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A reassessment of bone scintigraphy and commonly tested pretreatment biochemical parameters in newly diagnosed osteosarcoma

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Abstract *Purpose*: In patients with an osteosarcoma, the prognosis is still poor. The aim of the present study was to investigate whether routinely tested biochemical parameters or additional parameters on bone scintigraphy could be identified which can select prognostic subgroups at the time of diagnosis. *Methods*: A retrospective study was performed in 115 consecutive patients (70 male, 45 female) (mean age: 25.6 years; range: 3.50–78.0 years) who were referred for bone scintigraphy prior to treatment from March 1986 to September 2000 because of a newly diagnosed osteosarcoma. All bone scans were reassessed for the intensity and pattern of uptake and a bone-scan index. All pre-treatment general, histological, biochemical, and scintigraphic data were correlated with clinical outcome during follow-up. *Results*: During follow-up 54 patients died. Tumour volume and GGT showed significance as independent variables for metastases. Patients with metastases demonstrated a significantly lower survival rate (23% 5-year survival) than patients without metastases (98% 5-year survival). Tumours of the humerus and femur had a significantly lower survival rate. With respect to significant biochemical parameters (ALP, GGT, ASAT), it was not possible to determine a cut-off value that could be used to differentiate between high- and low-risk patients. Additional parameters assessed on bone scintigraphy were not important for prognostic stratification. *Conclusion*: The strongest predictor of survival in osteosarcoma is the presence or absence of metastasis. Some biochemical parameters have prognostic value, but they cannot be used for the unequivocal identification of subgroups. Additional scintigraphic parameters are irrelevant for prognostic stratification.

Keywords Bone scintigraphy · Biochemical parameters · Osteosarcoma · Metastasis

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Introduction

In the Netherlands, approximately 150 patients are diagnosed with osteosarcoma each year. Adjuvant and neoadjuvant chemotherapy combined with surgery substantially increased the cure rate of these patients. However, despite the success of this combined treatment, metastases still develop in approximately 30–40% of the patients. The 5-year event-free survival (EFS) rate is in the range of 38–70% at 5 years, whereas the 5-year survival rate is in the range of 20–79% [1, 2, 3]. Therefore, to improve the disease-free survival, it would be helpful to be able to identify patients with a poor prognosis who could be treated with more aggressive therapy.

A substantial number of clinical studies that have attempted to identify the prognostic factors in osteosarcoma have been published [1, 2, 4]. Due to the variation in methodology and patients selection used in these studies, data are not conclusive and, consequently, it is difficult to draw definite conclusions. The aim of the present study was to investigate whether a routinely tested biochemical parameter or an additional parameter on bone scintigraphy could be identified which can improve the selection of prognostic subgroups at the time of diagnosis.

Materials and methods

Patients and methods

We retrospectively studied 115 consecutive osteosarcoma patients (70 male, 45 female) (mean age: 25.6 years; range: 3.50–78.0 years) who were referred for bone scintigraphy prior to treatment from March 1986 to September 2000. In all patients, biopsy and histologic examination of the specimen established the diagnosis of osteosarcoma. Of all patients included sufficient data were available for further evaluation. Follow-up ranged from 2.3 months to 15.31 years (mean 4.1 years) during which 54 patients died. The staging procedure at the time of diagnosis included an X-ray and CT scan of the chest and bone scintigraphy. All patients without metastases were treated with three courses of chemotherapy including anthracyclines and cisplatin. In addition, surgery was performed followed by another three courses of chemotherapy. In eight patients, surgery was performed after two courses of chemotherapy, as progression of the primary tumour was seen. In patients with metastatic disease at initial stage, treatment consisted of chemotherapy only, which included cisplatin, ifosfamide, and anthracyclines. Finally, a resection of the primary tumour was performed in 103 patients.

Histological data

All histological data with respect to the primary tumour were extracted from the patients' records in the Hospital Information System. A complete data set including primary tumour site, extension, and malignancy grade was available. The diameter and height were measured during histological examination of the resection specimen ($n = 103$). Tumour volume was calculated using the formula: volume $= p \times r^2$ (mean diameter) $\times$ h (height).

