FULL PAPER

BJC

British Journal of Cancer (2015) 112, 455–460 | doi: 10.1038/bjc.2014.635

Keywords: soft tissue sarcoma; viable tumour; mitotic count; response assessment; neoadjuvant treatment

Prognostic relevance of the mitotic count and the amount of viable tumour after neoadjuvant chemotherapy for primary, localised, high-grade soft tissue sarcoma

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Background: We sought to examine whether mitotic count (MC) and the amount of viable tumour (VT) following neoadjuvant systemic chemotherapy (SC) for primary, localised, high-grade soft tissue sarcoma (STS) correlate with prognosis.

Methods: Retrospective analysis of 57 patients who underwent SC involving a combination of an anthracycline and an alkylating agent, followed by surgical resection between 2001 and 2011.

Results: The amount of VT after chemotherapy was significantly associated with disease-specific survival (DSS) and event-free survival (EFS). Patients with $<10\%$ VT had a DSS of 94% at 5 years, compared with 61% for patients with $\geqslant 10\%$ VT ($P=0.033$); EFS was 75%, compared with 48% ($P=0.030$). Patients with an MC of $\geqslant 20/10$ high power fields (HPF) after chemotherapy had a significantly lower DSS (33% *vs* 84% at 5 years, $P<0.001$) and EFS (40% *vs* 63% at 5 years, $P=0.019$) than patients with an MC of $<20/10$ HPF.

Conclusions: The MC and the amount of VT after neoadjuvant therapy for primary, localised, high-grade STS appear to correlate with prognosis. If these results are validated prospectively, then they could provide a rational for the design of neoadjuvant treatment modification/escalation studies, analogue to the EURAMOS-1 trial for bone sarcomas.

The treatment of high-grade soft tissue sarcomas can be challenging (Clarkson and Ferguson, 2004), and neoadjuvant modalities are often implemented to improve tumour resectability and disease outcome (Eilber *et al*, 2001). Preoperative systemic chemotherapy has been shown to be effective in downstaging large, high-grade tumours (Demetri *et al*, 2010) and can be considered for tumours that can only be resected by means of ablative or mutilating surgery (ESMO/European Sarcoma Network Working Group, 2012).

The assessment of tumour response to neoadjuvant treatment is an important tool in multidisciplinary treatment plans, as it helps

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Received 31 August 2014; revised 1 November 2014; accepted 1 December 2014; published online 23 December 2014

identify patients who might benefit from treatment modification or intensification (Schmidt *et al*, 1993; Eilber *et al*, 2001), potential candidates for experimental treatment options (Picci *et al*, 1993), as well as patients who can be spared the morbidity of continuing an ineffective treatment (Pezzi *et al*, 1990). Contrary to bone sarcomas, where the histological evaluation of treatment-induced necrosis has been validated as a very important prognostic factor (Picci *et al*, 1993; Bielack *et al*, 2002), there is no standardised approach for the histological response assessment of soft tissue sarcomas to neoadjuvant chemotherapy (Lucas *et al*, 2008). The most commonly used parameter has been tumour necrosis (Huth *et al*, 1985; Pezzi *et al*, 1990; Schmidt *et al*, 1993; Eilber *et al*, 2001; Menendez *et al*, 2007; Lucas *et al*, 2008; Novais *et al*, 2010) analogue to bone sarcomas. However, the definition of 'good' and 'poor' responders in published studies has not been uniform and the results have been conflicting, with some studies finding that tumour necrosis after neoadjuvant treatment is a good predictor of prognosis (Huth *et al*, 1985; Pezzi *et al*, 1990; Eilber *et al*, 2001; Novais *et al*, 2010), and other studies claiming that this is not the case (Menendez *et al*, 2007; Lucas *et al*, 2008). Although changes in mitotic count (MC), an important prognostic factor in soft tissue sarcomas (Trojani *et al*, 1984; Tsujimoto *et al*, 1988), have been described after induction chemotherapy (Coindre *et al*, 1985), the prognostic value of this parameter in assessing tumour response has been largely ignored.

