alveolar soft part sarcoma 1.

Table 2. Histological grades and response in 81 evaluable patients receiving doxorubicin for advanced soft tissue sarcomas

		Responders (CR + PR)		Failures (NC + PD)		χ^2 test for trend P
Total		24	(30%)	57	(70%)	
Highest grade	I	0		1		
	II	4	(29%)	10	(71%)	
	IIIA	9	(24%)	28	(76%)	
	IIIB	11	(38%)	18	(62%)	0.3
Lowest grade	I	2	(29%)	5	(71%)	
	II	5	(25%)	15	(75%)	
	IIIA	10	(31%)	22	(69%)	
	IIIB	7	(32%)	15	(68%)	0.7
Necrosis	None	7	(28%)	18	(72%)	
	< 50%	12	(26%)	34	(74%)	
	> 50%	5	(50%)	5	(50%)	0.35
Pleomorphism	Slight	5	(31%)	11	(69%)	
	Moderate	6	(17%)	29	(83%)	
	Severe	13	(43%)	17	(57%)	0.2
Cellularity	Low	0		1		
	Moderate	8	(23%)	27	(77%)	
	High	16	(36%)	29	(64%)	0.17
Vascularity	Low	4	(20%)	16	(80%)	
	Moderate	16	(36%)	28	(64%)	
	High	4	(24%)	13	(76%)	0.75
Mitoses (per 2.5 mm^2)	< 1	0		1		
	1–5	4	(29%)	10	(71%)	
	6–20	9	(24%)	28	(76%)	
	> 20	11	(38%)	18	(62%)	0.3

CR = Complete response; PR = partial response; NC = no change; PD = progressive disease.

RESULTS

The histological diagnoses are shown in Table 1. All 94 patients were evaluable with regard to survival; only 20 were alive at the time of last follow-up. 13 patients were not evaluable with regard to response and were therefore excluded from the response analysis. There were 4 CR and 20 PR, corresponding to a response rate of 30%; the failures included 30 patients with NC and 27 with PD.

Table 2 shows the results of the response analysis: no statistically significant correlations appeared, although there was a tendency towards a better response for tumours with high cellularity.

In the survival analysis (data not shown), there were no statistically significant differences between the sub-groups, and log-rank tests for trend were not significant.

DISCUSSION

Several studies have confirmed the clinical importance of histopathological grading of soft tissue sarcomas [11–16]. The investigators have used different grading systems (with from two to four grades), but all incorporate an evaluation of necrosis, cellularity, pleomorphism and mitotic activity. In multivariate (Cox) analyses, grade (with degree of necrosis and mitotic activity as the most important single parameters [17, 18] emerges as one of the strongest prognostic factors, surpassing other predictors such as age, tumour size and site [11, 14, 16, 18].

The importance of these factors is less well studied in the advanced stages of sarcomas. Our results do not indicate that they have any significant predictive value as regards response to

chemotherapy. This is in accordance with the experience from the CYVADIC regimen [3–5], and from a small phase II study of ifosfamide plus etoposide combined with regional hyperthermia [19]. However, in the ECOG experience with doxorubicin there was a slight, but not significant tendency towards higher response rates in the lower grades [6]. These findings are at variance with a recently published study which observed increasing response rates with increasing grade in four doxorubicin-containing regimens [23].

In our study, survival after recurrence was not significantly correlated with tumour grade; however, our sample size may have been too small to detect minor tendencies.

In addition, there are several inherent problems in a retrospective study like this, and in the grading itself. First, only selected slides were available for review and, e.g. necrosis may thus have been underestimated. Second, the histological picture of the primary tumour may not be representative of its recurrences, since dedifferentiation is known to occur. Third, the grading itself is subjective, although attempts have been made to define the grades better and thereby minimize interobserver variation [11, 20].

We have used the traditional method of counting mitoses/10 HPF/2.5 mm^2, because this has been proved to yield prognostic information [17, 18]. However, the mitotic count is also subject to interobserver variation [20], apart from being sensitive to delays in fixation [21]. New methods, such as staining for Ki-67 or PCNA, the counting of nucleolar organiser regions, or flow cytometric determination of growth fraction may, hopefully, turn out to be more reproducible and contain even more prognostic information [22].

In conclusion, our study did not show any statistically significant correlations between conventionally determined histological grade of the primary tumours and response to chemotherapy in the advanced stage. We, therefore, cannot support the view of van Haelst-Pisani *et al.* [23] that patients should be stratified according to grade. However, the current trend towards objectivisation and quantification together with new techniques may yet disclose parameters of prognostic importance. To test these, cooperative treatment trials with pathological review are still necessary because of the relative rarity of these tumours.

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