Biochemistry

Values of commonly tested biochemical parameters, such as lactate dehydrogenase (LDH: normal value < 160 IU/l), alkaline phosphatase (AP: normal range 15–60 IU/l), calcium (Ca: normal range 2.25–2.55 mmol/l), albumin (normal range 40–50 g/l), gamma-glutamyl transpeptidase (GGT: normal range men 6–28 IU/l, women 14–18 IU/l), aspartate aminotransferase (AST: normal range < 215 IU/l), and alanine aminotransferase (ALT: normal range < 216 IU/l) were gathered. All biochemical parameters measured at the time of diagnoses and before any kind of treatment were registered.

Bone scintigraphy

All bone scans were reassessed. The whole-body scans were performed after the intravenous injection of 550 MBq of Tc-99m HDP with dual-head gamma camera (Toshiba CGA-901, Japan) equipped with a LEGP-collimator. With respect to the primary tumour a tumour index was scored. This index is currently used to assess scintigraphically the percentage of bone involved, and which has been used extensively for dose calculations in prostate cancer treatment with radionuclides [5]. The uptake was scored as slightly ($= 1$), moderately ($= 2$) or intensely ($= 3$) increased, whereas the uptake pattern was scored as homogeneous ($= 0$) or heterogeneous ($= 1$). The presence or absence of metastases was scored as follows: 0 ($=$ no metastases), 1 ($= 1$ metastasis), 2 ($= 2$ metastases) or 3 (more than 2 metastases).

Statistical methods

All clinical and biochemical parameters were correlated with the development of metastases and survival. Patient groups were compared using the Student's t-test, the Mann Whitney U or χ^2 test where appropriate. Quantitative variables were summarised with their mean, standard deviation, and range. Multivariate and stepwise forward analyses with respect to tumour recurrence were done with the logistic regression model. The predictive value of the patient's characteristics for the result of follow-up was quantified with the odds ratio and its 95% confidence level. Multivariate and stepwise forward analysis with respect to survival was done with the Cox regression model. The prognostic value of the patients' characteristics was quantified with the hazard ratio and its 95% confidence level and illustrated with Kaplan-Meier curves. Throughout, a P-value of 0.05 or less was considered statistically significant.

Results

Table 1 lists the pre-treatment characteristics of the subgroups of patients studied. During follow-up 54 patients died. The mean disease-free survival was 3.15 years (range: 0.1–15.31 years). The maximum interval between initial diagnosis and tumour recurrence (i.e., the recurrence of metastases) was 5.29 years. In 19 patients, metastases were found at initial stage, whereas in 48 patients metastases were found during follow-up. The sites of metastases were: lung (59), bone (14), liver (5), lymph nodes (three), soft tissue (two), kidney (two), pleural (two), spleen (one), and brain (one). In eight patients (7%), bone was the only site of metastatic disease at initial stage: one metastasis and more than two metastases were seen in three and five patients, respectively. In all patients with metastases during follow-up, the lung was the presenting site of recurrence.

For all initial parameters tested, the hazard rates for death and metastases are listed in Table 2. Logistic regression analysis demonstrated that three parameters were significant predictors of metastases in the population studied. When all variables were evaluated by stepwise proportional hazard analysis, tumour volume and GGT were the most significant independent variables. Excluding patients with metastases at initial stage, both parameters kept their significance (tumour volume: $P = 0.0065$, GGT: $P = 0.001$). However, it was not possible to determine a cut-off value (ROC analysis) that could be used for further differentiation.

Kaplan-Meier analysis revealed that seven parameters were significant predictors of survival and when all variables were evaluated by stepwise proportional hazard analysis, six showed significance as independent variable. These parameters were age, primary tumour site, ALP, GGT, ASAT, and metastases. Patients with metastases demonstrated a significantly lower survival rate (52% at 2-year survival; 23% at 5-year survival) than patients without metastases (98% both at 2- and 5-year). For patients with metastatic disease the hazard rate for death was 168 times higher (95% CI: 17.47–1615.86) (Fig. 1). The humerus and femur as primary site demonstrated a significantly lower survival rate compared to the other sites (Fig. 2). With respect to the other parameters, it was not possible to determine a

Table 1. Characteristics of the population of patients correlated with final outcome. (*CR* complete response, *DIS* disseminated disease)