The objective of this study was to examine whether MC and the amount of viable tumour (VT) following neoadjuvant treatment for primary, localised, high-grade soft tissue sarcoma correlate with prognosis.

PATIENTS AND METHODS

Study design. Between 2001 and 2011, 58 consecutive patients with primary, locally advanced, non-metastatic, high-grade soft tissue sarcomas underwent neoadjuvant systemic chemotherapy followed by the surgical tumour resection at the sarcoma center Berlin-Brandenburg. One patient underwent induction chemotherapy followed by neoadjuvant isolated limb perfusion before surgical treatment and was excluded from our analysis leaving 57 patients as the subject of this study. All patients signed an informed consent form at hospital admission allowing the use of anonymised data for research purposes.

Data regarding patient demographics, tumour characteristics, first-line treatment, and follow-up were collected prospectively and entered into an electronic database. Further details regarding VT and MC before and after treatment were collected retrospectively from pathology reports. The tumours of patients with missing data were reexamined by a sarcoma pathologist (MW) who was blinded to the clinical characteristics and patients' outcome. Survival analysis was based on follow-up data as of May 2013. Follow-up information for patients who had stopped presenting at our outpatient clinic was gathered by contacting the referring physicians.

Neoadjuvant treatment. The decisions regarding neoadjuvant treatment were made by an interdisciplinary panel, based on tumour histology, localisation, and extent, as well as patient performance status and preference. Systemic chemotherapy protocols consistently involved the combination of an anthracycline (doxorubicin or epirubicin) and an alkylating agent (ifosfamide or dacarbazine). Some patients also received etoposide, while three young patients with pediatric-type sarcomas were additionally treated with vincristine and dactinomycin. Patients were treated with four to six cycles of induction chemotherapy; surgical resection was performed 3 weeks after the last cycle.

Histopathology. All surgical specimens were evaluated by pathologists specialising in bone and soft tissue sarcomas in a standardised manner, according to the technique established for bone sarcomas (Salzer-Kuntschik *et al*, 1983). Following fixation in a 4% formalin solution for 24 h, the surgical specimens were bisected along their greatest diameter. A longitudinal section was then obtained, followed by several transversal sections. A gross estimation of the residual amount of VT was recorded, taking into consideration areas of cystic degeneration, haemorrhage, or fibrosis. An amount of $<10\%$ residual VT was classified as 'low', while an amount of $\geqslant 10\%$ residual VT was classified as 'high', analogue to the Salzer-Kuntschik grading system for osteosarcoma patients (Salzer-Kuntschik *et al*, 1983). The number of mitoses was recorded both in the surgical specimen after neoadjuvant treatment and, when available, in the biopsy specimen. Mitoses were counted in at least 10 high power fields (HPF), which correspond to 3.06 mm^2 in our microscopes. An MC of 0–9 mitoses per 10 HPF was classified as 'low', an MC of 10–19 mitoses per 10 HPF as 'intermediate', while an MC of $\geqslant 20$ mitoses per 10 HPF was classified as 'high' (Trojani *et al*, 1984).

Statistical analysis. Non-parametric analyses were carried out with the Mann–Whitney U and the Wilcoxon signed-rank test. The duration of follow-up and the time to event (local recurrence, metastasis, or disease-related death) were calculated from the date of diagnostic biopsy. Survival curves were calculated with the Kaplan–Meier method (Kaplan and Maier, 1958) and compared with the log-rank test (Mantel, 1966). Statistical analyses were performed with the IBM SPSS Statistics for Windows software version 20.0 (IBM Corp., Armonk, NY, USA). All *P*-values are two-sided; a *P*-value <0.05 was considered significant.

RESULTS

Patient demographics, tumour characteristics and neoadjuvant treatment are listed in Table 1. The median patient age at diagnosis was 55 years (range, 16–73 years). The median tumour size at diagnosis, available for 53 patients, was 12 cm (range, 4–27 cm), while the median tumour size after neoadjuvant treatment was available for 54 patients and amounted to 9 cm (range, 0–26 cm; $P=0.043$). The median follow-up was 44 months for all patients (range, 5–134 months) and 55 months for survivors (range, 13–134 months).