	Clinical outcome					
	CR ($n=48$)	DIS ($n=67$)	*P*	Alive ($n=61$)	Death ($n=54$)	*P*
Age (years)	23 (14)	27 (15)	0.135	25 (15)	26 (15)	0.657
Male gender; *n* (%)	27 (56)	43 (64)	0.398	35 (57)	35 (65)	0.415
ALP (IU/l)	218 (596)	443 (870)	0.103	249 (526)	462 (949)	0.152
LDH (IU/l)	259 (152)	301 (231)	0.239	290 (183)	276 (223)	0.715
Ca (mmol/l)	2.48 (0.46)	2.43 (0.13)	0.479	2.45 (0.41)	2.45 (0.12)	0.971
GGT (IU/l)	16.9 (16.6)	23.1 (32.1)	0.183	18.7 (16.8)	22.4 (35.0)	0.482
AST (IU/l)	13.6 (8.6)	15.7 (10.9)	0.254	15.6 (9.3)	14.1 (10.8)	0.435
ALT (IU/l)	11.7 (8.9)	17.4 (14.4)	0.011	13.7 (10.9)	16.5 (14.3)	0.254
Albumin (g/l)	45.6 (4.3)	53.2 (62.0)	0.314	45.7 (4.6)	55.0 (69.0)	0.329
Bone scan index	0.93 (4.2)	0.33 (0.18)	0.338	0.82 (3.8)	0.32 (0.17)	0.307
Histotype (*n*)			0.196			0.717
Common	15	25		21	19	
Osteoblast	3	0		3	0	
Chondroblast	7	16		10	13	
Fibroblast	6	5		6	5	
Telangiectatic	12	14		14	12	
Sclerosing	1	4		3	2	
Small cell	4	2		3	3	
Extra-osseous	0	1		1	0	
Primary Site			0.015			0.022
Humerus	3	9		4	8	
Forearm	3	0		3	0	
Femur	20	40		26	34	
Lower leg	18	11		20	9	
Others	4	7		8	3	
Tumour volume (cm^3)	244	529	<0.001	302	533	0.002
Metastases (%)				14 (23)	53 (98)	<0.001

Table 2. Univariate hazard ratios of patient characteristics for death and for the development of metastases during follow-up. (*HR* hazard rate, *CI* confidence interval)

	HR (95% CI) for metastases	*P*	HR (95% CI) for survival	*P*
Gender	0.97 (0.83–1.16)	0.966	1.01 (0.90–1.36)	0.972
Age	1.01 (0.91–1.13)	0.228	1.03 (0.88–1.19)	0.048
Malignancy grade	0.84 (0.55–1.28)	0.733	4.51 (1.32–15.40)	0.021
Histology	0.93 (0.71–1.22)	0.273	1.08 (0.82–1.43)	0.369
Site	0.75 (0.44–1.28)	0.057	0.65 (0.34–1.26)	0.023
LDH	1.00 (0.96–1.04)	0.177	1.00 (0.97–1.03)	0.574
ALP	1.00 (0.98–1.03)	0.033	1.00 (0.96–1.04)	<0.001
Calcium	0.64 (0.33–1.25)	0.534	1.59 (0.81–3.16)	0.710
ASAT	0.98 (0.85–1.13)	0.563	0.93 (0.38–1.11)	0.067
ALAT	1.02 (0.89–1.17)	0.318	0.99 (0.93–1.07)	0.824
GGT	1.01 (0.90–1.14)	0.015	1.02 (0.88–1.18)	0.003
Albumin	1.00 (0.95–1.06)	0.095	1.00 (0.95–1.05)	0.282
Bone-scan index	0.79 (0.49–1.28)	0.795	0.10 (0.02–0.46)	0.159
Uptake pattern	1.33 (0.78–2.56)	0.367	1.01 (0.91–1.13)	0.974
Intensity of uptake	1.35 (0.78–2.35)	0.185	0.89 (0.63–1.25)	0.656
Tumour volume	1.00 (0.96–1.04)	0.001	1.00 (0.97–1.03)	0.020
Metastases	–	–	168.00 (17.47–1615.86)	<0.001

cut-off value that could be used to differentiate between high- and low-risk patients.

Regarding the reassessment of the bone scans, only the presence of metastases was highly important for prognostic stratification of the patients with an osteosarcoma. All additional parameters, such as the bone-scan index and uptake at the primary site, scored on the whole body scans were irrelevant and did not provide prognostic information. Due to the limited number of patients presenting with 2 (score 2) or more metastases (score 3), statistical analysis was not performed.