All patients underwent surgical resection of the primary tumour following neoadjuvant treatment. Limb-sparing surgery was performed in 45 of the patients with tumour in the extremities or pelvis, while an amputation was necessary in 8 patients. Surgical margins were clear in 50 patients and microscopically positive in 7 patients. Thirty-five patients underwent adjuvant radiation treatment.

The MC before neoadjuvant treatment was available for 32 patients and amounted to a median of 22/10 HPF (range, 0–150/10 HPF; mean, 32/10 HPF). The MC after neoadjuvant treatment, available for all patients, was significantly lower (median, 10/10 HPF; range, 0–120/10 HPF; mean, 17/10 HPF; $P=0.001$). The amount of VT after neoadjuvant treatment was available for 56 patients. Eighteen patients had $<10\%$ VT, while 38 patients had $\geqslant 10\%$ VT.

Six patients developed a local recurrence after a median time of 18 months (range, 10–25 months), five of which had clear margins at the surgical treatment of the primary tumour. Distant metastases were diagnosed in 20 patients after a median time of 15 months (range, 7–70 months). At the time of last follow-up, 36 patients were alive without evidence of disease, 3 patients were alive with disease, 15 patients had died of their disease, and 3 patients had died of other causes. Disease-specific survival (DSS) probability at 2 and 5 years amounted to 89% and 70%, respectively. Event-free

Table 1. Patient demographics and tumour characteristics

Variable	*n*	%
Eligible patients	57	100
Sex		
Male	29	51
Female	28	49
Primary tumour site		
Lower extremity	46	81
Upper extremity	5	9
Thoracic wall	4	7
Pelvis	2	3
Histology		
Undifferentiated sarcoma	24	42
Synovial sarcoma	10	17
Leiomyosarcoma	6	11
Rhabdomyosarcoma	5	9
Myxofibrosarcoma	5	9
Dedifferentiated liposarcoma	4	7
Myxoid liposarcoma	2	3
MPNST	1	2
Tumour grade (FNCLCC)		
2	11	19
3	46	81
Stage (AJCC)		
IIa	9	16
III	48	84

Abbreviations: AJCC = American Joint Committee on Cancer; FNCLCC = Fédération Nationale des Centres de Lutte Contre le Cancer; MPNST = malignant peripheral nerve sheath tumour.

survival (EFS) probability at 2 and 5 years was 64% and 57%, respectively.

The amount of VT after systemic chemotherapy was significantly associated with DSS and EFS (Figure 1). Patients with $<10\%$ VT had a DSS of 94% at 5 years, compared to 61% for patients with $\geqslant 10\%$ VT ($P=0.033$), while EFS amounted to 75% compared to 48% ($P=0.030$). The MC before neoadjuvant treatment had no statistically significant influence on DSS or EFS in this selected group of patients with high-grade tumours (Table 2). On the other hand, patients with a high MC after systemic chemotherapy had a significantly lower DFS ($P<0.001$) and EFS ($P=0.019$) than patients with a low and intermediate MC (Figure 2; Table 2).

To evaluate whether a more accurate prediction of prognosis could be achieved, we examined the combined influence of MC after treatment and VT on survival. Patients with an MC of $<20/10$ HPF and a VT of $<10\%$ had a strong trend for an improved DSS (100% *vs* 76% at 5 years, $P=0.068$) and an improved EFS (77% *vs* 54% at 5 years, $P=0.083$), compared with patients with an MC of $<20/10$ HPF and a VT of $\geqslant 10\%$ (Figure 3).

Finally, the MC after neoadjuvant treatment had a significant influence on post-relapse survival (PRS) probability. Following a local or systemic recurrence, patients with an MC of $<20/10$ HPF had a PRS of 70% at 2 years, compared to 22% for patients with an MC of $\geqslant 20/10$ HPF ($P=0.003$) (Figure 4). With the amount of patients available for this study, the amount of VT after chemotherapy did not correlate with PRS. Patients with $<10\%$ VT had a PRS of 75% at 2 years, compared to 49% for patients with $\geqslant 10\%$ VT ($P=0.423$).