Discussion

Primary osteosarcoma is a rare malignancy that is characterised by atypical cells that produce osteoid (matrix protein produced by bone cells). The typical

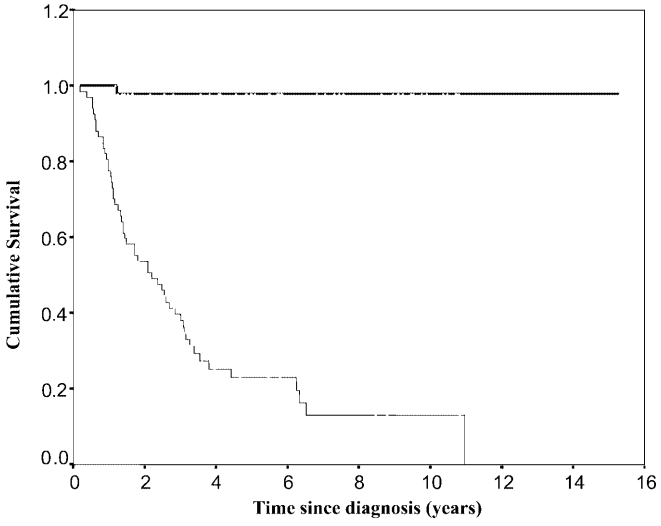

Fig. 1. Kaplan-Meier curves for osteosarcoma patients with and without metastases

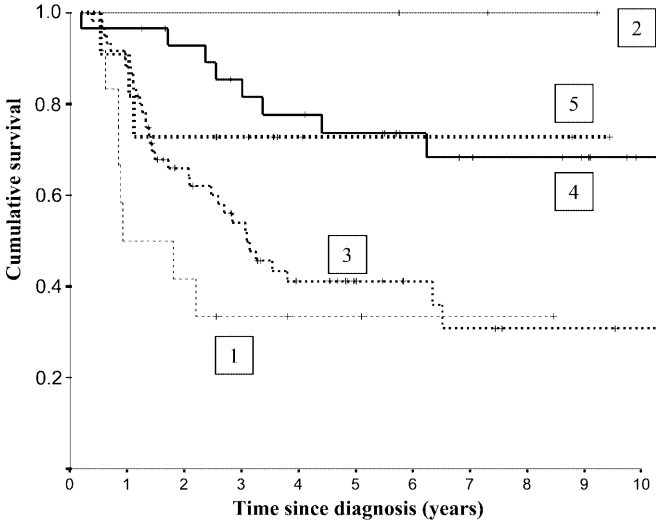

Fig. 2. Kaplan-Meier curves for primary tumour sites in osteosarcoma. *1* humerus, *2* forearm, *3* femur, *4* tibia, *5* not further specified

patient with osteosarcoma is between the ages of 10 years and 20 years, most commonly male, with complaints of pain for 1 month to 3 months. In a review of 600 cases by Dahlin et al. [6], 75% have these typical features. Despite the recognition that staging is valuable in both treatment planning and evaluation of treatments there is no universally accepted staging system in osteosarcoma. The surgical staging system proposed by Enneking, which is based on tumour grade, location, and the presence or absence of metastases, is easy to use clinically, but it suffers highly from significant oversimplification. The present study was performed to assess the prognostic value of commonly tested additional parameters in untreated osteosarcoma patients to differentiate low-risk from high-risk patients.

Presence of metastases

The overall survival found in the present study is in agreement with data found in the literature. In this respect, the presence of metastases is one of the most important prognostic factors [7]. Harris et al. [8] studied thirty patients aged younger than 30 years and demonstrated that patients with more than eight pulmonary metastases at diagnosis had an estimated 5-year event-free survival rate of 25% compared with 66.7% for patients with less than eight nodules. In a study by Meyers et al. [9], location of metastatic disease was significantly correlated with survival. Seven out of 48 patients with lung metastases survived with a median survival of 18 months for bilateral and 36 months for unilateral nodes. In contrast, none of the patients with bone metastases ($n=9$) survived. In the present study, the number of bone metastases was not evaluated as the number of patients presenting with bone metastases was limited ($n=8$). Nevertheless, for staging and prognostic stratification, bone scintigraphy and CT-scanning are highly important. During follow-up, CT-scanning is the first test of choice in case of suspected tumour recurrence as in all patients with tumour recurrence the lungs were involved. In contrast, bone scintigraphy appears not to be useful as routine follow-up investigation. This finding is in agreement with reports by Korholz et al. [10, 11]. As the maximum interval between initial diagnosis and tumour recurrence (i.e., the recurrence of metastases) is 5.3 years, intensive follow-up seems not to be required anymore after this period.