DISCUSSION

The histological assessment of tumour response to neoadjuvant chemotherapy is one of the most important prognostic factors in

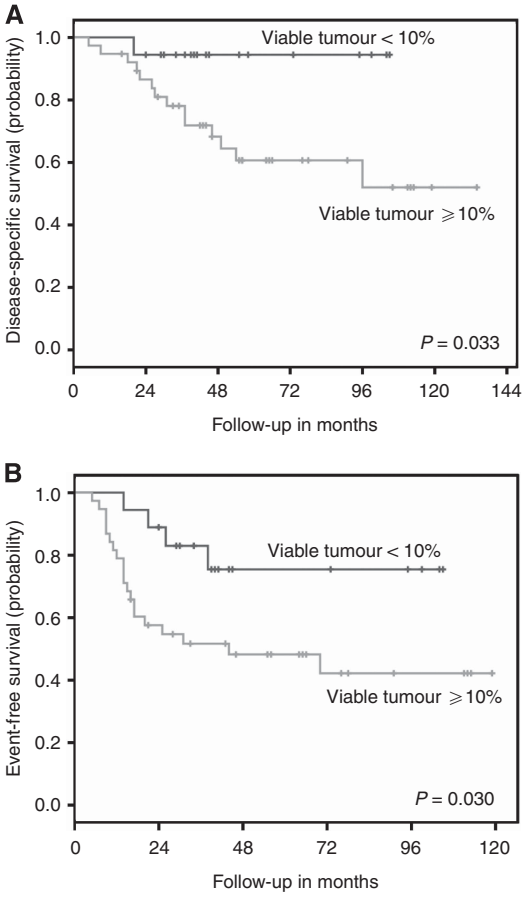

Figure 1. Disease-specific (**A**) and event-free survival (**B**) probability according to the amount of VT after treatment.

osteosarcoma (Bielack *et al*, 2002) and Ewing sarcoma (Picci *et al*, 1993). While several studies have sought to determine the prognostic value of histological response assessment in soft tissue sarcomas (Huth *et al*, 1985; Pezzi *et al*, 1990; Schmidt *et al*, 1993; Eilber *et al*, 2001; Menendez *et al*, 2007; Lucas *et al*, 2008), the definitions of 'good' and 'poor' response were varied and the results conflicting. Our study aimed at shedding new light on this issue, evaluating the impact of not only tumour necrosis but also MC, a factor that has been largely overlooked in previous analyses. Inevitably, the differences in systemic chemotherapy protocols used over the years caused for some inhomogeneity in our patient cohort, which is one of the limitations of this study, while another is its retrospective nature. Nevertheless, all patients received at least an anthracycline and an alkylating agent, both of which are regarded as active and are recommended for first-line treatment in most soft tissue sarcoma subtypes (Demetri *et al*, 2010; ESMO/European Sarcoma Network Working Group, 2012).

A further limitation of our study regards the lack of a validated technique to assess the response of soft tissue sarcomas to neoadjuvant treatment. As a result, many authors use techniques developed for and validated in osteosarcoma patients instead (Huth *et al*, 1985; Menendez *et al*, 2007). This approach has its drawbacks, as reductions in tumour volume under neoadjuvant chemotherapy are more common in soft tissue sarcoma than in osteosarcoma patients. These volume reductions can lead to a discrepancy between clinical and histopathological response – for example, a given tumour might reduce significantly in size during neoadjuvant treatment but show mostly viable areas in histopathology (Lucas *et al*, 2008). Due to these drawbacks, we chose to also evaluate the prognostic significance of the MC after treatment as a marker of histopathological tumour response to treatment.

Table 2. Mitotic count and survival

		Overall survival (%)			Event-free survival (%)		
	No. of patients	5 years	s.e.	*P* (log-rank)	5 years	s.e.	*P* (log-rank)
Mitotic count before SC							
Low (1–9/10 HPF)	7	66.7	19.2		71.4	17.1	
Intermediate (10–19/10 HPF)	8	85.7	13.2	0.551	46.9	18.7	0.399
High (⩾20/10 HPF)	17	62.9	13.8	0.156	52.2	13.5	0.845
Low *vs* high				0.549			0.411
Mitotic count after SC							
Low (1–9/10 HPF)	28	86.1	7.5		66.5	10.0	
Intermediate (10–19/10 HPF)	14	76.9	15.3	0.886	53.1	14.1	0.346
High (⩾20/10 HPF)	15	33.3	13.9	0.017	40.0	12.6	0.213
Low *vs* high				0.001			0.016
Low + intermediate *vs* high				<0.001			0.019

Abbreviations: HPF = high power fields; SC = systemic chemotherapy; s.e. = standard error.