Primary tumour: size, site, and bone scintigraphy

In this study, the primary tumour site was significantly correlated with survival. Patients with osteosarcoma of the humerus or femur had a lower survival rate compared with osteosarcoma at other sites. The anatomic site was not defined in a consistent manner among other studies. In a study by Saeter et al. [5], proximal extremity sarcomas have worse prognosis than more distal located tumours, but the type of extremity localisation has not been a significant factor in multivariate analysis. Pochanugool et al. [12] found a 9-year survival probability of 50%, 68%, 42%, and 75% for femurs ($n=65$), tibias ($n=35$), humerus ($n=13$), and other bones ($n=13$), respectively ($P=0.81$). However, non- or upper extremity sites are often associated with large tumour volumes, which is found to be a more important factor [13]. The reported relapse rate of tumours larger than 150 cm^3 is in the range of 40–60% compared with less than 10% in smaller tumours. In a study by Bielack et al. [14] including 925 osteosarcoma patients, tumour size and site were also significantly correlated with survival. One of the major limitations of their study is the selection of high-grade central tumours of the extremities only in patients aged younger than 40 years. In Ewing's sarcoma, a tumour diameter over 8–10 cm is a well-

established prognostic sign, irrespective of tumour localisation [15]. In the present study, logistic regression analysis of the parameters tested indeed demonstrated that tumour volume was significantly correlated with tumour relapse and survival. When all the variables were evaluated by stepwise proportional hazard analysis, however, it lost its significance as predictor of survival.

In the present study, we have reviewed all bone scans to reassess the uptake at the site of the primary tumour. The uptake of the radiopharmaceuticals used for bone scanning is dependent on both vascular supply and a network of osteocytes and, in general, areas of increased uptake correspond to areas of accelerated bone turnover. Bone scintigraphy is not only a valuable technique for the assessment of distant metastases, it is also helpful in monitoring therapy effects without any additional cost or radiation dose [16, 17]. In this respect, a decrease in tumour blood flow and an irregular uptake pattern following therapy is highly correlated with tumour necrosis and a good therapy response. Such features on bone scintigraphy prior to treatment, however, may also represent a poor vascular supply and necrosis and may be indicative for tumours that poorly respond to chemotherapy. In the present study, neither the pattern and intensity of uptake nor the bone-scan index were correlated with a poor outcome. Consequently, such parameters are clinically irrelevant and cannot be used for a better selection of prognostic subgroups.

Age

Some previous studies have indicated young age to be an adverse factor in osteosarcoma, whereas others have not [18, 19, 20, 21]. However, due to the limited age-ranges included in most of these studies – most did not include very young or very old patients – it was not possible to draw any conclusions regarding the prognostic value of age. In a study by Meyers et al. [9], survival was significantly better for older patients ($P = 0.04$), but patients with clinically detectable metastases at initial presentation were studied. In the present study, age ranged from 3.5 years to 78.0 years with a mean age of 25.6 years. Despite the significant but weak correlation between age and survival ($P = 0.048$), it is not helpful in further prognostic stratification. With respect to gender, histology, and malignancy grade, a limited number of studies found male gender and telangiectatic osteosarcoma to be independently significant factors, but they lost their significance by multivariate analysis in all but in a limited number of studies including a small number of patients.

Biochemical parameters

It is known that many patients with osteosarcoma have high serum alkaline phosphatase (ALP) levels. Thorpe et al. [22] described one of the first reports on the prognostic value of this parameter in 1979. In 12 out of 17 patients with elevated serum levels, recurrence was found compared to only four out of 13 patients with normal values. In a study by Bacci et al. [23] considering 656 patients with primary osteosarcoma, similar results were found. The percentage of patients with increased ALP levels was significantly higher in the metastatic group than in the group of patients with localised disease (91.5% vs 61.3%, $P < 0.001$). In addition, the relapse rate was significantly higher in the metastatic group with elevated serum ALP levels than in those with normal levels. In the present study, there was no significant difference in ALP levels between patients achieving complete remission and those demonstrating tumour recurrence ($P = 0.103$). Comparable results were seen with respect to survival ($P = 0.152$). Despite the fact that higher serum levels are correlated with a higher chance of tumour recurrence and a poor survival, ALP cannot be used to identify subgroups. Our findings are in agreement with the results reported by Pochanugool et al. [12]. They found a 9-year survival probability of 58% and 45% in patients with ALP levels < 400 IU/L and > 400 IU/L, respectively, which was not significantly different ($P = 0.63$).

In some reports, LDH is described to be of prognostic value with respect to survival. In a study by Bacci et al. [24], it was found that the percentage of patients who had an elevated serum LDH at the time of diagnosis was significantly higher in those patients with metastatic disease than in those who presented with localised disease (64% vs 33%, $P < 0.001$). For those who presented with localised disease and had an increased serum LDH level, far more patients ultimately developed a relapse of disease (60% vs 38%, $P < 0.001$). In a study by Link et al. [3], similar results were found. At 6 years, the event-free survival for patients with elevated LDH levels was 41% compared to 69% for the remaining patients who had normal LDH at diagnosis ($P < 0.001$). Their study, however, included patients younger than 30 years of age only without evidence of metastatic disease and high-grade osteosarcoma. Furthermore, LDH levels before definitive surgery were used for evaluation. In contrast to these results, our study revealed that LDH is not helpful in identifying subgroups at initial stage as the pre-treatment values in patients with a poor survival did not significantly differ from those with a good survival ($P = 0.715$).

Glycoproteins

Recent reports on prognostic factors in osteosarcoma have emphasised the expression of glycoproteins [25]. In this respect, tumour cell expression of the multidrug resistance (MDR) gene has recently been identified as a strong adverse prognostic factor. CD44, a transmembranous glycoprotein, seems to be related to the degree of tumour differentiation, tumour cell invasion, and metastasis. This may be an explanation for the reduced

chemosensitivity of metastatic osteosarcoma as found by Bacci et al. [2]. Data on long term follow-up, however, are scarce and therefore a relation between tumour response, glycoprotein expression, and event-free survival has not yet been established.

Treatment and prognosis

In one of the first studies in the 1970s on osteosarcoma treated by surgical ablation of the primary tumour, progression to metastatic disease was seen in 80–90% of the patients shortly after surgery. It is known that the rate of local recurrence significantly correlates with the surgical margins. In a recent report by Bacci et al. [26], again it was unequivocally shown that patients with a marginal (R1) or intralesional (R2) surgical margin, the local recurrence rate was significantly higher than for patients with radical or wide margins (R0): 38% vs 7%; $P < 0.02$. Therefore, the objective of surgery should be a complete resection of the primary tumour with wide margins. In this respect, there is no significant difference in outcome reported between patients who underwent limb-sparing surgery and the patients who had amputation [26, 27].

Currently, an aggressive chemotherapy regimen before (neoadjuvant) and/or after (adjuvant) surgery is the standard treatment in osteosarcoma patients [26, 28, 29, 30]. These regimens, which generally include high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide given in various combinations, are superior to single-agent treatments. In many reports, event-free or disease-free survival and survival appear to be directly related to the histological response to neoadjuvant chemotherapy. The difference between patients with total necrosis and patients without total necrosis is found to be statistically significant [14, 20, 28, 29, 30, 31]. A major problem in this respect, however, is that an objective scoring system has not yet been described and it is by no means clear that changing chemotherapy in non-responders can alter the prognosis. In addition, it is difficult or impossible to assess the response of osseous osteosarcoma radiographically as these lesions rarely change in size after chemotherapy. Consequently, the evaluation of histological response to chemotherapy in resected tumours remains the most reliable means of distinguishing two groups with different prognosis. Therefore, it was not evaluated in the present study on pre-treatment prognostic parameters.

Study limitations

One of the limitations of the present study is the retrospective analysis. It may have caused a selection bias as patients without any treatment were selected for evaluation. Due to the fact that chemotherapy definitely influences the value of most of the parameters tested, it was decided not to include patients that were already treated. Furthermore, patients that were referred for imaging, but not treated in our hospital, were also excluded. Based on the present results, however, a prospective study is initiated to assess the prognostic value of the expression of glycoproteins in addition to the other parameters tested. All patients referred to our hospital because of a newly diagnosed untreated osteosarcoma will be included in this study.

Currently, MRI is the most sensitive single method for non-invasively assessing medullar involvement by tumour. MRI is superior to CT in differentiating tumour from adjoining muscle and it is highly accurate in assessing extra-osseous soft tissue extension. In the present study, tumour volume and extension was assessed on histological examination. Despite the fact that histological examination is still the golden standard, the results may have been slightly underestimated as these primary tumour-related parameters were obtained after two or three courses of chemotherapy. In the initiated prospective study, tumour volume, diameters, and extension will be measured with MRI to obtain values that are not influenced by any kind of treatment.

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