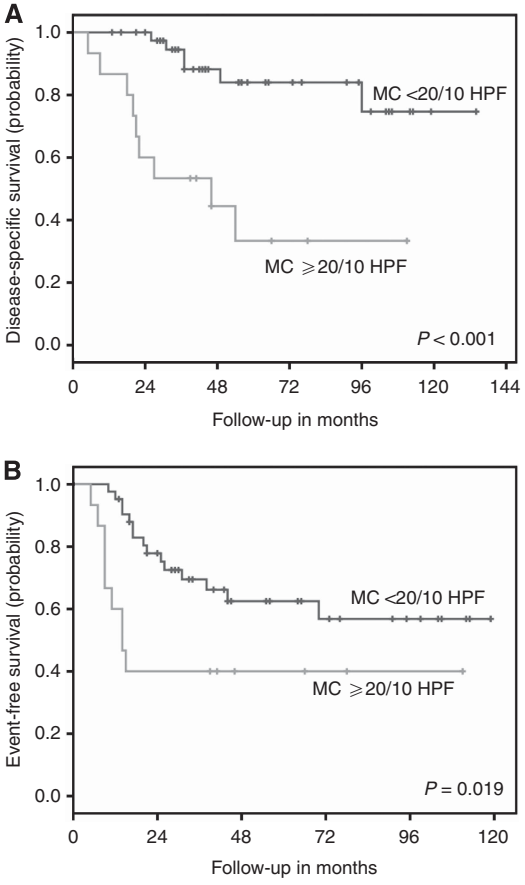

Figure 2. Disease-specific (**A**) and event-free survival (**B**) probability according to the MC after treatment.

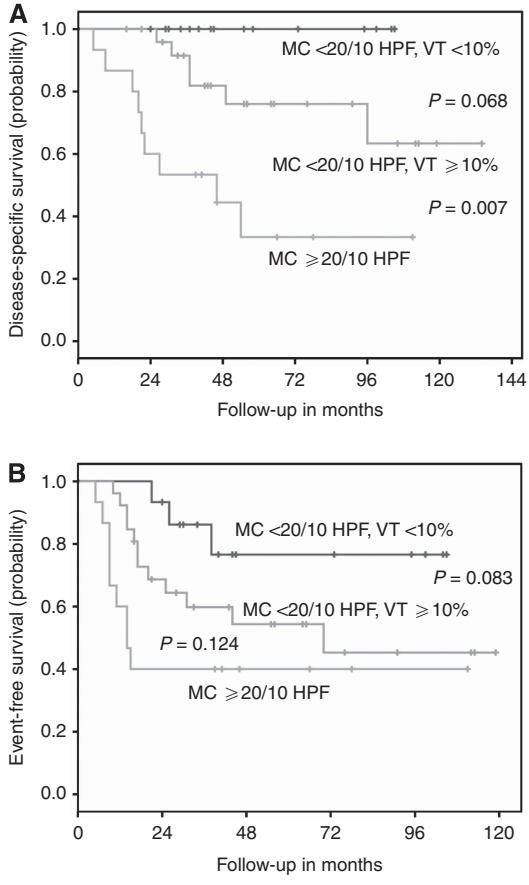

Figure 3. Disease-specific (**A**) and event-free survival (**B**) probability according to the MC and the amount of VT.

The amount of VT after systemic chemotherapy with a cutoff at 10% had a significant correlation to DSS and EFS. Our results are in accordance with those of several previous studies (Huth *et al*, 1985; Pezzi *et al*, 1990; Eilber *et al*, 2001; Novais *et al*, 2010), however some authors have reported a lack of association between the amount of VT and survival (Menendez *et al*, 2007; Lucas *et al*, 2008). One possible explanation for these conflicting findings might be the fact that the neoadjuvant treatment protocols used in the aforementioned studies greatly varied in terms of the chemotherapy agents that were applied, their dosage, as well as in the use of neoadjuvant radiation treatment and adjuvant chemotherapy.

While in this selected group of patients the MC before treatment had no influence on DSS or EFS, the most important predictor of survival was the MC after neoadjuvant treatment. To our knowledge, this is the first time this aspect of tumour response has been evaluated in detail in soft tissue sarcomas following systemic chemotherapy. Whether the histological response to neoadjuvant chemotherapy is a marker of the tumour's susceptibility to preoperative treatment or an indication of the tumour's biologic aggressiveness, as has been hypothesised in the past (Huth *et al*, 1985), remains unclear. However, the information provided can still be used to identify a group of high-risk patients who might benefit from second-line or experimental treatments (Pezzi *et al*, 1990).

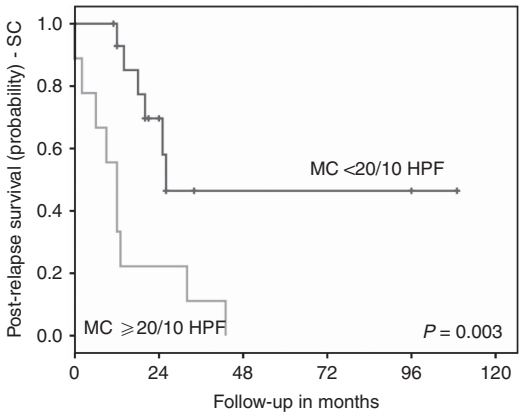

Figure 4. Post-relapse survival probability according to the MC after chemotherapy.

An obvious disadvantage of using the MC after neoadjuvant treatment as a prognostic factor is that it is subject to interobserver variation and sensitive to delays in fixation (Daugaard *et al*, 1993). Ki-67 staining has been proposed as an alternative method to measure cell proliferation (Daugaard *et al*, 1993), as it has been shown to have a somewhat higher reproducibility than MC (Hasegawa *et al*, 2002). Nevertheless, MC remains one of the parameters used in the most widely employed system to determine tumour grade in soft tissue sarcomas (Deyrup and Weiss, 2006) and has also been established in gastrointestinal stromal tumours (GIST) as one of the three prognostic factors used to identify patients at a high risk for recurrence who should be considered for adjuvant treatment (Joensuu *et al*, 2012).

The combined evaluation of the amount of VT and the MC after chemotherapy appears to separate patients into three prognostic groups (Figure 3). If these results are confirmed in a separate patient cohort, then they could provide a rationale for the design of neoadjuvant treatment modification/escalation studies, analogue to the EURAMOS-1 (Marina *et al*, 2009) and EURO-B.O.S.S. (Carrle and Bielack, 2006) trials for bone sarcomas. It should be noted that, contrary to osteosarcoma and Ewing sarcoma, neoadjuvant chemotherapy is not the standard of care in soft tissue sarcoma. However, the guidelines of the European Society for Medical Oncology (ESMO) recommend the use of neoadjuvant chemotherapy for the treatment of non-resectable tumours or tumours amenable only to mutilating surgery (ESMO/European Sarcoma Network Working Group, 2012). Furthermore, it has been shown that neoadjuvant chemotherapy may be associated with an improved DSS in patients with large (>10 cm), high-grade soft tissue sarcomas (Grobmyer *et al*, 2004).

In conclusion, the MC and the amount of VT after neoadjuvant therapy for primary, localised, high-grade soft tissue sarcoma appear to correlate with prognosis. Obviously, these findings need to be validated in larger, homogeneous studies. As the focus of systemic chemotherapy trials has been shifting to tailored studies for patients with different soft tissue sarcoma subtypes in recent years (Eriksson, 2010), we believe that this provides an excellent opportunity to prospectively examine the prognostic relevance of the MC and the amount of VT after treatment in different subgroups of soft tissue sarcoma patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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